World's Veterinary Journal

BOOKLET

Volume 14, Issue 3, September 2024

2024, Scienceline Publication

World's Veterinary Journal

World Vet J, 14(3): 293-475, September 25, 2024

ISSN 2322-4568

Editors-in-Chief

Managing Editor

Associate Editors

Language Editors

Statistical Editor

Technical Editor

Editorial Team

Advisory Board

Join WVJ Team

World's Veterinary Journal is always striving to add diversity to our editorial board and operations staff. Applicants who have previous experience relevant to the position they are applying for may be considered for more senior positions within WVJ. All other members must begin as section reviewer before progressing on to more senior roles. Editor and editorial board members do not receive any remuneration. These positions are voluntary.

If you are currently an undergraduate, MSc or PhD student at university and interested in working for WVJ, please fill out the application form below. Once your filled application form is submitted, the board will review your credentials and notify you within a week of an opportunity to membership in editorial board. If you are PhD, assistant, associate editors, distinguished professor, scholars or publisher of a reputed university, please rank the mentioned positions in order of your preference. Please send us a copy of your resume (CV) or your ORCID ID or briefly discuss any leadership positions and other experiences you have had that are relevant to applied poultry research, Researches or publications. This includes courses you have taken, editing, publishing, web design, layout design, and event planning. If you would like to represent the WVJ at your university, join our volunteer staff today! WVJ representatives assist students at their university to submit their work to the WVJ. You can also, registered as a member of journal for subsequent contacts by email and or invitation for a honorary reviewing articles.

Download WVJ Application Form

Contact us at editor [at] wvj.science-line.com

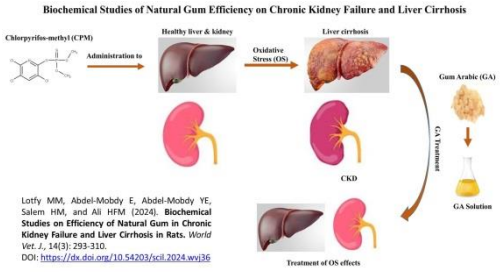
TABLE OF CONTENTS

Volume 14 (3); September 25, 2024

Research Paper

Biochemical Studies on Efficiency of Natural Gum in Chronic Kidney Failure and Liver Cirrhosis in Rats

World Vet. J. 14(3): 293-310, 2024; pii:S232245682400036-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj36



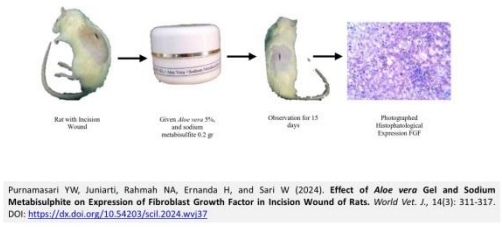
ABSTRACT: It is well-established that apoptosis, oxidative stress, and inflammation are associated with several disorders, including chronic renal disease and hepatic disease. Oxidative stress (OS) is a major cause of death from end-stage renal disease which also contributes to atherosclerosis and cardiac issues. The present study aimed to assess the efficacy of Gum Arabic (GA) in mitigating renal damage and hepatotoxicity in rats induced by Chloropyrifos-methyl (CPM). A total of 42 male Wistar rats were divided into seven groups, with four groups (group 2 [IC], group 5 [GA1+IC]a, group 6 [GA2+IC], and group 7 [GA1+IC]b treated with CPM for eight weeks to induce hepatic and renal damage. Two models of GA administration, including the standard oral model in drinking water (15% w/v) and the oral model by gavage at a dose of 1 g/kg body weight were administered. Physiological parameters of kidney and liver functions, including urea, creatinine, AST, and ALT along with anti-oxidant factors (Melaodialdehyde, superoxide dismutase, reduced glutathione, and catalase) were measured in plasma, and homogenates of renal and hepatic tissues on day 57 of the experiment. In addition, histopathological examination was conducted on liver and kidney tissues using hematoxylin and eosin stain to evaluate the efficacy of GA on damaged tissues. Gum Arabic was found to significantly reduce CPM toxic effects in the liver and kidney in groups treated with CPM as liver and kidney parameters were reduced to normal levels. Furthermore, GA reduced histological indicators of inflammation, fibrosis, and apoptosis, as well as renal morphological damage. Additionally, it reduced OS in liver and kidney homogenates. In conclusion, GA effectively reduced the damage that CPM inflicted on liver and kidney tissue by stabilizing physiological parameters to normal levels and repairing cellular structures damaged by OS.
Keywords: Antioxidant, Anti-inflammatory, Gum Arabic, Kidney, Liver, Oxidative stress

[Full text-PDF] [Crossref Metadata]

Research Paper

Effect of *Aloe vera* Gel and Sodium Metabisulphite on Expression of Fibroblast Growth Factor in Incision Wound of Rats

World Vet. J. 14(3): 311-317, 2024; pii:S232245682400037-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj37



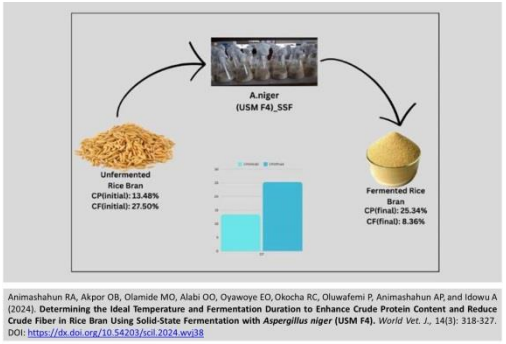
ABSTRACT: An incision wound is a wound caused by being sliced. Two ingredients that play a key role in the wound-healing process are glucomannan and acemannan, which are rich in polysaccharides and growth hormones. Growth hormones stimulate fibroblast activity and proliferation. The present study involved 35 Sprague Dawley male rats, aged 2-3 months old and weighing 200-300 grams. The study comprised seven groups including, negative control group (G1), positive control (aquades, G2), betadine 10% (G3), gel base (0.5 mg, G4), gel base + sodium metabisulfite 0.2 gr (G5), gel base + *Aloe vera* 5% (G6), and gel base + *Aloe vera* 5% + sodium metabisulfite 0.2 gr (G7). Each group had five replications. Initially, a 4-cm incision was made on the dorsal skin of each rat. The study lasted 15 days with observations made on days 3, 7, and 15. After the observation period, the rats were anesthetized and then terminated to collect skin tissues for microscopic examination. The tissue samples were then stained immunohistochemically to assess fibroblast growth factor (FGF) expressions. The results showed that the highest FGF expression was observed in the 5% *Aloe vera* + 2% metabisulfite group (G7), while the lowest FGF expression was in the negative control group (G1). It is concluded that *Aloe vera* L. extract gel at 5% + 2% metabisulfite (G7) significantly enhances the expression of FGF.
Keywords: *Aloe vera* L, Fibroblast growth factor, Incision wound, Skin, Sodium metabisulfite

[Full text-PDF] [Crossref Metadata]

Research Paper

Determining the Ideal Temperature and Fermentation Duration to Enhance Crude Protein Content and Reduce Crude Fiber in Rice Bran Using Solid-State Fermentation with *Aspergillus niger* (USM F4)

Animashahun RA, Akpor OB, Olamide MO, Alabi OO, Oyawoye EO, Okocha RC, Oluwafemi P, Animashahun AP, and Idowu A.
World Vet. J. 14(3): 318-327, 2024; pii:S232245682400038-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj38



Animashahun RA, Akpor OB, Olamide MO, Alabi OO, Oyawoye EO, Okocha RC, Oluwafemi P, Animashahun AP, and Idowu A (2024). **Determining the Ideal Temperature and Fermentation Duration to Enhance Crude Protein Content and Reduce Crude Fiber in Rice Bran Using Solid-State Fermentation with *Aspergillus niger* (USM F4).** *World Vet. J.*, 14(3): 318-327. DOI: https://dx.doi.org/10.54203/scil.2024.wvj38

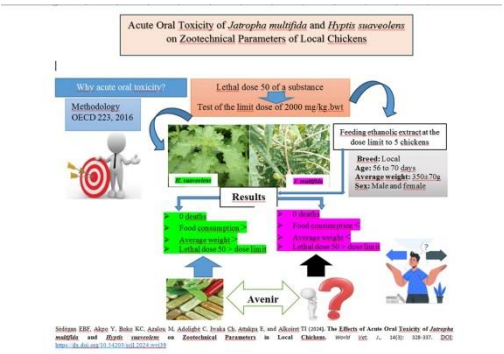
ABSTRACT: Solid-state fermentation (SSF) offers a sustainable method for enhancing the nutritional quality of agricultural residues such as red rice bran. This study aimed to determine the optimal temperature and duration for SSF of red rice bran, focusing specifically on increasing the crude protein (CP) content and reducing the crude fiber (CF) content. SFF of rice bran with *Aspergillus niger* (*A. niger*) USM F4 was conducted over 14 consecutive days at three different temperatures (25°C, 35°C, and 45°C). A total of 63 samples of rice bran were divided into three temperature groups, each containing 21 samples. Three samples per group were collected at 48-hour intervals over the 14-day fermentation period. The fermentation process for the collected samples at 48-hour intervals was halted by oven drying at 60°C for 24 hours. The fermented products were subjected to proximate analysis for crude protein (CP), ash, ether extract (EE), and crude fiber (CF) contents using the methods outlined by the Association of Official Analytical Chemists (AOAC). The results revealed a significant effect of temperature and fermentation duration on CP, ash, EE, and CF content when compared to the unfermented rice bran kept at room temperature (25°C). The peak values of CP and the highest degradation of CF across all temperature levels were observed on day 10 while the maximum increase in ash and EE content occurred on day 8. Among the temperature conditions, the highest CP values and the lowest CF values were recorded at 35°C. Conversely, the lowest improvements in CP and CF degradation were observed at 25°C on day 10. In conclusion, the optimal conditions for SSF of rice bran with *A. niger* to enhance CP content and degrade CF are a temperature of 35°C and a fermentation duration of 10 days.
Keywords: Alternate feed resource, Animal production, *Aspergillus niger*, Proximate component, Solid-state fermentation, Value- addition

[Full text-PDF] [Crossref Metadata]

Research Paper

The Effects of Acute Oral Toxicity of Jatropha multifida and Hyptis suaveolens on Zootechnical Parameters in Local Chickens

Sèdégan EBF, Akpo Y, Boko KC, Azalou M, Adoligbé C, Iwaka Ch, Attakpa E, and Alkoiret TI.
World Vet. J. 14(3): 328-337, 2024; pii:S232245682400039-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj39



Sèdégan EBF, Akpo Y, Boko KC, Azalou M, Adoligbé C, Iwaka Ch, Attakpa E, and Alkoiret TI (2024). **The Effects of Acute Oral Toxicity of *Jatropha multifida* and *Hyptis suaveolens* on Zootechnical Parameters in Local Chickens.** *World Vet. J.*, 14(3): 328-337. DOI: https://dx.doi.org/10.54203/scil.2024.wvj39

ABSTRACT: The substantial use of medicinal plants in traditional poultry farming is a well-established practice. The present study aimed to determine the median lethal dose (LD_{50}) of ethanolic extracts of *Jatropha multifida* and *Hyptis suaveolens* in local chickens and to assess the effects of these extracts on feed intake, water intake, and average body weight. The methodology followed OECD Directive 223, which involves administering the highest dose of the extract to the chickens and assessing any mortality. Three homogeneous groups of five chickens each were formed for the limit dose test. The control group received distilled water, while batches 1 and 2 received 700 mg of ethanolic extract of *J. multifida* and *H. suaveolens*, respectively. The average body weight of the chickens was 350g ± 20, and the extracts were administered via gavage at a suspension of 2000 mg/kg.bwt of the extract dissolved in water. The results of the phytochemical tests indicated the presence of several chemical compounds known for their therapeutic effects. The productivity of the extract was 2.75 ± 0.19 for *J. multifida* and 3.3 ± 0.27 for *H. suaveolens*. After administration of the suspensions, observation for 14 days revealed no mortality. This finding indicated that the LD_{50} of the utilized ethanolic extracts exceeds the limit dose (2000 mg/kg.bwt). However, feed intake (49 ± 3 > 46±4) and average body weight (436 ± 31 > 388 ± 37) in batch 2 were significantly higher than those in batch 1. Future research should explore the subacute toxicity of *J. multifida* and *H. suaveolens* across various chicken breeds.
Keywords: Body weight, Lethal Dose, Local chicken, Medicinal plant, Mortality, Toxicity

[Full text-PDF] [Crossref Metadata]

Research Paper

Effects of Butylated Hydroxytoluene and Sorbitol as Diluent Components on Structural and Surface Ultrastructural Changes of Gaga Chicken Sperm During Cryopreservation

Khaeruddin, Ciptadi G, Yusuf M, Suyadi, Natsir MH, Hermadi HA, and Wahjuningsih S.
World Vet. J. 14(3): 338-348, 2024; pii:S232245682400040-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj40

ABSTRACT: The Gaga chicken is an indigenous Indonesian breed that is important to preserve using semen cryopreservation technology. The study was conducted to determine the effect of adding sorbitol and butylated hydroxytoluene (BHT) in the diluent on the structural and surface ultrastructure of cryopreserved Gaga chicken sperm during cryopreservation /frozen storage. The study aimed to assess how adding sorbitol and butylated hydroxytoluene (BHT) to the diluent affects the structure and surface ultrastructure of cryopreserved Gaga chicken sperm. A completely randomized design was employed with four treatments and 10 replications including egg yolk-lactate ringer diluent (EYLR) as the control group, EYLR diluent with 3 mM BHT, EYLR diluent with 2% sorbitol, and EYLR diluent with both 3 mM BHT and 2% sorbitol. Semen was collected using a massage technique from 4 male chickens aged approximately 10 months, pooled semen was diluted, packaged in 0.25 mL straws, equilibrated for 2 hours at 5 °C, pre-freeze for 10 minutes, frozen for 24 hours, and thawed for 30 seconds at 37 °C. The parameters evaluated were sperm plasma membrane integrity, acrosome integrity, DNA damage, mitochondrial functionality, and surface ultrastructure. The results showed that the treatment had a significant effect on plasma membrane integrity and post-thawing mitochondrial functionality compared to the control, but no effect was observed on acrosome integrity or DNA damage. The results showed that the combination treatment of BHT with sorbitol had a significant effect on plasma membrane integrity and post-thawing mitochondrial function, but did not affect acrosome integrity or DNA damage when compared to the control group. Ultrastructural observations indicated that cryopreservation caused damage to the head, middle, and tail of the sperm in the control groups. However, these changes were prevented by the diluent containing a combination of BHT and sorbitol. The addition of both components (BHT 3 mM + sorbitol 2%) effectively maintained plasma membrane integrity, mitochondrial functionality, and surface ultrastructure of Gaga chicken sperm during cryopreservation.
Keywords: Butylated hydroxytoluene, Chicken sperm, Cryopreservation, Sorbito, Structure, Sperm ultrastructure

Khaeruddin, Ciptadi G, Yusuf M, Suyadi, Natsir MH, Hermadi HA, and Wahjuningsih S (2024). **Effects of Butylated Hydroxytoluene and Sorbitol as Diluent Components on Structural and Surface Ultrastructural Changes of Gaga Chicken Sperm During Cryopreservation.** *World Vet. J.*, 14(3): 338-348. DOI: https://dx.doi.org/10.54203/scil.2024.wvj40

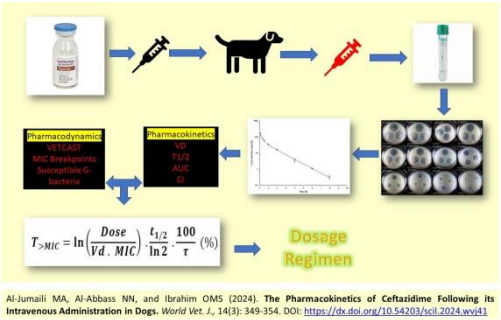
[Full text-PDF] [Crossref Metadata]

Research Paper

The Pharmacokinetics of Ceftazidime Following its Intravenous Administration in Dogs

Al-Jumaili MA, Al-Abbass NN, and Ibrahim OMS.
World Vet. J. 14(3): 349-354, 2024; pii:S232245682400041-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj41

ABSTRACT: Ceftazidime is a beta-lactam that is used in the treatment of bacterial infections in humans and companion animals, such as dogs and cats. It is prescribed to treat gram-negative infections, especially those caused by Pseudomonas aeruginosa. This study aimed to compare the pharmacokinetics of ceftazidime using a microbiological assay to evaluate the adequacy of the proposed dosage regimens for susceptible gram-negative bacteria. For this purpose, five healthy mongrel male dogs, with a mean age of four years and an average weight of 19.1 kg, were administered a single intravenous bolus dose of ceftazidime (20 mg/kg). Plasma concentrations were measured using a microbiological assay, and dosage regimens were established by integrating pharmacokinetics data with pharmacodynamics parameters. The results showed that ceftazidime was rapidly distributed to the peripheral tissues (0.189 L/kg), with a half-life of 1.15 hours and a clearance rate of 0.166 L/hr./kg. The results obtained from the pharmacokinetics-pharmacodynamic integration suggested 20 mg/kg q8 hours of ceftazidime for susceptible gram-negative bacteria with a Minimum Inhibitory Concentration of ≤ 8 µg/ml, and 20 mg /kg q12 hours of ceftazidime for susceptible gram-negative bacteria with a Minimum Inhibitory Concentration of ≤ 4 µg/ml. In conclusion, a mild correlation was observed between the dogs' weight and the ceftazidime half-life, which led to an adjustment of the proposed dosage regimen to 20 mg/kg q8 hours.
Keywords: Ceftazidime, Dog, Dosage regimen, Gram-negative microbe, Pharmacokinetic



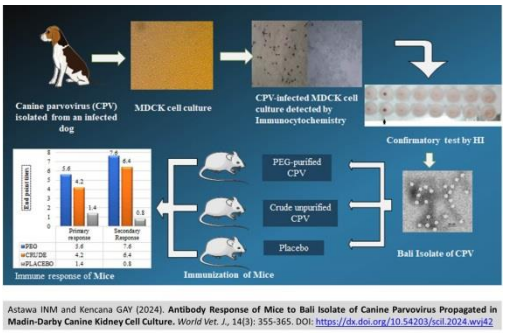
Al-Jumaili MA, Al-Abbass NN, and Ibrahim OMS (2024). **The Pharmacokinetics of Ceftazidime Following its Intravenous Administration in Dogs.** *World Vet. J.*, 14(3): 349-354. DOI: https://dx.doi.org/10.54203/scil.2024.wvj41

[Full text-PDF] [Crossref Metadata]

Research Paper

Antibody Response of Mice to Bali Isolate of Canine Parvovirus Propagated in Madin-Darby Canine Kidney Cell Culture

Astawa INM and Kencana GAY.
World Vet. J. 14(3): 355-365, 2024; pii:S232245682400042-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj42



Astawa INM and Kencana GAY (2024). **Antibody Response of Mice to Bali Isolate of Canine Parvovirus Propagated in Madin-Darby Canine Kidney Cell Culture.** *World Vet. J.*, 14(3): 355-365. DOI: https://dx.doi.org/10.54203/scil.2024.wvj42

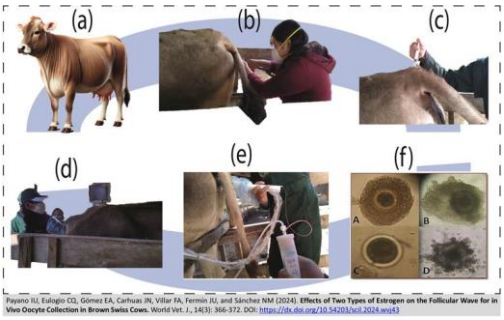
ABSTRACT: Canine parvovirus (CPV) infection is still common among dogs, leading to severe disease with high mortality. The potential of a local isolate of CPV as an effective vaccine to prevent the disease warrants investigation. This study aimed to determine the antibody response in mice against a Bali isolate of CPV propagated in the Madin-Darby Canine Kidney (MDCK) cell culture. The virus was purified using polyethylene glycol (PEG)-6000 and mixed with an Aluminum hydroxide adjuvant. Fifteen 7-week female mice were divided into three treatment groups: treatment group 1 (PEG-purified virus and Adjuvant), treatment group 2 (crude unpurified virus and adjuvant), and treatment group 3 (adjuvant without virus), with five replicates per group. The Bali isolate of CPV was successfully replicated in MDCK cells, achieving a titer of 2^{10}-2^{11} hemagglutination (HA) units after eight serial passages through the cell culture. The virus was confirmed as CPV by immunocytochemistry test using a monoclonal antibody and hemagglutination inhibition (HI) test using chicken anti-CPV polyclonal antibody. Following the first immunization, the antibody endpoint titer in mice immunized with PEG-purified CPV (5.6) was significantly higher than those immunized with crude unpurified CPV (4.2) and adjuvant without CPV (1.4). Similarly, after the second immunization, the antibody endpoint titer in mice immunized with PEG-purified CPV (7.6) also remained significantly higher than those immunized with crude unpurified CPV (6.4) and adjuvant without CPV (0.8). Significant increases in antibody endpoint titer were observed after the second immunization in mice immunized with PEG-purified CPV and crude unpurified CPV, but not in those given adjuvant without CPV. The Bali isolate of CPV propagated in MDCK cell culture induced a robust antibody response in mice, suggesting it's a potential as an alternative vaccine candidate for preventing CPV infection in dogs.
Keywords: Bali, Canine parvovirus, Madin-Darby Canine Kidney, Mice, Vaccines

[Full text-PDF] [Crossref Metadata]

Research Paper

Effects of Two Types of Estrogen on the Follicular Wave for in Vivo Oocyte Collection in Brown Swiss Cows

Payano IU, Eulogio CQ, Gómez EA, Carhuas JN, Villar FA, Fermín JU, and Sánchez NM.
World Vet. J. 14(3): 366-372, 2024; pii:S232245682400043-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj43



Payano IU, Eulogio CQ, Gómez EA, Carhuas JN, Villar FA, Fermín JU, and Sánchez NM (2024). **Effects of Two Types of Estrogen on the Follicular Wave for in Vivo Oocyte Collection in Brown Swiss Cows.** World Vet. J., 14(3): 366-372. DOI: https://dx.doi.org/10.54203/scil.2024.wvj43

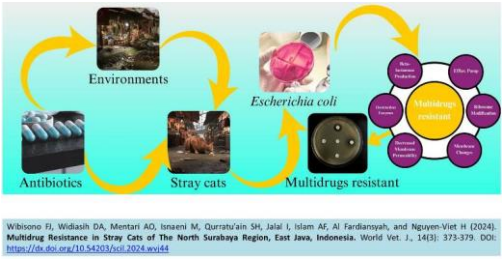
ABSTRACT: The manipulation of follicular waves through hormonal treatments, such as estrogen administration, plays a crucial role in optimizing *in-vivo* oocyte collection for assisted reproductive technologies. The present study aimed to evaluate the effect of two specific types of estrogen on follicular wave dynamics and their impact on *in-vivo* oocyte collection in Brown Swiss cows. Fourteen cows, in their first lactation, weighing approximately 340 kg were randomly assigned to one of two treatments including T1 (estradiol cypionate) and T2 (estradiol benzoate). Both treatments were administered at 1.2 mg of estrogen, at day 0 of the experiment. All Brown Swiss cows were provided with a diet entirely consisting of alfalfa grazing. On day 7, follicular wave dynamics were assessed using a DP-50 vet ultrasound device equipped with a 7.5 MHz transducer for transvaginal follicular aspiration guidance. Follicle counts were categorized into three size ranges including 2-4 mm (small), 4-8 mm (medium), and greater than 8 mm (large). Additionally, the quantity and quality (viable oocytes) of the collected oocytes were evaluated by the Ovum Pick Up (OPU) team for oocyte viability on day 7. The study assessed the follicular dynamics (number of follicles) and efficiency of oocyte collection (viable oocytes) in cows treated with Estradiol Cypionate (T1) and Estradiol Benzoate (T2). The average number of small, medium-sized, and large follicles size were 6.048 ± 6.037, 3.16 ± 2.01, and 0.53 ± 0.67 respectively. The total number of follicles was 9.59 ± 3.56. The mean number of viable oocytes recovered was 3.024 ± 1.66, while the mean number of non-viable oocytes was 1.47 ± 1.01. The results indicated no significant differences between treatments in the size of small, medium, and large follicles, nor in the total number of follicles and viable oocytes recovered. However, a significant difference was observed in the number of non-viable oocytes recovered, with a higher mean in T2 (1.86) compared to T1 (1.09). The results indicated an adequate follicular response and viable oocyte recovery in both treatment groups (estradiol cypionate and estradiol benzoate). However, variations in oocyte viability were observed, with estradiol cypionate showing a slight advantage.
Keywords: Follicle count, Follicular wave dynamic, Oocyte collection, Transvaginal follicular aspiration

[Full text-PDF] [Crossref Metadata]

Research Paper

Multidrug Resistance in Stray Cats of The North Surabaya Region, East Java, Indonesia

Wibisono FJ, Widiasih DA, Mentari AO, Isnaeni M, Qurratu'ain SH, Jalal I, Islam AF, Al Fardiansyah, and Nguyen-Viet H.
World Vet. J. 14(3): 373-379, 2024; pii:S232245682400044-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj44



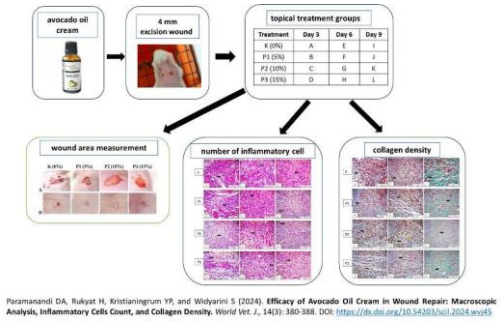
ABSTRACT: Stray cats survive by getting food or drink that is available in the environment, correspondingly, stray cats have relatively high exposure to antibiotic resistance obtained from resistant bacteria found in the environment. The present study was conducted to determine patterns of multidrug resistance and *Escherichia coli* resistance in stray cats. A total of 50 stray cat anal swab samples were taken randomly from the previously recorded stray cat population in the Surabaya area, East Java, Indonesia. Samples were brought using buffered peptone water. They were cultured on MacConkay Agar differential selective media, and all suspicious colonies of *Escherichia coli* were examined by biochemical tests. Isolates were then identified, and susceptibility testing was performed according to the Clinical and Laboratory Standards Institute. The results of the resistance test indicated that the multidrug resistance in *Escherichia coli* bacteria taken from cats was 14.6% (7/48). The high antibiotic resistance of *Escherichia coli* bacteria in stray cats, which were resistant to many drugs, provides an early warning of environmental health. Environmental health is closely related to animal and human health, especially antibiotic resistance.
Keywords: Antibiotic, Antimicrobial resistance, *Escherichia coli*, Multidrug resistance, Stray cat

[Full text-PDF] [Crossref Metadata]

Research Paper

Efficacy of Avocado Oil Cream in Wound Repair: Macroscopic Analysis, Inflammatory Cells Count, and Collagen Density

Paramanandi DA, Rukyat H, Kristianingrum YP, and Widyarini S.
World Vet. J. 14(3): 380-388, 2024; pii:S232245682400045-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj45



ABSTRACT: The wound healing process goes through a series of complex stages that are mutually continuous, namely inflammation, proliferation, and maturation. Following wound formation and the release of proinflammatory cytokines and growth factors, inflammation occurs immediately after the hemostasis phase. Proliferation occurs when products processed by the growth factors are present. The final stage, maturation, is portrayed by the plan of the extracellular network. This study utilized oil from avocado fruit (*Persea americana*), which contains linoleic and oleic acid content that supports skin tissue repair. The study aimed to explore the effects of using avocado oil cream on the area of wound healing, the number of inflammatory cells, and the collagen density. Twenty-four female mice, aged eight weeks, were used as experimental animals by making excision wounds using a 4 mm biopsy punch on the dorsal skin on the left and right sides. The mice were divided into four groups based on the percentage of avocado oil in the topical cream included Group K (control, topical cream without avocado oil), Group P1 (5% topical avocado oil cream), Group P2 (10% topical avocado oil cream), and Group P3 (15% topical avocado oil cream). Macroscopic examination of the wounds was conducted daily on days 3, 6, and 9 after topical cream treatment using a digital caliper. A total of 48 skin tissue samples were collected from days 3, 6, and 9 after cream application, which were then processed for histopathology evaluations using hematoxylin-eosin staining and Masson's Trichrome staining. Hematoxylin-eosin staining was used to count the inflammatory cells, and Masson's Trichrome staining was employed to assess collagen density. The results revealed that avocado oil had a great impact on wound closure after 9 days of 15% avocado oil cream treatment, reducing the inflammatory cells after 3-6 days of 10% avocado oil cream therapy, and increasing collagen density after 9 days of 15% avocado oil cream application, as compared to the control, non-avocado oil cream group. Avocado oil can help close wounds, reduced the number of inflammatory cells, and increased collagen density when used in topical pharmaceutical formulations. Avocado oil cream may, therefore, be considered a viable option for wound repair treatment.

Keywords: Avocado oil, Collagen, Inflammatory cell, Wound repair

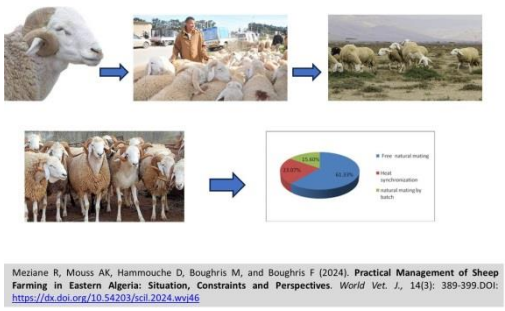
[Full text-PDF] [Crossref Metadata]

Research Paper

Practical Management of Sheep Farming in Eastern Algeria: Situation, Constraints and Perspectives

Meziane R, Mouss AK, Hammouche D, Boughris M, and Boughris F.
World Vet. J. 14(3): 389-399, 2024; pii:S232245682400046-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj46

ABSTRACT: Successful sheep farming requires hands-on management and a thorough understanding of the factors that influence it. This necessitates a practical, well-organized approach to ensure the flock's health, productivity, and profitability. Various factors, such as climate, available resources, and management strategies, play a crucial role in determining the success of sheep farming. This study, conducted in the Batna region of Algeria, aimed to assess sheep breeding practices and production performance through a questionnaire distributed to farmers and veterinarians. The study encompassed 14,124 sheep, including 9,435 ewes, from 33 Batna region farms to evaluate production and reproduction performance. The findings revealed that the Ouled Djellal breed is the most prevalent, comprising 61.02% of the sheep population. The results indicated that sheep farming in the region largely relies on traditional extensive grazing systems. It is worth noting that breeding practices have not consistently followed recommended guidelines, as evidenced by a suboptimal sex ratio of 36.28. Additionally, only 21.94% of breeders had employed heat synchronization methods, and artificial insemination was not utilized. The low adoption of artificial insemination is primarily attributed to factors, including a lack of knowledge regarding reproductive management, along with challenges related to illiteracy and limited access to essential resources. The analysis further demonstrated that all categories of sheep were profitable; however, profitability was influenced by factors, such as environmental conditions, feed availability, and the age of the animals. The study underscored significant findings, including the prevalence of the Ouled Djellal breed and the limited use of advanced breeding practices, such as artificial insemination, in the Batna region. The value of the current study lies in its comprehensive examination of traditional sheep farming practices and its recommendations for enhancing productivity. These include improving management practices, increasing access to resources, and promoting genetic improvement by adopting advanced breeding technologies.
Keywords: Ewe, Livestock management, Nutrition, Ouled Djellal, Performance



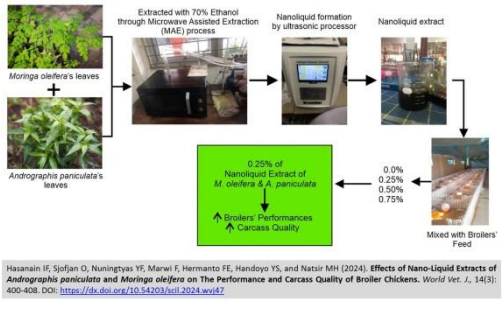
[Full text-PDF] [Crossref Metadata]

Research Paper

Effects of Nano-Liquid Extracts of Andrographis paniculata and Moringa oleifera on The Performance and Carcass Quality of Broiler Chickens.

Hasanain IF, Sjofjan O, Nuningtyas YF, Marwi F, Hermanto FE, Handoyo YS, and Natsir MH.
World Vet. J. 14(3): 400-408, 2024; pii:S232245682400047-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj47

ABSTRACT: Feed was crucial for achieving optimal productivity in broiler chickens, which required ongoing monitoring of its quantity and quality. The present study aimed to evaluate the effects of nanoliquid extracts from *Andrographis paniculata* and *Moringa oleifera* used as photobiotic on the performance and carcass quality of broiler chickens. The research involved 128 broiler chickens, which were divided into four treatment groups, each with four replications of eight broiler chickens. The treatments included a control group (T0), a 0.25% nano liquid extract mixture of *Andrographis paniculata and Moringa oleifera* (T1), a 0.50% nanoliquid extract mixture (T2), and a 0.75% nanoliquid extract mixture (T3). The study utilized an *in vivo* method and analysed the data using a completely randomized design. The optimal level of nano liquid extract was determined based on chicken performance (feed consumption, body weight, feed conversion ratio (FCR), income-over-feed cost (IOFC)) and carcass quality (carcass percentage, cooking loss, meat color, water-holding capacity, and texture). The findings indicated that the addition of combined *Andrographis paniculata* and *Moringa oleifera* (1:1, w/w) nano-liquid extract in the chickens' feed significantly influenced body weight, FCR, and IOFC. However, there was no significant effect on feed consumption. Furthermore, the use of *Andrographis paniculata* and *Moringa oleifera* combination had a significant impact on all carcass quality parameters beyond *b carcass color. It was concluded that the addition of 0.25% of combined *Andrographis paniculata* and *Moringa oleifera* nano liquid extract yielded the most favorable outcomes for the performance and carcass quality of broiler chickens.
Keywords: *Andrographis paniculata*, Broiler chicken, Carcass quality, *Moringa oleifera*, Nano liquid, Performance

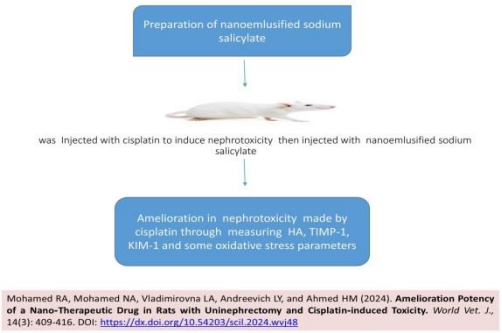


[Full text-PDF] [Crossref Metadata]

Research Paper

Amelioration Potency of a Nano-Therapeutic Drug in Rats with Uninephrectomy and Cisplatin-induced Toxicity

Mohamed RA, Mohamed NA, Vladimirovna LA, Andreevich LY, and Ahmed HM.
World Vet. J. 14(3): 409-416, 2024; pii:S232245682400048-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj48



Mohamed RA, Mohamed NA, Vladimirovna LA, Andreevich LY, and Ahmed HM (2024). **Amelioration Potency of a Nano-Therapeutic Drug in Rats with Uninephrectomy and Cisplatin-induced Toxicity.** *World Vet. J.*, 14(3): 409-416. DOI: https://dx.doi.org/10.54203/scil.2024.wvj48

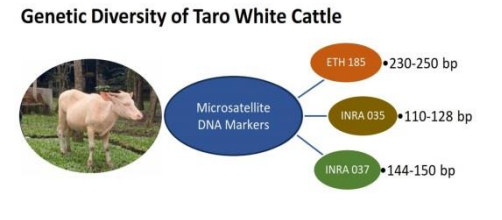
ABSTRACT: While physicians describe drugs to treat diseases, these medications may have cytotoxic effects on certain organs, necessitating the use of some drugs to ameliorate such adverse effects. The study was conducted to investigate the protective behavior of nanoemulsified sodium salicylate on uninephrectomized rats injected with cisplatin to induce nephrotoxicity. Fifty adult male albino rats, aged five weeks and weighing approximately 100-120 g, were divided into five groups. The first group received 200 mg/kg/day i.p normal saline for 30 days. The second group was administrated 200 mg/kg/day of nanoemulsified salt of salicylic acid for 30 days. The third group, comprising uninephrectomized rats, was injected with two doses of cisplatin (20 mg/kg body weight) on alternate days from the start of the experiment to induce nephrotoxicity. The fourth group, also uninephrectomized, received 200 mg/kg/day i.p of nanoemulsified sodium salicylate for 30 days. The fifth group, uninephrectomized and treated with 200 mg/kg/day sodium salicylate nanoemlusion for 21 days, was subsequently injected with two doses of cisplatin, followed by continued nanoemulsified sodium salicylate treatment until day 30 from the start of the study. The results showed a significant increase in tissue inhibitor metalloproteinase 1 (TIMP-1), Hyaluronic acid (HA), malondialdehyde, kidney injury molecule -1(KIM-1), and nitric oxide in the nephrotoxic group injected with cisplatin compared to the control group. Additionally, there was an elevation in the mRNA expression of nephrotoxic group with uninephrectomy. However, nephrotoxic rats treated with nanoemulsified sodium salicylate exhibited only a modest increase in TIMP-1, HA, and KIM-1 levels, along with elevated expressions of podocin and nephrin compared to the healthy control group. These findings suggest that nanoemulsified sodium salicylate exerts a protective effect against cisplatin-induced nephrotoxicity in uninephrectomized.
Keywords: Cisplatin, Lateral nephrectomy, Nanoemlusion, Podocyte, Rat, Surgery

[Full text-PDF] [Crossref Metadata]

Research Paper

Identification of Genetic Diversity of Taro White Cattle Using Microsatellite DNA Markers

Heryani LGSS, Susari NNW, Yusmalinda NLA, and Merdana IM.
World Vet. J. 14(3): 417-423, 2024; pii:S232245682400049-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj49



Heryani LGSS, Susari NNW, Yusmalinda NLA, and Merdana IM (2024). **Identification of Genetic Diversity of Taro White Cattle Using Microsatellite DNA Markers.** *World Vet. J.*, 14(3): 417-423. DOI: https://dx.doi.org/10.54203/scil.2024.wvj49

ABSTRACT: Taro white cattle have a small population found in Taro Village, Tegalalang, and Gianyar, Bali, which must be protected as one of the most valuable biological resources for the Hindu community in Bali. White cattle require conservation efforts, with morphometric characterization and genetics providing the first phases of an animal conservation program. The purpose of this study was to identify the genetic diversity of Taro white cattle based on allele frequency, heterozygosity, and fixation index (FIT) using different microsatellite DNA loci so that it can be used as a study and reference in determining policies for the conservation of Taro white cattle in Indonesia, particularly in Bali. This cross-sectional observational study utilized *ETH 185*, *INRA 035*, and *INRA 037* microsatellite DNA markers. Polymerase chain reaction amplified a total of 22 Taro white cattle blood samples with an average of 4.33 alleles; the average observed heterozygosity and expected heterozygosity were 0.288 and 0.637, respectively, with an average fixation index value of 0.55. In conclusion, there is a significant deviation from the Hardy-Weinberg equilibrium with the likelihood of inbreeding, as indicated by the Hardy-Weinberg balance. The microsatellite loci used in this study can be further used to evaluate the genetic diversity of Taro white cattle.
Keywords: Conservation, Genetic diversity, Microsatellite DNA, Taro white cattle

[Full text-PDF] [Crossref Metadata]

Research Paper

Microorganisms' Growth Inhibition in Poultry Meat Using *Bacillus* spp.

Kolchyk OV, Borovuk IV, Buzun AI, Illarionova TV, and Zazharska NM.
World Vet. J. 14(3): 424-434, 2024; pii:S232245682400050-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj50

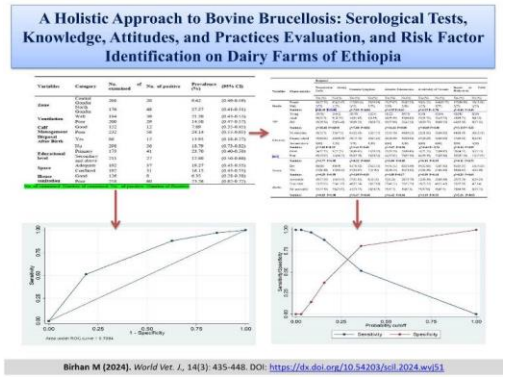
ABSTRACT: Meat processing enterprises are currently seeking ways to improve the efficiency of their operations. This study aimed to assess the presence of harmful microorganisms in poultry meat treated with a probiotic complex of *Bacillus* spp. bacteria during storage. Of the 2,516 meat samples collected from broiler chickens across six poultry processing enterprises in the Dnipropetrovsk region over three years, 1,845 samples tested positive for pathogens. *Listeria* spp. were isolated in 52.7% of meat samples, S. aureus in 28.7%, *P. aeruginosa* – in 6.9%, *E. coli* in 4.2%, and *Salmonella* spp. in 7.5%. The next stage of the study was the infection of 10 samples of poultry meat with pathogens of test cultures (*Escherichia coli UNCSM* - 007, *Pseudomonas aeruginosa UNCSM* - 012, *Staphylococcus aureus UNCSM* - 017, *Listeria ivanovii UNCSM* - 042, *Salmonella* Enteritidis *UNCSM* - 081), followed by aerosol treatment with a probiotic complex of *Bacillus* spp. (1.5×10^8 in ml (0.5 Mac Farland) administered at a dose of 1 ml per sample with daily registration of colony growth. Following pathogen contamination and a single aerosol treatment with the probiotic complex of *Bacillus* spp., the growth *of E. coli* and *S. aureus* was already suppressed on the second day of meat storage. The probiotic complex of *Bacillus* spp. was able to displace *Salmonella* Enteritidis on the third day and *P. aeruginosa* on day 4, but the growth of *L. ivanovii* could be observed only on day 5. The probiotic complex of *Bacillus* spp. formed visible biofilms from the five strains of microorganisms and remained viable for five days, forming a dense biofilm with a high accumulation rate of 4.73 D620. A distinctly noticeable ability to form microbial biofilms within three days was observed in planktonic forms of *L. ivanovii* up to 2.88 D620, followed by *P. aeruginosa* at 2.28 D620. Low biofilm density was observed for *Salmonella* Enteritidis (1.77 D620) and *S. aureus* (1.76 D620). The probiotic complex of bacteria of the genus *Bacillus* spp. shows potential for use in meat processing plants to prevent the growth of harmful microbial biofilms on meat products stored under refrigeration.
Keywords: Antagonistic activity, Biofilm formation, Microbial biofilm, Pathogen, Probiotic complex of *Bacillus* spp.

[Full text-PDF] [Crossref Metadata]

Research Paper

A Holistic Approach to Bovine Brucellosis: Serological Tests, Knowledge, Attitudes, and Practices Evaluation, and Risk Factor Identification on Dairy Farms of Ethiopia

Birhan M.
World Vet. J. 14(3): 435-448, 2024; pii:S232245682400051-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj51



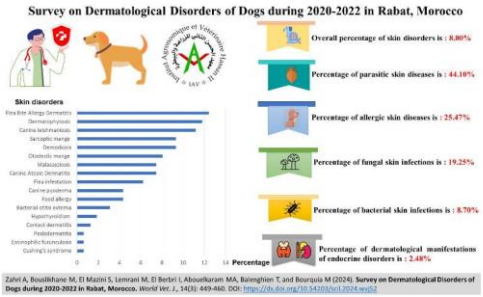
ABSTRACT: Brucellosis, a contagious bacterial disease affecting animals globally presents a substantial zoonotic risk that is frequently underestimated, hinders animal trade, and endangers livestock and human health. The present study was conducted from November 2023 to June 2024 in Central and North Gondar Zone, Ethiopia. The current cross-sectional study aimed to evaluate the prevalence of brucellosis and to explore the related knowledge, attitudes, and practices within the specified region. A total of 384 serum samples were collected via random sampling from 20 dairy farms located in Ethiopia. Both local and cross-breed samples screened using the Rose Bengal Plate test and confirmed through an enzyme-linked immunosorbent assay. The seroprevalence of bovine brucellosis for both tests was 17.71% overall, with 9.62% for Central Gondar and 27.27% for North Gondar. Extensive farms exhibited notably higher odds of brucellosis compared to intensive farms, with unadjusted crude odds ratios of 3.01 and adjusted odds ratios of 2.37, respectively. Medium-sized herds also demonstrated increased odds in the multivariate analysis compared with small herds. Young respondents displayed the highest awareness levels, followed by adults and older individuals, with statistically significant differences observed across all categories. Regarding a semi-structured survey from 150 farmers on the association between sociodemographic data and knowledge, females exhibited higher awareness levels, with 117 (80.14%) responding positively. Young respondents showed a higher positive response rate of 58% compared to adults (52.7%) and the elderly (36.54%). In conclusion, these results emphasize the need for comprehensive strategies to address the factors influencing bovine brucellosis prevalence and respondent awareness.
Keywords: Brucellosis, Central Gondar, Dairy Farm, North Gondar, Seroprevalence

[Full text-PDF] [Crossref Metadata]

Research Paper

Survey on Dermatological Disorders of Dogs during 2020-2022 in Rabat, Morocco

Zahri A, Bouslikhane M, El Mazini S, Lemrani M, El Berbri I, Abouelkaram MA, Balenghien T, and Bourquia M.
World Vet. J. 14(3): 449-460, 2024; pii:S232245682400052-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj52



ABSTRACT: Dermatology is an important specialty in veterinary medicine, focusing on the skin and its appendages. Therefore, the present study highlighted the percentage of skin disorders, as well as their associated risk factors, from cases received at the Parasitology-Dermatology clinic of the Hassan II Institute of Agronomy and Veterinary Medicine (IAV Hassan II), Rabat, Morocco for two years. A total of 1561 dogs (1450 dogs were in 28 different pure breeds and 111 dogs were mongrels, 805 males, and 756 females, with an average age of 6.5 years old) were presented at the University Veterinary Teaching Hospital (UVTH) of the IAV Hassan II from the end of October 2020 to the end of May 2022 (including vaccinations) and 125 dogs were assessed and 161 skin diseases were found (a few dogs had more than one skin disease). Dermatological examinations represented an average of 8.00% (125/1561) of all canine cases received at the University Veterinary Teaching Hospital. The most common clinical signs were pruritus, alopecia, erythema, onychogryphosis, and visible ectoparasites. Parasitic dermatoses were the most frequent, representing 44.10% of all dermatological cases, followed by allergic dermatoses (25.47%) and fungal skin infections (19.25%). Bacterial skin infections and dermatological manifestations of endocrine disorders were infrequent, representing 8.70% and 2.48%, respectively of all observed cases. Risk factors contributing to the occurrence of canine skin disorders included age and lifestyle for sarcoptic mange. An apparent predilection for the living environment was observed in the case of canine leishmaniosis, and an apparent predilection for sex regarding otodectic mange was also demonstrated. Similar results were found for the living environment and lifestyle concerning canine atopic dermatitis. Data reported herein fill gaps in knowledge of skin disorders and their associated risk factors in dogs in Morocco, demonstrating the dominance of skin diseases of zoonotic interest, including flea bite allergy dermatitis (FBAD), dermatophytosis, and canine leishmaniosis.
Keywords: Dog, Morocco, Rabat, Skin disease, Survey

[Full text-PDF] [Crossref Metadata]

Mini Review

How to Design a Biomonitoring Study – A Practical Guide for Veterinary Professionals under a One Health approach

Jota Baptista C, Seixas F, Gonzalo-Orden JM, and Oliveira PA.
World Vet. J. 14(3): 461-463, 2024; pii:S232245682400053-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj53

ABSTRACT: Currently, veterinarians can see their daily practice and medical tasks as constant opportunities for passive surveillance of One Health threats, such as infectious zoonotic diseases and chemical pollution effects on living beings. The present study aimed to provide a practical guide to designing a biomonitoring study during veterinary clinical practice without time-consuming procedures or significant costs. The constant access to several species' specimens provides the necessary samples to perform a biomonitoring study of environmental pollutants at the regional or national level. Generally, most health professionals know what to do (or where to find information) to report a disease outbreak. However, a summarized background to perform a biomonitoring study of a chemical hazard is missing. The authors of the current study provided a flow chart with the main steps to conduct a biomonitoring study in different fields of veterinary medicine. Thus, a biomonitoring study might give veterinarians (as other health professionals) a positive contribution to the clinical cases' resolution, while improving the general knowledge about the impact of environmental contamination on animals and human health.
Keywords: Contamination, Guideline, Monitoring, One Health, Pollution

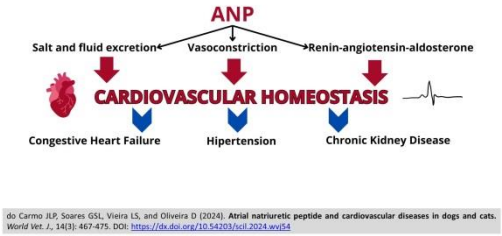
[Full text-PDF] [Crossref Metadata]

Review

Atrial Natriuretic Peptide and Cardiovascular Diseases in Dogs and Cats

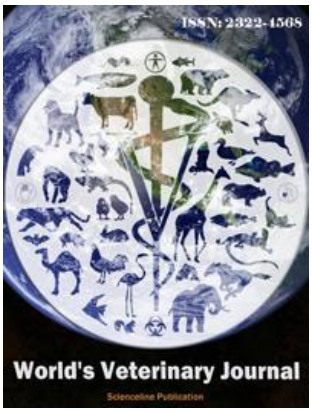
do Carmo JLP, Soares GSL, Vieira LS, and Oliveira D.
World Vet. J. 14(3): 467-475, 2024; pii:S232245682400054-14
DOI: https://dx.doi.org/10.54203/scil.2024.wvj54

ABSTRACT: The term biomarker encompasses various biological indicators that objectively reflect a patient's medical status with precision and reproducibility. These indicators range from basic measurements like pulse and blood pressure to more intricate laboratory tests. Cardiac markers are crucial for accurate and prompt diagnosis of heart diseases in animals. Given the challenge of diagnosing cardiac diseases in small animals due to nonspecific clinical signs, cardiac markers provide quantitative indicators of biological processes. These markers include cardiac troponins for myocardial injury, natriuretic peptides for myocardial function, lipoproteins for serum homeostasis, and markers for inflammation of the cardiovascular system. Among natriuretic peptides, atrial natriuretic peptide (ANP) has emerged as a significant tool in diagnosing and monitoring cardiac diseases. ANP, primarily synthesized in cardiac atria, regulates salt and fluid excretion, counteracts vasoconstriction, and inhibits the renin-angiotensin-aldosterone system, contributing to the maintenance of cardiovascular homeostasis. Additionally, it functions as a biomarker for ventricular hypertrophy and congestive heart failure (CHF) in animals. Furthermore, it protects against hypertension and cardiac remodeling by demonstrating antagonism to the same system. This review addresses the definition of biomarkers within the context of molecular biology, elucidates their multifaceted functions in the animal organism in light of integrative physiology, and explores the pathologies correlated with ANP, with an emphasis on its etiopathogenesis and clinical manifestations.
Keywords: Biomarker, Canine, Feline, Heart physiology



[Full text-PDF] [Crossref Metadata]

Previous issue | Archive



World's Veterinary Journal

E-ISSN: 2322-4568

Frequency: Quarterly

DOI Prefix: 10.54203

Current Issue: 2024, Vol: 14, Issue: 3 (September 25)

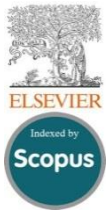
Publisher: SCIENCELINE

www.wvj.science-line.com

World's Veterinary Journal (ISSN 2322-4568) is an international, English language, peer reviewed open access journal aims to publish the high quality material from veterinary scientists' studies ... View full aims and scope

Editors-in-Chief:

- WVJ indexed/covered by SCOPUS (CiteScore=1.0), NLM Catalog, AGRIS, ScopeMed, NAAS (Score: 3.96), Ulrich's™/ProQuest, UBTIB, SHERPA/RoMEO, Genamic, INFOBASE, ...full index information



- Open access full-text articles is available beginning with Volume 1, Issue 1.
- Digital Archiving: Journal Repository (eprints)
- Full texts and XML articles are available in Crossref and AGRIS.
- High visibility of articles over the Internet through Gold Open Access.
- This journal is in full compliance with Budapest Open Access Initiative and International Committee of Medical Journal Editors' Recommendations (ICMJE).
- This journal encourage the academic institutions in low-income countries to publish high quality scientific results, free of charges... view Review/Decisions/Processing/Policy
- Publisher Item Identifier ...details

Scienceline Publication, Ltd.
Editorial Office:
Ömer Nasuhi Bilmen Road, Dönmez Apart., G Block, No:1/6, Yakutiye, Erzurum/25100, Turkey
Homepage: www.science-line.com
Email: administrator@science-line.com
Phone: +90 538-7708824 (Turkey)

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 293-310.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj36
PII: S232245682400036-14

Biochemical Studies on Efficiency of Natural Gum in Chronic Kidney Failure and Liver Cirrhosis in Rats

Manal Mohamed Lotfy[1]*, Emam Abdel-Mobdy[1], Yasmin Emam Abdel-Mobdy[2], Hassan Mohamed Salem[3], and Hanaa Fawzy Mohamed Ali[1]

[1]*Biochemistry Department, Faculty of Agriculture, Cairo University, Cairo, Egypt*
[2]*Economic Entomology and Pesticides Department, Faculty of Agriculture, Cairo University, Cairo, Egypt*
[3]*Faculty of Agriculture, Cairo University, Cairo, Egypt*

*Corresponding author's Email: manal.lotfy@post.agr.cu.edu.eg

ORIGINAL ARTICLE
Received: June 17, 2024
Revised: July 4, 2024
Accepted: July 24, 2024
Published: September 25, 2024

ABSTRACT

It is well-established that apoptosis, oxidative stress, and inflammation are associated with several disorders, including chronic renal disease and hepatic disease. Oxidative stress (OS) is a major cause of death from end-stage renal disease which also contributes to atherosclerosis and cardiac issues. The present study aimed to assess the efficacy of Gum Arabic (GA) in mitigating renal damage and hepatotoxicity in rats induced by Chloropyrifos-methyl (CPM). A total of 42 male Wistar rats were divided into seven groups, with four groups (group 2 [IC], group 5 [GA1+IC]a, group 6 [GA2+IC], and group 7 [GA1+IC]b treated with CPM for eight weeks to induce hepatic and renal damage. Two models of GA administration, including the standard oral model in drinking water (15% w/v) and the oral model by gavage at a dose of 1 g/kg body weight were administered. Physiological parameters of kidney and liver functions, including urea, creatinine, AST, and ALT along with anti-oxidant factors (Melaodialdehyde, superoxide dismutase, reduced glutathione, and catalase) were measured in plasma, and homogenates of renal and hepatic tissues on day 57 of the experiment. In addition, histopathological examination was conducted on liver and kidney tissues using hematoxylin and eosin stain to evaluate the efficacy of GA on damaged tissues. Gum Arabic was found to significantly reduce CPM toxic effects in the liver and kidney in groups treated with CPM as liver and kidney parameters were reduced to normal levels. Furthermore, GA reduced histological indicators of inflammation, fibrosis, and apoptosis, as well as renal morphological damage. Additionally, it reduced OS in liver and kidney homogenates. In conclusion, GA effectively reduced the damage that CPM inflicted on liver and kidney tissue by stabilizing physiological parameters to normal levels and repairing cellular structures damaged by OS.

Keywords: Antioxidant, Anti-inflammatory, Gum Arabic, Kidney, Liver, Oxidative stress

INTRODUCTION

In many organisms, oxidative stress (OS) induced by oxygen radicals is a typical response and a crucial biological mechanism in the production of energy (Hajam et al., 2022). Nonetheless, a substantial body of research indicates that the aging process and several diseases including cataracts, cancer, rheumatoid arthritis, the Alzheimer's disease, and atherosclerosis are caused by the unchecked production of free radicals derived from oxygen (Finkel and Holbrook, 2000; Zhu et al., 2004; Luo et al., 2008). Furthermore, individuals with end-stage renal disease (ESRD) may have an accelerated oxidative process (Ali et al., 2020). Chronic kidney disease (CKD), also known as chronic renal failure (CRF), is a serious and expanding public health concern in both industrialized and developing countries (Hussien et al., 2021). Poor health outcomes associated with major non-communicable diseases are thought to be largely caused by CKD (Ayodele and Alebiosu, 2010). The high mortality and distress rates in CKD patients are primarily caused by cardiovascular disease. Inflammation, oxidative stress, and apoptosis are the pathophysiological causes of CKD and its effects (Okamura and Pennathur, 2015). These pathophysiological causes are common in both animals and humans and are important in mediating disease processes (Hajam et al., 2022). The liver is a vital organ that is frequently the target of many toxicants and is actively involved in metabolic processes. One of the main functions of the liver is the detoxification of toxins and xenobiotics (Fan et al., 2021. The chronic liver condition known as hepatic cirrhosis, or cirrhosis, leads to the gradual decline of liver function and the formation of scar tissue rather than healthy liver tissue (David and Hamilton, 2010).

Chlorpyrifos-methyl (CPM; O, O-Dimethyl 0-3, 5, 6-trichloro-2-pyridyl) is an organophosphate (OP) pesticide widely used in agricultural and public health (Hites, 2021). Among agricultural workers and individuals close to application sites, exposure often occurs by accidental ingestion, inhalation, and skin absorption (Ferrer, 2003).

To cite this paper: Lotfy MM, Abdel-Mobdy E, Abdel-Mobdy YE, Salem HM, and Ali HFM (2024). Biochemical Studies on Efficiency of Natural Gum in Chronic Kidney Failure and Liver Cirrhosis in Rats. *World Vet. J.*, 14(3): 293-310. DOI: https://dx.doi.org/10.54203/scil.2024.wvj36

Through oxidative desulfuration, chloropyrifos is physiologically activated to its potentially hazardous form, i.e., chloropyrifos-oxon, by specific cytochrome P450 oxidases found mostly in liver tissue. This may lead to the inhibition of acetylcholinesterase, which is one of the main causes of poisoning from chloropyrifos (Eaton et al., 2008). According to a study, one of the molecular reasons for CPM poisoning is the generation of damaging reactive oxygen species (ROS), which is demonstrated by elevated levels of thiobarbituric acid reactive substances (TBARS) and the oxidative stress that follows in their target organs (Uchendu, et al., 2012). A few examples of ROS are superoxide, hydroxyl radicals, and hydrogen peroxide which are produced during metabolic reactions and interact with lipids, proteins, and DNA in cells to alter their operation (Uzun et al., 2010). Moreover, CPM exhibits lipophilicity and broad binding to biological membranes, specifically to phospholipid bilayers (Morita et al., 2023). It has the potential to cause membrane damage by inducing peroxidation of lipid (LPO; Kalender et al., 2012). Free radical generation is known to cause organ damage, especially hepatotoxicity, prompting research into antioxidant supplementation as a potential substitute for chelating therapy (Heikal, 2012). Consequently, there is a pressing need for natural chemicals that are safe and efficient at scavenging free radicals as an additional tool in the fight against oxidative damage (Patel et al., 2010). The kidneys are extremely fragile and susceptible to damage, as both the renal parenchyma and vasculature can be adversely affected, leading to the loss of some or all renal functions (Russo et al., 2023). Antioxidant research, particularly the discovery of effective natural molecules with low cytotoxicity from plants, has become a significant focus in biomedicine (Tejchman et al., 2021).

The mature sap of *Acacia Senegal* or *Acacia seyal* trees, mostly found in the Sahel region of Sudan, is used to make gum Arabic (GA), a consumable biopolymer. This exudate, which is a thick liquid with a high soluble fiber content, is released by the stems and branches in response to stress factors such as drought, low soil fertility, or damage (Williams and Phillips, 2000). Branching chains of β-d-galactopyranosyl units make up the polysaccharide GA (branches: 21, 22, and 23). Gum Arabic is one of the safest dietary fibers and a valuable food supplement because intestinal bacteria convert it to short-chain fatty acids (SCFAs). Additionally, it contains a lot of calcium, potassium, and magnesium (Ali et al., 2022). Dietary supplementation with GA has been shown to improve high nitrogen discharge and lower serum urea nitrogen levels in patients with chronic renal failure (Hussein et al., 2022). Dietary supplementation with GA also resuts in low serum butyrate, which supports the generation of profibrotic cytokines in the kidneys (Al-Baadani et al., 2021). GA is a combination of macromolecules, mostly proteins, and carbohydrates (Mohammed, 2015). It is utilized as a medication carrier and in pharmaceutical preparations since it is a food supplement that is safe for the body. Additionally, many studies have demonstrated the antioxidant capabilities of GA (Hinson et al., 2004; Trommer and Neubert, 2005; Ali and Al Moundhri, 2006), as well as its involvement in the metabolism of lipids (Evans et al., 1992; Tiss et al., 2001).

The results of treatment for some degenerative diseases, like renal failure, have been positive (Matsumoto et al., 2006; Ali et al., 2008). This is also true for heart implications (Glover et al., 2009) and digestive disorders (Rehman et al., 2003; Wapnir et al., 2008). Thus, there is compelling evidence that in addition to its well-known emulsifying properties, GA can have beneficial health effects as well (Montenegro et al., 2012). In studies assessing the risk associated with the liver, heart, and kidneys, research has indicated that GA decreases blood pressure and plasma cholesterol in rats, promotes tooth demineralization, inhibits microbial development, and enhances intestinal absorption, all of which contribute to a decrease in diarrhea (Kamal et al., 2021).

The administration of GA in mice has been found to decrease intestinal sodium expression, leading to delayed glucose transport, hyperglycemia, hyperinsulinemia, and increased body weight (Dashtdar and Kardi, 2018). Additionally, GA has shown promise in the treatment of colorectal carcinogenesis, a condition marked by the progressive accumulation of several genetic changes. Tumor suppressor genes become inactive due to mutations in the *p53* and *Kras* genes, while oncogenes (antagonists) become active, directly contributing to the occurrence of colorectal cancer (Melo et al., 2023).

The present study aims to assess the antioxidant capacity of GA against oxidative stress in hepatic and renal tissues in Wistar lab rats.

MATERIALS AND METHODS

Ethical approval

The World Organization for Animal Health (WOAH), Cairo University's Institutional Animal Care and Use Committee (CU-IACUC) guidelines at Cairo University, Cairo, Egypt, official Ministry of Agriculture Decree No. 27, 1967, and the Guide for the Care and Use of Laboratory Animals, 8th Edition 2011 (the Guide) were all consulted in the preparation of this report.

Animals

A total of 42 male Wistar albino rats (n = 42), approximately 200 ± 10 g, 9–10 weeks old, were obtained from the Animal House of the Agricultural Research Center in Dokki, Giza Governorate, Egypt. The rats were maintained under a 12-hour light-dark cycle, at a temperature of 24 ± 2°C, and a relative humidity of 60%. They were provided with regular mouse food and had unrestricted access to water.

Experimental design

The acclimatization period was two weeks to stabilize all metabolic conditions. A total of 42 rats were treated for eight weeks using the following protocol after being assigned to seven equal groups (6 rats per group). Group 1 (Normal control; NC) was fed a normal diet until the end of the study (8 weeks). Group 2 (Injury control; IC) received a normal diet plus an oral dose of CPM (O, O-Dimethyl 0-3, 5, 6-trichloro-2-pyridyl phosphorothioate; Reldan® 22.5% Emulsifiable Concentrate "EC"), at 1/50 of LD_{50} (3129 mg/kg) daily for eight weeks. Group 3 (gum Arabic1; GA1) was fed a regular diet plus oral GA at a rate of 1 g/kg body weight daily for eight weeks. Group 4 (gum Arabic2; GA2) was given normal rat feed and 15% weight/volume of GA in drinking water daily for eight weeks. Group 5 (GA1+IC)a received the same daily amount of CPM as Group 2 (IC), a normal feed, and GA as Group 3 (GA1) for the same period of time. Group 6 (GA2+IC) received normal feed and CPM as in Group 2 (IC), plus GA as in Group 4 (GA2) for eight weeks. Group 7 (GA1+IC)b was given normal feed plus CPM as in Group 2 (IC) for four weeks, followed by the GA dose used in Group 3 (GA1) for an additional four weeks. Throughout the treatment phase, rats underwent weekly weighting.

Blood sampling and tissue retrieval

On day 57, 3 ml blood samples were collected from overnight-fasted rats using capillary glass tubes from the retro-orbital plexus. Plain tubes were kept dry and clean for 30 minutes to allow blood clotting before being centrifuged at 3000 rpm. The resulting serum was then utilized to measure the lipid profile, liver function, and kidney function by assaying the physiological parameters using a Cobas C11 blood chemistry analyzer. Liver and kidney tissue samples were retrieved from all animals in the seven groups of the experiment after anesthetizing them with ketamine (Ketalar 10 mg/ml injection by Pfizer Limited) at a dose of 100 mg/kg body weight, and then all 42 animals were dissected.

Evaluation of kidney and liver antioxidant enzymes

Malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) levels in renal and hepatic tissues were determined using Total Antioxidant Capacity (TAC) Assay Kit by Abbexa, United Kingdom.

Histopathological examination

Small portions of kidney and liver tissue were cut, quickly fixed with 10% neutral buffered formalin, and then embedded in paraffin wax. Subsequently, paraffin blocks from the kidney and liver were sectioned using a microtome to create 4-µm thin slices. These slices were placed on slides and stained with hematoxylin-eosin. Then slides were then examined using a Leica DM 2500 with a mounted Leica DFC 290HD digital camera, Germany.

Statistical analysis

Data are presented as the mean ± standard error. Statistical comparisons between different groups were conducted using one-way analysis of variance (ANOVA). Statistical analyses were performed using the Statistical Package for the Social Science (SPSS) software, version 22.0 (SPSS Inc., Chicago, IL, USA). Significant differences were determined using T-test at a significance level of $p < 0.05$. Values are expressed as the mean ± standard error of the mean (Levesque, 2007).

RESULTS

Physiological data

The results in Figure 1 show that the injury control (IC) rats treated with CPM and the rats treated with GA separately or in combination with other groups experienced a significant decrease in weight gain ($p < 0.05$). In contrast to group 1 (NC), the organ weights of the rats administered with CPM alone increased significantly compared to Group 1 (NC) and the other groups that received GA alone or CPM ($p < 0.05$, Table 1).

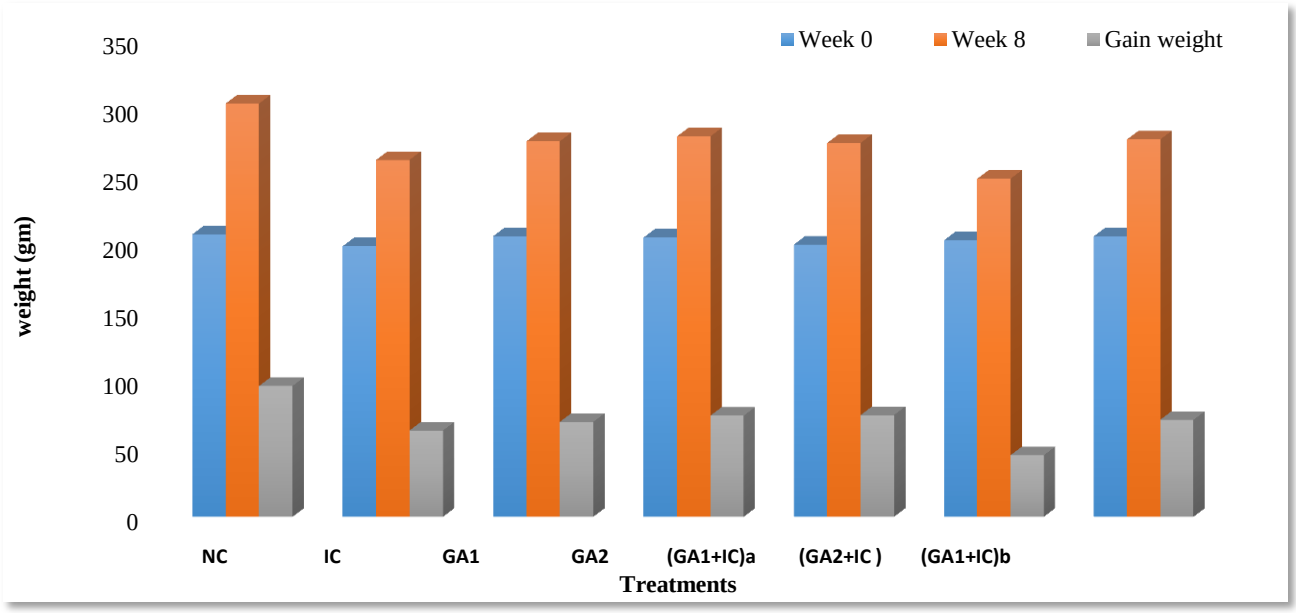


Figure 1. The affected rats' weight by administrating chlorpyrifos-methyl to induce oxidative stress and treatment by different groups. GA: Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

Table 1. Administration effect of chlorpyrifos-methyl and gum Arabic on weight gained of rats aged 4 months in the experimental groups

Treatments / Parameters	Group 1 (NC)	Group 2 (IC)	Group 3 (GA1)	Group 4 (GA2)	Group 5 (GA1+IC)a	Group 6 (GA2+IC)	Group 7 (GA1+IC)b
Week 0 weight (g)	207.2	198.5	205.8	204.8	199.7	202.8	205.7
Week 8 weight (g)	303.2	261.8	275.5	279	274.3	248	276.8
Gain weight (g)	96	63.33	69.67	74.17	74.67	45.17	71.17

Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

Liver biochemical parameters

The results in Figures 2A and 2B show that CPM administration in Group 2 (IC) significantly increased the activities of liver function parameters such as ALT, AST, and Total Bilirubin compared to control Group 1 (NC; Figure 3A, $p < 0.05$). However, the CPM Group 2 (IC) revealed a significant decrease in Total Protein in comparison with Group 1 (NC), indicating hepatocellular damage ($p < 0.05$). This negative effect was mitigated in the groups that received CPM combined with GA (Figure 3B). A slight increase was observed in ALT activity in Group 3 (GA1) which received GA (Figure 2A, $p > 0.05$). In summary, the results indicate a positive effect of GA on liver functions in groups that received GA treatment either alone or in combination with CPM, compared to Group 2 (IC), which received only CPM and exhibited liver toxicity (Table 2).

Renal biochemical parameters

Given that Group 2 (IC) has significantly higher serum creatinine and urea values than the other groups, data in Figure 4 shows that CPM significantly increased these parameters ($p < 0.05$). This increase is indicative of renal damage and kidney dysfunction caused by toxicity from CPM. The renal parameters significantly improved in all groups that drank water containing GA compared to Group 2 (IC) ($p < 0.05$, Table 2).

Lipids profile in serum of the experimental groups

In comparison to Group 1 (NC), the CPM Group 2 (IC) data showed a significant decrease in HDL but a notable increase in triglycerides (TG), LDL, and VLDL ($p < 0.05$). However, the groups that received GA treatment had a

significant improvement in lipid profile characteristics, indicating that GA has a promising potential for treating elevated lipid profile parameters (Figure 5, $p < 0.05$, Table 2).

Antioxidant parameters in liver and kidney tissues

The results represented in figures 6 and 7 show a notable decrease in GSH, SOD and Catalase levels in the liver and kidney tissues of the CPM Group 2 (IC) compared to Group 1 (NC). This decrease provides clear evidence of improvement as a result of the GA administration in other groups ($p < 0.05$). Administration of GA showed insignificant changes compared to the NC Group 1 ($p > 0.05$), with a slight increase in Catalase (Figure 6A) and GSH (Figure 6B) compared to the NC Group ($p > 0.05$). On the other hand, the CPM Group showed increased levels of MDA and SOD in the liver and kidney tissues, indicating heightened oxidative stress via the administration of GA in other groups (GA1; GA2; (GA1+IC)a; (GA2+IC): (GA1+IC)b) as represented in Figure 7 ($p < 0.05$).

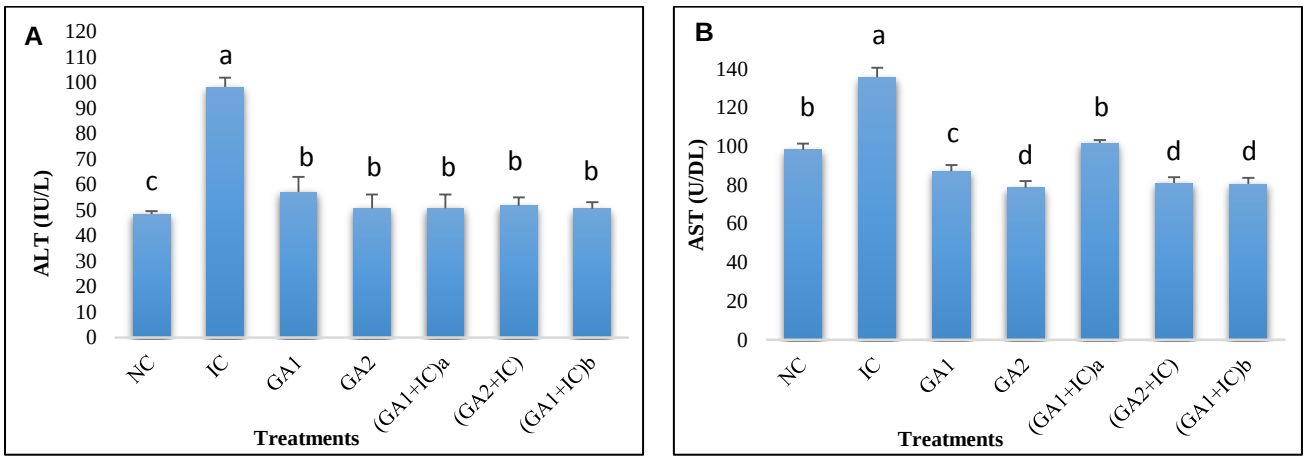


Figure 2. The rats' liver enzymes as a result of administrating chlorpyrifos-methyl to induce oxidative stress, and gum Arabic treatment. A: ALT level difference in serum, B: AST level difference in serum, Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

Table 2. Physiological parameters of the experimental groups as a result of chlorpyrifos-methyl administration and gum Arabic treatment in rats aged 4 months

Parameters \ Treatments	Group 1 (NC)	Group 2 (IC)	Group 3 (GA1)	Group 4 (GA2)	Group 5 (GA1+IC)a	Group 6 (GA2+IC)	Group 7 (GA1+IC)b
Urea (mmol/L)	58 ± 1^{b}	76 ± 3^{a}	42 ± 7^{c}	43 ± 5^{c}	57 ± 3^{b}	56 ± 3^{b}	59 ± 1^{b}
Creatinine (mg/dL)	0.56 ± 0.11^{c}	1.71 ± 0.12^{a}	0.50 ± 0.09^{c}	0.47 ± 0.12^{c}	0.47 ± 0.03^{c}	0.5 ± 0.04^{c}	0.68 ± 0.02^{b}
Cholesterol (Chol) (mg/dl)	56.90 ± 1.5^{d}	99.35 ± 1.4^{a}	47.52 ± 0.94^{f}	52.22 ± 0.82^{e}	69.81 ± 1.0^{b}	58.27 ± 0.96^{d}	64.43 ± 0.68^{c}
Triglycerides (TG) (mg/dl)	45.82 ± 0.4^{d}	79.88 ± 2.5^{a}	37.45 ± 2.8^{e}	47.37 ± 2.8^{d}	56.04 ± 2.7^{bc}	50.65 ± 2.7^{cd}	62.00 ± 2.6^{b}
Total protein (TP) (g/dl)	5.56 ± 0.17^{a}	4.29 ± 0.11^{d}	5.44 ± 0.15^{ab}	5.24 ± 0.33^{ab}	4.65 ± 0.47^{cd}	5.53 ± 0.34^{a}	4.96 ± 0.32^{bc}
Albumin (Alb) (g/dl)	2.86 ± 0.07^{a}	2.53 ± 0.04^{c}	2.79 ± 0.02^{b}	2.75 ± 0.02^{b}	2.73 ± 0.04^{b}	2.72 ± 0.07^{b}	2.58 ± 0.05^{c}
Total Bilirubin	0.19 ± 0.02^{d}	0.61 ± 0.02^{a}	0.23 ± 0.01^{c}	0.26 ± 0.00^{b}	0.16 ± 0.02^{e}	0.16 ± 0.00^{de}	0.23 ± 0.00^{bc}
Alanine transaminase (ALT) (U/L)	48.48 ± 1.1^{c}	98.27 ± 3.7^{a}	57.12 ± 5.9^{b}	50.70 ± 5.5^{bc}	50.70 ± 5.5^{bc}	51.80 ± 3.2^{bc}	50.60 ± 2.6^{bc}
Aspartate aminotransferase (AST) (U/L)	98.33 ± 3.1^{b}	135.69 ± 4.9^{a}	87.30 ± 3.1^{c}	78.67 ± 3.4^{d}	101.74 ± 1.6^{b}	81.06 ± 3.0^{d}	80.57 ± 3.1^{d}

[a, b, c, d, e, f]: Indicate that different letters in the same row differ at ($p < 0.05$). Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

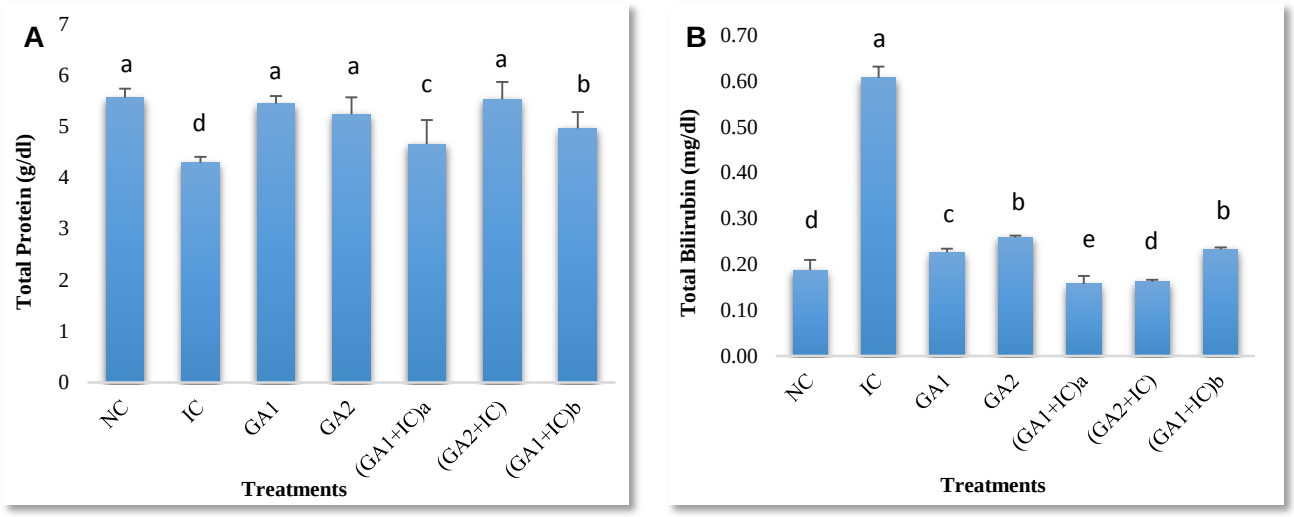


Figure 3. The rats' liver parameters were affected by chlorpyrifos-methyl administration as oxidative stress and gum Arabic treatment. A: Total protein level in serum, B: Total bilirubin level in serum, Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

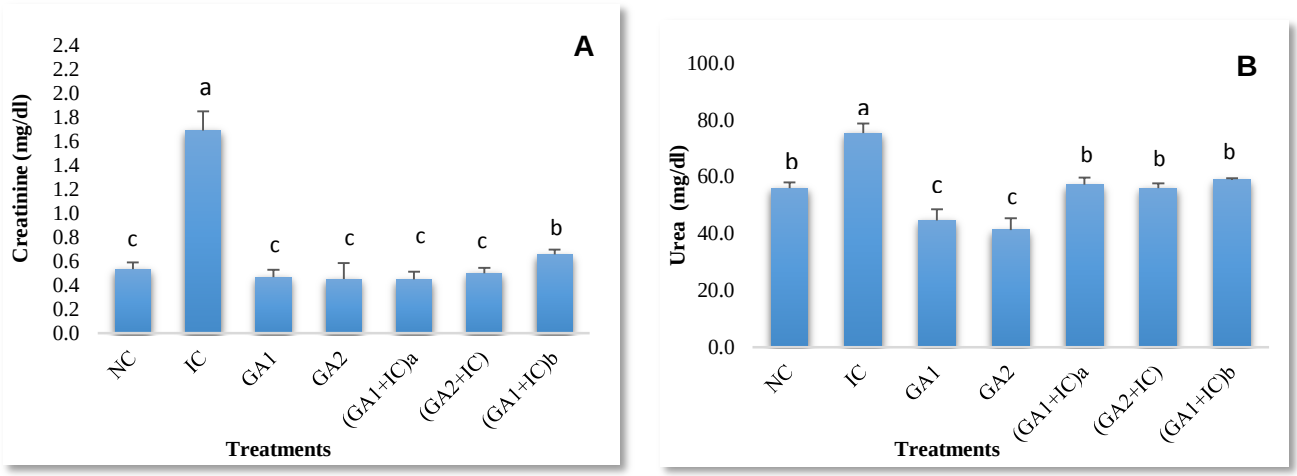


Figure 4. Renal function of rats administered chlorpyrifos-methyl to induce oxidative stress in the kidney, and its effects were countered by gum Arabic treatment. A: Serum creatinine level, B: Blood urea level, Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

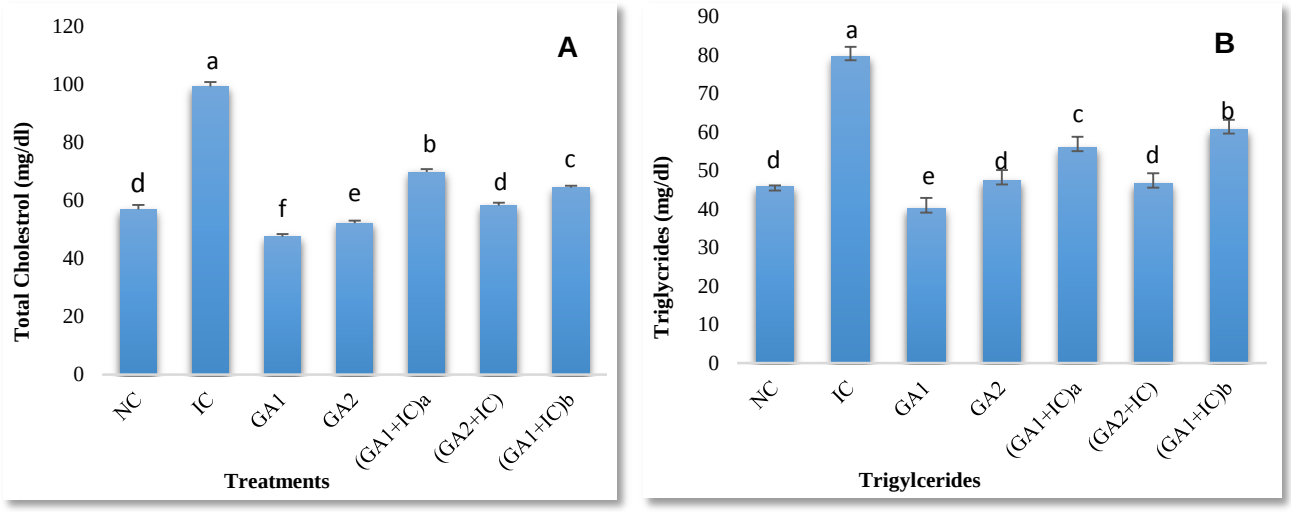


Figure 5. The effects on lipid profile of rats affected by chlorpyrifos-methyl administration countered by gum Arabic treatment. A: Total cholesterol in serum, B: Triglycerides in serum, Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

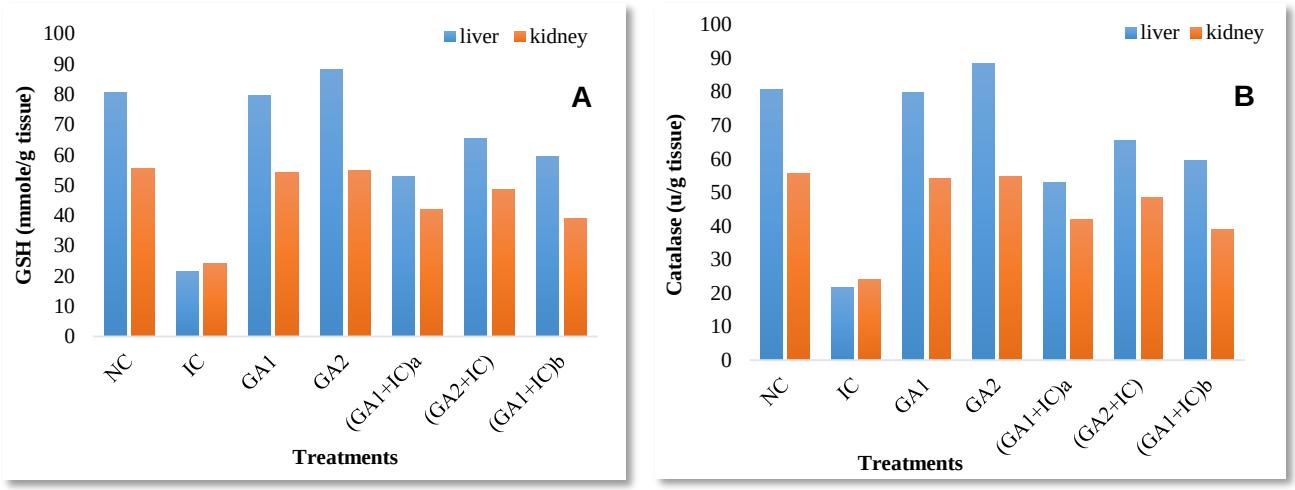


Figure 6. Reduced glutathione and catalase level differences in liver and kidney homogenates of rats in rats treated with chlorpyrifos-methyl in comparison with rats treated with gum Arabic. A: Reduced glutathione (GSH) level difference, B: Catalase (CAT) level difference, Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

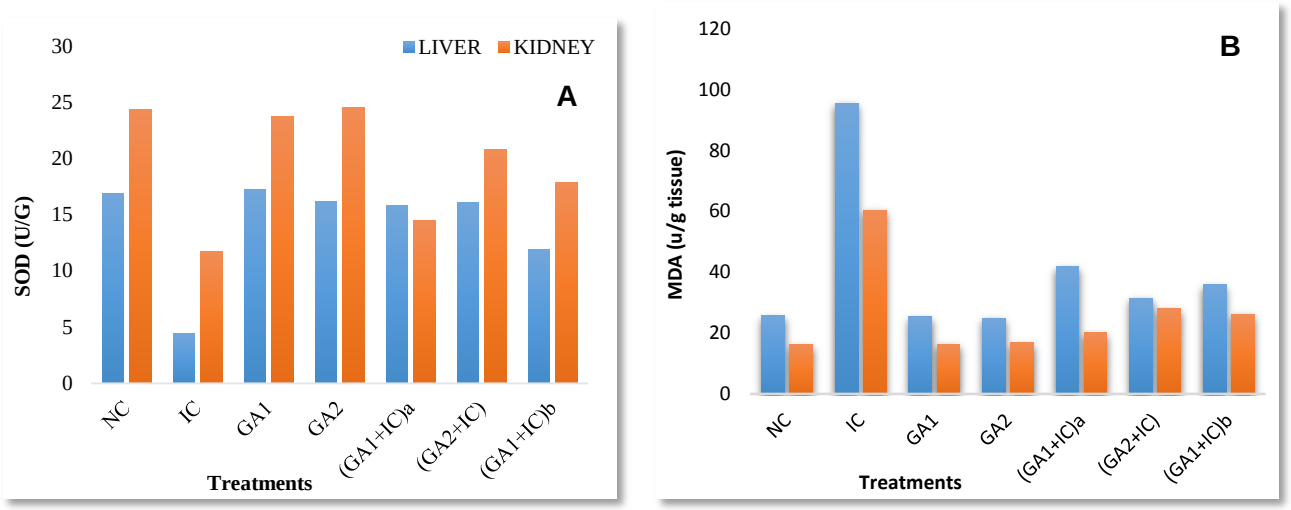


Figure 7. Superoxide dismutase and Melondialdehyde level in liver and kidney homogenates of rats treated with chlorpyrifos-methyl in comparison with rats treated with gum Arabic. A: Superoxide dismutase (SOD) level difference, B: Melondialdehyde (MDA) level difference, Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks, Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

Histopathological examination of liver tissue

The histopathological analysis of liver tissues in Group 1 (NC) revealed a normal hepatic lobule structure (Figure 8A). In contrast, the liver tissue in Group 2 (IC) displayed significant alterations, including fibroplasia in the portal triad (Figure 8D), mononuclear cell infiltration associated with focal hepatocellular necrosis (Figure 8C), and portal infiltration with mononuclear cells (Figure 8E). These findings indicate that CPM administration in Group 2 (IC) has severely damaged the tissue by increasing toxicity levels. However, no histological changes were observed in the liver tissues in Group 3 (GA1) and Group 4 (GA2) (Figures 8F and 9A). Yet, examining certain sections from Group 5 (GA1+IC)a demonstrated improvement as they showed only slight activation of Kupffer cells (Figure 9D). These sections also showed small focal hepatocellular necrosis associated with mononuclear cells infiltration (Figure 9B) and apoptosis of hepatocytes associated with infiltration of mononuclear cells (Figure 9C). Furthermore, the liver tissue in Group 6 (GA2+IC) showed improved histological features in comparison with Group 2 (IC). Liver tissues did not exhibit any histological changes (as depicted in Figure 10A) except a minor Kupffer cell activation and hepatoportal blood channel congestion shown in Figure 10C. The liver tissue in Group 7 (GA1+IC)b exhibited a regression of histological abnormalities, similar to Group 6 (GA2+IC), except for a minor activation of Kupffer cells in the evaluated sections (Figures 10D, E, and F). In this histological examination, the groups that received GA treatment showed repairing effects in their liver tissue, suggesting that the antioxidant effect imposed by GA elevates antioxidant levels, thereby reducing the oxidative stress caused by CPM administration. Consequently, liver function parameters such as ALT, AST, Total Bilirubin, and ALP were improved.

Histopathological examination of kidney tissue

The normal histological structure of the renal parenchyma was revealed by the histopathological examination of kidney tissue in Group 1 (NC) (Figures 11A and B). However, the kidney tissue in Group 2 (IC) displayed severe damage, as evidenced by periglomerular fibroblast proliferation (Figure 11D), thickening of the glomerular basement membrane (Figure 11E), and interstitial inflammatory cell infiltration, and vacuolization of the renal tubular epithelium (Figure 11F). Additionally, localized necrosis of the renal tubules linked to inflammatory cell infiltration was observed (Figure 11C). On the other hand, no histological changes were seen in kidney tissue in Group 3 (GA1) and Group 4 (GA2) (Figures 12A, B, C, and D). However, the kidney tissue in Group 5 (GA1+IC)a showed improvement in comparison with Group 2 (IC), with sections displaying renal tubular epithelium with granular or vacuolar degeneration, congested glomerular tufts, and congested renal blood vessels (Figures 11E and F). Sections in Group 6 (GA2+IC) similarly showed renal tubular epithelial vacuolar degeneration and glomerular tuft congestion (Figures 13A, and B). Similarly, the kidney tissue in Group 7 (GA1+IC)b showed notable regressive lesions with no other significant histological changes observed in the evaluated sections (Figures 13C and D). The kidney tissues in this examination showed repairing effects as a result of GA administration. The oxidative stress induced by CPM administration was countered by elevating antioxidant levels which, in turn, improved kidney tissue cells and reduced urea and creatinine levels.

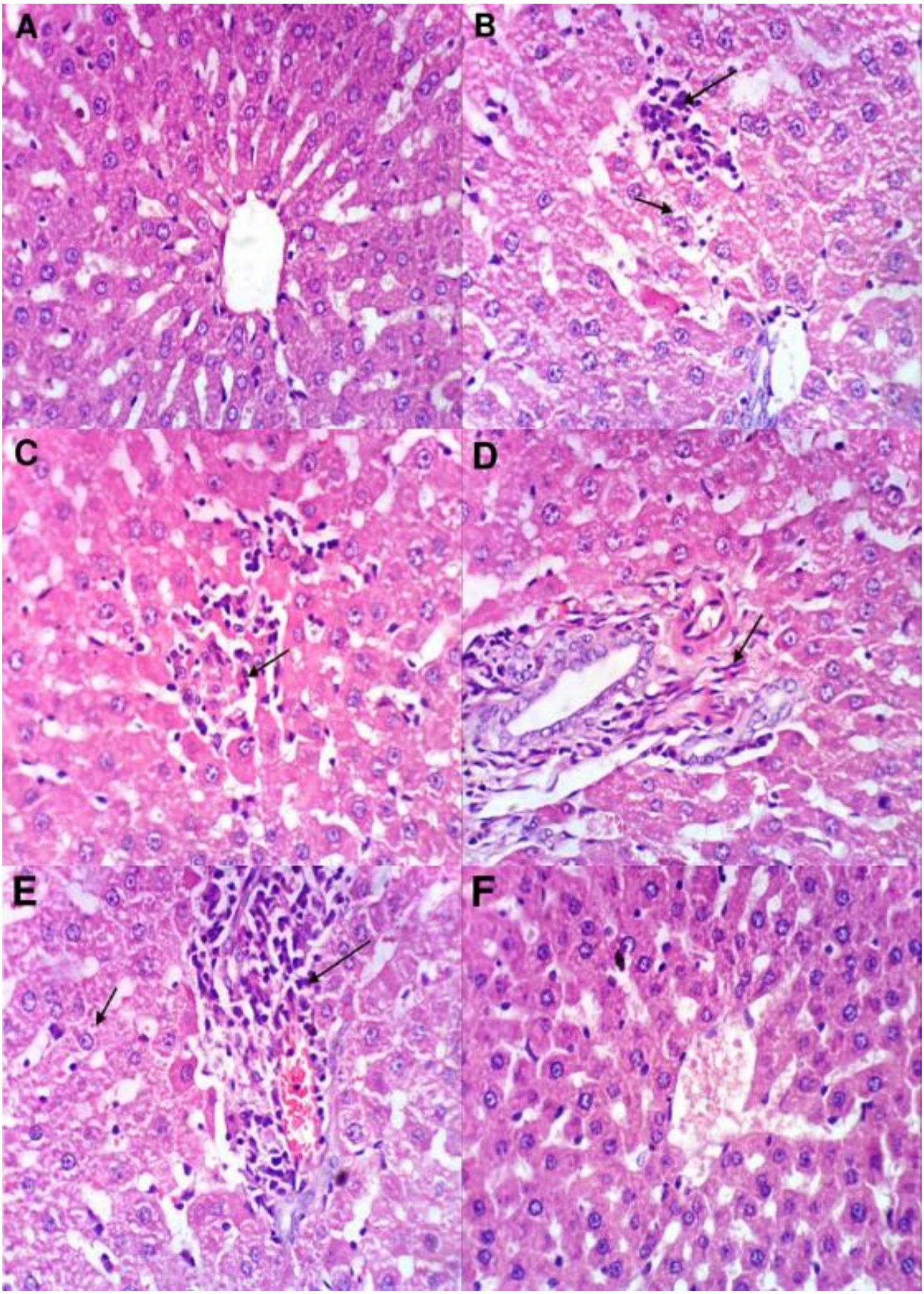


Figure 8. The histopathological changes in liver tissue of rats from group 1, group 2, and group 3 (H&E X 400).

A: Group 1 (NC) showing the normal histological structure of hepatic lobule, **B**: Group 2 (IC) showing cytoplasmic vacuolation of hepatocytes and focal hepatocellular necrosis associated with mononuclear cells infiltration (arrow indicated), C: Group 2 (IC) showing focal hepatocellular necrosis associated with mononuclear cells infiltration in a different area of the tissue (arrow indicated), D: Group 2 (IC) showing fibroplasia in the portal triad, E: Group 2 (IC) showing cytoplasmic vacuolation of hepatocytes and portal infiltration with mononuclear cells infiltration caused by CPM administration (arrow indicated), F: Group 3 (GA1) showing no histopathological alterations, Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks, Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks.

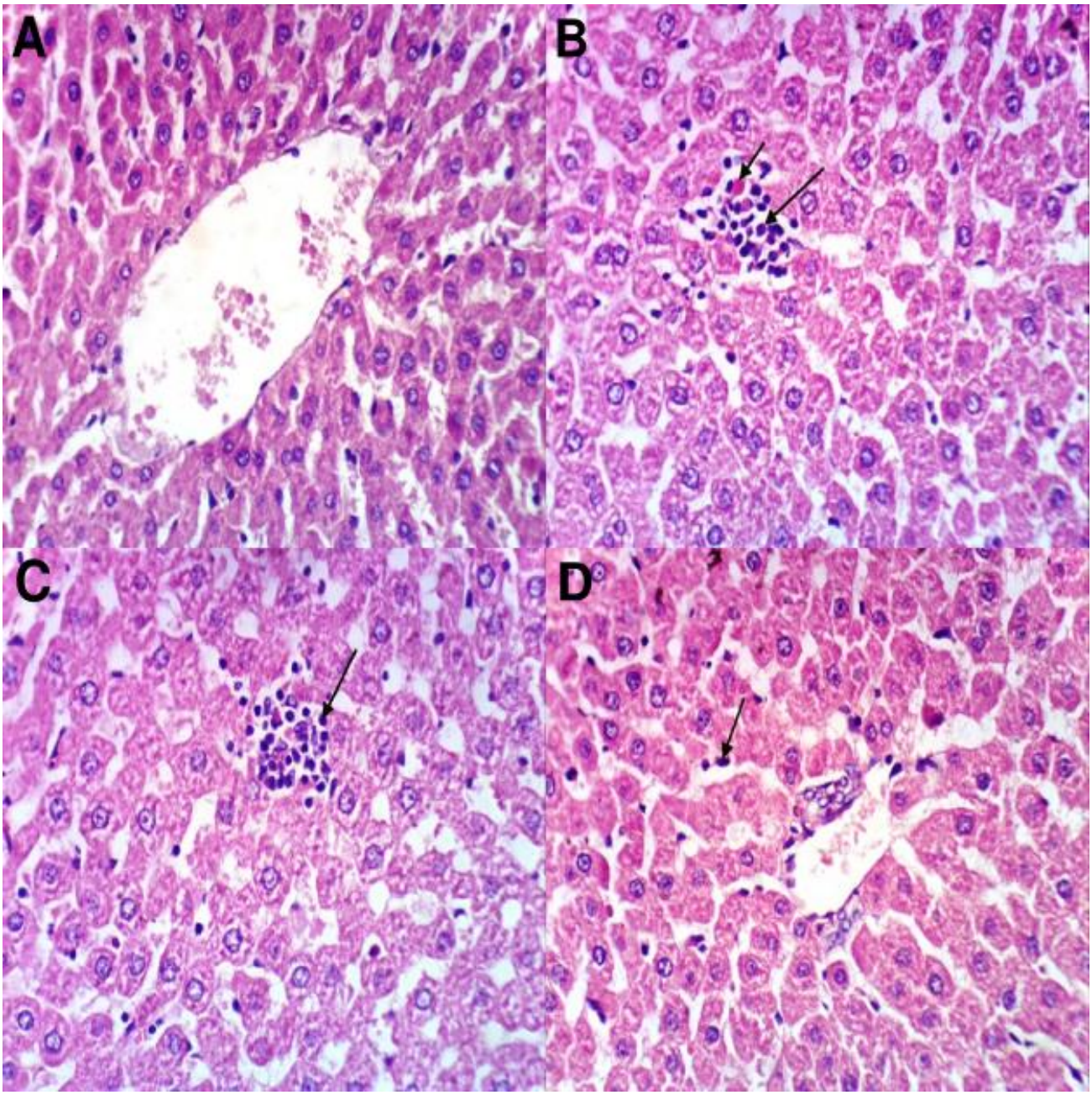


Figure 9. The histopathological changes in rat's liver tissue from group 4, and group 5 (H&E X 400).

A: Group 4 (GA2) showing no histopathological alterations, B: Group 5 (GA1+IC)a showing apoptosis of hepatocytes associated with mononuclear cells infiltration (arrow indicated), C: Group 5 (GA1+IC)a showing small focal hepatocellular necrosis associated with mononuclear cells infiltration (arrow indicated), D: Group 5 (GA1+IC)a showing slight Kupffer cells activation (arrow indicated), Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks.

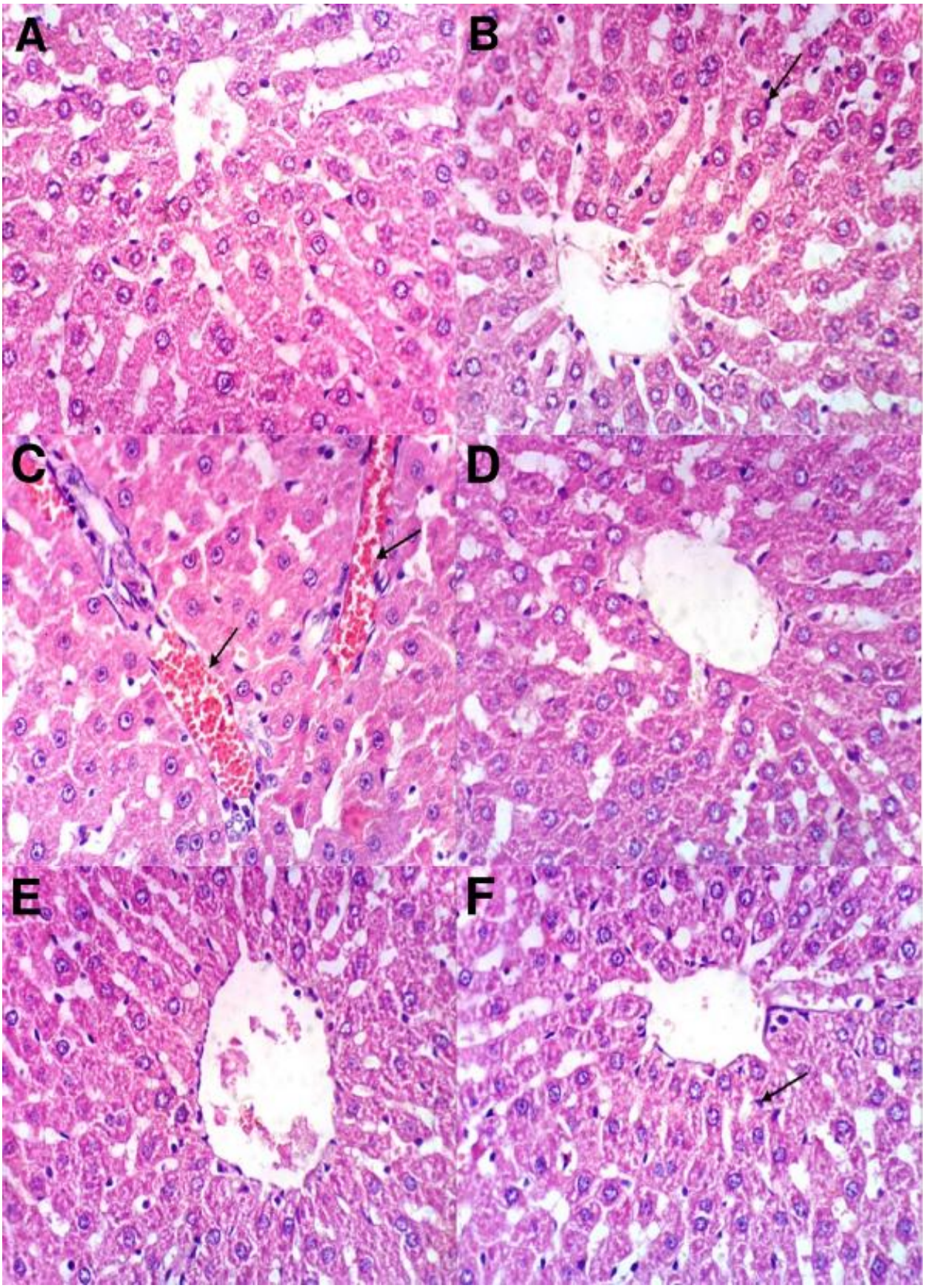


Figure 10. The histopathological changes in rats' liver tissue from group 6, and group 7 (H&E X 400).

A: Group 6 (GA2+IC) showing no histopathological alterations, B: Group 6 (GA2+IC) showing slight Kupffer cells activation (arrow indicated), C: Group 6 (GA2+IC) showing congestion of hepatoportal blood vessels (arrow indicated), D: Group 7 (GA1+IC)b showing no histopathological alterations, E: Group 7 (GA1+IC)b showing no tissue damage, F: Group 7 (GA1+IC)b showing slight Kupffer cells activation (arrow indicated), Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

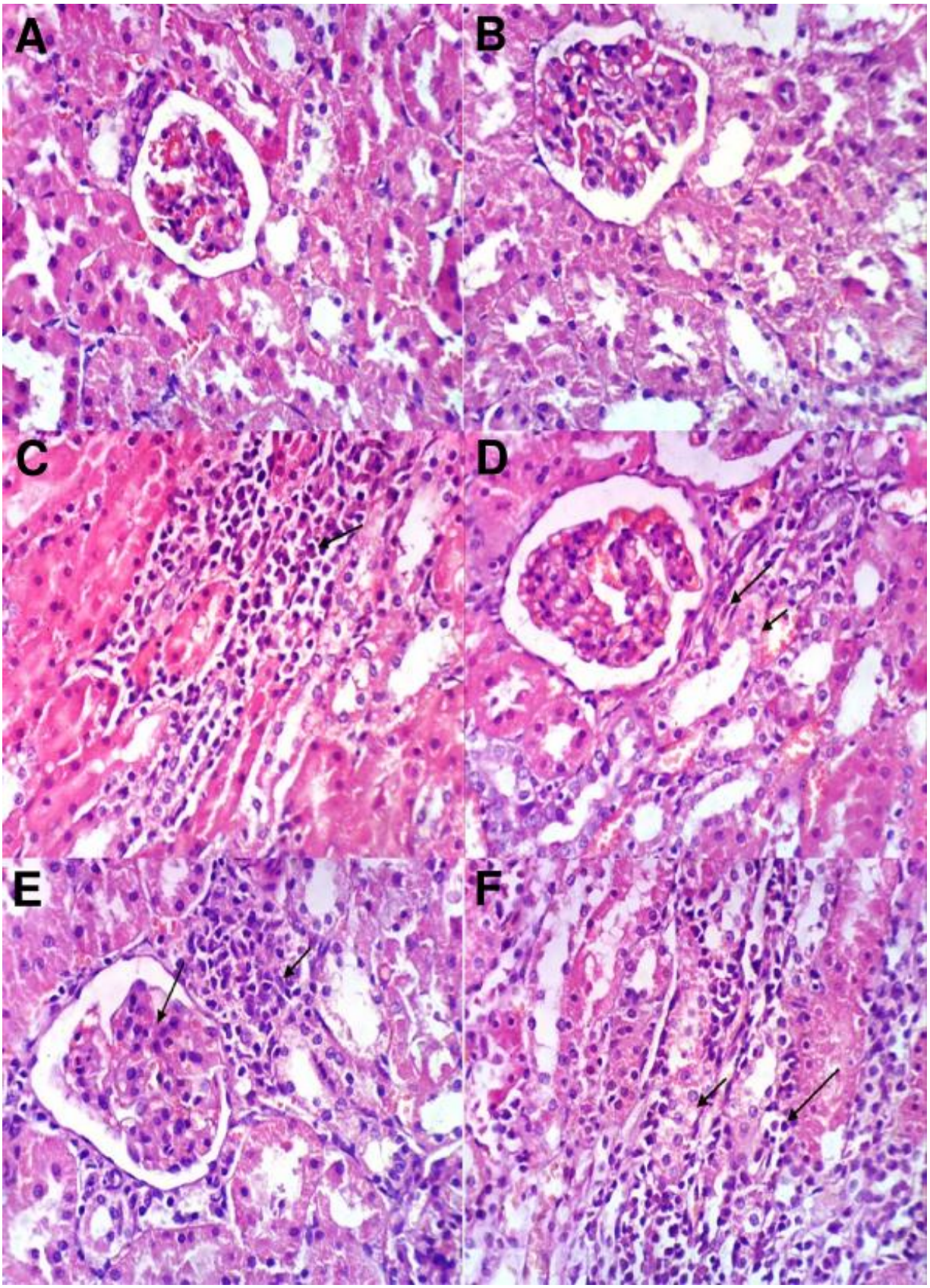


Figure 11. The histopathological changes in rats' kidney tissue from group 1, and group 2 (H&E X 400).

A: Group 1 (NC) showing the normal histological structure of renal parenchyma, B: Group 1 (NC) showing the normal histological structure of renal tissue, C: Group 2 (IC) showing focal necrosis of renal tubules associated with inflammatory cells infiltration (arrow indicated), D: Group 2 (IC) showing vacuolation of renal tubular epithelium and periglomerular fibroblasts proliferation (arrow indicated), E: Group 2 (IC) showing focal necrosis of renal tubules associated with inflammatory cells infiltration and thickening of the glomerular basement membrane (arrow indicated), F: Group 2 (IC) showing vacuolization of renal tubular epithelium and interstitial inflammatory cells infiltration (arrow indicated), Group 1 (NC): Control group, Group 2 (IC): Rats which administered CPM (3129 mg/kg) daily for 8 weeks.

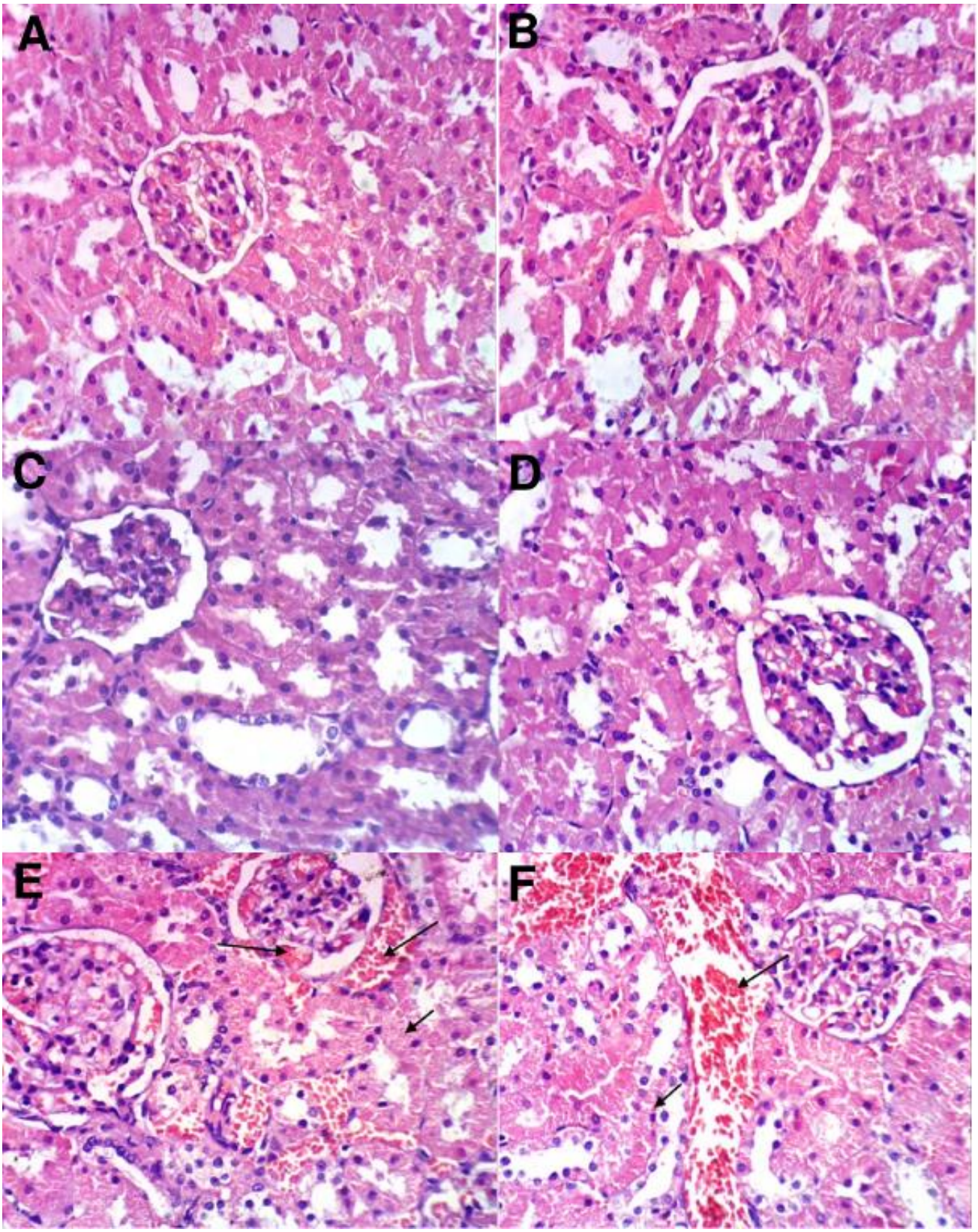


Figure 12. The histopathological changes in rats' kidney tissue from group 3, group 4, and group 5 (H&E X 400).
A: Group 3 (GA1) showing no histopathological alterations, B: Group 3 (GA1) showing also with no tissue damage, C: Group 4 (GA2) showing no histopathological alterations, D: Group 4 (GA_2) showing no histopathological alterations in different area in the tissue, E: Group 5 (GA1+IC)a showing granular degeneration of renal tubular epithelium with congestion of glomerular tufts and renal blood vessels (arrow indicated), F: Group 5 (GA1+IC)a showing granular degeneration of renal tubular epithelium and congestion of renal blood vessels indicating damage done by CPM treatment (arrow indicated), Group 3 (GA1): Rats which received normal GA feed 1 gm/kg body weight daily for 8 weeks, Group 4 (GA2): Rats which administered GA 15% w/v in drinking water daily for 8 weeks, Group 5 (GA1+IC)a: Rats which received normal feed and CPM (3129 mg/kg) plus GA (1 gm/kg body weight) daily for 8 weeks.

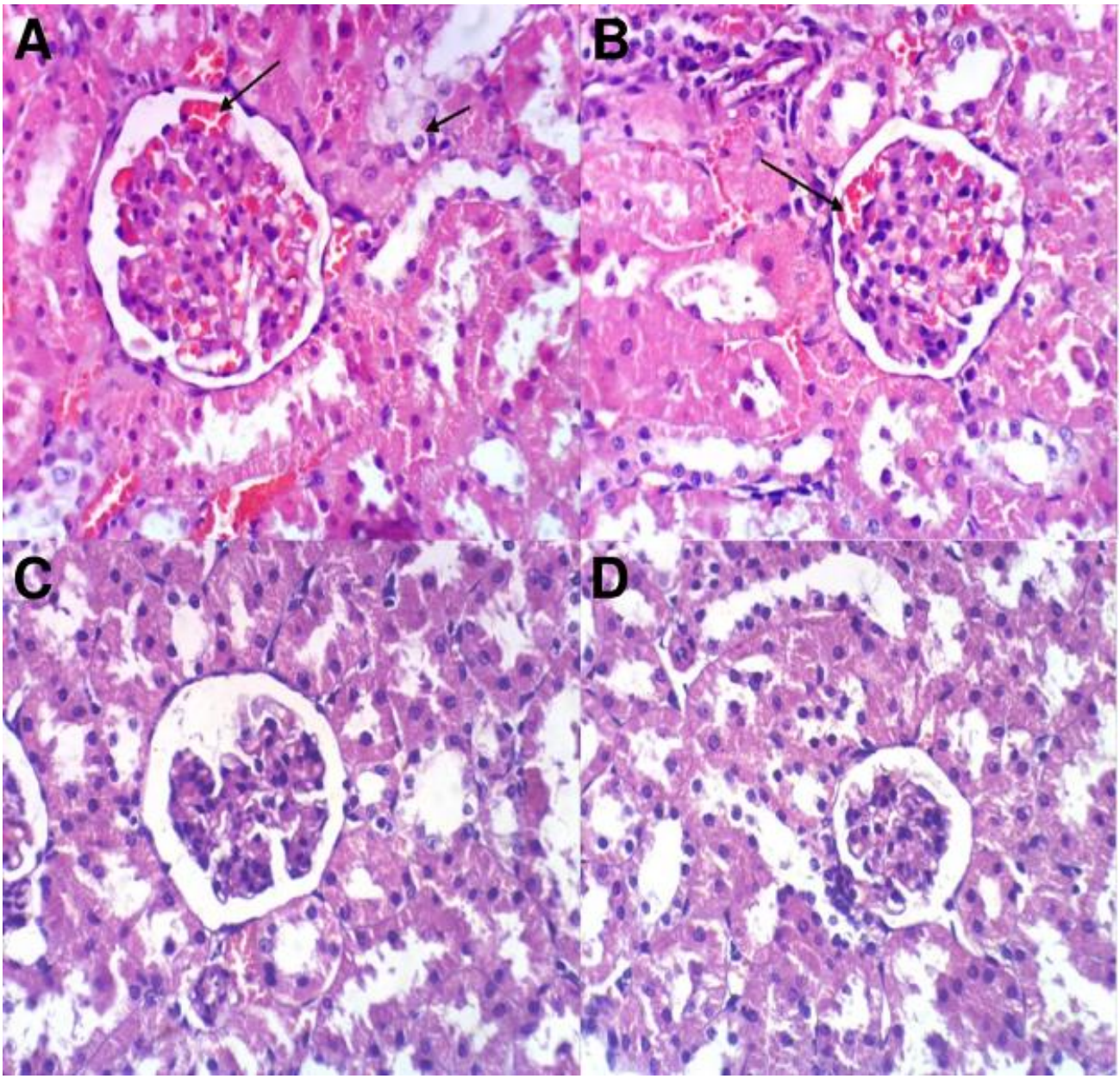


Figure 13. The histopathological changes in rats' kidney tissue from group 6, and group 7 (H&E X 400).
A: Group 6 (GA2+IC) showing vacuolar degeneration of renal tubular epithelium and congestion of glomerular tufts (arrow indicated), B: Group 6 (GA2+IC) showing congestion of glomerular tufts, C and D: Group 7 (GA1+IC)b showing no histopathological alterations (arrow indicated), Group 6 (GA2+IC): Rats which received normal feed and CPM (3129 mg/kg) plus GA 15% w/v in drinking water daily for 8 weeks, Group 7 (GA1+IC)b: Rats which received normal feed plus CPM for 4 weeks, then treated with GA (1 gm/kg body weight) for 4 weeks.

DISCUSSION

As more zones with high CKD risk are identified among agricultural, the focus on environmental exposures as causes of kidney disease is expanding. Since agricultural laborers are more likely to develop CKD, research on agriculture with special attention on herbicides and insecticides has increased (Tejchman et al., 2021). Acute kidney damage has been associated with specific agents; nevertheless, chronic exposure to industrial pollutants and agrichemicals can cause CKD (Prudente et al., 2021). Another possible cause of kidney illness is occupational exposure to infectious agents such as insecticides (Twombley et al., 2011). Hepatic damage, which can eventually result in liver cirrhosis, and renal dysfunction, which can progress to CKD, end-stage renal disease, or cardiovascular disease, can be avoided by using natural products such as GA for nephron and hepatic protection (Kamal et al., 2021). GA contains antioxidant macromolecules, including lysine, tyrosine, and histidine, which enhance its antioxidant activity (Naiel et al., 2021). It has been shown to lower urea nitrogen and creatinine levels, thus improving renal function in patients with diabetic nephropathy (Alshelleh et al., 2023). Additionally, GA significantly reduces fasting blood glucose, HbAc1 levels, blood

uric acid, and total protein levels. Moreover, it facilitates the passage of minerals and water from the intestinal lumen into the circulation (Salama et al., 2021).

One of the several health benefits of GA is its renal protection; it is believed that the main mechanism is GA's ability to increase nitrogen excretion, which lowers serum urea nitrogen levels (Alshelleh et al., 2023). GA is also recognized as a rich source of K^+, Mg^{2+}, and Ca^{2+} dietary fiber (Prasad et al., 2022). Furthermore, GA enhances creatinine clearance in healthy mice and promotes the excretion of antidiuretic hormone (ADH) and Ca2+ in the kidneys and intestines (Farman et al., 2020). One of the key advantages of GA is its role in increasing nitrogen excretion in stool, thereby reducing serum urea nitrogen levels. This occurs because the bacterial flora in the large intestine slowly ferments GA to form short-chain fatty acids (Salama et al., 2021). Additionally, GA can raise the quantity of lactic acid bacteria and Bifidus in healthy individuals (Yousefi et al., 2023). A previous study found that rats consuming 25–30 g of GA daily for 21-30 days experienced a reduction in total cholesterol by 6% and 10.4%, respectively. There was no impact on HDL or triglycerides; the reduction was restricted to LDL and VLDL, which aligns with the present results (Sharma, 1985).

GA has been used as a dental hygiene agent; after GA therapy, the gingival and plaque index scores were dramatically reduced when an herbal mixture containing GA was applied directly to the teeth and gums (Al-Jubori et al., 2023). The present study found that GA treatment at different doses in drinking water had beneficial hepatic and renal effects. After four weeks of treatment, improvements in kidney and liver function parameters were observed, along with enhanced measurements of antioxidant enzymes. GA treatment effectively mitigated the negative effects of CMP administration on most of kidney and liver parameters.

In terms of kidney tests, the study found that administering GA at doses of 1 g/kg body weight and a 15% weight/volume solution in drinking water for eight weeks resulted in a considerable reduction in urea and creatinine levels, consistent with Tichati et al. (2020). The increase in ALT level by GA administration in Group 3 (GA1) at 1g/kg body weight rate is in accordance with Fareed et al. (2022). However, unlike the present findings, another study reported that 0.5 g/kg b.wt/rat/day GA for 45 days increased AST and alkaline phosphatase levels (Kamal et al., 2021). A study employing a 3% GA solution for six days at a 100 mg/kg body weight dose indicated that low dosages (100 and 500 mg/day) were associated with high levels of AST and ALP, while higher doses (10 grams, 10%, and 15% daily) led to lower levels of these parameters (Babiker et al., 2017). These findings suggest that the efficacy of GA on liver function is dosage-dependent concerning alterations in lipid profile (Al-Jubori et al., 2023). Similar to the findings of El-Deeb et al. (2007), the present data demonstrated significant increases in total cholesterol, triglycerides, LDL, and VLDL, along with a significant reduction in HDL with CPM administration (3129 mg/kg) compared to the NC Group 1. In terms of antioxidant parameters, Group 2 (IC) showed a considerable decrease in antioxidant enzymes (free radical scavengers) and a significant increase in lipid peroxidation. However, the current study discovered that catalase, reduced glutathione (GSH), and superoxide dismutase (SOD) had a strong antioxidant activity, which GA markedly increased, while it decreased malondialdehyde (MDA) levels. These findings are similar to those reported by Ahmed et al. (2022).

Colon cancer results from oxidative stress caused by 1, 2-dimethylhydrazine (DMH), which damages DNA in animals' colon cells, liver, kidneys, heart, stomach, and lungs (Jelic et al., 2021). This will, in turn, result in an insufficient immune response, increased oxidative damage to lipids and proteins, lung metastasis, and the formation of alkylated DNA adducts in kidneys. Gum Arabic (GA) and Eugenol (EUG), administered either alone or combined, effectively prevent and cure rat kidney tissue and spleen geno-toxicity (Dutra et al., 2022). According to a study, rats with colorectal carcinogenesis exhibited a synergistic effect of GA and EUG on genotoxicity in the spleen and kidney tissues (Melo et al., 2023).

Histopathological results in the present study showed the anti-inflammatory and antioxidant effects of GA by reversing histopathological changes such as inflammatory cell infiltration, necrosis, apoptosis, and fibroplasia caused by oxidative stress in liver and kidney tissues. These findings align with the results of Ayaz et al. (2017).

CONCLUSION

The cytotoxic effects of chlorpyrifos-methyl, which cause oxidative stress and inflammation in liver and kidney tissues, were found to be significantly reduced by GA's strong antioxidant ability. The histological analysis of GA revealed enhanced efficacy on both liver and kidney tissues, as well as improvements in antioxidants such as Catalase, GSH, SOD, and MDA in the treated groups. Ultimately, GA (1 g/kg) demonstrated its ability to protect renal and hepatic organs against oxidative stress induced by chlorpyrifos-methyl (3129 mg/kg) administration. Future studies should evaluate the targeted genes affected by oxidative stress due to chlopyrifos-methyl administration, assess the reparative effects of GA as an antioxidant on those genes. and show how its antioxidant properties contribute to physiological mechanisms in liver and kidney tissues.

DECLARATIONS

Acknowledgments

The authors gratefully acknowledge the resources and support for this study provided by the Faculty of Agriculture at Cairo University.

Funding

The study was self-funded and there was no funding source.

Authors' contributions

The study design and experiment scheduling were contributed by Professors Hanaa Fawzy and Yasmin Emam, while the data analysis was done by Professor Emam Abd Al-Mobdy. All authors reviewed the analyzed data and gave their approval to the final draft of the manuscript.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time. Additionally, all authors checked the originality of data and sentences via plagiarism checkers.

Availability of data and materials

The original data presented in the study are included in the article. For inquiries, please contact the corresponding author/s.

Conflict of interests

No conflicts of interest have been disclosed by the authors.

REFERENCES

Ahmed N, El-Rayes SM, Khalil WF, Abdeen A, Abdelkader A, Youssef M, Maher ZM, Ibrahim AN, Abdelrahman SM, Ibrahim SF et al. (2022). Arabic gum could alleviate the aflatoxin B1-provoked hepatic injury in rat: The involvement of oxidative stress, inflammatory, and apoptotic pathways. Toxins, 14(9): 605. DOI: https://www.doi.org/10.3390/toxins14090605

Ali BH and Al Moundhri MS (2006). Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: A review of some recent research. Food and Chemical Toxicology, 44(8): 1173-1183. DOI: https://www.doi.org/10.1016/j.fct.2006.01.013

Ali AA, Ali KE, Fadlalla A, and Khalid KE (2008). The effects of GA oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in Central Sudan. Natural Product Research, 22(1): 12-21. DOI: https://www.doi.org/10.1080/14786410500463544

Ali NE, Kaddam LA, Alkarib SY, Kaballo BG, Khalid SA, Higawee A, AbdElhabib A, AlaaAldeen A, Phillips AO, and Saeed AM (2020). Gum Arabic (Acacia Senegal) augmented total antioxidant capacity and reduced C-reactive protein among haemodialysis patients in phase II trial. International Journal of Nephrology, 1: 1-7. DOI: https://www.doi.org/10.1155/2020/7214673

Ali I, Raza A, Ahmad MA, and Li L (2022). Nutrient sensing mechanism of short-chain fatty acids in mastitis control. Microbial Pathogenesis, 170: 105692. DOI: https://www.doi.org/10.1016/j.micpath.2022.105692

Al-Baadani HH, Al-Mufarrej SI, Al-Garadi MA, Alhidary IA, Al-Sagan AA, and Azzam MM (2021). The use of gum Arabic as a natural prebiotic in animals: A review. Animal Feed Science and Technology, 274: 114894. DOI: https://www.doi.org/10.1016/j.anifeedsci.2021.114894

Al-Jubori Y, Ahmed NTB, Albusaidi R, Madden J, Das S, and Sirasanagandla SR (2023). The efficacy of gum Arabic in managing diseases: A systematic review of evidence-based clinical trials. Biomolecules, 13(1): 138. DOI: https://www.doi.org/10.3390/biom13010138

Alshelleh SA, Alhawari H, Oweis AO, and Alzoubi KH (2023). Arabic gum as a natural therapeutic agent for diabetic patients with CKD: A retrospective study. Electronic Journal of General Medicine, 20(4): 1-6. DOI: https://www.doi.org/10.29333/ejgm/13183

Ayodele OE and Alebiosu CO (2010). Burden of chronic kidney disease: An international perspective. Advances in Chronic Kidney Disease, 17(3): 215-224. DOI: https://www.doi.org/10.1053/j.ackd.2010.02.001

Ayaz NO, Ramadan KS, Farid HE, and Alnahdi HS (2017). Protective role and antioxidant activity of Arabic gum against trichloroacetate-induced toxicity in liver of male rats. Indian Journal of Animal Research, 51(2): 303-309. Available at: https://www.indianjournals.com/ijor.aspx?target=ijor:ijar1&volume=51&issue=2&article=019

Babiker M, Abbas T, and Mohammed MEA (2017). Effect of gum Arabic on liver function and antioxidant enzymes of Sprague Dawley rats. IOSR Journal of Pharmacy and Biological Sciences, 12(2): 29-33. DOI: https://www.doi.org/10.9790/3008-1202032933

David S and Hamilton JP (2010). Drug-induced liver injury. US Gastroenterology and Hepatology Review, 6: 73. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3160634/

Dashtdar M and Kardi K (2018). Benefits of gum Arabic for a solitary kidney under adverse conditions: A case study. Chinese Medicine and Culture, 1(2): 88-96. DOI: https://www.doi.org/10.4103/CMAC.CMAC_22_18

Dutra BDAL, Carneiro CLB, Melo NDOR, de Oliveira GB, Jamacaru FVF, Ribeiro JC, and Dornelas CA (2022). Gum Arabic and Eugenol in a silica-induced inflammatory angiogenesis model in Wistar rat. Journal of Pharmacy and Nutrition Sciences, 12: 11-19. DOI: https://www.doi.org/10.29169/1927-5951.2022.12.02

Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, Coyle J, McKhann G, Mobley WC, Nadel L, and Neubert D (2008). Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Critical Reviews in Toxicology, 38(2): 1-125. DOI: https://www.doi.org/10.1080/10408440802272158

El-Deeb AEA, Abd El-Aleem IM, and Sherin SG (2007). Harmful effect of some insecticides on vital parameters of albino rats. Journal of the Egyptian Society of Toxicology, 36: 53-60. Available at: https://pesquisa.bvsalud.org/portal/resource/pt/emr-83713

Evans AJ, Hood RL, Oakenfull DG, and Sidhu GS (1992). Relationship between structure and function of dietary fibre: A comparative study of the effects of three galactomannans on cholesterol metabolism in the rat. British Journal of Nutrition, 68(1): 217-229. DOI: https://www.doi.org/10.1079/BJN19920079

Fan T, Xie Y, and Ma W (2021). Research progress on the protection and detoxification of phytochemicals against aflatoxin B1-Induced liver toxicity. Toxicon, 195: 58-68. DOI: https://www.doi.org/10.1016/j.toxicon.2021.03.007

Farman MS, Salman MI, and Hamad HS (2020). Effect of gum arabic administration on some physiological and biochemical parameters in chronic renal failure patients. Systematic Reviews in Pharmacy, 11(6): 697-701. DOI: https://www.doi.org/10.31838/srp.2020.6.103

Fareed SA, Almilaibary AA, Nooh HZ, and Hassan SM (2022). Ameliorating effect of gum Arabic on the liver tissues of the uremic rats: A biochemical and histological study. Tissue and Cell, 76: 101799. DOI: https://www.doi.org/10.1016/j.tice.2022.101799

Ferrer A (2003). Intoxicación por plaguicidas [Pesticide poisoning]. Anales del Sistema Sanitario de Navarra, 26: 155-171. Available at: https://scielo.isciii.es/pdf/asisna/v26s1/nueve.pdf

Finkel T and Holbrook N J (2000). Oxidants, oxidative stress and the biology of aging. Nature, 408: 239-247. DOI: https://www.doi.org/10.1038/35041687

Glover DA, Ushida K, Phillips AO, and Riley SG (2009). Acacia (Sen) SUPERGUM™ (Gum Arabic): An evaluation of potential health benefits in human subjects. Food Hydrocolloids, 23(8): 2410-2415. DOI: https://www.doi.org/10.1016/j.foodhyd.2009.06.020

Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, Pramodh S, Alsulimani A, Alkhanani MF, Harakeh S, and Hussain A (2022). Oxidative stress in human pathology and aging: Molecular mechanisms and perspectives. Cells, 11(3): 552. DOI: https://www.doi.org/10.3390/cells11030552

Heikal TM, El-Sherbiny MA, Hassan SA, Arafa A, and Ghanem HZ (2012). Antioxidant effect of selenium on hepatotoxicity induced by chlorpyrifos in male rats. International Journal of Pharmacy and Pharmaceutical Sciences, 4(4): 603-609. Available at: https://www.innovareacademics.in/journal/ijpps/Vol4Suppl4/4694.pdf

Hinson JA, Reid AB, McCullough SS, and James LP (2004). Acetaminophen-induced hepatotoxicity: Role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. Drug Metabolism Reviews, 36(3-4): 805-822. DOI: https://www.doi.org/10.1081/DMR-200033494

Hites RA (2021). The rise and fall of chlorpyrifos in the United States. Environmental Science & Technology, 55(3): 1354-1358. DOI: https://www.doi.org/10.1021/acs.est.0c06579

Hussien H, Apetrii M, and Covic A (2021). Health-related quality of life in patients with chronic kidney disease. Expert Review of Pharmacoeconomics & Outcomes Research, 21(1): 43-54. DOI: https://www.doi.org/10.1080/14737167.2021.1854091

Hussein SA, Fadlalmola HA, Salama SM, Osman EG, and Mariod AA (2022). Efficacy and safety of gum Arabic on renal failure patients: Systematic review and meta-analysis. Sudan Journal of Medical Sciences, 17(4): 459-475. Available at: https://www.ajol.info/index.php/sjms/article/view/241764

Jelic MD, Mandic AD, Maricic SM, and Srdjenovic BU (2021). Oxidative stress and its role in cancer. Journal of Cancer Research and Therapeutics, 17(1): 22-28. DOI: https://www.doi.org/10.4103/jcrt.jcrt_862_16

Kalender Y, Kaya S, Durak D, Uzun FG, and Demir F (2012). Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testis-histoarchitecture induced by chlorpyrifos in male rats. Environmental Toxicology and Pharmacology, 33(2): 141-148. DOI: https://www.doi.org/10.1016/j.etap.2011.12.008

Kamal E, Kaddam LA, Alagib A, and Saeed A (2021). Dietary fibers (gum Arabic) supplementation modulates hepatic and renal profile among rheumatoid arthritis patients, phase II trial. Frontiers in Nutrition, 8: 552049. DOI: https://www.doi.org/10.3389/fnut.2021.552049

Levesque R (2007). SPSS programming and data management. A guide for SPSS and SAS users, 4th Edition. Available at: https://dl.acm.org/doi/abs/10.5555/1199139

Luo JP, Deng YY, and Zha XQ (2008). Studies on the intervening mechanism of polysaccharides from Dendrobium huoshanense on streptozotocin-induced diabetic cataract. Pharmaceutical Biology, 46(4): 243-249. DOI: https://www.doi.org/10.1080/13880200701739397

Naiel MA, Abd El-hameed SA, Arisha AH, and Negm SS (2022). Gum Arabic-enriched diet modulates growth, antioxidant defenses, innate immune response, intestinal microbiota and immune related genes expression in tilapia fish. Aquaculture, 556: 738249. DOI: https://www.doi.org/10.1016/j.aquaculture.2022.738249

Matsumoto N, Riley S, Fraser D, Al-Assaf S, Ishimura E, Wolever T, Phillips GO, and Phillips AO (2006). Butyrate modulates TGF-β1 generation and function: Potential renal benefit for Acacia (sen) SUPERGUM™ (gum Arabic)? Kidney International, 69(2): 257-265. DOI: https://www.doi.org/10.1038/sj.ki.5000028

Melo NDOR, Silva MDS, Pires W, Lima FVFJ, Cavalcanti BC, Silva AADS, and Dornelas CA (2023). Effect of Eugenol and gum Arabic on oxidative stress and genotoxicity in rat spleen, kidney and lung tissue following colorectal carcinogenesis. International Journal of Herbal Medicine, 11: 22-29. DOI: https://www.doi.org/10.22271/flora.2023.v11.i1a.849

Montenegro MA, Boiero ML, Valle L, and Borsarelli CD (2012). Gum Arabic: More than an edible emulsifier. In: C. J. Reinhard Verbeek, Products and applications of biopolymers. IntechOpen, pp. 953-978. Available at: https://www.intechopen.com/chapters/31731

Mohammed AME (2015). Estimation of the active components in gum Arabic collected from western Sudan. International Journal of Science and Research, 6(3): 1262-1282. Available at: https://www.ijsr.net/archive/v6i3/ART20171695.pdf

Morita S, Sakai C, Sakamoto M, and Nishimoto M (2023). Evaluating pesticide ecotoxicity using a stimuli-response model in liposomes. JCIS Open, 10: 100082.DOI: https://www.doi.org/10.1016/j.jciso.2023.100082

Okamura DM and Pennathur S (2015). The balance of powers: Redox regulation of fibrogenic pathways in kidney injury. Redox Biology, 6: 495-504. DOI: https://www.doi.org/10.1016/j.redox.2015.09.039

Patel V, Upaganlawar A, Zalawadia R, and Balaraman R (2010). Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitectural evaluation. European Journal of Pharmacology, 644(1-3): 160-168. DOI: https://www.doi.org/10.1016/j.ejphar.2010.06.065

Prasad N, Thombare N, Sharma SC, and Kumar S (2022). Gum Arabic–A versatile natural gum: A review on production, processing, properties and applications. Industrial Crops and Products, (187): 115304. DOI: https://www.doi.org/10.1016/j.indcrop.2022.115304

Prudente IRG, Souza BRS, Nascimento LDC, Gonçalves VSDS, Silva DSD, Rabelo TK, Alves O, Santos AC, de Rezende Neto JM, Tavares DDS et al. (2021). Nephrotoxic effects caused by occupational exposure to agrochemicals in a region of Northeastern Brazil: A cross-sectional study. Environmental Toxicology and Chemistry, 40(4): 1132-113.DOI: https://www.doi.org/10.1002/etc.4962

Rehman KU, Wingertzahn MA, Teichberg S, Harper RG, and Wapnir RA (2003). Gum Arabic (GA) modifies paracellular water and electrolyte transport in the small intestine. Digestive Diseases and Sciences, 48(4): 755-760. DOI: https://www.doi.org/10.1023/A:1022845011192

Russo E, Bussalino E, Macciò L, Verzola D, Saio M, Esposito P, Leoncini G, Pontremoli R, and Viazzi F (2023). Non-haemodynamic mechanisms underlying hypertension-associated damage in target kidney components. International Journal of Molecular Sciences, 24(11): 9422. DOI: https://www.doi.org/10.3390/ijms24119422

Salama SM, Fadlalmola HA, Hafeez MMA, Ahmed SAM, Mohamed RA, Elatta NMA, and Mariod AA (2021). Effectiveness of gum Arabic in diabetes and its complications: A narrative review. Sudan Journal of Medical Sciences, 16(3): 436-453. DOI: http://www.doi.org/10.18502/sjms.v16i3.9703

Sharma RD (1985). Hypocholesterolaemic effect of Gum acacia in men. Nutrition Research, 5(12): 1321-1326. DOI: https://www.doi.org/10.1016/S0271-5317(85)80042-7

Tejchman K, Kotfis K, and Sieńko J (2021). Biomarkers and mechanisms of oxidative stress-Last 20 years of research with an emphasis on kidney damage and renal transplantation. International Journal of Molecular Sciences, 22(15): 8010. DOI: https://www.doi.org/10.3390/ijms22158010

Tichati L, Trea F, and Ouali K (2020). Potential role of selenium against hepatotoxicity induced by 2, 4-dichlorophenoxyacetic acid in albino Wistar rats. Biological Trace Element Research, 194(1): 228-236. DOI: https://www.doi.org/10.1007/s12011-019-01773-9

Tiss A, Carrière F, and Verger R (2001). Effects of gum Arabic on lipase interfacial binding and activity. Analytical Biochemistry, 294(1): 36-43. DOI: https://www.doi.org/10.1006/abio.2001.5095

Trommer H and Neubert RH (2005). The examination of polysaccharides as potential antioxidative compounds for topical administration using a lipid model system. International Journal of Pharmaceutics, 298(1): 153-163. DOI: https://www.doi.org/10.1016/j.ijpharm.2005.04.024

Twombley K, Baum M, and Gattineni J (2011). Accidental and iatrogenic causes of acute kidney injury. Current Opinion in Pediatrics, 23(2): 208-214. DOI: https://www.doi.org/10.1097/mop.0b013e328343cd16

Uchendu C, Ambali SF, and Ayo JO (2012). The organophosphate, chlorpyrifos, oxidative stress and the role of some antioxidants: A review. African Journal of Agricultural Research, 7(18): 2720-2728. DOI: https://www.doi.org/10.5897/AJAR11.2510

Uzun FG, Demir F, Kalender S, Bas H, and Kalender Y (2010). Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food and Chemical Toxicology, 48(6): 1714-1720. DOI: https://www.doi.org/10.1016/j.fct.2010.03.051

Wapnir RA, Sherry B, Codipilly CN, Goodwin LO, and Vancurova I (2008). Modulation of rat intestinal nuclear factor NF-kappaB by gum arabic. Rat small intestine by gum Arabic. Digestive Diseases and Sciences, 53(1): 80-87. DOI: https://www.doi.org/10.1007/s10620-007-9826-0

Williams PA and Phillips GO (2000). Handbook of physiology of hydrocolloids. CRC Press., Cambridge, pp. 155-168. Available at: https://www.yumpu.com/en/document/view/63233637/handbook-of-hydrocolloids-by-g-o-phillips-pa-williams-z-liborg

Yousefi M, Farsani MN, Ghafarifarsani H, and Raeeszadeh M (2023). Dietary Lactobacillus helveticus and gum Arabic improves growth indices, digestive enzyme activities, intestinal microbiota, innate immunological parameters, antioxidant capacity, and disease resistance in common carp. Fish & Shellfish Immunology, 135: 108652. DOI: https://www.doi.org/10.1016/j.fsi.2023.108652

Zhu X, Raina AK, Lee HG, Casadesus G, Smith MA, and Perry G (2004). Oxidative stress signaling in Alzheimer's disease. Brain Research, 1000: 32-39. DOI: https://www.doi.org/10.1016/j.brainres.2004.01.012

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 311-317.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj37
PII: S232245682400037-14

Effect of *Aloe vera* Gel and Sodium Metabisulphite on Expression of Fibroblast Growth Factor in Incision Wound of Rats

Yulia Wiji Purnamasari[1], Juniarti[2*], Nunung Ainur Rahmah[2], Hafid Ernanda[1], and Wening Sari[2]

[1]*Biomedical Sciences Department, Universitas Yarsi, Jakarta, Indonesia*
[2]*Department of Pharmacology, School of Medicine, Universitas Yarsi, Jakarta, Indonesia*

*Corresponding's author's Email: jiezka1701@gmail.com

ORIGINAL ARTICLE
Received: June 24, 2024
Revised: July 20, 2024
Accepted: August 26, 2024
Published: September 25, 2024

ABSTRACT

An incision wound is a wound caused by being sliced. Two ingredients that play a key role in the wound-healing process are glucomannan and acemannan, which are rich in polysaccharides and growth hormones. Growth hormones stimulate fibroblast activity and proliferation. The present study involved 35 Sprague Dawley male rats, aged 2-3 months old and weighing 200-300 grams. The study comprised seven groups including, negative control group (G1), positive control (aquades, G2), betadine 10% (G3), gel base (0.5 mg, G4), gel base + sodium metabisulfite 0.2 gr (G5), gel base + *Aloe vera* 5% (G6), and gel base + *Aloe vera* 5% + sodium metabisulfite 0.2 gr (G7). Each group had five replications. Initially, a 4-cm incision was made on the dorsal skin of each rat. The study lasted 15 days with observations made on days 3, 7, and 15. After the observation period, the rats were anesthetized and then terminated to collect skin tissues for microscopic examination. The tissue samples were then stained immunohistochemically to assess fibroblast growth factor (FGF) expressions. The results showed that the highest FGF expression was observed in the 5% *Aloe vera* + 2% metabisulfite group (G7), while the lowest FGF expression was in the negative control group (G1). It is concluded that *Aloe vera* L. extract gel at 5% + 2% metabisulfite (G7) significantly enhances the expression of FGF.

Keywords: *Aloe vera* L, Fibroblast growth factor, Incision wound, Skin, Sodium metabisulfite

INTRODUCTION

An incision wound is an injury caused by a sharp object slicing the skin. The wound healing process consists of four different phases including homeostasis, inflammation, proliferation, and maturation (LaiCheong and McGrath, 2017). The migration and proliferation of fibroblasts in the wound area play a crucial role in this process. An increase in fibroblast cells affects the amount of collagen fibers, which can be linked to the edges of wounds, form connective tissue, and provide strength and integrity to the wound.

Conventional Treatments for incision wounds typically include povidone-iodine and topical antibiotics (Wilantari, 2020). However, povidone Iodine 10% has several side effects such as bacterial resistance and hypersensitivity, inhibiting the wound granulation process (Amiruddin et al., 2015). To mitigate these drawbacks, herbal remedies such as *Aloe vera* can be recommended as an alternative and complementary treatment.

Aloe vera L. is a plant that contains materials for wound healing. According to Nurhidayanti (2022), *Aloe vera* has several vitamins, minerals, enzymes, polysaccharides, glucomannans, acemannans, polypakaride compounds, and organic acids that are water-soluble and fat-soluble. Glucomannans and acemannas plays an important role in the wound healing phase, as they activate macrophages, which play a key role in regulating tissue repair. Macrophages release cytokines and growth factors (PDGF, TGF-a, TGF-b, EGF VEGF) that facilitate tissue repair. Additionally, glucomannas and acemannas activate and proliferate fibroblasts, accelerating the expression of vascular endothelial growth factor (VEGF), which stimulates the formation of new capillaries in the wound-healing process. *Aloe vera* gel also contains lignin, which can penetrate and absorb into the skin, playing a significant role in the proliferation phase by promoting new cells and preventing excess fluid loss from the skin surface (Hekmatpou et al., 2019). Mustaqim et al. (2018) reported that the administration of *Aloe vera* gel on mouse wounds improved wound healing, as evidenced by the parameters of epithelial thickness and the average number of fibroblasts.

Sodium metabisulfite is a preservative substance. It is used as an antioxidant and antimicrobial agent which functions as a preservative in foodstuff, syrup medicines, cosmetic preparations, and hair nourishers (Ilie-mihai et al.,

To cite this paper: Purnamasari YW, Juniarti, Rahmah NA, Ernanda H, and Sari W (2024). Effect of *Aloe vera* Gel and Sodium Metabisulphite on Expression of Fibroblast Growth Factor in Incision Wound of Rats. *World Vet. J.,* 14(3): 311-317. DOI: https://dx.doi.org/10.54203/scil.2024.wvj37

2022). In the process of healing incisional wounds, sodium metabisulfite functions as an antimicrobial that inhibits bacterial contamination of skin tissue. As a preservative, it also helps maintain the stability of the *Aloe vera* content in the preparation. The present study aimed to evaluate the effect of the formulation of *Aloe vera* extract gel with the addition of sodium metabisulfite preservatives on the expression of FGF in wound healing.

MATERIALS AND METHODS

Ethical approval

The animal experimentation was approved by the Ethical Clearance Committee of YARSI University Research Institute, Jakarta, with registration number 135/KEP-UY/EA.10/VI/2023. This study was conducted from July to December 2023 at the Animal Research Facility, Jakarta, Indonesia.

Study design

The manufacture of the gel base was carried out at the Pharmacy Laboratory of PT., Derma Beauty Indonesia, Tangerang. The *Aloe vera* samples were washed, and the skin of the leaf gel was separated, cut into small pieces, and macerated overnight in ethanol solvent. The filtrate was separated from the residue and then evaporated using a rotary evaporator (Japan) under vacuum pressure at a temperature of ± 40°C to obtain an ethanol extract (Baldi et al., 2021).

Gel base production

The tools used included a homogenizer, a beaker, a gel placement container, and a digital scale. There was a 5% extract of *Aloe vera* L in 100-gram preparations. The gel base was prepared using *Aloe vera* extract, where 5% of the extract was calculated to be added. This means that after adding 5 g of extract to 100 grams of the gel base, the resulting amount of the gel base was 95 grams. This mixture was homogenized using a Thinky mixer (USA), at a speed of 2000 rpm for 3 minutes until homogeneous. Sodium metabisulfite (Indonesia) was added at a rate of 0.2 grams and mixed at the final stage. Next, the gel base was mixed with *Aloe vera* L extract and sodium metabisulfite at a predetermined dose using a mixer at a speed of 2000 rpm for 3 minutes. The gel base was then evaluated organoleptically for its physical stability, acidity, viscosity, and total plate and yeast mold counts for 8 weeks (Retnowati et al., 2021).

The study used male Sprague Dawley rats, 2-3 months old, weighing 200-300 grams. The rats were divided into seven groups with five repetition rats in each group including, Group 1 (negative control), Group 2 (positive control given aquades 0.5 ml), Group 3 (positive control given betadine 10 %, 0.5 ml, Indonesia), Group 4 group (given the gel base), Group 5 (given the gel base and sodium metabisulfite 0.2 gr), Group 6 (given the gel base and *Aloe vera* 5%), and Group 7 (given *Aloe vera* 5%, and sodium metabisulfite 0.2 gr). Treatment for all groups was conducted over 15 days. To begin, the rats were given anesthesia with ketamine (10 mg/kg BW) and xylazine (2 mg/kg BW, Sotoudeh and Namavar, 2022) intraperitoneally. After anesthesia, the fur on the rats' backs was shaved to a size of 5 x 3 cm. The rats were then placed on the operating table and fixed according to the procedure (Kartika et al., 2013). The skin was disinfected with betadin, rubbed with 70% alcohol, and then a 4cm long incision was made on the back. Observations were made on days 3, 7, and 15. At the end of the observation period, the rats were anesthetized and terminated using cervical dislocation. Finally, the skin tissue was taken for microscopic examination.

Sample collection

The rats were maintained for 15 days. On day 15, the skin samples were collected and fixed in 10% neutral buffer formalin.

Laboratory test

The skin samples were dehydrated using graded alcohol and xylene and then blocked using liquid paraffin (Alturkistani et al., 2015). The samples were cut using a microtome. The skin sections were then stained by immunohistochemia (IHC) antibody for fibroblast growth factor (Haid et al., 2020). The slides were then dehydrated and treated with endogenous peroxidase and protein block. Next, they were incubated using antibody primer FGF2 (Vantec bio) for several minutes. Following this, the slides were incubated with a post-primary antibody and diethylaminobenzidine (DAB). They were finally photographed using an Olympus microscope (CX33, Japan) at 400 × magnification and analyzed using ImageJ software (NIH, USA).

Data analysis

Data were analyzed quantitatively using SPSS version 25. The analysis was conducted using the Kruskal Wallis non-parametric test followed by the Mann-Whitney post hoc test. Correlation analysis was performed using the Spearman test with a confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

The results of the observation of organoleptic tests on the formulation of gel preparations on day 1 did not show any changes in color, odor, or texture. Organoleptic tests (Table 1 and Figure 1a) on the formulation of gel preparations for 8 weeks indicated no changes in the texture, color, and odor of the preparations. This stability is because of the presence of sodium metabisulfite. The addition of sodium metabisulfite was proven to increase the shelf life of the gel-based *Aloe vera* L. extraction formulation. This is due to the role of sodium metabisulfite as a preservative when added to drug formulations because sodium metabisulfite has the function of preventing the growth of microbes (Yusuf et al., 2020). The acidity test in the gel formulation of *Aloe vera* extract shown a pH of 5.1 (Table 1 and Figure 1b). This indicates that preparations have the same pH value of 4.5-6.5 as human skin (Yusuf et al., 2020). The viscosity test on the formulation of gel preparations showed a viscosity value of 5420 centipoise (cps) (Table 1 and Figure 1c). This value indicates that preparations had a low viscosity (5640 cps). Mixing sodium metabisulfite may dilute the formulation, as the small molecular weights of sodium metabisulfite and *Aloe vera* cause a decrease in the overall consistency of the preparation. However, the viscosity results for the preparations remain within the normal range because they are still in the range of 2000–50000 cps (Yusuf et al., 2020). The test of the total plate count and yeast mold count showed that the formulations had low microbial counts (Table 1 and Figure 1d). This finding proves that the addition of sodium metabisulfite to formulations can prevent the development of microbes in the preparations. This is in accordance with research by Kristantri et al. (2022), who reported that microbial agents developed more quickly in preparations without sodium metabisulfite. The reason is that sodium metabisulfite, apart from being an antioxidant, also acts as an inhibitor of microbial agents in preparations (Table 1).

In this study, the results indicated a significant increase in FGF expression after treatment with *Aloe vera* L extract gel and sodium metabisulfite ($p < 0.05$). The increase in FGF expression on day 7 was higher than on day 3. The highest FGF expression intensity was observed on days third and seventh, in the *Aloe vera* + sodium metabisulfite group compared to other groups. In this study, FGF expression increased on days 3 and 7 but decreased on day 15 (Figure 2).

Table 1. Gel stability test (pH, viscosity, total plate count, and yeast mold count)

Test	Results
Acidity degree (pH)	5.1
Viscosity	5420 cps
Total plate count	< 10^1 cfu/g
Yeast mold count	1×< 10^cfu/g

Description: Stability test results of 5% *Aloe vera* gel + 0.2 gr of sodium metabisulfite

Figure 1. Stability test results of gel preparations. **a:** Organoleptic test, **b:** pH test, **c:** Viscosity test, and **d:** Total plate test and yeast mold test

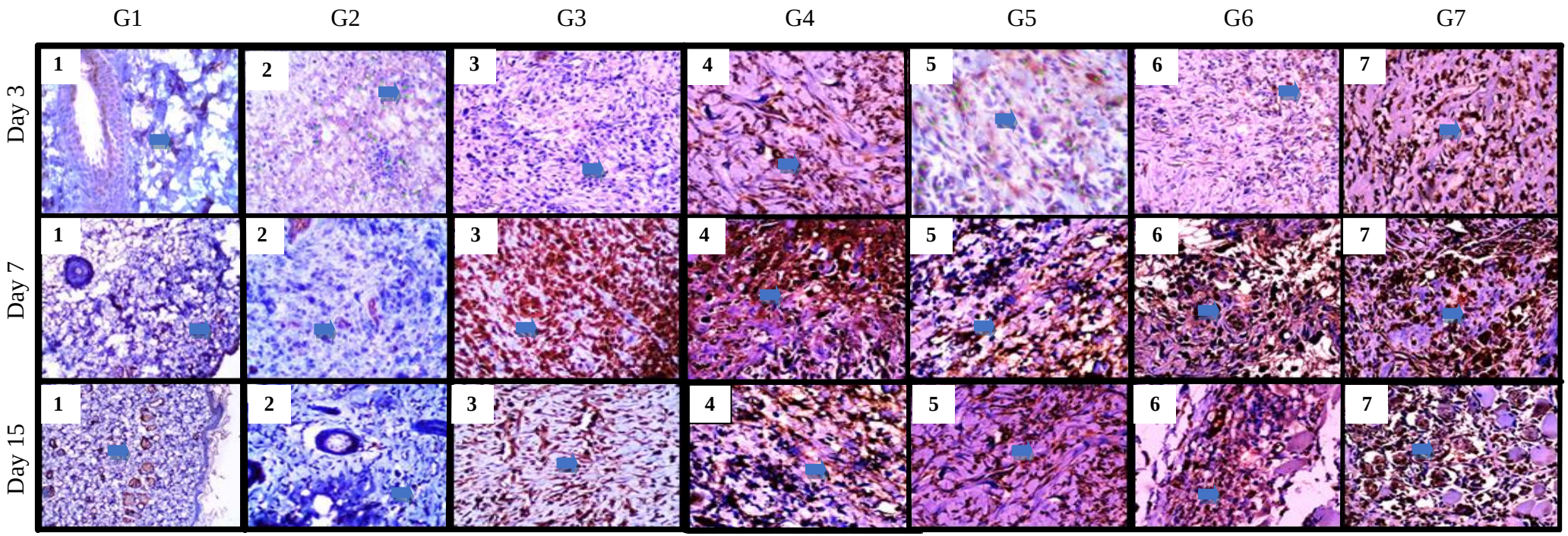


Figure 2. Histopathological description of fibroblast growth factor (FGF) expression using immunohistochemical staining of skin tissue on the backs of Sprague Dawley rats in various groups on days 3,7 and 15 with 400x magnification. **G1:** Negative control; **G2:** Distilled water; **G3:** Betadine 10 %; **G4:** Gel base; **G5:** Gel base + sodium metabisulfite 0.2 gr; **G6:** Gel base + *Aloe vera* 5%; and **G7:** Gel base + *Aloe vera* 5% + sodium metabisulfite 0.2 gr

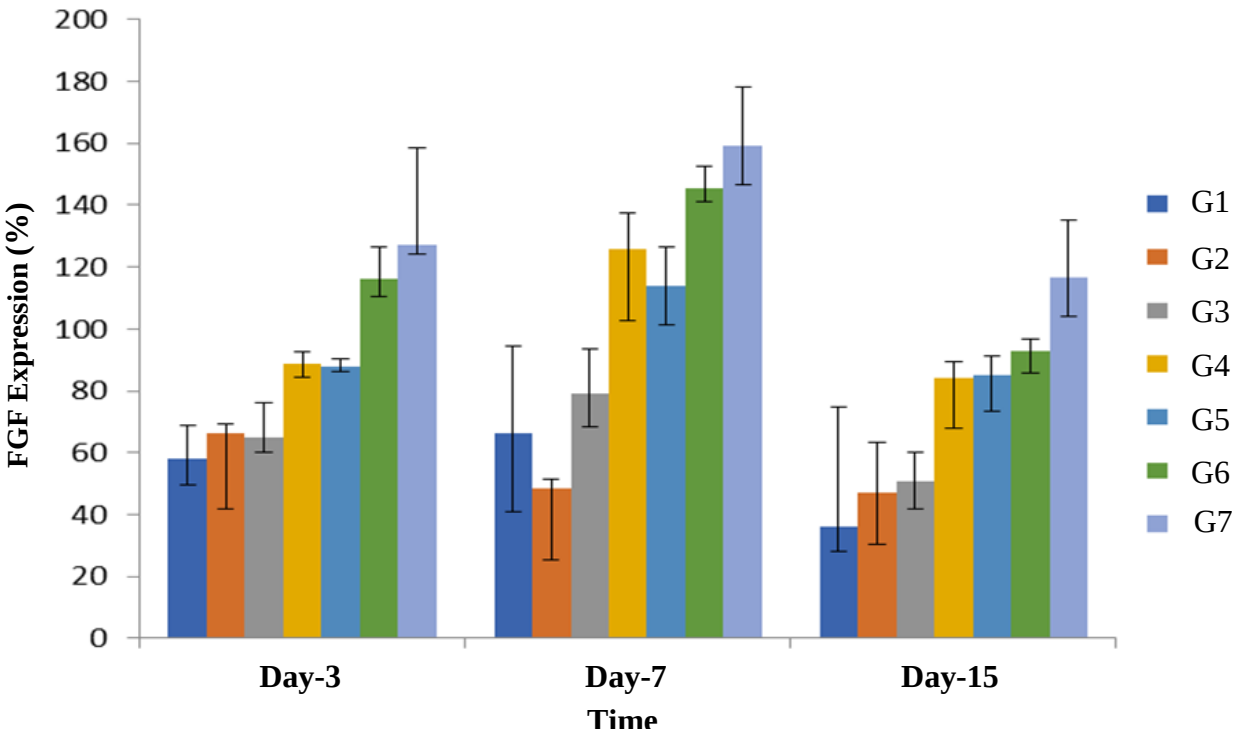


Figure 3. Comparison of fibroblast growth factor (FGF) expressions. **G1:** Negative control; **G2:** Distilled water; **G3:** Betadine 10 %; **G4:** Gel base; **G5:** Gel base + sodium metabisulfite 0.2 gr; **G6:** Gel base + *Aloe vera* 5%; and **G7:** Gel base + *Aloe vera* 5% + sodium metabisulfite 0.2 gr

The image shows a comparison of FGF expressions among different treatment groups on days 3, 7, and 15. Data are presented as median values, with minimum and maximum ranges. On day 3, G7 exhibited the highest FGF expression compared to other groups, while G1 had the lowest expression ($p < 0.05$). G2 did not differ significantly from G3 ($p > 0.05$), and there was no significant difference between G4 and G5 ($p > 0.05$). By day 7, the highest FGF expression remained in G7, followed by G6, while G5 had a lower FGF expression compared to G4 ($p < 0.05$). G3 showed a higher expression compared to G2 and G1. On day 15, FGF expression in G7 was still the highest, with G1 maintaining the lowest expression. G2 did not show a significant difference compared to G3 ($p > 0.05$). Also, there was no significant difference between G4 and G5. These results indicate that the addition of *Aloe* vera and sodium metabisulfite (AVM) significantly increases FGF expression compared to other treatments (Figure 3).

Fibroblast growth factor-2 (FGF-2) plays a crucial role in angiogenesis in wounds, the formation of granulation tissue, and the regulation of re-epithelialization (Takaya et al., 2022). Besides activating fibroblasts, FGF-2 activates other cells originating from the mesoderm, including vascular endothelium and smooth muscle cells, osteoblasts, and chondrocytes. FGF-2 can also accelerate the healing of acute and chronic wounds (Cowin, 2019). In this study, FGF expressions increased in all groups on days 3 and 7 but decreased on day 15. FGF-2 increases in the acute phase of wounds because it plays a role in the formation of granulation tissue, re-epithelialization, and tissue remodeling. FGF-2 also regulates the synthesis and deposition of extracellular matrix components, increasing keratinocyte movement, accelerating fibroblast migration, and stimulating collagen production. FGF-2 levels decreased in the chronic wound phase. This is characterized by an increase in FGF and fibroblasts on day 3 and reaching a peak on day 7 (Wei et al., 2022). Other studies have shown that local administration of FGF-2 can have anti-fibrotic effects in wounds, fighting myofibroblast differentiation and reducing fibrosis in wound tissue (Koike et al., 2020).

The results of this study are in line with several previous studies. In the study by Koike et al. (2020), local administration of FGF-2 also had anti-fibrotic effects showing the same functions in fighting myofibroblast differentiation and reducing fibrosis in wound tissue. They also reported that FGF-2 stimulates re-epitheliation in epidermal defect wound models. The results of the study by Takzaree et al. (2016) pointed to a significant increase in fibroblast formation and accelerated wound healing in the group that received *Aloe vera* gel, accompanied by an increase in TGF-β and FGF gene expression. Moreover, Atiba et al. (2022) found that topical application of *Aloe vera* gel not only accelerated wound healing but also increased the expression of vascular endothelial growth factor, basic fibroblast growth factor, and antioxidants. Additionally, Hormozi et al. (2017) reported that the administration of *Aloe vera* increased the expression of TGFβ1 and FGF *in vitro.*

To cite this paper: Purnamasari YW, Juniarti, Rahmah NA, Ernanda H, and Sari W (2024). Effect of *Aloe vera* Gel and Sodium Metabisulphite on Expression of Fibroblast Growth Factor in Incision Wound of Rats. *World Vet. J.,* 14(3): 311-317. DOI: https://dx.doi.org/10.54203/scil.2024.wvj37

CONCLUSION

The combination of *Aloe vera* L. extract gel at 5% concentration and sodium metabisulfite (0.2 gr) was more effective in increasing FGF expression than *Aloe vera* or sodium metabisulfite alone in the treatment of incision wounds on rat backs. Future research should consider comparing optimal treatment durations, examining different concentrations of *Aloe vera* extract, exploring the long-term effects of *Aloe vera* gel and sodium metabisulfite, and testing different wound models.

DECLARATIONS

Acknowledgments

All authors acknowledge the research participants at the Animal Research Facility, Yarsi University for their assistance.

Authors' contributions

Yulia Wiji Pirnama Sari performed an experiment and data analysis. Muhammad Hafid Ernanda designed and drafted the manuscript. Juniarti designed monitored, evaluated the data analysis, and revised the draft of the manuscript. Nunung Ainur Rahmah and Wening Sari performed data analysis and corrected the paper. The last edition of the manuscript was read and approved by all authors.

Funding

This study did not receive any financial support.

Competing interests

The authors have no conflict of interest.

Ethical considerations

This paper was originally written by the authors and has not been published elsewhere. The authors checked the text of the article for plagiarism index and confirmed that the text of the article is written based on their original scientific results.

Availability of data and materials

The data to support this study finding is available upon reasonable request to the corresponding author.

REFERENCES

Alturkistani HA, Tashkandi FM, and Mohammedsaleh ZM (2015). Histological stains: A literature review and case study. Global Journal of Health Science, 8(3): 72-79. DOI: https://www.doi.org/10.5539/gjhs.v8n3p72

Amiruddin, Syafruddin, Zuraidawati, Desky R, Siregar TN, Sayuti A, and Harris A (2015). The effect of papaya latex (*Carica papaya*) latex and povidone iodine on castration wound healing of male cat (*Felis domestica*). Jurnal Medika Veterinaria, 9(1): 44-47. DOI: https://www.doi.org.10.21157/j.med.vet..v9i1.2994

Atiba A, Abdo WS, kamal Ali E, Abd-Elsalam MM, Amer M, Monsef AA, Taha RS, Antar SA, and Mahmoud AI (2022). Topical and oral applications of *Aloe vera* improve healing of deep second-degree burns in rats via modulation of growth factors. Biomarkers, 27: 608-617. DOI: https://www.doi.org/10.1080/1354750x.2022.2085800

Baldi A, Sommella E, Campiglia P, and Daglia M (2021). *Aloe* gel-base food products: Chemical, toxicological, and regulatory aspects. Regulatory Toxicology and Pharmacology, 119: 104818. DOI: https://www.doi.org/10.1016/j.yrtph.2020.104818

Cowin AJ (2019). New innovations in wound healing and repair. International Journal of Molecular Sciences, 20(7): 1724. DOI: https://www.doi.org/10.3390/ijms20071724

Haid B, Pechriggl E, Nägele F, Dudas J, Webersinke G, Rammer M, Fritsch H, and Oswald J (2020). FGF8, FGF10 and FGF receptor 2 in foreskin of children with hypospadias: An analysis of immunohistochemical expression patterns and gene transcription. Journal of Pediatric Urology, 16(1): 41.e1-41.e10. DOI: http://www.doi.org.10.1016/j.jpurol.2019.10.007

Hekmatpou D, Mehrabi F, Rahzani K, and Aminiyan A (2019). The effect of *Aloe vera* clinical trials on prevention and healing of skin wound: A systematic review. Iranian Journal of Medical Sciences, 44(1): 1-9. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6330525/

Hormozi M, Assaei R, and Boroujeni MB (2017). The effect of *Aloe vera* on the expression of wound healing factors (TGFβ1 and bFGF) in mouse embryonic fibroblast cell: *In vitro* study. Biomedicine & Pharmacotherapy, 88: 610-616. DOI: https://www.doi.org/10.1016/j.biopha.2017.01.095

Ilie-Mihai RM, Ion BC, and van Staden JKF (2022). Sodium metabisulfite in food and biological samples: A rapid and ultra-sensitive electrochemical detection method. Micromachines, 13(10). DOI: https://www.doi.org/10.3390/mi13101707

Kartika AA, Siregar HC, and Fuah AM (2013). Business development strategy raising rats (*Rattus noevegicus*) and mice (mus musculus) at the faculty IPB animal husbandry. Journal of Production Science and Product Technology Farm, 1(3): 147-154. Available at: https://journal.ipb.ac.id/i ndex.php/ipthp/article/view/15543

Koike Y, Yozaki M, Utani A, and Murota H (2020). Fibroblast growth factor 2 accelerates the epithelial-mesenchymal transition in keratinocytes during wound healing process. Scientific Reports, 10(1): 1-13. DOI: https://www.doi.org/10.1038/s41598-020-75584-7

Kristantri R, Sari W, and Pebriani TH (2022). Test the total plate number (ALT) and yeast mold number (AKK) of sunscreen spray gel preparations for ethanol extract of cinnamon bark (Cinnamomum burmanii Ness. BI. Syn). Journal of Pharmaceutical Sciences, 3(2): 300-301. Available at: https://journal.ummat.ac.id/index.php/farmasi/article/view/9397/pdf

LaiCheong JE and McGrath JA (2017). Structure and function of skin, hair and nail. Journal Medicine Abingdon, 41(6): 317-320. DOI: https://www.doi.org/10.1016/j.mpmed.2013.04.017

Mustaqim A, Asri A, and Almurdi (2018). Effect of administering *Aloe vera* gel (*Aloe vera*) on the histopathological picture of the stomach of Wistar rats induced by indomethacin. Andalas Health Journal, 6(3): 641-645. DOI: https://www.doi.org/10.25077/jka.v6.i3.p641-646.2017

Nurhidayanti N (2022). The effectiviness test of *Aloe vera* extract inhibiting the growth of *Staphylococcus aureus* bacteria. Jurnal Biologi Lingkungan Industri Kesehatan, 8(2): 146-151. DOI: https://www.doi.org/10.31289/biolink.v8i2.5942

Retnowati D, Sari R, Hendradi E, and Septiani S (2021). The stability and irritability study of the chitosan-*Aloe vera* spray gel as wound healing. Journal of basic and clinical physiology and pharmacology, 32(4): 651-656. DOI: https://www.doi.org/10.1515/jbcpp-2020-0407

Sotoudeh N and Namavar MR (2022). Optimisation of ketamine-xylazine anaesthetic dose and its association with changes in the dendritic spine of CA1 hippocampus in the young and old male and female Wistar rats. Veterinary Medicine and Science, 8(6): 2545-2552. DOI: https://www.doi.org/10.1002/vms3.936

Takaya K, Aramaki-Hattori N, Sakai S, Okabe K, Asou T, and Kishi K (2022). Fibroblast growth factor 7 suppresses fibrosis and promotes epithelialization during wound healing in mouse fetuses. International Journal of Molecular Sciences, 23(13): 7087. DOI: https://www.doi.org/10.3390/ijms23137087

Takzaree N, Hadjiakhondi A, Hassanzadeh G, Rouini MR, Manayi A, and Zolbin MM (2016). Transforming growth factor-β (TGF-β) activation in cutaneous wounds after topical application of *Aloe vera* gel. Canadian Journal of Physiology and Pharmacology, 94(12): 1285-1290. DOI: https://www.doi.org/10.1139/cjpp-2015-0460

Wei L, Tan J, Li L, Wang H, Liu S, Chen J, Weng Y, and Liu T (2022). Chitosan/Alginate hydrogel dressing loaded FGF/VE-cadherin to accelerate full-thickness skin regeneration and more normal skin repairs. International Journal of Molecular Sciences, 23(3): 1249. DOI: https://www.doi.org/10.3390/ijms23031249

Wilantari P (2020). Incision wound healing activity from binahong leaf ointment (*Anredera scandens* (L.) moq.). Journal Udayana Pharmacy, 8(2): 78-89. DOI: https://www.doi.org/10.24843/jfu.2019.v08.i02.p04

Yusuf AL, Nugraha D, Wahlanto P, Indriastuti M, and Lestari NI (2020). Activity test of *Aloe Vera* skin extract gel for healing light burns in rabbits (*Oryctolagus cuniculus*). Jurnal Wiyata, 7(2): 133-137. Available at: https://ojs.iik.ac.id/index.php/wiyata/article/view/379

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 318-327.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj38
PII: S232245682400038-14

Determining the Ideal Temperature and Fermentation Duration to Enhance Crude Protein Content and Reduce Crude Fiber in Rice Bran Using Solid-State Fermentation with *Aspergillus niger* (USM F4)

Animashahun Razaq Adekunle[1]*, Akpor Oghenerobor Benjamin[2], Olamide Musa[1], Alabi Olayinka Olubunmi[1], Oyawoye Enoch Olayiwola[1], Okocha Reuben[1], Oluwafemi Precious[1], Animashahun Adedeji Peculiar[3], and Idowu Abiodun[1]

[1]*Animal Science Programme, College of Agricultural Sciences, Landmark University, P.M.B. 1001, Omu Aran, Nigeria*
[2]*Department of Biological Sciences, College of Sciences, Afe Babalola University, P. M. B. 5454 Ado Ekiti, Ekiti State, Nigeria*
[3]*Department of Animal Breeding and Genetics, Federal University of Agriculture Abeokuta, P.M.B 2240, Abeokuta, Nigeria*

*Corresponding author's Email: animashaun.rasaq@lmu.edu.ng; kunleannies@yahoo.com

ORIGINAL ARTICLE
Received: June 29, 2024
Revised: July 23, 2024
Accepted: August 13, 2024
Published: September 25, 2024

ABSTRACT

Solid-state fermentation (SSF) offers a sustainable method for enhancing the nutritional quality of agricultural residues such as red rice bran. This study aimed to determine the optimal temperature and duration for SSF of red rice bran, focusing specifically on increasing the crude protein (CP) content and reducing the crude fiber (CF) content. SFF of rice bran with *Aspergillus niger* (*A. niger*) USM F4 was conducted over 14 consecutive days at three different temperatures (25°C, 35°C, and 45°C). A total of 63 samples of rice bran were divided into three temperature groups, each containing 21 samples. Three samples per group were collected at 48-hour intervals over the 14-day fermentation period. The fermentation process for the collected samples at 48-hour intervals was halted by oven drying at 60°C for 24 hours. The fermented products were subjected to proximate analysis for crude protein (CP), ash, ether extract (EE), and crude fiber (CF) contents using the methods outlined by the Association of Official Analytical Chemists (AOAC). The results revealed a significant effect of temperature and fermentation duration on CP, ash, EE, and CF content when compared to the unfermented rice bran kept at room temperature (25°C). The peak values of CP and the highest degradation of CF across all temperature levels were observed on day 10 while the maximum increase in ash and EE content occurred on day 8. Among the temperature conditions, the highest CP values and the lowest CF values were recorded at 35°C. Conversely, the lowest improvements in CP and CF degradation were observed at 25°C on day 10. In conclusion, the optimal conditions for SSF of rice bran with *A. niger* to enhance CP content and degrade CF are a temperature of 35°C and a fermentation duration of 10 days.

Keywords: Alternate feed resource, Animal production, *Aspergillus niger*, Proximate component, Solid-state fermentation, Value- addition

INTRODUCTION

Food insecurity in Africa poses a significant threat to the continent's sustainability. Boosting livestock production levels is imperative for augmenting the supply of animal protein for human consumption, thereby contributing to global efforts to address food scarcity, hunger, and food security challenges. The high cost and scarcity of conventional feed ingredients pose a significant challenge to the sustainability of animal production. Consequently, the industry has increasingly focused on identifying alternative feed resources (Akintan et al., 2024; Dou et al., 2024).

Along the same line, agro-industrial by-products have turned into a primary focus of animal scientists seeking potential alternatives to conventional feed resources (Ayojimi et al., 2023). Many of these by-products, including rice bran (RB), are characterized by low crude protein content, high crude fiber levels, and the presence of anti-nutritive factors such as low digestibility and nutrient lock-up, which limit their suitability for livestock production (Obaniyi et al., 2019; Yang et al., 2021).

Fermentation has long served as a method for preserving and enhancing the quality attributes of foods (Siddiqui et al., 2023). Solid-state fermentation (SSF) occurs without free water and presents a technological solution for processing various foods, enhancing their nutritional value, and creating edible products with favorable sensory traits (Senanayake et al., 2023). During SSF, microorganisms metabolize the substrate, producing various metabolites such as organic acids, enzymes, vitamins, antibiotics, and bioactive compounds. These metabolites have potential applications in the food, pharmaceutical, and cosmetics industries, to name a few. The liberated enzymes also help increase the nutritional value of the substrate by increasing the crude protein content and reducing the crude fiber level, thereby transforming the agro-industrial waste into value-added products (Sadh et al., 2018; Senanayake et al., 2023). Yang et al. (2021) assert that the

To cite this paper: Animashahun RA, Akpor OB, Olamide MO, Alabi OO, Oyawoye EO, Okocha RC, Oluwafemi P, Animashahun AP, and Idowu A (2024). Determining the Ideal Temperature and Fermentation Duration to Enhance Crude Protein Content and Reduce Crude Fiber in Rice Bran Using Solid-State Fermentation with *Aspergillus niger* (USM F4). *World Vet. J.*, 14(3): 318-327. DOI: https://dx.doi.org/10.54203/scil.2024.wvj38

SSF of feed represents a promising strategy for bridging the gap between feed supply and demand, enhancing food safety, conserving energy, and lowering emissions.

Fermentation, as proposed by Osemwegie et al. (2020), can enhance the nutritional value of ingredients before they are consumed by animals. Solid-state fermented feed production yields various beneficial components, such as organic acids, enzymes (amylase, cellulase, etc.), vitamins, β-Carotene, γ-linolenic acid, citric acid, peptides, and growth factors, all of which can positively impact animal performance (Yang et al., 2021).

The nutritional characteristics of fermented feed are influenced by several factors, including the fermentation starter (the culture initiating fermentation), the substrate type, the initial moisture content, and the fermentation conditions, such as temperature and duration (Mengesha et al., 2022). Recent research has consistently shown that adjusting the moisture content of fermentation substrates is crucial and should be tailored to the specific properties of the substrates (Sun et al., 2022; Wang et al., 2023), microbial attributes, duration, and temperature (Park et al., 2018).

Optimal temperature is crucial for promoting microorganism growth and metabolism, which in turn reduces the fermentation time and improves the product quality (Yang et al., 2021). Elevated temperatures can accelerate reactions and microbial growth (Li et al., 2024). However, they may also deactivate enzymes and cause excessive heat due to the rapid proliferation of microorganisms (Yang et al., 2021). Moisture content is another key factor that significantly impacts the quality of solid-state fermented feed. Low moisture in solid-state fermentation hampers the movement of nutrients, reduces enzyme activity, and consequently limits the growth of microorganisms, thereby affecting the efficiency of the fermentation process (He et al., 2019). Inadequate water availability within the system can prevent nutrients from spreading effectively throughout the substrate, limiting their availability to microorganisms. The enzymes require water to function optimally, and their activity may be hindered when water is scarce. Also, low moisture conditions impede nutrient diffusion and enzyme activity, creating an unfavorable environment for microbial growth (Yang et al., 2021). As a result, the growth of microorganisms is restricted or slowed down, impacting the overall fermentation process (Cruz-Parede et al., 2021; Gonzalez and Aranda, 2023). Conversely, excessive moisture can reduce substrate porosity, impair oxygen transfer, and increase mycotoxin risk. Improper moisture levels disrupt microbial growth, destabilize pH, and reduce dry matter recovery. Laure et al. (2021) opted that incubation duration is another critical factor in the SSF process, as it influences the quality of the end products. In the early stages of SSF, an ample supply of food supports the growth of microorganisms and the production of the desired end product. However, in case the fermentation process is terminated too soon, the organisms may not have completed the conversion of all available nutrients into an end product, leading to incomplete fermentation and potentially lower concentrations of the desired end products (Yang et al., 2021). Extended fermentation periods, on the contrary, diminish nutrient availability, leading to a decline in bacterial population and the triggering of autophagy (Zhang et al., 2015).

Different microorganisms can be used to valorize various substrates for the production of specific products (Pranay et al., 2019; de Oliveira et al., 2020; Melnichuk et al., 2020; Putri et al., 2020). Filamentous fungi from genera such as *Aspergillus, Fusarium, Penicillium, Rhizopus, and Trichoderma* are particularly favored for solid-state fermentation, as highlighted by Munishamanna et al. (2017). This preference stems from their hyphal structure, which enables them to penetrate solid matrices effectively. Additionally, these fungi exhibit efficient digestion of solid organic substrates even under low moisture conditions, rendering them particularly suitable for solid-state fermentation, as emphasized by Abu Yazid et al. (2017). The selection of microorganisms is influenced by several critical factors, including their growth traits, the yield of the intended product, their ability to metabolize particular substrates, their resilience to temperature and pH fluctuations, their susceptibility to genetic alterations, and the safety of the resulting fermented product for human and animal consumption (Upadhyaya et al., 2016).

Yafetto et al. (2020), for instance, showcased that the protein levels in cassava and yam substrates could be enhanced through the application of mono- and co-cultures involving *Aspergillus niger* and *Trichoderma viride*. Similarly, Aruna et al. (2018) utilized a mono-culture approach using the yeast *Saccharomyces cerevisiae* to increase the protein content of yam peels.

Maximizing the potential of solid-state fermentation in enhancing agro-industrial by-products in the livestock industry requires the determination of optimal conditions. Hence, this study aimed to identify the ideal temperature and duration for solid-state fermentation of rice bran (RB) using *Aspergillus niger* (USM F4).

MATERIALS AND METHODS

Study location

The research was conducted at the Animal Science and Microbiology Laboratories of Landmark University, Omu-Aran, Kwara State, Nigeria.

Source of rice bran

The rice bran was purchased from a feed mill in Omu Aran town, Kwara State, Nigeria.

Source of candidate organism for solid-state fermentation

The fully typed strain of filamentous *Aspergillus niger* (USM F4) was obtained from the stock culture at the Microbiology Laboratory of Landmark University, Omu Aran, Kwara State, Nigeria. The organism was sub-cultured on Potato Dextrose Agar slants and maintained at 4°C for a week (Ogbonna et al., 2015; Ajibo and Said, 2023). To inhibit bacterial growth, the agar was supplemented with 0.5 ml of gentamycin (40 mg/ml) After 7 days, the mycelia had completely covered the surface of the petri dish. Spores of *Aspergillus niger* were harvested by gently tapping and inverting the plate. Subsequently, the spores were washed with sterile saline solution, and their quantification was performed using a hemocytometer (Model No: 8100110, AS ONE Japan) following the Fuchs-Rosenthal technique (Wolk et al., 2000), resulting in an estimated concentration of approximately 2.0×10^7 spore-forming units (sfu) per milliliter

Substrate preparation and inoculation for solid-state fermentation

Thirty grams of rice bran (RB) were weighed and added to each of the sixty-three 250 ml Erlenmeyer flasks. Subsequently, the moisture content was adjusted to 80%, and the pH was set to 5.0, as described by Teixeira da Silva et al. (2016). The mouths of the flasks were sealed with water-resistant cotton wool and covered with aluminum foil. All the samples were then autoclaved for 15 minutes at a pressure of 103.421 KPa and a temperature of 121°C. After autoclaving, the samples were cooled at 25°C before inoculation with the fungus. In each flask, 0.1 g of urea $[CO(NH_2)_{/2}]$, serving as a nitrogen supplement, was added per gram of the sample. After sterilization, rice bran samples in each flask were aseptically inoculated with 4 ml of the prepared *A. niger* (USM F4), containing 2.0 x 10^7 spore-forming units per milliliter, within a laminar flow chamber. The incubation temperature varied at 25°C, 35°C, and 45°C, with 24 inoculated samples incubated at each temperature.

Triplicate samples were collected at 2-day intervals over 14 days (specifically on days 2, 4, 6, 8, 10, 12, and 14) at each temperature to determine the optimal fermentation period. The fermentation process was terminated by oven drying the samples at 60°C for 24 hours, following the method outlined by Lasekan and Shittu (2019).

Determination of proximate values

The unfermented RB and the fermented samples were analyzed for crude protein (CP), ether extract (EE), ash, and crude fiber (CF) using standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2012). Crude protein content was analyzed by the Kjeldahl Method (AOAC Method 968.06). It was then calculated by multiplying the value of nitrogen by a conversion factor of 6.25.

Crude protein (%) = N X 6.25 (Formula 1)

Ether extract (EE) analysis was performed using Soxhlet Extraction **(**AOAC Method 920.39). Lipids in the samples were extracted utilizing petroleum ether as the solvent. The samples were placed in an extraction thimble and introduced into the Soxhlet apparatus. As the ether was heated to its boiling point, it passed through the samples, dissolving the lipids, which were then transported into a collection flask. The extraction continued until all fat was thoroughly removed. Following this, the lipid residue underwent drying to eliminate any lingering traces of ether. The weight of the dried residue was measured to determine the quantity of lipids extracted from the samples using Formula 2.

Ether extract (%) = (initial weight – final weight) ÷ initial weight X 100 (Formula 2)

Ash analysis was conducted using the Dry Ashing method (AOAC Method 942.05), where the organic material underwent complete combustion, leaving behind inorganic residue (ash). One gram of the sample was heated in a muffle furnace at 550°C until thorough combustion occurred. After cooling, the ash was weighed using a sensitive scale (Chaux Corp. Pine Brook, NJ USA. Model PAS 12).

The analysis of crude fiber (CF) was conducted using AOAC method 978.10. It involved sequential acid and alkali extractions to eliminate protein, sugar, starch, lipids, and portions of structural carbohydrates and lignin. Initially, the sample underwent digestion with sulfuric acid to extract sugar and starch. Subsequently, secondary digestion in sodium hydroxide was aimed at removing proteins, hemicellulose, and lignin. The determination of crude fiber was achieved gravimetrically by examining the residue remaining after the completion of acid and alkaline digestions.

Statistical analysis

All experiments were conducted in triplicate, with the proximate parameters expressed as the mean ± standard deviation of three measurements. Statistical analysis was performed using SPSS version 18, employing analysis of

variance (ANOVA) to compare the data. Mean values were compared using the Duncan multiple range test, with significance set at $p < 0.05$. The optimization of temperature and fermentation period was performed using a one-factor-at-a-time design. This approach is straightforward to implement, making it suitable for preliminary or exploratory studies where simplicity is prioritized over complexity.

RESULTS AND DISCUSSION

Table 1 presents the proximate analysis of the unfermented rice bran. The values obtained are similar to those in previous studies on the proximate composition of rice-bran meal (Mishra, 2017; Devi et al., 2021).

Table 1. Proximate components of unfermented rice bran

Parameters	Percentage
Moisture	9.45
Crude protein	13.48
Ether extract	12.35
Ash	10.50
Crude fiber	27.50
Nitrogen free extract	29.72

Effect of temperature and fermentation period on proximate components of rice bran

The findings of this study indicated that the proximate components of rice bran were significantly influenced ($p < 0.05$) by varying solid-state fermentation temperatures and durations, as compared to unfermented rice bran.

Effect of temperature and fermentation period on crude protein

The crude protein (CP) content is a crucial parameter affecting the overall quality of animal feed products. The CP level can vary depending on factors such as the microorganism used, their access to carbon and nutrients, and cultivation conditions (Shi et al., 2021). Figure 1 illustrates the changes in the CP content of RB following SSF at three temperature levels and with different durations. In this study, the CP content exhibited a progressive increase with fermentation duration across all temperature levels, albeit at varying rates. Various authors have reported comparable results using rice bran as a substrate with different fungal species. Specifically, Omarini et al. (2019) utilized *Pleurotus sapidus*, Wolayan and Mandey (2019) employed *A. niger*, Sukma et al. (2021) utilized *Rhizopus oryzae*, and Huervana et al. (2024) employed *Tichoderma harzianum.*

In SSF, the fungus releases a range of extracellular enzymes, enabling it to acquire nutrients while altering the chemical composition of the substrate and producing various metabolites (El-Gendi et al., 2021). The rise in protein levels may stem from the proliferation of fungus mycelium driven by microbial growth and reproduction throughout fermentation (Shi et al., 2021). The increased CP content of the RB suggests that the treated substrate may serve as a valuable protein source for livestock (Huervana et al., 2024). The greatest enhancements in CP content occurred on the 10th day across all temperature levels, followed by a decline. Specifically, CP values reached 18.23%, 25.34%, and 22.35% at 25°C, 35°C, and 45°C, respectively, representing respective improvements of 35.23%, 87.98%, and 65.80% compared to the 13.48% CP content in unfermented RB. The notable 87.98% improvement observed at 35°C suggests that *A. niger* USM F4 thrives best at this temperature. During fermentation, the fungus may release proteins from the substrate to utilize it as a carbon source, thereby increasing the CP of the fermented product. Variations in CP among different temperatures may be attributed to *A. niger* USM F4 releasing a higher number of enzymes during fermentation at 35°C.

Huervana et al. (2024) had earlier reported a 169.2% enhancement of CP in rice bran fermented with *Tichoderma harzianum* at 30^{o}C for 4 days under solid-state fermentation. Wolayan and Mandey (2019) conducted a similar study, fermenting RB with *A. niger* at 30^{o}C for 10 days, and reported a crude protein enhancement of 41.24%. This enhancement is notably lower than the values observed at 35°C on the 10th day in the current study. Similarly, Sukma et al. (2021) conducted an SSF experiment using the fungus *Rhizopus oryzae* (*R. oryzae*) at varying incubation temperatures (28°C, 30°C, and 32°C) for 5 days and achieved the highest percentage increase in CP content of 62.51% at 30°C. Also, Oliveira et al. (2010) reported a 40% enhancement in CP using the same fungus (*R. oryzae)* at 30°C. Omarini et al. (2019) reported a 72.97% increment in crude protein when RB underwent 10 days of solid-state fermentation assisted by *Pleurotus sapidus* at 25°C for 5 days.

The choice of variables, such as the fermentation temperature, the duration, and the microorganism used for the fermentation process significantly influenced the crude protein enrichment in RB in this study (Sukma et al., 2021).

Furthermore, differences between the values obtained in this study and those in the previous studies may be attributed to various factors, including the origin of *A. niger*, its enzyme production capacity, the amount of inoculum, the duration and conditions of fermentation, the pretreatment method, the origin of the substrate, etc. (Altop et al., 2018).

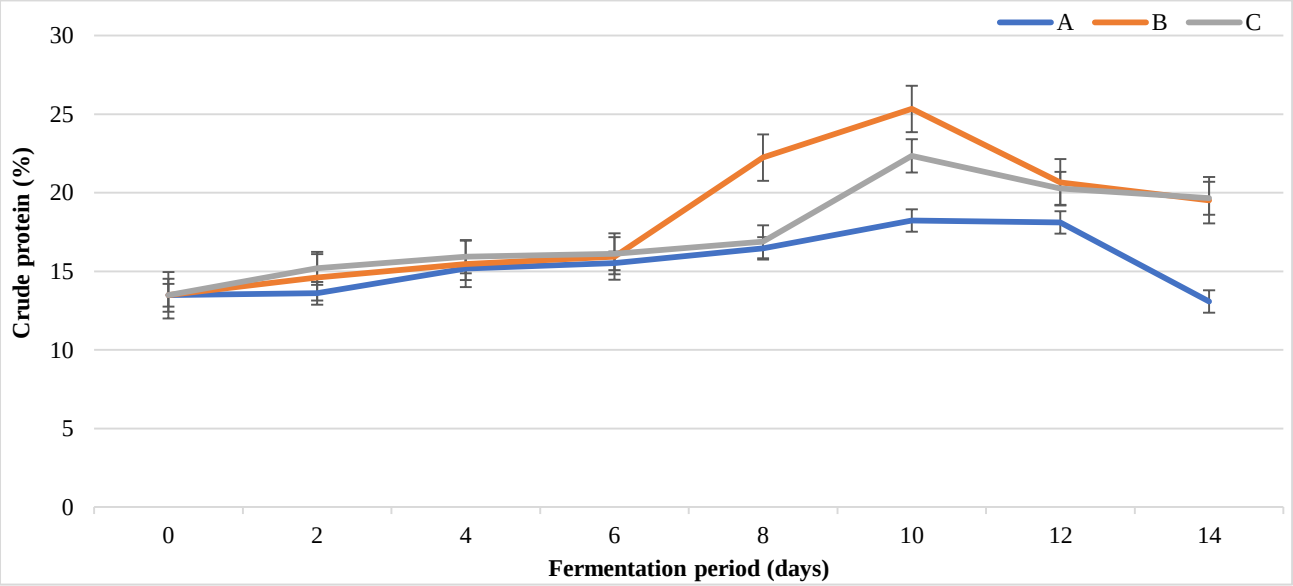


Figure 1. Influence of temperature and fermentation period on the crude protein of rice bran .**A**: 25°C, **B**: 35°C, **C**: 45°C.

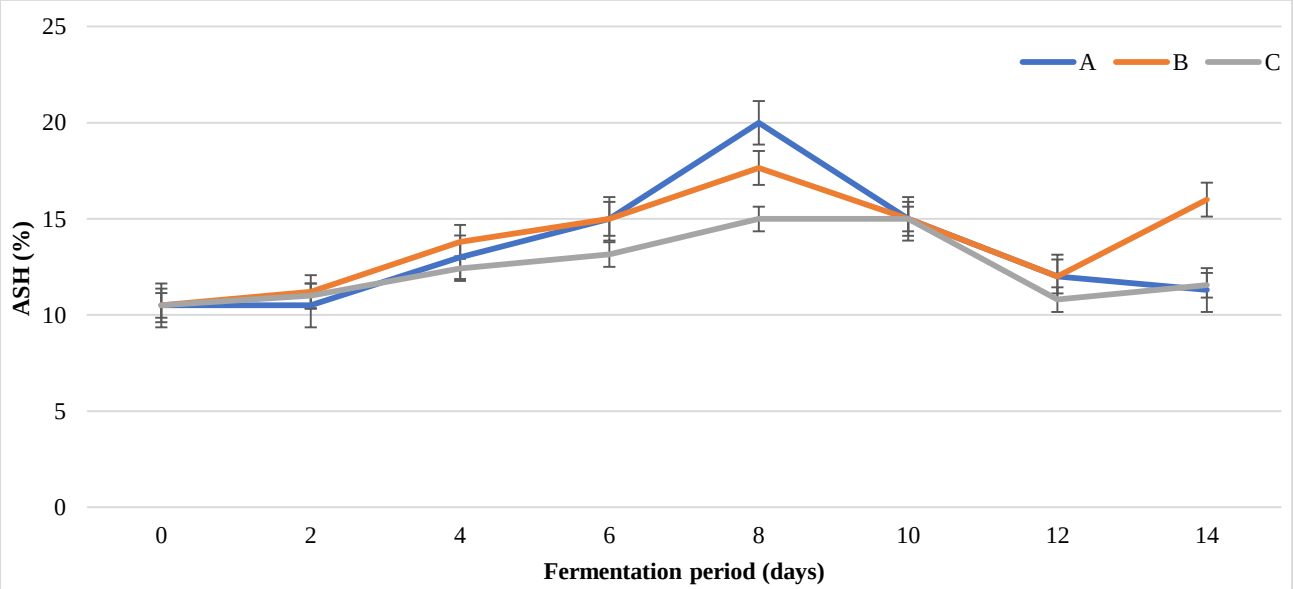


Figure 2. Effect of temperature and fermentation period on the ash content of rice bran .A: 25°C; B: 35°C; C: 45°C

Effect of temperature and fermentation period on ash component of rice bran

It was noted that the solid-state fermentation process led to an increase in the ash value of rice bran. As illustrated in Figure 2, both temperature and fermentation duration exerted significant effects on the ash content of RB ($p < 0.05$). Across all temperature levels (25°C, 35°C, and 45°C), the ash content exhibited a consistent rise from 10.50% in unfermented rice bran until day 8, followed by a gradual decline. The maximum ash contents recorded were 20.00% (25°C), 17.65% (35°C), and 15.00% (45°C), representing enhancements of 90.48%, 68.10%, and 42.10% of the ash value in unfermented RB, respectively.

The rise in ash content indicates the potential release of enzymes such as phytase by the organism during fermentation, contributing to the breakdown of antinutrients present in the unfermented substrate. This release may have liberated minerals previously bound or chelated by these antinutrients (Altop et al., 2018). *A. niger* USM F4 may induce the production of enzymes such as hydrolase, hemicellulase, lipase, and pectinase during SSF, potentially altering the structure of anti-nutritional factors (Kaur et al., 2020). Simultaneously, SSF led to an augmentation in substrate ash content, likely attributed to microbial metabolic activity or dry matter loss. Similar results have been reported in

previous studies, where SSF led to increased ash content in various substrates. Instances of such studies include a 43.90% increase in ash content of RB after 4 days of SSF with *A. niger* (Ribeiro et al., 2017), a 50.34% rise in ash content of olive leaves following 2 days of SSF with *A. niger* (Altop et al., 2018), and a 51.31% increment in ash content of rice bran after 10 days of SSF with *Pleurotus sapidus* (Omarini et al., 2019).

The percentages of enhancement in ash content observed in this study at both 25°C and 35°C surpass the 51.31% reported by Omarini (2019) after 10 days of SSF of RB at 25°C with *Pleurotus sapidus* Dk3174. Likewise, the ash improvement in this study exceeds the findings of Oliveira et al. (2010), who observed an enhancement in ash content when RB was fermented for 5 days using *Rhizopus oryzae* at 30°C. However, Bonilla Loaiza et al. (2022) documented a reduction in ash content during solid-state fermentation, noting that fermentation at 25°C yielded the highest ash improvement.

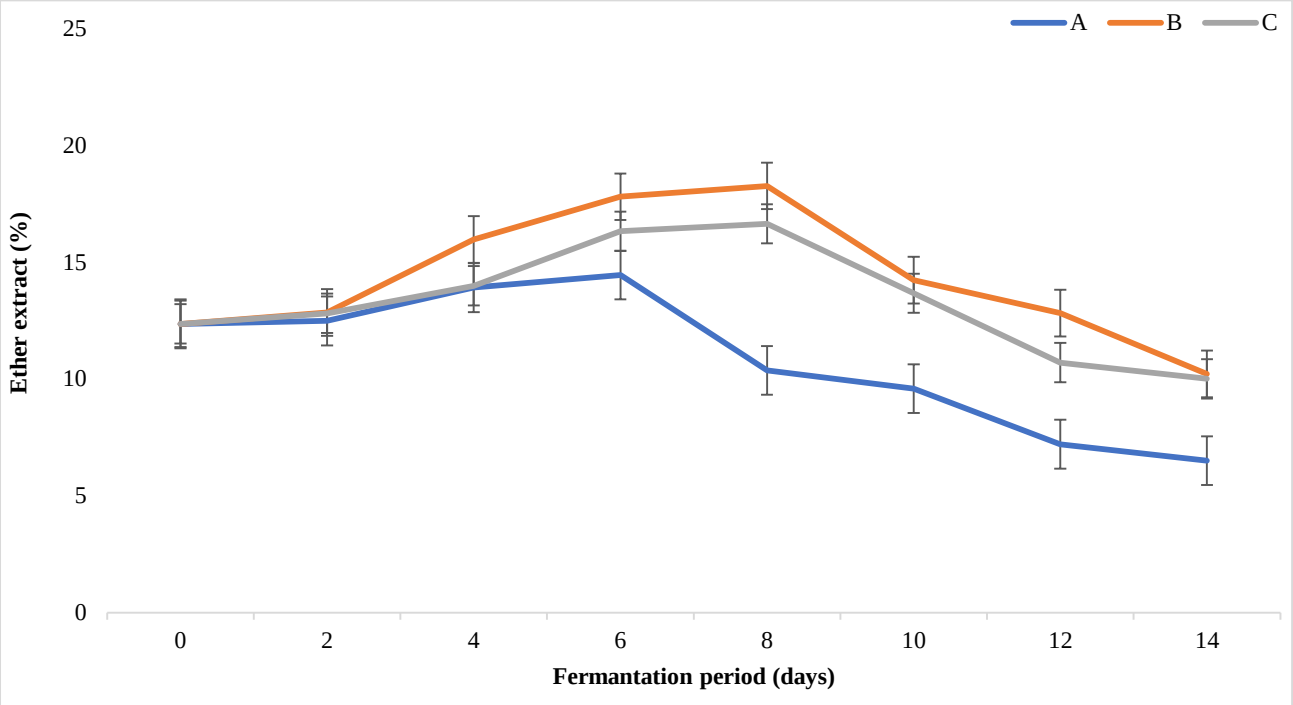


Figure 3. Effect of temperature and fermentation period on the ether extract content of rice bran. **A**: 25°C, **B**: 35°C, **C**: 45°C.

Effect of temperature and fermentation duration on the ether extract of rice bran

Figure 3 depicts how temperature and fermentation duration influence the EE component of RB during SSF. Significant enhancements in the EE component of RB were observed under various temperatures and fermentation durations ($p < 0.05$), as compared to the unfermented RB (Table 1). The highest enhancement occurred on day 8, with EE peaking at 15.25% and a 63.10% increase recorded at 35°C.

The rise in EE content is influenced by various factors, such as the production of lipolytic enzymes by *A. niger* in the RB matrix, microbial growth utilizing nutrients, and enzymatic breakdown of cell walls releasing trapped lipids. Moreover, the SSF process can modify RB's physicochemical properties, making lipids more soluble (Christ-Ribeiro et al., 2021; Spaggiari et al., 2021). *A. niger* demonstrates a remarkable ability to transform substrates into value-added compounds, thereby significantly augmenting the lipid content in fermented rice bran.

Previous studies have reported varying effects of SSF on EE content. Ribeiro et al. (2017) conducted a study where rice bran was fermented with *A. niger* at 30°C for 4 days, while Altop et al. (2018) investigated the fermentation of olive leaves with *A. niger* at the same temperature for 2 days. Both studies reported an increase in EE. Conversely, Oliveira et al. (2010) and Omarini et al. (2019) observed a reduction in EE during SSF. In Omarini et al.'s (2019) study, a substantial 74.45% decrease in the EE content was observed when rice bran underwent solid-state fermentation for 10 days using *Pleurotus sapidus* at 25°C. Similarly, Shi et al. (2021) documented a 30% reduction in EE when Moringa oleifera leaves were subjected to solid-state fermentation by *A. niger* at 32°C for 7 days.

Effect of temperature and fermentation period on crude fiber degradation

In the present study, the crude fiber content of the unfermented RB (27.50%) significantly decreased by solid-state fermentation (SSF) using *A. niger* USM F4 ($p < 0.05$) (Figure 4). The lowest CF values were observed on the 10th day

across all temperature levels. Specifically, CF values were 12.45 (25°C), 8.36 (35°C), and 10.33 (45°C), indicating CF degradation by 54.73%, 69.60%, and 62.44% respectively. The highest degradation of CF (69.60%) occurred at 35°C on the 10th day.

A. niger, known for its cellulase production (Altop et al., 2018), shows promising prospects for single-cell protein production. The breakdown of crude fiber (CF) during fermentation by *A. niger* USM F4 is believed to entail the secretion of enzymes like cellulases, hemicellulases, and ligninases. The degradation of crude fiber (CF) during fermentation by *A. niger* USM F4 likely involves the secretion of enzymes such as cellulases, hemicellulases, and ligninases. These enzymes break down the complex polysaccharides in CF into simpler sugars for fungal growth. Additionally, the metabolic activities and by-products of *A. niger* may directly or indirectly degrade CF (Cui et al., 2021; El-Gendi et al., 2021). Fermentation alters substrate pH, moisture, and other parameters, making CF more susceptible to enzymatic breakdown (Siddiqui et al., 2023; Kasprowicz-Potocka et al., 2024). Physical disruption of rice bran during fermentation enhances CF degradation by increasing enzyme accessibility (Dilaga et al., 2022; Ibrahim et al., 2023). Moreover, *A. niger* has the potential to selectively utilize various components of rice bran, such as CF, for its growth (Yu et al., 2021). This preference often leads to the breakdown of more fermentable CF components into degradation products. In a similar study, Shi et al. (2021) conducted SSF of *Moringa oleifera* leaf using *A. niger* GIM 3.576 at 32°C for 7 days and reported a 70% degradation of CF. Oliveira et al. (2010) fermented RB with *Rhizopus oryzae* in a solid-state setting at 30°C over 5 days, noting a 50% reduction in CF. Similarly, Wolayan and Mandey (2019) fermented RB with *A. niger* for 10 days at 30°C, yielding a 47.81% reduction in CF.

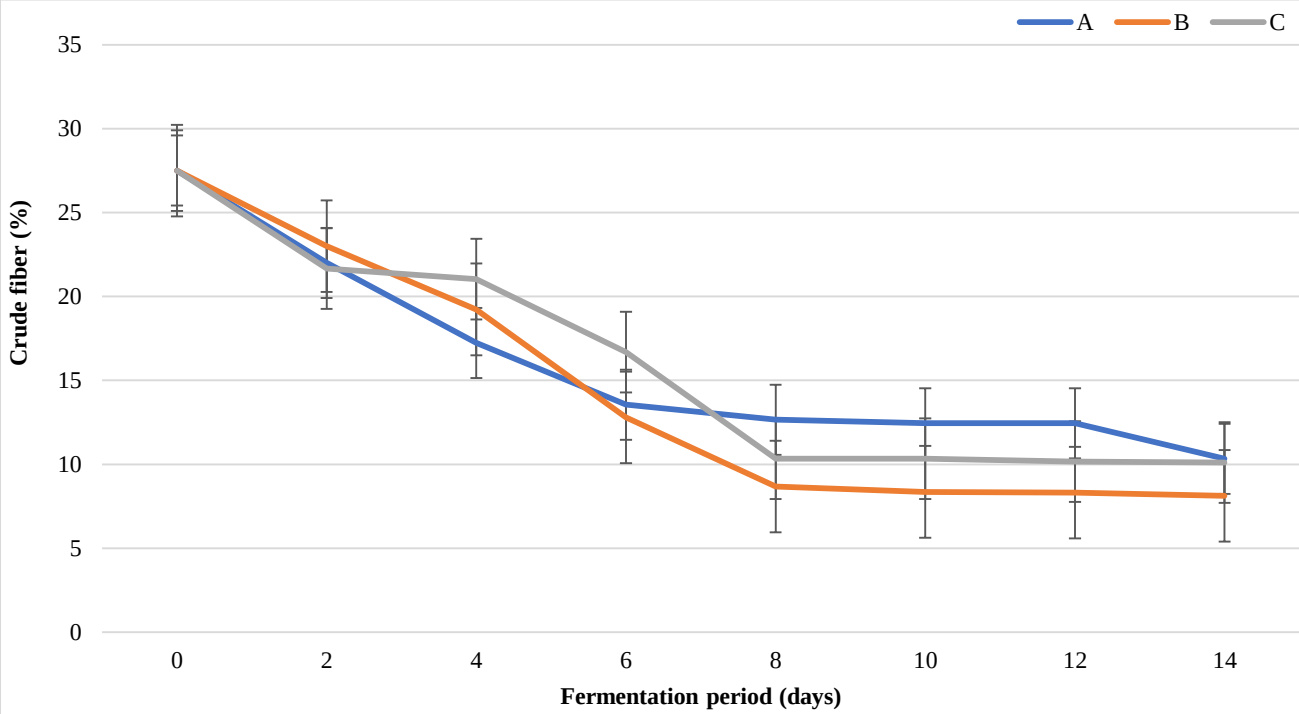


Figure 4. Effect of temperature and fermentation period on the crude fiber of rice bran. **A**: 25°C, **B**: 35°C, **C**: 45°C.

CONCLUSION

The current study demonstrated that solid-state fermentation can enhance the bioavailability of nutrients in rice bran, potentially improving its digestibility in animals. The optimal media temperature and duration for solid-state fermentation using *A. niger* USM F4 to boost crude protein levels and degrade crude fiber in rice bran are determined to be 35°C and 10 days, respectively. Moreover, the utilization of SSF assisted with *A. niger* USM F4 can elevate the quality of agro-industrial by-products by enhancing crude protein content and degrading crude fiber. This, in turn, facilitates increased utilization of rice bran in livestock feed, particularly in monogastric animals. Further research is recommended to explore optimal fermentation conditions for *A. niger* USM F4 in rice bran, enzyme characterization for industrial applications, nutritional evaluations for livestock feed, confirmation of antinutrient reduction, and pilot-scale trials for industrial feasibility.

DECLARATIONS

Acknowledgments

The authors are grateful to the management of Landmark University for providing the facilities necessary for this study.

Authors' contributions

Animashahun Razaq Adekunle and Akpor Oghenerobor Benjamin conceptualized the study, while Olamide Musa, Oluwafemi Precious, Animashahun Adedeji Peculiar and Idowu Abiodun experimented. Alabi Olayinka Olubunmi, Oyawoye Enoch Olayiwola, and Animashahun Razaq Adekunle provided supervision and data curation. Laboratory analyses were performed by Animashahun Razaq, Akpor Oghenerobor Benjamin, and Olamide Musa, with data analysis conducted by Oghenerobor Benjamin, Okocha Reuben, and Olamide Musa. Olamide Musa and Oluwafemi Precious drafted the manuscript, which was revised by Animashahun Razaq Adekunle, Akpor Oghenerobor, Oyawoye Enoch Olayiwola, and Alabi Olayinka Olubunmi. All authors reviewed and approved the final version of the manuscript for publication in the current journal.

Funding

The study was solely funded by the authors.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon request.

Competing interests

No conflicts of interest have been disclosed by the authors.

Ethical considerations

Every author has reviewed ethical considerations such as plagiarism, duplicate publication or submission, redundancy, data fabrication or falsification, consent for publication, and misconduct.

REFERENCES

Abu Yazid N, Barrena R, Komilis D, and Sánchez A (2017). Solid-state fermentation as a novel paradigm for organic waste valorization: A review. Sustainability, 9(2): 224. DOI: https://www.doi.org/10.3390/su9020224

Akintan O, Gebremedhin KG, and Uyeh DD (2024). Animal feed formulation — Connecting technologies to build a resilient and sustainable system. Animals, 14(10): 1497. DOI: https://www.doi.org/10.3390/ani14101497

Altop A, Coskun I, Filik G, Kucukgul A, Bekiroglu YG, Cayan H, Gungor E, Sahin A, and Erener G (2018). Amino acid, mineral, condensed tannin, and other chemical contents of olive leaves (*Olea europaea* L.) processed via solid-state fermentation using selected *Aspergillus niger* strains. Ciencia e Investigación Agraria, 45(3): 220-230. Available at: https://www.scielo.cl/scielo.php?pid=S0718-16202018000300220&script=sci_arttext

Ajibo AE and Said RO (2023). Production of gluconic acid from sweet potato peels using naturally occurring fungi by submerged fermentation. Bio-Research, 21(1): 1828-1837. DOI: https://www.doi.org/10.4314/br.v21i1.6

Aruna TE, Aworh OC, and Ezekiel OO (2018). Effect of administration of *Saccharomyces cerevisiae* and *Trichoderma viride* fermented yam and pineapple peels on essential organs of Wistar rats. Annals: Food Science & Technology, 19(2): 369-377. Available at: https://afst.valahia.ro/wp-content/uploads/2022/09/V.1_Aruna.pdf

Association of official and analytical chemists (AOAC) (2012). Association of official and analytical chemists. Official methods of analysis, 21st Edition. Washington D.C., pp. 121-130. Available at: https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1819676

Ayojimi W, Bamiro OM, Otunaiya AO, Shoyombo AJ, and Matiluko OE (2023). Sustainability of poultry egg output and efficiency: A risk-mitigating perspective. Frontiers in Sustainable Food Systems, 7: 1195218. DOI: https://www.doi.org/10.3389/fsufs.2023.1195218

Bonilla Loaiza AM, Rodríguez-Jasso RM, Belmares R, López-Badillo CM, Araújo RG, Aguilar CN, Chávez ML, Aguilar MA, and Ruiz HA (2022). Fungal proteins from *Sargassum* spp. using solid-state fermentation as a green bioprocess strategy. Molecules, 27(12): 3887. DOI: https://www.doi.org/10.3390/molecules27123887

Christ-Ribeiro A, Chiattoni LM, Mafaldo CRF, Badiale-Furlong E, and de Souza-Soares LA (2021). Fermented rice-bran by Saccharomyces cerevisiae: Nutritious ingredient in the formulation of gluten-free cookies. Food Bioscience, 40: 100859. DOI: https://www.doi.org/10.1016/j.fbio.2020.100859

Cruz-Parede C, Tájmel D, and Rousk J (2021). Can moisture affect temperature dependences of microbial growth and respiration?. Soil Biology and Biochemistry, 156: 108223. DOI: https://www. doi.org/10.1016/j.soilbio.2021.108223

Cui Y, Li J, Deng D, Lu H, Tian Z, Liu Z, and Ma X (2021). Solid-state fermentation by *Aspergillus niger* and *Trichoderma koningii* improves the quality of tea dregs for use as feed additives. Plos One, 16(11): e0260045. DOI: https://www.doi.org/10.1371/journal.pone.0260045

Devi R, Veliveli VL, and Devi SS (2021). Nutritional composition of rice bran and its potentials in the development of nutraceuticals rich products. Journal of Pharmacognosy and Phytochemistry, 10(2): 470-473. Available at:

https://www.phytojournal.com/archives/2021.v10.i2.13842/nutritional-composition-of-rice-bran-and-its-potentials-in-the-development-of-nutraceuticals-rich-products

Dilaga SH, Putra RA, Pratama ANT, Yanuarianto O, Amin M, and Suhubdy S (2022). Nutritional quality and in vitro digestibility of fermented rice bran based on different types and doses of inoculants. Journal of Advanced Veterinary and Animal Research, 9(4): 625-633. DOI: https://www.doi.org/10.5455/javar.2022.i632

Dou Z, Dierenfeld ES, Wang X, Chen X, and Shurson GC (2024). A critical analysis of challenges and opportunities for upcycling food waste to animal feed to reduce climate and resource burdens. Resources, Conservation and Recycling, 203: 107418. DOI: https://www.doi.org/10.1016/j.resconrec.2024.107418

El-Gendi H, Saleh AK, Badierah R, Redwan EM, El-Maradny YA, and El-Fakharany EM (2021). A comprehensive insight into fungal enzymes: structure, classification, and their role in mankind's challenges. Journal of Fungi, 8 (1): 23. DOI: https://www.doi.org/10.3390/jof8010023

Gonzalez JM and Aranda B (2023). Microbial growth under limiting conditions-future perspectives. Microorganisms, 11 (7): 1641. DOI: https://www.doi.org/10.3390/microorganisms11071641

He Q, Peng H, Sheng M, Hu S, Qiu J, and Gu J (2019). Humidity control strategies for solid-state fermentation: Capillary water supply by water-retention materials and negative-pressure auto-controlled irrigation. Frontiers in Bioengineering and Biotechnology, 7: 263. DOI: https://www.doi.org/10.3389/fbioe.2019.00263

Huervana FH, Traifalgar RFM, and Dionela CS (2024). Solid-state fermentation converts rice bran into a high-protein feed ingredient for Penaeus monodon. Frontiers in Marine Science, 11: 1384492. DOI: https://www.doi.org/10.3389/fmars.2024.1384492

Ibrahim D, El-Sayed HI, Mahmoud ER, El-Rahman GIA, Bazeed SM, Abdelwarith A, Elgamal A, Khalil SS, Younis EM, Kishawy ATY et al. (2023). Impacts of solid-state fermented barley with fibrolytic exogenous enzymes on feed utilization, and antioxidant status of broiler chickens. Veterinary Sciences, 10(10): 594. DOI: https://www.doi.org/10.3390/vetsci10100594

Kasprowicz-Potocka M, Zaworska-Zakrzewska A, Łodyga D, and Józefiak D (2024). The effect of enzymatic fermentation on the chemical composition and contents of antinutrients in rapeseed meal. Fermentation, 10(2): 107. DOI: https://www.doi.org/10.3390/fermentation10020107

Kaur A, Rishi V, Soni SK, and Rishi P (2020). A novel multi-enzyme preparation produced from *Aspergillus niger* using biodegradable waste: A possible option to combat heterogeneous biofilms. Amb Express, 10(36): 1-16. DOI: https://www.doi.org/10.1186/s13568-020-00970-3

Lasekan O and Shittu R (2019). Effect of solid-state fermentation and drying methods on the physicochemical properties of flour of two plantain cultivars grown in *Malaysia.* International Food Research Journal, 26(5): 1485-1494. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20193510007

Laure NN, Dawan J, and Ahn J (2021). Effects of incubation time and inoculation level on the stabilities of bacteriostatic and bactericidal antibiotics against *Salmonella Typhimurium.* Antibiotics, 10(8): 1019. DOI: https://www.doi.org/10.3390/antibiotics10081019

Melnichuk N, Braia MJ, Anselmi PA, Meini MR, and Romanini D (2020). Valorization of two agroindustrial wastes to produce alpha-amylase enzyme from *Aspergillus oryzae* by solid-state fermentation. Waste Management, 106: 155-161. DOI: https://www.doi.org/10.1016/j.wasman.2020.03.025

Mengesha Y, Tebeje A, and Tilahun B (2022). A review on factors influencing the fermentation process of teff (*Eragrostis teff*) and other cereal-based Ethiopian injera. International Journal of Food Science, 2022(1): 4419955. DOI: https://www.doi.org/10.1155/2022/4419955

Mishra N (2017). Utilization of waste defatted rice bran in formulation of functional cookies and its effect on physiochemical characteristic of cookies. International Journal of Advanced Science and Research, 2(5): 64-68. Available at: https://allsciencejournal.com/assets/archives/2017/vol2issue5/2-5-25-530.pdf

Munishamanna KB, Suresha KB, Veena R, and Subramanya S (2017). Solid state fermentation of mango peel and mango seed waste by different yeasts and bacteria for nutritional improvement. International Journal of Food and Fermentation Technology, 7(1): 111-118. DOI: https://www.doi.org/10.5958/2277-9396.2017.00011.3

Obaniyi KS, Kolawole AE, Ajala AO, Owolabi AO, and Akangbe JA (2019). Environmental change impacts on agribusiness and food security in sub sahara Africa: A practical way forward medwell. Journal of Engineering and Applied Science, 14(24): 9639-9644. DOI: http://www.doi.org/10.36478/jeasci.2019.9639.9644

de Oliveira RL, da Silva MF, Converti A, and Porto TS (2020). Production of β-fructofuranosidase with transfructosylating activity by *Aspergillus tamarii* URM4634 Solid-State Fermentation on agroindustrial by-products. International Journal of Biological Macromolecules, 144: 343-350. DOI: https://www.doi.org/10.1016/j.ijbiomac.2019.12.084

Ogbonna AI, Onyimba IA, Chuku A, Nwadiaro PO, Ogbonna C, and Onwuliri FC (2015). Growth assessment and amylase production by *Aspergillus niger* and A. terreus isolated from soils of Artemisia annua l. Plantation. European Journal of Biotechnology and Bioscience 2015, 3(1): 10-16. Available at: http://hdl.handle.net/123456789/1032

Oliveira MD S, Feddern V, Kupski L, Cipolatti EP, Badiale-Furlong E, and de Souza-Soares LA (2010). Physico-chemical characterization of fermented rice bran biomass Caracterización fisico-química de la biomasa del salvado de arroz fermentado. CyTA. Journal of Food, 8(3): 229-236. DOI: https://www.doi.org/10.1080/19476330903450274

Omarini AB, LabuckasD, Zunino MP, Pizzolitto R, Fernández-Lahore M, Barrionuevo D, and Zygadlo JA (2019). Upgrading the nutritional value of rice by solid-state fermentation with *Pleurotus sapidus.* Fermentation, 5(2): 44. DOI: https://www.doi.org/10.3390/fermentation5020044

Osemwegie OO, Adetunj CO, Ayeni EA, Adejobi OI, Arise RO Nwonuma CO, and Oghenekaro AO (2020). Exopolysaccharides from bacteria and fungi: Current status and perspectives in Africa. Heliyon, 6(6): e04205. DOI: https://www.doi.org/10.1016/j.heliyon.2020.e04205

Park SE, Seo SH, Kim EJ, Na CS and Son HS (2018). Effects of different fermentation temperatures on metabolites of Kimchi. Food Bioscience, 23: 100-106. DOI: https://www.doi.org/10.1016/j.fbio.2018.03.009

Pranay K, Padmadeo SR, and Prasad B (2019). Production of amylase from Bacillus subtilis sp. strain KR1 under solid state fermentation on different agrowastes. Biocatalysis and Agricultural Biotechnology, 21: 101300. DOI: https://www.doi.org/10.1016/j.bcab.2019.101300

Putri DN, Khootama A, Perdani MS, Utami TS, and Hermansyah H (2020). Optimization of *Aspergillus niger* lipase production by solid state fermentation of agro-industrial waste. Energy Reports, 6: 331-335. DOI: https://www.doi.org/10.1016/j.egyr.2019.08.064

Ribeiro AC, Graça CD, Chiattoni LM, Massarolo KC, Duarte FA, Mellado M, and Soares LA (2017). Fermentation Process in the Availability of Nutrients in Rice Bran. Research and Reviews: Journal of Microbiology and Biotechnology, 6: 45-52. Available at: www.rroij.com/open-access/fermentation-process-in-the-availability-of-nutrients-in-rice-bran-.pdf

Sadh PK, Duhan S, and Duhan JS (2018). Agro-industrial wastes and their utilization using solid state fermentation: A review. Bioresources and Bioprocessing, 5(1): 1. DOI: https://www.doi.org/10.1186/s40643-017-0187-z

Shi H, Yang E, Li Y, Chen X, and Zhang J (2021). Effect of solid-state fermentation on nutritional quality of leaf flour of the drumstick tree (*Moringa oleifera* Lam.). Frontiers in Bioengineering and Biotechnology, 9: 626628. DOI: https://www.doi.org/10.3389/fbioe.2021.626628

Senanayake D, Torley PJ, Chandrapala J, and Teref NS (2023). Microbial fermentation for improving the sensory, nutritional and functional attributes of legumes. Fermentation, 9(7): 635. DOI: https://www.doi.org/10.3390/fermentation9070635

Siddiqui SA, Erol Z, Rugji J, Taşçı F, Kahraman HA, Toppi V, Musa, Di Giacinto G, Mohammad NA, Mehdizadeh M, and Castro-Muñoz R (2023). An overview of fermentation in the food industry-looking back from a new perspective. Bioresources and Bioprocessing, 10(1): 85. DOI: https://www.doi.org/10.1186%2Fs40643-023-00702-y

Spaggiari M, Dall'Asta C, Galaverna G, and del Castillo Bilbao MD (2021). Rice bran by-product: From valorization strategies to nutritional perspectives. Foods, 10(1): 85. DOI: https://www.doi.org/10.3390/foods10010085

Sukma ACT, Herwati Oktavianty HO, and Siswo Sumardiono SS (2021). Optimization of solid-state fermentation condition for crude protein enrichment of rice bran using *Rhizopus oryzae* in tray bioreactor. Indonesian Journal of Biotechnology, 26(1): 33-40. DOI: https://www.doi.org/10.22146/ijbiotech.57561

Sun W, Shahrajabian MH, and Lin M (2022). Research progress of fermented functional foods and protein factory-microbial fermentation technology. Fermentation, 8(12): 688. DOI: https://www.doi.org/10.3390/fermentation8120688

Upadhyaya S, Tiwari S, Arora NK, and Singh DP (2016). Microbial protein: a valuable component for future food security. Microbes and environmental management. Studium Press., New Delhi, pp. 259-279.

Teixeira da Silva VDC, de Souza Coto AL, de Carvalho Souza R, Bertoldi Sanchez Neves M, Gomes E, and Bonilla-Rodriguez GO (2016). Effect of pH, temperature, and chemicals on the endoglucanases and β-Glucosidases from the thermophilic fungus *myceliophthora heterothallica* F. 2.1. 4. obtained by solid-state and submerged cultivation. Biochemistry Research International, 2016: 9781216. DOI: https://www.doi.org/10.1155/2016/9781216

Wang H, Sun C, Yang S, Ruan Y, Lyu L, Guo X, Wu X, and Chen Y (2023). Exploring the impact of initial moisture content on microbial community and flavor generation in Xiaoqu baijiu fermentation. Food Chemistry, 20: 100981. DOI: https://www.doi.org/10.1016/j.fochx.2023.100981

Wolayan FR and Mandey JS (2019). Nutritional value of rice bran fermented by *Aspergillus niger* and its effect on nutrients digestibility of broiler chickens. Journal of Advanced Agricultural Technologies, 6(1): 53-56. Available at: https://www.joaat.com/uploadfile/2019/0329/20190329041354792.pdf

Wolk DM, Johnson CH, Rice EW, Marshall MM, Grahn KF, Plummer CB, Sterling CR (2000). A spore counting method and cell culture model for chlorine disinfection studies of Encephalitozoon syn. *Septata intestinalis.* Applied and Environmental Microbiology, 66(4): 1266-1273. DOI: https://www.doi.org/10.1128/AEM.66.4.1266-1273.2000

Yafetto L, Odamtten GT, Birikorang E, and Adu S (2020). Protein enhancement of yam (*Dioscorea rotundata*) peels with single-or co-inoculation of *Aspergillus niger* van Tieghem and *Trichoderma viride* Pers ex Fr. under solid-state fermentation. Ghana Journal of Science, 61(2): 27-37. DOI: https://www.doi.org/10.4314/gjs.v61i2.3

Yang K, Qing Y, Yu Q, Tang X, Chen, G, Fang R, and Liu H (2021). By-product feeds: Current understanding and future perspectives. Agriculture, 11(3): 207. DOI: https://www.doi.org/10.3390/agriculture11030207

Yu R, Liu J, Wang Y, Wang H, and Zhang H (2021). *Aspergillus niger* as a secondary metabolite factory. Frontiers in Chemistry, 9: 701022. DOI: https://www.doi.org/10.3389/fchem.2021.701022

Zhang QQ, Li W, Li HK, Chen XH, Jiang M, and Dong MS (2017). Low-field nuclear magnetic resonance for online determination of water content during sausage fermentation. Journal of Food Engineering, 212: 291-297. DOI: https://www.doi.org/10.1016/j.jfoodeng.2017.05.021

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 328-337.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj39
PII: S232245682400039-14

The Effects of Acute Oral Toxicity of *Jatropha multifida* and *Hyptis suaveolens* on Zootechnical Parameters in Local Chickens

Enagnon Bienvenue Florent Sèdégan[1], Yao Akpo[1], Kadoéito Cyrille Boko[2], Maximilien Azalou[1], Camus Adoligbé[2], Christophe Iwaka[1], Eloi Attakpa[1], and Ibrahim Alkoiret Traoré [1]

[1]*Laboratoire d'Ecologie, de Santé et de Production Animales (LESPA) Université de Parakou. 01 BP: 123 Parakou, Bénin*
[2]*Unité de Recherches sur les Maladies Transmissibles (URMAT) 01BP 2009 Cotonou. Université d'Abomey-Calavi, Bénin. Enseignent chercheur à l'Ecole Polytechnique de l'Université d'Abomey-calavi, Bénin*

*Corresponding author's Email: sedeganbienvenue@gmail.com

ORIGINAL ARTICLE
Received: June 25, 2024
Revised: July 30, 2024
Accepted: August 19, 2024
Published: September 25, 2024

ABSTRACT

The substantial use of medicinal plants in traditional poultry farming is a well-established practice. The present study aimed to determine the median lethal dose (LD_{50}) of ethanolic extracts of *Jatropha multifida* and *Hyptis suaveolens* in local chickens and to assess the effects of these extracts on feed intake, water intake, and average body weight. The methodology followed OECD Directive 223, which involves administering the highest dose of the extract to the chickens and assessing any mortality. Three homogeneous groups of five chickens each were formed for the limit dose test. The control group received distilled water, while batches 1 and 2 received 700 mg of ethanolic extract of *J. multifida* and *H. suaveolens*, respectively. The average body weight of the chickens was 350g ± 20, and the extracts were administered via gavage at a suspension of 2000 mg/kg.bwt of the extract dissolved in water. The results of the phytochemical tests indicated the presence of several chemical compounds known for their therapeutic effects. The productivity of the extract was 2.75 ± 0.19 for *J. multifida* and 3.3 ± 0.27 for *H. suaveolens*. After administration of the suspensions, observation for 14 days revealed no mortality. This finding indicated that the LD_{50} of the utilized ethanolic extracts exceeds the limit dose (2000 mg/kg.bwt). However, feed intake (49 ± 3 > 46±4) and average body weight (436 ± 31 > 388 ± 37) in batch 2 were significantly higher than those in batch 1. Future research should explore the subacute toxicity of *J. multifida* and *H. suaveolens* across various chicken breeds.

Keywords: Body weight, Lethal Dose, Local chicken, Medicinal plant, Mortality, Toxicity

INTRODUCTION

Jatropha multifida of the Euphorbiaceae family and *Hyptis suaveolens* of the Lamiaceae family are medicinal plants in Benin's traditional pharmacopoeia, which are widely used by traditional therapists (Agban et al., 2012; Koudokpon et al., 2015) as traditional recipes in the treatment of several animal diseases. In southern Benin, Sèdégan et al. (2023a) reported that traditional poultry farmers use the leaves and stems of *J. multifida* and *H. suaveolens* in the treatment of fowl pox with inconclusive results, although they have been cited as one of the plants with proven veterinary uses (Adjanohoun et al., 1989; Dassou et al., 2015). The use of the phytotherapeutic heritage cannot, therefore, remain static and be limited solely to the collection of traditional recipes (Fokunang et al., 2011). Enhancing the value of medicinal flora requires phytochemical, pharmacological, and clinical studies (Kouchadé et al., 2017), as well as pharmaco-toxicological studies to rationalise administration doses (Sèdégan et al., 2023b; 2024), with a view to developing improved traditional medicines (Agban et al., 2020). Previous studies have demonstrated the biological effects and pharmacological activities of these medicinal plants in *in vitro* tests on germs responsible for common diseases. For instance, ethanolic and hydroethanolic extracts of *J. multifida* are thought to have very strong inhibitory activity against *C. albicans* (Agban et al., 2012), which justifies the use of the plant in the management of candidiasis in certain African countries such as Nigeria (Adesola and Adetunji, 2007). The bark and leaves are used to treat itchy skin and eczema (Hamza et al., 2006), while the sap has been shown to have strong healing properties (Philippe et al., 2012; Klotoe et al., 2014). However, the fruits can cause severe diarrhoea, dehydration and liver failure (Levin et al., 2000). The sap and aqueous extract of *J. multifida* organs have been studied for their toxicity in Wistar rats (Dougnon et al., 2012; Falodun et al., 2014; Senou et al., 2022). In contrast, no toxicity has been reported in humans or animals from the use of *H. suaveolens* organs (Santos et al., 2007; Sèdégan et al., 2024), whose efficiency in the treatment of candidiasis is proven with its leaves used in food and feed (Kouchadé et al., 2017).

To cite this paper: Sèdégan EBF, Akpo Y, Boko KC, Azalou M, Adoligbé C, Iwaka Ch, Attakpa E, and Alkoiret TI (2024). The Effects of Acute Oral Toxicity of *Jatropha multifida* and *Hyptis suaveolens* on Zootechnical Parameters in Local Chickens. *World Vet. J.,* 14(3): 328-337. DOI: https://dx.doi.org/10.54203/scil.2024.wvj39

In southern Benin, 29.1% of traditional poultry farmers use medicinal plants to treat diseases in their flocks (Sèdégan et al., 2023a), often without guidance on safe doses of administration and the potential toxicity risks induced by the used plants. Furthermore, the toxicity of medicinal plants *(J. multifida* and *H. suaveolens*) in both traditional and modern poultry farming is poorly documented despite their growing use in traditional pharmacopoeia in general, and in traditional poultry farmers in particular (Sèdégan et al., 2023b). As a result, this study aimed to address this gap by determining the median lethal dose (LD_{50}) of ethanolic extracts from *J. multifida* and *H. suaveolens* in local chickens. Specifically, it evaluated the impact of these extracts on feed and water consumption as well as the body weight of the chickens.

MATERIALS AND METHODS

Ethical approval

All authors declare that the experiments were examined and approved by the Ethics Committee of the Communicable Diseases Research Unit at the Applied Biology Research Laboratory of the University of Abomey-Calavi under approval number 002 2023/EPAC/LARBA/URMAT/CE/R. The experiments were carried out in accordance with the ethical standards defined in the 1964 Declaration of Helsinki.

Study setting

The study was conducted at Lumière Agropastorale, a farm breeding site in the locality of Massi, commune of Zogbodomey, department of Zou, Republic of Benin. Figure 1 showed the geolocation of the clinical trial sites and the collection sites for the used plants.

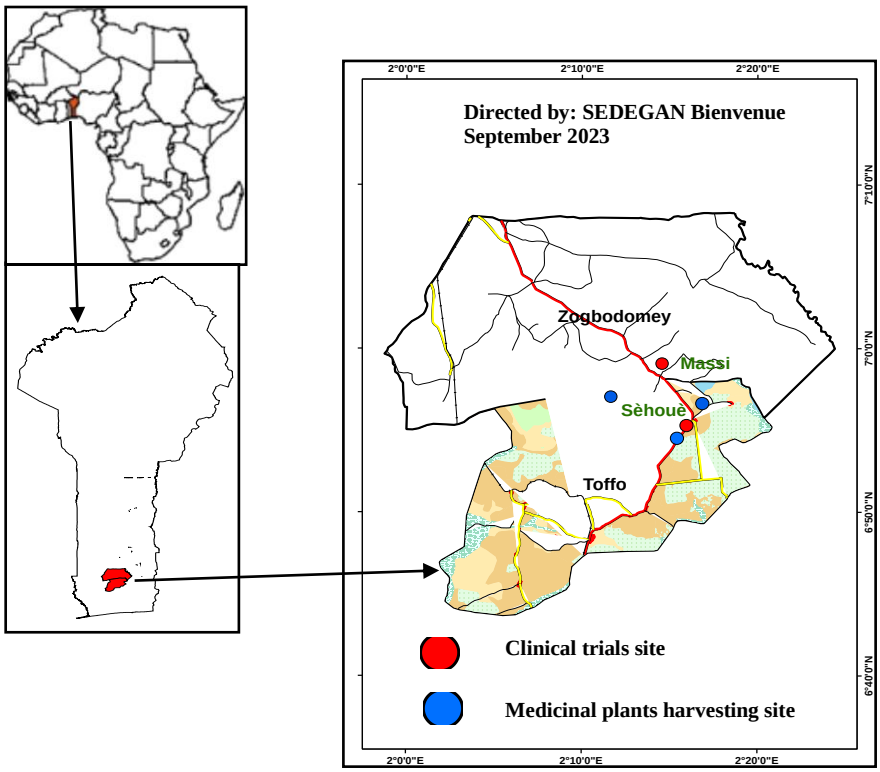


Figure 1. Clinical trial and medicinal plant harvesting sites for evaluating acute oral toxicity of *Jatropha multifida* and *Hyptis suaveolens* in Benin local chickens

Material

Plant materials consisted of the leaves and stems of *J. multifida* and *H. suaveolens*, collected in April 2022 in the Toffo region following an ethnobotanical survey of local traditional practitioners. The plants were identified at the national herbarium of the University of Abomey-Calavi under the identification number YH 775/HNB for *J. multifida* and YH 774/HNB for *H. suaveolens*.

Preparation of extracts and identification of the main chemical groups

Fresh leaves and stems were weighed, washed, disinfected with VIRUNET$^{@}$, rinsed with running water and then air-dried in a dark room at ambient temperature (25°C on average) (Agban et al., 2020). The dried plants were ground using

a RETSCH mill (type SK 100, Verder Scientific, Germany). The two powders obtained were used to prepare the extracts. The dry matter content was determined by weighing the powders using an electronic balance, applying the the following formula.

$$T(MS) = \frac{\text{Masse de feuille sèche}}{\text{Masse de feuille fraîche}} \times 100 \qquad \text{(Formula 1)}$$

To identify the main chemical groups, present in the plants, five grams of dry powder from each plant was boiled in fifty milliliters of water for 15 minutes. The resulting decoctate was filtered to obtain an aqueous extract. Qualitative phytochemical screening was conducted on aqueous extracts using the standard method based on colorimetric and precipitation reactions by different reagents (Adjatin et al., 2013). The phytochemical analyses were carried out at the Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA) of the Ecole Polytechnique d'Abomey-Calavi (EPAC/UAC). The presence or absence of these secondary metabolites was indicated by the presence of legend (+) or its absence (-). To prepare the ethanolic extract, 200 grams of each powder was placed in 500 milliliters of 95% ethanol supplied by the Biochemistry Laboratory of the Faculty of Health Sciences (FSS) at the University of Abomey-Calavi (UAC). The mixture was stirred for 15 to 20 minutes and then allowed to stand for 24 hours to facilitate the extraction the chlorophyll and its derivatives (Agban et al., 2012). The mixture was filtered and the extraction process was repeated on the residue for three more consecutive times at 24-hour intervals. Each obtained extract was evaporated using a rotavapor at the Pharmacology and Essential Oils Laboratory of the Institute of Advanced Bio-Medical Sciences (ISBA-FAST/UAC) in order to obtain dry extracts. The productivity of each extract was determined by the following formula.

$$R = \frac{\text{Dry extract mass}}{\text{Dry sheet mass}} \times 100 \qquad \text{(Formula 2)}$$

Experimental design

Young local breed chickens (*Gallus gallus domesticus*), aged between 8 and 10 weeks and weighing 350±70 g, were used. The chickens were obtained after the natural hatching of chicks reared in a building specially fitted out for the purpose and complying with chick hygiene standards. They were fed *ad libitum*, with a commercial feed, produced and certified in Benin by Groupe Veto Services (GVS). The feed consisted of 2830 Kcal/kg metabolizable energy, 4.9% crude fat, and 19% crude protein. The chickens were given distilled water to drink. Prevention against New Castle disease was carried out by subcutaneous injection with the inactivated ITA NEW ND$^{@}$ vaccine in oily emulsion, manufactured and marketed by LAPROVET, under Marketing Authorization N°UEMOA/V/00021/2014/04/30. For the acute toxicity test, three batches of five chickens were formed and housed in three different cages, each with a surface area of 1m^2 laced. The groups were treated as follows:

- Control Group: consisting of five chickens given distilled water
- Treatment 1: consisting of five chickens, each administered 700 mg of ethanolic extract of *J. multifida*
- Treatment 2: consisting of five chickens, each given 700 mg of ethanolic extract of *H. suaveolens*

The trial which lasted 14 days was conducted at room temperature (24°C to 26°C) with sufficient ventilation allowing at least ten air changes per hour. The chickens were exposed to 12-hour light cycles daily.

Acute oral toxicity test (Lethal Dose 50) and limit dose test

The acute oral toxicity test was conducted in accordance with OECD (2016), Guideline 223, for the testing of chemicals. This *in vivo* test evaluated the toxicity of ethanolic extracts from the leaves and stems of *J. multifida* and *H. suaveolens* in chickens. The extracts were weighed, suspended, and administered via gavage. The chickens were fasted, with no access to feed or water, for 12 to 15 hours during overnight prior to the administration of the suspensions (Zann, 1996). Feed and water were reintroduced two hours post-administration. The trial involved administering a single, high dose of ethanolic extract (2000 mg/kg.bwt) to each chicken in the respective treatment groups of *J. multifida* and *H. suaveolens*. The administered volume remained constant in relation to the body weight of the chickens, not exceeding 10 ml/kg body weight (Zann, 1996). The control group received 2 ml of distilled water. The test was conducted at ambient temperature and humidity, with sufficient ventilation to allow at least ten air changes per hour. Administration of medications was avoided for 14 days before and after the test (OECD 223, 2016). Average weight of the chickens and average feed consumption were calculated over a period of 14 days as indices for assessing the acute toxicity of the extracts (Pissang et al., 2018).

Observation of test chicks and measurement of study parameters

Chickens were observed continuously for the first two hours following the administration of the extracts. Observations focused on signs such as aggression, mobility, vigilance, droppings, regurgitation, abnormal behavior, and time to death. Three more observations were made at intervals during the daylight hours on the first day. From the second day onwards, two observations during the day were required. The body weight of the chickens was recorded

using a precision electronic balance before the administration of each extract on days 3, 7, and 14 to track weight variations. Daily feed and water consumptions were also measured throughout the 14-day trial period.

Statistical analysis

Data were compiled into an Excel database, and statistical analyses were performed using R software version 3.6.2, 2019. Means were calculated using the Summary procedure, and relative frequencies were assessed using the t-test procedure. Confidence intervals (CI) for the percentages were calculated using the following formula.

$$CI = 1{,}96\sqrt{\frac{[p(1-p)]}{N}} \qquad \text{(Formula 3)}$$

where p was the observed percentage and N was the total number of participants. The results from the experimental groups were compared with the control group treated with distilled water, as well as between each experimental group. In order to determine the presence of significant differences among the groups, p values less than 0.05 were considered significant.

RESULTS

Ethanolic extraction yield and phytochemical compositions

The obtained dry matter content (TMS) was 16±1.3% for *J. multifida* and 20±2.3% for *H. suaveolens*. The productivity of ethanolic extract was 2.75 ± 0.19 % for *J. multifida* and 3.3 ± 0.27 % for *H. suaveolens*. Table 1 presented; the results of phytochemical screening carried out on the aqueous extracts of these plants. As was seen, the harvested leaves and stems were rich in secondary metabolites, including alkaloids, catechic tannins, flavonoids, anthocyanins, mucilages, and coumarin quinones. Only *J. multifida* contained leuco-anthocyanins. Neither plant contained gall tannins, reducing compounds, saponosides, cyanogenic derivatives, and anthraquinones.

Table1. Effects of phytochemical test results of *Jatropha multifida* and *Hyptis suaveolens* on zootechnical parameters of local chickens

Chemical groups	***J. multifida***	***H. suaveolens***
Alkaloids	+	+
Gall tannins	-	-
Catechin tannins	+	+
Flavonoids	+	+
Anthocyanins	+	+
Leuco-anthocyanins	+	-
Reducing compounds	-	-
Mucilages	+	+
Saponosides	-	-
Cyanogenic derivatives	-	-
Anthraquinones	-	-
Coumarin quinones	+	+

+ Present; - Absent

Toxicological study

Administering a single limit dose of 2000 mg/kg.bwt of the ethanolic extract from *J. multifida* and *H. suaveolens* did not cause any mortality in the treated chickens (treatment group 1 and treatment group 2) during the fourteen-day observation period. Except for some signs of distress observed in chickens from batch 1 on the first day, no signs of aggression, regurgitation, or abnormal behavior were observed in either treatment group during the experiment.

Effect of ethanolic extract of *Jatropha multifida* on feed consumption, water consumption, and body weight

Figure 2 illustrated the variations in feed consumption, water consumption, and average body weight of chickens fed with *J. multifida* ethanolic extract. The average feed (54 ± 6.43 g) and water (88.5 ± 9.69 ml) consumption in the control treatment was higher than the average feed (46.3 ± 4.04 g) and water (76.7 ± 8.12 ml) consumption in Treatment 1. There was a remarkable drop in feed and water consumption in treatment 1 over the 14 days of observation. The average body weight of the control group (422 ± 55.5 g) was higher than that of Treatment 1 (388 ± 37 g) from the third day of observation onwards. In addition, the mean difference in feed consumption (FC) between the control group and Treatment 1 was 7.78 g with a 95% confidence interval of [4.52 g; 11 g]. Also, the t-test confirmed a significant difference (p = 0.001) in feed consumption between the control group and Treatment 1. Similarly, the mean difference in water consumption between the control group and Treatment 1 was 11.7 ml with a 95% confidence interval of [3.89 ml;

19.6 ml], indicating a significant difference in water consumption of the two compared groups (p = 0.001). While the average body weights of the chickens increased during the observation period, the mean difference in body weight between the control group and Treatment 1 was 33.7 g with a 95% confidence interval of [-20.7 g ; 87.7 g]. However, no significant difference in average weight (p = 0.14) was found in treatment 1 compared to the control group on the fourteenth day.

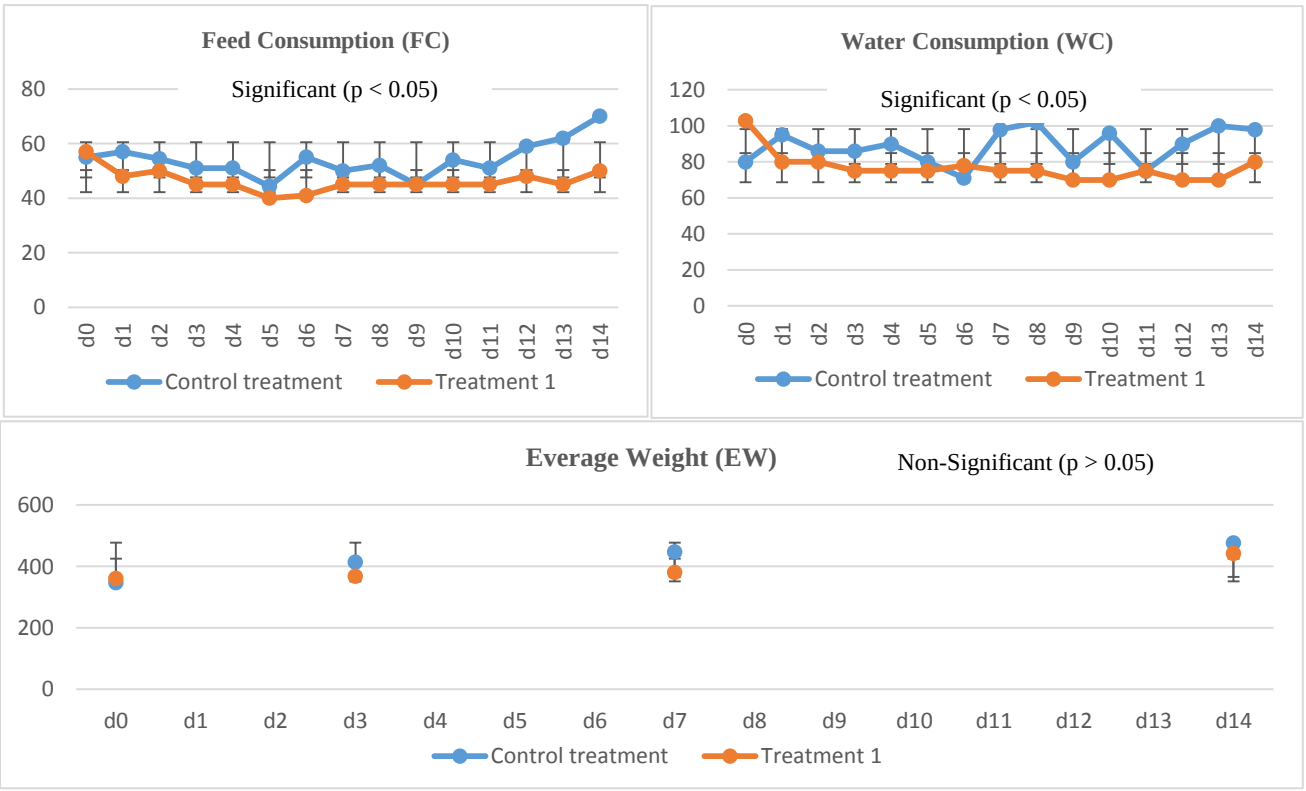


Figure 2. Effects of ethanolic extract of *J.multifida* on feed and water consumption and average weight in treatment 1 of local chicken

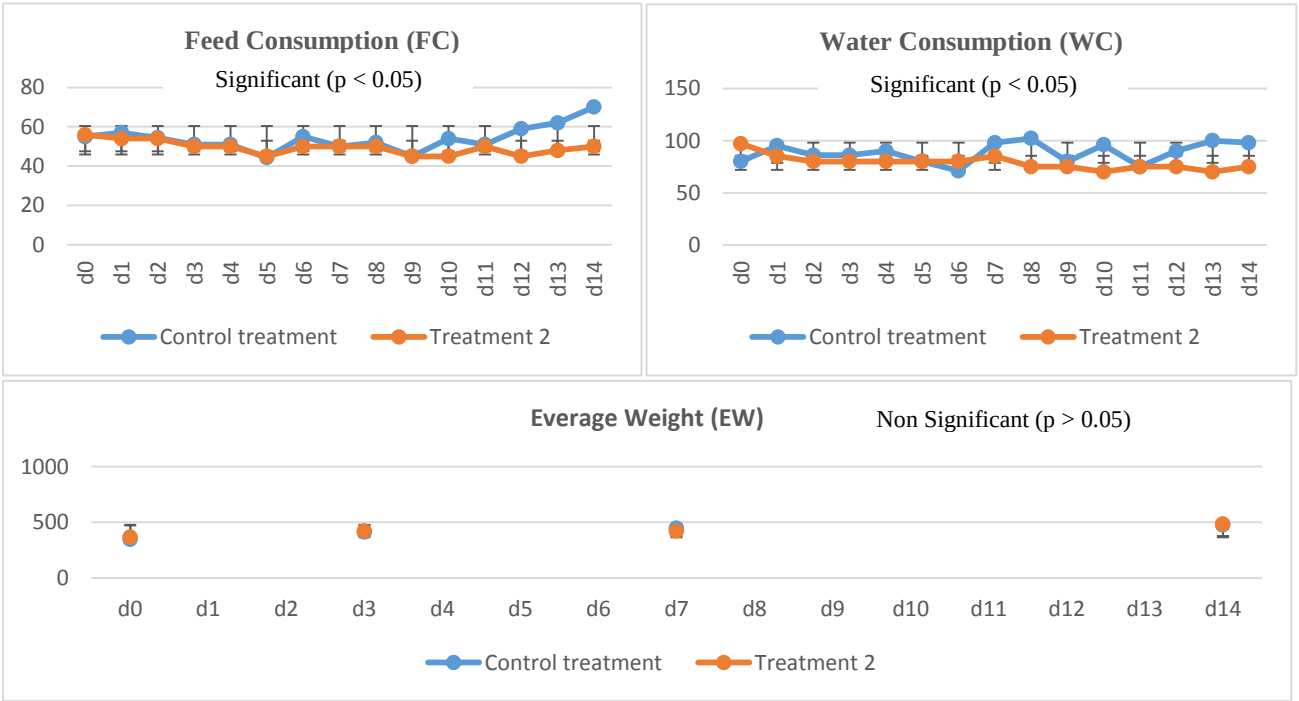


Figure 3. Effects of ethanolic extract of *H. suaveolens* on feed and water consumption and average weight in treatment 2 of local chicken

Effect of ethanolic extract of *Hyptis suaveolens* on feed consumption, water consumption and body weight

Figure 3 illustrated the variations in feed consumption, water consumption and average body weight of the chickens administered the ethanolic extract of *H. suaveolens*. The control group exhibited higher average feed consumption (54 ± 6.43 g) and water intake (88.5 ± 9.7 ml) compared to Treatment 2, which recorded 49.5 ± 3.5 g for feed and 78.8 ± 6.8 g for water consumption of. In Treatment 2, there was a drop in feed consumption from the tenth day, while a slight decrease in water consumption began in this group from the seventh day. The mean difference in feed consumption between Treatment 2 and the control group was 4.6 g with a 95% confidence interval of [0.9 g; 8.2 g]. Thus, the t-test established a significant difference (p = 0,002) in feed consumption between the two groups. Similarly, the mean difference in water consumption was 9.7 ml, with a 95% confidence interval of [2.3 ml; 17.1 ml], also showing a significant difference (p = 0.001) between Treatment 2 and the control group on the fourteenth day. Whereas the average weights on the third and seventh days were approximately equal, the average weight of Treatment 2 (436 ± 31.6 g) surpassed that of the control group (422 ± 55.5 g) on the fourteenth day.

The average weight difference between Treatment 2 and the control group was -14.6 g with a 95% confidence interval of [-78.9 g; 49.7 g]. Despite these variations, no significant difference in average weight was detected between the treatment groups (p = 0.012).

Comparison of parameters studied between experimental batches

Table 2 presented the average values for feed and water consumption, average weights, confidence intervals, and p-values comparing treatment groups 1 and 2. The data indicated that chickens in Treatment 2 performed better than those in Treatment 1 in terms of feed consumption, water consumption, and average weight. However, a significant difference was observed in feed consumption and average weight between Treatment groups 1 and 2, suggesting that the ethanolic extract of *J. multifida* may have reduced the appetite of chickens in Treatment 1, leading to decreased feed intake and, consequently, lower body weight. In contrast, the ethanolic extract of *H. suaveolens* may have enhanced the appetite of chickens in Treatment 2, resulting in increased feed and water consumption and subsequent weight gain.

Table 2. Effects of *Jatropha multifida* and *Hyptis suaveolens* on feeding and body weight of local chickens aged 14 days

	Treatment 1	Treatment 2	Dif moy	CI1	CI2	p-value	Décision
FC (g)	46.3 ± 4.04	49.5 ± 3.46	-3.2	-5	-1.39	0.001	S
WC (ml)	76.7 ± 8.12	78.8 ± 6.77	-2.06	-4.41	0.27	0.079	NS
AW(g)	388 ± 37.1	436 ± 31.6	-48.3	-61.7	-34.8	0.001	S

FC: Feed consumption; WC: Water consumption; AW: Average weight; Dif moy: Average difference between treatment 1 and treatment 2; CI: Confidence interval; S: Significant; NS: Not significant. Treatment 1 : 700 mg of ethanolic extract of *J. multifida*, Treatment 2 : 700 mg of ethanolic extract of *H. suaveolens*

DISCUSSION

This study represented a pioneering evaluation of the acute oral toxicity of the ethanolic extracts from *Jatropha multifida* and *Hyptis suaveolens* in local breed chickens. The plant materials selected for the present study were chosen due to their common traditional use in treating infectious diseases such as candidiasis and enteritis (Dougnon et al., 2012; Klotoe et al., 2014; Kouchadé et al., 2017; Agban et al., 2020), Additionally, the two plants were the most commonly mentioned plants by traditional poultry farmers for treating fowl pox (Sèdégan et al., 2023a). Given the increasing use of medicinal plants for managing certain infectious diseases (candidiases and enteritis) and parasitic diseases (Intestinal parasitosis and ectoparasitosis) (Iwaka et al., 2022a; 2022b; Sèdégan et al., 2023b), ongoing evaluation of therapeutic doses and potential side effects induced by these plants remains crucial.

Phytochemical screening of the powdered leaves and stems of *J. multifida* and *H. suaveolens* revealed the presence of several secondary metabolites known for their therapeutic and toxic effects. For *J. multifida*, the results corroborated those of Aiyelaagbe et al. (2008), Biswanath et al. (2008), Bruneton (2009), Agban et al. (2012; 2020), and Senou et al. (2022), which confirmed the presence of alkaloids, tannins, and flavonoids in *J. multifida* leaves and stems. In contrast to present findings, Senou et al. (2022) confirmed the presence of gall tannins and saponosides in *J. multifida* leaves, but rejected the presence of quinone derivatives (coumarin quinones), which were toxic compounds (Kollin and Uziel 2006). For *H. suaveolens*, the results were similar to those of Koné (2009), Traoré (2016), and corroborated those reported by Kouchadé et al. (2017) and Soumahoro et al. (2020), who confirmed the presence of chemical compounds such as gall tannins, leuco-anthocyanins, and anthraquinones in *H suaveolens*. In addition, these secondary metabolites contribute to a range of beneficial properties, including antiplasmodic, antioxidant, antibacterial, antifungal, antidiabetic,

antirheumatic, antisuporific, antiinflammatory, and antiseptic effects helping in burns and multiple skin complications (Grassi et al., 2006). However, the absence or non-existence of secondary metabolites in a plant could be due to factors such as the place and season of harvest, the handling conditions (quality of reagents used) and the professionalism of the operator, the variation in genetic make-up, the weather conditions, the part of the plant studied, its good preservation (water content in the dry state), its purity (ash content), and the employed extraction method (Koné 2009; Akhtar and Ihsan-ul-Haq, 2018).

The absence of mortality after the administration of ethanolic extracts suggests that the Lethal Dose 50 (LD_{50}) for ethanolic extracts of *J. multifida* and *H. suaveolens* exceeds the limit dose of 2,000 mg/Kg.bwt. This finding was in line with those of earlier studies on *J. multifida* extract (Agban et al., 2020; Senou et al., 2022) and *H. suaveolens* extract (Attawish et al., 2005; Santos et al., 2007). However, *Jatropha multifida* extract could be considered toxic in that it caused a reduction in feed consumption and consequently in average weight in chickens, in contrast to an increase in feed consumption associated with a loss of average weight (Agban et al., 2020). These results indicated that *J. multifida* extract was a cause of the drop in body weight of chickens in Treatment 1. This supported the claim that *J. multifida* intoxication was similar to organophosphate intoxication, which caused muscle paralysis in animal species (Kollin and Uziel, 2006). However, the use of *H. suaveolens* as an aperitif in traditional medicine (Moreira et al., 2010) justified the significant increase in feed consumption and average weight of chickens in Treatment 2 compared with chickens in Treatment 1. It was therefore certain that the toxicity of *H. suaveolens* extract has never been proved (Attawish et al., 2005; Santos et al., 2007; Traoré, 2009; Ansm, 2021; Sèdégan et al., 2024). Overall, this study indicated that, at a dose of 2,000 mg/Kg.bwt, the ethanolic extract of *H. suaveolens* was preferable to that of *J. multifida* for *in vivo* use in local chickens for all purposes.

CONCLUSION

The results of this study demonstrated the use of ethanolic extracts of *Jatropha multifida* and *Hyptis suaveolens* at a dose limit of 2,000 mg/kg.bwt would not result in mortality among chickens. This indicated that the Lethal Dose 50 (LD_{50}) for both of these plant extracts taken from Benin's traditional pharmacopoeia was higher than the limited dose. Notwithstanding, whereas the *J. multifida* extract had a negative impact on feed consumption and the average body weight of the chickens, thereby reflecting its toxicity, the *H. suaveolens* extract improved feed consumption and the average body weight of the chickens. These findings highlight the need for further research to explore the subacute toxicity as well as potential hematological and biochemical disturbances induced by these plant extracts in both local and commercial breeds of chickens.

DECLARATIONS

Funding

This study received no financial support.

Availability of data and materials

All data from the current study are available in this article.

Authors' contributions

Enagnon Bienvenue Florent Sèdégan, Yao Akpo, and Kadoéito Cyrille Boko designed and planned the study, supervised data collection, and analyzed the data. Enagnon Bienvenue Florent Sèdégan, Maximilien Azalou, and Christophe Iwaka collected data and drafted the first version of the manuscript. Camus Adoligbé, Christophe Iwaka, and Enagnon Bienvenue Florent Sèdégan wrote the final version of the document and carried out the critical review. Eloi Attakpa, Ibrahim Alkoiret Traoré, Yao Akpo, and Kadoéito Cyrille Boko revised the document. All authors read and approved the final version of the article.

Competing interests

The authors declare that they have no conflict of interest.

Ethical considerations

The authors took ethical concerns and farmers' consent into account prior to the surveys. This article was originally written without copying from other articles.

REFERENCES

Adesola A and Adetunji A (2007). L'efficacité de *Jatropha multifida* dans la gestion de la candidose orale: Etude préliminaire. [The efficacy of *Jatropha multifida* in the management of oral candidiasis: Preliminary study]. Le Journal Internet de La Médecine Alternative, 4: 66-68. Available at: https://www.ajol.info/index.php/jab/article/view/233746/220778

Adjanohoun EJ, Adjakidje V, Ahyi MRA, Aké Assi L, Akoegninou A, D'Almeida J, Apovo F, Boukef K, Chadare M, Cusset G et al. (1989). Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin [Contribution to ethnobotanical and floristic studies in the People's Republic of Benin]. Médecine traditionnelle et pharmacopée ACCT., Paris, pp. 1-895. Available at: https://www.scirp.org/reference/referencespapers?referenceid=2437685

Adjatin A, Dansi A, Badoussi E, Loko YL, Dansi M, Azokpota P, Gbaguidi F, Ahissou H, Akoègninou A, Akpagana K et al. (2013). Phytochemical screening and toxicity studies of Crassocephalum rubens (Juss. ex Jacq.) S. Moore and Crassocephalum crepidioides (Benth.) S. Mooreconsumed as vegetable in Benin. International Journal of Current Microbiology and Applied Sciences, 5(6): 160-167. Available at: https://www.jocpr.com/abstract/phytochemical-screening-and-toxicity-studies-of-crassocephalum-rubens-juss-ex-jacq-s-moore-and-crassocephalum-crepidioid-1896.html

Agban A, Hoekou Y, Pissang P, Tchacondo T, and Batawila K (2020). Evaluation du potentiel antimicrobien et de la toxicité des extraits de *Jatropha multifida* Linn, (Euphorbiaceae) [Evaluation of the antimicrobial potential and toxicity of extracts of *Jatropha multifida* Linn, (Euphorbiaceae)]. Journal of Applied Biosciences, 151: 15550-15558. DOI: https://www.doi.org/10.35759/JABs.151.4

Agban A, Gbogbo KA, Amana EK, Tegueni K, Batawila K, Koumaglo K, and Akpagana K (2012). Evaluation des activités antimicrobiennes de *Tridax procumbens* (Asteraceae), *Jatropha multifida* (Euphorbiaceae) et de *Chromolaena odorata* (Asteraceae) [Evaluation of the antimicrobial activities of *Tridax procumbens* (Asteraceae), *Jatropha multifida* (Euphorbiaceae) and *Chromolaena odorata* (Asteraceae)]. European Scientific Journal, 9(36): 278-290. Available at: https://core.ac.uk/download/pdf/328023948.pdf

Aiyelaagbe OO, Oguntuase BJ, Arimah BD, and Adeniyi BA (2008). The antimicrobial activity of *Jatropha multifida* extracts and chromatographic fractions against sexually transmitted infections. Journal of Medical Sciences, 8(2): 143-147. Available at: https://scialert.net/abstract/?doi=jms.2008.143.147

Ansm (2021). Liste des plantes médicinales utilisées traditionnellement. Pharmacopée française [List of medicinal plants used traditionally. French pharmacopoeia], pp. 1-36. Available at: https://ansm.sante.fr/uploads/2021/03/25/liste-a-des-plantes-medicinales-utilisees-traditionnellement-4.pdf

Akhtar N and Ihsan-ul-Haq BM (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arabian Revues of Chemistry, 11: 1223-1235. DOI: https://www.doi.org/10.1016/j.arabjc.2015.01.013

Attawish A, Chivapat S, Chavalittumrong P, Phadungpat S, Bandiddhi J, and Chaorai B (2005). Chronic toxicity study of *Hyptis suaveolens* (L.) Poit in rats. Songklanakarin Journal of Science and Technology, 27(5): 1027-1036. Available at: https://www.thaiscience.info/Journals/Article/SONG/10986985.pdf

Batista I, Ramos C, Coutinho J, Bandarra NM, and Nunes ML (2010). Characterization of protein hydrolysates and lipids obtained from black scabbardfish (Aphanopus carbo) by-products and antioxidative activity of the hydrolysates produced. Process Biochemistry, 45(1): 18-24. DOI: https://www.doi.org/10.1016/j.procbio.2009.07.019

Bhaskar N and Mahendrakar NS (2008). Protein hydrolysate from visceral waste proteins of Catla (Catla catla): Optimization of hydrolysis conditions for a commercial neutral protease. Bioresource Technology, 99(10): 4105-4111. DOI: https://www.doi.org/10.1016/j.biortech.2007.09.006

Biswanath D, Bommena R, Kongara RR, and Ponnaboina T (2008). Diterpenoids from *Jatropha multifida*. Phytochemistry, 69(14): 2639-2641. DOI: https://www.doi.org/10.1016/j.phytochem.2008.08.011

Bruneton J (2009). Phytothérapie, les données de l'évaluation. Techniques et documentation [Phytotherapy, evaluation data. Techniques and documentation]. Lavoisier., Paris, pp. 1-242. Available at: https://www.scirp.org/reference/referencespapers?referenceid=3287261

Dassou GH, Adomou AC, Yédomonhan H, Ogni CA, Tossou M, Dougnon JT, and Akoègninou A (2015). Flore médicinale utilisée dans le traitement des maladies et symptômes animaux au Bénin. [Medicinal flora used to treat animal diseases and symptoms in Benin]. Journal of Animal & Plant Sciences, 26(1): 4036-4057. Available at: https://www.m.elewa.org/JAPS/2015/26.1/4.%20dassou.pdf

Dougnon TV, Klotoé JR, Dougnon TJ, Sègbo J, Atègbo J, Edorh PA, Sodipo O, Dougnon F, Dandjesso C, Loko Fb et al. (2012). Hemostatic activity screening and skin toxicity of sap of *Jatropha multifida* L. (Euphorbiaceae) used in traditional medicine (Benin). Asian Pacific Journal of Tropical Disease, 2(2): S927-S932. DOI: https://www.doi.org/10.1016/S2222-1808(12)60293-X

Falodun A, Imieje V, Erharuyi O, Joy A, Langer P, Jacob M, Khan S, Abaldry M, and Hamann M (2014). Isolation of antileishmanial, antimalarial and antimicrobial metabolites from *Jatropha multifida*. Asian Pacific Journal of Tropical Biomedicine, 4(5): 374-378. DOI: https://www.doi.org/10.12980/APJTB.4.2014C1312

Fokunang CN, Ndikum V, Tabi OY, Jiofack RB, Ngameni B, Guedje NM, Tembe-Fokunang EA, Tomkins P, Barkwan S, Kechia F et al. (2011) Traditional medicine: Past, present and future research and development prospects and integration in the national health system of Cameroon. African Journal of Traditional Complementary and Alternative Medecines, 8(3): 284-295. DOI: https://www.doi.org/10.4314/ajtcam.v8i3.65276

Grassi P, Reyesb TSU, Sosac S, Tubaroc A, Hoferd O, and Zitterl-Eglseera K (2006). Anti-inflammatory activity of two diterpenes of *Hyptis suaveolens* from El Salvador. Zeitschrift fur Naturforschung, C 61(3-4): 165-70. DOI: https://www.doi.org/10.1515/znc-2006-3-402

Hamza OJM, Van Den Bout-Van Den Beukel CJP, Matee MIN, Moshi MJ, Mikx FHM, Selemani HO, Mbwambo ZH, Van Der Ven AJAM, and Verwij PE (2006). Antifungal activity of some Tanzanian plants used traditionally for treatment of fungal infections. Journal of Ethnopharmacology, 108(1): 124-132. DOI: https://www.doi.org/10.1016/j.jep.2006.04.026

Iwaka C, Azando EV, Tchetan E, Assani AS, Olounladé AP, and Hounzangbé-Adoté MS (2022a). Propriétés biologiques et pharmacologiques de *Kigelia africana* (Lam.) Benth en médecine traditionnelle humaine et vétérinaire [Biological and pharmacological properties of *Kigelia africana* (Lam.) Benth in traditional human and veterinary medicine]. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 10(4): 567-579. Available at: https://agrimaroc.org/index.php/Actes_IAVH2/article/view/1257

Iwaka C, Azando EVB, Hountondji FCC, Worogo HSS, Attakpa EY, Olounlade PA, and Hounzangbe-Adote MS (2022b). Medicinal plants of the African traditional pharmacopoeia in the management of bovine trypanosomosis: A review. Journal of Medicinal Plants Research, 16: 214-229. DOI: https://www.doi.org/10.5897/JMPR2022.7232

Klotoe JR, Dougnon TV, Dougnon TJ, Agbodjento E, Ategbo JM, and Loko F (2014). *Jatropha multifida* linn (Euphorbiaceae): exploration des proprietes antibacteriennes et du pouvoir cicatrisant de la sève de cette plante chez le rat albinos de souche wistar [*Jatropha multifida* linn (Euphorbiaceae): Exploration of the antibacterial properties and healing power of the sap of this plant in albino wistar rats]. Revue de Microbiologie Industrielle, Sanitaire, et Environnementa, 8(2): 120-132. Available at: https://www.scirp.org/reference/referencespapers? referenceid=3287252

Kollin D and Uziel Y (2006). A case of *Jatropha multifida* poisoning resembling organophosphate intoxication. Journal of Toxicology-Clinical Toxicology, 44: 337-338. Available at: https://www.doi.org/10.1080/15563650600584584

Koné MD (2009). Etude de la phytochimie et des activités larvicide, anticholinesterasique et antioxydante des extraits de quatre plantes du Mali: Acacia nilotica Guill. et Perr. (Mimosaceae), Calotropis procera (Ait.) Ait. f. (Asclepiadaceae), Euphorbia sudanica A. Chev (Euphorbiaceae) et *Hyptis suaveolens* (L.) Poit (Lamiaceae) [Study of the phytochemistry and larvicidal, anticholinesterase and antioxidant activities of extracts from four plants from Mali: *Acacia nilotica* Guill. et Perr. (Mimosaceae), *Calotropis procera* (Ait.) Ait.f. (Asclepiadaceae), *Euphorbia sudanica* A. Chev (Euphorbiaceae) and *Hyptis suaveolens* (L.) Poit (Lamiaceae)]. Thèse de Doctorat, University of Bamako, Mali, pp. 1-83 Available at: https://bibliosante.ml/bitstream/handle/123456789/7082/09P48.pdf?sequence=1&isAllowed=y

Kouchadé SA, Adjatin AR, Adomou AC, Dassou HG, and Akoègninou A (2017). Phytochimiques des plantes médicinales utilisées dans la prise en charge des maladies infantiles au Sud Bénin [Phytochemicals of medicinal plants used in the treatment of childhood illnesses in southern Benin.] European Scientific Journal, 3(1): 471-488. DOI: https://www.doi.org/10.19044/esj.2017.v13n3p471

Koudokpon H, Dougnon TV, Bankolé HS, Fah L, Hounmanou YMG, Baba-moussa L, and Loko F (2015). Enquête ethnobotanique sur les plantes utilisées dans le traitement des infections au Sud-Bénin [Ethnobotanical survey of plants used to treat infections in southern Benin]. Health Science and Diseases, 18(2): 92-99. DOI: https://www.doi.org/10.5281/hsd.v18i2.809

Levin Y, Sherer Y, Bibi H, Schlesinge M, and Hay E (2000). Rare *Jatropha multifida* intoxication in two children. The Journal of Emergency Medicine, 19(2): 173-175. DOI: https://www.doi.org/10.1016/S0736-4679(00)00207-9

Moreira ACP, de Oliveira Lima E, Wanderley PA, Carmo ES, and de Souza EL (2010). Chemical composition and antifungal activity of *Hyptis suaveolens* (L.) Poit leaves essential oil against Aspergillus species. Brazilian Journal of Microbiology, 41(1): 28-33. DOI: https://www.doi.org/10.1590/S1517-83822010000100006

Organisation for economic co-operation and development (OECD) (2016). Test N°. 223: Birds, acute oral toxicity. OECD guidelines for testing of chemicals, section 2. Publishing OECD, Paris, pp.1-28. DOI: https://www.doi.org/10.1787/9789264264533-fr

Pissang P, Tchacondo T, Hoekou YP, Maman I, Effoe S, and Agban A (2018). Subacute toxicity of *Pteleopsis suberosa Engl*. & Diels (Combretaceae): Effect of time of administration in Mice. Afrique Science, 14(6): 428-439.

Philippe MABA, Tobada P, Aboh AB, Pomalègni SCB, Mensah SEP, Kpéra GN, Farougou S, and Mensah GA (2012). Efficacité de *Jatropha multifida* dans le traitement des blessures externes des aulacodes (*Thryonomys swinderianus*) d'élevage [Efficacy of Jatropha multifida in the treatment of external wounds of farmed aulacodes (*Thryonomys swinderianus*)]. Bulletin de la Recherche Agronomique du Bénin, pp. 30-37. Available at: https://publications-chercheurs.inrab.bj/publications/details_publication/284

Santos TC, Marques MS, Menezes IAC, Dias KS, Silva ABL, Mello ICM, Carvalho ACS, Cavalcanti SCH, Antoniolli AR, and Marçal RM (2007). Antinociceptive effect and acute toxicity of the *Hyptis suaveolens* leaves aqueous extract on mice. Fitoterapia, 78(5): 333-336. DOI: https://www.doi.org/10.1016/j.fitote.2007.01.006

Sèdégan B, Akpo Y, Boko C, Attakpa E, and Alkoiret I (2023a). Perception des aviculteurs traditionnels sur la maladie de variole aviaire au sud Bénin [Traditional poultry farmers' perceptions of fowl pox in southern Benin]. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 11(1): 107-112. Available at: https://core.ac.uk/download/552579295.pdf

Sèdégan EBF, Akpo Y, Boko KC, Iwaka C, Attakpa E, and Alkoiret IT (2023b). Méthodes chimiques et alternatives de lutte contre la variole dans les élevages de poulet: Synthèse bibliographique [Chemical and alternative methods for controlling smallpox on chicken farms: Literature review]. Bulletin de la Recherche Agronomique du Bénin (BRAB), 33(3): 79-88. Available at: https://publications-chercheurs.inrab.bj/uploads/fichiers/recents/18027c76fc5ac2113975623f5c783d12.pdf

Sèdégan EBF, Akpo Y, Boko KC, Seidou AA, Iwaka C, Attakpa E, Alkoiret IT, and Mensah GA (2024). Biological activities and traditional use of *Hyptis suaveolens* in human and veterinary medicine: A review. Journal of Veterinary Physiology and Patholog, 3(1): 11-19. DOI: https://www.doi.org/10.58803/jvpp.v3i1.41

Senou M, Lokonon JE, Abissi EOTR, Agbogba F, Dehou RJ, Medoatinsa E, Tchogou P, Cachon BF, Houngbeme A, Attakpa E et al. (2022). Antibacterial activity and toxicity of the sap and aqueous extract of the leaves of Jatropha multifida Linn. Journal of Biosciences and Medicines, 10(7): 171-182. DOI: https://www.doi.org/10.4236/jbm.2022.107013

Soumahoro B, Soro Y, Kassi ABB, and Siaka S (2020). Etude comparative des caractéristiques phytochimiques des feuilles de *Hyptis suaveolens* avant et après extraction de l'huile essentielle [Comparative study of the phytochemical characteristics of *Hyptis suaveolens* leaves before and after extraction of the essential oil]. Journal de la Société Ouest-Africaine de Chimie, 49: 1-8. Available at: https://www.soachim.info/document/journal-soachim/article/Vol-049Num-1-f9c453dc515f01983959ccc4f0643fac.pdf

Traoré A (2016). Plantes insecticides et inhibition de l'acetylcholinestérase [Comparative study of the phytochemical characteristics of *Hyptis suaveolens* leaves before and after extraction of the essential oil]. Thèse de doctorat d'Etat en pharmacie Université des sciences, des techniques et des technologies de Bamako, Mali, pp. 1-82 Available at: https://bibliosante.ml/bitstream/handle/123456789/ 5307/16P22.pdf

Traoré P (2009). Etude de la toxicité et activité antioxydant de *Calotropis procera*, *Centaurea perrottetii*, *Euphorbia sudanica* et *Hyptis suaveolens* [Study of the toxicity and antioxidant activity of *Calotropis procera*, *Centaurea perrottetii*, *Euphorbia sudanica* and *Hyptis suaveolens*]. Thèse d'exercice en Pharmacie. Université de Bamako, Mali, pp. 1-112. Available at: https://bibliosante.ml/handle/123456789/7067 3

Zann RA (1996). Feeding ecology. The zebra finch: A synthesis of field and laboratory studies. Oxford University Press., page 44-59. DOI: https://www.doi.org/10.1093/oso/9780198540793.003.0004

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 338-348.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj40
PII: S232245682400040-14

Effects of Butylated Hydroxytoluene and Sorbitol as Diluent Components on Structural and Surface Ultrastructural Changes of Gaga Chicken Sperm During Cryopreservation

Khaeruddin[1], Gatot Ciptadi[2], Muhammad Yusuf[3], Suyadi[2], Muhammad Halim Natsir[2], Herry Agoes Hermadi[4], and Sri Wahjuningsih[2*]

[1]*Doctoral Program in Animal Science, Faculty of Animal Science, University of Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia*
[2]*Department of Animal Science, Faculty of Animal Science, University of Brawijaya, Jl. Veteran, Malang 65145, East Java, Indonesia*
[3]*Livestock Reproduction Laboratory, Faculty of Animal Science, Universitas Hasanuddin Jl. Perintis Kemerdekaan, Makassar 90245, South Sulawesi, Indonesia*
[4]*Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Surabaya 60115, Indonesia*
*Corresponding author's Email: yuning@ub.ac.id

ORIGINAL ARTICLE
Received: June 29, 2024
Revised: July 20, 2024
Accepted: August 20, 2024
Published: September 25, 2024

ABSTRACT

The Gaga chicken is an indigenous Indonesian breed that is important to preserve using semen cryopreservation technology. The study was conducted to determine the effect of adding sorbitol and butylated hydroxytoluene (BHT) in the diluent on the structural and surface ultrastructure of cryopreserved Gaga chicken sperm during cryopreservation /frozen storage. The study aimed to assess how adding sorbitol and butylated hydroxytoluene (BHT) to the diluent affects the structure and surface ultrastructure of cryopreserved Gaga chicken sperm. A completely randomized design was employed with four treatments and 10 replications including egg yolk-lactate ringer diluent (EYLR) as the control group, EYLR diluent with 3 mM BHT, EYLR diluent with 2% sorbitol, and EYLR diluent with both 3 mM BHT and 2% sorbitol. Semen was collected using a massage technique from 4 male chickens aged approximately 10 months, pooled semen was diluted, packaged in 0.25 mL straws, equilibrated for 2 hours at 5 °C, pre-freeze for 10 minutes, frozen for 24 hours, and thawed for 30 seconds at 37 °C. The parameters evaluated were sperm plasma membrane integrity, acrosome integrity, DNA damage, mitochondrial functionality, and surface ultrastructure. The results showed that the treatment had a significant effect on plasma membrane integrity and post-thawing mitochondrial functionality compared to the control, but no effect was observed on acrosome integrity or DNA damage. The results showed that the combination treatment of BHT with sorbitol had a significant effect on plasma membrane integrity and post-thawing mitochondrial function, but did not affect acrosome integrity or DNA damage when compared to the control group. Ultrastructural observations indicated that cryopreservation caused damage to the head, middle, and tail of the sperm in the control groups. However, these changes were prevented by the diluent containing a combination of BHT and sorbitol. The addition of both components (BHT 3 mM + sorbitol 2%) effectively maintained plasma membrane integrity, mitochondrial functionality, and surface ultrastructure of Gaga chicken sperm during cryopreservation.

Keywords: Butylated hydroxytoluene, Chicken sperm, Cryopreservation, Sorbito, Structure, Sperm ultrastructure

INTRODUCTION

Gaga chicken, an original Indonesian breed, is renowned for a relatively long sound resembling human laughter, leading to the preference by hobbyists for competitions. In this context, longer-duration vocalizations often fetch higher prices (Khaeruddin et al., 2024a). Gaga chicken mating system in society occurs uncontrollably, allowing the decline in purity due to natural mating with other local chicken species (Bugiwati and Ashari, 2013). The Decree of the Minister of Agriculture number 2920/Kpts/OT.140/6/2011, Indonesia has mandated the protection and preservation of Gaga chicken as a rich livestock genetic resource (Khaeruddin et al., 2022).

Cryopreservation is a method to preserve germplasm in various fields including endangered animal conservation, through freezing spermatozoa (Sharafi et al., 2022). Storing chicken sperm by freezing (-196 °C) for 18 years does not reduce the ability to fertilize egg cells (Thélie et al., 2019). However, several reports indicated low sperm quality in chickens after freeze-thawing (Kumar et al., 2019; Masoudi et al., 2021; Ratchamak et al., 2023).

Cell damage during the cryopreservation process is caused by the formation of ice crystals as well as chemical and physical processes, including the denaturation of proteins and lipids in membranes, which trigger the formation of reactive oxygen species (ROS, Pini et al., 2018). Cryopreservation causes low plasma membrane and acrosome integrity as well as post-thawing mitochondrial activity in chicken sperm (Partyka et al., 2012). Ultrastructural analysis by Zong et al. (2023) found that cryopreservation also caused structural damage, particularly in the mitochondria, midpiece, and perforatorium.

To cite this paper: Khaeruddin, Ciptadi G, Yusuf M, Suyadi, Natsir MH, Hermadi HA, and Wahjuningsih S (2024). Effects of Butylated Hydroxytoluene and Sorbitol as Diluent Components on Structural and Surface Ultrastructural Changes of Gaga Chicken Sperm During Cryopreservation. *World Vet. J.,* 14(3): 338-348. DOI: https://dx.doi.org/10.54203/scil.2024.wvj40

Damage to spermatozoa during the cryopreservation process can be prevented by adding special ingredients to the semen diluent. Cryoprotectants in retailers are needed to protect spermatozoa cells both extracellularly and intracellularly. Examples of extracellular cryoprotectants are dimethyl sulfoxide and glycerol, while extracellular cryoprotectants include several types of sugar, such as trehalose (Murray and Gibson, 2022), sucrose, lactose, glucose or fructose (Gómez-Fernández et al., 2012). The addition of sugar has proven effective as a cryoprotectant agent on chicken spermatozoa (Thananurak et al., 2019; Stanishevskaya et al., 2021). Apart from being a cryoprotectant, sugar also acts as an energy source (Zhang et al., 2023; Gholami et al., 2023). Previous reports showed that sugar alcohols such as sorbitol were more effective in maintaining mammalian sperm quality during cryoprotection (Pojprasath et al., 2011; Wu et al., 2016), but this finding has not been reported for avian sperm.

Poultry sperm cell membranes, rich in polyunsaturated fatty acids (PUFA, Mussa et al., 2021), are susceptible to damage from ROS (Collodel et al., 2022). Additional antioxidants can reduce the impact of sperm damage due to ROS and cold shock, improving post-thawing semen quality (Amidi et al., 2016). Butylated hydroxytoluene (BHT), an antioxidant, effectively reduces ROS levels (Zhao et al., 2018), as evidenced by the ability to maintain the quality of mammalian sperm during the cryopreservation process (Merino et al., 2015; Seifi-Jamadi et al., 2016; Jara et al. 2019; Sun et al., 2020). Previous studies reported the effectiveness in maintaining the quality of Gaga chicken sperm during liquid storage (Khaeruddin et al., 2024a). Despite the widespread usage, the effect of sorbitol and combination with BHT on the quality of chicken sperm during the cryopreservation process has not been reported. Therefore, the current study aimed to assess how adding sorbitol and butylated hydroxytoluene (BHT) to the diluent affects the structure and ultrastructure of Gaga chicken sperm during freeze-thawing.

MATERIALS AND METHODS

Ethical approval

The University of Brawijaya Research Ethics Committee, Indonesia, granted Approval No: 020-KEP-UB-2023 for the procedures and animals used in this study.

Diluent preparation

The primary diluent utilized was egg yolk-Ringer's lactate (EYRL), consisting of 90% Ringer's lactate and 10% chicken egg yolk, which was homogenized and centrifuged at 3000 rpm for 15 minutes. The Ringer's lactate solution (PT. Widatra Bakti, Indonesia) contained 3 g sodium chloride, 1.55 g sodium lactate, 0.1 g calcium chloride, and 0.155 g potassium chloride dissolved in 500 ml of sterile water. After centrifugation, the supernatant was collected, and 1000 IU penicillin (PT Meiji, Indonesia), 1 mg/ml streptomycin (PT Meiji, Indonesia), and 7% dimethyl sulfoxide (Merck KGaA, Germany) were added. Subsequently, the diluent was divided into 4 tubes with 3 mM BHT (Sigma, US) and 2% sorbitol (Merck KGaA, Germany) added. The treatment composition was EYRL (control, osmolality 1586 mOsm/kg), EYRL + BHT (osmolality 1605 mOsm/kg), EYRL + sorbitol (osmolality 1615 mOsm/kg), and EYRL + BHT + sorbitol (osmolality 1682 mOsm/kg).

Chicken maintenance and semen collection

Four Gaga chickens aged 12 months obtained from breeders in Malang Regency, East Java, Indonesia were used as a source of semen for cryopreservation. The chickens were kept in individual cages measuring 55 x 60 x 60 cm3 and were given commercial feed (PT New Hope, East Java, Indonesia, 17% crude protein, 2800-2900 kcal/kg energy metabolism, 3% crude fat, and 14% ash) at 100 gr/day alongside drinking water *ad libitum*. Semen was collected using tuberculin spoid by massaging each individual (Kucera and Heidinger, 2018). The Semen collected from each individual was evaluated macroscopically and microscopically, and semen that met the requirements (motility > 70%, viability > 90%, and abnormalities < 20%) were used for the next stage. The semen collected from each individual ranged from 0.1 to 0.2 ml, which was mixed (pooled) was processed further to the dilution and cryopreservation stages.

Cryopreservation stages

Semen was divided into 4 microtubes and diluted based on treatment followed by packaging with a minimum concentration of 200 million per straw (IMV, France). The straw was equilibrated at a temperature of 5 °C for 2 hours (Wahjuningsih et al., 2024), then pre-freezing was carried out in a styrofoam box by placing the straw 3 cm above the surface of liquid nitrogen (Madeddu et al., 2016) for 10 minutes (Mosca et al., 2016). The straw was equilibrated at 5°C for 2 hours (Wahjuningsih et al., 2024). Following this, pre-freezing was performed by placing the straw 3 cm above the surface of liquid nitrogen in a styrofoam box (Madeddu et al., 2016) for 10 minutes (Mosca et al., 2016). Subsequently, the straw was stored in a tank filled with liquid nitrogen (-196 °C) for 24 hours and thawed in a water bath for 30 seconds in water at 37°C (Shah et al., 2016; Wahjuningsih et al., 2024).

Semen evaluation

Plasma membrane integrity

Plasma membrane integrity was assessed using a hypoosmotic swelling test (HOST) solution, which contained 0.9 g of fructose and 0.49 g of sodium citrate in 100 µl of distilled water. Approximately 10 µl of liquid semen was mixed with 100 µl of HOST solution and incubated at 37°C for 30 minutes (Mehdipour et al., 2016; Najafi et al., 2019). The solution was then fixed onto a glass slide using eosin-nigrosin solution, dried, and observed under a light microscope (Olympus CX23, Japan) at 400x magnification (Figure 1). Sperm with intact plasma membranes were identified according to the criteria outlined by Santiago-Moreno et al. (2009), including bent tail, knotted tail tip, bent midpiece, as well as shortened and thickened tail.

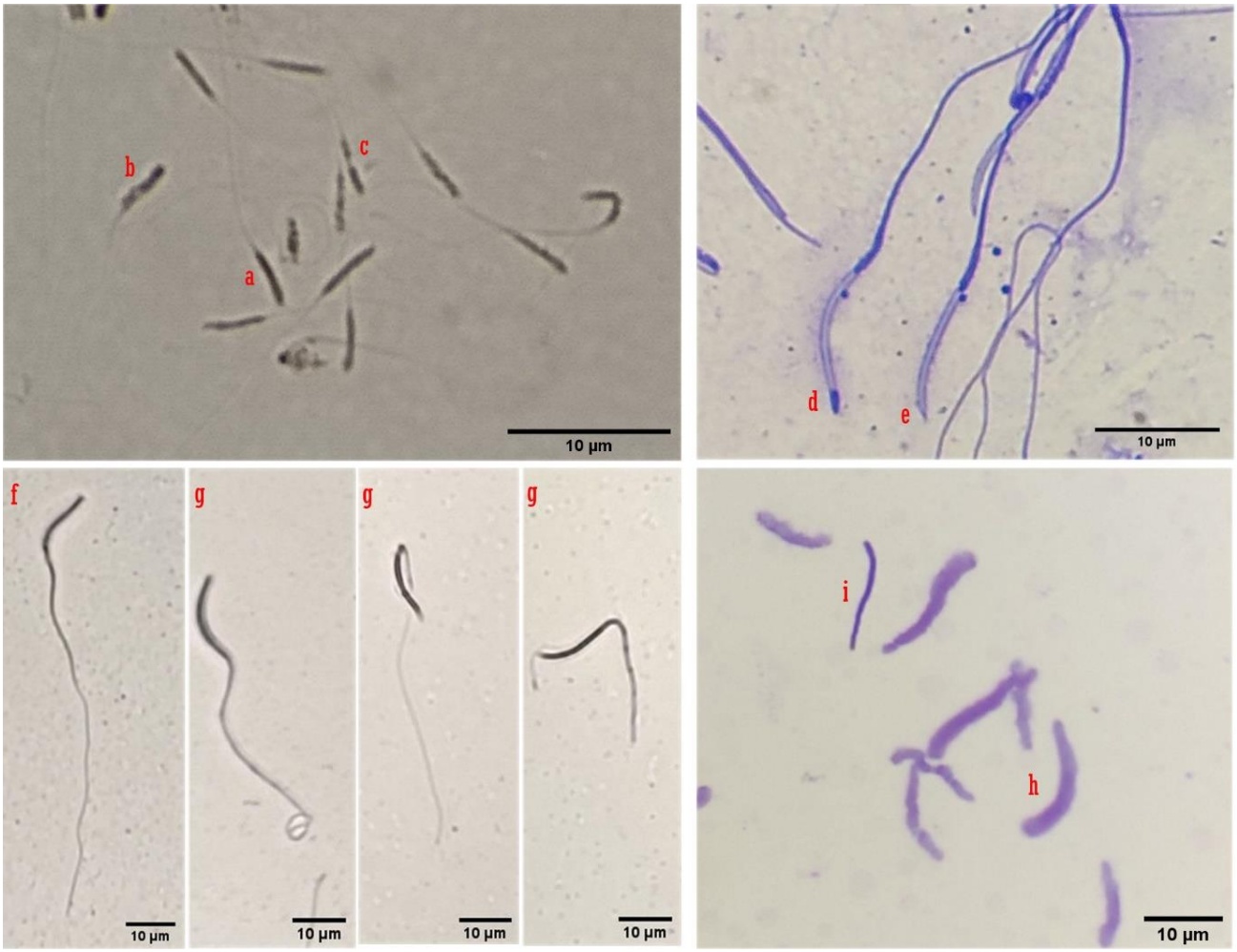


Figure 1. Gaga chicken sperm structure using a light microscope (with 1000x magnification). Mitochondrial functionality (**a**: DAB I, **b**: DAB II, **c**: DAB III), acrosome integrity (**d**: Intact, **e**: Damaged), plasma membrane integrity (**f**: intact membrane, **g**: damaged membrane) and DNA damage (**h**: Intact, **i**: Damaged)

Acrosome integrity

A drop of semen was placed on a glass slide, mixed with 5% formalin, and then thinly smeared on the slide. The samples were re-fixed in a 5% formalin solution for 30 minutes at 37°C, then rinsed and air-dried (Khaeruddin et al., 2024a). Following this, the samples were stained using Coomassie Brilliant Blue solution [0.25% Coomassie Brilliant Blue R 250 (BBI Life Sciences, Canada) in a solution of 25% methanol and 10% glacial acetic acid] for 5 minutes, followed by rinsing and air-drying. The observation was conducted under a light microscope at 1000x magnification (dropped with immersion oil) (Figure 1), with intact acrosomes appearing blue and thick while incomplete acrosomes were less colored or thin (Silyukova et al., 2022).

Mitochondrial functionality

Mitochondrial functionality was observed using 3,3'-diaminobenzidine (DAB) dye (Sigma-Aldrich, US). Semen was diluted in a DAB solution (1 mg/mL phosphate-buffered saline), covered with aluminum foil, and incubated for 1 hour at 37°C. Approximately 10 µl of the solution was then smeared on a glass slide and air-dried. Subsequently,

observation was conducted using a light microscope with 1000x magnification (dropped with immersion oil) on 200 sperm cells (Figure 1). Mitochondrial activity was divided into 4 categories, namely DAB I (100% active), DAB II (> 50% active), DAB III (< 50% active), and DAB IV (inactive) (Rui et al., 2017).

DNA damage

DNA damage was observed using toluidine blue O dye (Merck KGaA, Germany), in this process, semen was spread thinly on a glass object and air-dried. The preparation was fixed for 30 minutes in a solution of 96% ethanol-acetone (1:1) at 4 °C and air-dried. Subsequently, hydrolysis was carried out for 5 minutes in 0.1 N HCl solution at a temperature of 4 °C, followed by rinsing three times with an interval of 2 minutes and air drying. The preparation was dripped with toluidine blue O and left at room temperature for 20 minutes, then rinsed and air dried. Observations were carried out under a light microscope at 400x magnification (Figure 1). Sperm heads with damaged DNA were marked in dark blue while intact DNA was colored bright blue (Rui et al., 2017; Khaeruddin et al., 2024a).

Sperm ultrastructural analysis

Sperm ultrastructural analysis was performed using field-emission scanning electron microscopy (FE-SEM) (FEI Quanta 600, US). The sample preparation involved washing the semen with physiological NaCl, and then fixing it with 2.5% glutaraldehyde for 3-4 hours. This was followed by washing with pH 8 phosphate buffer saline (PBS) three times for 5 minutes each, dehydrating with graded alcohol, and then attaching to a stub. Subsequently, the stub was attached and coated with gold. The sample was analyzed by FE-SEM (FEI Quanta 600, US) at 20,000x magnification with an acceleration voltage of 10 kV.

Statistical analysis

This study used a completely randomized design with 4 treatments and 10 replications. Sperm quality data was analyzed for variance (ANOVA), and Duncan's multiple range test was performed when a significant effect ($p < 0.05$) was found. Ultrastructural data was analyzed descriptively and all analyses were conducted using IBM SPSS Statistics 25 software.

RESULTS

Plasma membrane integrity

The results indicated that adding sorbitol and BHT to EYLR did not significantly impact ($p > 0.05$) the plasma membrane integrity of Gaga chicken sperm before freezing, but a significant effect was observed after thawing ($p < 0.05$, Graph 1). The addition of BHT and sorbitol in the diluent effectively maintained post-thawing plasma membrane integrity (65.47-70.02%) compared to control groups (58.43%). Before freezing, the plasma membrane integrity was in the range of 97.22-98.35%, but a decrease was found post-thawing with a range of 58.43-70.02%.

Acrosome integrity

The addition of BHT and sorbitol in the diluent did not affect the acrosome integrity of Gaga chicken sperm before freezing and post-thawing ($p > 0.05$, Table 1). The range of acrosome integrity before freezing was 97.23-97.9% which decreased to 40.95-42.57% post-thawing.

DNA damage

The results showed that the addition of BHT and sorbitol in EYLR diluent did not affect sperm DNA damage before freezing and post-thawing ($p > 0.05$, Table 2). The range of DNA damage before freezing was relatively low, namely 1.49-1.86, which increased slightly post-thawing to 2.59-3.14%.

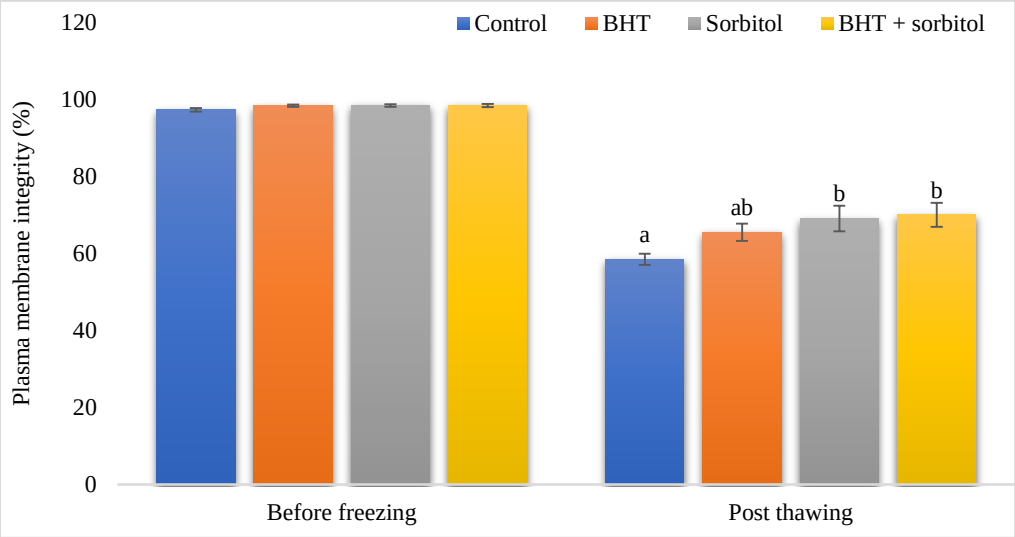
Mitochondria functionality

The addition of BHT and sorbitol in the diluent did not affect the mitochondrial functionality of sperm ($p > 0.05$) before freezing but had a significant effect on post-thawing ($p < 0.05$, DAB I and DAB II, Graph 2). The range of DAB I (100% active mitochondria) before freezing decreased post-thawing, where the addition of sorbitol and combination with BHT in the diluent produced a higher percentage, namely 58.9-63.59%.

Sperm surface ultrastructure

The ultrastructural analysis results of spermatozoa using FE-SEM (Figure 2 a-i) showed that chicken sperm before freezing (fresh semen) had an intact head and acrosome (Figure 2a), as well as sperm post thawing with the addition of BHT + sorbitol (Figure 2b), while post-thawing sperm without BHT and sorbitol experienced damage to the head (Figure 2c). The midpiece of the sperm before freezing looked intact (Figure 2d), as did the post thawing sperm with the addition of BHT + sorbitol (Figure 2e), while the midpiece of the sperm post thawing without BHT and sorbitol

was damaged (Figure 2f). The sperm tail before freezing looked intact (Figure 2g), as did the sperm post thawing with the addition of BHT + sorbitol (Figure 2h), while the sperm tail post thawing without BHT and sorbitol tended to experience damage (Figure 2i).



Graph 1. Plasma membrane integrity percentage of Gaga chicken sperm during cryopreservation with the addition of butylated hydroxytoluene (BHT) and sorbitol in the diluent

Table 1. Acrosome integrity percentage of Gaga chicken sperm before freezing and post-thawing with the addition of butylated hydroxytoluene (BHT) and sorbitol to the diluent

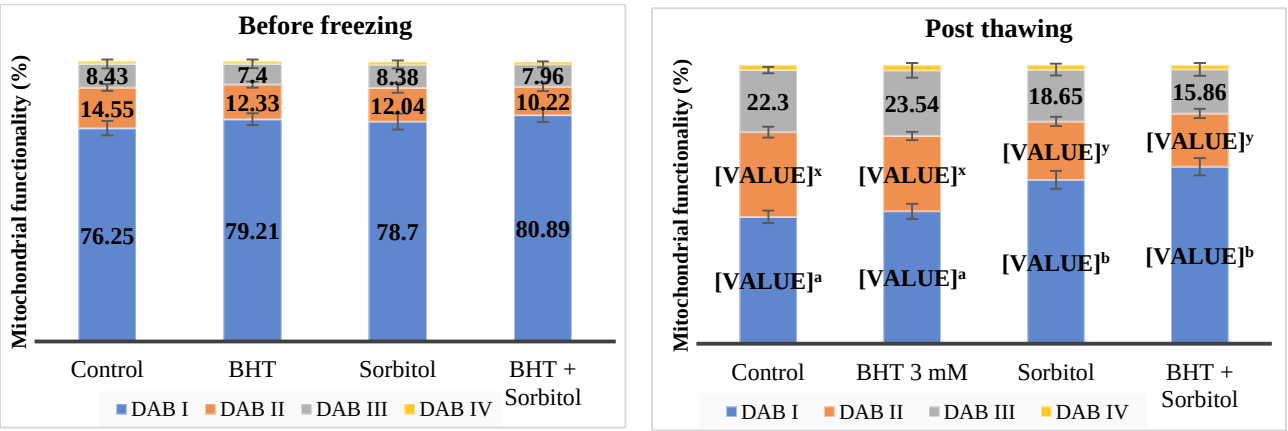
Treatments	Before freezing	Post thawing
Control	97.23 ± 0.69	40.95 ± 2.68
BHT	97.53 ± 0.50	42.22 ± 3.29
Sorbitol	97.78 ± 0.48	42.57 ± 3.33
BHT + Sorbitol	97.90 ± 0.52	42.55 ± 2.54

Control: EYLR diluent, BHT: EYLR diluent + 3 mM BHT, sorbitol: EYLR diluent + 2% sorbitol, and BHT + sorbitol: EYLR diluent + 3 mM BHT + 2% sorbitol.

Table 2. DNA damage percentage of Gaga chicken sperm before freezing and post-thawing with the addition of butylated hydroxytoluene (BHT) and sorbitol to the diluent

Treatments	Before freezing	Post thawing
Control	1.86 ± 0.20	3.14 ± 0.38
BHT	1.49 ± 0.11	3.01 ± 0.39
Sorbitol	1.74 ± 0.22	2.67 ± 0.35
BHT + Sorbitol	1.61 ± 0.23	2.59 ± 0.36

Control: EYLR diluent, BHT: EYLR diluent + 3 mM BHT, sorbitol: EYLR diluent + 2% sorbitol, and BHT + sorbitol: EYLR diluent + 3 mM BHT + 2% sorbitol.



Graph 2. Mitochondrial functionality percentage of Gaga chicken sperm during cryopreservation with the addition of butylated hydroxytoluene (BHT) and sorbitol in the diluent. **DAB I**: 100% mitochondria active, **DAB II**: > 50% mitochondria active, **DAB III**: < 50% mitochondria active, and **DAB IV**: Mitochondria inactive

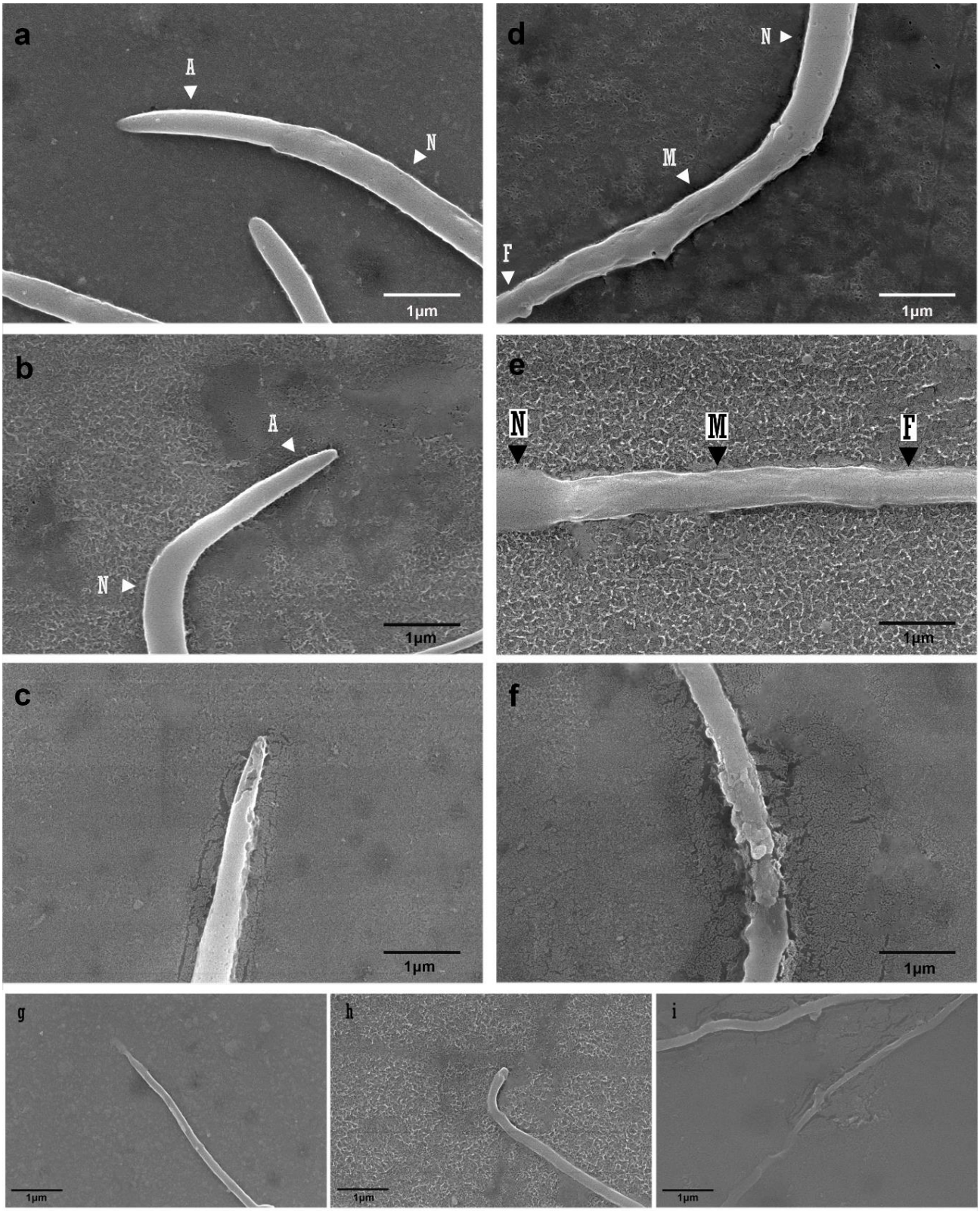


Figure 2. Surface ultrastructural observations of the Gaga sperm surface using the FE-SEM instrument with 20000x magnification. **a**: Sperm heads (fresh semen), **b**: Post-thawing sperm heads (butylated hydroxytoluene + sorbitol), **c**: Post-thawing sperm heads (control), **d**: Sperm midpiece (fresh semen), **e**: Sperm midpiece post thawing (butylated hydroxytoluene + sorbitol), **f:** Sperm midpiece post thawing (control), **g**: Sperm tail (fresh semen), **h**: Post thawing sperm tail (butylated hydroxytoluene + sorbitol), **i**: Post thawing sperm tail (control)

DISCUSSION

Analyzing the structure and ultrastructure of sperm after freezing is crucial as intact sperm are vital for successful fertilization in artificial insemination. This study was one of the first to examine the effects of sorbitol and BHT combination as a diluent on the structure and surface ultrastructure of Gaga chicken sperm in the cryopreservation process. Based on the results, the addition of sorbitol and combination with BHT effectively maintained the integrity of the plasma membrane post-thawing. Sorbitol acts as an antioxidant by donating hydrogen to free radicals, thereby preventing lipid peroxidation in the membrane.

According to Peshev et al. (2013), sugar can act as a hydroxyl (OH) scavenger through the transfer of hydrogen atoms. Sugar alcohol acts as an antioxidant by scavenging oxy-radicals thereby preventing oxidative stress in cells (Kang et al., 2007). More specifically, sorbitol functions by scavenging OHl radicals (Faraji and Linsay, 2004) and superoxide radicals up to 28% (Truffin et al., 2021). As cryoprotectants, sugar alcohols protect proteins from aggregation by inhibiting denaturation caused by ice crystal growth and preventing intermolecular hydrophobic interactions as cryoprotectants, sugar alcohols safeguard proteins from aggregation by preventing denaturation due to ice crystal formation and blocking intermolecular hydrophobic interactions (Zhu et al., 2023). Santivarangkna et al. (2010) suggest that sorbitol protects cells by suppressing the membrane phase transition temperature through interactions with phosphate groups. Takahashi and Hatta (2001) also stated that the compound reduced the interface area between the lipid and water phases. According to Hincha and Hageman (2004), sorbitol showed better protection against membrane leakage than disaccharides. It was proven more effective than other types of sugar in maintaining the integrity of sperm membranes after freezing (Wu et al., 2016). Sorbitol may play an important role in bacterial cell protection, by stabilizing the membrane bilayer or fluidity, and cell recovery from stress (Sootsuwan et al., 2013). Meanwhile, BHT, a phenolic compound is a synthetic antioxidant that eliminates or deactivates free radicals formed during initiation or propagation reactions, inhibiting chain reactions (Fasihnia et al., 2020).

The freezing process in this study caused a decrease in sperm membrane integrity post-thawing. The formation of ice crystals is one of the main sources of physical damage to sperm membranes (Upadhyay et al., 2021; Lee et al., 2023). Another cause of membrane damage in the cryopreservation process is excessive ROS production which triggers changes in membrane proteins, lipids, and carbohydrates due to reduced disulfide bonds between proteins, peroxidation of membrane phospholipids, and modification of the sperm glycocalyx. These changes cause sperm to become brittle and lose their semipermeable properties (Peris-Frau et al., 2020). The average post-thawing plasma membrane integrity value was higher than previous studies, which used Beltsville Poultry Semen Extender (BPSE) diluent added with lycopene-loaded nanoliposomes (42.46-62.71%, Najafi et al., 2018), and Lake diluent added with MitoQ (55-64%, Alipour-Jenaghard et al., 2023). However, the result was almost the same as Stanishevskaya et al. (2021) which obtained a value of 67.2-69.3% using Leningrad Cryoprotective Medium diluent added with disaccharides.

Based on the results, the addition of sorbitol and BHT to the diluent had no significant effect on acrosome integrity and DNA damage. Freezing caused a decrease in acrosome integrity and slightly increased DNA damage. Cryopreservation harms acrosome morphology, motility, and enzyme activity in sperm (Sun et al., 2021b). Freezing affects the acrosomal membrane and induces pre-acrosomal reactions (Khan et al., 2021). Acrosome integrity in this study was higher compared to values obtained in previous studies including 22.8-30.46% using Sasaki diluent (Ruiz et al., 2024) and 35.16-53.16% with Lake diluent (Sun et al., 2021a). Freezing caused a slight increase in DNA damage as reported by Gliozzi et al. (2011) which recorded low sensitivity of chicken sperm to DNA fragmentation during the freezing process. Post-thawing sperm DNA damage in this study was lower than the results of previous studies using Lake diluent, namely 8-13.6% (Masoudi et al., 2021) and 19.82% (Gliozzi et al., 2011).

The cryopreservation process caused a decrease in mitochondrial functionality in this study. Post-thawing sperm ROS accumulation causes damage to the mitochondrial structure, changes in membrane potential difference and respiratory function, as well as decreased adenosine triphosphate (ATP) production and sperm motility (Fang et al., 2014; Słowińska et al., 2018; Zhang et al., 2021; Song et al., 2024). Abnormal ROS concentrations can disrupt the relationship between electron transport and oxidative phosphorylation leading to loss of mitochondrial function (Marcantonini et al., 2022). Continuous accumulation of ROS in cells damages mt-DNA and causes mitochondrial dysfunction (Song et al., 2024).

The addition of sorbitol and combination with BHT effectively maintained post-thawing spermatozoa mitochondrial functionality. This may be due to the ability of the treatment to maintain plasma membrane integrity thereby limiting the entry of excessive amounts of calcium into cells. Mitochondria actively contribute to cellular calcium buffering, but excess accumulation beyond physiological requirements leads to the opening of the mitochondrial permeability transition pore (mPTP), triggering apoptotic or necrotic cell death (Matuz-Mares et al., 2022). Calcium

accumulation can disrupt mitochondrial function, causing a decrease in ATP production as well as an increase in the release of ROS (Santulli et al., 2015). Defects in spermatozoa mitochondrial function greatly disrupt energy production required for motility. This explains the low sperm motility in the treatment without sorbitol and BHT observed (Khaeruddin et al., 2024b).

Ultrastructural observations offer clearer insights into the condition of the sperm surface. The freezing process damaged the sperm at the head, midpiece, and tail region but the addition of sorbitol and BHT in the diluent mitigated this damage. The results are in line with several ultrastructural analyses of cryopreserved chicken sperm in previous studies which found acrosome disruption, neck damage, loss of plasma membrane in the head (Heng et al., 2022), reduced mitochondrial matrix density (Zong et al., 2023), as well as uneven distribution of plasma and mitochondrial sheaths (Chen et al., 2024). Severe damage occurred in the middle part after cryoprepression in the control treatment. The midpiece contains mitochondria which contribute to energy metabolism (Blesbois, 2018), crucial for the progressive movement of spermatozoa (Sangani et al., 2017). The ROS can attack the plasma membrane and sperm mitochondria leading to dysfunction, release of cytochrome C into the cytoplasm, and initiation of cell apoptosis (Zhang et al., 2021).

Damage to sperm structure after freezing may be caused by ice crystallization, oxidative stress, heat shock, and osmotic shock (O'Neill et al., 2019). Poultry sperm have a higher amount of polyunsaturated fatty acids in the plasma membrane than several mammalian species (Najafi et al., 2020), increasing susceptibility to damage during the freezing process (Zong et al., 2023). An imbalance between the cellular antioxidant defense system and the production of ROS during freezing triggers oxidative stress which is the main cause of damage to sperm (Peña et al., 2019).

The combination of sorbitol and BHT prevents damage to sperm structure by maintaining membrane integrity due to their properties as an extracellular cryoprotectant and antioxidant respectively. The plasma membrane is a semipermeable barrier that protects the extracellular environment (Ray et al., 2016). Therefore, an intact plasma membrane is crucial to protect cell organelles such as mitochondria, nucleus, and acrosome from damage by external factors. The ability of cryoprotectant materials to maintain the ultrastructure of chicken sperm during freezing was also reported by Heng et al. (2022).

CONCLUSION

In conclusion, the addition of sorbitol 2% and combination with butylated hydroxytoluene (BHT) 3 mM effectively maintained plasma membrane integrity, mitochondrial functionality, and surface ultrastructure of Gaga chicken sperm during cryopreservation. Further studies are needed to evaluate the effects of sorbitol and BHT in diluents on sperm fertility after artificial insemination.

DECLARATIONS

Acknowledgments

The author thanks the Center for Higher Education Funding (BPPT) and Indonesia Endowment Funds for Education (LPDP). The author also thanks the Integrated Research Laboratory, Brawijaya University.

Funding

This research was funded by the Center for Higher Education Funding (BPPT) and Indonesia Endowment Funds for Education (LPDP), with grant number 1184/j5.2.3./bpi.06/10/2021.

Authors' contributions

Khaeruddin conducted research, laboratory work, and data analysis, and wrote the first version of the manuscripts. Gatot Ciptadi conducted laboratory work. Muhammad Yusuf revised the manuscript. Suyadi conducted sampling. Muhammad Halim Natsir conducted the statistical analysis. Herry Agoes Hermadi conducted the methodology and approved the final revision. Sri Wahjuningsih conducted the methodology and sampling. All the authors read and approved the final version of the manuscript.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

Availability of data and materials

All data of the current study are available upon reasonable requests from the authors.

REFERENCES

Alipour-Jenaghard P, Daghigh-Kia H, Masoudi R, Moghaddam G, and Qasemi-Panahi B (2023). Mitochondria-targeted antioxidant MitoQ improves rooster's cooled sperm quality indicators and reproductive performance. Theriogenology, 197: 26-30. DOI: https://www.doi.org/10.1016/j.theriogenology.2022.11.034

Amidi F, Pazhohan A, Shabani Nashtaei M, Khodarahmian M, and Nekoonam S (2016). The role of antioxidants in sperm freezing: A review. Cell and Tissue Banking, 17(4): 745-756. DOI: https://www.doi.org/10.1007/s10561-016-9566-5

Blesbois E (2018). Bird reproduction overview. In: M. K. Skinner (Editor), Encyclopedia of reproduction. Vol. VI. Academic Press., Cambridge, pp. 579-585. DOI: https://www.doi.org/10.1016/B978-0-12-809633-8.20636-5

Bugiwati SRA and Ashari F (2013). Crowing sound analysis of Gaga' chicken: Local chicken from South Sulawesi Indonesia. International Journal of Plant, Animal and Environmental Sciences, 3(2): 162-168. Available at: https://www.fortunejournals.com/ijpaes/admin/php/uploads/330_pdf.pdf

Chen X, Liu J, Liu Y, Li X, An D, Liu X, and Zhang L (2024). Alpha-lipoic acid improves cryopreservation of rooster semen by reducing oxidative stress. Poultry Science, 103(5): 103632. DOI: https://www.doi.org/10.1016/j.psj.2024.103632

Collodel G, Moretti E, Noto D, Corsaro R, and Signorini C (2022). Oxidation of polyunsaturated fatty acids as a promising area of research in infertility. Antioxidants, 11(5): 1002. DOI: https://www.doi.org/10.3390/antiox11051002

Fang L, Bai C, Chen Y, Dai J, Xiang Y, Ji X, Huang C, and Dong Q (2014). Inhibition of ROS production through mitochondria-targeted antioxidant and mitochondrial uncoupling increases post-thaw sperm viability in yellow catfish. Cryobiology, 69(3): 386-393. DOI: https://www.doi.org/10.1016/j.cryobiol.2014.09.005

Faraji H and Lindsay RC (2004). Characterization of the antioxidant activity of sugars and polyhydric alcohols in fish oil emulsions. Journal of Agricultural and Food Chemistry, 52(23): 7164-7171. DOI: https://www.doi.org/10.1021/jf035291k

Fasihnia SH, Peighambardoust SH, Peighambardoust SJ, Oromiehie A, Soltanzadeh M, and Peressini D (2020). Migration analysis, antioxidant, and mechanical characterization of polypropylene-based active food packaging films loaded with BHA, BHT, and TBHQ. Journal of Food Science, 85(8): 2317-2328. DOI: https://www.doi.org/10.1111/1750-3841.15337

Gómez-Fernández J, Gómez-Izquierdo E, Tomás C, Mocé E, and de Mercado E (2012). Effect of different monosaccharides and disaccharides on boar sperm quality after cryopreservation. Animal Reproduction Science, 133(1-2): 109-116. DOI: https://www.doi.org/10.1016/j.anireprosci.2012.06.010

Gholami D, Sharafi M, Esmaeili V, Nadri T, Alaei L, Riazi G, and Shahverdi A (2023). Beneficial effects of trehalose and gentiobiose on human sperm cryopreservation. PLoS One, 18(4): e0271210. DOI: https://www.doi.org/10.1371/journal.pone.0271210

Gliozzi TM, Zaniboni L, and Cerolini S (2011). DNA fragmentation in chicken spermatozoa during cryopreservation. Theriogenology, 75(9): 1613-1622. DOI: https://www.doi.org/10.1016/j.theriogenology.2011.01.001

Jara B, Merino O, Sánchez R, and Risopatrón J (2019). Positive effect of butylated hydroxytoluene (BHT) on the quality of cryopreserved cat spermatozoa. Cryobiology, 89: 76-81. DOI: https://www.doi.org/10.1016/j.cryobiol.2019.05.003

Heng N, Zhao ZX, Guo Y, Gao S, Cai DL, Fu BF, Sheng XH, Wang XG, Xing K, Xiao LF et al. (2022). RhoA improves cryopreservation of rooster sperm through the Rho/RhoA-associated kinase/cofilin pathway. Poultry Science, 101(10): 102051. DOI: https://www.doi.org/10.1016/j.psj.2022.102051

Hincha DK and Hagemann M (2004). Stabilization of model membranes during drying by compatible solutes involved in the stress tolerance of plants and microorganisms. Biochemical Journal, 383(Pt2): 277-283. DOI: https://www.doi.org/10.1042/BJ20040746

Kang KW, Kwak SH, Yun SY, and Kim SK (2007). Evaluation of antioxidant activity of sugar alcohols using TOSC (total oxy-radical scavenging capacity) assay. Toxicological Research, 32(2): 143-150. DOI: https://www.doi.org/10.5487/TR.2007.23.2.143

Khan IM, Cao Z, Liu H, Khan A, Rahman SU, Khan MZ, Sathanawongs A, and Zhang Y (2021). Impact of cryopreservation on spermatozoa freeze-thawed traits and relevance omics to assess sperm cryo-tolerance in farm animals. Frontiers in Veterinary Science, 8: 609180. DOI: https://www.doi.org/10.3389/fvets.2021.609180

Khaeruddin K, Ciptadi G, Yusuf M, Iswati I, Udrayana SB, and Wahjuningsih S (2024a). Effectiveness of butylated hydroxytoluene in maintaining the quality of Gaga chicken sperm in liquid storage for 72 hours. Advanced in Animal and Veterinary Sciences, 12(2): 371-380. DOI: https://www.doi.org/10.17582/journal.aavs/2024/12.2.371.380

Khaeruddin K, Ciptadi G, Yusuf M, Sawitri W, Chotimah C, and Wahjuningsih S (2024b). Effects of sorbitol and butylated hydroxytoluene on quality, lipid peroxidation, and intracellular calcium concentration of Gaga chicken frozen sperm. International Journal of Agriculture and Biology, 32: 62-70. DOI: https://www.doi.org/10.17957/IJAB/15.2177

Khaeruddin K, Wahjuningsih S, Ciptadi G, Yusuf M, Hermawansyah H, and Sahiruddin S (2022). Cryopreservation of Gaga' chicken semen from South Sulawesi, Indonesia with the addition of L-carnitine, hyaluronic acid, sucrose and their combination in diluent. Biodiversitas Journal of Biological Diversity, 23(6): 3297-3302. DOI: https://www.doi.org/10.13057/biodiv/d230659

Kucera AC and Heidinger BJ (2018). Avian semen collection by cloacal massage and isolation of DNA from sperm. Journal of Visualized Expperiments, 132: e55324. DOI: https://www.doi.org/10.3791/55324

Kumar KP, Swathi B, and Shanmugam M (2019). Effect of supplementing vitamin E analogues on post-thaw semen parameters and fertility in chicken. British Poultry Science, 60(3): 340-345. DOI: https://www.doi.org/10.1080/00071668.2019.1602249

Lee S, Kim YM, Cheong HT, Park CK, and Lee SH (2023). Effect of magnetized freezing extender on membrane damages, motility, and fertility of boar sperm following cryopreservation. Animals, 13(4): 634. DOI: https://www.doi.org/10.3390/ani13040634

Madeddu M, Mosca F, Sayed AA, Zaniboni L, Mangiagalli MG, Colombo E, and Cerolini S (2016). Effect of cooling rate on the survival of cryopreserved rooster sperm: Comparison of different distances in the vapor above the surface of the liquid nitrogen. Animal Reproduction Science, 171: 58-64. DOI: https://www.doi.org/10.1016/j.anireprosci.2016.05.014

Marcantonini G, Bartolini D, Zatini L, Costa S, Passerini M, Rende M, Luca G, Basta G, Murdolo G, Calafiore R et al. (2022). Natural cryoprotective and cytoprotective agents in cryopreservation: A focus on melatonin. Molecules, 27(10): 3254. DOI: https://www.doi.org/10.3390/molecules27103254

Masoudi R, Asadzadeh N, and Sharafi M (2021). Effects of freezing extender supplementation with mitochondria-targeted antioxidant Mito-TEMPO on frozen-thawed rooster semen quality and reproductive performance. Animal Reproduction Science, 225: 106671. DOI: https://www.doi.org/10.1016/j.anireprosci.2020.106671

Matuz-Mares D, González-Andrade M, Araiza-Villanueva MG, Vilchis-Landeros MM, and Vázquez-Meza H (2022). Mitochondrial calcium: Effects of its imbalance in disease. Antioxidants, 11(5): 801. DOI: https://www.doi.org/10.3390/antiox11050801

Mehdipour M, Kia HD, Najafi A, Dodaran HV, and García-Álvarez O (2016). Effect of green tea (*Camellia sinensis*) extract and pre-freezing equilibration time on the post-thawing quality of ram semen cryopreserved in a soybean lecithin-based extender. Cryobiology, 73(3): 297-303. DOI: https://www.doi.org/10.1016/j.cryobiol.2016.10.008

Merino O, Aguagüiña WE, Esponda P, Risopatrón J, Isachenko E, Isachenko V, and Sánchez R (2015). Protective effect of butylated hydroxytoluene on sperm function in human spermatozoa cryopreserved by vitrification technique. Andrologia, 47(2): 186-193. DOI: https://www.doi.org/10.1111/and.12246

Mosca F, Madeddu M, Sayed AA, Zaniboni L, Iaffaldano N, and Cerolini S (2016). Combined effect of permeant and non-permeant cryoprotectants on the quality of frozen/thawed chicken sperm. Cryobiology, 73(3): 343-347. DOI: https://www.doi.org/10.1016/j.cryobiol.2016.10.001

Mussa NJ, Ratchamak R, Ratsiri T, Vongpralub T, Boonkum W, Semaming Y, and Chankitisakul V (2021). Lipid profile of sperm cells in Thai native and commercial roosters and its impact on cryopreserved semen quality. Tropical Animal Health and Production, 53(2): 321. DOI: https://www.doi.org/10.1007/s11250-021-02664-9

Murray KA and Gibson MI (2022). Chemical approaches to cryopreservation. Nature Reviews Chemistry, 6(8): 579-593. DOI: https://www.doi.org/10.1038/s41570-022-00407-4

Najafi A, Taheri RA, Mehdipour M, Farnoosh G, and Martínez-Pastor F (2018). Lycopene-loaded nanoliposomes improve the performance of a modified Beltsville extender broiler breeder roosters. Animal Reproduction Science, 195: 168-175. DOI: https://www.doi.org/10.1016/j.anireprosci.2018.05.021

Najafi, A, Kia HD, Mehdipour M, Hamishehkar H, and Álvarez-Rodríguez M (2020). Effect of quercetin loaded liposomes or nanostructured lipid carrier (NLC) on post-thawed sperm quality and fertility of rooster sperm. Theriogenology, 152: 122-128. DOI: https://www.doi.org/10.1016/j.theriogenology.2020.04.033

Najafi A, Taheri RA, Mehdipour M, Martínez-Pastor F, Rouhollahi AA, and Nourani MR (2019). Improvement of post-thawed sperm quality in broiler breeder roosters by ellagic acid-loaded liposomes. Poultry Science, 98(1): 440-446. DOI: https://www.doi.org/10.3382/ps/pey353

O'Neill HC, Nikoloska M, Ho H, Doshi A, and Maalouf W (2019). Improved cryopreservation of spermatozoa using vitrification: comparison of cryoprotectants and a novel device for long-term storage. Journal of Assisted Reproduction and Genetics, 36: 1713-1720. DOI: https://www.doi.org/10.1007/s10815-019-01505-x

Partyka A, Łukaszewicz E, and Niżański W (2012). Effect of cryopreservation on sperm parameters, lipid peroxidation and antioxidant enzymes activity in fowl semen. Theriogenology, 77: 1497-1504. DOI: https://www.doi.org/10.1016/j.theriogenology.2011.11.006

Peña FJ, O'Flaherty C, Rodríguez JMO, Cano FEM, Gaitskell-Phillips GL, Gil MC, and Ferrusola CO (2019). Redox regulation and oxidative stress: The particular case of the stallion spermatozoa. Antioxidants, 8(11): 567. DOI: https://www.doi.org/10.3390/antiox8110567

Peshev D, Vergauwen R, Moglia A, Hideg É, and Van den Ende W (2013). Towards understanding vacuolar antioxidant mechanisms: a role for fructans?. Journal of Experimental Botany, 64(4): 1025-1038. DOI: https://www.doi.org/10.1093/jxb/ers377

Peris-Frau P, Soler AJ, Iniesta-Cuerda M, Martín-Maestro A, Sánchez-Ajofrín I, Medina-Chávez DA, Fernández-Santos MR, García-Álvarez O, Maroto-Morales A, Montoro V et al. (2020). Sperm cryodamage in ruminants: Understanding the molecular changes induced by the cryopreservation process to optimize sperm quality. International Journal of Molecular Sciences, 21(8): 2781. DOI: https://www.doi.org/10.3390/ijms21082781

Pini T, Leahy T, and de Graaf SP (2018). Sublethal sperm freezing damage: Manifestations and solutions. Theriogenology, 118: 172-181. DOI: https://www.doi.org/10.1016/j.theriogenology.2018.06.006

Pojprasath T, Lohachit C, Techakumphu M, Stout T, and Tharasanit T (2011). Improved cryopreservability of stallion sperm using a sorbitol-based freezing extender. Theriogenology, 75(9): 1742-1749. DOI: https://www.doi.org/10.1016/j.theriogenology.2011.01.014

Ratchamak R, Authaida S, Boonkum W, and Chankitisakul V (2023). Improvement of rooster semen freezability and fertility rate after sericin supplementation in freezing semen extender. Animal Bioscience, 36(10): 1530. DOI: https://www.doi.org/10.5713/ab.23.0016

Ray S, Kassan A, Busija AR, Rangamani P, and Patel HH (2016). The plasma membrane as a capacitor for energy and metabolism. American Journal of Physiology-Cell Physiology, 310(3): C181-C192. DOI: https://www.doi.org/10.1152/ajpcell.00087.2015

Rui BR, Angrimani DS, Losano JDA, de Cássia Bicudo L, Nichi M, and Pereira RJ (2017). Validation of simple and cost-effective stains to assess acrosomal status, DNA damage and mitochondrial activity in rooster spermatozoa. Animal Reproduction Science, 187: 133-140. DOI: https://www.doi.org/10.1016/j.anireprosci.2017.10.017

Ruiz ED, Ariza AG, Jurado JML, Arbulu AA, Guzmán JFB, Oria AB, Bermejo JVD, and González FJN (2024). Evaluation of the effect of the addition of an olive oil-derived antioxidant (Pectoliv-80A) in the extender for cryopreservation of rooster sperm through the use of a discriminant statistical tool. Poultry Science, 103(5): 103630. DOI: https://www.doi.org/10.1016/j.psj.2024.103630

Sangani AK, Masoudi AA, and Torshizi RV (2017). Association of mitochondrial function and sperm progressivity in slow- and fast-growing roosters. Poultry Science, 96: 211-219. DOI: https://www.doi.org/10.3382/ps/pew273

Santiago-Moreno J, Castaño C, Coloma MA, Gómez-Brunet A, Toledano-Díaz A, López-Sebastián A, and Campo JL (2009). Use of the hypo-osmotic swelling test and aniline blue staining to improve the evaluation of seasonal sperm variation in native Spanish free-range poultry. Poultry Science, 88(12): 2661-2669. DOI: https://www.doi.org/10.3382/ps.2008-00542

Santivarangkna C, Naumann D, Kulozik U, and Foerst P (2010). Protective effects of sorbitol during the vacuum drying of Lactobacillus helveticus: An FT-IR study. Annals of Microbiology, 60: 235-242. DOI: https://www.doi.org/10.1007/s13213-010-0032-5

Santulli G, Xie W, Reiken SR, and Marks AR (2015). Mitochondrial calcium overload is a key determinant in heart failure. Proceedings of the National Academy of Sciences, 112(36): 11389-11394. DOI: https://www.doi.org/10.1073/pnas.1513047112

Seifi-Jamadi A, Kohram H, Zareh-Shahne A, Dehghanizadeh P, and Ahmad E (2016). Effect of various concentrations of butylated hydroxyanisole and butylated hydroxytoluene on freezing capacity of Turkman stallion sperm. Animal Reproduction Science, 170: 108-113. DOI: https://www.doi.org/10.1016/j.anireprosci.2016.04.010

Shah SAH, Andrabi SMH, and Qureshi IZ (2016). Effect of equilibration times, freezing, and thawing rates on post-thaw quality of buffalo (Bubalus bubalis) bull spermatozoa. Andrology, 4(5): 972-976. DOI: https://www.doi.org/10.1111/andr.12214

Sharafi M, Borghei-Rad SM, Hezavehei M, Shahverdi A, and Benson JD (2022). Cryopreservation of semen in domestic animals: A review of current challenges, applications, and prospective strategies. Animals, 12(23): 3271. DOI: https://www.doi.org/10.3390/ani12233271

Silyukova Y, Fedorova E, and Stanishevskaya O (2022). Influence of technological stages of preparation of rooster semen for short-term and long-term storage on its quality characteristics. Current Issues in Molecular Biology, 44(11): 5531-5542. DOI: https://www.doi.org/10.3390/cimb44110374

Słowińska M, Liszewska E, Judycka S, Konopka M, and Ciereszko A (2018). Mitochondrial membrane potential and reactive oxygen species in liquid stored and cryopreserved turkey (Meleagris gallopavo) spermatozoa. Poultry Science, 97(10): 3709-3717. DOI: https://www.doi.org/10.3382/ps/pey209

Sootsuwan K, Thanonkeo P, Keeratirakha N, Thanonkeo S, Jaisil P, and Yamada M (2013). Sorbitol required for cell growth and ethanol production by Zymomonas mobilis under heat, ethanol, and osmotic stresses. Biotechnology for Biofuels and Bioproducts, 6: 1-13. DOI: https://www.doi.org/10.1186/1754-6834-6-180

Song J, Xiao L, Zhang Z, Wang Y, Kouis P, Rasmussen LJ, and Dai F (2024). Effects of reactive oxygen species and mitochondrial dysfunction on reproductive aging. Frontiers in Cell and Developmental Biology, 12: 1347286. DOI: https://www.doi.org/10.3389/fcell.2024.1347286

Stanishevskaya O, Silyukova Y, Pleshanov N, and Kurochkin A (2021). Role of mono-and disaccharide combination in cryoprotective medium for rooster semen to ensure cryoresistance of spermatozoa. Molecules, 26(19): 5920. DOI: https://www.doi.org/10.3390/molecules26195920

Sun L, He M, Wu C, Zhang S, Dai J, and Zhang D (2021a). Beneficial influence of soybean lecithin nanoparticles on rooster frozen-thawed semen quality and fertility. Animals, 11(6): 1769. DOI: https://www.doi.org/10.3390/ani11061769

Sun W, Jiang S, Su J, Zhang J, Bao X, Ding R, Shi P, Li S, Wu C, Zhao G, and Cao G (2021b). The effects of cryopreservation on the acrosome structure, enzyme activity, motility, and fertility of bovine, ovine, and goat sperm. Animal Reproduction, 17(4): e20200219. DOI: https://www.doi.org/10.1590/1984-3143-ar2020-0219

Sun L, Wu C, Xu J, Zhang S, Dai J, and Zhang D (2020). Addition of butylated hydroxytoluene (BHT) in tris-based extender improves post-thaw quality and motion dynamics of dog spermatozoa. Cryobiology, 97: 71-75. DOI: https://www.doi.org/10.1016/j.cryobiol.2020.10.006

Takahashi H and Hatta I (2001). Interactions of sugars with phosphatidylcholines. Studies in Surface Science and Catalysis, 132: 557-560. DOI: https://www.doi.org/10.1016/S0167-2991(01)82152-2

Thananurak P, Chuaychu-Noo N, Thélie A, Phasuk Y, Vongpralub T, and Blesbois E (2019). Sucrose increases the quality and fertilizing ability of cryopreserved chicken sperms in contrast to raffinose. Poultry Science, 98(9): 4161-4171. DOI: https://www.doi.org/10.3382/ps/pez196

Thélie A, Bailliard A, Seigneurin F, Zerjal T, Tixier-Boichard M, and Blesbois E (2019). Chicken semen cryopreservation and use for the restoration of rare genetic resources. Poultry Science, 98(1): 447-455. DOI: https://www.doi.org/10.3382/ps/pey360

Truffin D, Häusler O, Blouet E, Ramnath M, Martin M, and Cotier S (2021). Determination of antioxidant effect of polyols in a cell free environment. In Proceedings of the 12th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Vienna, Austria. pp. 11-14. DOI: https://www.doi.org/10.13140/RG.2.2.25602.81606

Upadhyay VR, Ramesh V, Dewry RK, Kumar G, Raval K, and Patoliya P (2021). Implications of cryopreservation on structural and functional attributes of bovine spermatozoa: An overview. Andrologia, 53(8): e14154. DOI: https://www.doi.org/10.1111/and.14154

Wahjuningsih S, Arif AA, Khaerudin K, Pratiwi H, and Putri ARI (2024). The effects of equilibration time and post-thawing temperatures in cryopreservation of Gaga chicken semen. Advanced in Animal and Veterinary Sciences, 12(5): 807-811. DOI: https://www.doi.org/10.17582/journal.aavs/2024/12.5.807.814

Wu GQ, Lv CR, Jiang YT, Wang SY, Shao QY, Hong QH, and Quan GB (2016). The replacement of monosaccharide by mannitol or sorbitol in the freezing extender enhances cryosurvival of ram spermatozoa. Biopreservation and Biobanking, 14(5): 357-366. DOI: https://www.doi.org/10.1089/bio.2015.0080

Zhang B, Wang Y, Wu C, Qiu S, Chen X, Cai B, and Xie H (2021). Freeze-thawing impairs the motility, plasma membrane integrity and mitochondria function of boar spermatozoa through generating excessive ROS. BMC Veterinary Research, 17: 127. DOI: https://www.doi.org/10.1186/s12917-021-02804-1

Zhang L, Wang Y, Sun X, Kang Y, Sohail T, Wang J, and Li Y (2023). Effects of different diluents on semen quality of hu ram stored at 4°C. Animals, 13(18): 2823. DOI: https://www.doi.org/10.3390/ani13182823

Zhao Y, Yue C, Ding W, Li T, Xu JW, Zhao P, Ma H, and Yu X (2018). Butylated hydroxytoluene induces astaxanthin and lipid production in Haematococcus pluvialis under high-light and nitrogen-deficiency conditions. Bioresource Technology, 266: 315-321. DOI: https://www.doi.org/10.1016/j.biortech.2018.06.111

Zhu H, Li J, Su Y, Gu L, Chang C, and Yang Y (2023). Sugar alcohols as cryoprotectants of egg yolk: Inhibiting crystals and interactions. Journal of Food Engineering, 342: 111360. DOI: https://www.doi.org/10.1016/j.jfoodeng.2022.111360

Zong Y, Li Y, Sun Y, Mehaisen GM, Ma T, and Chen J (2023). Chicken sperm cryopreservation: Review of techniques, freezing damage, and freezability mechanisms. Agriculture, 13(2): 445. DOI: https://www.doi.org/10.3390/agriculture13020445

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 349-354.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj41
PII: S232245682400041-14

The Pharmacokinetics of Ceftazidime Following its Intravenous Administration in Dogs

Mustafa A. Al-Jumaili *[1], Nibras N. Al-Abbass [2], and Orooba M. S. Ibrahim [2]

[1]*Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Diyala, Iraq*
[2]*Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq*

*Corresponding author's Email: mustafa.a@uodiyala.edu.iq

ORIGINAL ARTICLE
Received: July 10, 2024
Revised: July 29, 2024
Accepted: August 21, 2024
Published: September 25, 2024

ABSTRACT

Ceftazidime is a beta-lactam that is used in the treatment of bacterial infections in humans and companion animals, such as dogs and cats. It is prescribed to treat gram-negative infections, especially those caused by *Pseudomonas aeruginosa*. This study aimed to compare the pharmacokinetics of ceftazidime using a microbiological assay to evaluate the adequacy of the proposed dosage regimens for susceptible gram-negative bacteria. For this purpose, five healthy mongrel male dogs, with a mean age of four years and an average weight of 19.1 kg, were administered a single intravenous bolus dose of ceftazidime (20 mg/kg). Plasma concentrations were measured using a microbiological assay, and dosage regimens were established by integrating pharmacokinetics data with pharmacodynamics parameters. The results showed that ceftazidime was rapidly distributed to the peripheral tissues (0.189 L/kg), with a half-life of 1.15 hours and a clearance rate of 0.166 L/hr./kg. The results obtained from the pharmacokinetics-pharmacodynamic integration suggested 20 mg/kg q8 hours of ceftazidime for susceptible gram-negative bacteria with a Minimum Inhibitory Concentration of ≤ 8 µg/ml, and 20 mg /kg q12 hours of ceftazidime for susceptible gram-negative bacteria with a Minimum Inhibitory Concentration of ≤ 4 µg/ml. In conclusion, a mild correlation was observed between the dogs' weight and the ceftazidime half-life, which led to an adjustment of the proposed dosage regimen to 20 mg/kg q8 hours.

Keywords: Ceftazidime, Dog, Dosage regimen, Gram-negative microbe, Pharmacokinetic

INTRODUCTION

Dogs are the most common companion animals to humans because of their positive impacts on their owner's physical and mental status (Overgaauw et al., 2020). However, many bacterial diseases can affect the dogs' health and lead to a potentially unfavorable prognosis (Marks et al., 2011; De Sousa et al., 2023). Rational antibiotic therapy seems a solution to avoid undesirable complications (Wayne et al., 2011).

Ceftazidime belongs to the third generation of cephalosporins with a broad-spectrum bactericidal effect and high accessibility to most tissues, including hard and CNS tissues with unchanged excretion through the kidney (Budde and McCluskey, 2023). Ceftazidime is used to manage bacterial infections in dogs having bactericidal spectrum, including the susceptible pathogenic gram-negative bacteria, with additional activity against *Pseudomonas aeruginosa* (Papich, 2020).

Previous studies on the pharmacokinetics of ceftazidime in dogs, involving intravenous administration at doses ranging from 20 to 25 mg/kg in animals weighing 10.5-15.6 kg, have demonstrated that ceftazidime has rapid distribution, short half-life, and rapid elimination (Matsui et al., 1984; Kita et al., 1992; Sakamoto et al., 1993; Monfrinotti et al., 2010; Papich et al., 2022).

Pharmacokinetics plays a crucial role in establishing rational dosage regimens for antibiotics through integration with pharmacodynamics (PK/PD integration), which is considered a suitable solution to obtain the desired therapeutic response, minimizing adverse reactions, decreasing the risk of bacterial resistance, and reducing treatment costs (Guardabassi et al., 2018).

It is known that the diversity of dog breeds presents a challenge that could lead to a non-standardized pharmacokinetic profile of drugs due to potential unmatched digestive physiology, metabolic profile, kidney function, and protein binding (Tibbitts, 2003; Toutain et al., 2010). Therefore, this study aims to assess the pharmacokinetics of ceftazidime in local Mongrel dogs, compare the results with the previous studies conducted exclusively on Beagles, and determine whether adjustments to ceftazidime dosage regimen make any difference in treating susceptible gram-negative bacterial infections.

To cite this paper: Al-Jumaili MA, Al-Abbass NN, and Ibrahim OMS (2024). The Pharmacokinetics of Ceftazidime Following its Intravenous Administration in Dogs. *World Vet. J.*, 14(3): 349-354. DOI: https://dx.doi.org/10.54203/scil.2024.wvj41

MATERIALS AND METHODS

Ethical approval

The study was approved by the Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq (Approval No. VM 301; November 2022).

Animals

Five healthy male Mongrel dogs, provided by the Dogs Kennel of the College of Veterinary Medicine, University of Diyala, with an average age of 4 (± 0.5) years and an average weight of 19.1 kg (± 1.3), were utilized in this study. A comprehensive physical examination, including assessments of physical appearance, skin and coat integrity, lymph nodes, respiration rate and rhythm, pulse, and body temperature as well as general mouth and teeth examination was done by a certified veterinarian to ensure the dog was in good health. All dogs were free of antibiotics and other medications and were kept in an isolated kennel in the College of Veterinary Medicine, University of Diyala, for a week for behavioral adaptation. They were provided with free access to water and a balanced diet.

Drug administration

Ceftazidime (LDP Laboratories Torlan, Barcelona, Spain) was injected as an intravenous bolus via the right cephalic vein at 20 mg/kg dose for each dog (Monfrinotti et al., 2010).

Samples collection and analysis

One milliliter of blood was obtained from the left cephalic vein on 0.08, 0.16, 0.33, 0.5, 1, 2, 4, 8, 12, and 24 hours post-administration. The samples were kept in heparinized tubes, and plasma was separated by centrifugation and stored at -20C^{o} for further drug analysis. The microbiological assay was applied to estimate the concentration of ceftazidime using spores of *Bacillus subtilis* ATCC 6633 provided by the Department of Biology at the College of Science, University of Diyala, prepared as previously described by Sabath (1976). A drug-free plasma sample was used to prepare the standard curve for further determination of ceftazidime concentrations. additionally, the protein binding of ceftazidime was determined by calculating the partitioning ratio of ceftazidime between phosphate-buffered saline and plasma (Craig and Suh, 1991).

Pharmacokinetic analysis

The pharmacokinetic parameters of ceftazidime were calculated using Microsoft Excel®, following the equations outlined in Rosenbaum (2017). The Aikake Information Criterion (AIC) was applied to identify the most suitable model to fit the data points (Yamaoka et al., 1978).

Dosage regimen

The Minimum Inhibitory Concentration (MIC) breakpoints of ceftazidime against susceptible gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were obtained from the Clinical and Laboratory Standards Institute (CLSI, 2024). The breakpoints were integrated with the pharmacokinetic parameters obtained in this study (PK/PD integration) to establish a dosage regimen for ceftazidime (Fratoni et al., 2021).

Statistical analysis

Pearson correlation coefficient (r) was applied to determine the relationship between body weight and the half-life of ceftazidime across different studies (Matsui et al., 1984; Kita et al., 1992; Sakamoto et al., 1993; Monfrinotti et al., 2010; Papich et al., 2022). Statistical analyses were performed using GraphPad Prism 8.0 for Windows (GraphPad Software, Boston, Massachusetts USA).

RESULTS

Clinical observations indicated no adverse effects from the intravenous administration of ceftazidime in the dogs. The microbiological assay employed to construct the ceftazidime standard curve in plasma showed a linear pattern, with an acceptable coefficient of determination (R^2 = 0.973), The limit of detection (LOD) for ceftazidime in plasma was 0.23 µg/ml, while the accuracy of the assay of ceftazidime analysis qualified by the limit of quantification (LOQ) was 0.78 µg/ml as listed (Table 1).

The two-compartment model selected to fit time-concentration points was based on AIC. The data points, depicted in Figure 1, show a clear bi-exponential decay of ceftazidime concentration over time. All the calculated primary and secondary pharmacokinetic parameters are summarized in Table 2. Briefly, ceftazidime was quickly distributed to peripheral tissues (0.189 L/kg), with a half-life of 1.15 hours and a clearance rate of 0.166 L/hour/kg.

The PK/PD integration results, reported in Table 3, suggested a dosage regimen of 20 mg /kg q8 hours of ceftazidime for susceptible *Pseudomonas aeruginosa* (MIC ≤ 8 µg/ml), while it proposed a dosage regimen of 20 mg /kg q12 hours of ceftazidime for susceptible *Enterobacterales* (MIC ≤ 4 µg/ml).

The correlation between the average body weight and the half-life of ceftazidime was established by analyzing data from previous works (Table 4) in conjunction with the present study. The analysis revealed a moderate positive relationship (r = 0.62) between body weight and ceftazidime half-life, as illustrated in Figure 2.

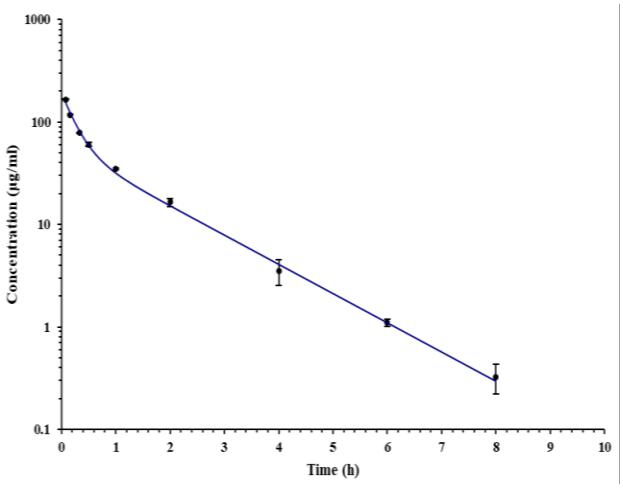


Figure 1. Ceftazidime concentrations in the plasma of dogs after a single Intravenous bolus administration (20 mg/kg).

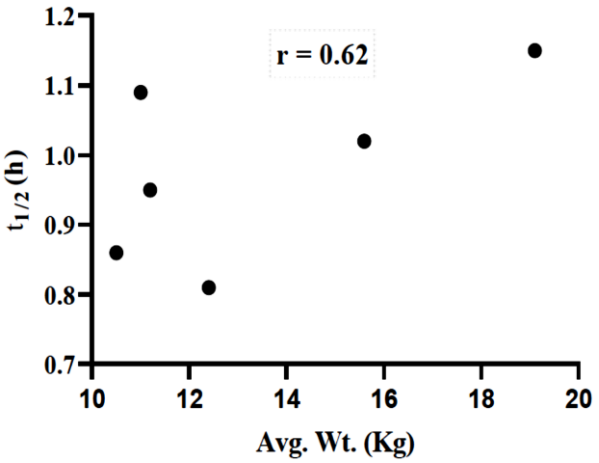


Figure 2. Relationship between dog body weight and the half-life of Ceftazidime through different studies. $t_{1/2}$, half-life; Avg. wt.: Average weight

Table 1. Standard curve of Ceftazidime microbiological assay

Parameter	Value
CV%	3
Slope	13.87
Intercept	12.871
R^2	0.97
LOD (µg/ml)	0.23
LOQ (µg/ml)	0.78

CV: Variation coefficient; R^2: Determination coefficient; LOD: The limit of detection; LOQ: The limit of quantification

Table 2. Pharmacokinetics of Ceftazidime administration in plasma of dogs

Parameter	Unit	Mean	SD
A	µg/ml	135.75	13.41
α	1/h	3.83	2.04
$t_{1/2\alpha}$	h	0.24	0.16
AUC	(h*µg)/ml	120.01	7.07
AUMC	µg/ml*h^2	137.61	13.75
B	µg/ml	48.39	26.67
β	1/h	0.61	0.11
$t_{1/2\beta}$	h	1.15	0.21
Cl_T	L/hr./kg	0.166	0.009
Cp^0	µg/ml	184.14	39.75
K_{12}	1/h	1.37	1.05
K_{21}	1/h	1.56	0.84
MRT	h	1.13	0.05
V_C	L/kg	0.113	0.026
Vd_{ss}	L/kg	0.189	0.004
Protein binding	%	12.3	1.02

A: Distribution intercept; α: Distribution rate constant; $t_{1/2\alpha}$: Distribution half-life; AUC: Area under the curve; AUMC: Area under the moment curve; B: Elimination intercept; β: Elimination rate constant; $t_{1/2\beta}$: Elimination half-life; Cl_T: Total body clearance; Cp^0: Zero-time concentration; K_{12} and K_{21}: Micro-distribution rate constants; MRT: Mean residence time; Vc: Volume of distribution of central compartment; Vd: Volume of distribution at steady state

Table 3. Dosage regimens of Ceftazidime in dogs

τ (h.)	%T>MIC (1 µg/ml)	%T>MIC (2 µg/ml)	%T>MIC (4 µg/ml)	%T>MIC (8 µg/ml)	No. doses/day
8	96.68	82.30	67.93	53.55	3
12	64.45	54.87	45.29	35.70 [N.A.]	2

τ: Time interval; %T > MIC: Percentage of time over the minimum inhibitory concentration; N.A.: Not applicable (T > MIC is below 45 %). No: Number

Table 4. Comparison across different studies of Ceftazidime pharmacokinetics in dogs (IV Bolus)

Study	Avg. Wt. (Kg)	VDss (L/kg)	$t_{1/2}$ (h)	AUC (µg.h/ml)	CL (L/kg/h)
Matsui et al. (1984)	12.4	0.218	0.81	93	0.215
Kita et al. (1992)	10.5	0.210	0.86	105	0.192
Sakamoto et al. (1993)	11.0	0.353	1.09	89	0.228
Monfrinotti et al. (2010)	15.6	0.206	1.02	126	0.159
Papich et al. (2022)	11.2	0.171	0.95	142.4	0.176
Average	12.14	0.232	0.95	111.08	0.194
Present study	19.1	0.189	1.15	120.01	0.166

Avg. wt.: Average weight; Vdss, Volume of distribution at steady state; t1/2: Elimination half-life; AUC: Area under the curve; Cl: Total body clearance

DISCUSSION

The accuracy of the assay of drug analysis, determined by the limit of quantification (LOQ), was higher than what the was quantified in the current study. This issue, known as data below the limit of quantification (BLOQ), is typically managed by discarding values below LOQ (Barnett et al., 2021).

The current study found that the volume of distribution at steady state (VD_{ss}), the area under the curve (AUC), and the drug clearance (Cl) values of ceftazidime were within the range as reported in previous studies. However, the half-life observed in this study was slightly longer than that reported in most previous research (Matsui et al., 1984; Kita et al., 1992; Sakamoto et al., 1993; Monfrinotti et al., 2010; Papich et al., 2022).

The half-life correlates proportionally to the volume of distribution and inversely to the clearance (Smith et al., 2018). In the current study, the clearance was lower than the average reported in previous studies (Matsui et al., 1984; Kita et al., 1992; Sakamoto et al., 1993; Monfrinotti et al., 2010; Papich et al., 2022), which may explain the moderately long half-life of ceftazidime (Lieberman and Murti Vemuri, 2015). According to the obtained data, the observed difference could be attributed to the marginally greater body weight of the dogs in the current study in comparison to those in earlier research (Table 4). The increment in the body weight linearly increases the volume of the distribution and extends the half-life as denoted previously in other beta-lactams such as aminopenicillins (Lashev and Pashov, 1992), and subsequently slow elimination (Zamboni et al., 2023).

The PK/PD integration results from this study align with most dosage regimens recommended by different texts (Grayson et al., 2017; Riviere and Papich, 2018; Papich, 2020). Specifically, the authors of this study suggest a dosage regimen of 20 mg /kg q12 hours of ceftazidime for susceptible *Enterobacterales* (MIC ≤ 4 µg/ml) and a dosage regimen of 20 mg /kg q8 hours of ceftazidime for susceptible *Pseudomonas aeruginosa* (MIC ≤ 8 µg/ml). This suggestion is based on the conclusion of Muller et. al. (2013) who recommend a 45 % time over MIC ratio for ceftazidime to achieve a favorable bactericidal effect against gram-negative microbes (Muller et al., 2013).

CONCLUSION

The pharmacokinetics of ceftazidime in the local Mongrel dogs used as a model in this study were comparable to the mean of those observed in Beagle dogs in similar studies, except for a slightly longer half-life. This finding may be attributed to the larger average body weight of the dogs in the current study, which could impact the recommended dosage regimen of 25 mg/kg q8 hours for ceftazidime in dogs as suggested by veterinary texts. However, the dosage

regimen suggested in the current study still requires further validation due to the small sample size used, which was limited by ethical considerations, and the lack of multiple statistical simulations.

DECLARATIONS

Funding
This study is self-funded by the authors.

Acknowledgment
The authors would like to thank the Laboratory of Pharmacology at the College of Veterinary Medicine, University of Diyala, for their invaluable assistance with sample handling and analysis.

Availability of data and materials
All data for the current study are available from the corresponding author upon reasonable request.

Authors' contributions
All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Mustafa A. Al-Jumaili, Nibras N. Al-Abbass, and Orooba M. S. Ibrahim. The first draft of the manuscript was written by Mustafa A. Al-Jumaili and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interests
The authors have no competing interests to declare.

Ethical considerations
The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors.

REFERENCES

Barnett HY, Geys H, Jacobs T, and Jaki T (2021). Methods for non-compartmental pharmacokinetic analysis with observations below the limit of quantification. Statistics in Biopharmaceutical Research, 13(1): 59-70. DOI: https://www.doi.org/10.1080/19466315.2019.1701546

Budde JA and McCluskey DM (2023). Plumb's veterinary drug handbook, 10th Edition. Wiley-Blackwell., New Jersey, pp. 219-221. Available at: https://www.wiley.com/en-sg/Plumb's+Veterinary+Drug+Handbook%2C+10th+Edition-p-9781394172207

Clinical and laboratory standards institute (CLSI) (2024). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 7th Edition. CLSI supplement VET01S. Clinical and laboratory standards institute. Available at: http://vet01s.edaptivedocs.net/GetDoc.aspx?doc=CLSI%20VET01S%20ED7:2024&xormat=SPDF&src=BB

Craig WA and Suh B (1991). Protein binding and the antimicrobial effects: Methods for the determination of protein binding. In: V. Lorian (Editor), Antibiotics in laboratory medicine, 3rd Edition. Williams and Wilkins., Baltimore MD, pp. 367-402. available at: https://www.goodreads.com/book/show/10305461-antibiotics-in-laboratory-medicine

De Sousa T, Garcês A, Silva A, Lopes R, Alegria N, Hébraud M, Igrejas G, and Poeta P (2023). The impact of the virulence of pseudomonas aeruginosa isolated from dogs. Veterinary Sciences, 10(5): 343-357. DOI: https://www.doi.org/10.3390/vetsci10050343

Fratoni AJ, Nicolau DP, and Kuti JL (2021). A guide to therapeutic drug monitoring of β-lactam antibiotics. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 41(2): 220-233. DOI: https://www.doi.org/10.1002/phar.2505

Grayson ML, Cosgrove SE, Crowe S, Hope W, McCarthy JS, Mills J, Mouton JW, and Paterson DL (2017). Kucers' the use of antibiotics: A clinical review of antibacterial, antifungal, antiparasitic, and antiviral drugs. CRC Press., Florida, pp. 615-621. available at: https://www.routledge.com/Kucers-The-Use-of-Antibiotics-A-Clinical-Review-of-Antibacterial-Antifungal-Antiparasitic-and-Antiviral-Drugs-Seventh-Edition---Three-Volume-Set/Grayson-Cosgrove-Crowe-Hope-McCarthy-Mills-Mouton-Paterson/p/book/9781498747950

Guardabassi L, Apley M, Olsen JE, Toutain PL, and Weese S (2018). Optimization of antimicrobial treatment to minimize resistance selection. In: S. Schwarz, L. M. Cavaco, and J. Shen (Editors), Antimicrobial resistance in bacteria from livestock and companion animals. ASM press., Washington DC, pp. 637-673. Available at: https://onlinelibrary.wiley.com/doi/book/10.1128/9781555819804

Kita Y, Yamazaki T, and Imada A (1992). Comparative pharmacokinetics of SCE-2787 and related antibiotics in experimental animals. Antimicrobial Agents and Chemotherapy, 36(11): 2481-2486. DOI: https://www.doi.org/10.1128/AAC.36.11.2481

Lashev LD and Pashov DA (1992). Interspecies variations in plasma half-life of ampicillin, amoxycillin, sulphadimidine and sulphacetamide related to variations in body mass. Research in Veterinary Science, 53(2): 160-164. DOI: https://www.doi.org/10.1016/0034-5288(92)90104-A

Lieberman H and Murti Vemuri N (2015). Chemical and physicochemical approaches to solve formulation problems. In: C. G. Wermuth, D. Aldous, P. Raboisson, and D. Rognan (Editors), The practice of medicinal chemistry, 4th Edition. Academic Press., California, pp. 767-791. Available at: https://www.sciencedirect.com/book/9780124172050/the-practice-of-medicinal-chemistry

Marks SL, Rankin SC, Byrne BA, and Weese JS (2011). Enteropathogenic bacteria in dogs and cats: Diagnosis, epidemiology, treatment, and control. Journal of Veterinary Internal Medicine, 25(6): 1195-1208. DOI: https://www.doi.org/10.1111/j.1939-1676.2011.00821.x

Matsui H, Komiya M, Ikeda C, and Tachibana A (1984). Comparative pharmacokinetics of YM-13115, ceftriaxone, and ceftazidime in rats, dogs, and rhesus monkeys. Antimicrobial Agents and Chemotherapy, 26(2): 204-207. DOI: https://www.doi.org/10.1128/AAC.26.2.204

Monfrinotti A, Ambros L, Prados AP, Kreil V, and Rebuelto M (2010). Pharmacokinetics of ceftazidime after intravenous, intramuscular and subcutaneous administration to dogs. Journal of Veterinary Pharmacology and Therapeutics, 33(2): 204-207. DOI: https://www.doi.org/10.1111/j.1365-2885.2009.01104.x

Muller AE, Punt N, and Mouton JW (2013). Optimal exposures of ceftazidime predict the probability of microbiological and clinical outcome in the treatment of nosocomial pneumonia. Journal of Antimicrobial Chemotherapy, 68(4): 900-906. DOI: https://www.doi.org/10.1093/jac/dks468

Overgaauw PAM, Vinke CM, Van Hagen MAE, and Lipman LJA (2020). A one health perspective on the human–companion animal relationship with emphasis on zoonotic aspects. International Journal of Environmental Research and Public Health, 17(11): 3789-3817. DOI: https://www.doi.org/10.3390/ijerph17113789

Papich MG (2020). Papich handbook of veterinary drugs, 5th Edition. Elsevier Inc., St. Louis, Missouri, pp. 156-157. Available at: https:/shop.elsevier.com/books/papich-handbook-of-veterinary-drugs/papich/978-0-323-70957-6

Papich MG, Madsen M, Messenger K, and Enomoto H (2022). Ceftazidime pharmacokinetics in dogs after intravenous injection and delivered with the RxActuator Mini-Infuser infusion pump. Journal of Veterinary Emergency and Critical Care, 32(5): 608-615. DOI: https://www.doi.org/10.1111/vec.13205

Riviere JE and Papich MG (2018). Veterinary pharmacology and therapeutics, 10th Edition. John Wiley and Sons., New Jersey, pp. 838-848. available at: https:/www.wiley.com/en-us/Veterinary+Pharmacology+and+Therapeutics%2C+10th+Edition-p-9781118855881

Rosenbaum S (2017). Basic pharmacokinetics and pharmacodynamics: An integrated textbook and computer simulations, 2nd Edition. John Wiley and Sons., New Jersey, pp. 529-537. Available at: https:/www.wiley.com/en-us/Basic+Pharmacokinetics+and+Pharmacodynamics%3A+An+Integrated+Textbook+and+Computer+Simulations%2C+2nd+Edition-p-9781119143154

Sabath LD (1976). The assay of antimicrobial compounds. Human Pathology, 7(3): 287-295. DOI: https://www.doi.org/10.1016/S0046-8177(76)80039-1

Sakamoto H, Hatano K, Higashi Y, Mine Y, Nakamoto S, Tawara S, Kamimura T, Matsumoto F, and Kuwahara S (1993). Animal pharmacokinetics of FK037, a novel parenteral broad-spectrum cephalosporin. The Journal of Antibiotics, 46(1): 120-130. DOI: https://www.doi.org/10.7164/antibiotics.46.120

Smith DA, Beaumont K, Maurer TS, and Di L (2018). Relevance of half-life in drug design: Miniperspective. Journal of Medicinal Chemistry, 61(10): 4273-4282. DOI: https://www.doi.org/10.1021/acs.jmedchem.7b00969

Tibbitts J (2003). Issues related to the use of canines in toxicologic pathology-issues with pharmacokinetics and metabolism. Toxicologic Pathology, 31(1_suppl): 17-24. DOI: https://www.doi.org/10.1080/01926230390174896

Toutain PL, Ferran A, and Bousquet-Mélou A (2010). Species differences in pharmacokinetics and pharmacodynamics. In: F. Cunningham, J. Elliott, P. Lees (Editors), Comparative and Veterinary pharmacology. Handbook of Experimental Pharmacology, Springer., Berlin, Heidelberg, pp. 19-48. DOI: https://www.doi.org/10.1007/978-3-642-10324-7_2

Wayne A, McCarthy R, and Lindenmayer J (2011). Therapeutic antibiotic use patterns in dogs: Observations from a veterinary teaching hospital. Journal of Small Animal Practice, 52(6): 310-318. DOI: https://www.doi.org/10.1111/j.1748-5827.2011.01072.x

Yamaoka K, Nakagawa T, and Uno T (1978). Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. Journal of Pharmacokinetics and Biopharmaceutics, 6(2): 165-175. DOI: https://www.doi.org/10.1007/BF01117450

Zamboni WC, Charlab R, Burckart GJ, and Stewart CF (2023). Effect of obesity on the pharmacokinetics and pharmacodynamics of anticancer agents. The Journal of Clinical Pharmacology, 63(Suppl 2): S85-S102. DOI: https://www.doi.org/10.1002/jcph.2326

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 355-365.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj42
PII: S232245682400042-14

Antibody Response of Mice to the Bali Isolate of Canine Parvovirus Propagated in Madin-Darby Canine Kidney Cell Culture

I Nyoman Mantik Astawa* **and Gusti Ayu Yuniati Kencana**

Veterinary Virology Laboratory, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia

*Corresponding author's Email: mantik.astawa@unud.ac.id

ORIGINAL ARTICLE
Received: June 26, 2024
Revised: July 17, 2024
Accepted: August 19, 2024
Published: September 25, 2024

ABSTRACT

Canine parvovirus (CPV) infection is still common among dogs, leading to severe disease with high mortality. The potential of a local isolate of CPV as an effective vaccine to prevent the disease warrants investigation. This study aimed to determine the antibody response in mice against a Bali isolate of CPV propagated in the Madin-Darby Canine Kidney (MDCK) cell culture. The virus was purified using polyethylene glycol (PEG)-6000 and mixed with an Aluminum hydroxide adjuvant. Fifteen 7-week female mice were divided into three treatment groups: treatment group 1 (PEG-purified virus and Adjuvant), treatment group 2 (crude unpurified virus and adjuvant), and treatment group 3 (adjuvant without virus), with five replicates per group. The Bali isolate of CPV was successfully replicated in MDCK cells, achieving a titer of 2^{10}-2^{11} hemagglutination (HA) units after eight serial passages through the cell culture. The virus was confirmed as CPV by immunocytochemistry test using a monoclonal antibody and hemagglutination inhibition (HI) test using chicken anti-CPV polyclonal antibody. Following the first immunization, the antibody endpoint titer in mice immunized with PEG-purified CPV (5.6) was significantly higher than those immunized with crude unpurified CPV (4.2) and adjuvant without CPV (1.4). Similarly, after the second immunization, the antibody endpoint titer in mice immunized with PEG-purified CPV (7.6) also remained significantly higher than those immunized with crude unpurified CPV (6.4) and adjuvant without CPV (0.8). Significant increases in antibody endpoint titer were observed after the second immunization in mice immunized with PEG-purified CPV and crude unpurified CPV, but not in those given adjuvant without CPV. The Bali isolate of CPV propagated in MDCK cell culture induced a robust antibody response in mice, suggesting it's a potential as an alternative vaccine candidate for preventing CPV infection in dogs.

Keywords: Bali, Canine parvovirus, Madin-Darby Canine Kidney, Mice, Vaccine

INTRODUCTION

Canine parvovirus (CPV) infection in dogs is characterized by severe gastroenteritis with a high mortality rate (Voorhees et al., 2019). Persistent vomiting and diarrhea, which can lead to dehydration and damage to intestinal mucosa, are the main causes of death in infected dogs (Nandi and Kumar, 2010). Although dogs of all ages can be affected, the disease tends to be more serious in puppies compared to adult dogs. In addition, myocarditis, commonly found in young dogs, contributes to the fatality of CPV infection (Decaro et al., 2020). The case fatality rate of CPV infection can reach 91% in untreated dogs. However, if the disease is treated properly and in the early stage of the disease, its survival rates can reach 80-95% (Prittie, 2004: Ling et al., 2012). Effective biosecurity measures, such as disinfection of kennel equipment and environments, along with proper vaccination, are essential to prevent CPV infection in dogs (Mazzaferro, 2020). Despite the availability of various vaccines, cases of CPV infection remain prevalent among dogs indicating that current vaccines do not provide complete prevention (Harelas et al., 2022).

Vaccination failure appears to be the main factor contributing to the prevalence of CPV infection among dogs in Indonesia (Harelas et al., 2022). Such failure can be due to different variants of CPV field isolate with virus strain used for preparation of vaccine, high titer of maternal antibody at the time of vaccination, and the low potency of available vaccines, which fail to induce protective immunity in dogs. The presence of antigenic variants among CPV isolates has been widely reported (Alexis et al., 2021; Maganga et al., 2023). The antigenic disparity between field isolates and CPV vaccine has long been under investigation, with combo vaccines consisting of both local isolates and classical CPV strains showing improved protection rates against field CPV isolates (Mittal et al., 2014; Woolford et al., 2017). Previous studies have also demonstrated that anti-CPV antibodies neutralize the homologous type of CPV in a much higher titer than the heterologous type of the virus (Cavalli et al., 2008; Kour et al., 2023), indicating that vaccines prepared using local isolates are likely to induce better protection against local wild-type CPV.

Two main CPV types (CPV-1 and CPV-2) have been identified, each comprising several subtypes based on their genetic, antigenic, and pathogenic characteristics in dogs (Decaro and Buonavoglia, 2012). However, CPV-2 is more

To cite this paper: Astawa INM and Kencana GAY (2024). Antibody Response of Mice to Bali Isolate of Canine Parvovirus Propagated in Madin-Darby Canine Kidney Cell Culture. *World Vet. J.*, 14(3): 355-365. DOI: https://dx.doi.org/10.54203/scil.2024.wvj42

heterogenous and includes many variants, such as CPV2a, CPV2b, and CPV2c (Decaro et al., 2020; Maganga et al., 2023). Mutation in the gene encoding for its major viral protein 2 (VP2) has contributed to the presence of new CPV variants in the fields (Alexis et al., 2021). The CPV vaccines available for vaccination of dogs are generally prepared using original CPV-2. It is possible then that the vaccine is not capable of inducing a fully protective immune response against CPV infection when new CPV variants are present in the field (Decaro et al., 2020).

Exploration of novel CPV strains in Indonesia has not yet been conducted. However, several CPV variants have been identified in Asia, such as CPV-2a in China (Zhao et al., 2013) and CPV-2b in India (Nandi et al., 2010). An isolate of CPV from an infected dog in Bali was confirmed as CPV by polymerase chain reaction (PCR). This Bali isolate of CPV replicates effectively in Madin-Darby Canine Kidney (MDCK) cell culture and yields a high titer after eight serial passages. The present study was therefore conducted to evaluate this virus isolate for its potential use as a vaccine candidate to control CPV infection in dogs.

MATERIALS AND METHODS

Ethical approval

The current study was approved by the Animal Ethics Committees of the Faculty of Veterinary Medicine, Udayana University, Indonesia with the Animal Ethics Approval Certificate number: B/282/UN14.2.9/PT.01.04/20023.

Virus and cells

The virus used in the present study was isolated from a deceased Bali dog exhibiting signs of gastroenteritis. The dog was submitted for necropsy to the Veterinary Pathology Laboratory by a co-assistant student during his co-assistantship in the Veterinary Pathobiology Department (registration number: Koas/409/2021). Following necropsy, specimens, such as feces, organs, and blood were collected and sent to the Veterinary Virology Laboratory, Faculty of Veterinary Medicine, Udayana University, Indonesia for confirmatory PCR testing. The dog was recorded in the student report as a positive case of CPV infection. Organ samples, including the intestine, liver, and spleen were processed and used as inoculum for MDCK cells. The MDCK cells were obtained from Veterinary Investigation Center Regional II, Bukittinggi, West Sumatera, Indonesia.

Adaptation and propagation of canine parvovirus in Madin-Darby canine kidney cell culture

The propagation of the Bali isolate of CPV in MDCK cell culture was carried out as explained in the following steps. Organs such as the intestines, spleen, and liver were collected from the infected dog and homogenized in a mortar to prepare 20% organ suspension in serum-free cell culture media. After centrifugation at 3000 x g for 10 minutes, the supernatant fluid was collected, filtered through 0.2 µm syringe filter, and used as inoculum for MDCK cells. The MDCK cell cultures, grown in minimum essential medium (MEM) with 5% fetal bovine serum (FBS) (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) at approximately 70% confluency, were prepared in 25 cm^2 tissue culture flasks. The medium was discarded and the cells were inoculated with 1 ml of the prepared inoculum. After incubation for 90 minutes at 37^oC, the inoculum was discarded and replaced with MEM containing 3% FBS. The cells were incubated at 37^oC for 7-8 days until clear cytopathic effects were observed. The infected cells underwent three freezing and thawing steps (Parthiban et al., 2011). The cells and the medium were collected in a 15 ml centrifuge tube and centrifuged at 5000 x for 10 minutes.

Titration and identification of canine parvovirus by hemagglutination and hemagglutination inhibition test

The titer of CPV in the infected MDCK cells was determined by hemagglutination (HA) test. Following the processing of CPV-infected MDCK cells, the CPV titer was assessed using the HA test, and the virus was subsequently identified using hemagglutination inhibition (HI) test (Cavalli et al., 2008). For the titration of CPV, 50 µl supernatant fluid was subjected to serial two-fold dilution with phosphate buffered saline (PBS: pH 6.8) in 96-well V-bottomed microtitration plates. Fifty µl of 0.5% porcine red blood cells in PBS was added to each well and incubated overnight at 4^oC. Normal uninfected MDCK cell culture medium was used as a negative control. A well-characterized CPV isolate published by Suartini et al. (2014) was used as a positive control. The HA titer of CPV was expressed as the antilog of the highest virus dilution capable of completely hemagglutinating 0.5% porcine red blood cells. To identify CPV, the HI test was performed. A serial two-fold dilution of chicken anti-CPV antibody in PBS (pH 6.8) was performed in 96-well V-bottomed microtitration plates (Suartini et al., 2014). Twenty-five µl of each antibody dilution was mixed with 25 µl CPV containing 8 HA units in each well and incubated for one hour at room temperature. Fifty µl of 0.5% porcine red blood cells in PBS was then added to each well and incubated at 4^oC overnight. The virus isolate was confirmed as CPV if the antibody inhibited the hemagglutination of porcine red blood cells by the virus.

Detection of canine parvovirus replication in Madin-Darby canine kidney cells

Replication of CPV in MDCK cell culture was detected by immunocytochemistry (ICC) following a similar but modified procedure based on Astawa et al. (2018). The MDCK cells grown in 24-well plates at approximately 70% confluency were inoculated with CPV at 1 MOI (multiplicity of infection). Normal uninfected MDCK cells were used as a negative control. After three days of incubation at 37°C, the supernatant medium was removed and the cells were fixed with cold 80% acetone in PBS for 30 minutes at -20°C. The acetone was then removed and the cells were treated with 3% H_2O_2 for 20 minutes. Subsequently, 200 µl of anti-CPV monoclonal antibody (Santa Cruz, sc-57961, Texas, USA) diluted 1:100 in Da Vinci Green antibody diluent (Biocare Medical, Pacheco, USA) was added to each well. Following incubation for 1 hour at room temperature, the cells were washed twice with Phosphate-buffered Saline-Tween (PBS-T: PBS with 0.5% tween 20. pH 7.4) and 200 µl of Histofine Simple Max PO (multi) (Nichirei Biosciences INC, Tokyo, Japan) was added. Following another hour of incubation at room temperature, the cells were washed five times as described above and 200 µl of 4-chloro-1-naphthol substrate solution (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) was added. CPV-infected cells were identified by the presence of dark blue-purple precipitate, mainly in the nuclei of the infected cells (Ulyashova et al., 2011).

Preparation of virus for immunization of mice

Several flasks of MDCK cells at approximately 80% confluency were infected with CPV. Seven days after infection, the infected cells underwent four freeze-thaw cycles. The infected cell culture was then centrifuged at 5000 x g for 5 minutes. The supernatant was collected and used as a virus stock for the immunization of mice. The virus titer was then determined by the HA test and confirmed again by the HI test. For the preparation of PEG-purified CPV, the same method described below for ELISA antigens was used. The virus was inactivated with binary ethylenimine (BEI) and mixed with Alum Alhydrogel adjuvant (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) in a 50:50 ratio of virus to adjuvant.

Inactivation of virus

The virus was inactivated using binary ethylenimine (BEI) according to the methods described by Mondal et al. (2005) and Elbagory et al. (2021). The BEI solution was prepared by dissolving 0.6 M of 2-bromoethylamine hydrobromide (BEA) in 0.5 M of sodium hydroxide (NaOH) and incubation at 37°C for 1 hour to convert BEA into BEI. The resulting BEI was mixed with the virus stock at a final BEI concentration of 0.03 M. After incubation for 30 hours at room temperature, 2% sodium thiosulfate ($Na_2S_2O_3$) was added to neutralize the BEI in the virus stock.

Immunization of mice

A total of 15 female mice, aged seven weeks with an average body weight of 20 grams, were used in the present study. They were divided into three treatment groups included treatment group 1 (PEG-purified CPV + adjuvant), treatment group 2 (crude CPV + adjuvant), and treatment group 3 (adjuvant without virus), five 5 mice in each group. Each mouse was immunized twice with 0.2 ml of vaccine at two-week intervals. Approximately 200 µl blood was collected from the orbital vein 10 days after each vaccination and incubated for 2 hours at room temperature. After centrifugation at 1000 x g for 5 minutes, serum was collected in 1.5 ml Eppendorf tubes and stored at -20°C until use. The titer of anti-CPV antibody in mouse serum was determined by enzyme-linked immunosorbent assay (ELISA) test.

Titration of antibody by enzyme-linked immunosorbent assay

The antigen for the ELISA test was prepared using CPV grown in MDCK cell culture as described above. The virus was purified by using polyethylene glycol (PEG) and sodium chloride (NaCl) precipitation method (Boisvert et al., 2010). On day 8 post-infection, the cells were freeze-thawed 4 times, after which chloroform was added at a ratio of 1 ml chloroform to 9 ml infected cell stock. The mixture was vortexed and centrifuged at 10,000 x for 30 minutes. The supernatant was collected and the virus in the supernatant was precipitated by adding 7.5% PEG 6000 and 1.5 M NaCl (final concentration) for 16 hours at 4°C with shaking. The mixture was then centrifuged at 15,000 x for 45 minutes at 4°C. The pellet was diluted with 5 ml of 10 mM Tris-HCl (pH 7.5) and dialyzed overnight against the same buffer. Checkerboard titration was then conducted to determine the optimal dilution of the PEG-purified CPV for use in the ELISA test. The purified virus was then used as an antigen for the ELISA test at the dilution of 1:100 in the coating buffer.

Each well of 96-well ELISA microplates was coated with 100 ul purified parvovirus antigen diluted 1:100 in coating buffer (0.05 M Carbonate-Bicarbonate, pH 9.6) at 4°C for 18 hours. After washing twice with PBS, each well was blocked with 200 µl of 5% skim milk diluted in PBS and left at 37°C for 1 hour. Serial two-fold dilutions of mice sera, starting at 1:100, were prepared in PBS-T containing 2% skim milk (Oxoid, Hampshire, UK). Additionally, each well received 100 ul of sera samples from each dilution, followed by a 1-hour incubation at 37°C. Three normal mouse

sera samples were also prepared to calculate the cutoff value. The microplate wells were then washed three times and 100 ul of anti-mouse IgG-HRP (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) diluted 1:2000 in PBS-T containing 3% skim milk was added into each well. The microplate was incubated for one more hour at 37°C. Following five washes with PBS-T, 100 µl of TMB (3,3', 5,5'-Tetramethylbenzidine) substrate (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) was added to each well. After incubation for 15 minutes at 37°C, 50 µl of stop solution (2 N H_2SO_4) was added to each well.

The absorbance values of the substrate in microplate wells were then determined using an ELISA reader with a 450 nm filter (BioTek ELX800 Microplate reader, Cole-Palmer, Illinois City, USA). The cutoff point value was calculated by adding three standard deviations (SDs) to the average optical density (OD) value of 3 negative samples run on the ELISA (Larsen et al., 2021). This cutoff value was then used to determine the dilution endpoint antibody in each serum sample. Given that each serum sample was diluted in a serial two-fold dilution starting at 1:100, the dilution endpoint of each serum sample was determined as $1/100 \times 2^x$, where x is $\log_2$ of serum dilution. The antibody endpoint titer is defined as the reciprocal of the highest dilution of a serum that gives a reading above the cutoff value (Makarova et al., 2011). The antibody endpoint titer was determined by utilizing the dilution endpoint as 100×2^x, where x is $\log_2$ of antibody dilution. In the current study, the antibody endpoint titer was expressed as the antilog_2 of the dilution endpoint using 1:100 dilution as the starting point.

Statistical analysis

Antibody titers were analyzed using one-way ANOVA and Tukey's multiple comparisons tests to determine the significant differences among treatment groups, utilizing SPSS v.23 (Armonk, NY, USA: IBM Corp). A p-value less than 0.05 was considered statistically significant.

RESULTS

Growth characteristics of canine parvovirus in Madin-Darby canine kidney cells

The replication of CPV in MDCK cells was characterized by cytopathic effects (CPEs), observable at day 4 post-infection and becoming more evident after day 6 post-infection. Optimum CPEs were observed at day 7 post-infection, although not all MDCK cells died due to Canine parvovirus infection. The CPEs in MDCK cells induced by Canine parvovirus infection were characterized by the detachment of cells from the walls of the flasks (Figure 1). Other forms of CPE such as syncytium were not observed in MDCK cells infected with the Bali isolate of CPV. The initial titer of CPV grown in MDCK cells was 2^8 and increased gradually following several passages in MDCK cells. After passage 8, the HA titer of the virus reached 2^{11} HA units (Table 1).

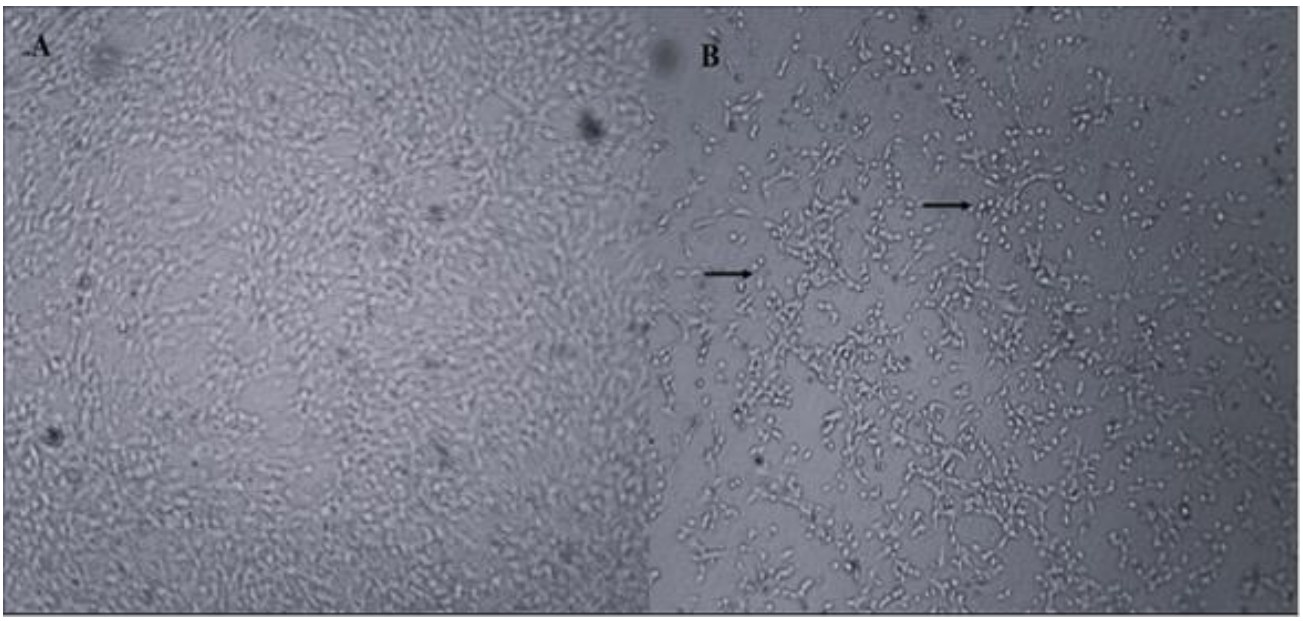


Figure 1. Cytopathic effects induced by canine parvovirus infection in Madin-Darby Canine Kidney cell culture. **A**: Normal MDCK cells, **B**: CPV-infected MDCK cells. Cytopathic effects are characterized by detaching cells from the surface flask where the cells were grown (arrow).

Table 1. Titers of the Bali isolate of canine parvovirus following passages in Madin-Darby Canine Kidney cell culture

Passage 1	Passage 2	Passage 4	Passage 6	Passage 8
2^8 HA units	2^9 HA units	2^9 HA units	$2^{9.5}$ HA units	2^{11} HA units

HA: Hemagglutination

Evidence for the replication of canine parvovirus in Madin-Darby Canine kidney cells

The replication of the CPV Bali isolate in MDCK cell culture was further confirmed by an immunocytochemistry test using an anti-CPV monoclonal antibody as a detection antibody. CPV-infected MDCK cells were visible as dark blue-purple stains, predominantly in the nuclei and, to a lesser extent, in the cytoplasm of the infected cells. In contrast, uninfected cells appeared as unstained cells in the nuclei and cytoplasm of the cells (Figure 2). Approximately 40% of cells were infected by day 3 post-infection. Further evidence for CPV replication in MDCK cells was obtained using an HI test by chicken anti-CPV antibody. As it is shown in Figure 3, the anti-CPV antibody was capable of inhibiting CPV to hemagglutinate porcine red blood cells at the antibody titer of titer 2^2 HI units (Figure 3).

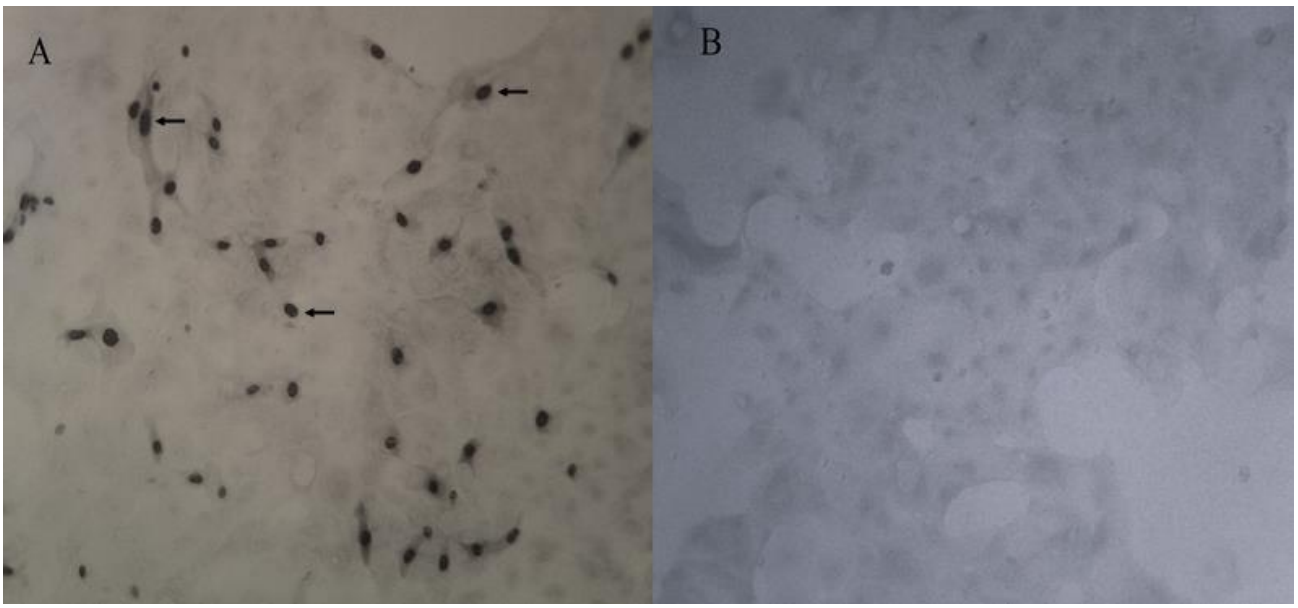


Figure 2. Madin-Darby Canine Kidney cells Infected with CPV at day 3, detected by immunocytochemistry using a monoclonal antibody against CPV. **A**: Infected cells appear bluish purple mainly in the nuclei and, to a lesser extent, in the cytoplasm (arrow), **B**: Uninfected cells.

Figure 3. Inhibition of porcine red blood cell hemagglutination by chicken anti-CPV antibody. Inhibition of hemagglutination is indicated by settling porcine red blood cells at the bottom of a V-shaped microtitration plate (arrow).

Antibody response of mice against inactivated Canine parvovirus antigen

Sera from mice were analyzed for anti-CPV antibodies following the first (primary) and the second (booster) immunizations. The average dilution endpoints of 5 serum samples after the first immunization were $1/100 \times 2^{5.6}$, $1/100 \times 2^{4.2}$, and $1/100 \times 2^{1.4}$ for PEG-purified CPV, crude unpurified CPV, and adjuvant without CPV, respectively (Figure 4).

Following the second immunization, dilution endpoints of those samples were $1/100 \times 2^{7.6}$, $1/100 \times 2^{6.2}$, and $1/100 \times 2^{0.8}$ for PEG-purified CPV, crude unpurified CPV, and adjuvant without CPV, respectively (Figure 5). The endpoint titers of anti-CPV antibody were expressed as the antilog_2 of the dilution endpoints (antilog $1/100 \times 2^{x}$). Following the first immunization, the antibody endpoint titer of the mice immunized with PEG-purified CPV (5.6) was significantly higher than those of the mice immunized with crude-unpurified CPV (4.2) and adjuvant without CPV (1.4) ($p < 0.05$). After the second immunization, the antibody endpoint titer of the mice immunized with PEG-purified CPV (7.6) remained significantly higher than those of the mice immunized with crude unpurified CPV (6.4) and adjuvant without CPV (0.8) ($p < 0.05$). A significant increase in antibody endpoint titers was observed after the second immunization compared to the first ($p < 0.05$), with notable enhancement in the mice immunized with PEG-purified CPV and crude unpurified CPV, but not in those immunized with adjuvant without CPV (Figure 6).

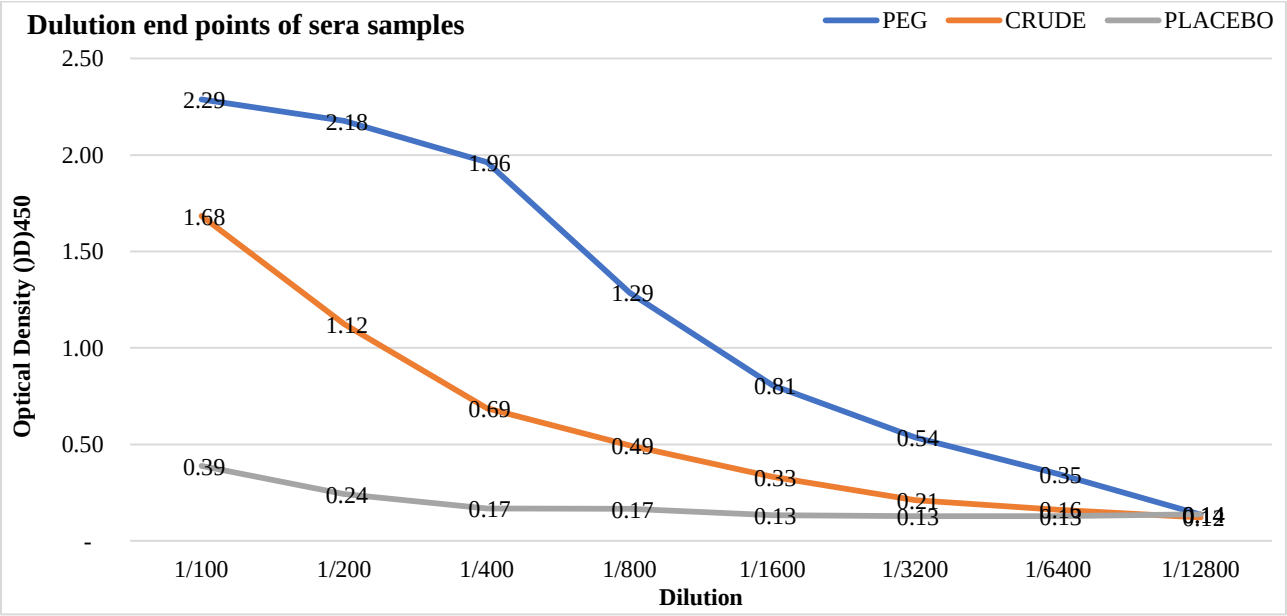


Figure 4. Dilution endpoints of mouse serum samples following the first immunization with CPV antigen determined by the ELISA test. PEG: Immunized with PEG-purified CPV antigen, CRUDE: Immunized with crude unpurified CPV-antigen, and PLACEBO: Immunized with PBS without CPV antigen

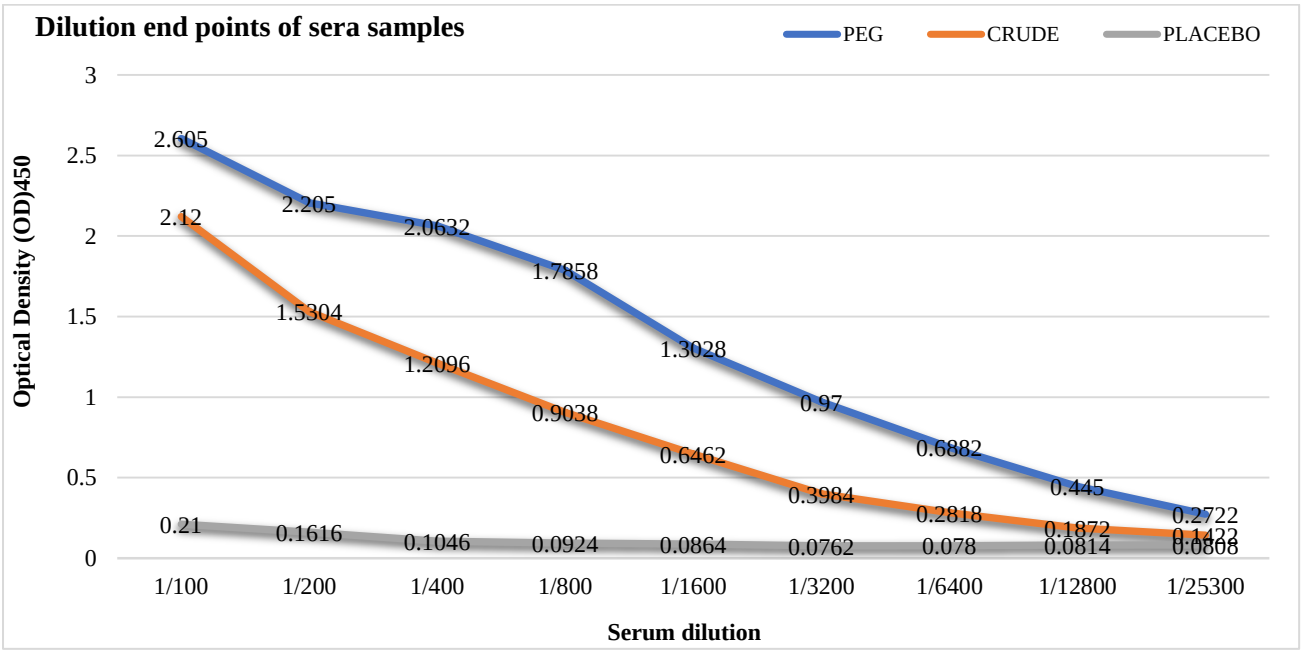


Figure 5. Dilution endpoint of mice sera samples following second immunization with CPV antigen examined by ELISA test. PEG: Immunized with PEG-purified CPV antigen, CRUDE: Immunized with crude unpurified CPV-antigen, PLACEBO: Immunized with PBS without CPV antigen.

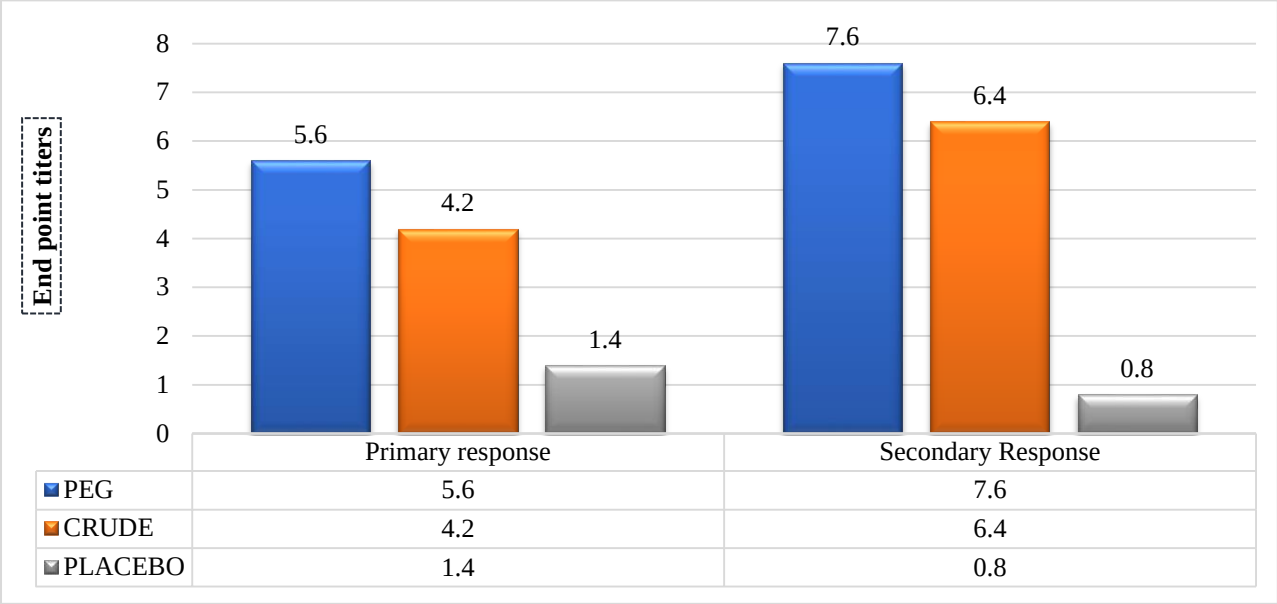


Figure 6. Antibody endpoint titers of mouse serum samples following the first and second immunizations with CPV antigens. PEG: Immunized with PEG-purified CPV antigen, CRUDE: Immunized with crude unpurified CPV-antigen, and PLACEBO: Immunized with PBS without CPV antigen

DISCUSSION

Madin-Darby Canine Kidney (MDCK) cells are epithelial mammalian cell lines derived from dog kidneys. They have been widely used in various studies including viral infections, cell stress responses, and vaccine production. These cells have undergone an immortalization process, allowing them to grow unlimitedly in culture (Capellini et al., 2020). MDCK cells were initially designed for the production of influenza virus vaccine (WHO, 1995). They have been widely used to propagate many viruses such as influenza virus (Kim et al., 2018; Wu et al., 2020; 2021) and Canine parvovirus (Kaur et al., 2015; Sharma et al., 2016).

In the present study, the Bali isolate of CPV efficiently replicated in MDCK cells, inducing clear cytopathic effects characterized by cell rounding and detachment. However, many cells did not detach from the surface of the flasks at day 7 post-infection, indicating that some cells may have developed insensitivity to CPV infection. This phenomenon is also observed in other viruses grown in MDCK cell culture. In the influenza virus, for instance, re-cloning MDCK cells from a single cell has shown to enhance their sensitivity to influenza virus infection (Zinnecker et al., 2024). Similarly, obtaining cells with heightened sensitivity to CPV infection may necessitate the re-cloning of MDCK cells from a single cell. Previous studies have demonstrated that CPV replicates in MDCK cell cultures (Kaur et al., 2015; Sharma et al., 2016). However, virus replication in MDCK cell cultures is generally less efficient compared to Crandell-Rees Feline Kidney (CrFK) cell culture (Jaune et al., 2019). Therefore, the use of MDCK cells to produce CPV vaccine has not been reported. Researches by Hyeon et al. (2023) and Dai et al. (2020) indicated that CPV is more adaptable to replicate in CrFK cells as compared to the same in MDCK cells. Consequently, when CPV is replicated in MDCK cells, the resulting titer is typically lower than that achieved in CrFK cells.

The reason is not known why CrFK cells are more sensitive to CPV infection compared to MDCK cells. Whereas MDCK cells were initially designed for the propagation and production of the influenza virus vaccine (WHO, 1995), CrFK cells were initially used for the isolation and production of the feline virus vaccine (Lappin et al., 2005). CrFK cells have been shown to be highly sensitive to CPV infection (Parthiban et al., 2011: Hyeon et al., 2023). This suggests that while MDCK cell clones are more suitable for influenza viruses as compared with CPV, CrFK clones are more so for both feline and Canine viruses.

The titer of CPV propagated in MDCK cells was assessed using the HA test. The test is a simple and widely-used method to determine the titer of CPV in fecal and tissue samples of infected dogs. However, the test is less reliable when there are high levels of non-specific hemagglutinating factors in the samples (Nandi et al., 2019). The results of present study showed that the HA titer of the Bali isolate of CPV propagated in MDCK cells was comparable to the titer of CPV propagated in CrFK cells. In the previous studies, the HA titers of CPV obtained from infected CrFK cells at passage three varied from 2^6 to 2^{11} HA units (Parthiban et al., 2011: Hyeon et al., 2023). Additionally, the results of the present study indicated that serial passages in MDCK cells increased the titer of the virus in MDCK cells. A report detailing the methods by which passages can enhance CPV titer in MDCK cells is currently unavailable. However, in the influenza

virus, serial passages can increase the titers of the influenza virus adapted to grow in MDCK cells. Generally, after a series of passages, a stable titer of the influenza virus was obtained after three passages in MDCK cells, and a high titer of the virus was obtained after passage 20 (Genzel et al., 2010). A similar phenomenon appeared in CPV adapted to grow in MDCK cells and a higher titer of the Bali isolate of CPV might be obtained after further passages.

Evidence supporting the replication of the Bali CPV isolates in MDCK cells was shown by the immunocytochemistry test. This test, which detects the viral antigen in cells, has widely been used for the detection of virus replication in infected cell cultures. The test is simple and very useful to detect the virus in cell cultures when CPEs are not present, as seen with rabies virus replication in BHK-21 cells (Astawa et al., 2018). The results of the present study showed that CPV replication was characterized by the presence of viral antigens predominantly in the nuclei and, to a lesser extent, in the cytoplasm of infected cells (Figure 3). It was clear that by day three, some 40% of cells seemed to have been infected. Parvovirus is a small DNA virus that replicates in nuclei of infected cells and the presence of CPV antigen in the nuclei of MDCK cells provides further evidence for viral replication in the cells (Mattola et al., 2022). However, the process of virus replication and the synthesis of viral proteins takes place in the cytoplasm of cells (Mäntylä et al., 2020), matching with the ICC test that the CPV protein was also detected in the cytoplasm of the infected cells.

The replication of the Bali isolate of CPV in MDCK cells was also confirmed by the HI test in which chicken anti-CPV IgY could inhibit the virus to hemagglutinate porcine red blood cells (Figure 5). The HI test is commonly used to detect anti-CPV antibodies in the serum of vaccinated dogs (Cavalli et al., 2020). and can also be employed to detect the presence of CPV in the suspected samples, such as feces and tissues from infected dogs (Nandi and Kumar, 2010). It is important to note that the HA test may be affected by non-specific hemagglutinating factors, particularly when using serum as the antibody source, which can interfere with test results (Cavalli et al., 2021).

Active attenuated CPV vaccines are generally considered more effective and efficient in inducing a protective immune response in dogs. However, inactivated CPV vaccines still remain in use and BEI is a viral inactivating agent commonly used for the preparation of inactive vaccines (Hasan et al., 2017; Elbagory et al., 2021). The use of BEI-inactivated CPV for preparation of vaccine has been reported in previous studies. Previous studies have reported that BEI-inactivated CPV can induce antibody response in puppies (Elbagory et al., 2021). Similarly, it was found in this study that the BEI-inactivated Bali isolate of CPV induces antibody response in mice. Nevertheless, there is a need to further boost the virus concentration to improve the immune response. The current study clearly indicated that the virus concentration was elevated by PEG-precipitation of CPV, leading to a higher antibody titer in mice. Instances of using PEG as a precipitating agent for concentrating viruses such as infectious bursal disease virus (Leong et al., 2023), and SARS-CoV-2 have been widely reported (Lucansky et al., 2023).

Vaccines, whether active attenuated or inactivated, are available to prevent CPV infection in dogs (Hedgespeth and Fogle, 2021). The present study evaluated a local CPV isolate inactivated with BEI as a potential vaccine candidate. Since a local and virulent virus provides a vaccine with better antigenic homology to the field virus, utilizing the inactivated virus as a vaccine is preferable. For example, during the foot-and-mouth disease outbreaks in Pakistan, the vaccine to control the disease was prepared using inactivated local isolate propagated in adherent BHK-21 cell culture (Razak et al., 2023). The ability of local CPV isolate to induce immune response in mice indicates that this isolate holds promise as a candidate for developing a vaccine to control CPV infection in dogs.

CONCLUSION

The Bali isolate of CPV was able to grow in MDCK cell culture reaching the HA titer of 2^{11} HA units after 8 passages. The virus was confirmed as Canine parvovirus both by immunocytochemistry test using monoclonal antibody against CPV and by hemagglutination inhibition test using chicken anti-CPV antibody. The Bali isolate of CPV induced antibody response in mice with the highest response observed using PEG-purified CPV antigen compared to crude-unpurified CPV antigen. However, as this is an initial stage of vaccine development, further research is necessary to explore its potential for use as a vaccine, possibly using dogs as experimental animals instead of mice. In addition, it appeared that further passages of the virus in MDCK cells are still required to increase the titer of the virus obtained from infected MDCK cell cultures.

DECLARATIONS

Acknowledgments

The authors would like to extend their sincere gratitude and appreciation to co-assistant students for conducting a necropsy and providing us with organ samples of CPV-infected dogs which have enabled us to undertake this study.

Funding

This study was funded by the Institute of Research and Community Service, Udayana University (Hibah Unggulan Udayana Scheme) under grant number B/1.486/UN14.4. A/PT.01.03/2023.

Authors' contributions

I Nyoman Mantik Astawa and Gusti Ayu Yuniati Kencana conceived and designed the experiment, collection, and data analysis. Both authors read and approved the final draft of the manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors.

Availability of data and materials

All data of this study are available upon reasonable requests from authors

REFERENCES

Alexis VA, Sonia V, Daniela S, Miguel G, Timothy H, Valentina F, Lisette L, and Leonardo S (2021). Molecular analysis of full-length VP2 of Canine parvovirus reveals antigenic drift in CPV-2b and CPV-2c variants in Central Chile. Animals, 11(8): 2387. DOI: https://www.doi.org/10.3390/ani11082387

Astawa INM, Agustini NLP, Masa Tenaya IW, and Aryawiguna IPGW (2018). Protective antibody response of Balb/c mice to Bali rabies virus isolate propagated in BHK-21 cells. Journal of Veterinary Medical Science, 80(10): 1596-1603. DOI: https://www.doi.org/10.1292/jvms.17-0385

Boisvert M, Fernandes S, and Tijssen P (2010). Multiple pathways involved in porcine parvovirus cellular entry and trafficking toward the nucleus. Journal of Virology, 84(15): 7782-7792. DOI: https://www.doi.org/10.1128/JVI.00479-10

Capellini FM, Vencia W, Amadori M, Mignone G, Parisi E, Masiello L, Vivaldi B, Ferrari A, and Razzuoli E (2020). Characterization of MDCK cells and evaluation of their ability to respond to infectious and non-infectious stressors. Cytotechnology, 72(1): 97-109. DOI: https://www.doi.org/10.1007/s10616-019-00360-z

Cavalli A, Martella V, Desario C, Camero M, Bellacicco AL, De Palo P, Decaro N, Elia G, and Buonavoglia C (2008). Evaluation of the antigenic relationships among Canine parvovirus type 2 variants. Clinical and Vaccine Immunology, 15(1): 534-539. DOI: https://www.doi.org/10.1128/CVI.00444-07

Cavalli A, Desario C, Marinaro M, Losurdo M, Camero M, Decaro N, Catella C, Lanave G, and Buonavoglia C (2020). Oral administration of modified live Canine parvovirus type 2b induces systemic immune response. Vaccine, 38(2): 115-118. DOI: https://www.doi.org/10.1016/j.vaccine.2019.10.016

Cavalli A, Martella V, Desario C, Camero M, Lanave G, Barrs VR, Decaro N, and Buonavoglia C (2021). Modified haemagglutination inhibition assay for the detection of Canine parvovirus type 2 antibodies in dog sera. The Veterinary Journal, 274: 105709. DOI: https://www.doi.org/10.1016/j.tvjl.2021.105709

Dai X, Zhang X, Miao Y, Han P, and Zhang J (2020). Canine parvovirus induces G1/S cell cycle arrest that involves EGFR Tyr1086 phosphorylation. Virulence, 11(1): 1203-1214. DOI: https://www.doi.org/10.1080/21505594.2020.1814091

Decaro N and Buonavoglia C (2012). Canine parvovirus-A review of epidemiological and diagnostic aspects, with emphasis on type 2c. Veterinary Microbiology, 155(1): 1-12. DOI: https://www.doi.org/10.1016/j.vetmic.2011.09.007

Decaro N, Buonavoglia C, and Barrs VR (2020). Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication?. Veterinary Microbiology, 247: 108760. DOI: https://www.doi.org/10.1016/j.vetmic.2020.108760

Elbagory GF, Elkhatib S, and Khodier MH (2021). Efficacy of live attenuated and inactivated bivalent vaccine against Canine distemper and Canine parvoviruses in dogs. Benha Veterinary Medical Journal, 40(2): 168-173. DOI: https://www.doi.org/10.21608/bvmj.2021.68131.1377

Genzel Y, Dietzsch C, Rapp E, Schwarzer J, and Reichl U (2010). MDCK and Vero cells for influenza virus vaccine production: A one-to-one comparison up to lab-scale bioreactor cultivation. Applied Microbiology and Biotechnology, 88(2): 461-475. DOI: https://www.doi.org/10.1007/s00253-010-2742-9

Harelas GC, Arimbi, Hamid IS, Praja RN, and Yunita MN (2022). Faktor risiko parvovirus pada anjing di de' lab pet clinic, Bekasi [Risk factors of parvovirus in dogs at De' Lab Pet Clinic, Bekasi]. Jurnal Medik Veteriner, 5(2): 170-177. DOI: https://www.doi.org/10.20473/jmv.vol5.iss2.2022.170-177

Hasan K, Rathnamma, D, Narayanaswamy HD, Malathi V, Tomar N, Gupta S, and Singh SV (2017). Current scenario and future perspectives of CPV-2 vaccines in India. Advances in Animal and Veterinary Sciences, 5: 446-448. DOI: http://www.doi.org/10.17582/journal.aavs/2017/5.11.446.448

Hedgespeth BA and Fogle JE (2021). Canine parvovirus vaccination. Vaccination station, North Carolina State University College of Veterinary Medicine, USA, pp. 1-5. Available at: https://todaysveterinarypractice.com/wp-content/uploads/sites/4/2021/02/TVP-2021-0304_Canine_Parvovirus.pdf

Hyeon L, Yang D, Kim E, Park Y, Lee HJ, and Hyun B (2023). Isolation and biomolecular analysis of Canine parvovirus 2a from Korean dogs. Journal of Bacteriology and Virology, 53(1): 43-50. DOI: https://www.doi.org/10.4167/jbv.2023.53.1.043

Jaune FW, Taques IIGG, Dos Santos Costa J, Araújo JP Jr, Catroxo MHB, Nakazato L, and de Aguiar DM (2019). Isolation and genome characterization of Canine parvovirus type 2c in Brazil. Brazilian Journal of Microbiology, 50(1): 329-333. DOI: https://www.doi.org/10.1007/s42770-018-0036-z

Kaur G, Chandra M, Dwivedi PN, and Sharma NS (2015). Isolation of Canine parvovirus with a view to identify the prevalent serotype on the basis of partial sequence analysis. Veterinary World, 8(1): 52-56. DOI: https://www.doi.org/10.14202/vetworld.2015.52-56

Kim EH, Kwon HI, Park SJ, Kim YI, Si YJ, Lee IW, Kim SM, Kim SI, Ahn DH, and Choi YK (2018). Generation of a high-growth influenza vaccine strain in MDCK cells for vaccine preparedness. Journal of Microbiology and Biotechnology, 28(6): 997-1006. DOI: https://www.doi.org/10.4014/jmb.1712.12007

Kour H, Kaur G, and Chandra M (2023). Antigenic relationship of Canine parvovirus type 2c with other variants and vaccine strain using *in-vitro* cross neutralization assay. Indian Journal of Veterinary Sciences and Biotechnology, 19(4): 20-23. DOI: https://www.doi.org/10.48165/ijvsbt.19.4.04

Lappin MR, Jensen WA, Jensen TD, Basaraba RJ, Brown CA, Radecki SV, and Hawley JR (2005). Investigation of the induction of antibodies against Crandell-Rees feline kidney cell lysates and feline renal cell lysates after parenteral administration of vaccines against feline viral rhinotracheitis, calicivirus, and panleukopenia in cats. American Journal of Veterinary Research, 66: 506-511. DOI: https://www.doi.org/10.2460/ajvr.2005.66.506

Larsen SE, Berube BJ, Pecor T, Cross E, Brown BP, Williams B, Johnson E, Qu P, Carter L, Wrenn S et al. (2021). Qualification of ELISA and neutralization methodologies to measure SARS-CoV-2 humoral immunity using human clinical samples. Journal of Immunological Methods, 499: 113160. DOI: https://www.doi.org/10.1016/j.jim.2021.113160

Leong HY, Fu X, Liu X, Yao S, and Lin D (2023). Characterisation and separation of infectious bursal disease virus-like particles using aqueous two-phase systems. Chinese Journal of Chemical Engineering, 57: 72-78. DOI: https://www.doi.org/10.1016/j.cjche.2022.08.019

Ling M, Norris JM, Kelman M, and Ward MP (2012). Risk factors for death from Canine parvoviral-related disease in Australia. Veterinary Microbiology, 158(3-4): 280-290. DOI: https://www.doi.org/10.1016/j.vetmic.2012.02.034

Lucansky V, Samec M, Burjanivova T, Lukacova E, Kolkova Z, Holubekova V, Turyova E, Hornakova A, Zaborsky T, Podlesniy P et al. (2023). Comparison of the methods for isolation and detection of SARS-CoV-2 RNA in municipal wastewater. Frontiers in Public Health, 11: 1116636. DOI: https://www.doi.org/10.3389/fpubh.2023.1116636

Maganga GD, Labouba I, Milendz Ikapi SZ, Nkili-Meyong AA, Ngonga Dikongo AM, Boundenga L, Ngoubangoye B, Memvie C, and Kumulungui BS (2023). Molecular characterization of Canine parvovirus variants CPV-2a and CPV-2c, associated with vaccinated dogs at Libreville, Gabon. Viruses, 5(5): 1169. DOI: https://www.doi.org/10.3390/v15051169

Makarova N, Zhao C, Zhang Y, Bhosle S, Suppiah S, Rhea JM, Kozyr N, Arnold RS, Ly H, Molinaro RJ et al. (2011) Antibody responses against xenotropic murine leukemia virus-related virus envelope in a murine model. PLoS One, 6(4): e18272. DOI: https://www.doi.org/10.1371/journal.pone.0018272

Mäntylä E, Aho V, Kann M, and Vihinen-Ranta M (2020). Cytoplasmic parvovirus capsids recruit importin beta for nuclear delivery. Journal of Virology, 94(4): e01532-19. DOI: https://www.doi.org/10.1128/jvi.01532-19

Mattola S, Aho V, Bustamante-Jaramillo LF, Pizzioli E, Kann M, and Vihinen-Ranta M (2022). Nuclear entry and egress of parvoviruses. Molecular Microbiology, 118(4): 295-308. DOI: https://www.doi.org/10.1111/mmi.14974

Mazzaferro EM (2020). Update on Canine parvoviral enteritis. Veterinary Clinics of North America: Small Animal Practice, 50(6): 1307-1325. DOI: https://www.doi.org/10.1111/mmi.14974

Mittal M, Chakravarti S, Mohapatra JK, Chug PK, Dubey R, Upmanuyu V, Narwal PS, Kumar A, Churamani CP, and Kanwar NS (2014). Molecular typing of Canine parvovirus strains circulating from 2008 to 2012 in an organized kennel in India reveals the possibility of vaccination failure. Infection, Genetic and Evolution, 23: 1-6. DOI: https://www.doi.org/10.1016/j.meegid.2014.01.015

Mondal SK, Neelima M, Seetha Rama Reddy K, Ananda Rao K, and Srinivasan VA (2005). Validation of the inactivant binary ethylenimine for inactivating rabies virus for veterinary rabies vaccine production. Biologicals, 33(3): 185-189. DOI: https://www.doi.org/10.1016/j.biologicals.2005.05.003

Nandi S, Chidri S, Kumar M, and Chauhan RS (2010). Occurrence of Canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. Research in Veterinary Science, 88: 169-171. DOI: https://www.doi.org/10.1016/j.rvsc.2009.05.018

Nandi S and Kumar M (2010). Canine parvovirus: Current perspective. Indian Journal of Virology, 21(1): 31-44. DOI: https://www.doi.org/10.1007/s13337-010-0007-y

Nandi S, Sharma GK, Gupta V, Deol P, Chander V, UK De UK, and Gupta VK (2019). Global scenario of Canine parvovirus mutants: Epidemiology, diagnostics and immunoprophylactic agents. JSM Veterinary Medical Research, 1: 12. Available at: https://www.jsmcentral.org/VeterinaryMedicine/jsmvmr560327.pdf

Parthiban S, Mukhopadhyay HK, Panneer D, Antony PX, and Pillai RM (2011). Isolation and typing of Canine parvovirus in CRFK cell line in Puducherry, South India. Indian Journal of Microbiology, 51(4): 456-460. DOI: https://www.doi.org/10.1007/s12088-011-0105-6

Prittie J (2004). Canine parvoviral enteritis: A review of diagnosis, management, and prevention. Journal of Veterinary Emergency and Critical Care, 13: 167. DOI: https://www.doi.org/10.2147/VMRR.S80971

Razak A, Altaf I, Ahmad Anjum A, and Raza Awan A (2023). Preparation of purified vaccine from local isolate of foot and mouth disease virus and its immune response in bovine calves. Saudi Journal of Biological Sciences, 30(7): 103709. DOI: https://www.doi.org/10.1016/j.sjbs.2023.103709

Sharma S, Dhar P, Thakur A, Sharma V, and Sharma M (2016). First detection of Canine parvovirus type 2b from diarrheic dogs in Himachal Pradesh. Veterinary World, 9(9): 964-969. DOI: http://www.doi.org/10.14202/vetworld.2016.964-969

Suartini GA, Suprayogi A, Wibawan WT, Sendow I, and Mahardika GN (2014). Intravenous administration of chicken immunoglobulin has a curative effect in experimental infection of Canine parvovirus. Global Veterinaria, 13: 801-808. Available at: https://erepo.unud.ac.id/id/eprint/5714

Ulyashova MM, Rubtsova MY, and Egorov AM (2011). Colorimetric detection of immobilized horseradish peroxidase based on the co-oxidation of benzidine derivatives and 4-chloro-1-naphthol. Russian Chemical Bulletin, 60: 656-661. DOI: https://www.doi.org/10.1007/s11172-011-0101-3

Voorhees IEH, Lee H, Allison AB, Lopez-Astacio R, Goodman LB, Oyesola OO, Omobowale O, Fagbohun O, Dubovi EJ, Hafenstein SL et al. (2019). Limited intrahost diversity and background evolution accompany 40 years of Canine parvovirus host adaptation and spread. Journal of Virology, 94(1): e01162. DOI: https://www.doi.org/10.1128/jvi.01162-19

Woolford L, Crocker P, Bobrowski H, Baker T, and Hemmatzadeh F (2017). Detection of the Canine parvovirus 2c subtype in Australian dogs. Viral Immunology, 30(5): 371-376. DOI: https://www.doi.org/10.1089/vim.2017.0019

World health organization (WHO) (1995). Cell culture as a substrate for the production of influenza vaccines: Memorandum from a WHO meeting. Bulletin of the World Health Organization, 73(4): 431-435. Available at: https://iris.who.int/handle/10665/264114

Wu Y, Bissinger T, Genzel Y, Liu X, Reichl U, and Tan WS (2021). High cell density perfusion process for high yield of influenza A virus production using MDCK suspension cells. Applied Microbiology and Biotechnology, 105(4): 1421-1434. DOI: https://www.doi.org/10.1007/s00253-020-11050-8

Wu Y, Jia H, Lai H, Liu X, and Tan W (2020). Highly efficient production of an influenza H9N2 vaccine using MDCK suspension cells. Bioresources and Bioprocessing, 7: 63. DOI: https://www.doi.org/10.21203/rs.3.rs-58786/v2

Zhao Y, Lin Y, Zeng X, Lu C, and Hou J (2013). Genotyping and pathobiologic characterization of Canine parvovirus circulating in Nanjing, China. Virology Journal, 10: 272. DOI: https://www.doi.org/10.1186/1743-422X-10-272

Zinnecker T, Badri N, Araujo D, Thiele K, Reichl U, and Genzel Y (2024). From single-cell cloning to high-yield influenza virus production-implementing advanced technologies in vaccine process development. Engineering in Life Sciences, 24(4): 2300245. DOI: https://www.doi.org/10.1002/elsc.202300245

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 366-372.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj43
PII: S232245682400043-14

Effects of Two Types of Estrogen on the Follicular Wave for *in Vivo* Oocyte Collection in Brown Swiss Cows

Ide Unchupaico Payano[1], Carlos Quispe Eulogio[2], Edith Ancco Gómez[2], Jordan Ninahuanca Carhuas[1,2]*, Fernando Arauco Villar[1], Jorge Unchupaico Fermín[1], and Noemí Mayorga Sánchez[1]

[1]*Departamento Académico de Zootecnia, Universidad Nacional del Centro del Perú. Av. Mariscal Castilla N° 3909 – El Tambo, Huancayo, Junín, Perú*
[2]*Escuela Profesional de Medicina Veterinaria y Zootecnia, Universidad Peruana Los Andes. Av. Mártires del periodismo cuadra 20 (Ex Calmell del Solar) Chorrillos – Huancayo, Junín, Perú*

*Corresponding author's Email: jninahuanca@uncp.edu.pe

ORIGINAL ARTICLE
Received: July 20, 2024
Revised: August 16, 2024
Accepted: September 01, 2024
Published: September 25, 2024

ABSTRACT

The manipulation of follicular waves through hormonal treatments, such as estrogen administration, plays a crucial role in optimizing *in-vivo* oocyte collection for assisted reproductive technologies. The present study aimed to evaluate the effect of two specific types of estrogen on follicular wave dynamics and their impact on *in-vivo* oocyte collection in Brown Swiss cows. Fourteen cows, in their first lactation, weighing approximately 340 kg were randomly assigned to one of two treatments including T1 (estradiol cypionate) and T2 (estradiol benzoate). Both treatments were administered at 1.2 mg of estrogen, at day 0 of the experiment. All Brown Swiss cows were provided with a diet entirely consisting of alfalfa grazing. On day 7, follicular wave dynamics were assessed using a DP-50 vet ultrasound device equipped with a 7.5 MHz transducer for transvaginal follicular aspiration guidance. Follicle counts were categorized into three size ranges including 2-4 mm (small), 4-8 mm (medium), and greater than 8 mm (large). Additionally, the quantity and quality (viable oocytes) of the collected oocytes were evaluated by the Ovum Pick Up (OPU) team for oocyte viability on day 7. The study assessed the follicular dynamics (number of follicles) and efficiency of oocyte collection (viable oocytes) in cows treated with Estradiol Cypionate (T1) and Estradiol Benzoate (T2). The average number of small, medium-sized, and large follicles size were 6.048 ± 6.037, 3.16 ± 2.01, and 0.53 ± 0.67 respectively. The total number of follicles was 9.59 ± 3.56. The mean number of viable oocytes recovered was 3.024 ± 1.66, while the mean number of non-viable oocytes was 1.47 ± 1.01. The results indicated no significant differences between treatments in the size of small, medium, and large follicles, nor in the total number of follicles and viable oocytes recovered. However, a significant difference was observed in the number of non-viable oocytes recovered, with a higher mean in T2 (1.86) compared to T1 (1.09). The results indicated an adequate follicular response and viable oocyte recovery in both treatment groups (estradiol cypionate and estradiol benzoate). However, variations in oocyte viability were observed, with estradiol cypionate showing a slight advantage.

Keywords: Follicle count, Follicular wave dynamic, Oocyte collection, Transvaginal follicular aspiration

INTRODUCTION

The efficient and sustainable production of cattle is a key objective in the global agricultural industry. In the central region of Peru, dairy farming primarily revolves around the efforts of local producers, who are at the heart of this practice (Estremadoyro et al., 2024). Within this framework, reproductive biotechnology emerges as an essential tool to enhance herds' productivity and genetic quality (Dahlen et al., 2014). *In-vivo* oocyte collection followed by *in-vitro* fertilization has facilitated significant advancements in assisted reproduction, optimizing the selection and proliferation of desirable genetic traits (Lonergan and Fair, 2014). Specifically, the manipulation of the follicular wave through estrogen administration, including the use of different types of estradiol such as Estradiol Cypionate and Estradiol Benzoate, has become a common practice to maximize the efficiency of oocyte collection. These types of estradiol differ in their effects on follicular development, with variations in dosage, timing, and duration influencing follicle size progression, oocyte maturation, and overall follicular dynamics (Lima et al., 2011). Additionally, cloning has made significant strides, allowing the creation of uniform herds with high productivity and disease resistance (Gray et al., 2020). Genomic selection has revolutionized genetic improvement in livestock by enabling the identification and selection of animals with superior genetic traits from an early age (Gutierrez-Reinoso et al., 2021). Genetic editing, using technologies, such as CRISPR-Cas9, allows precise modifications in animal DNA to enhance specific traits, including increased muscle growth, improved milk production, and enhanced fertility. It also targets disease resistance, particularly against common bovine ailments like mastitis, bovine respiratory disease (BRD), and foot-and-mouth disease, and improves feed efficiency by optimizing nutrient absorption and reducing methane emissions (Perisse et al., 2021).

To cite this paper: Payano IU, Eulogio CQ, Gómez EA, Carhuas JN, Villar FA, Fermín JU, and Sánchez NM (2024). Effects of Two Types of Estrogen on the Follicular Wave for *in Vivo* Oocyte Collection in Brown Swiss Cows. *World Vet. J.,* 14(3): 366-372. DOI: https://dx.doi.org/10.54203/scil.2024.wvj43

Brown Swiss cows, known for their high milk production and adaptability to various environmental conditions, represent an excellent candidate for studies in reproductive biotechnology (Baykan and Özcan, 2017). However, the physiological response of these cows to different types of estrogens concerning follicular dynamics and *in-vivo* oocyte collection efficiency is not yet fully understood (Mikkola et al., 2020). Understanding these aspects will not only provide valuable insights into best practices for estrogen administration to improve reproductive efficiency but also contribute to the development of more effective and sustainable reproductive management strategies in bovine livestock (Boneya, 2021).

Estrogen plays a crucial role in oocyte collection by influencing follicular wave dynamics, Estradiol Cypionate, with its longer half-life, provides a sustained release of estrogen, which leads to more prolonged and controlled synchronization of follicular waves (Abdulghani, 2022). Estrogen administration improves synchronization and stimulates follicular development in the ovary, promoting the selection, and growth of dominant follicles and thereby increasing the number of mature oocytes available for collection. Additionally, estrogen can enhance oocyte quality and optimize the hormonal environment, which is essential for efficient and effective *in-vivo* oocyte collection. Therefore, the present study aimed to evaluate the effect of two specific types of estrogen on follicular wave dynamics and their impact on *in-vivo* oocyte collection in Brown Swiss cows.

MATERIALS AND METHODS

Ethical approval

The Animal Ethics Committee of the Dirección Regional de Agricultura Junín, Perú (LETTER N° 008-GRJ-DRA-AAC-PERÚ-2023) approved all animal handling procedures employed in this study.

Study area

The study was carried out at the "Estación Experimental el Mantaro", Mantaro, Jauja, Junin of Peru (Figure 1). Positioned at an elevation of 3,320 meters above sea level, the station experiences average temperatures between 4°C and 8°C and receives an annual rainfall of approximately 749 mm (Senamhi, 2023). Due to its strategic placement in the Junín region, this station is pivotal for research, offering an in-depth understanding of follicular dynamics in local cattle populations.

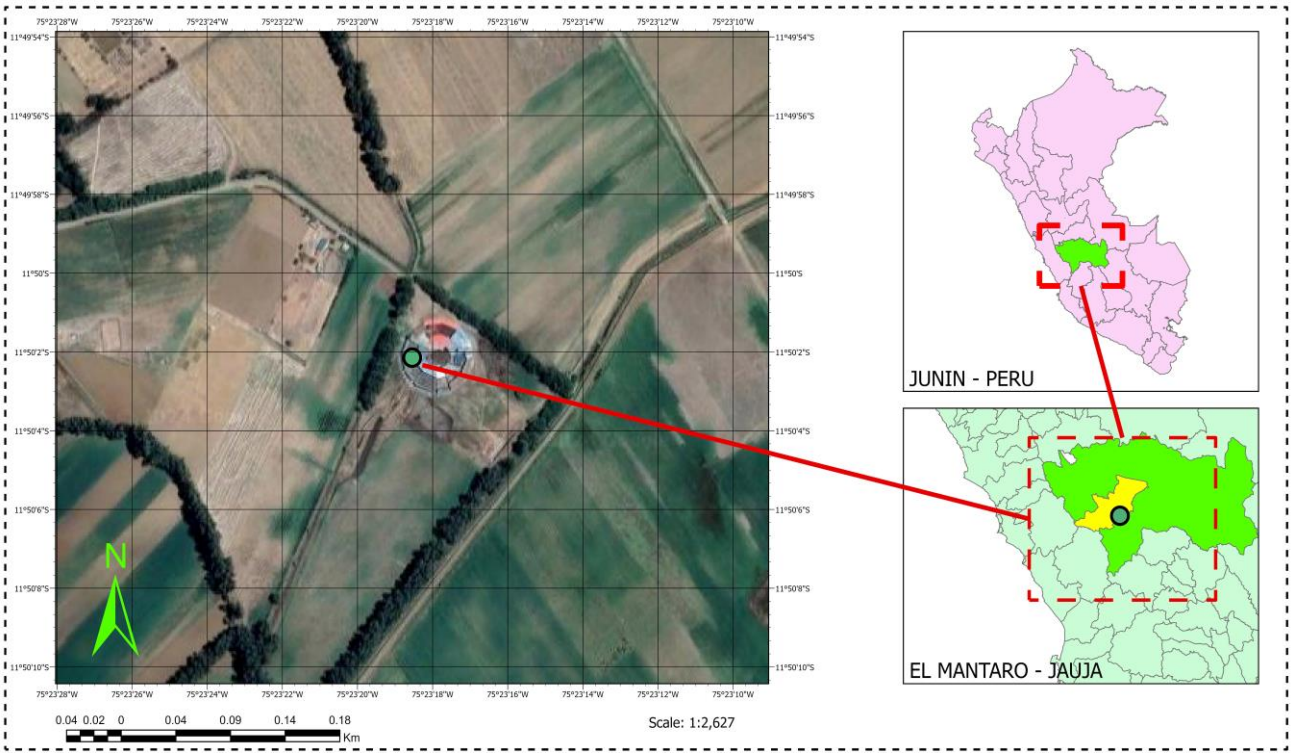


Figure 1. The location of study in country of Peru. Junín region (green color), district of El Mantaro (yellow), place of execution

Animal and distribution

A total of 14 cows (7 for each group) of an approximate weight of 350 kg and a body condition of 3 (Figure 2a), in the first third of lactation, were selected and randomly distributed among the treatments (T1: estradiol cypionate and T2 : estradiol benzoate). All of the Brown Swiss breeds were fed 100% alfalfa grazing.

Data collection

Follicular wave

For both experimental groups, an intravaginal DIB (Bovine intravaginal device), was purchased from Argentina (Figure 2b). The first group (T1) received 1.2 mg of Estradiol Benzoate (Estrovet, Montana, Peru) and 0.524 mg of Cloprostenol Sodium, was implemented on day "0", intramuscular route. The second group (T2) received 1.2 mg of Estradiol Cypionate and 0.524 mg of Cloprostenol Sodium (Figure 2c), which was implemented on day "0", one month after calving. In both groups, intravaginal ultrasonography was performed on days 0 and 7 to evaluate follicular dynamics and follicle growth. For imaging, a DP-50 vet ultrasound of Peru device equipped with a 7.5 MHz transducer for transvaginal follicular aspiration guidance (WTA) was used (Figure 2d). Follicle counts were categorized into three size ranges including 2-4 mm (small), 4-8 mm (medium), and greater than 8 mm (large, Figure 2e) (Haadsma et al., 2007). Additionally, the quantity and quality of the collected oocytes were evaluated (Figure 2f).

Oocyte collection by ovum pick-up

Epidural anesthesia with Xylazine (0.05 mg/kg, LIDOCAINA OVER, PERU) was administered to facilitate the manipulation of the ovaries (Lima et al., 2011). Subsequently, the rectum was manually emptied, followed by the cleaning and disinfection of the vulva and perineal area (Simões et al., 2021). A transducer was introduced into the vagina using a sanitary latex cover. The Ovum Pick-Up (OPU) handle was held in the right hand. Visualization was achieved using an ultrasound machine fitted with a transvaginal probe and a 60 cm OPU handle (Figure 2e). A disposable puncture needle (18 G, 0.9 x 70 mm) attached to a sterile 50 ml collection tube was then inserted through the guide. The OPU setup was completed with a foot-pedal-operated vacuum pump, applying a constant aspiration of 75 mm Hg. To observe the follicle sizes, the ovaries were positioned rectally in front of the probe. Before starting the puncture session, the system was flushed by aspirating a small amount of collection medium. After aspirating 3-4 follicles, the follicular fluid in the aspiration (Figure 2e) needle and collection system was thoroughly washed with washing and collection medium (PBS supplemented with sodium heparin, 2.2 IU/ml, and fetal bovine serum, 1%) (Landeo et al., 2022).

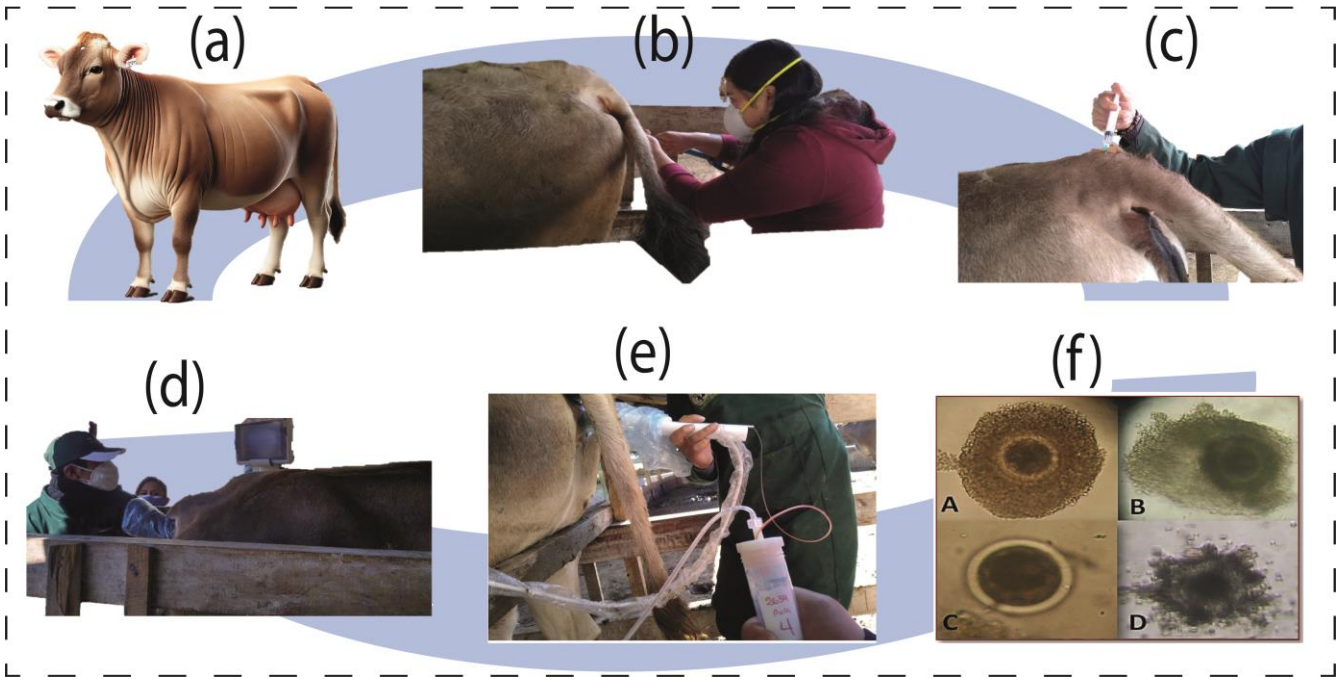


Figure 2. Oocyte collection procedure for Brown Swiss cows. Sequence of steps; **a**: Swiss brown cattle, **b**: Application of the synchronization protocol (estradiol cypionate and estradiol benzoate) to the study cows Brown Swiss, **c**: Application of anesthetic via epidural to the cow, **d**: Oocyte collection using the Ovum Pick Up technique, **e**: Oocyte aspiration guide and oocyte collection tube, **f**: Oocyte qualities evaluation (viable oocytes)

Oocyte search and sorting

After collection, the conical tubes were transported to the laboratory, maintaining the appropriate temperature throughout (20 °). The process began using a 100 µm diameter filter to separate the oocytes and cumulus cells from other cellular debris and blood. The filtered liquid was transferred to a stereoscopic microscope (LSM-B10 Labtron, Peru) at 20X magnification to visualize the cumulus-oocyte complexes (COCs). The COCs were observed at 40X magnification and evaluated based on their morphology to classify them into four categories including A (surrounded by ≥ 3 layers of

cumulus cells with homogeneous cytoplasm), B (oocytes partially surrounded by cumulus cells and irregular cytoplasm), C (denuded oocytes), and D (oocytes surrounded by fibrin). Oocytes of grades A and B were defined as viable, while those of grades C and D were considered non-viable (Figure 2f).

Statistical analysis

Normality and homogeneity tests (Shapiro-Wilk) were conducted on the data. Variables that followed a normal distribution included follicle size of 2-4 mm, total follicles, and total recovered oocytes; for these variables, T-tests (T-Student) were performed. However, the variables follicle size of 4-8 mm, size greater than 8 mm, viable oocytes, and non-viable oocytes did not meet the normality assumptions, so Mann-Whitney tests were applied. All analyses at a confidence level of 95% ($p < 0.05$), were conducted using R-Studio (Team et al., 2018) using version 4.3.0.

RESULTS AND DISCUSSION

According to Table 1, the following data were reported, the follicle size of 2-4 mm (small) showed an average of 6.048 ± 6.037 units, with a maximum of 13 follicles; the follicle size of 4-8 mm (medium) presented an average of 3.16 ± 2.01 units, with a maximum of 8 follicles; and the follicle size greater than 8 mm (large) had an average of 0.53 ± 0.67 units, with a maximum of 2 follicles. The average total number of follicles was 9.59 ± 3.56 units, with a maximum of 15. For viable recovered oocytes, an average of 3.024 ± 1.66 units was observed, with a minimum of 0 and a maximum of 8. Non-viable oocytes had an average of 1.47 ± 1.01 units, ranging from 0 to 4. Finally, the total average number of recovered oocytes was 4.5 ± 1.98, with a minimum of 1 and a maximum of 9 (Viable oocyte refers to the oocyte that is suitable, oocyte retrieved refers to the number of oocytes retrieved).

According to Table 2, there are no significant statistical differences between the treatments (T1 and T2). For follicle sizes of 2-4 mm, the averages were 5.67 for T1 (Estradiol Cypionate) and 6.43 for T2 (Estradiol Benzoate), showing similar results ($p > 0.05$). Similarly, for follicles sized 4-8 mm, the averages were 3.52 for T1 and 2.81 for T2, with no significant statistical differences ($p > 0.05$). For follicles larger than 8 mm, no significant differences were evident ($p > 0.05$), with averages of 0.66 for T1 and 0.38 for T2. For the total number of follicles, no significant differences were found ($p > 0.05$), with averages of 9.81 for T1 and 9.38 for T2. Similarly, no significant differences were found in viable oocytes recovered or the total oocytes recovered ($p > 0.05$). However, the variable of non-viable oocytes recovered caused statistically significant differences, with averages of 1.09 units for T1 and 1.86 units for T2.

Table 1. The mean of follicle sizes and oocyte recovery in the follicular collection of Brown Swiss cows (estradiol cypionate and estradiol benzoate)

Variable	Mean	SD	min	max	median
2-4 mm size	6.048	6.037	0	13	5.5
4-8 mm size	3.16	2.01	0	8	3
8 mm (Larger size)	0.53	0.67	0	2	0.0
Total follicles	9.59	3.56	2	15	10
Viable oocytes recovered	3.024	1.66	0	8	3
Non-viable oocytes recovered	1.47	1.01	0	4	1
Total oocytes recovered	4.5	1.98	1	9	4

SD: Standard deviation, Median: Mean value of the data

Table 2. The comparison of the two treatments means' in follicle sizes and oocyte recovery of follicular collection in Brown Swiss cows (estradiol cypionate and estradiol benzoate).

Variable	T1	T2	P-value
Size 2-4 mm	$5.67^{a} \pm 2.99$	$6.43^{a} \pm 3.75$	0.471^{T}
Size 4-8 mm	$3.52^{a} \pm 2.14$	$2.81^{a} \pm 1.87$	0.302^{M}
Larger size 8 mm	$0.66^{a} \pm 0.73$	$0.38^{a} \pm 0.58$	0.184^{M}
Total follicles	$9.81^{a} \pm 3.41$	$9.38^{a} \pm 3.79$	0.702^{T}
Viable Oocytes Recovered	$3.47^{a} \pm 1.94$	$2.57^{a} \pm 1.20$	0.132^{M}
Non-viable Oocytes Recovered	$1.09^{a} \pm 0.94$	$1.86^{b} \pm 0.96$	0.008^{M}
Total Oocytes Recovered	$4.57^{a} \pm 2.08$	$4.43^{a} \pm 1.91$	0.818^{T}

[a, b] Similar letters in the same row indicate similarity ($p > 0.05$) and different letters imply statistical differences ($p < 0.05$). [T], implies a T-Student test, due to its normal distribution. [M], implies a Mann-Whitney test, because it does not meet the assumptions of normality; T1: Estradiol cypionate group, T: Estradiol benzoate group

The obtained results provide information on follicular dynamics and the efficiency of oocyte collection in cows (Brown Swiss) treated with different types of estrogens. The follicle size of 2-4 mm (small) showed an average of 6.048 ± 6.037, with a maximum of 13 follicles. This follicle size range is crucial, as it represents follicles in the initial growth phase, this range of follicle sizes is critical because it reflects the early growth phase, during which follicles are most responsive to hormonal stimulation (Ferst et al., 2020). For follicles sized 4-8 mm (medium), an average of 3.16 ± 2.01 was observed, with a maximum of 8 follicles. This size range is important because follicles within these dimensions are at a more advanced stage of development. At this point, they are nearing the final stages of maturation and are therefore more likely to be selected for ovulation. This makes them crucial targets in reproductive protocols, as they have a higher potential for yielding viable oocytes, which are essential for successful fertilization and subsequent embryo development. Understanding the development of follicles in this size range helps optimize the timing and efficiency of oocyte collection procedures (Richard et al., 2024). The variability in follicle size may be influenced by factors such as estrous cycle synchronization and the administration of exogenous hormones (Quirino et al., 2020). For follicles larger than 8 mm, the average was 0.53 ± 0.67 units, with a maximum of 2 follicles. These results are comparable to those reported by Gomez-Leon et al. (2023), who found that dominant follicles are usually few but essential for ovulation and subsequent fertilization. The lower number of follicles in this category suggests that most follicles are in earlier stages of development during the collection process. The average total number of follicles was 9.59 ± 3.56, with a maximum of 15, indicating a good response to hormonal treatments in terms of follicular recruitment. This finding is consistent with Reineri et al. (2023), who observed a similar follicular response in estrogen synchronization protocols in beef cattle.

Regarding viable recovered oocytes, an average of 3.024 ± 1.66 was observed, with a range of 0 to 8. This result is important as viable oocytes are crucial for the success of *in vitro* fertilization (IVF). Compared to other studies, this average is slightly lower than that reported by Zago et al. (2023), who observed higher viable oocyte recovery in superovulation protocols in eight Flemish and eight Holstein females. The observed difference may stem from variations in study design, including differences in hormone dosages, timing of oocyte collection, and the specific breed of cattle used. For non-viable oocytes, the average was 1.47 ± 1.01, with a range of 0 to 4. The proportion of non-viable oocytes could be influenced by factors such as the precision of handling during collection and the hormonal conditions within the follicular environment, which can significantly impact oocyte quality and viability (Sartori et al., 2023). Finally, the average number of oocytes recovered was 4.5 ± 1.98, with a range from 1 to 9. This result is in line with previous studies that have reported similar recovery rates in bovine follicular collection protocols, suggesting that the methods used in this study are effective and consistent with established practices. The consistency of these findings with existing literature reinforces the reliability of the oocyte collection techniques employed and underscores their applicability in routine reproductive procedures (Souza-Fabjan et al., 2023).

CONCLUSION

The obtained results demonstrated a similar follicular response and viable oocyte recovery in both treatment groups (estradiol cypionate and estradiol benzoate). However, variations in oocyte viability were observed, with estradiol cypionate showing a slight advantage. It is suggested that while both estrogens can be effectively used in follicular wave programs, estradiol cypionate may offer a marginal improvement in the recovery of viable oocytes. Although the type of estrogen used does not appear to significantly affect follicular dynamics in terms of follicle size and number, but it may influence oocyte viability, with the use of estradiol cypionate being slightly superior. The findings underscore the importance of carefully selecting hormonal agents, considering the specific objectives of the reproductive protocol and the individual characteristics of the cattle. Precise selection of hormonal agents can enhance the quality of recovered oocytes, thereby increasing the efficiency and success of oocyte collection programs, further studies are suggested to explore the long-term effects of different types of estrogens on subsequent fertility and embryo development after oocyte retrieval, as well as possible interactions with other reproductive hormones.

DECLARATIONS

Funding

This work was supported through the Annual Funding track (CANON AND SOBRECANON) by the Universidad Nacional del Centro del Perú.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

Acknowledgment to Universidad Nacional del Centro for its valuable collaboration in the logistics and development of this research.

Authors' contributions

Ide Unchupaico Payano supervised the research, Carlos Quispe Eulogio executed the research, Edith Ancco Gómez did laboratory analysis, Jordan Ninahuanca Carhuas did statistical analysis, Fernando Arauco Villar did animal non-monitoring, Jorge Unchupaico Fermín collected data, and Noemí Mayorga Sánchez did the laboratory analysis. All authors confirmed the last edition of the manuscript before submission to the journal.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

The authors confirm that the manuscript has been reviewed and submitted to this journal for the first time and all content of article have been checked via a well-known plagiarism checker software before submission.

REFERENCES

Abdulghani MAM (2022). The ovulation assessment of regular cyclic rats following subacute oral administration of monosodium glutamate: An *in vivo* study. Journal of Pharmacy Pharmacognosy Research, 10(3): 397-405. DOI: https://www.doi.org/10.56499/jppres21.1312_10.3.397

Baykan ZK and Özcan M (2017). Determination of reproduction and lactation parameters in the first production year of brown Swiss and Simmental cows imported from Austria. İstanbul Üniversitesi Veteriner Fakültesi Dergisi 43(2): 132-139. DOI: https://www.doi.org/10.16988/iuvfd.324052

Boneya G (2021). Sexed semen and major factors affecting its conception rate in dairy cattle. International Journal of Advanced Research in Biological Sciences, 8(1): 99-107. DOI: http://www.doi.org/10.22192/ijarbs.2021.08.01.012

Dahlen C, Larson J, and Lamb GC (2014). Impacts of reproductive technologies on beef production in the United States. In: G. Lamb and N. DiLorenzo (Editors), Current and future reproductive technologies and world food production. Advances in experimental medicine and biology. Springer., New York, pp. 97-114. DOI: https://www.doi.org/10.1007/978-1-4614-8887-3_5

Estremadoyro LJG, Salome PH, Carhuas JN, Guzman SO, Tacza AA, Guillen MAF, and Garcia-Olarte E (2024). Effects of different seasons on milk quality: A study on two cattle breeds in rainy and drought contexts. World's Veterinary Journal, 14(2): 213-219. DOI: https://www.doi.org/10.54203/scil.2024.wvj25

Ferst JG, Rovani MT, Dau AM, Gasperin BG, Antoniazzi AQ, Bordignon V, Oliveira DE, Gonçalves PB, and Ferreira R (2020). Activation of PPARG inhibits dominant follicle development in cattle. Theriogenology, 142: 276-283. DOI: https://www.doi.org/10.1016/j.theriogenology.2019.10.032

Gomez-Leon VE, Ginther O, Domingues RR, Sanglard LP, and Wiltbank MC (2023). Temporality of ovarian steroids and lh/fsh pulse profiles encompassing selection of the dominant follicle in heifers. Biology of Reproduction, 108(2): 269-278. DOI: https://www.doi.org/10.1093/biolre/ioac201

Gray A, Bradbury AR, Knappik A, Plückthun A, Borrebaeck CA, and Dübel S (2020). Animal-free alternatives and the antibody iceberg. Nature Biotechnology, 38(11): 1234-1239. DOI: https://www.doi.org/10.1038/s41587-020-0687-9

Gutierrez-Reinoso MA, Aponte PM, and Garcia-Herreros M (2021). Genomic analysis, progress and future perspectives in dairy cattle selection: A review. Animals, 11(3): 599. DOI: https://www.doi.org/10.3390/ani11030599

Haadsma M, Bukman A, Groen H, Roeloffzen E, Groenewoud E, Heineman M, and Hoek A (2007). The number of small antral follicles (2-6 mm) determines the outcome of endocrine ovarian reserve tests in a subfertile population. Human Reproduction, 22(7): 1925-1931. DOI: https://www.doi.org/10.1093/humrep/dem081

Landeo L, Zuñiga M, Gastelu T, Artica M, Ruiz J, Silva M, and Ratto MH (2022). Oocyte quality, *in vitro* fertilization and embryo development of alpaca oocytes collected by ultrasound-guided follicular aspiration or from slaughterhouse ovaries. Animals, 12(9): 1102. DOI: https://www.doi.org/10.3390/ani12091102

Lima F, Ayres H, Favoreto M, Bisinotto RS, Greco LF, Ribeiro E, Baruselli P, Risco C, Thatcher W, and Santos J (2011). Effects of gonadotropin-releasing hormone at initiation of the 5-d timed artificial insemination (AI) program and timing of induction of ovulation relative to AI on ovarian dynamics and fertility of dairy heifers. Journal of dairy science, 94(10): 4997-5004. DOI: https://www.doi.org/10.3168/jds.2011-4240

Lonergan P and Fair T (2014). The art of studying early embryo development: Progress and challenges in ruminant embryo culture. Theriogenology, 81(1): 49-55. DOI: https://www.doi.org/10.1016/j.theriogenology.2013.09.021

Mikkola M, Hasler JF, and Taponen J (2020). Factors affecting embryo production in superovulated bos taurus cattle. Reproduction, Fertility and Development, 32(2): 104-124. DOI: https://www.doi.org/10.1071/RD19279

Perisse IV, Fan Z, Singina GN, White KL, and Polejaeva IA (2021). Improvements in gene editing technology boost its applications in livestock. Frontiers in Genetics, 11: 614688. DOI: https://www.doi.org/10.3389/fgene.2020.614688

Quirino M, da Rosa Ulguim R, Bernardi ML, Pereira VN, Magoga J, Gianluppi RDF, Mellagi APG, Gasperin BG, and Bortolozzo FPJT (2020). Follicular dynamic and reproductive performance of gilts submitted to estrous cycle synchronization using two different progestogen sources. Theriogenology, 158: 31-38. DOI: https://www.doi.org/10.1016/j.theriogenology.2020.08.035

Reineri PS, Bernhard SDR, Principi SA, Gerlero GD, and Aller JF (2023). Effects of two protocols of ovulation synchronization on corpus luteum size and blood flow, progesterone concentration, and pregnancy rate in beef heifers. Animal Reproduction Science, 251: 107223. DOI: https://www.doi.org/10.1016/j.anireprosci.2023.107223

Richard S, Zhou Y, Jasoni CL, and Pankhurst MW (2024). Ovarian follicle size or growth rate can both be determinants of ovulatory follicle selection in mice. Biology of Reproduction, 110(1): 130-139. DOI: https://www.doi.org/10.1093/biolre/ioad134

Sartori R, Consentini C, Alves R, Silva L, and Wiltbank M (2023). Manipulation of follicle development to improve fertility of cattle in timed-artificial insemination programs. Animal, 17: 100769. DOI: https://www.doi.org/10.1016/j.animal.2023.100769

Senamhi (2023). Datos hidrometeorológicos a nivel nacional en Perú [Hydrometeorological data at the national level in Peru]. Available at: https://www.senamhi.gob.pe/?&p=estaciones

Simões J, Stilwell G, Simões J, and Stilwell G (2021). Puerperal complications in the dam. Calving management and newborn calf care: An interactive textbook for cattle medicine and obstetrics. Springer., Cham, pp. 209-237. DOI: https://www.doi.org/10.1007/978-3-030-68168-5_9

Souza-Fabjan JMG, Leal GR, Monteiro CAS, Batista RITP, Barbosa NO, and Freitas VJF (2023). *In vitro* embryo production in small ruminants: What is still missing?. Animal Reproduction, 20(3): e20230055. DOI: https://www.doi.org/10.1590/1984-3143-AR2023-0055

Team RC, Team MRC, Suggests M, and Matrix S (2018). Package stats. The R stats package.

Zago FC, Schütz LF, Gerger RPdC, Aguiar LHd, Pinzón-Osorio CA, Mezzallira A, Rodrigues JL, Forell F, and Bertolini M (2023). *In vitro* and *in vivo* embryo production efficiency in flemish and Holstein donor females. Animal Reproduction Science, 20: e20230080. DOI: https://www.doi.org/10.1590/1984-3143-AR2023-0080

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 373-379.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj44
PII: S232245682400044-14

Multidrug Resistance in Stray Cats of The North Surabaya Region, East Java, Indonesia

Freshinta Jellia Wibisono[1], Dyah Ayu Widiasih[2*], Andi Oktaviana Mentari[1], Mutia Isnaeni[1], Seryna Hasna Qurratu'ain[1], Ismul Jalal[1], Afif Fajrul Islam[1], Al Fardiansyah[1], and Hung Nguyen-Viet[3]

[1]*Departement Veterinary Public Health, Veterinary Medicine Faculty, Universitas Wijaya Kusuma Surabaya, Indonesia*
[2]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[3]*International Livestock Research Institute, Nairobi, Kenya*

*Corresponding author's Email: dyahaw@ugm.ac.id

ORIGINAL ARTICLE
Received: June 27, 2024
Revised: July 19, 2024
Accepted: August 21, 2024
Published: September 25, 2024

ABSTRACT

Stray cats survive by getting food or drink that is available in the environment, correspondingly, stray cats have relatively high exposure to antibiotic resistance obtained from resistant bacteria found in the environment. The present study was conducted to determine patterns of multidrug resistance and *Escherichia coli* resistance in stray cats. A total of 50 stray cat anal swab samples were taken randomly from the previously recorded stray cat population in the Surabaya area, East Java, Indonesia. Samples were brought using buffered peptone water. They were cultured on MacConkay Agar differential selective media, and all suspicious colonies of *Escherichia coli* were examined by biochemical tests. Isolates were then identified, and susceptibility testing was performed according to the Clinical and Laboratory Standards Institute. The results of the resistance test indicated that the multidrug resistance in *Escherichia coli* bacteria taken from cats was 14.6% (7/48). The high antibiotic resistance of *Escherichia coli* bacteria in stray cats, which were resistant to many drugs, provides an early warning of environmental health. Environmental health is closely related to animal and human health, especially antibiotic resistance.

Keywords: Antibiotic, Antimicrobial resistance, *Escherichia coli*, Multidrug resistance, Stray cat

INTRODUCTION

Stray cats which inhabit unregulated and open environments, demonstrate a remarkable adaptability, enabling them to thrive in diverse settings despite lacking human ownership (Vasileva and McCulloch, 2023). Cats are potentially exposed to pathogen infection from the environment (Agustin and Ningtyas, 2022). Previous research by Mauwalan et al. (2022) showed the existence of *Escherichia coli* resistance in an environment in Indonesia. The level of antibiotic resistance in the ampicillin 100%, streptomycin 73.3%-86.7%, gentamicin 100%, and kanamycin 33.3%. Pathogen contamination is generally caused by the entry of feces, animal waste, and garbage into water bodies, such as the return of wastewater into wells and leaky water pipes in dirty areas. Stray cats have a significant potential to interact with such environments, triggering the spread of pathogens such as *Escherichia coli* bacteria (Gargano et al., 2022). *Escherichia coli* is a gram-negative known as a normal flora found naturally in the digestive tract of humans and animals. *Escherichia coli* is considered pathogenic when its concentration surpasses typical levels (Mueller and Tainter, 2023).

Antibiotic drug use in the animal sector reached 80% (Hosain et al., 2021). The effectiveness of antibiotics against some animals, such as cats has decreased currently, this is indicated by an increase in multidrug resistance observed in 9% of isolated cats (Li et al., 2021). Improper dosage, indications, and antibiotic use can lead to animal resistance (Rahman et al., 2022). Antibiotic resistance occurs when bacteria adapt and thrive in an environment exposed to antimicrobials, posing a severe threat to public health globally (Salam et al., 2023). Multidrug resistance refers to resistance to three or more different groups of antibiotics (Mandal et al., 2022). High levels of multidrug resistance in *Escherichia coli* were detected in healthy and diseased cats, raising concerns about transmission to humans and supporting the need for a one-health approach to address the potential threat of cats as a reservoir for AMR (Fayez et al., 2023).

Since cats interact with humans, studying antibiotic resistance is crucial whether domestic or stray, facilitating the transfer of genes for resistance to the common bacterium *Escherichia coli* (Li et al., 2021; Fayez et al., 2023). It is crucial to determine the resistance levels of *Escherichia coli* in stray cats to ampicillin, streptomycin, and tetracycline due to the significant antibiotic resistance present in the environment. Stray cats are exposed to the environment, which

To cite this paper: Wibisono FJ, Widiasih DA, Mentari AO, Isnaeni M, Qurratu'ain SH, Jalal I, Islam AF, Al Fardiansyah, and Nguyen-Viet H (2024). Multidrug Resistance in Stray Cats of The North Surabaya Region, East Java, Indonesia. *World Vet. J.,* 14(3): 373-379. DOI: https://dx.doi.org/10.54203/scil.2024.wvj44

puts them at risk. The present study aimed to determine multidrug resistance *Escherichia coli* isolated from anal samples of stray cats in the North Surabaya region of Indonesia.

MATERIALS AND METHODS

Ethical approval

All research methods and procedures, along with the utilization of animals, have been approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, Indonesia, with certificate number 123-KKE/III/FKH-UWKS/2023.

Sample collection

In the present cross-sectional study, 50 stray cats, both male and female, were used randomly. Samples were collected from North Surabaya, East Java, Indonesia, from March to May 2023. The sample size was taken proportionally based on the stray cat population in that district. The samples were collected from five districts in North Surabaya, including Bulak (12 samples), Kenjeran (12 samples), Semampir (9 samples), Pabean (11 samples), and Krembangan (6 samples). Samples were collected directly by anal swab and incubated buffered peptone water (BPW) in cold conditions (4°C) to transport to the laboratory for detecting *Escherichia coli* at the Faculty of Veterinary Medicine Laboratory, Wijaya Kusuma University Surabaya, Indonesia.

Isolation and identification

The isolation of *Escherichia coli* was carried out following the Indonesian National Standard, SNI 7388:2009. Anal swab samples were incubated in buffered peptone water broth (BPW, Merck, Germany) to enrich *Escherichia coli* in samples. After incubation at 37°C for 24 hours, each loop of enrichment broth samples was cultured on MacConkey agar (MCA, HiMedia MH081, India) for further incubation at 37°C for 24 hours. *Escherichia coli* colonies were subcultured on nutrient agar (NA, Merck, Germany) for further incubation at 37°C for 24 hours to check biochemical tests. The confirmed *Escherichia coli* strains are cultured on Triple Sugar Iron Agar (TSIA, HiMedia M021, India) and incubated at 37°C for 24 hours.

Antimicrobial susceptibility testing

After identifying *Escherichia coli*, the Kirby-Bauer agar plate diffusion method was used to test the sensitivity of bacteria to antibiotics on Muller Hinton agar (MHA, HiMedia, India). The sensitivity of bacteria to antibiotics was evaluated by comparing the results with the CLSI standard (2022). *Escherichia coli* ATCC 25922 was utilized as a quality control, and the findings were then compared with the CLSI standards (2022). The antibiotic discs used in the present study included ampicillin (AMP 10µg, Oxoid™), streptomycin (S 10µg, Oxoid™), and tetracycline (TE 30µg, Oxoid™).

Data analysis

According to the evaluation's post-incubation results, the presence of inhibition zones, namely susceptible, intermediate, and resistant, was interpreted according to CLSI guidelines. Data analysis of the current study was carried out descriptively (Effendi et al., 2018).

RESULTS AND DISCUSSION

The results of the present study indicated that 48/50 (96%) of the samples tested positive for *Escherichia coli* from stray cats in North Surabaya. The *Escherichia coli* was isolated from Bulak, Semampir, and Pabean districts at 100%, Kenjeran at 91.7%, and Kembangan at 83.3%. Although *Escherichia coli* bacteria are not pathogenic, these bacteria are opportunistic bacteria that can cause pathogens in cats (Núñez-Samudio et al., 2024). The pathogenicity of *Escherichia coli* can be influenced by several predisposing factors, including feed, environment, and the intensity of the pathogen strain (EFSA AHAW Panel, et al., 2022; Damborg et al., 2023). *Escherichia coli* has several pathogenic strains that cause diarrhea, namely diarrheagenic *Escherichia coli* strains, which can produce toxins (Das et al., 2023). Humans and cats can be infected by *Escherichia coli* (Li et al., 2021). Farizqi et al. (2023) have indicated that cats and their owners could be infected with dangerous *Escherichia coli* strains. Despite the absence of symptoms in the cat, it has the potential to act as a reservoir or carrier of enterohemorrhagic *Escherichia coli* in humans (Gargano et al., 2022).

Antibiotic sensitivity test on positive *Escherichia coli* isolates was conducted using three different antibiotic groups, including beta-lactam (ampicillin), aminoglycoside (streptomycin), and tetracycline group (Tetracycline; Table

1). The percentage of isolates exhibiting multidrug resistance in this test was 14.6% (7/48). Based on the tested isolates, resistance was observed in all used antibiotic disks, indicating multidrug resistance, as shown in Figure 1. The high results of antibiotic resistance were for ampicillin (35.41%), streptomycin (18.75%), and tetracycline (22.92%), respectively in North Surabaya that are following previous research which showed resistance to ampicillin (31%) and tetracycline (21%) (Gargano et al., 2022). However, the results of this study are higher than previous studies, which show in stray cat ampicillin resistance (19%), tetracycline (15%), and streptomycin (8%) (Wibisono et al., 2024). Stray cats can develop antibiotic resistance through the environment. The water contaminated with resistant *Escherichia coli* bacteria can be a bridge for the spread of antibiotic resistance in stray cats (Janke et al., 2023). Resistance genes could be exchanged between animals and humans, or environmental bacteria (Saraiva et al., 2022). A previous study by Chen et al. (2019), reported a significant risk of the spread of resistant *Escherichia coli* bacteria from pets to humans, and consequently using antibiotic in pets should be strictly regulated.

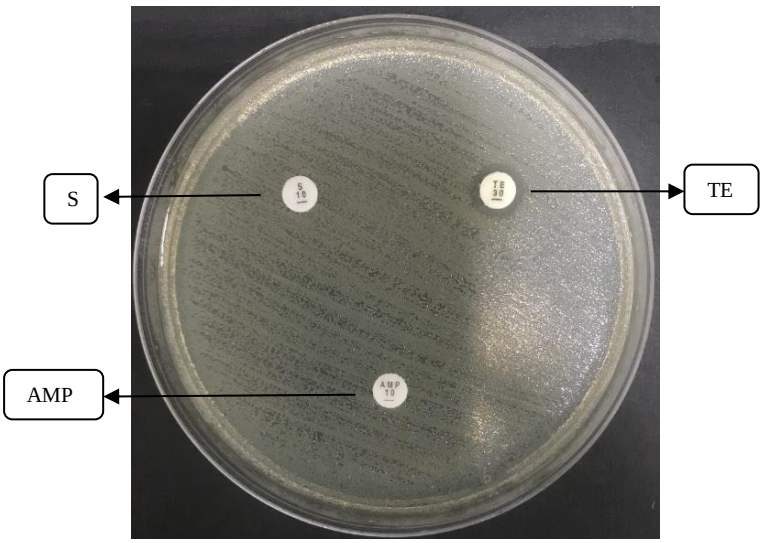


Figure 1. Multidrug resistance of *Escherichia coli* in stray cats. S: Streptomycin, TE: Tetracycline, AMP: Ampicillin

Ampicillin, a beta-lactam antibiotic, has encountered the most significant resistance. Ampicillin is a versatile antibiotic that is utilized in the treatment of various infections, including gastrointestinal infections (Kim et al., 2019), and urinary tract infections (Kang et al., 2018) is a crucial antibiotic that has been most widely used in humans and animals health (Hardiati et al., 2021). Ampicillin, belonging to the aminopenicillin group, is a semisynthetic antibiotic that is widely known for its effectiveness against *Escherichia coli*, the bacterium commonly associated with resistance (Monaghan et al., 2021). The agricultural use or contamination of antibiotics directly or indirectly exposes soil, water, livestock, and plant foods to these substances (Skandalis et al., 2021). A recent revealed that 57.1% of *Escherichia coli* strains isolated from the wastewater of a hospital in Banda Aceh, Indonesia, exhibited resistance to ampicillin (Anelia et al., 2023). The results of sensitivity testing for antibiotics from the tetracycline group (tetracycline) indicated 22.92% (11/48). The obtained result was higher than previous research on stray cats in Poland (53%) (Rzewuska et al., 2015). The percentage of resistance to the aminoglycoside group in stray cats in the North Surabaya region showed a resistance result of 18.75% (9/48). Cats can encounter resistance to the antibiotic streptomycin, as well as resistance to ampicillin and tetracycline, from their environment (Fayez et al., 2023). According to Mandal et al. (2022), beta-lactam, tetracycline, and aminoglycoside antibiotics are widely used to treat bacterial infections in humans and the livestock industry, therefore, an environment polluted by resistant bacteria can spread resistance genes in stray cats. The resistance of stray cats to various antibiotics may be attributed to the food they consume or the environment they inhabit. This is evident in the high levels of resistance found in the environment and traditional markets where stray cats reside in the Northern Surabaya area (Dewi et al., 2023).

As can be seen in Table 2, according to the resistance pattern, the percentage of positive isolates of *Escherichia coli* exhibiting the highest resistance at a level of 14.6% (7/48), specifically against the antibiotics ampicillin, tetracycline, and streptomycin (AMP–TE-S). The resistance pattern of *Escherichia coli* indicates multidrug resistance. High dosages of antibiotics used in the medical field, particularly in the fisheries, for the treatment of humans and animals might lead to the emergence of antibiotic-resistant harmful microorganisms. One of the two pathways mentioned below has the potential to result in multidrug resistance. Initially, within a single cell, bacteria can accumulate numerous genes responsible for drug resistance. Resistance plasmids are often the site of this buildup. Additionally, increased expression of genes producing multidrug efflux pumps, which enable them to secrete a range of medicines, can lead to a second

mechanism of multidrug resistance (Das et al., 2023; Fayez et al., 2023). Animals, such as cats, dogs, calves, pigs, turkeys, chickens, and rodents have all been found to harbor multidrug-resistant bacteria (Harijani et al., 2020; Wibisono et al., 2021; Zhao et al., 2021). A multidrug-resistant *Escherichia coli* strain with an AMP–TE–S resistance pattern to ampicillin, tetracycline, and streptomycin was found in 14.6% (7/48) of the isolates based on the findings of the antibiotic sensitivity test. *Escherichia coli* often exhibits multidrug resistance when exposed to beta-lactam, tetracycline, and aminoglycoside antibiotics (Nguyen et al., 2021; Saraiva et al., 2022).

Table 1. Antibiotic sensitivity test of *Escherichia coli* in stray cats at North Surabaya, Indonesia

Sub-district	*Escherichia coli* (%)	Antibiotic Sensitivity Test (%)									
		Ampicillin			Streptomycin			Tetracycline			MDR
		R	I	S	R	I	S	R	I	S	
Kenjeran	11/12 (91.66 %)	36.4% 4/11	0 (0/11)	63.6% (7/11)	18.2% (2/11)	54.5% (6/11)	27.3% (3/11)	9.1% (1/11)	0 (0/11)	90.9% (10/11)	0 (0/11)
Semampir	(9/9) (100 %)	22.2% (2/9)	0 (0/9)	77.8% (7/9)	22.2% (2/9)	22.2% (2/9)	55.6% (5/9)	33.3% (3/9)	0 (0/9)	66.7% (6/9)	22.2% (2/9)
Krembangan	(5/6) (83.3 %)	20% (1/5)	0 (0/5)	80% (4/5)	20% (1/5)	0 (0/5)	80% (4/5)	20% (1/5)	0 (0/5)	80% (4/5)	20 (1/5)
Bulak	(12/12) (100 %)	50% (6/12)	0 (0/12)	50% (6/12)	25% (3/12)	8.3% (1/12)	66.7% (8/12)	33.3% (4/12)	25 (3/12)	41.7% (5/12)	25% (3/12)
Pabean	(11/11) (100 %)	36.4% (4/11)	0 (0/11)	63.6% (7/11)	9.1% (1/11)	9.1% (1/11)	81.8% (9/11)	18.2% (2/11)	9.1% (1/11)	72.7% (8/11)	9.1% (1/11)
Total	(48/50) (96 %)	35.4% (17/48)	0 (0/48)	64.6% (31/48)	18.7% (9/48)	20.8% (10/48)	60.4% (29/48)	22.9% (11/48)	8.3% (4/48)	68.7% (33/48)	14.6% (7/48)

R: Resistant, I: Intermediate, S: Susceptible, MDR: Multidrug resistance

Table 2. Resistance pattern of *Escherichia coli* in stray cats at North Surabaya, Indonesia

Number	Resistance pattern	Percentage
1	AMP-TE-S	14.6 (7/48)
2	AMP-TE	6.2 (3/48)
3	AMP-S	4.2 (2/48)
4	TE-S	0 (0/48)

S: Streptomycin, TE: Tetracycline, AMP: Ampicilline

Antibiotic-resistant bacteria can be transmitted through various means, including feces, food, and animal products (Eltai et al., 2020). The livestock environment, the slaughterhouse environment, and the environment during meat processing can all be contaminated by resistant bacteria found in the feces of food animals (Indrawati et al., 2019; Osman et al., 2021). Test obtained results indicate that multidrug resistance can be dangerous and need to be handled. Cats can contaminate their surroundings and act as a reservoir for the propagation of resistant genes (Gargano et al., 2022). *Escherichia coli* bacteria can transfer antibiotic-resistance genes between animals and owners through horizontal transfer (Fayez et al., 2023). Stray cats can serve as reservoirs by consuming vectors, such as rats and cockroaches, as well as consuming raw meat (Koutsoumanis et al., 2021; Rafiq et al., 2022). Proper use of antibiotics and awareness of their side effects are essential in preventing antibiotic resistance, as excessive use can lead to the presence of antibiotic residues (Handayani et al., 2017). Moreover, the issue of antibiotic resistance can be addressed by educating the public to enhance awareness and knowledge about antibiotic resistance, influence public behaviour toward the responsible use of antibiotics, and adopt appropriate sanitation and hygiene measures (Suherman et al., 2023). Three distinct antibiotic groups were used in this study: ampicillin (penicillin), streptomycin (aminoglycoside), and tetracycline. To determine if *Escherichia coli* is sensitive to antibiotics or resistant, it would be ideal if testing on a variety of drugs could be conducted. This would reveal any potential resistance of *Escherichia coli* germs in stray cats. This can surely help to improve the management of antibiotic use. The Kirby-Bauer disk diffusion method, which is employed in this work, is the gold standard for confirming bacterial susceptibility. More information would be available if the resistance genes of the bacterium were identified.

CONCLUSION

According to the present study, 96% of anal swab samples originate from stray cats in the Northern region of Surabaya, Indonesia. Sensitivity testing revealed 14.6% multidrug resistance in *Escherichia coli* from anal swab samples of stray cats in North Surabaya. The most significant antibiotic resistance was observed with ampicillin, then tetracycline, and

finally streptomycin. It is crucial to recognize the significance of understanding the utilization of antibiotics in animals and humans to effectively combat antibiotic resistance in the environment and prevent it from posing a threat to stray cats.

DECLARATIONS

Funding

The UGM Quality Assurance Office funded this study according to the assignment letter, Decree of the Chancellor of Gadjah Mada University Number 323/UN1. P. II/KPT/HUKOR/2023, dated March 30, 2023.

Acknowledgments

The authors would like to acknowledge the UGM Research Directorate and UGM Reputation Improvement Team toward World Class University for funding this research.

Authors' contributions

Freshinta Jellia Wibisono and Dyah Ayu Widiasih conceived and designed the study. Mutia Isnaeni, Afif Fajrul Islam, Andi Oktaviana Mentari, Ismul Jalal, Seryna Hasna Qurratu'ain, and Al Fardiansyah contributed to antimicrobial agent and susceptibility testing. Freshinta Jellia Wibisono, Dyah Ayu Widiasih, and Hung Nguyen-Viet contributed to the interpretation of the results, and AOM led the writing of the manuscript. All authors provided critical feedback and helped shape the research, analysis, and writing of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Ethical considerations

This paper was written originally by the authors. The authors were not submitting this paper to the other journal or publisher.

Availability of data and materials

All data of the current study are available upon reasonable request from the authors.

REFERENCES

Agustin ALD and Ningtyas NSI (2022). Resistance of *Escherichia coli* to various antibiotics of cat patients in educational veterinary hospital, Mandalika University of Education. Media Kedokteran Hewan, 33(2): 63-71. DOI: https://www.doi.org/10.20473/mkh.v33i2.2022.63-71

Anelia N, Suhartono S, and Hayati Z (2023). Abundance and phenotypic-genotypic analysis of antibiotic-resistant *Escherichia coli* isolated from wastewater of the Zainoel Abidin Hospital, Banda Aceh, Indonesia. Biodiversitas, 24(2): 2513-2520. DOI: https://www.doi.org/10.13057/biodiv/d240502

Chen Y, Liu Z, Zhang Y, Zhang Z, Lei L, and Xia Z (2019). Increasing prevalence of ESBL-producing multidrug resistance *Escherichia coli* from diseased pets in Beijing, China from 2012 to 2017. Frontiers in Microbiology, 10: 2852. DOI: https://www.doi.org/10.3389/fmicb.2019.02852

Damborg P, Pirolo M, Schøn Poulsen L, Frimodt-Møller N, and Guardabassi L (2023). Dogs can be reservoirs of *Escherichia coli* strains causing urinary tract infection in human household contacts. Antibiotics, 12(8): 1269. DOI: https://www.doi.org/10.3390/antibiotics12081269

Das S, Kabir A, Chouhan CS, Shahid MAH, Habib T, Rahman M, and Nazir KNH (2023). Domestic cats are potential reservoirs of multidrug-resistant human enteropathogenic *Escherichia coli* strains in Bangladesh. Saudi Journal of Biological Sciences, 30(10): 103786. DOI: https://www.doi.org/10.1016/j.sjbs.2023.103786

Dewi RR, Berutu KM, and Sihombing JM (2023). Antibiotic resistance pattern of *Escherichia coli* derived from retail raw meat in North Sumatera, Indonesia. Jurnal Ilmu dan Teknologi Hasil Ternak, 18(2): 87-99. DOI: https://www.doi.org/10.21776/ub.jitek.2023.018.02.2

Effendi MH, Harijani N, Yanestria SM, and Hastutiek P (2018). Identification of Shiga toxin-producing *Escherichia coli* in raw milk samples from dairy cows in Surabaya, Indonesia. Philippine Journal of Veterinary Medicine, Special Issue, 55: 109-114. Available at: https://scholar.unair.ac.id/en/publications/identification-of-shiga-toxin-producing-*escherichia-coli*-in-raw-m

EFSA Panel on animal health and welfare (AHAW), Nielsen SS, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Rojas JLG, Gortázar C, Herskin M, Michel V et al. (2022). Assessment of listing and categorisation of animal diseases within the framework of the animal health law (Regulation (EU) No. 2016/429): Antimicrobial-resistant *Escherichia coli* in dogs and cats, horses, swine, poultry, cattle, sheep, and goats. European Food Safety Authority Journal, 20(10): 8327. DOI: https://www.doi.org/10.2903/j.efsa.2023.8327

Eltai NO, Yassine HM, El-Obeid T, Al-Hadidi SH, Al Thani AA, and Alali WQ (2020). Prevalence of antibiotic-resistant *Escherichia coli* isolates from local and imported retail chicken carcasses. Journal of Food Protection, 83(12): 2200-2208. DOI: https://www.doi.org/10.4315/JFP-20-113

Farizqi MTI, Effendi MH, Adikara RTS, Yudaniayanti IS, Putra GDS, Khairullah AR, Kurniawan C, Silaen OSM, Ramadhani S, Millannia SK et al. (2023). Detection of extended-spectrum β-lactamase-producing *Escherichia coli* genes isolated from cat rectal swabs at Surabaya Veterinary Hospital, Indonesia. Veterinary World, 16(9): 1917-1925. DOI: http://www.doi.org/10.14202/vetworld.2023.1917-1925

Fayez M, Elmoslemany A, Al Romaihi AA, Azzawi AY, Almubarak A, and Elsohaby I (2023). Prevalence and risk factors associated with multidrug resistance and extended-spectrum β-lactamase producing *Escherichia coli* isolated from healthy and diseased cats. Antibiotics, 12(2): 229. DOI: https://www.doi.org/10.3390/antibiotics12020229

Gargano V, Gambino D, Orefice T, Cirincione R, Castelli G, Bruno F, Interrante P, Pizzo M, Spada E, Proverbio D et al. (2022). Can stray cats be reservoirs of antimicrobial resistance?. Veterinary Sciences, 9(11): 631. DOI: https://www.doi.org/10.3390/vetsci9110631

Handayani RS, Siahaan S, and Herman MJ (2017). Antimicrobial resistance and its control policy implementation in hospital in Indonesia. Jurnal penelitian dan pengembangan pelayanan kesehatan, 1(2): 131-140. Available at: http://download.garuda.kemdikbud.go.id/article.php?article=1706690&val=12397&title=Antimicrobial%20Resistance%20and%20Its%20Control%20Policy%20Implementation%20in%20Hospital%20in%20Indonesia

Hardiati A, Safika S, Wibawan IWT, Indrawati A, and Pasaribu FH (2021). Isolation and detection of antibiotics resistance genes of *Escherichia coli* from broiler farms in Sukabumi, Indonesia. Journal of Advanced Veterinary and Animal Research, 8(1): 84-90. DOI: http://www.doi.org/10.5455/javar.2021.h489

Harijani N, Oetama SJT, Soepranianondo K, Effendi MH, and Tyasningsih W (2020). Biological hazard on multidrug resistance (MDR) of *Escherichia coli* collected from cloacal swab of broiler chicken on wet markets Surabaya. Indian Journal of Forensic Medicine and Toxicology, 14(4): 3239-3244. DOI: https://www.doi.org/10.37506/ijfmt.v14i4.12125

Hosain MZ, Kabir SML, and Kamal MM (2021). Antimicrobial uses for livestock production in developing countries. Veterinary World, 14(1): 210-221. DOI: http://www.doi.org/10.14202/vetworld.2021.210-221

Indrawati A, Kurnia RS, and Mayasari N (2019). Detection of gene encoding resistance AMPC and MCR-1 in *Escherichia coli* causes avian colibacillosis in Sukabumi. Jurnal Veteriner, 20(4): 495-503. DOI: https://www.doi.org/10.19087/jveteriner.2019.20.4.495

Janke N, Stone EA, Coe JB, and Dewey CE (2023). Companion animal veterinarians discuss aspects of one health with pet owners during most veterinary appointments. Journal of the American Veterinary Medical Association, 261(12): 1-9. DOI: https://www.doi.org/10.2460/javma.23.05.0287

Kang CI, Kim J, Park DW, Kim BN, Ha US, Lee SJ, Yeo JK, Min SK, Lee H, and Wie SH (2018). Clinical practice guidelines for the antibiotic treatment of community-acquired urinary tract infections. Infection and Chemotherapy, 50(1): 67-100. DOI: https://www.doi.org/10.3947/ic.2018.50.1.67

Kim YJ, Park KH, Park DA, Park J, Bang BW, Lee SS, Lee EJ, Lee HJ, Hong SK, and Kim YR (2019). Guideline for the antibiotic use in acute gastroenteritis. Infection and Chemotherapy, 51(2): 217-243. DOI: https://www.doi.org/10.3947/ic.2019.51.2.217

Koutsoumanis K, Allende A, Álvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F et al. (2021). Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain. European Food Safety Authority Journal, 19(6): e06651. DOI: https://www.doi.org/10.2903/j.efsa.2021.6651

Li Y, Fernandez R, Duran I, Molina-Lopez RA, and Darwich L (2021). antimicrobial resistance in bacteria isolated from cats and dogs from the Iberian Peninsula. Frontiers in Microbiology, 11: 621597. DOI: https://www.doi.org/10.3389/fmicb.2020.621597

Mandal AK, Talukder S, Hasan MM, Tasmim ST, Parvin MS, Ali MY, and Islam MT (2022). Epidemiology and antimicrobial resistance of *Escherichia coli* in broiler chickens, farmworkers, and farm sewage in Bangladesh. Veterinary Medicine and Science, 8(1): 187-199. DOI: https://www.doi.org/10.1002/vms3.664

Mauwalan YEC, Kallau NH, and Laut MM (2022). Study of antibiotic resistant *Escherichia coli* in the water environment in Indonesia. Jurnal Veteriner Nusantara, 5: 1-11. DOI: https://www.doi.org/10.35508/jvn.v5i1.3264

Monaghan KN, Labato MA, and Papich MG (2021). Ampicillin pharmacokinetics in azotemic and healthy dogs. Journal of Veterinary Internal Medicine, 35(2): 987-992. DOI: https://www.doi.org/10.1111/jvim.16026

Mueller M and Tainter CR (2023). *Escherichia coli* infection. StatPearls-NCBI Bookshelf. pp. 1-11. Available at: https://www.ncbi.nlm.nih.gov/books/NBK564298/

Nguyen TK, Nguyen LT, Chau TTH, Nguyen TT, Tran BN, Taniguchi T, Hayashidani H, and Ly KTL (2021). Prevalence and antibiotic resistance of *Salmonella* isolated from poultry and its environment in the Mekong Delta, Vietnam. Veterinary World, 14(12): 3216-3223. DOI: www.doi.org/10.14202/vetworld.2021.3216-3223

Núñez-Samudio V, Peralta G, De La Cruz A, and Landires I (2024). Multidrug-resistant phenotypes of genetically diverse *Escherichia coli* isolates from healthy domestic cats. Scientific Reports, 14(1): 1-9. DOI: https://www.doi.org/10.1038/s41598-024-62037-8

Osman AY, Elmi SA, Simons D, Elton L, Haider N, Khan MA, Othman I, ZumlaA, McCoy D, and Kock R (2021). Antimicrobial resistance patterns and risk factors associated with salmonella spp. Isolates from poultry farms in the east coast of peninsular Malaysia: A cross-sectional study. Pathogens, 10(9): 1160. DOI: https://www.doi.org/10.3390/pathogens10091160

Rafiq K, Islam MR, Siddiky NA, Samad MA, Chowdhury S, Hossain KMM, Rume FI, Hossain MK, Mahbub-E-Elahi ATM, Ali MZ et al. (2022). Antimicrobial resistance profile of common foodborne pathogens recovered from livestock and poultry in Bangladesh. Antibiotics, 11(11): 1551. DOI: https://www.doi.org/10.3390/antibiotics11111551

Rahman MM, Tumpa MAA, Zehravi M, Sarker MT, Yamin M, Islam MR, Harun-Or-rashid M, Ahmed M, Ramproshad S, Mondal B et al. (2022). An overview of antimicrobial stewardship optimization: The use of antibiotics in humans and animals to prevent resistance. Antibiotics, 11(5): 667. DOI: https://www.doi.org/10.3390/antibiotics11050667

Rzewuska M, Czopowicz M, Kizerwetter-Świda M, Chrobak D, Błaszczak B, and Binek M (2015). Multidrug resistance in *Escherichia coli* strains isolated from infections in dogs and cats in Poland (2007-2013). Scientific World Journal, 2015: 1-8. DOI: https://www.doi.org/10.1155/2015/408205

Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, and Alqumber MAA (2023). Antimicrobial resistance: A growing serious threat for global public health. Healthcare, 11(13): 1946. DOI: https://www.doi.org/10.3390/healthcare11131946

Saraiva MMS, Lim K, do Monte DFM, Givisiez PEN, Alves LBR, de Freitas Neto OC, Kariuki S, Júnior AB, de Oliveira CJB, and Gebreyes WA (2022). Antimicrobial resistance in the globalized food chain: A one health perspective applied to the poultry industry. Brazilian Journal of Microbiology, 53: 465-486. DOI: https://www.doi.org/10.1007/s42770-021-00635-8

Skandalis N, Maeusli M, Papafotis D, Miller S, Lee B, Theologidis I, and Luna B (2021). Environmental spread of antibiotic resistance. Antibiotics, 10(6): 640. DOI: https://www.doi.org/10.3390/antibiotics10060640

Suherman DA, Sudarnika E, and Purnawarman T (2023). Penicillin resistance against *Escherichia coli* in raw milk from North Cianjur cattle farmers cooperative, West Java. Jurnal Sain Veteriner, 41(2): 170-179. DOI: https://www.doi.org/10.22146/jsv.83050

Vasileva I and McCulloch SP (2023). Attitudes and behaviours towards cats and barriers to stray cat management in Bulgaria. Journal of Applied Animal Welfare Science, 18: 1-15. DOI: https://www.doi.org/10.1080/10888705.2023.2186787

Wibisono FJ, Wirjaatmadja R, Candra AYR, Rahmaniar RP, Trirahayu RA, Diningrum DP, Rianto V, and Kendek IA (2024). The detection of resistant *Escherichia coli* isolated from cats in Dukuh Kupang Sub-District, Surabaya. Acta Veterinaria Indonesiana, 12(1): 1-7 DOI: https://www.doi.org/10.29244/avi.12.1.1-7

Wibisono FM, Faridah HD, Wibisono FJ, Tyasningsih W, Effendi MH, Witaningrum AM, and Ugbo EN (2021). Detection of inva virulence gene of multidrug-resistant Salmonella species isolated from the cloacal swab of broiler chickens in Blitar district, East Java, Indonesia. Veterinary World, 14(12): 3126-3131. DOI: https://www.doi.org/10.14202%2Fvetworld.2021.3126-3131

Zhao X, Ju Z, Wang G, Yang J, Wang F, Tang H, and Sun S (2021). Prevalence and antimicrobial resistance of *Salmonella* isolated from dead-in-shell chicken embryos in Shandong, China. Frontiers in Veterinary Science, 8: 581946. DOI: https://www.doi.org/10.3389/fvets.2021.581946

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 380-388.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj45
PII: S232245682400045-14

Efficacy of Avocado Oil Cream in Wound Repair: Macroscopic Analysis, Inflammatory Cells Count, and Collagen Density

Dini Agusti Paramanandi[1,2], Husnur Rukyat[1], Yuli Purwandari Kristianingrum[3], and Sitarina Widyarini[3]*

[1]*Master student, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[2]*Laboratory of Veterinary Histology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia*
[3]*Department of Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia*

*Corresponding author's Email: sitarina@ugm.ac.id

ORIGINAL ARTICLE
Received: July 24, 2024
Revised: August 17, 2024
Accepted: September 08, 2024
Published: September 25, 2024

ABSTRACT

The wound healing process goes through a series of complex stages that are mutually continuous, namely inflammation, proliferation, and maturation. Following wound formation and the release of proinflammatory cytokines and growth factors, inflammation occurs immediately after the hemostasis phase. Proliferation occurs when products processed by the growth factors are present. The final stage, maturation, is portrayed by the plan of the extracellular network. This study utilized oil from avocado fruit (*Persea americana*), which contains linoleic and oleic acid content that supports skin tissue repair. The study aimed to explore the effects of using avocado oil cream on the area of wound healing, the number of inflammatory cells, and the collagen density. Twenty-four female mice, aged eight weeks, were used as experimental animals by making excision wounds using a 4 mm biopsy punch on the dorsal skin on the left and right sides. The mice were divided into four groups based on the percentage of avocado oil in the topical cream included Group K (control, topical cream without avocado oil), Group P1 (5% topical avocado oil cream), Group P2 (10% topical avocado oil cream), and Group P3 (15% topical avocado oil cream). Macroscopic examination of the wounds was conducted daily on days 3, 6, and 9 after topical cream treatment using a digital caliper. A total of 48 skin tissue samples were collected from days 3, 6, and 9 after cream application, which were then processed for histopathology evaluations using hematoxylin-eosin staining and Masson's Trichrome staining. Hematoxylin-eosin staining was used to count the inflammatory cells, and Masson's Trichrome staining was employed to assess collagen density. The results revealed that avocado oil had a great impact on wound closure after 9 days of 15% avocado oil cream treatment, reducing the inflammatory cells after 3-6 days of 10% avocado oil cream therapy, and increasing collagen density after 9 days of 15% avocado oil cream application, as compared to the control, non-avocado oil cream group. Avocado oil can help close wounds, reduced the number of inflammatory cells, and increased collagen density when used in topical pharmaceutical formulations. Avocado oil cream may, therefore, be considered a viable option for wound repair treatment.

Keywords: Avocado oil, Collagen, Inflammatory cell, Wound repair

INTRODUCTION

The skin, as the largest organ in the animal's body, plays an essential role as the first line of defence against external threats. Damage to the skin, such as wounds, results in a loss of anatomical and functional integrity (Poljsak et al., 2019). Closing wounds and repairing damaged tissues are critical for restoring skin function to its original state, with an emphasis on minimizing healing time and side effects (Landen et al., 2016). In recent decades, research related to wound healing has continued to be carried out both from the molecular systematics of wound healing and the choice of therapies that can be used to obtain the best healing conditions. The treatments using natural ingredients to repair damaged skin tissue have recently gained popularity. In this line, phytomedicine is a field of science that studies using plants as ingredients for therapy or preventing damage to the body (Sichani et al., 2021). In Indonesia, a country rich in natural biodiversity and resources, research on herbal therapies has grown significantly, providing opportunities to explore alternative wound healing treatments using locally available natural resources.

Avocado (*Persea americana*), a fruit native to the Americas, is rich in vitamin B12, calcium, iron, magnesium, protein, and fibres. Avocado also acts as an anti-aging agent, prevents stroke, has analgesic and anti-inflammatory effects, is anti-diabetic and anti-ulcer, helps wound healing, and contains antioxidants (Ranade and Thiagarajan, 2015; Gupta et al., 2018). The study conducted by de Oliveira et al. (2013) was on utilizing avocado oil for wound healing therapy in mice. Avocado oil has been used in treating psoriasis, wrinkles, and stretch marks (Ranade and Thiagarajan, 2015). Moreover, avocado oil is rich in oleic acid and linoleic acid, found also in olives and olive oil, and helpful in skin repair (de Oliveira et al., 2013; Li et al., 2013).

The wound healing process involves a series of complex and continuous stages involved inflammation, proliferation, and maturation. Inflammation begins immediately after the hemostasis phase following the formation of

To cite this paper: Paramanandi DA, Rukyat H, Kristianingrum YP, and Widyarini S (2024). Efficacy of Avocado Oil Cream in Wound Repair: Macroscopic Analysis, Inflammatory Cells Count, and Collagen Density. *World Vet. J.,* 14(3): 380-388. DOI: https://dx.doi.org/10.54203/scil.2024.wvj45

the wound and the release of proinflammatory cytokines and growth factors. Proliferation occurs with the presence of products processed by the growth factors. The final stage of maturation is characterized by the arrangement of the extracellular matrix and scar tissue (Velnar et al., 2009). The overall goal of these wound-healing stages is to achieve normal skin conditions regarding anatomical and physiological functions (Landen et al., 2016). The present study aimed to determine the effects of topical avocado oil cream therapy on the area of wound healing, the number of inflammatory cells, and the collagen density.

MATERIALS AND METHODS

Ethical approval

The Research Ethics Commissions of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia, approved this research under document number 128/EC-FKH/Int./2023.

Study period and location

This research was conducted from November 2023 to February 2024 at the Department of Pathology, Faculty of Veterinary Medicine, Faculty of Dentistry, and Faculty of Medicine, Universitas Gadjah Mada, Indonesia.

Cream preparation

The cream formulation consisted of a mixture of avocado oil and bio-cream. The concentrations of avocado oil cream used in this study were 0%, 5%, 10% and 15%. The avocado oil cream was an oil-in-water (o/w) type of cream in which water was used as a solvent that can dissolve the dissolved components formulated in the cream. Water which was free from toxins, pollutants, and microbes was used in making the cream. The emulsion formed by the water helps to stabilize the oil-in-water mixture, classifying it as an oil-in-water emulsion (Rai et al., 2019).

Animal preparation

The study involved 24 eight-week-old female Deutschland Denken Yoken (DDY) mice, with an average body weight of 30 grams. The mice were divided into four treatment groups with three different time points, with two mice per group. Each mouse had two excisional wounds, resulting in four wounds in each group. The animals were purchased from the Integrated Laboratory for Research and Testing at Universitas Gadjah Mada, Indonesia. The mice were kept individually, in 12h light/dark cycle, and at constant temperature (25^0C). They were given food and water *ad libitum* throughout the experiment.

Wound model and treatments

The laboratory animals were wounded by making excision wounds using a 4 mm biopsy punch on the dorsal skin on the left and right parts (Lin et al., 2003; Masson-Meyers et al., 2020). The excisional wounds were suitable for histological assessment (de Oliveira et al., 2013). The 24 mice were divided into Groups K, P1, P2 and P3. Groups P1, P2, and P3 were treated topically with avocado oil cream at 5%, 10%, and 15% respectively, once daily on the wound sites (Bessera et al., 2020). The control group (Group K) received a topical application of a 0% avocado oil cream. A digital caliper measured the wound area on days 3, 6, and 9 after treatment. After the final measurements, the mice were euthanized by cervical dislocation and necropsied (Liu et al., 2022).

Histological study

The 48 wound tissues were collected and fixed in 10% formalin for 24 hours, and subsequently dehydrated in ethanol, cleared in xylol, embedded in paraffin wax, and sectioned by a microtome (Bessera et al., 2020). The tissue sections (5 µm thickness) were placed on slides and stained with hematoxylin-eosin for inflammatory cell observation and Masson's Trichrome for collagen density assessment. A binocular light microscope (Olympus CX-23, Japan) with 400x magnification was used to observe the number of inflammatory cells and collagen density, with three images captured per tissue. Observation of the number of inflammatory cells in histopathological slides was conducted using a microscope at 400x magnification across 3 fields of view, followed by counting using Image Raster software. Collagen density analysis was performed through microscopic observation (400x) of histopathological slides stained with Masson's Trichrome. Three fields of view were used to obtain the average collagen density using ImageJ software.

Macroscopic study

Measurement of wound areas and wound healing areas were conducted daily from the first day to the ninth day of topical administration by a digital caliper. The wound area was calculated using the Formula 1.

$$A = \pi . rx . ry, \qquad \text{(Formula 1)}$$

where "A" represented the area (mm^2) of the wound, "rx" was the x-axis, and "ry" was the y-axis of the wound.

The wounds were measured and the data were collected on days 3, 6 and 9.

Statistical analysis

The data for the wound areas was analysed statistically using SPPS (ver. 27, 2020). Inflammatory cell counts were performed in the dermis area from three different fields of view using Image Raster 3 software. The collagen density was determined using ImageJ software. The average number of inflammatory cells and collagen density from each group were analysed using the One-way ANOVA test or Kruskall-Wallis's test (SPSS, version 27, 2020, Statistic), with $p < 0.05$ as considered statistically significant.

RESULTS

Wound area

The wound area was measured using a caliper at three time points included 3,6, and 9 days after treatment with cream in Groups K, P1, P2, and P3. In general, the average area of wound healing showed a decrease in wound size after three days of treatment (Figure 1). Topical therapy after six days revealed a declining trend in wound size (Figure 2). Moreover, after nine days of topical medication, the wounds were the most closed in Groups P1, P2, and P3 (Figure 3). Topical treatment with 15% avocado oil cream (Group P3) showed a significantly better reduction in wound size compared to the group without avocado oil (Group K) after three days of therapy ($p < 0.05$). The wound area after 6 days of treatment showed no difference between the groups ($p > 0.05$). However, after nine days of topical application, treatments with 10% (Group P2) and 15% (Group P3) avocado oil cream resulted in significantly better wound healing ($p < 0.05$) compared to the group without avocado oil therapy (Table 1).

Inflammation cells

Inflammatory cells were counted in three different areas of the dermis layer in histopathological images (Figure 4) for all groups. Overall, the average number of inflammatory cells after 3, 6, and 9 days of therapy in the treatment groups declined, as compared to Group K (Table 2), indicating an improved condition. Moreover, significant differences ($p < 0.05$) were observed after treatment with avocado oil cream. After three days, Groups P2 and P3 had significantly fewer inflammatory cells than Group K ($p < 0.05$). Similar results were found for the 6-day medication, where Groups P2 and P3 showed a significantly better reduction in inflammatory cells ($p < 0.05$) compared to the group with 0% avocado oil. Meanwhile, there were no differences ($p < 0.05$) between groups after nine days of therapy.

Collagen density

The density of collagen was assessed from three different regions in Trichrome Masson's histopathological images (Figure 5). Briefly, after three days of therapy, collagen density in Groups P1, P2, and P3 showed an increasing trend compared to Group K, although there were no significant differences between the groups ($p > 0.05$). On days 6 and 9, collagen density increased significantly in the groups treated with avocado oil cream ($p < 0.05$). Six days after treatment, Group P3 had significantly higher collagen density ($p < 0.05$) than Group K. Meanwhile, by the ninth day of treatment, Group P2 showed significantly greater collagen density ($p < 0.05$) compared to Group K (Table 3).

Table 1. Means number of wound sizes at 3, 6, and 9 days of treatment (mm^2) with avocado oil cream

Group (Avocado oil cream concentration) \ Day of treatment	3	6	9
K (0%)	11.61 ± 1.46^{a}	4.71 ± 1.29^{e}	2.55 ± 0.32^{i}
P1 (5%)	10.97 ± 2.00^{ab}	4.49 ± 1.22^{e}	1.99 ± 0.41^{ij}
P2 (10%)	8.41 ± 1.78^{ab}	5.56 ± 1.98^{e}	1.41 ± 0.50^{jk}
P3 (15%)	8.06 ± 0.81^{b}	4.26 ± 2.56^{e}	0.95 ± 0.20^{kl}
P value	0.016	0.339	0.009

K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: cream with 15% avocado oil. ^{f} to ^{e} and ^{jk} to ^{i}: Mean within a column with different lowercase superscripts differ significantly ($p < 0.05$).

Table 2. Means number of inflammatory cell measurements (cell) at 3, 6, and 9 days of treatment with avocado oil cream

Group (Avocado oil cream concentration) \ Day of treatment	3	6	9
K (0%)	401.33 ± 50.19^{a}	258.75 ± 14.98^{e}	119.92 ± 13.85^{i}
P1 (5%)	284.83 ± 37.76^{ab}	191.17 ± 12.68^{ef}	103.83 ± 9.02^{i}
P2 (10%)	168.08 ± 6.19^{b}	155.08 ± 15.65^{f}	104.58 ± 11.14^{i}
P3 (15%)	169.50 ± 41.42^{b}	152.08 ± 22.83^{f}	89.75 ± 13.12^{i}
P value	0 < 0.001	0.003	0.4

K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: cream with 15% avocado oil. ^{f} to ^{e} and ^{jk} to ^{i}: Mean within a column with different lowercase superscripts differ significantly ($p < 0.05$).

Table 3. Means number of collagen density measurements (%) at 3, 6, and 9 days of treatment with avocado oil cream

Group (Avocado oil cream concentration) \ Day of treatment	3	6	9
K (0%)	16.97 ± 1.26^{a}	23.06 ± 4.35^{e}	44.13 ± 2.91^{i}
P1 (5%)	17.67 ± 3.75^{a}	25.83 ± 3.56^{ef}	46.27 ± 3.05^{ij}
P2 (10%)	18.59 ± 5.30^{a}	29.84 ± 3.38^{ef}	51.78 ± 1.06^{jk}
P3 (15%)	19.11 ± 1.33^{a}	31.55 ± 2.66^{f}	49.23 ± 3.28^{ij}
P value	0.81	0.02	0.09

K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil. ^{f} to ^{e} and ^{jk} to ^{i}: Mean within a column with different lowercase superscripts differ significantly ($p < 0.05$).

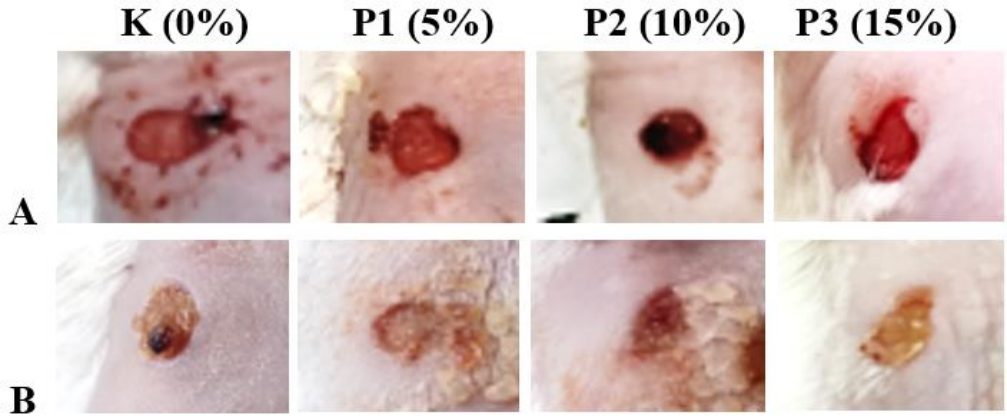


Figure 1. Wound healing after three days of avocado oil cream treatment in mice. K: Cream with no avocado oil; P1: cream with 5% avocado oil; P2: Cream with 10% avocado oil; P3: Cream with 15% avocado oil; **A**: Day 0, **B**: Day 3

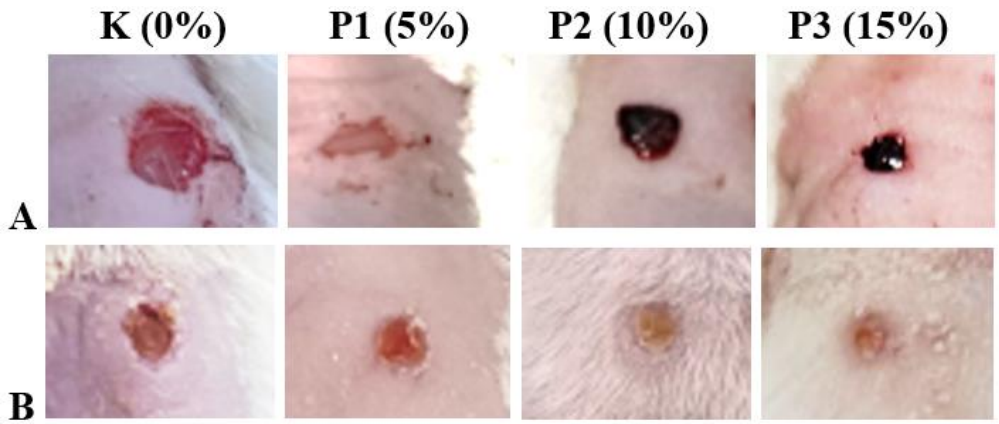


Figure 2. Wound healing after six days of treatment with avocado oil cream in mice. K: Cream with no avocado oil; P1: cream with 5% avocado oil; P2: Cream with 10% avocado oil; P3: Cream with 15% avocado oil; **A**: Day 0, **B**: Day 6

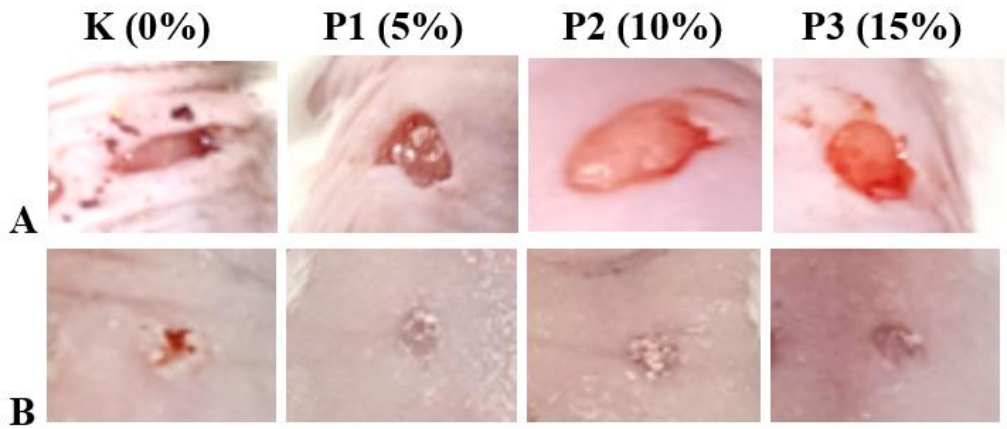


Figure 3. Wound healing after nine days of treatment with avocado oil cream in mice. K: Cream with no avocado oil; P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil; **A**: Day 0, **B**: Day 9.

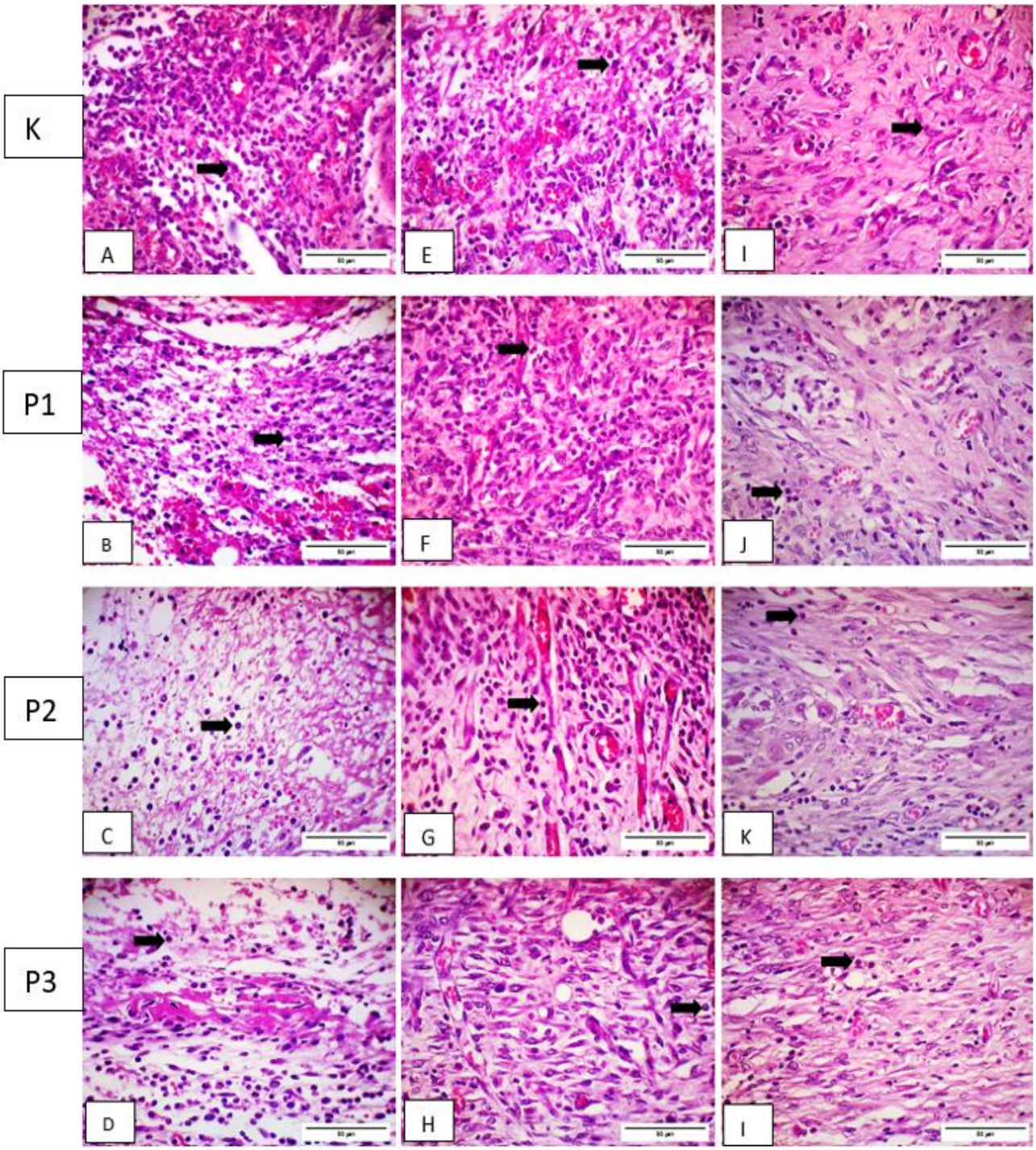


Figure 4. Inflammatory cell in wound healing with avocado oil cream in mice on days 3, 6 and 9. Day 3 (**A-D**), day 6 (**E-H**), day 9 (**I-L**). K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil, black arrow: Inflammatory cell, magnification 400x.

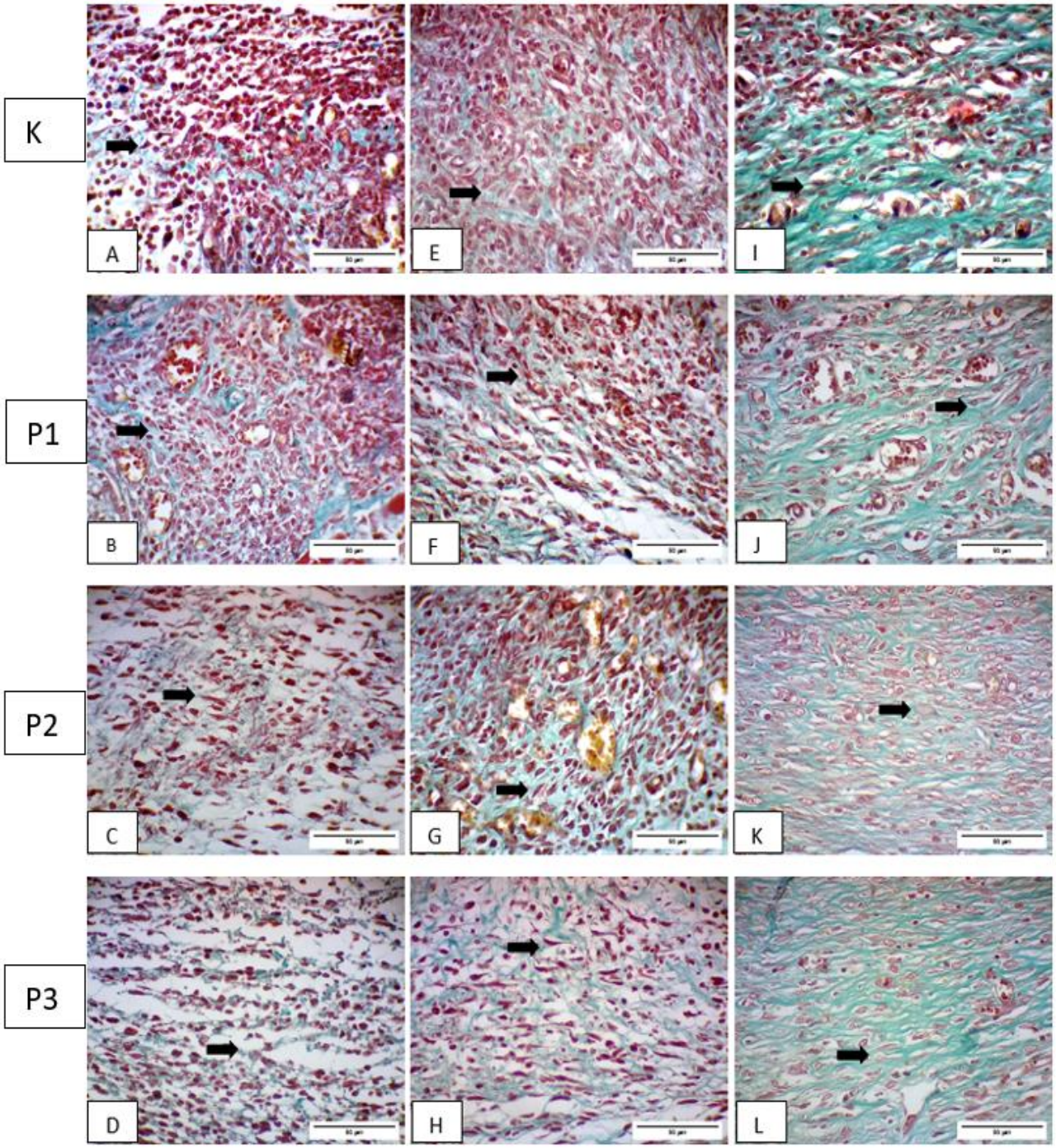


Figure 5. Collagen density (Trichrome Masson) in wound healing with avocado oil cream in mice on days 3, 6, and 9. Day 3 (**A-D**), day 6 (**E-H**), day 9 (**I-L**). K: Cream with no avocado oil, P1: Cream with 5% avocado oil, P2: Cream with 10% avocado oil, P3: Cream with 15% avocado oil. Black arrow: Collagen, 400x, 2024.

DISCUSSION

The present study provided evidence that avocado oil cream significantly affects wound healing. The wound healing process included the three stages of inflammation, proliferation, and maturation. The inflammation stage occured from the time of wound formation until three days later (Kaifa et al., 2021). The proliferation stage typically begins on the fourth day of wound healing (Wahyuni et al., 2024). The maturation stage was the final stage of wound healing, characterized by wound closure and the restoration of tissue strength to 20–80% of its original state (Wahyuni et al., 2024). The wound area in Groups P1, P2, and P3 throughout the 9 days yielded data that corroborate the macroscopic image of a closed wound with a wound size of 1.99 + 0.41 mm^2, 1.41 $\pm$ 0,50 mm^2 and 0.95 $\pm$ 0.20 mm^2. These results suggested that avocado oil had the potential to be an alternative wound therapy. According to research by de Oliveira et al. (2013), avocado oil possesses anti-inflammatory properties. The oil was rich in essential fatty acids, such as linoleic acid and oleic acid, which act as anti-inflammatory agents. These two acids prevent the inflammatory process from going beyond the standard wound healing time so that chronic wound conditions do not occur. The mechanism inhibited

the multiplication of inflammatory cells (Lin et al., 2018; Pegoraro et al., 2021). Linoleic acid enhances wound closure by shortening the bleeding time and stabilizing fibrin and fibroblast migration. Another role of linoleic acid was to accelerate the inflammatory phase allowing the proliferation phase to commence sooner, thereby shortening the wound healing period (Silva et al., 2018). According to Pegoraro et al. (2021), oleic acid can inhibit the occurrence of oedema, showing its anti-inflammatory effect. Moreover, topical administration of linoleic and oleic acid in the wound healing process in rats had resulted in significant closure in the wound area (Ishak et al., 2019).

The findings of the current study reveal that avocado oil cream reduced the number of inflammatory cells in the process of wound healing. At 3 - 6 days post-treatment, both 10% and 15% avocado oil creams significantly reduced the number of inflammatory cells compared to the group without avocado oil ($p < 0.05$). However, after 9 days of treatment, there were no significant differences ($p > 0.05$) between the groups. Oedema and infiltration of inflammatory cells were the main changes in wound healing in the dermis layer (Widyarini et al., 2023). The proliferation stage can begin when the number of inflammatory cells decreases (Lin et al., 2003; Velnar et al., 2009). When the inflammatory response was working well and the number of inflammatory cells was higher, as observed on days 3 and 6 in comparison with day 9, the wound is healing well (Wosgrau et al., 2015). According to the study by Pegoraro et al. (2021), topical oleic acid therapy has anti-inflammatory effects; therefore, it can be an alternative to cutaneous inflammation therapy. Linoleic and oleic acids were two types of compounds that were involved in the inflammatory process. The wound can receive a significant influx of neutrophils from both acids. Linoleic corrosive effect influences aggravation through neutrophil movement (Silva et al., 2018; Guidoni et al., 2019). Neutrophils control the beginning of wound healing through cell migration to the inflammatory area, phagocytosis of cellular debris and microorganisms, and the release of pro-inflammatory cytokines (Pereira et al., 2008).

Collagen was one of the main components in the wound healing process. The results of this study indicated a significant difference ($p < 0.05$) in collagen density after 6 days of treatment with 15% avocado oil cream compared to the control group (Group K). Furthermore, after 9 days of therapy with avocado oil cream, a significant increased ($p < 0.05$) in collagen density was observed with 10% avocado oil cream compared to the group without avocado oil. Collagen was a connective tissue that provides a structural framework for tissue regeneration (Al-Henhena et al., 2011). Fibroblasts were cells responsible for synthesizing collagen. Progressive collagen synthesis will support the formation of excellent and optimal connective tissue (Sembiring et al., 2021). Collagen production in wound healing will increase because it repairs damaged or lost tissue (Gunawan et al., 2019). Collagen induced platelet activation and aggregation, resulting in the deposition of fibrin clots at the wound site. The inflammatory phase encourages the proliferation of fibroblasts that synthesize collagen and extracellular matrix. Collagen produced a robust inflammatory response, thus paving the way for faster wound healing (Mathew-Steiner et al., 2021). The initial process of the proliferation stage was assisted by fibroblasts that will produce collagen. Collagen will connect the tissues in the wound to help restore the strength of the skin tissue and accelerate wound closure (Sentat and Permatasari, 2015). During the maturation stage, young collagen will develop into mature collagen. Young collagen undergoes degradation or restructuring to become more prominent, organized, and bundle-shaped, eventually forming dermal tissue (Bodas and Shinde, 2021).

Previous research by de Oliveira et al. (2013) and Lin et al. (2018) demonstrated that topical application of avocado oil on injured rat skin significantly increased collagen levels. Oleic and linoleic acids were active ingredients in avocado oil that support wound healing. Oleic acid has been shown to restore and enhance collagen levels in both acute and chronic wounds (Ventura et al., 2021). Linoleic acid played a vital role in the expression of fibrinolytic system components that regulate collagen production (Ferreira et al., 2011). The release of cytokines was enhanced by linoleic acid, which also promotes the growth and differentiation of fibroblasts, keratinocytes, and endothelial cells. These processes accelerated the synthesis of collagen, which in turn aids in tissue healing. The composition of these two acids promotes the production of new collagen to replace damaged collagen, which aids in the healing process of wounds (Ishak et al., 2019). The notable findings of this study included the efficacy of 15% avocado oil cream in achieving significant wound closure after 9 days of treatment, 10% avocado oil cream in effectively reducing inflammatory cells within 3-6 days, and 15% avocado oil cream in significantly increasing collagen density after 9 days of application.

CONCLUSION

The application of avocado oil has demonstrated a great impact on wound closure after 9 days of treatment with 15% avocado oil cream, reducing the inflammatory cells after 3-6 days of 10% avocado oil cream therapy, and increasing collagen density at 15% of avocado oil application after 9 days. Future studies should focus on extending treatment duration, exploring higher concentrations of avocado oil, and assessing specific cytokine expressions to provide more detailed insights into its wound-healing mechanisms.

DECLARATIONS

Authors' contributions

Dini Agusti Paramanandi conceptualized, managed, and conducted data analysis and interpretation. Dini Agusti Paramanandi and Husnur Rukyat performed all the experimental procedures. Yuli Purwandari Kristianingrum

supervised the study. Sitarina Widyarini conceptualized and supervised the study. All authors read and approved the final manuscript.

Competing interests

The authors have not declared any conflict of interest.

Acknowledgments

The authors would like to thank the Indonesia Endowment Fund for Education Agency, Faculty of Veterinary Medicine, Faculty of Dentistry, and Faculty of Medicine, University Gadjah Mada Yogyakarta, Indonesia.

Ethical consideration

The authors confirm that the manuscript has been checked for plagiarism and submitted to this journal originally.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. For inquiries, please contact the corresponding author.

Funding

This research was funded by the Indonesia Endowment Fund for Education Agency Scholarship.

REFERENCES

Al-Henhena N, Mahmood AA, Al-magrami A, Syuhada ABN, Zahra AA, Summaya MD, Suzi MS, and Salmah I (2011). Histological study of wound healing potential by ethanol leaf extract of strobilanthes crispus in rats. Journal of Medicinal Plants Research, 5(16): 3660-3666. Available at: https://academicjournals.org/journal/JMPR/article-full-text-pdf/17D81E520829

Bessera FP, Gushiken LF, Vieira AJ, Bergamo DA, Bergamo PL, Oliveira de Souza M, Hussni CA, Takahira RK, Nobrega RH, Martinez ERM et al. (2020). From inflammation to cutaneous repair: Topical application of Lupeol improves skin wound healing in rats by modulating the cytokine levels, NF-Kb, Ki-67, growth factor expression, and distribution of collagen fibers. International Journal of Molecular Science, 21(14): 4952. DOI: https://www.doi.org/10.3390/ijms21144952

Bodas K and Shinde V (2021). Healing of wounds: A detailed review on models, biomarkers, biochemical and other wound assessment parameters. International Journal of All Research Education and Scientific Methods, 9(3): 2069-2085. Available at: https://www.ijaresm.com/healing-of-wounds-a-detailed-review-on-models-biomarkers-biochemical-and-other-wound-assessment-parameters

de Oliveira AP, de Souza Franco E, Barreto RR, Cordeiro DP, de Melo RG, de Aquino CMF, de Silva AAR, de Medeiros PL, da Silva TG, da Silva Góes AJ et al. (2013). Effect of semisolid formulation of *Persea americana* Mill (avocado) oil on wound healing in rats. Evidence-Based Complementary and Alternative Medicine, 2013: 472382. DOI: https://www.doi.org/10.1155/2013/472382

Ferreira AM, de Souza BMV, Rigotti MA, and Loureiro MRD (2011). The use of fatty acids in wound care: An integrative review of the Brazilian literature. Revista da Escoka de Enfermagem da USP, 46(3): 745-753. DOI: https://www.doi.org/10.1590/s0080-62342012000300030

Guidoni M, Scherer MMD, Figueria MM, Schmitt EFP, Almeida LC, Scherer R, Bogusz S, and Fronza M (2019). Fatty acid composition of vegetable oil blend and *in vitro* of pharmacotherapeutic skin care applications. Brazilian Journal of Medical and Biological Research, 52(2): 1-8. DOI: https://www.doi.org/10.1590/1414-431x20188209

Gunawan SA, Berata IK, and Wirata IW (2019). Histopathology of skin recovery incision wound in rar post giving extracellular matrix (ECM) from Prok's vesica uruniaria. Indonesia Medicus Veterinus, 8(3): 313-324. Available at: https://ojs.unud.ac.id/index.php/imv/article/view/51218

Gupta Sk, Singhal P, Singh A, Chauhan R, and Kumar B (2018). Nutritional and pharmaceutical benefits of avocado plant. Journal of Advanced Scientific Research, 9(2): 4-11. Available at: https://www.sciensage.info/index.php/JASR/article/download/284/533

Ishak WMW, Katas H, Yuen NP, Abdullah MA, and Zulfakar MH (2019). Topical application of omega-3, omega-6, and omega-9-rich oil emulsion for cutaneous wound healing in rats. Drug Delivery and Translational Research, 9: 9418-9433. DOI: https://www.doi.org/10.1007/s13346-018-0522-8

Kaifa A, Irramah M, and Aliska G (2021). The effect of carrot extract (*Daucus carota* L.) ointment on burn healing in rats (*Rattus novergicus*) in the proliferative phase. Archives Pharmacia, 3(2): 94-108. Available at: https://ejurnal.esaunggul.ac.id/index.php/AP/article/view/4476/3157

Landen NX, Li D, and Stahle M (2016). Transition from inflammation to proliferation: A critical step during wound healing. Cellular and Molecular Life Science, 73: 3861-3885. DOI: https://www.doi.org/10.1007/s00018-016-2268-0

Li Z, Wong A, Henning SM, Zhang Y, Jones A, Zerlin A, Thames F, Bowerman S, Tseng C, and Heber D (2013). Hass avocado modulates prosprandial vascular reactivity and postprandial inflammatory response to a hamburger meal in healthy volunteers. Food & Function, 4: 384-391. DOI: https://www.doi.org/10.1039/c2fo30226h

Lin T, Zhong L, and Santiago JL (2018). Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. International Journal of Molecular Sciences, 19(70): 1-20. DOI: https://www.doi.org/10.3390/ijms19010070

Lin ZQ, Kondo T, Ishida Y, Takayasu T, and Mukaida N (2003). Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. Journal of Leukocyte Biology, 73(6): 713-721. DOI: https://www.doi.org/10.1189/jlb.0802397

Liu C, Xu Y, Lu Y, Du P, Li X, Wang C, Guo P, Diao L, and Lu G (2022). Mesenchymal stromal cells pretreated with proinflammatory cytokines enhance skin wound healing via IL-6-dependent M2 polarization. Stem Cells Research & Therapy, 13: 1-17. DOI: https://www.doi.org/10.1186/s13287-022-02934-9

Masson-Meyers DS, Andrade TA, Caetano GF, Guimares FR, Leite MN, Leite SN, and Frade MAC (2020). Experimental models and methods for cutaneous wound healing assessment. International Journal of Experimental Pathology, 101(1-2): 21-37. DOI: https://www.doi.org/10.1111/iep.12346

Mathew-Steiner SS, Roy S, and Sen CK (2021). Collagen in wound healing. Bioengineering, 8(63): 1-15. DOI: https://www.doi.org/10.3390%2Fbioengineering8050063

Pegoraro NS, Componogara C, Cruz L, and Oliveira SM (2021). Oleic acid exhibit and expressive anti-inflammatory effect in croton oil-induced irritant contact dermatitis without the occurrence of toxicological effects in mice. Journal of Ethnopharmacology, 267: 113486. DOI: https://www.doi.org/10.1016/j.jep.2020.113486

Pereira LM, Hatanaka E, Martins EF, Oliveira F, Liberti E, Farsky SH, Curi R, and Pithon-Curi TC (2008). Effect of oleic and linoleic acids on the inflammatory phase of wound healing in rats. Cell Biochemical Function, 26: 197-204. DOI: https://www.doi.org/10.1002/cbf.1432

Poljsak N, Kreft S, and Glavac NK (2019). Vegetable butters and oils in skin wound healing: Scientific evidence for new opportunities in dermatology. Phytotherapy Research, 34(2): 254-269. DOI: https://www.doi.org/10.1002/ptr.6524

Sentat T and Permatasari R (2015). Activity test of ethanol extract of avocado leaves (*Persea americana* mill.) on healing burn wounds on the backs of male white mice (*Mus musculus*). Jurnal Ilmiah Manuntung, 1(2): 100-106. DOI: http://www.doi.org/10.53675/jsfar.v10i2.985

Sichani MR, Farid M, and Khorasgani EM (2021). Histomorphological examination of skin wound healing under the effect of avocado oil in Wistar rats. Acta Veterinaria Eurasia, 47: 121-128. DOI: http://www.doi.org/10.5152/actavet.2021.20096

Rai P, Poudyl AP, and Das S (2019). Pharmaceutical creams and their use in wound healing: A review. Journal of Drug Delivery & Therapeutics. 9(3): 907-912. DOI: http://www.doi.org/10.22270/jddt.v9i3-s.3042

Ranade SS and Thiagarajan P (2015). A review on *Persea americana* Mill (avocado) – Its fruit and oil. International Journal of PharmTech Research, 8(6): 72-77. Available at: https://www.researchgate.net/publication/283777915_A_review_on_Persea_Americana_Mill_Avocado-_Its_fruit_and_oil

Sembiring ICB, Jayawardhita AAG, and Adi AAAM (2021). Muntingia calabura's leaves extract ointment increased collagen density and enhanced healing of skin incision wound in hyperglycemic mice. Indonesia Medicus Veterinus, 10(2): 189-199. DOI: http://www.doi.org/10.19087/imv.2021.10.2.189

Silva JR, Burger B, Kuhl CMC, Candreva T, dos Anjos MBP, and Rodrigues HG (2018). Wound healing and omega-6 fatty acids: From inflammation to repair. Mediators of Inflammation, 2018: 2503950. DOI: https://www.doi.org/10.1155/2018/2503950

Velnar R, Bailey T, and Smrkolj V (2009). The wound healing process: An overview of the cellular and molecular mechanisms. The Journal of International Medical Research, 37(5): 1528-1542. DOI: https://www.doi.org/10.1177/147323000903700531

Ventura ACSSB, de Paula T, Goncalves, JP, Soley BS, Cretella, ABM, Otuki MF, and Cabrini DA (2021). The oil from *Moringa oleifera* seeds accelerates chronic skin wound healing. Phytomedicine Plus, 1(3): 100099. DOI: https://www.doi.org/10.1016/j.phyplu.2021.100099

Wahyuni S, Notodiputro KA, Oktarani SD, and Mualifah LNA (2024). The effect of *Chlorella vulgaris* ointment aplicated on incision wound of mice (*Mus musculus* albinus). Jurnal Veteriner dan Biomedis, 2(1): 16-21. DOI: https://www.doi.org/10.29244/jvetbiomed.2.1.16-21.

Widyarini S, Sugiyono, Akrom AM, and Paryuni AD (2023). Carrageenan-induced acute inflammation on back-skin of mice: Histopathological features, number of inflammatory cells, and expression of COX-2, COX-1, and IL-6. World Veterinary Journal, 13(4): 520-530. DOI: https://www.doi.org/10.54203/scil.2023.wvj55

Wosgrau ACC, Jeremias TD, Leonardi DF, Pereima MJ, Giunta GD, and Trentin AG (2015). Comparative experimental study of wound healing in mice: Pelnac versus integra. Plos One, 10(3): e0120322. DOI: https://www.doi.org/10.1371/journal.pone.0120322

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 389-399.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj46
PII: S232245682400046-14

Practical Management of Sheep Farming in Eastern Algeria: Situation, Constraints and Perspectives

Rahla Meziane[1]*, Abdelhak Karim Mouss[2], Dalila Hammouche[2], Mouzdalifa Boughris[1], and Farid Boughris[3]

[1]*Department of Veterinary Sciences, Institute of Veterinary Sciences and Agronomic Sciences, University of Batna1, Batna 05000, Algeria*
[2]*Faculty of Natural and Life Sciences and Earth Sciences, Djilali Bounaama University, Khemis Miliana, Ain Defla 44000, Algeria*
[3]*Practicing veterinarian, Ain Yagout, Batna 05000, Algeria*

*Corresponding author's Email: rahla.meziane@univ-batna.dz

ORIGINAL ARTICLE
Received: July 13, 2024
Revised: August 17, 2024
Accepted: September 12, 2024
Published: September 30, 2024

ABSTRACT

Successful sheep farming requires hands-on management and a thorough understanding of the factors that influence it. This necessitates a practical, well-organized approach to ensure the flock's health, productivity, and profitability. Various factors, such as climate, available resources, and management strategies, play a crucial role in determining the success of sheep farming. This study, conducted in the Batna region of Algeria, aimed to assess sheep breeding practices and production performance through a questionnaire distributed to farmers and veterinarians. The study encompassed 14,124 sheep, including 9,435 ewes, from 33 Batna region farms to evaluate production and reproduction performance. The findings revealed that the Ouled Djellal breed is the most prevalent, comprising 61.02% of the sheep population. The results indicated that sheep farming in the region largely relies on traditional extensive grazing systems. It is worth noting that breeding practices have not consistently followed recommended guidelines, as evidenced by a suboptimal sex ratio of 36.28. Additionally, only 21.94% of breeders had employed heat synchronization methods, and artificial insemination was not utilized. The low adoption of artificial insemination is primarily attributed to factors, including a lack of knowledge regarding reproductive management, along with challenges related to illiteracy and limited access to essential resources. The analysis further demonstrated that all categories of sheep were profitable; however, profitability was influenced by factors, such as environmental conditions, feed availability, and the age of the animals. The study underscored significant findings, including the prevalence of the Ouled Djellal breed and the limited use of advanced breeding practices, such as artificial insemination, in the Batna region. The value of the current study lies in its comprehensive examination of traditional sheep farming practices and its recommendations for enhancing productivity. These include improving management practices, increasing access to resources, and promoting genetic improvement by adopting advanced breeding technologies.

Keywords: Ewe, Livestock management, Nutrition, Ouled Djellal, Performance

INTRODUCTION

Sheep farming is a valuable national asset in Algeria. Historically, it has been vital to the livelihoods of Algerian nomadic and semi-nomadic tribes, providing meat, milk, wool, and hides—resources essential for survival in arid and semi-arid environments. Today, it remains a primary source of income for populations in regions, such as the Algerian steppe (Slimani et al., 2021). It ranks among the most critical sectors of agriculture (Douh et al., 2024), as demonstrated by its substantial growth from 22.87 million to over 30.90 million heads between 2010 and 2020 (FAO, 2021).

Despite the increasing number of sheep, inadequate breeding management practices result in low yields (Saidi et al., 2020). Most sheep are concentrated in steppe regions and high semi-arid cereal plains (Aidoud et al., 2006), particularly in environments, such as the Saharan Atlas and the High Plateaus (Saidi et al., 2020). The predominant farming system is extensive, making it vulnerable to climatic risks and lacking proper healthcare infrastructure. The primary breeds raised include "Ouled Djellal," "Hamra," "Rembi", and "D'men" (Moula, 2018). Food availability determines the rationing mechanism, which is currently not well-defined. Typically, the ration consists of dry feed and concentrate, while transhumant herds benefit from access to green forage (Saidi et al., 2020). Furthermore, the diversity of sheep breeds, adapted to harsh environmental conditions, represents a crucial advantage, ensuring a stable and secure livestock resource for the country (Dekhili, 2010).

Despite the challenges posed by climate and food availability, sheep farming remains widely practiced in Batna Province. This region accounts for more than 50% of the national red meat production and significantly contributes to job creation (MARD, 2021). However, sheep farming is conducted on most farms using traditional methods (Bencherif, 2011). As a result, there is only limited knowledge of the significant constraints hindering the development of this industry (Missoko et al., 2020).

The success of sheep farming largely depends on effective management, particularly in understanding the key factors influencing productivity. However, current practices need to be improved, especially regarding nutrition and

To cite this paper: Meziane R, Mouss AK, Hammouche D, Boughris M, and Boughris F (2024). Practical Management of Sheep Farming in Eastern Algeria: Situation, Constraints and Perspectives. *World Vet. J.,* 14(3): 389-399.DOI: https://dx.doi.org/10.54203/scil.2024.wvj46

reproduction. The present study aimed to characterize sheep farming in the semi-arid region of eastern Algeria (Province of Batna) using data collected from farms and livestock monitoring. It also sought to identify the regional strengths and challenges of sheep farming and to propose solutions for improving management practices and promoting the sustainable development of this sector.

MATERIALS AND METHODES

Ethical approval

The present study adhered to the ethical guidelines established by the Institutional Animal Care Committee of Algeria's National Administration of Higher Education and Scientific Research (Law 98-11, August 22, 1998). The welfare of the animals was prioritized throughout the investigation, and all animal rights concerns were appropriately addressed. No animals experienced distress during the study. All participating farmers and veterinarians did so voluntarily and were informed they could withdraw anytime. Verbal consent was obtained after thoroughly explaining the purpose and significance of the study to the participants.

Study design and locations

The study was conducted across various locations within Batna Province, Algeria, from October 2020 to October 2021, spanning 12 months. Data on herd structure, feeding practices, reproduction, production, pathologies, and treatments were collected daily through documentation and interviews with resource persons, such as farmers and veterinarians. The data collection method depended on the participants' availability and ease of providing information. The methodology primarily relied on information gathered through investigations conducted over the year. A structured survey, incorporating both closed-ended and open-ended questions, was employed. The closed-ended questions gathered quantitative data on sheep breeding practices and production performance. In contrast, the open-ended questions provided qualitative insights into the experiences and challenges faced by farmers and veterinarians. This mixed-method approach facilitated a comprehensive analysis of numerical data alongside in-depth narrative responses.

Study area

Batna Province, Algeria, is a semi-arid region characterized by cold, humid winters and hot summers (DPSB, 2021). The region is located at an altitude of approximately 1,050 meters (3,445 feet) above sea level, with an average annual temperature of 13.5°C (56.4°F) and annual rainfall of around 496 mm (DPSB, 2021). Agriculture, particularly livestock farming, plays a vital role in the local economy, with sheep farming especially prominent. Data were collected over 12 months through field visits to communes, including Ain Yagout, Djerma, and El Madher, recognized for their dairy production. The region supports a substantial sheep population of 1,137,361 (DSA, 2021).

Animal component

The selection of farms and livestock numbers was determined by the availability of farmers and accessibility to the farms. The study involved 14,124 sheep, including 9,435 ewes of varying ages and healthy breeds, from 33 private, traditional farms located across multiple municipalities within the study area.

Questionnaire development and data collection

To analyze the sheep farms in the study region data was collected during routine farm visits. A structured questionnaire comprising closed- and open-ended questions was developed and distributed to farmers and veterinarians. The survey aimed to gather essential information regarding sheep farm management, including breeds, diets, breeding methods, and production details.

Statistical analysis

Averages and histograms for each studied parameter were calculated and plotted using Excel 2007. Descriptive statistics were employed to assess the quantitative parameters.

RESULTS AND DISCUSSION

Census of farmers

The analysis of Table 1 indicates that the surveyed farmers were between 40 and 65 years old, and all participants were male. Notably, 60% of the farmers were over 60, 33% were between 50 and 60, and only 6% were aged 40 to 50. This age distribution reflects a general disinterest among younger generations in sheep farming as they pursue other activities outside of animal husbandry. This trend limits the development of the sheep farming sector within the study

area. Younger generations may view sheep farming as less profitable than other career options, with urbanization and modern lifestyles contributing to a growing detachment from traditional agricultural practices. Additionally, sheep farming can be physically demanding, and younger individuals may prefer career paths that are more technology-driven or less labor-intensive. These factors collectively explain the declining interest in sheep farming as a viable career choice among the younger population.

Most of the farmers (90%) did not engage in other professional activities and had an average level of education. They have practiced sheep farming for over 20 years, motivated by economic reasons and the desire to preserve their family heritage passed down from father to son. Only 10% of the surveyed farmers pursued other professions, including teaching or commerce. These findings indicate that farmers possess significant experience in sheep farming. However, the relatively low level of education among them poses a challenge, as it may hinder the adoption of modern technologies and practices that could enhance the efficiency and sustainability of the sector.

Addressing this issue requires targeted interventions. Ensuring that farmers have access to education and training programs is crucial for promoting the adoption of innovative practices. Providing subsidies or incentives to encourage biotechnological methods and establishing cooperatives or farmer networks could facilitate the sharing of knowledge and resources. Such initiatives empower farmers to learn about new technologies and improve their skills. According to Faradji et al. (2023), herding in Algeria's steppe region is predominantly managed by older, illiterate individuals, further underscoring the need for education-focused solutions.

Table1. Distribution of sheep farmers in the study area

Age (years)	40 to 50	50 to 60	More than 60	Total
Farmers	2	11	20	33
Percentage	6.06%	33.33%	60.61%	100%

Sheep farming practices

According to the obtained results, sheep farming practices in the region are extensive and primarily based on seasonal nomadism, known locally as *Azaba*. The sheep graze year-round, returning to the farm only in the evening or during the hottest hours of summer and the coldest periods of winter. Flocks typically range in size from 80 to 300 animals. Lambs are raised and later fattened for sale at the market or slaughtered to supply meat. The results indicate that sheep farming in the study region remains traditional and dependent on extensive grazing. Livestock are allowed to roam freely on pasturelands and are brought back to the farm only during specific hours of the day. In the Batna region, livestock management remains traditional mainly, relying on low-quality rangelands with minimal feed supplementation and limited access to veterinary care. This management style is consistent with the extensive practices observed in similar regions, as noted by Boujenah (2023). However, such traditional practices and limited resources may negatively impact animal productivity (Boujenah, 2023).

Breeds

Sheep farming in the study area is deeply embedded in local tradition. It is primarily dominated by the Ouled Djellal breed, which accounted for 61.02% of the sheep population in the survey. Farmers favor this breed due to its superior breeding performance, resilience to harsh conditions (Faradji et al., 2023), excellent meat yield, and exceptional fattening capacity. The Ouled Djellal breed is also the most in-demand among Algerian consumers for livestock on the hoof (MeftiKorteby, 2017).In addition to the Ouled Djellal, other breeds, such as Hamra (15.32%) and mixed breeds, including Rembi and crossbreeds (23.66%), were present. Despite originating from uncontrolled crossings, these breeds are known for producing high-quality meat (Figure 1).

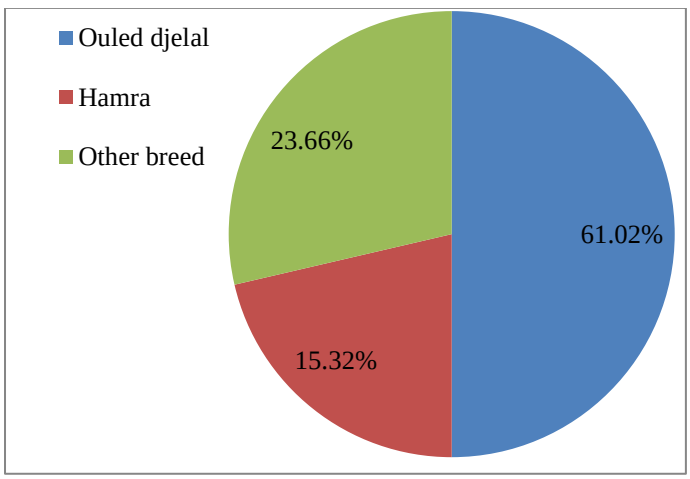


Figure 1. Distribution of sheep breeds in the study region, Batna Province, Algeria

Housing

In the Batna region, most sheep flocks were housed in traditional *zeriba*-style sheep sheds constructed from lightweight and cost-effective materials, such as wood, wire mesh, and sheet metal. A *zeriba* is an open-air animal pen traditionally made from tree and shrub branches, date palm leaves, or even stones (Boujenane, 2023). These enclosures are typically outside the main dwelling and often lack adequate shade (Boujenane, 2023). A minority of flocks were accommodated in modern, semi-covered sheep sheds. In the present study, approximately 72% of farmers housed lambs with adult sheep. Additionally, over half (52%) sheltered their sheep alongside other farm animals, consistent with the results reported by Boujenane (2023), where the percentages were 78.3% and 55.7%, respectively.

The hygiene conditions of these buildings were found to be suboptimal. Approximately 35% of the housing facilities maintained an average standard of hygiene, while the majority (65%) were below acceptable standards. These conditions predispose the flocks to the spread of germs and increase the risk of disease transmission. According to Dechicha et al. (2020), there is a correlation between abortion rates and the use of *zeriba* housing, which is predominantly found in steppe areas where herds are typically managed.

Feeding

Sheep feeding practices in the study region primarily relied on straw as the main roughage feed, supplemented by barley provided by the National Animal Feed Office (ONAB: Office National des Aliments de Bétail). Feed resources varied seasonally, including grazing, straw, hay, grass silage, and barley. These rations were designed to meet the animal's nutritional requirements and promote optimal zootechnical performance (Meradi et al., 2016). Sheep were fed twice daily, once in the morning and once in the evening. During the winter months, their diet consisted of grass, hay, and cereals, while in summer; it primarily comprised hay, cereals, and occasionally grass silage.Watering was generally provided midday, with an average consumption of 2.5 liters per head daily. However, in summer, water access was unrestricted. Feed supplements, such as maize and mineral-vitamin concentrates (CMV), were purchased from the private market. The average feed allocation was typically 1 kilogram per head per day, depending on the size of the flock.

Feeding management

A combination of grazing, straw, hay, cereals, and feed supplements characterized sheep feeding management in the study region. Approximately 10% of farmers opted for concentrated feed, occasionally incorporating cereals like wheat bran due to its cost-effectiveness. Barley was the preferred choice for fattening, with an average of 300 g per head per day. This contrasts with the findings of Boussaada and Benabdelli (2021), who reported quantities closer to 0.75–1 kg per head per day. Small concentrates were typically purchased during spring and summer when pastures were available, while more significant amounts were used in preparation for the sacrifice feast, even during high prices. These results align with those of Mouhous et al. (2015), who similarly reported seasonal fluctuations in concentrate usage, with a notable increase in the lead-up to the sacrifice feast despite elevated costs.

Feed supplementation was uncommon outside the breeding season, although approximately 5% of farmers distributed complementary rations, including 300 g of barley, before breeding (flushing) and during the final third of gestation. These practices were consistent with findings by Mebirouk-Boudechiche et al. (2015), who noted that flushing positively influenced lamb birth weight and ewe milk production, particularly colostrum. Banchero et al. (2023) also reported that supplementing ewes' diets before and during breeding enhanced ovulation and prolificacy rates, with improved nutrition, particularly energy intake, significantly boosting ovulation rates. However, it is essential to emphasize that feed supplements should be administered judiciously, with farmers consulting veterinarians or nutritionists to develop appropriate feeding regimens.

Feeding practices not only affected flock productivity and health but were also critical to the overall success of livestock farming (Deghnouche et al., 2011). High-quality feed during mating significantly improved ewe fertility (Abbas et al., 2004; Paquay et al., 2004; Karfel et al., 2005; Deghnouche et al., 2011). Optimal fertility was achieved when mating coincided with periods of abundant and nutritious feed resources (Slimani et al., 2021). Data analysis highlighted that while farmers adapted their feeding practices, these did not always meet the animals' nutritional needs. The region's extensive feeding systems, marked by limited concentrate use, particularly during periods of forage scarcity, had a detrimental impact on animal production (Khaldi and Dahane, 2011). This issue was exacerbated by degraded pastures, a decline in forage cultivation, severe weather conditions, and recent low rainfall. In such contexts, concentrated feed supplements became essential, although their use varied depending on each farmer's resources.

While some animals could adapt to harsh environments, others struggled to survive, requiring alternative strategies, including migrating to more favorable habitats, changing diet preferences, and activity patterns, or seeking refuge in protected areas (Alary, 2015). Farmers expressed concern over these challenges, particularly after an arid spring. Many sought sustainable solutions to reduce reliance on expensive concentrated feeds due to the scarcity of food

resources and the rising cost of raw materials.

The feeding strategies adopted—barley, barley bran, straw, hay, and supplements—were intended to meet sheep's nutritional requirements at different growth stages. However, it is critical that these methods sufficiently address nutritional needs during growth, gestation, and lactation. Seasonal fluctuations in feed availability can compromise nutrition consistency, affecting overall sheep health and productivity. Therefore, maintaining a balanced diet with appropriate energy levels, protein, vitamins, and minerals is essential for optimal results.

Reproduction

Ram selection

Farmers in the study population primarily prioritized the size of the ram, particularly the length of the tail and horns, as well as its breed, overall size, and age (minimum of two years) when selecting rams for slaughter. Their focus was mainly on external conformation, often neglecting the animal's performance and sexual function. Notably, 40% of farmers did not consider wool color, focusing on meat production. These findings are consistent with those Belaid (2017) reported, who emphasized the significant impact that breeding ram selection can have on sheep farm productivity. Rams must be 18 to 30 months old to ensure optimal fertility (Ungerfeld and Lacuesta, 2010). However, some farmers rely solely on younger rams, thereby limiting the potential benefits of selective breeding. This practice poses challenges, as studies by Kridli et al. (2006) and Rege et al. (2000) indicate that ejaculates from young Ouled Djellal sheep at puberty often display low motility due to sperm maturation disorders, which can reduce fertility.

Sex ratio and number of rams

The survey counted 14,124 sheep, including 260 rams and 9,435 ewes. The sex ratio was calculated using the following formula, yielding an average of 1 ram per 36 ewes:

Sex-ratio = Number of ewes / Number of rams (Castonguay, 2018).

The findings indicate that, on average, one ram was assigned to 36 ewes. However, 5% of farmers sometimes consider young antenaise (young female sheep) as breeders, which may need improvement to cover all ewes adequately. On extensive farms, the number of breeding males was below the recommended standard, with 260 rams for 9,435 ewes. Castonguay (2018) suggested a ratio of 1 RAM for every ten ewes to achieve optimal results, compared to the current ratio of 1 for 36 to 37 ewes. Kabbali and Berger (1990) suggest a ratio of 20 ewes per antenaise and 25 to 30 per adult ram. Taherti et al. (2024) recommend a sex ratio of 1 ram per 25 to 30 sheep for optimal reproductive efficiency.

Distribution of ewes

The survey indicated that most ewes in the studied farms were adults between 5 and 6 years of age, representing 71.02% of the population (Table 2). Younger ewes under four years old made up only 28.98%. This age distribution highlights an imbalance, as older ewes should ideally be bred one final time before being replaced by younger sheep to improve reproductive performance, as noted by Baa et al. (2020). Maintaining this balance is essential for sustaining productivity and ensuring the long-term viability of the flock.Farm sizes varied within the region, with 45% of farms having fewer than 100 sheep, 35% holding between 100 and 200 sheep, and 20% managing over 200 sheep. These differences in flock size were attributed mainly to the socio-economic status of the farmers. The composition of the flock must be adjusted by integrating new reproductive candidates and culling older, sick, or weakened animals to maintain productivity (Koycegiz et al., 2009).

Table 2. Age distribution of ewes in Batna region, Algeria during 2020

Age category	Number of Ewes	Percentage (%)
Antenaise	1240	13.14
Young ewes	1495	15.84
Adult ewes	6700	71.02
Total	9435	100%

Age of breeding

The study revealed that the sheep's breeding age varied between 6 and 10 months, with most ewes being bred at approximately nine months. This aligns with findings from Boussena (2016), who noted that the average breeding age ranges from 5 to 12 months. Research by Jainudeen et al. (2000) demonstrated that some sheep reach puberty as late as 12 months or more. Factors influencing the breeding age include the season of birth, exposure to photoperiodism, breed, and food availability, as indicated by Deghnouche et al. (2017). In some cases, ewes were bred as early as seven months, which can negatively impact their health if sufficient body weight is not achieved before breeding.

Mortality of lambs

Lamb mortality, defined as the ratio of dead lambs to those born (Castonguay, 2018), was recorded at 4.44% in the current study, with a higher concentration of deaths occurring within the first seven days of life. This neonatal mortality rate falls within acceptable parameters, meeting the recommended target of not exceeding 10% (Bedrane, 2019). This rate was primarily attributed to the hardiness and adaptability of the sheep population despite unfavorable hygiene conditions during lambing, as documented by Chellig (1992) and Dekhili and Aggoun (2007).

Mode of reproduction

Reproduction in the surveyed region was predominantly natural, with 64.78% of farmers relying on breeding rams and forgoing artificial insemination techniques. Males remained continuously with the flock, and breeding typically began in February, extending through spring into early summer, with additional breeding occurring in the fall (September to October) to maximize female coverage. Only 21.94% of breeders utilized heat synchronization, and artificial insemination was absent (0%) in the region. Artificial insemination is considered "haram" (forbidden) by the local population (Figure 2). Additionally, inseminators cited a lack of fresh semen and the considerable distance to insemination centers as significant barriers affecting reproductive practices.

Reproductive control techniques like heat synchronization allow farmers to choose the calving period and optimize litter size (Chemineau et al., 1988). Unfortunately, artificial insemination is not employed in the study area due to perceived disadvantages associated with the practice and the cultural mentality of breeders towards this technique.

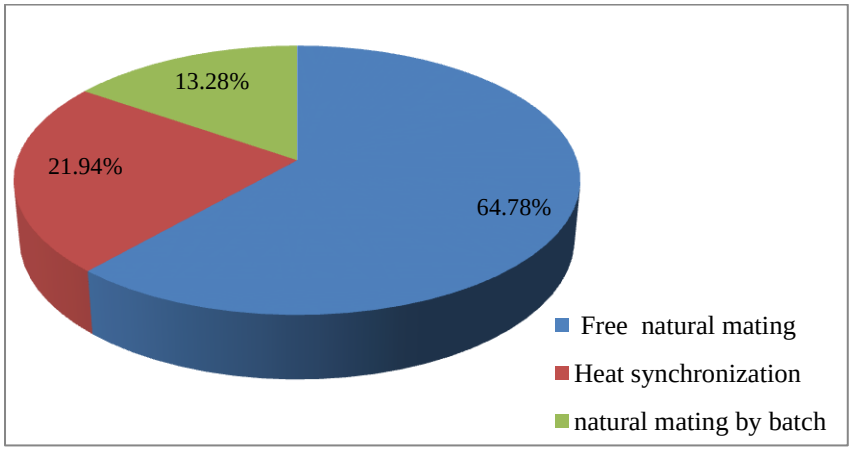


Figure 2. Reproduction methods practiced in sheep in Batna region, Algeria during 2020

Mode of birth

Approximately 65.2% of lambs were born individually, while 33.73% were born in multiple births, with a distribution of 54% for lambs and 46% for ewes. These figures align with the findings of Douh et al. (2024). A significantly lower incidence of double and triple births in ewes than single births was observed (Table 3). Several factors contributed to this variation, including feeding practices, the genetic breed type of the ewes, and the management practices employed by farmers throughout the sheep production cycle. Notably, the lack of readily available biotechnological methods in the study region may have also influenced these outcomes (Lamrani and Benyounes, 2015).

Heat synchronization, a biotechnological method, can increase the frequency of multiple births, particularly twinning (Castonguay, 2018). Moreover, the Ouled Djellal breed is known for its prolificacy, and proper nutrition during the breeding season (flushing) can promote double births (Clément et al., 1997; Douh et al., 2024). Several studies have highlighted that intra- and extra-animal factors influence prolificacy variations (Rebai et al., 2023). These studies emphasize the roles of genetic markers (Castonguay, 2018), nutritional influences (Clément et al., 1997), and environmental management strategies (Douh et al., 2024).

Table 3. Distribution of lambing by mode of birth in sheep in Batna region (Algeria).

Birth Type	Number	Percentage
Single birth	6152	65.2%
Double birth	3183	33.73%
Triple birth	100	1.05%
Total	9435	100%

Pregnancy diagnosis

The most common method for diagnosing pregnancy in sheep within the study region was abdominal palpation, typically performed in the third month post-mating. While this technique is simple and inexpensive, it has limitations, particularly its anatomical applicability to sheep (Ganaie et al., 2009). Although widely used, it does not yield optimal results. Alternative methods, such as ultrasound—widely recognized as the most reliable, rapid, and easy-to-perform technique for confirming pregnancy—were not utilized due to their high cost (Barbagianni et al., 2017). Laboratory-based methods were also absent for similar reasons. This limitation may hinder effective monitoring and management of reproduction, particularly in cases of early or late embryonic death. Early and accurate pregnancy diagnosis is essential for implementing effective management strategies for pregnant ewes (Ganaie et al., 2009).

Production

According to the breeders surveyed, sheep production in the region primarily focused on meat, with a small portion dedicated to family milk consumption. Historically, the region has concentrated on lamb meat production for the past three decades. This study revealed that 90% of breeders aimed to produce meat, while only 8% pursued meat and wool production. Only 2% of breeders had alternative objectives, such as sheep trading or butchery. These findings are consistent with the broader purpose of sheep farming in Algeria, where red meat production is a reliable income source for most breeders, as Natorp (2013) noted.

In the present study, meat production is primarily derived from adult animals. Lambs were born at a healthy weight, and typically reach market readiness between 8 and 14 months of age or as early as six months for religious celebrations, such as Eid al-Adha. At birth, lambs weigh an average of 2 kg, increasing to 16.1 kg by 90 days and reaching 35 kg by one-year-old. During their 100-day fattening period, lambs achieve a carcass weight of 18 kg. The dominant breed for meat production in Algeria is the Ouled Djellal population (Taherti et al., 2023).

The primary criteria for evaluating meat production in sheep breeds include growth rate, meat yield, and carcass and meat quality (Gurgeira et al., 2022). In Algeria, meat production generally involves adult animals, but young lambs are favored for their rapid growth and efficient feed conversion ratios (MARD, 2021). However, the high feed cost negatively impacts sheep meat production's competitiveness. Current breeding practices do not allow for self-sufficiency in animal proteins, and the high price of meat makes it less accessible to many consumers. All breeders in the region expressed concerns about the sustainability of their sheep flocks and the preservation of the national heritage.

Although wool production in the region was not considered a significant activity, it remains a constant source of income for breeders (DSA, 2021). Shearing is typically performed annually in late spring, between mid-May and mid-June, either by traditional methods or with shearing machines. The wool is then sold directly to artisans. The average wool yield was 2.5 kg per ram and 1.5 kg per ewe, with higher yields observed in young lambs than adult sheep. However, these figures remain relatively low compared to other regions of the country, where the average annual production per head reaches 1.78 kg (Mohammedi et al., 2022). For instance, the Ouled Djellal breed can produce up to 3.5 kg of wool per fleece (Khelifi, 1999).

Health management

Prophylaxis, hygiene, and health of sheep

From a health management perspective, annual sheep mortality in the region was primarily attributed to conditions, including dystocia, respiratory disorders, and gastroenteritis, with lambs being the most affected at birth. Fatal neonatal viral diarrhea was a leading cause of death in this group. The Province of Batna had 150 private veterinary clinics that provided comprehensive veterinary services to address these common pathologies. Vaccination against contagious viral diseases, including coxsackie disease, clostridial diseases, peste des petits ruminants (PPR), and foot-and-mouth disease, was implemented by private veterinarians on some farms as part of effective awareness campaigns (DSA, 2021). This vaccination effort was essential for maintaining animal health.As a result, 77% of breeders in the study region followed vaccination programs targeting clostridial diseases, PPR, and foot-and-mouth disease established by regional veterinary services. These services required proof of vaccination for breeders to access animal feed (DSA, 2021), a measure designed to enhance the success of vaccination campaigns. However, hygiene and sanitation practices in sheep housing were found to be inadequate, as breeders did not commonly practice regular cleaning and disinfection. Hygiene in managing feeding and drinking systems should have been emphasized more strongly. Some breeders did, however, engage in zootechnical practices, such as hoof trimming, dehorning, and animal marking (DSA, 2021).

Treatments and common pathologies

Analysis of the questionnaire responses revealed that respiratory diseases, particularly pneumonia, were the most prevalent health issue in sheep, affecting 60% of the population. Digestive disorders, including acidosis, indigestion, and

diarrhea, followed at 30%, while locomotor problems, such as arthritis, affected 10% of the sheep. These diseases were typically considered collective health challenges within sheep flocks. Similar patterns of disease prevalence have been observed in herds across the Drâa-Tafilalet region of Morocco (Boujenane, 2023). In that region, the year-round prevalence of diseases is 40%, with seasonal peaks occurring in winter (25%), summer (20.1%), and spring (12.5%). These findings are consistent with the present study, where disease rates during winter, summer, and spring were 22.4%, 21.3%, and 15.8%, respectively. Although animals of all ages are affected, young animals are particularly vulnerable, especially during winter when preventive measures are insufficient.

Veterinarians commonly prescribe tetracyclines and β-lactams for treating sheep, which is consistent with the findings of Boukit (2024). However, 80% of breeders reported relying on self-medication for their sheep, and in 99% of these cases, they did not observe the recommended withdrawal periods for antibiotics. Belhaj et al. (2023) have identified this practice as a significant contributor to the widespread occurrence of antibiotic resistance. Only 20% of breeders sought private veterinarians' assistance in treating their animals. Additionally, traditional remedies were commonly employed, with 80% of breeders using substances, such as vinegar, soda drinks, and baking soda for indigestion, pomegranate peels for stomatitis, and "Gatrane" for skin conditions, as reported by Boujenane (2023).

CONCLUSION

Despite numerous challenges, sheep farming in the study area plays a significant role in production and preserving local traditions. Shepherds rely heavily on ancestral knowledge, yet feed shortages, insufficient technical support, and the lack of a comprehensive sheep farming policy hinder the industry. Reproductive management practices still need to be improved, with artificial insemination rarely utilized due to illiteracy and limited access to necessary resources. Additionally, farmers should consult veterinarians more frequently and adhere to the withdrawal periods for prescribed antibiotics. Efforts to enhance flock productivity should focus on improving feed quality, housing conditions, disease control, and implementing genetic improvement programs. The analysis of the results demonstrated that all categories of sheep produced in this region are profitable. However, various factors influence profitability, including the environment, feed availability, and the age of the animals. Establishing production objectives tailored to each breed's specific characteristics maximizes economic profitability and improves overall production.

DECLARATIONS

Acknowledgments

The authors sincerely thank the shepherds and veterinary practitioners in the region for their invaluable collaboration in data collection and technical support.

Funding

The authors declare that no funds, grants, or any financial support were received during the preparation of this manuscript.

Availability of data and materials

The datasets generated during the current study are available upon reasonable request of the corresponding author.

Conflict of interests

The authors declare no conflict of interest.

Authors' Contributions

Farid Boughris investigated farmers and contributed to the collection of veterinary surveys. Mouzdalifa Boughris was responsible for writing and translating the manuscript. Rahla Meziane provided conceptualization and supervision and reviewed the manuscript. Abdelhak Karim Mouss and Dalila Hammouche contributed to the data analysis and study design. All authors reviewed the data from this research and approved the final version of the manuscript.

Ethical considerations

All authors have addressed ethical issues, such as plagiarism, duplicate publication, redundancy, data fabrication, publication consent, and misconduct.

REFERENCES

Abbas K, Madani T, and Djennane AH (2004). Amélioration des performances de reproduction des brebis Ouled Djellal en zones semi-arides algériennes avec un implant de mélatonine [Improvement of reproduction performances in Algerian semiarid zones with a melatonin implant].Rencontre Recherche Ruminants, 11: 403. Availableat:https://www.journees3r.fr/IMG/pdf/2004_reproduction_07_Abbas.pdf

Aidoud A, Le Floc'h E, and Le Houérou HN (2006). Les steppes arides du nord de l'Afrique [The arid steppes of North Africa]. Sécheresse, 17(1): 19-30. Available at: https://www.jle.com/fr/revues/sec/e-docs/les_steppes_arides_du_nord_de_lafrique_270083/article.phtml

Alary V (2015). Changement climatique: Impacts et adaptations. Agropolis International, 20: 75. Available at: https://agritrop.cirad.fr/602927/1/ID602927.pdf

Baa A, Bara Y, Ben Laalam L, and Bensba W (2020). Facteurs influençant la croissance d'agneaux OuledDjellal type Hodna» dans la wilaya de Sétif (Algérie) [Factors influencing the growth of Ouled Djellal lambs of the Hodna type in the Wilaya of Sétif (Algeria)]. Livestock Research for Rural Development, 32(11): 182. Available at: https://www.lrrd.org/lrrd32/11/abdel32182.html

Banchero GE, Stefanova K, Lindsay DR, Quintans G, Baldi F, Milton JTB, and Martin GB (2023). Ovulation and ovulation rate in ewes under grazing conditions: Factors affecting the response to short-term supplementation animal. The International Journal of Animal Biosciences, 15(2): 100100. DOI: https://www.doi.org/10.1016/j.animal.2020.100100

Barbagianni MS, Ioannidi KI, Vasileiou NGC, Mavrogianni VS, Orfanou DC, Fthenakis GC, and Valasi I (2017). Ultrasonographic examination of pregnant ewes: From early diagnosis of pregnancy to early prediction of dystocia. Small Ruminant Research, 152: 41-55. DOI: https://www.doi.org/10.1016/j.smallrumres.2016.12.008

Bedrane MA (2019). Paramètres de reproduction chez les ovins [Reproductive parameters in sheep]. Agronomie. Available at: https://agronomie.info/fr/parametres-de-reproduction-chez-ovins

Belaid D (2017). Techniques d'amélioration du troupeau [Livestockimprovement techniques: Sheepselectionmanual]. Manuel de sélection ovine, Algérie, pp. 1-12.

Belhaj K, Mansouri F, Darouiche O, Khamri M, Belbachir C, and Elamrani A (2023). Prevalence and antimicrobial resistance of bacteria isolated from Beni-Guillamb carcasses in eastern Morocco. Asian Journal of Agriculture and Biology, 8(3): 368-376. DOI: https://www.doi.org/10.35495/ajab.2019.07.325

Bencherif S (2011). L'élevage pastoral et la céréaliculture dans la steppe algérienne Évolution et possibilités de développement [Pastoral farming and cereal cultivation in the Algerian steppe: Evolution and development prospects]. Doctoral thesis, Agro Paris Tech, Paris, France. DOI: https://www.doi.org/10.13140/RG.2.2.18767.92321

Boujenane I (2023). Husbandry practices of livestock farming in the DrâaTafilalet region of Morocco. Journal of Animal Science and Veterinary Medicine, 8(4): 144-152.DOI: https://www.doi.org/ 10.31248/JASVM2023.395

Boukit FZ (2024). Enquête sur l'utilisation des anti-infectieux dans le traitement des ovins et poulets de chair et l'impact de leursrésidus sur la santéhumaine dans la région de Mascara [Survey on the use of antimicrobials in the treatment of sheep and broiler chickens and the impact of their residues on human health in the Mascara region]. Master's thesis, University of Tlemcen, Algeria. Available at:http://dspace1.univ-tlemcen.dz/handle/112/22856

Boussaada D and Benabdelli K (2021). Steppe paths in agro-pastoralism and pastoralism and improvement strategy case of the M'ila region. Ukrainian Journal of Ecology, 11(4): 48-56. DOI: https://www.doi.org/10.15421/2021_198

Boussena S, Bouaziz O, Hireche S, Derqaoui L, Dib AL, and Moula N (2016). Apparition de la puberté chez les agneaux mâles de race OuledDjellal [Onset of puberty in male OuledDjellallambs]. Revue de Médecine Vétérinaire, 167: 274-282. Available at https://orbi.uliege.be/bitstream/2268/202497/1/RMV167_274_282.pdf

Castonguay F (2018). La reproduction chez les ovins [Reproduction in sheep], Edition 2018. Université Laval, Québec, Canada, pp.10-145. Available at: https://ovins.fsaa.ulaval.ca/fileadmin/Fichiers/Conferences/La%20reproduction_chez_les_ovins_2018

Chellig R (1992). Les races ovines algériennes. Office des Publications Universitaires [Algerian sheepbreeds. Office of University Publications]. Alger, 1 Place centrale de Ben Aknoun (Alger), pp. 50-80. Available at: https://bibliotheque.ensv.dz/index.php?lvl=notice_display&id=1818

Chemineau P, Pelletier J, Guérin Y, Colas G, Ravault JP, Touré G, Almeida G, Thimonier J, and Ortavant R (1988). Photoperiodic and melatonin treatments for the control of seasonal reproduction in sheep and goats. Reproduction Nutrition Development, 28(2B): 409-422. DOI: https://www.doi.org/10.1051/rnd:19880307

Clément V, Poivey JP, Fougère O, Tillard E, Lancelot R, Gueye A, and Bibé B (1997). Étude de la variabilité des caractères de reproduction chez les petits ruminants en milieu d'élevage traditionnel au Sénégal [Study of the variability of reproductive traits in small ruminants in traditional farming environments in Senegal]. Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, 50: 235-249. DOI: https://www.doi.org/10.19182/remvt.9576

Dechicha AS, Moula N, Gharbi I, Baazize-Ammi D, Akloul K, and Guetarni D (2020). Abortions in cattle and sheep herds in Algeria, descriptive study and risk factors. Agricultura, 2: 113-114. DOI:https://www.doi.org/10.15835/agrisp.v113i1-2.13597

Deghnouche K, Aissaoui M, Meziane T, and Tlidjane M (2017). Reproductive performance of Ouled Djellal ewes in an arid area of Algeria. Journal of New Sciences, Agriculture and Biotechnology, CSIEA, 29: 2815-2819. Available athttps://www.jnsciences.org/agri-biotech/70-volume-sp%C3%A9cial-csiea-2017/425-reproductive-performance-of-ouled-djellal-ewes-in-an-arid-area-of-algeria.html

Deghnouche K, Tlidjane M, Meziane T, and Touabtia (2011). Effects of the physiological stage on some blood biochemical parameters in Ouled Djellal ewes from arid South East Algeria. Revue de Médecine Vétérinaire, 162: 3-7. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20113086380

Dekhili M (2010). Fertilité des élevagesovins type hodnamenésenextensif [Extensive farming and fertility of Hodna type sheep husbandry]. Agronomie, 1: 1-7. Available at:https://www.asjp.cerist.dz/en/article/5259

Dekhili M and Aggoun A (2007). Performances reproductives de la brebis de race OuledDjellal dans deux milieux contrastés [Reproductive performances of Ouled Djellal ewes in two contrasting environments]. Archivos De Zootechnia, 56: 963-966. Available at: https://www.redalyc.org/pdf/495/49521620.pdf

Directorate of agricultural services (DSA) (2021). Collection of statistical data for the Wilaya of Batna for the year 2020. Directorate of agricultural services for the Wilaya of Batna.

Directorate of budget programming and monitoring (DPSB) (2021). Monograph of the Wilaya of Batna. Directorate of programming and budget monitoring of the Wilaya Batna. Available at: https://interieur.gov.dz/Monographie/article_detail.php?lien=1810&wilaya=5

Douh M, Rebai N Y, Gouasmia Y, Rebai S, and Aissaoui C (2024). Productive performance of Ouled Djellal Ewes in Arid and Semi-arid Zones of Eastern Algeria. Annals of Arid Zone, 63: 1-8. Available at: https://epubs.icar.org.in/index.php/AAZ/article/view/143429

Faradji K, Slimani N, and Senoussi A (2023). Sheep breeding and agropastoralism in the region of Ain Ben Khelile (Nâama-Western Algeria): Typology of breeding strategies. Pakistan Journal of Agricultural Research, 36: 36-45. DOI: https://www.doi.org/10.17582/journal.pjar/2023/36.1.36.45

Food and agriculture organization of the United Nations (FAO) (2021). Statistics of food and agriculture organization of the United Nations. Statistiques sur le bétail. Available at: http://www.fao.org/faostat/en/#data

Ganaie BA, Khan MZ, Islam R, Makhdoomi DM, Qureshi S, and Wani GM (2009). Evaluation of different techniques for pregnancy diagnosis in sheep. Small Ruminant Research, 85(2-3): 135-141. DOI:https://www.doi.org/10.1016/j.smallrumres.2009.09.003

Gurgeira DN, Crisóstomo C, Coelho Sartori LV, Paro de Paz CC, Delmilho G, Chay-Canul AJ, Narvaez Bedoya HJ, Ortiz Vega WH, Sartori Bueno M, and Dias da Costa RL (2022). Characteristics of growth, carcass and meat quality of sheep with different feed efficiency phenotypes. Meat Science, 194: 108959. DOI: https://www.doi.org/10.1016/j.meatsci.2022.108959

Jainudeen MR, Wahid H, and Hafez ESE (2000). Sheep and goats. In: E. S. E. Hafez and B. Hafez (Editors), Reproduction in farm animals, 7th Edition. Lippincott, Williams & Wilkins., Maryland, pp. 172-181. DOI: https://www.doi.org/10.1002/9781119265306.ch12

Kabbali A and Berger YM (1990). L'élevage du mouton dans un pays à climat méditerranéen [Sheepfarming in a Mediterraneanclimate country]. Actes Edition, Casablanca, Maroc, Hassan II Agronomic Veterinary Institute, pp. 184-185. Available at: https://www.inra.org.ma/sites/default/files/publications/ouvrages/elevagemouton.pdf

Karfel M, Chikhi A, and Boulanouar B (2005). Performances de reproduction et décroissance de la race D'man au domaine expérimental de l'INRAd'Errachidia au Maroc [Reproductive performance and decline of the D'man breed at the experimental domain of INRA in Errachidia, Morocco]. Rencontre Recherches Ruminants, 12: 206. Available at: https://www.journees3r.fr/IMG/pdf/2005_systemes_10_kerfal.pdf

Khaldi A and Dahane A (2011). Elevage et processus de désertification de la steppe algérienne [Livestockfarming and desertificationprocesses in the Algerian steppe]. Revue d'Ecologie et Environnement, 7: 70-79. Available at: http://fsnv.univ-tiaret.dz/ELEVAGE%20ET%20PROCESSUS%20DE%20DESERTIFICATION%20DE%20LA%20STEPPE%20ALGERIENNE.pdf

Khelifi Y (1999). Les productions ovines et caprines dans les zones steppiques algériennes [Sheep and goat productions in Algerian steppe areas]. In: R. Rubino and P. Morand-Fehr (Editors), Systems of sheep and goat production: Organization of husbandry and role of extension services. Zaragoza (CIHEAM) Options Méditerranéennes, 25: 245-247. Available at: https://om.ciheam.org/om/pdf/a38/99600166.pdf

Koycegiz E, Emsen E, Diaz CAG, and Korkmaz MK (2009). Effects of lambing season, lamb breed and ewe parity on production traits of fat tailed sheep and their lambs. Journal of Animal and Veterinary Advances, 8: 195-198. Available at: http://www.medwelljournals.com/fulltext/java/2009/195198.pdf

Kridli RT, Abdullah AY, Shaker MM, and Almomani AQ (2006). Age at puberty and some biological parameters of Awassi and its first crosses with Charollais and Romanov rams. Italian Journal of Animal Science, 5: 193-202. DOI: https://www.doi.org/10.4081/ijas.2006.193

Lamrani F and Benyounes A (2015). Croissance d'agneaux OuledDjellal élevés sur chaumes de céréales dans la région de Guelma, Algérie [Growth of Ouled Djellal lambs raised on cereal straw in the Guelma region]. Livestock Research for Rural Development, 27(3): 43. Available at https://www.lrrd.org/lrrd27/3/lamr27043.html

Mebirouk-Boudechiche L, Boudechiche L, and Miroud K (2015). Essaid'une complementation alimentaire au flushing et au steaming de brebis Ouled Djellalpar la caroube [Trial of dietary supplementation with carob during flushing and steaming of Ouled Djellal ewes]. Archivos de Zootecnia, 64: 449-455. DOI:https://www.doi.org/10.21071/az.v64i248.433

MeftiKorteby H, Koudri Z, and Saadi MA (2017). Caractérisation des performances de la race ovine Algérienne Ouled Djellal type Djellalia dans des conditions steppiques [Characterization of performance of the Algerian Ouled Djellal sheep breed (Djellalia type) under steppe conditions]. Agronomic & Biological Sciences, 17: 1-5. Available at:https://www.caractérisation-des-performances-de-la-race-ovine-algérienne-ouled-djellal-type-djellalia-dans-des-conditions-steppiques.pdf

Meradi S, Benguigua Z, Chekkal F, Aouachria M, and Ziad MS (2016). L'élevage ovin dans les zones oasiennes; Cas de la wilaya de Biskra (Algérie) [Sheepfarming in oasis zones: Case study of Biskra province, Algeria]. Journal Algérien des Régions Arides, 13(1): 90-96. Available at: https://www.asjp.cerist.dz/en/article/76119

Ministry of agriculture and rural development (MARD) (2021). Agricultural development in arid and semi-arid areas. Available at: http://www.minader.com

Missoko R M, Akouango P, Ognika A J, Gallion I, and Mbabala RJ (2020). Paramètres de reproduction des ovins Djallonké en milieu villageois dans le département du pool (Congo Brazzaville) [Reproductive parameters of Djallonké sheep in village settings in the Pool department (Congo Brazzaville)]. Journal of Animal & Plant Sciences, 46: 8141-8148. Available at: https://www.m.elewa.org/Journals/wp-content/uploads/2020/10/7.Missoko-1.pdf

Mohammedi K, Laiadi Z, and Titaouine M (2022). Phenotypic characterization of sheep population in Aures, Hodna and Ziban Zones, Algeria. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies, 79: 32-39. DOI:https://www.doi.org/ 10.15835/buasvmcn-asb:2022.0004

Mouhous A, Kadi SA, and Brabez F (2015). Analyse préliminaire des pratiques de production des élevages ovins en zone de montagne de Tizi-Ouzou (Algérie): Cas de l'alimentation [Preliminary analysis of sheep farming practices in the mountainous area of Tizi-Ouzou (Algeria): Focus on feeding]. Livestock Research for Rural Development, 27(7): 132. Available at: http://www.lrrd.org/lrrd27/7/mouh27132.html

Moula N (2018). Caractérisation de la race ovine algérienne Tazegzawth [Characterization of the Algerian sheep breed Tazegzawth]. Tropicultura, 36: 43-53. Available at: http://www.tropicultura.org/text/v36n1/43.pdf

Natorp JC (2013). Intérêt, limites et conditions des suivisd'élevageenmédecinevétérinaire ovine [Benefits, limitations, and conditions of livestock monitoring in veterinary medicine for sheep]. Bulletin de l'Académie Vétérinaire de France (France, tome 166 n°1, 2013). France, pp. 55-65. Available at: https://www.persee.fr/doc/bavf_0001-4192_2013_num_166_1_9691

Paquay R, Bister JL, Wergifosse F, and Pirotte C (2004). Effet de l'évolution du poids vif sur les performances de reproduction des brebis [The effect of live weightevolution on ewe reproductive performance]. Rencontre Recherche Ruminants, 11: 397. Available at: https://www.évaluation-des-performances-de-reproduction-et-de-productivité-chez-la-race-rembi-dans-la-région-de-médéa-(algérie).pdf

Rebai N, Rahmani A, Chacha F, Douh M, Ahmed Laloui H, Cherb N, Derdour M, and Abbas K (2023). Effets de régimes contenant différentes proportions de féverole (Vicia faba var. minor) sur les performances d'engraissement, les caractéristiques des carcasses et la qualité de la viande d'agneaux mâles de race OuledDjellal [Effects of diets containing different proportions of fababean (*Vicia faba* var. minor) on fattening performance, carcasscharacteristics, and meat quality of male Ouled Djellal lambs]. Livestock Research for Rural Development, 35(5): 35-38. Available at: https://www.lrrd.org/lrrd35/5/3538n.reb.html

Rege JEO, Toe F, Mukasa-Mugerwa E, Tembely S, Anindo D, Baker R L, and Lahloukassi A (2000). Reproductive characteristics of Ethiopian highland sheep. II. Genetic parameters of semen characteristics and their relationships with testicular measurements in ram lambs. Small Ruminant Research, 37: 173-187. DOI: https://www.doi.org/10.1016/S0921- 4488(00)00140-1

Saidi A, Moula N, Kaidi R, and BoumahdiMerad Z (2020). Typological characteristics of sheep farms in relation to a potential risk of contamination with Neospora Caninum in the Central region of Algeria. Agricultura, 3(4): 115-116. Available at: https://orbi.uliege.be/bitstream/2268/299825/1/_13956-Article%20Text-54838-1-10-20210109.pdf

Slimani A, Huguenin J, Bairi A, Bensouiah R, and Allaoua A (2021). Sheep feeding strategies in the face of declining steppe fodder resources in Tebessa Region/Algeria. Annals of Arid Zone, 60: 33-43. Available at:https://epubs.icar.org.in/index.php/AAZ/article/view/131410/48761

Taherti M, AitIssad N, and Mimoune N (2024). Breeding season and body condition influence on zootechnical parameters of Ouled Djellal ewes in semi-arid areas. Veterinarska Stanica, 55: 5.DOI:https://www.doi.org/ 10.46419/vs.55.5.10

Taherti M, Aitissad N, Khelef D, and Mimoune N (2023). Reproduction characteristics of Ouled Djellal Rams in a Semi-arid Area in Algeria. KafkasUniversitesiVeterinerFakultesiDergisi, 29: 717-726.DOI:https://www.doi.org/ 10.9775/kvfd.2023.30329

Ungerfeld R and Lacuesta L (2010). Social rank during pre-pubertal development and reproductive performance of adult rams. Animal Reproduction Science, 121(1-2): 101-105. DOI: https://www.doi.org/10.1016/j.anireprosci.2010.05.007

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 400-408.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj47
PII: S232245682400047-14

Effect of Nano-Liquid Extracts of *Andrographis paniculata* and *Moringa oleifera* on The Performance and Carcass Quality of Broiler Chickens

Ilham Fithrah Hasanain, Osfar Sjofjan, Yuli Frita Nuningtyas, Filoza Marwi, Feri Eko Hermanto, Yuanita Salsabilla Handoyo, and Muhammad Halim Natsir*

Department of Nutrition and Animal Feed, Faculty of Animal Science, University of Brawijaya, Malang City, East Java 65145, In donesia

*Corresponding author's Email: emhanatsir@ub.ac.id

ORIGINAL ARTICLE
Received: July 24, 2024
Revised: August 18, 2024
Accepted: September 01, 2024
Published: September 30, 2024

ABSTRACT

Feed was crucial for achieving optimal productivity in broiler chickens, which required ongoing monitoring of its quantity and quality. The present study aimed to evaluate the effects of nanoliquid extracts from *Andrographis paniculata* and *Moringa oleifera* used as photobiotic on the performance and carcass quality of broiler chickens. The research involved 128 broiler chickens, which were divided into four treatment groups, each with four replications of eight broiler chickens. The treatments included a control group (T0), a 0.25% nano liquid extract mixture of *Andrographis paniculata and Moringa oleifera* (T1), a 0.50% nanoliquid extract mixture (T2), and a 0.75% nanoliquid extract mixture (T3). The study utilized an *in vivo* method and analysed the data using a completely randomized design. The optimal level of nano liquid extract was determined based on chicken performance (feed consumption, body weight, feed conversion ratio (FCR), income-over-feed cost (IOFC)) and carcass quality (carcass percentage, cooking loss, meat color, water-holding capacity, and texture). The findings indicated that the addition of combined *Andrographis paniculata* and *Moringa oleifera* (1:1, w/w) nano-liquid extract in the chickens' feed significantly influenced body weight, FCR, and IOFC. However, there was no significant effect on feed consumption. Furthermore, the use of *Andrographis paniculata* and *Moringa oleifera* combination had a significant impact on all carcass quality parameters beyond *b carcass color. It was concluded that the addition of 0.25% of combined *Andrographis paniculata* and *Moringa oleifera* nano liquid extract yielded the most favorable outcomes for the performance and carcass quality of broiler chickens.

Keywords: *Andrographis paniculata*, Broiler chicken, Carcass quality, *Moringa oleifera*, Nano liquid, Performance

INTRODUCTION

Feed consumption is the primary cost for broiler chickens in production, and improving feed efficiency is a key strategy in broiler chicken management. However, few studies have focused on slow-growing broiler chickens (Wen et al., 2018). The use of antibiotic growth promoters (AGPs) as feed additives for livestock has been banned by the Indonesian government through Ministerial Regulation No. 14 of 2017 due to concerns about antibiotic resistance. This issue poses a significant problem for the poultry industry, leading to increased morbidity and mortality rates in broilers during outbreaks (Untari et al., 2021). Consequently, there are side effects, such as residue accumulation on broiler carcasses and the emergence of antibiotic-resistant bacteria (Mehdi et al., 2018). The use of AGPs has been reported to increase nutrient utilization, reduce intestinal pH, reduce pathogen bacterial populations, and increase nonpathogenic bacterial populations (Natsir et al., 2017). Singh et al. (2020) emphasized the need to find alternatives to synthetic antimicrobial growth promoters (AGPs) because of their related issues. They highlighted natural growth promoters (NGPs) as promising options. These NGPs, which include *Andrographis paniculata*, *Moringa oleifera*, and their derivatives, are recognized for their beneficial effects on the health of broiler chickens. These benefits include antimicrobial, antioxidant, anti-inflammatory (Okhuarobo et al., 2014; Chhikara et al., 2021).

The chemical compounds found in *Moringa oleifera* include alkaloids, flavonoids, saponins, terpenoids, tannins, and steroids (Bhattacharya et al., 2018). According to phytochemical tests conducted by Oladeji et al. (2020), these compounds function as antibacterials, antioxidants, and anti-inflammatory agents. *Moringa oleifera* is known for its natural antioxidant content and is a rich source of dietary fiber and essential macro- and micronutrients (Islam et al., 2021). *Andrographis paniculata* contains orthosiphon glucose, essential oils, saponins, polyphenols, flavonoids, sapogenins, potassium salts, and myoinositol (Hossain et al., 2014). Flavonoids in *Andrographis paniculata* are known to reduce free radicals, flavonoids, such as chalcones, flavones, flavonols, flavanones, and catechins exhibit antioxidant activity (Ahmad et al., 2020), and they have also been reported to be important for antiviral activity (Nithya et al., 2021).

To cite this paper: Hasanain IF, Sjofjan O, Nuningtyas YF, Marwi F, Hermanto FE, Handoyo YS, and Natsir MH (2024). Effects of Nano-Liquid Extracts of *Andrographis paniculata* and *Moringa oleifera* on The Performance and Carcass Quality of Broiler Chickens. *World Vet. J.,* 14(3): 400-408. DOI: https://dx.doi.org/10.54203/scil.2024.wvj47

Both *Andrographis paniculata* and *Moringa oleifera* can be extracted via nanoparticles to improve their poor bioavailability. Nanosized particles not only increase the surface area but also possess better physical and chemical characteristics, including enhanced reactivity and solubility (Martínez-Ballesta et al., 2018), which is due to the increased stability of the active compounds within the nanoparticles.

These issues highlight the need for alternatives to AGPs and improvements in carcass quality while exploring the potential of *Andrographis paniculata* and *Moringa oleifera* as replacements. The objective of this study was to evaluate the effects of administering *Andrographis paniculata* and *Moringa oleifera* at different concentrations on the performance and carcass quality of broiler chickens. This research was crucial for enhancing carcass quality affected by parasites through the use of active compounds from herbal plants and for identifying alternatives to antibiotic growth promoters (AGPs) in broiler chicken production.

MATERIALS AND METHODS

Ethics approval

This experiment was carried out in accordance with Brawijaya University regulations, and the Indonesian Animal Care and Utilization Committee gave ethical approval for this research with the number 099-KEP-UB-2023.

Experimental design and treatments

In the current study, day-old chick (DOC) of the Lohmann Japfa Platinum MB 202 strain, aged 1 day with an average body weight of 38 grams, was reared for 35 days without sex differentiation. The DOC Platinum strain was used to vaccinate against Newcastle disease (ND) and infectious bursal disease (IBD). A total of 128 DOC were allocated into four treatment groups, with each group being replicated four times and each replicate consisting of eight chicks. Throughout the study, lighting was maintained at a maximum intensity of 20-40 lux, and heating was provided during the 10-day incubation period, with six gasolec heaters positioned on the sides of the cage with a temperature range of 25-30°C and a humidity level of 50-70%. Tube feeders and drinkers were provided to ensure unlimited access to food and water. The feed additive used was a mixture of *Andrographis paniculata* and *Moringa oleifera*, which were extracted and nanoextracted in liquid form. These feed additives were mixed into the feed at concentrations of 0.25%, 0.50%, and 0.75% of the total feed given. The composition of the feed ingredients is detailed in Table 1, and the percentage of the phytogenic mixture is presented in Table 2.

Table 1. Nutritional contents of the basal feeds of starter and finisher in broiler chickens, after the addition of nanolnano-iquid extracts of *Andrographis paniculata* and *Moringa oleifera*

Nutrients of starter	
Crude Protein	21.00-22.00%
Fat	4.00-8.00%
Crude Fibre	3.00-5.00%
Ash	5.00-7.00%
Calcium	0.90-1.20%
Phospor	0.70-0.90%
ME	2950-3050 Kcal/kg
Nutrients of finisher	
Moisture	12%
Crude Protein	19%
Crude Fat	5%
Crude Fibre	5%
Calx	7%
Calsium	0.8-1.1%
PhosporUS	0.45%
Enzyme	Phytase 400 FTU/kg
Aflatoxin	50 ppb

Table 2. Percentage of phytogenic blend produced by the addition of nanolnano-iquid extracts of *Andrographis paniculata* and *Moringa oleifera* to broiler chickens' diet

Treatment	
T0: *Andrographis Paniculata* + *Moringa Oleifera*	Basal feed
T1: *Andrographis Paniculata* + *Moringa Oleifera*	Use 0.25% nanoliquid extract mixture
T2: *Andrographis Paniculata* + *Moringa Oleifera*	Use 0.50% nanoliquid extract mixture
T3: *Andrographis Paniculata* + *Moringa Oleifera*	Use 0.75% nanoliquid extract mixture

T: treatment

***Moringa oleifera* and *Andrographis paniculata* nano liquid preparation**

The feed additive used was a combination of *Andrographis paniculata* and *Moringa* extracted in a nanoliquid form. This feed additive was mixed with the feed at concentrations of 0.25%, 0.50%, and 0.75% of the total feed provided to the broilers from day 1 to day 35. *Andrographis paniculata* and *Moringa oleifera* extracts were prepared in the Nutrition Laboratory of the Faculty of Animal Husbandry, Brawijaya University, via a maceration process. The process involved soaking *Andrographis paniculata* and *Moringa oleifera* in a 1:1 ratio in a container and then adding 70% ethanol at a ratio of 1:5 (100 grams of sambiloto powder and 500 ml of ethanol; Prasetyaningrum et al., 2022). This mixture was stirred at room temperature for 24 hours. Extraction was then performed via microwave-assisted extraction (MAE) at a temperature of 50-60°C for 15 minutes. Once extraction was complete, the mixture was allowed to settle briefly and then filtered through filter paper to obtain the filtrate. The filtrate was subsequently distilled via a microwave at a controlled temperature for 30 minutes until all the ethanol had evaporated. Finally, nanoparticle-based extraction was conducted via ultrasonic assisted extraction (UAE) for 10 minutes, which was repeated three times, for a total of 30 minutes. The detailed process of extracting nano-liquid of the feed additive was illustrated in Figure 1.

Figure 1. The process of extracting a nano liquid of the feed additive used was a combination of *Andrographis paniculata* and *Moringa,* and microwave-assisted extraction (MAE) was performed at a temperature of 50-60°C for 15 minutes. Nanoparticle-based extraction was conducted via ultrasonic-assisted extraction (UAE) for 10 minutes, which was repeated three times, for a total of 30 minutes.

Preparation of the cage and equipment

The first stage of cage preparation involved cleaning the remaining dirt from the previous broiler chicken farm and trimming the bushes around the cage. The cage floor was then mopped until it was clean and dry for 1 week. The cage used was an open-house type with bamboo slats, partitions made of bamboo, and sacks measuring 100 cm × 100 cm × 70 cm, creating a total of 28 plots, each containing 8 broiler chickens. A sack was placed as a base, and the rice husks were evenly spread to a height of 5-10 cm in the treatment cages. Tarpaulins were placed around the plots to regulate the temperature and air conditions in the cage during the starter phase (Zhai et al., 2020). The number of feed and water containers provided was in accordance with the number of plots, totaling 28, with sizes adjusted according to the age of the livestock. Baby chick feeders were used for DOC (1-14 days), and feed containers with a capacity of 7 kg were used for older chickens. Drinking containers with a 2-liter capacity were also provided. The cage of the *in vivo* experiment can be seen in Figure 2. The heater system used 6 gas molecules, which were placed on the sides of the cage. The light was provided for 12 hours per day, from 18:00 to 06:00. Once all the equipment was set up, the gas was turned on for 3 hours before introducing the DOC to ensure that the cage temperature was adequately warm. A solution of brown sugar was prepared to prevent dehydration and minimize stress. During the starter phase, the broiler chickens were arranged in 5 separate plots, each designated for a different treatment, without any replication. After the brood hens were released, they were distributed according to the research cage repetitions for 35 days.

Figure 2. The open house type of cage used in the present study. A total of 28 plots, each containing 8 broiler chickens

Data collection

The initial data collection was conducted when the DOC arrived at the poultry house. All DOCs were weighed together to obtain the average body weight. Subsequent average body weight measurements were taken weekly. Feed consumption was also recorded weekly by subtracting the amount of feed remaining from the amount of feed given during weighing. At 35 days of age, two broiler chickens from each experimental unit, whose weight was close to the average weight of the unit, were then marked on their legs according to the treatment they received so that a total of 28 broiler chickens were used as samples. Broiler chickens were first weighed to determine their live weight and then slaughtered by hanging them upside down and severing three neck vessels, including the esophagus, carotid artery, and jugular vein. After slaughter, the blood was drained as much as possible, and the broiler chickens were immersed in hot water for 10 seconds to facilitate manual feather plucking. The head and feet were detached, the internal organs were removed, and the weights of the broiler chicken carcasses were recorded. A breast meat sample, measuring 5 cm in length, 3 cm in width, and 0.5 cm in thickness, was collected for texture and color analysis. The procedures for assessing color, texture, and cooking loss were conducted in the Laboratory of Animal Product Technology at the Faculty of Animal Science, Brawijaya University.

Statistical analysis

The software used was Microsoft Excel, and the data obtained were then analyzed via analysis of variance (ANOVA) with respect to the percentage of date pit flour usage. When different results were observed between treatments, Duncan's multiple range test was performed. Significantly different results were considered at the level of $p < 0.05$, and highly significantly different results were considered at the level of $p < 0.01$.

RESULTS

Effects of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* on broiler chicken performance

The average effects of the treatments on the feed conversion ratio (FCR), income-over-feed cost (IOFC), body weight, and feed consumption of broiler chickens are shown in Table 3. Statistical analysis revealed that the liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* had a significant effect ($p < 0.05$) on the feed conversion ratio (FCR), income over feed cost (IOFC), and body weight of broilers. The highest FCR was observed in the T0 (control) treatment at 1.57%, whereas the lowest FCR was found in the T1 treatment (0.25% nanoliquid extract mixture of *Andrographis paniculata* and *Moringa oleifera*) at 1.49%. The highest IOFC was recorded in the T1 treatment (9,530/kg), whereas the lowest IOFC was recorded in the T0 treatment (6,036/kg). The highest body weight was achieved in the T1 treatment (3,833.33 g/head), whereas the lowest body weight was achieved in the T0 treatment (3,553.9 g/head). The effects of nano *Andrographis paniculata* and *Moringa oleifera* did not significantly affect ($p > 0.05$) the feed consumption of broilers chicken. The highest feed consumption occurred in the T1 treatment (3.57 kg/broiler), whereas the lowest feed consumption occurred in the T2 treatment (3.45 kg/broiler).

Effects of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* on broiler chicken carcass quality

The average impact of the treatments on broiler chicken carcass quality was presented in Table 4. According to the statistical analysis, the liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* did not have a significant effect ($p > 0.05$) on carcass percentage, meat color (L*a*b*), water holding capacity, or meat texture. The highest carcass percentage was observed in the T1 treatment (75.52%), whereas the lowest carcass percentage was observed in the T0 treatment (67.83%). The highest value for meat color was recorded in the T3 treatment (5.75), whereas the lowest a value was recorded in the T0 treatment (3.18). The highest b value for meat color was found in the T3 treatment (13.61), whereas the lowest b value was found in the T0 treatment (11.56). The highest WHC was observed in the T2 treatment (44.85%), whereas the lowest WHC was observed in the T0 treatment (41.44%). The highest meat texture was found in the T0 treatment (4.59 N), whereas the lowest meat texture was found in the T3 treatment (3.65 N). The effects of the liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* on broiler chicken carcass quality significantly affected ($p < 0.05$) cooking loss and meat color L. The highest cooking loss was in the T3 treatment (36.25%), whereas the lowest cooking loss was in the T0 treatment (33.37%). The highest L value for meat color was recorded in the T3 treatment (54.79%), whereas the lowest L value was recorded in the T0 treatment (50.36%).

Table 3. Effects of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* on the feed conversion ratio, income over feed cost, body weight and feed consumption in broiler chickens

Treatments	T0	T1	T2	T3	*p-value*
Feed Consumption (g/broiler)	3494 ± 59.32	3579 ± 26.33	3455 ± 83.97	3510 ± 40.04	0.096
Body Weight (g/broiler)	2221.19 ± 45.33	2351.41 ± 71.12	2283.61 ± 58.65	2215.19 ± 59.59	0.044
IOFC (IDR/kg)	6036 ± 1046.08^{a}	9530 ± 1154.85^{b}	6053.13 ± 1430.22^{a}	6584.37 ±1360.59^{a}	0.009
FCR	1.57 ± 0.04^{b}	1.49 ± 0.04^{a}	1.55 ± 0.01^{b}	1.55 ± 0.03^{b}	0.019

Superscripts with different letters in the same columns indicate significant differences ($p < 0.05$). FCR: Feed conversion ratio, IOFC: Income-over-feed cost. T0: basal feed, 0% of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract, T1: 0.25% of of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract, T2: 0.50% of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract, and T3: 0.75% of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract.

Table 4. Effects of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* on the carcass quality of broiler chickens

Treatments		T0	T1	T2	T3	*p-value*
Carcass percentage (%)		67.83 ± 0.46^{a}	75.52 ± 1.27^{c}	72.57 ± 1.52^{b}	72.16 ± 1.37^{b}	2.81E-06
Cooking loss (%)		33.37 ± 0.24^{a}	34.09 ± 0.83^{ab}	35.69 ± 1.91^{bc}	36.25 ± 1.30^{c}	0.016
Meat color	L*	50.36 ± 0.34^{a}	52.67 ± 1.34^{ab}	53.24 ± 2.44^{b}	54.79 ± 2.09^{b}	0.020
	a*	3.18 ± 0.28^{a}	5.18 ± 1.07^{b}	5.66 ± 0.90^{b}	5.75 ± 0.29^{b}	0.0005
	b*	11.56 ± 0.66	11.95 ± 0.57	12.89 ± 2.55	13.61 ± 0.36	0.181
Water holding capacity (%)		41.44 ± 0.44^{a}	42.77 ± 1.01^{ab}	44.85 ± 0.38^{b}	43.4 ± 0.34^{b}	9.39E-06
Meat texture. (N)		4.59 ± 0.44^{b}	4.6 ± 0.29^{b}	4.46±0.24^{b}	3.65±0.29^{a}	0.002

Superscripts with different letters in the same columns indicate significant differences ($p < 0.05$). T0: basal feed, 0% of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract, T1: 0.25% of of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract, T2: 0.50% of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract, and T3: 0.75% of *Andrographis paniculata* and *Moringa oleifera* nanoliquid extract.

DISCUSSION

Performance

The success of broiler production was evaluated based on performance indicators such as feed consumption, final body weight, and the feed conversion ratio (FCR; Shafey et al., 2014). Research findings have shown that a nano mixture of *Andrographis paniculata* and *Moringa oleifera* contains active compounds with natural antimicrobial and antioxidant properties. An evaluation of performance factors against specific livestock parameters, such as FCR, revealed the ratio between the feed consumed and the weight gained by broiler chickens (Pierozan et al., 2016). A lower FCR value signified greater efficiency, which was evident from the FCR values in the T2 treatment, where T2 had a lower value than the control and was similar to T1. Nevertheless, all the treatments achieved a targeted low average feed conversion ratio (FCR) because the liquid nano extracts of *Andrographis paniculata* and *Moringa oleifera* have pharmacological activities as natural antibiotics, antivirals (Udikala et al., 2017), antimicrobials, anti-inflammatory agents, anti-cholesterol agents, anticancer agents, appetite stimulants, and digestive enhancers for broilers (Bagheri et al., 2020). The addition of liquid nano extracts of *Andrographis paniculata* and *Moringa oleifera* resulted in relatively high feed conversion ratios due to the physical and chemical properties of *Moringa oleifera*. As mentioned, the concentration of antinutritional factors such as tannins and saponins led to reduced feed consumption (Steven et al., 2015), making the feed more efficient, as observed in T2.

The results revealed that the nano mixture of *Andrographis paniculata* and *Moringa oleifera* presented the highest IOFC in the T1 treatment. The results from the T1 treatment demonstrated that higher levels of herbal mixture usage were associated with increased feed costs. According to Utami et al. (2023), factors influencing IOFC include broiler chicken body weight, feed intake, feed costs during the rearing period, and the selling price of the broiler chicken at harvest. Improved management practices lead to a higher IOFC, which implies more efficient broiler chicken rearing. The T2 treatment demonstrated that the average IOFC value was similar to that of T0 and T3, with nearly identical body weights, indicating that more efficient conversion of nutrients into meat results in better IOFC values. This result was related to the advantages of the liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera*, which include greater particle absorption in the form of nanoparticles. Greater surface area for improved interactions, extending their time in the intestines, minimizing intestinal cleansing processes, enhancing tissue penetration, and improving penetration of the epithelial layer, resulting in more effective cellular absorption (Cao et al., 2019).

The results of the present study indicated that the T1 treatment, which involved the liquid nano extracts of *Andrographis paniculata* and *Moringa oleifera* leaves, resulted in the highest body weight. However, this result led to

high IOFC. Therefore, the most stable treatment was T2, owing to its efficient feed conversion ratio, which reduced feed costs and contributed to body weight formation. Owoade et al. (2021) reported that low feed consumption was influenced by the antioxidant compounds present in *Andrographis paniculata* and *Moringa oleifera*, which affected organ performance and growth promoters in broilers, thus improving nutrient absorption and resulting in better body weight (Alwaleed et al., 2020). Compared with the other treatments, the addition of 0.50% nano *Andrographis paniculata* and *Moringa oleifera* resulted in slightly lower feed consumption but higher body weight again because *Andrographis paniculata* and *Moringa oleifera* were recognized as excellent sources of nutrients with high protein content and many benefits for monogastric livestock (Astuti and Irawati, 2022).

The results of the present study indicated that the consumption of 0.50% liquid nanoextracts from *Andrographis paniculata* and *Moringa oleifera* was the most effective, as it resulted in increased body weight gain in broiler chickens and decreased feed consumption. This was balanced by a lower feed conversion ratio, lower IOFC, and increased body weight. The addition of *Andrographis paniculata* and *Moringa oleifera* to the diet could reduce feed consumption. Oraibi and Ali (2021) noted that bioactive compounds in nano *Andrographis paniculata* and *Moringa oleifera* could reduce feed consumption compared with normal levels. Secondary compounds such as tannins and saponins, which have a bitter taste, were found to reduce palatability (Kholif et al., 2018). However, from a biological perspective, feed consumption, the protein efficiency ratio, and the feed conversion ratio were optimal in the T2 treatment. The bioactive compounds present in *Andrographis paniculata* and *Moringa oleifera* were present at optimal dosages within the herbal mixture. Additionally, the synergistic effects of the combined ingredients positively influenced various performance parameters.

Carcass quality

The average carcass percentage values with the addition of the nanoliquid additive extracts of *Andrographis paniculata* and *Moringa oleifera* were, in descending order, 75.52% for the 0.25% addition (T1), 72.57% for the 0.50% addition (T2), 72.16% for the 0.75% addition (T3), and 67.83% for the control (T0). In the present study, the ideal carcass percentage value was achieved with the addition of 0.25% nanoliquid to *Andrographis paniculata* and *Moringa oleifera*. This result was due to the advantages of nanoparticles, which have an increased surface area (Alkhtib et al., 2020). According to Abd El-Hack et al. (2018), greater particle absorption in the form of nanoparticles was due to their larger surface area, which allows for better interactions, prolongs the residence time in the intestines, reduces intestinal cleansing mechanisms, increases tissue penetration, and enhances epithelial layer penetration, leading to more efficient cellular absorption.

The cooking loss values in this study ranged from an average of 33.37% to 36.25%, which was considered normal. This aligns with the findings of Sari et al. (2021), who reported that broiler meat with a cooking loss of approximately 35% was of very good quality because of its relatively low cooking loss. Meat with a cooking loss of less than 35% was deemed to have acceptable quality, as it reflects minimal nutrient loss during cooking. The cooking loss values in this study were very similar, indicating that the addition of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* did not result in significant differences.

Meat color was assessed via a Minolta Chromatometer Color Reader to obtain International Commission on Illumination (CIE) laboratory values (L*: Lightness, a*: Redness, b*: Yellowness). The average L* values in this study ranged from 50.36-54.79, which was considered to be within the normal range. This finding was consistent with that of Hayat et al. (2024), who reported that lightness levels showed significant sensitivity to color measurement fluctuations via three-dimensional color values, which were correlated with higher L* values. The use of high dimensions could induce the release of stress-related hormones, including adrenaline, noradrenaline, and corticosterone. Downing et al. (2017) categorized broiler breast meat with an L* value greater than 53 as pale. This occurred because the additives used, *Andrographis paniculata* and *Moringa oleifera*, did not significantly differ. However, the addition of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera*, which have much smaller particle sizes, resulted in a more stable L* value of 52.67 in the T1 treatment.

Compared with those of the control treatment, the a* color values of the addition of liquid nanofeed additives to *Andrographis paniculata* and *Moringa oleifera* were also greater. However, the addition of 0.25%, 0.50%, and 0.75% liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* did not result in significant differences. Additionally, the a* color values were related to the L* color values; in this study, both the a* and L* color values increased with increasing concentrations of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera*, because *Andrographis paniculata* can maintain erythrocyte levels in the body, and *Moringa oleifera* significantly increased the number of micronucleated polychromatic erythrocytes originating from the bone marrow of rodents (Bagri and Kumar, 2024). Myoglobin oxidation caused a decrease in a* values across all treatments, as iron atoms can oxidize

or denature myoglobin molecules during oxidation, resulting in a negative color change in the product and conversion of myoglobin to methemoglobin (Çelebi, 2024).

In this study, the addition of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* did not have a significant effect on the b* values, because *Andrographis paniculata* and *Moringa oleifera* lack xanthophyll, which means that they do not affect the b* color of the carcass. Orkusz et al. (2024) reported that some bacteria can produce pigments through their metabolic processes, leading to alterations in meat color, such as an increase in yellowness to 58.59. Additionally, changes in meat color, including heightened yellowness, have been linked to bacterial spoilage, myoglobin autoxidation, and protein oxidation. With the addition of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera*, the water-holding capacity values, listed from highest to lowest, were 44.85% for the 0.50% addition (T2), 43.4% for the 0.75% addition (T3), 42.77% for the 0.25% addition (T1), and 41.44% for the 0% addition (T0). The WHC increased with the addition of nanoliquid extracts of *Andrographis paniculata* and *Moringa oleifera*, which was attributed to the presence of tannins in both *Andrographis paniculata* and *Moringa oleifera*, which can inhibit fat absorption. Shahlehi et al. (2024) reported that tannins react with mucosal and epithelial proteins in the intestines, thereby inhibiting the absorption of fats from ingested food.

The addition of liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera* to the feed resulted in texture values listed from highest to lowest as follows, including 4.6 for the 0.25% addition (T1), 4.4 for the 0.50% addition (T2), 4.5 for the 0% addition (T0), and 3.6 for the 0.75% addition (T3). The texture values of the *Andrographis paniculata* and *Moringa oleifera* extracts were greater than those of the control treatment, which was attributed to the alkaloid and tannin contents of *Andrographis paniculata* and *Moringa oleifera*, which inhibited fat absorption, leading to an increase in the meat protein content and consequently higher texture values (Ivanova et al., 2024).

CONCLUSION

The administration of 0.25% liquid nano extracts of *Andrographis paniculata* and *Moringa oleifera* effectively improved performance parameters, such as FCR, IOFC, body weight, and feed consumption and also effectively enhanced the carcass quality of broiler chickens. Moreover, additional studies were needed to explore the use of liquid and nanoliquid extracts of *Andrographis paniculata* and *Moringa oleifera* in feed to assess their potential for improving outcomes.

DECLARATIONS

Funding

This work is financially supported by Brawijaya University through the Professor Grant Scheme 2023.

Acknowledgments

The authors would like to acknowledge financial support from Research grant funding for Profesors from Brawijaya University.

Authors' contributions

Ilham Fithrah Hasanain contributed to the data analysis and wrote the manuscript. Muhammad Halim Natsir and Osfar Sjofjan developed the research methodology and reviewed the manuscript. Yuli Frita Nuningtyas analysed the data. Filoza Marwi developed the technical research and edited the manuscript. Feri Eko Hermanto analyzed liquid nanoextracts of *Andrographis paniculata* and *Moringa oleifera*. All the authors performed the validation and investigation and approved the final manuscript.

Availability of data and materials

The authors of this article confirm that all the data supporting the findings of this research are available upon reasonable request from the authors.

Competing interests

The authors declare that they have no conflicts of interest.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

REFERENCES

Abd El-Hack ME, Alagawany M, Elrys AS, Desoky ESM, Tolba HMN, Elnahal ASM, Elnesr SS, and Swelum AA (2018). Effect of forage *Moringa oleifera* L. (moringa) on animal health and nutrition and its beneficial applications in soil, plants and water purification. Agriculture, 8(9): 145. DOI: https://www.doi.org/10.3390/agriculture8090145

Ahmad M, Mohammad N, Aziz MA, Alam MA, Hossain MS, Islam MR, and Uddin MG (2020). Comparison of antioxidant role of methanol, acetone and water extracts of *Andrographis paniculata* Nees. Journal of Medicinal Plants Research, 14(8): 428-437. DOI: https://www.doi.org/10.5897/JMPR2020.6999

Alkhtib A, Scholey D, Carter N, Cave GW, Hanafy BI, Kempster SRJ, Mekapothula S, Roxborough ET, and Burton EJ (2020). Bioavailability of methionine-coated zinc nanoparticles as a dietary supplement leads to improved performance and bone strength in broiler chicken production. Animals, 10(9): 1482. DOI: https://www.doi.org/10.3390/ani10091482

Alwaleed S, Mickdam E, Ibrahim A, and Sayed A (2020). The effect of dried *Moringa oleifera* leaves on growth performance, carcass characteristics and blood parameters of broiler chicken. SVU-International. Journal of Veterinary Sciences, 3(1): 87-99. DOI: https://www.doi.org/10.21608/svu.2020.20685.1038

Astuti P and Irawati DA (2022). Broiler chicken performance given *Moringa* (*Moringa oliefera* Lam) and Sambiloto (*Andrographis paniculata*) leaf extract in drinking water. Jurnal Ilmiah Peternakan Terpadu, 10(1): 92-100. DOI: https://www.doi.org/10.23960/jipt. v10i1.p92-100

Bagheri G, Martorell M, Ramírez-Alarcón K, Salehi B, and Sharifi-Rad J (2020). Phytochemical screening of *Moringa oleifera* leaf extracts and their antimicrobial activities. Cellular and Molecular Biology, 66(1): 20-26. DOI: http://www.doi.org/10.14715/cmb/2019.66.1.3

Bagri P and Kumar V (2024). Determination of genoprotection against cyclophosphamide induced toxicity in bone marrow of Swiss albino mice by *Moringa oleifera* leaves and *Tinospora cordifolia* stem. Journal of Toxicology and Environmental Health, Part A, 87(16): 647-661. DOI: https://www.doi.org/10.1080/15287394.2024.2356861

Bhattacharya A, Tiwari P, Sahu PK, and Kumar S (2018). A review of the phytochemical and pharmacological characteristics of *Moringa oleifera*. Journal of Pharmacy and Bioallied Sciences, 10(4): 181-191. DOI: https://www.doi.org/10.4103/JPBS.JPBS_126_18

Cao SJ, Xu S, Wang HM, Ling Y, Dong J, Xia RD, and Sun HH (2019). Nanoparticles: Oral delivery for protein and peptide drugs. Pharmaceutical Science and Technology, 20: 190. DOI: https://www.doi.org/10.1208/s12249-019-1325-z

Çelebi Y (2024). Improvement of quality characteristics and shelf life extension of raw chicken meat by using black mulberry leaf (*Morus nigra* L.) Extracts. Journal of Food Quality, 2024(1): 5822690. DOI: https://www.doi.org/10.1155/2024/5822690

Chhikara N, Kaur A, Mann S, Garg MK, Sofi SA, and Panghal A (2021). Bioactive compounds, associated health benefits and safety considerations of *Moringa oleifera* L.: An updated review. Nutrition & Food Science, 51(2): 255-277. DOI: http://www.doi.org/10.1108/NFS-03-2020-0087

Downing JA, Kerr MJ, and DL Hopkins (2017). The effects of pretransport supplementation with electrolytes and betaine on performance, carcass yield and meat quality of broilers in summer and winter. Livestock Science, 205: 16-23. DOI: https://www.doi.org/10.1071/AN23186

Hayat MN, Ismail-Fitry MR, KakaU, Rukayadi Y, Ab Kadir MZA, Radzi MAM, Kumar P, Nurulmahbub NA, and Sazili AQ (2024). Assessing meat quality and textural properties of broiler chickens: The impact of voltage and frequency in reversible electrical water-bath stunning. Poultry Science, 103(7): 103764. DOI: https://www.doi.org/10.1016/j.psj.2024.103764

Hossain MS, Urbi Z, Sule A, and Rahman KH (2014). *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. The Scientific World Journal, 2014(1): 274905. DOI: https://www.doi.org/10.1155/2014/274905

Islam Z, Islam SR, Hossen F, Mahtab-ul-Islam K, Hasan MR, and Karim R (2021). *Moringa oleifera* is a prominent source of nutrients with potential health benefits. International Journal of Food Science, 2021(1): 6627265. DOI: https://www.doi.org/10.1155/2021/6627265

Ivanova S, Sukhikh S, Popov A, Shishko O, Nikonov I, Kapitonova E, Krol O, Larina V, Noskova S, and Babich O (2024). Medicinal plants: A source of phytobiotics for the feed additives. Journal of Agriculture and Food Research, 16: 101172. DOI: https://www.doi.org/10.1016/j.jafr.2024.101172

Kholif AE, Gouda GA, Anele UY, and Galyean ML (2018). Extract of *Moringa oleifera* leaves improves feed utilization of lactating Nubian goats. Small Ruminant Research, 158: 69-75. DOI: https://www.doi.org/10.1016/j.smallrumres.2017.10.014

Martínez-Ballesta M, Gil-Izquierdo Á, García-Viguera C, and Domínguez-Perles R (2018). Nanoparticles and controlled delivery for bioactive compounds: Outlining challenges for new smart-foods for health. Foods, 7(5): 72. DOI: https://www.doi.org/10.3390/foods7050072

Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, Brar SK, Cote C, Ramirez AA, and Godbout S (2018). Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition, 4(2): 170-178. DOI: https://www.doi.org/10.1016/j.aninu.2018.03.002

Natsir MH, Sjofjan O, and Muharlien M (2017). The effect of used form and level green cincau leaves (Cycleabarbata L. Miers) as feed additive on broiler performance production. Research Journal of Life Science, 4(2): 87-96. DOI: https://www.doi.org/10.21776/ub.rjls.2017.004.02.1

Nithya P, Mekala P, and Gopalakrishnamurthy TR (2021). Qualitative phytochemical analysis of leaf extracts of *Andrographis paniculata* and its antibacterial activity on poultry pathogens. Journal of Pharmacognosy and Phytochemistry, 10(1): 1942-1944. DOI: https://www.doi.org/10.22271/phyto.2021.v10.i1aa.13634

Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, and Langer P (2014). Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: A review of its phytochemistry and pharmacology. Asian Pacific Journal of Tropical Disease, 4(3): 213-222. DOI: https://www.doi.org/10.1016/S2222-1808(14)60509-0

Oladeji OS, Odelade KA, and Oloke JK (2020). Phytochemical screening and antimicrobial investigation of *Moringa oleifera* leaf extracts. African Journal of Science, Technology, Innovation and Development, 12(1): 79-84. DOI: https://www.doi.org/10.1080/20421338.2019.1589082

Oraibi DH and Ali NAL (2021). Effect of adding alcoholic and nano alcoholic extract of *Moringa oleifera* leaves to drinking water on some blood parameters for laying hens Lohmann Brown. Biochemical & Cellular Archives, 21(1): 2765-2769. Available at: https://connectjournals.com/03896.2021.21.2765

Orkusz A, Rampanti G, Michalczuk M, Orkusz M, and Foligni R (2024). Impact of refrigerated storage on microbial growth, color stability, and pH of turkey thigh muscles. Microorganisms, 12(6): 1114. DOI: https://www.doi.org/10.3390/microorganisms12061114

Owoade AO, Alausa AO, Adetutu A, Olorunnisola OS, and Owoade AW (2021). Phytochemical characterization and antioxidant bioactivity of Andrographis paniculata (Nees). Pan African Journal of Life Sciences, 5(2): 246-256. DOI: https://www.doi.org/10.36108/pajols/1202.50.0220

Pierozan CR, Agostini PDS, Gasa J, Novais AK, Dias CP, Santos RSK, PereiraJr M, Nagi JG, Alves JB, and Silva CA (2016). Factors affecting the daily feed intake and feed conversion ratio of pigs in grow-finishing units: The case of a company. Porcine Health Management, 2: 7. DOI: https://www.doi.org/10.1186/s40813-016-0023-4

Prasetyaningrum A, Jos B, Ratnawati R, Rokhati N, Riyanto T, and Prinanda GR (2022). Sequential microwave-ultrasound assisted extraction of Flavonoid from *Moringa oleifera*: Product characteristic, antioxidant and antibacterial activity. Indonesian Journal of Chemistry, 22(2): 303-316. DOI: https://www.doi.org/10.22146/ijc.65252

Sari TV, Zalukhu P, and Mirwandhono RE (2021). Water content, pH and cooking loss of broiler meat with garlic-based herbs solution on drinking water. E3S Web of Conferences, 332: 01011. DOI: https://www.doi.org/10.1051/e3sconf/202133201011

Shafey TM, Mahmoud AH, Alsobayel AA, and Abouheif MA (2014). Effects of *in ovo* administration of amino acids on hatchability and performance of meat chickens. South African Journal of Animal Science, 44(2): 123-130. DOI: https://www.doi.org/10.4314/sajas.v44i2.4

Shahlehi S, Kasah R, Petalcorin MI, and Najim MMM (2024). Phytochemical analysis and anthelmintic activity of *Andrographis paniculata*, *Azadirachta indica*, and *Litsea elliptica* leaves extracts against *Caenorhabditis elegans*. Journal of Wildlife and Biodiversity, 8(3): 365-388. DOI: https://www.doi.org/10.5281/zenodo.12580790

Stevens CG, Ugese FD, Otitoju GT, and Baiyeri KP (2015). Proximate and anti-nutritional composition of leaves and seeds of *Moringa oleifera* in Nigeria: A comparative study. Agro-Science, 14(2): 9-17. DOI: http://www.doi.org/10.4314/as.v14i2.2

Udikala M, Verma Y, Sushma SL, and Lal S (2017). Phytonutrient and pharmacological significance of Moringa oleifera. International Journal of Life-Sciences Scientific Research, 3(5): 1387-1391. DOI: https://www.doi.org/10.21276/ijlssr.2017.3.5.21

Untari T, Herawati O, Anggita M, Asmara W, Wahyuni AETH, and Wibowo MH (2021). The effect of antibiotic growth promoters (AGP) on antibiotic resistance and the digestive system of broiler chicken in Sleman, Yogyakarta. In BIO Web of Conferences, 33: 04005. DOI: https://www.doi.org/10.1051/bioconf/20213304005

Utami CMP, Sjofjan O, and Natsir MH (2023). Effects of bioherbal compounds on performance and intestinal characteristics of laying chickens. Journal of World's Poultry Research, 13(2): 199-205. DOI: https://www.doi.org/10.36380/jwpr.2023.22

Wen C, Yan W, Zheng J, Ji C, Zhang D, Sun C, and Yang N (2018). Feed efficiency measures and their relationships with production and meat quality traits in slower growing broilers. Poultry Science, 97(7): 2356-2364. DOI: https://www.doi.org/10.3382/ps/pey062

Zhai R, Fu B, Shi X, Sun C, Liu Z, Wang S, Shen Z, Walsh TR, Cai C, Wang Y, et al. (2020). Contaminated in-house environment contributes to the persistence and transmission of NDM-producing bacteria in a Chinese poultry farm. Environment International, 139: 105715. DOI: https://www.doi.org/10.1016/j.envint.2020.105715

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 409-416.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj48
PII: S232245682400048-14

Amelioration Potency of a Nano-Therapeutic Drug in Rats with Uninephrectomy and Cisplatin-induced Toxicity

Rehab A. Mohamed*[1], Nadia A. Mohamed[1], Luneva Albina Vladimirovna[2], Lysenko Yuri Andreevich[2], and Hend M. Ahmed[1]

[1]*Medical Biochemistry Department, National Research Centre, Dokki, Giza, Egypt*
[2]*Department of Veterinary Medicine of the Russian State Agrarian University, Moscow Agricultural Academy named after K. A. Timiryazev, Moscow, Russian Federation*

*Corresponding author's Email: roroanas30@yahoo.com

ORIGINAL ARTICLE
Received: July 26, 2024
Revised: August 23, 2024
Accepted: September 15, 2024
Published: September 30, 2024

ABSTRACT

While physicians describe drugs to treat diseases, these medications may have cytotoxic effects on certain organs, necessitating the use of some drugs to ameliorate such adverse effects. The study was conducted to investigate the protective behavior of nanoemulsified sodium salicylate on uninephrectomized rats injected with cisplatin to induce nephrotoxicity. Fifty adult male albino rats, aged five weeks and weighing approximately 100-120 g, were divided into five groups. The first group received 200 mg/kg/day i.p normal saline for 30 days. The second group was administrated 200 mg/kg/day of nanoemulsified salt of salicylic acid for 30 days. The third group, comprising uninephrectomized rats, was injected with two doses of cisplatin (20 mg/kg body weight) on alternate days from the start of the experiment to induce nephrotoxicity. The fourth group, also uninephrectomized, received 200 mg/kg/day i.p of nanoemulsified sodium salicylate for 30 days. The fifth group, uninephrectomized and treated with 200 mg/kg/day sodium salicylate nanoemlusion for 21 days, was subsequently injected with two doses of cisplatin, followed by continued nanoemulsified sodium salicylate treatment until day 30 from the start of the study. The results showed a significant increase in tissue inhibitor metalloproteinase 1 (TIMP-1), Hyaluronic acid (HA), malondialdehyde, kidney injury molecule -1(KIM-1), and nitric oxide in the nephrotoxic group injected with cisplatin compared to the control group. Additionally, there was an elevation in the mRNA expression of nephrotoxic group with uninephrectomy. However, nephrotoxic rats treated with nanoemulsified sodium salicylate exhibited only a modest increase in TIMP-1, HA, and KIM-1 levels, along with elevated expressions of podocin and nephrin compared to the healthy control group. These findings suggest that nanoemulsified sodium salicylate exerts a protective effect against cisplatin-induced nephrotoxicity in uninephrectomized.

Keywords: Cisplatin, Lateral nephrectomy, Nanoemlusion, Podocyte, Rat, Surgery

INTRODUCTION

Drug toxicity is a major factor contributing to the withdrawal of medications from market. Between 1953 and 2013, over 400 medications were withdrawn, with 10% of causes related to neurotoxicity and 16% to cardiotoxicity (Onakpoya et al., 2016).

The considerable cost of drug development and research from 2009 to 2018 amounted to billions of dollars. A significant contributor to these costs is the high rate of failure during clinical trials and developmental studies (Wouters eta al., 2020; Rennane et al., 2021). Despite a 15.6% decline in sales in the pharmaceutical industry from 2008 to 2019, there was an increase in research and development intensity from 11.9% to 17.7%. Development and research spending for large pharmaceutical companies rose from 16.6% to 19.3%, while sales increased by 10.0% (from $380.0 to $418.0 billion) during the same period, even though the cost of drug development remained relatively stable or even decreased (Sertkaya et al., 2024). For instance, only about 15% of central nervous system drugs and 25% of cardiovascular drugs progress to phase 1 clinical trials (Amanat et al., 2022).

Antineoplastic drugs, widely used to treat various cancers, including ovarian, head, cervical, testicular carcinoma, and neck cancers, have demonstrated significant efficacy (Gómez-Sierra et al., 2018). Although the activity of antineoplastic drugs has been reported to be great, their use is often limited by severe side effects, such as hepatotoxicity,

To cite this paper: Mohamed RA, Mohamed NA, Vladimirovna LA, Andreevich LY, and Ahmed HM (2024). Amelioration Potency of a Nano-Therapeutic Drug in Rats with Uninephrectomy and Cisplatin-induced Toxicity. *World Vet. J.*, 14(3): 409-416. DOI: https://dx.doi.org/10.54203/scil.2024.wvj48

neurotoxicity, myelosuppression, ototoxicity, and nephrotoxicity (Pellacani and Eleftheriou, 2020). Several studies have revealed that cisplatin, a commonly used antineoplastic agent, accumulates in organs such as the liver and kidney (Palipoch and Punsawad, 2013; Apaydin et al., 2018; Aldossary, 2019).

Drug-induced toxicity, particularly nephrotoxicity, is a significant concern, leading to substantial health and economic losses (Frezza et al., 2010). The nephrotoxicity of cisplatin is attributed to its formation of highly active platinum complexes that bind to nucleophilic sites on DNA through an inter-strand and intra-strand crosslinking by nucleotide guanine. These symptoms result in denaturation and arrest cell cycle dewspite the mechanism of cisplatin nephrotoxicity change from its anticancer activity (Hanigan and Devarajan, 2003).

Cisplatin through cytochrome P450 mitochondrial dysfunction and microsome lead to the formation of reactive oxygen species (ROS) and damage to renal tissues. Elevated ROS levels contribute to acute failure of kidney through the induction of inflammatory cytokines, oxidative damage, tubule-interstitial inflammation, and necrosis of kidney tubular cells (Saisyo et al., 2016). Various adjuvants, including antioxidants, anti-apoptotic agents, and modulators of nitric oxide have been investigated for their potential protective effects against cisplatin-induced kidney injury (Ali and Al Moundhri, 2006).

Nasr (2014) demonstrated that cisplatin-induced hepatotoxicity in male experimental animals at a dose of 7.5 mg/kg i.p, altering lipid peroxidation and liver biomarkers, along with significant changes in antioxidant enzymes such as glutathione peroxidase and catalase. The formulation of nanoemulsified sodium salicylates, which offers a sustained effect due to its nanoformulation, has shown promise. Nanoemulsions based on silica nanoparticles are utilized for encapsulating sodium salicylates for several reasons, including the ability of nanoemulsified silica to carry bioactive ingredients and reduce their degradation. Encapsulating sodium salicylates move free to target through the bloodstream, induce disease targeting, and minimize the side effects on healthy organs.

This study explores the ameliorative effects of nanoemlusified sodium salicylates against nephrotoxicity induced by cisplatin in uninephrectomized experimental animals.

MATERIALS AND METHODS

Ethical approval

The study protocols and guide were approved by the National Research Centre Ethics Committee (Cairo, Egypt) under the registered ethical number 16/370.

Chemical kits

Sodium salicylate, cisplatin, and TRIzol reagent were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was prepared using a Millipore Milli-Q Plus system.

Experimental animal

Fifty adult male Wister albino rats, five weeks old and weighing about 100-120 g, were procured from the Animal Housing Laboratory at the National Research Centre (NRC), Giza, Egypt, and were divided into five groups. The rats were housed under standard conditions, with a temperature of 25±2 °C and suitable ventilation. They were kept in qualified polypropylene cages with free access to standard diet and water.

Experimental design

Following a 10-day acclimatization period, the fifty rats were randomly divided into five groups: The first (control) group received 200 mg/kg/day i.p normal saline for 30 days. The second (nanoemulsion) group was administrated 200 mg/kg/day of nanoemulsified salt of salicylic acid for 30 days. The third (nephrotoxic) group, including the uninephrectomized rats, were injected with two doses of cisplatin (20 mg/kg body weight) on alternate days from the start of the experiment to induce nephrotoxicity. The fourth (treatment) group, comprising uninephrectomized rats, were injected with 200 mg/kg/day i.p nano emulsion of sodium salicylate for 30 days. Finally, the fifth (pre-treatment and cisplatin) group, including uninephrectomized rats, were injected with 200 mg/kg/day sodium salicylate nanoemlusion for 21 days, followed by two doses of cisplatin (20 mg/kg, administered on alternate days). The nanoemulsified sodium salicylate treatment continued until the end of the 30-day study period.

Surgical nephrectomy

Seven days before the experiment, the rats were anesthetized by inhalation of diethyl ether for 5 to 12 minutes, depending on their weight, with an ether volume of approximately 6 ml per hour. The surgical site was shaved, washed with soap and water, and sterilized with 70% alcohol followed by 4% tincture iodine. The incision in the muscles and

skin was done using a sterile cutter. The wound was widened till the kidney appeared using forceps. Separate ligation of the renal vein and renal artery was done with kidney excision. Lamberts sutures were used to close the wound.

Sampling of kidney tissue and blood

Thirty days after the start of the study, the rats were fasted for 12 hours. Blood samples were collected from the venous plexus of the eyes using a capillary tube with heparin. About 3.5 ml of blood was collected in tubes for biochemical analysis of the study's relevant biological parameters. One kidney was quickly removed from each rat, perfused, and rinsed with ice-cold saline to remove blood cells (Wu et al.,2017). The renal tissue was preserved in TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA) for gene expression analysis (Saleh et al., 2022).

Investigation of biological markers

The mRNA expression levels of various genes in renal tissues were quantified using real-time quantitative PCR (qRT-PCR). The serum levels of tissue inhibitor of metalloproteinases-1 (TIMP-1), hyaluronic acid (HA), and kidney injury molecule-1 (KIM-1) were measured in samples collected 30 days after the start of the experiment.

Preparation of genes

Expression analysis of nephrotoxicity related genes

RNA isolation

Total RNA was isolated from renal tissues of all experimental groups using TRIzol® reagent. The isolated RNA was then dissolved in deionized water. To eliminate any contaminating DNA, RNA samples were treated with RNAse-free DNAse. The purity of the RNA was assessed using spectrophotometry.

Reverse transcription reaction

First-strand cDNA synthesis was performed to generate cDNA copies from the isolated RNA. The reverse transcription reaction was carried out using the following program: about 600 seconds at room temperature, followed by 60 minutes at 42 °C, and 300 seconds at 95°C. The resulting cDNA was then collected in tubes and kept in tubes inside snow cups (Khalil and Booles, 2011).

Quantitative Real Time-PCR

Quantitative Real-Time PCR was conducted in a 0.025 mL reaction volume, which included 0.0125 mL of SYBR® Premix Ex TaqTM, 0.005 mL 0.002 mM antisense primer, 0.005 mL 0.002 mM sense primers, 0.0065 mL distilled water, and 0.005 mL of cDNA template. The qRT-PCR program consisted of the following steps: Step 1 involved an initial denaturation for 180 seconds at 95.0°C. Step 2 included 40 cycles. Step 3 involved generating a melting curve profile with about 71 turns, starting at 60°C and increasing by 0.5°C every 0.6 minutes until reaching 95.0°C (Khalil and Booles, 2011).

Statistical investigations

Statistical Analysis was carried out using one-way analysis of variance (ANOVA), followed by Bonferroni post hoc analysis. All data were expressed as mean ± standard error. The data were analyzed using SPSS software (Version 16). A p-value of less than 0.001 was considered statistically significant.

RESULTS

Cisplatin-induced nephrotoxicity in the experimental animals led to a significant increase in serum levels of HA, TIMP-1, and KIM-1 ($p < 0.05$). However, in uninephrectomized animals treated with ameliorate, there was a significant but comparatively smaller elevation in the levels of HA, KIM-1, and TIMP-1 compared to healthy control animals ($p < 0.05$, Table 1).

Table 1. Effect of nano emulsified sodium salicylate on adult male albino rats weighing about 100-120g aged 5 weeks

Treatments	HA (Pg\ml)	TIMP-1 (Pg\ml)	KIM-1 (Pg\ml)
Control	40.5 ± 1.07	68 ± 1.04	24 ± 1.18
Carrier	42.9 ± 2.02	78 ± 1.06	25 ± 1.13
CisP	90.9 [a,b,d] ± 3. 5	128[a,b,d] ± 2.16	158 [a,b,d] ± 1.18
SiNPs@sod	43.6 [c,,e] ±1.17	86[c,,e] ± 1.04	99 [c,e] ±1.19
SiNPs@sod-CisP	49.4[a,b,c] ± 3.6	77 [a,b,c] ± 1.07	53 [a,b,c] ± .99

*Results are expressed as means ± SD for 10 animals in certain 5 sets. [a] all results in comparison with the normal group, [b]all result in comparison to SiNPs group. [c] Significant difference compared to the Cisplatin group. [d] Significant difference compared to SiNPs@sod group [e] Significant difference compared to SiNPs@sod- Cisplatin group significant at $P < 0.05$. The superscript (a,b,c,d) shows a significant difference at $p < 0.05$. **HA: Hyaluronic acid; TIMP -1: Tissue inhibitor metalloproteinase 1; KIM -1: kidney injury molecule -1, First group: Control, second group: Nanoemulsion, third (nephrotoxic) group: Injected with two doses of cisplatin. Fourth (treatment) group: Uninephrectomized rats, were injected with 200 mg/kg/day i.p nanoemulsion of sodium salicylate. Fifth (pre-treatment and cisplatin) group: Uninephrectomized rats, injected with 200 mg/kg/day sodium salicylate nanoemlusion, followed by two doses of cisplatin.

Oxidative stress markers in kidney homogenate

Kidney malondialdhyde and nitric oxide levels were significantly elevated by 116% and 128%, respectively, in the Cisplatin group compared to the control, indicating increased oxidative stress. However, both renal malondialdhyde and nitric oxide levels showed a significant decrease ($p < 0.05$) in the group treated with nano-emulsified sodium salicylate compared to the normal ($p < 0.05$) Conversely, rats treated with sodium salicylate alone or with the nanoemulsified form exhibited no significant changes in oxidative stress markers (Table 2).

Nephrin and Podocin genes

The expression levels of podocin and nephrin showed a significant decrease in the Cisplatin-only group with a 1.2- and 1.3-fold reduction, respectively ($p < 0.05$). However, uni nephrectomized animals treated with nano emulsified exhibited only a modest reduction in the expression levels of nephrin and podocin genes with a 0.2-fold decrease compared to healthy animals. In contrast, rats treated with either silicate or the nanoemulsified form of salicylic acid showed no significant variation in gene expression levels compared to the normal control group ($p < 0.05$) (Table 3).

Table 2. Effects of nano emulsified sodium salicylate on oxidative stress indicators of adult male albino rats weighing about 100-120g aged 5 weeks

Treatments	MD (nmol/l)	No(nmol/l)
Control	90.9 ± 5.2	10 ± 0.45
Carrier	91.57 ± 2.8	9.1 ± 0.3
CisP	$190.1^{a,b,d,e}$ ± 12.5	$50.2^{a,b,d,e}$ ± 0.69
SiNPs@sod	$100.6^{c,e}$ ± 5.04	$12.02^{c,e}$ ± 0.42
SiNPs@sod-CisP	$105.2^{a,b}$ ± 4.1	$19.9 \pm 0.79^{a,b}$

*Results are revealed as means ± SD for 10 animals in certain 5 sets. The superscript (^{a,b,c,d}) shows a significant difference at $p < 0.05$. ** MD: Malondialdehyde; NO: Nitric oxide; CisP: Cisplatin; SiNPs@sod: Sodium salicylate nanoparticles; SiNPs@sod-CisP: Sodium salicylate nanoparticle with cisplatin, first group: Control, second group: Nanoemulsion, third (nephrotoxic) group: Injected with two doses of cisplatin. Fourth (treatment) group: Uninephrectomized rats, were injected with 200 mg/kg/day i.p nano emulsion of sodium salicylate. Fifth (pre-treatment and cisplatin) group: Uninephrectomized rats, injected with 200 mg/kg/day sodium salicylate nanoemlusion, followed by two doses of cisplatin.

Table 3. Effect of nano emulsified sodium salicylate 0n expression of Podocin and Nephrin genes in adult male albino rats weighing 100 -120 g aged 5 weeks

Treatments	Nephrin	Podocin
Control	1.74 ± 0.012	2.1 ± 0.03
Carrier	1.8 ± 0.004	2.2 ± 0.008
CisP	0.9^{abde} ± 0.002	0.76^{abde} ± 0.004
SiNPs@sod	1.07 ± 0. 14	1.9 ± 0.012
SiNPs@sod-CisP	1.16^{ab} ± 0.017	1.5^{ab} ± 0.016

*Values are represented as means ± SD for 10 rats in each group and 5 sets. The superscript (^{a,b,c,d}) shows a significant difference at $p < 0.05$. CisP: Cisplatin; SiNPs@sod: Sodium salicylate nanoparticles; SiNPs@sod-CisP: Sodium salicylate nanoparticle with cisplatin, first group: Control, second group: Nanoemulsion, third (nephrotoxic) group: Injected with two doses of cisplatin. Fourth (treatment) group: Uninephrectomized rats, were injected with 200 mg/kg/day i.p nano emulsion of sodium salicylate. Fifth (pre-treatment and cisplatin) group: Uninephrectomized rats, injected with 200 mg/kg/day sodium salicylate nanoemlusion, followed by two doses of cisplatin.

DISCUSSION

Many systemic approaches with selective protection of normal cells from the toxic effects of chemotherapy also have been investigated (Brizel, 1998). The current study investigated the nephro-protective potentials using the technique of nanotechnology drug delivery as a cytoprotector. According to the obtained results, cisplatin injection significantly increased the serum HA. The nephrotoxic effect of cisplatin on serum HA was previously reported (Akin et al., 2017). Extracellular matrix markers undergo both qualitative and quantitative alterations during the process of fibrosis. Potential markers of fibrosis include glycosaminoglycans and glycoproteins found in the extracellular matrix, as well as the synthesis of matrix and enzymes associated with degradation, along with collagen synthesis and the enzymes involved in its breakdown. One of the direct markers of fibrosis is HA, which is found to be elevated in chronic kidney disease. Results in the present study revealed that the cisplatin group recorded a significant increase in serum TIMP-1 compared to control. Additionally, serum TIMP- 1 mean value was significantly increased in the cisplatin group compared to the carrier group. The result is in agreement with Tianhui et al. (2012), who suggested that the activation of TIMP1 is responsible for the deposition of extracellular matrix following kidney damage. Protection of nephrotoxic rats with

nanoemlusified salt of salicylic acid resulted in a deceased TIMP-1 relative nonprotected group, however, it was still higher than its matched value in the control group. The ameloriting effect of encapsulated sodium salycsilate on serum TIMP-1 was reported previously (Cheng et al., 2020). In this work increased serum KIM-1 in the cisplatin group was noted and agrees with Takaharu et al., (2004). Takaharu et al., (2004) reported that KIM-1 may be a sensitive general renal injury or early repair biomarker in animals exposed to nephrotoxicants. Measurement of KIM-1 protein expression may be beneficial for the detection of abnormalities of tubular epithelial cells during injury/repair in the kidney. KIM-1 may be useful in preclinical and clinical studies vital to drug development and evaluation. It may also serve in the monitoring of disease states that manifest as injury to the proximal tubule and be useful in guiding interventional strategies (Tanase et al., 2019; Chalo and Aydilek, 2022). Administration of nano-emulsified sodium salicylate ameliorated Cisplatin-induced changes in multiple blood and urine variance, contributing to a notable recovery. Results of the present study revealed no significant change in kidney Malondialdhyde and nitric oxide in the carrier group compared to the control. Furthermore, a significant increase in kidney MDA and NO in the cisplatin cisplatin-injected group was observed compared to the control and carrier groups. These results are in agreement with Naqshbandi et al., (2013). Oxidative stress plays a pivotal role in Cisplatin-induced toxicity, leading to various changes in blood and urine parameters. Under normal physiological conditions, the generation and elimination of ROS in tissues are balanced by endogenous scavenging systems such as catalases, reduced glutathione, and superoxide dismutase. Nanoemulsified sodium salicylate appears to mimic these mechanisms. Elevated ROS levels damage multiple macromolecules, including DNA, lipids, and proteins. Oxygen radicals play a role in the nephrotoxicity enhanced by cisplatin lead to damage of macromolecules in cells. Elevating oxidative stress causes necrosis and cellular injury in the liver, kidney, and other tissues (Naqshbandi et al., 2013). Antioxidant enzymes, such as paraoxinase scavenge free radicals or convert them into non-toxic forms (Zheng et al., 2008).

The results of the present study showed that cisplatin injection elevated the levels of lipid peroxidation markers, such as nitric oxide and malondialdhyde (Ognjanović et al., 2012; Koyuncu et al., 2017). In contrast, nano emulsified sodium salicylate decreased lipid peroxidation levels in the kidney and helped retain normalized antioxidant enzyme levels. Patients with chronic renal failure exhibited multiple changes in amino acid and protein metabolism, including increased levels of homocysteine amino acid (Guldener, 2006). Elevated homocysteine levels are frequently observed in individuals suffering from renal failure, with multiple studies establishing a correlation between heightened homocysteine levels and a significant reduction in glomerular filtration rate (Ninomiya et al., 2004). These observations align with the results of the present study, where homocysteine levels increased in the cisplatin toxic group compared to the healthy group. Acute kidney damage increases homocystein due to alterations in the disposal of homocystein or impaired metabolism (Guldener, 2006). The enhancement observed with nano-emlusified salt of salicylic acid in the present study could reduce the cisplatin-induced elevation of TIMP1 of Cis- c, indicating the significant protective role of this nano-formulated medication in preventing renal injury (Al Nahyah, 2021). The TIMP-1 is responsible for the precipitation of the extracellular matrix (Cong et al., 2013).

The filtration of blood components via the formation of urine in the glomerulus capsule is one of the key functions of the kidney. The filtration pores between adjacent foot processes are crucial for maintaining the integrity of the glomerular filter (Murray and Paolini, 2023). In experimental animals, several studies have highlighted the direct role of the slit diaphragm in the progression of proteinuria, suggesting its importance in preserving the glomerular filtration barrier (Kawachi and Shimizu, 2000; Kocylowski et al., 2022).

The current study indicated that the level of tissue nephrin mRNA and podocin mRNA significantly decreased in the cisplatin group compared to the normal control and carrier groups, which was previously reported by Kaveripakam and Adikay (2017). Podocin and nephrin are essential proteins located in the podocyte slit diaphragm, significantly contributing to the functionality of the glomerular filter (Huber et al., 2003). Researchers indicated the downregulation of nephrin mRNA in the cisplatin group, including the characterization of structural alteration in glomerular epithelial cells, alteration of foot processes, and subsequent filtration slits, as well as the loss of podocytes. From the current perspective and alongside the results of the present study, the loss of podocytes is closely related to progressive glomerular injury or both. Protection with carrier recorded upregulation of nephrin mRNA compared to the cisplatin group, indicating an improvement in kidney functions (Wang et al., 2002).

CONCLUSION

The use of unsaturated lipid and polar solvents in the formulation of nanoemlusified sodium salicylate as a drug delivery system has shown significant potential in improving kidney disorders. These findings suggest that nanoemulsified sodium salicylate exerts a protective effect against cisplatin-induced nephrotoxicity in uninephrectomized. Further studies are required to evaluate the effects of nano-emulsified sodium salicylate on the toxicity of other internal organs.

DECLARATIONS

Funding

This study is part of a project financially supported by the National Research Center, Cairo, Egypt (Project No.11010132).

Availability of data and materials

Raw data or additional supporting information is available upon request from the corresponding author.

Acknowledgments

The authors are grateful to the National Research Centre of Egypt in Dokki, Giza, and the Department of Veterinary Medicine of the Russian State Agrarian University.

Authors' contributions

This study was completed with the assistance and participation of all authors. The methods and design of the study (especially laboratory works) were developed by Rehab A. Mohamed, Nadia A. Mohamed, and Hend M. Ahmed. The drafting, analysis and proofreading were conducted by Rehab A. Mohamed, Luneva Albina Vladimirovna, and Lysenko Yuri Andreevich. All authors checked and approved the final edition of the manuscript before publication.

Competing interests

The authors do not have any conflict of interest.

Ethical considerations

The article was checked for plagiarism during the writing of the draft of the manuscript and also the final edition was originally prepared for submission. The authors confirm that all authors have reviewed and submitted the original manuscript to this journal for the first time.

REFERENCES

Akin D, Ozmen S, Yilmaz ME. Hyaluronic Acid as a New Biomarker to Differentiate Acute Kidney Injury from Chronic Kidney Disease. Iranian journal of kidney diseases,11(6):409-413. Available at: https://pubmed.ncbi.nlm.nih.gov/29190600/

Al Nahyah KS (2021). Utilization of nanotechnology and hepatoprotective agents to improve the bioavailability and to reduce the toxicity associated with isotretinoin. Doctoral Dissertation, King Abdulaziz University, Saudi Arabia. Available at: https://platform.almanhal.com/Files/4/235749

Aldossary SA (2019). Review on pharmacology of cisplatin: Clinical use, toxicity and mechanism of resistance of cisplatin. Biomedical and Pharmacology Journal, 12(1): 7-15. DOI: https://www.doi.org/10.13005/bpj/1608

Ali BH and Al Moundhri MS (2006). Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: A review of some recent research. Food and Chemical Toxicology, 44(8): 1173-1183. DOI: https://www.doi.org/10.1016/j.fct.2006.01.013

Amanat M, Nemeth CL, Fine AS, Leung DG, and Fatemi A (2022). Antisense oligonucleotide therapy for the nervous system: From bench to bedside with emphasis on pediatric neurology. Pharmaceutics, 14(11): 2389. DOI: https://www.doi.org/10.3390/pharmaceutics14112389

Apaydin FG, Kaltalıoğlu K, Balabanlı B, and Cevher ŞC (2018). Morin and hesperidin ameliorate cisplatin-induced hepatotoxicity and nephrotoxicity in rats: A histopathological study. Gazi University Journal of Science, 31(2): 399-406. Available at: https://dergipark.org.tr/en/pub/gujs/issue/37206/337235

Brizel DM (1998). Future directions in toxicity prevention. In Seminars in Radiation Oncology, 8 (Suppl 1): 17-20. PMID: 9794997.

Chalo S, and Aydilek İB (2022). A New Preprocessing Method for Diabetes and Biomedical Data Classification. Qubahan Academic Journal, 2(4):6-18. DOI: https://www.doi.org/10.48161/qaj.v2n4a135

Cheng H, Lv M, Mi R, and Xue G (2020). Amifostine ameliorates cerebral ischaemia-reperfusion injury via p38-mediated oxidative stress and mitochondrial dysfunction. Folia neuropathologica, 58(4): 334-346. DOI: https://www.doi.org/10.5114/fn.2020.102436

Cong M, Liu T, Wang P, Fan X, Yang A, Bai Y, Peng Z, Wu P, Xiaofei Tong, Jing Chen et al. (2013). Antifibrotic effects of a recombinant adeno-associated virus carrying small interfering RNA targeting TIMP-1 in rat liver fibrosis. The American Journal of Pathology, 182(5): 1607-1616. DOI: https://www.doi.org/10.1016/j.ajpath.2013.01.036

Frezza M, Hindo S, Chen D, Davenport A, Schmitt S, Tomco D, and Ping Dou Q (2010). Novel metals and metal complexes as platforms for cancer therapy. Current Pharmaceutical Design, 16(16): 1813-1825. DOI: https://www.doi.org/10.2174/138161210791209009

Gómez-Sierra T, Eugenio-Pérez D, Sánchez-Chinchillas A, and Pedraza-Chaverri J (2018). Role of food-derived antioxidants against cisplatin induced-nephrotoxicity. Food and Chemical Toxicology, 120: 230-242. DOI: https://www.doi.org/10.1016/j.fct.2018.07.018

Guldener C (2006). Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering?. Nephrology Dialysis Transplantation, 21(5): 1161-1166. DOI: https://www.doi.org/10.1093/ndt/gfl044

Hanigan MH and Devarajan P (2003). Cisplatin nephrotoxicity: Molecular mechanisms. Cancer Therapy, 1: 47. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2180401/

Huber TB, Simons M, Hartleben B, Sernetz L, Schmidts M, Gundlach E, Saleem MA, Walz G, Benzing T (2003). Molecular basis of the functional podocin-nephrin complex: Mutations in the NPHS2 gene disrupt nephrin targeting to lipid raft microdomains. Human Molecular Genetic, 12(24): 3397-3405. DOI: https://www.doi.org/10.1093/hmg/ddg360

Kaveripakam S and Adikay S (2017). Amelioration of Cisplatin- Induced Nephrotoxicityby Roots of Anthocephalus cadamba. Biomedical & Pharmacology Journal; 10(3): 1433-1439. DOI: https://www.doi.org/10.13005/bpj/1250

Khalil W and Booles H (2011). Protective role of selenium against over-expression of cancer-related apoptotic genes induced by-cresol in rats. Archives of Industrial Hygiene and Toxicology, 62(2): 121-129. DOI: https://www.doi.org/10.2478/10004-1254-62-2011-2074

Kawachi H, and Shimizu F (2000). Molecular composition and function of the slit diaphragm: nephrin, the molecule responsible for proteinuria. Clinical and experimental nephrology, 4:161-72. DOI: https://www.doi.org/10.1007/s101570070017

Kocylowski MK, Aypek H, Bildl W, Helmstädter M, Trachte P, Dumoulin B, and et al. (2022). A slit-diaphragm-associated protein network for dynamic control of renal filtration. Nature Communications, 13(1):6446. Available at: https://www.nature.com/articles/s41467-022-33748-1

Koyuncu I, Kocyigit A, Gonel A, Arslan E, and Durgun M (2017). The protective effect of Naringenin-oxime on cisplatin-induced toxicity in rats. Biochemistry Research International, 2017: 9478958. DOI: https://www.doi.org/10.1155/2017/9478958

Mondi S, Kvsrg P, Jhansi D, Vijay R, and Rao VU (2014). Prophylactic and curative effect of ethanolic extract of Bassiamalabaricabark against cisplatin induced nephrotoxicity. Asian Journal of Pharmaceutical and Clinical Research, 7(4): 143-146. Available at: https://journals.innovareacademics.in/index.php/ajpcr/article/view/1205

Murray IV, and Paolini MA (2023). Histology, kidney and glomerulus. StatPearls [Internet]. Medline. Available at: https://pubmed.ncbi.nlm.nih.gov/32119431/

Naqshbandi A, Rizwan S, and Khan F (2013). Dietary supplementation of flaxseed oil ameliorates the effect of cisplatin on rat kidney. Journal of Functional Foods, 5(1): 316-326. DOI: https://www.doi.org/10.1016/j.jff.2012.11.002

Nasr AY (2014). Aged garlic extract protects against cisplatin-induced hepatotoxicity in adult male rats: biochemical and ultrastructure study. Life Science Journal, 11(12): 92-101. Available at: http://www.lifesciencesite.com/lsj/life1112/016_26087life111214_92_101.pdf

Naziroğlu M, Karaoğlu A, and Aksoy AO (2004). Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. Toxicology, 195(2-3): 221-230. DOI: https://www.doi.org/10.1016/j.tox.2003.10.012

Ninomiya T, Kiyohara Y, Kubo M, Tanizaki Y, Tanaka K, Okubo K, Nakamura H, Hata J, Oishi Y, Kato I et al. Hyperhomocysteinemia and the development of chronic kidney disease in a general population: The Hisayama study. American Journal of Kidney Diseases, 44(3): 437-445. DOI: https://www.doi.org/10.1053/j.ajkd.2004.05.024

Ognjanović BI, Djordjević NZ, Matić MM, Obradović JM, Mladenović JM, Štajn AŠ, and Saičić ZS (2012). Lipid peroxidative damage on cisplatin exposure and alterations in antioxidant defense system in rat kidneys: A possible protective effect of selenium. International Journal of Molecular Sciences, 13(2): 1790-1803. DOI: https://www.doi.org/10.3390/ijms13021790

Onakpoya IJ, Heneghan CJ, and Aronson JK (2016). Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. BMC Medicine, 14: 10. DOI: https://www.doi.org/10.1186/s12916-016-0553-2

Palipoch S and Punsawad C (2013). Biochemical and histological study of rat liver and kidney injury induced by cisplatin. Journal of Toxicologic Pathology, 26(3): 293-299. DOI: https://www.doi.org/10.1293/tox.26.293

Pellacani C and Eleftheriou G (2020). Neurotoxicity of antineoplastic drugs: Mechanisms, susceptibility, and neuroprotective strategies. Advances in Medical Sciences, 65(2): 265-85. DOI: https://www.doi.org/10.1016/j.advms.2020.04.001

Rennane S, Baker L, and Mulcahy A (2021). Estimating the cost of industry investment in drug research and development: a review of methods and results. INQUIRY: The Journal of Health Care Organization, Provision, and Financing, 58: 1-11. DOI: https://www.doi.org/10.1177/00469580211059731

Saisyo A, Nakamura H, Fang J, Tsukigawa K, Greish K, Furukawa H, and Maeda H (2016). pH-sensitive polymeric cisplatin-ion complex with styrene-maleic acid copolymer exhibits tumor-selective drug delivery and antitumor activity as a result of the enhanced permeability and retention effect. Colloids and Surfaces B: Biointerfaces, 138: 128-137. DOI: https://www.doi.org/10.1016/j.colsurfb.2015.11.032

Saleh H, Salama M, and Hussein RM (2022). Polyethylene glycol capped gold nanoparticles ameliorate renal ischemia–reperfusion injury in diabetic mice through AMPK-Nrf2 signaling pathway. Environmental Science and Pollution Research, 29(51):77884-907. DOI: https://www.doi.org/10.1007/s11356-022-21235-5

Sertkaya A, Beleche T, Jessup A, and Sommers BD (2024). Costs of drug development and research and development intensity in the US, 2000-2018. JAMA Net Open, 7(6): e2415445. DOI: https://www.doi.org/10.1001%2Fjamanetworkopen.2024.15445

Takaharu I, Hung CC, Yang SA, Stevens JL, Bonventre JV. Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. American Journal of Physiology-Renal Physiology, 286(3): F552-F563. Available at: https://journals.physiology.org/doi/full/10.1152/ajprenal.00285.2002

Tanase DM, Gosav EM, Radu S, Costea CF, Ciocoiu M, Carauleanu A, and et al. (2019). The predictive role of the biomarker kidney molecule-1 (KIM-1) in acute kidney injury (AKI) cisplatin-induced nephrotoxicity. International journal of molecular sciences, 20(20): 5238. DOI: https://www.doi.org/10.3390/ijms20205238

Wang SX, Rastaldi MP, Pätäri A, Ahola H, Heikkilä E, and Holthöfer H (2002). Patterns of nephrin and a new proteinuria-associated protein expression in human renal diseases. Kidney International, 61(1): 141-147. DOI: https://www.doi.org/10.1046/j.1523-1755.2002.00114.x

Wouters OJ, McKee M, and Luyten J (2020). Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009-2018. JAMA, 323(9): 844-853. Available at: https://jamanetwork.com/journals/jama/article-abstract/2762311

Wu J, Pan X, Fu H, Zheng, Dai Y, Yin Y, Chen Q, Hao Q, Bao D, and Hou D (2017). Effect of curcumin on glycerol-induced acute kidney injury in rats. Scientific Reports, 7: 10114. DOI: https://www.doi.org/10.1038/s41598-017-10693-4

Zheng W, Huang LZ, Zhao L, Wang B, Xu HB, and Wang GY (2008). Superoxide dismutase activity and malondialdehyde level in plasma and morphological evaluation of acute severe hemorrhagic shock in rats. The American Journal of Emergency Medicine, 26(1): 54-58. DOI: https://www.doi.org/10.1016/j.ajem.2007.02.007

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 417-423.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj49
PII: S232245682400049-14

Identification of Genetic Diversity of Taro White Cattle Using Microsatellite DNA Markers

Luh Gde Sri Surya Heryani[1*], Ni Nyoman Werdi Susari[1], Ni Luh Astria Yusmalinda[2], and I Made Merdana[3]

[1]*Department of Veterinary Basic Science, Faculty of Veterinary Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*
[2]*Yayasan Biodiversitas Indonesia (BIONESIA), Denpasar 80234, Bali, Indonesia*
[3]*Departemnt of Veterinary Pharmacy and Pharmacology, Faculty of Veterinary Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*

*Corresponding's author's Email: surya_heryani@unud.ac.id

ORIGINAL ARTICLE
Received: June 30, 2024
Revised: July 27, 2024
Accepted: August 21, 2024
Published: September 25, 2024

ABSTRACT

Taro white cattle have a small population found in Taro Village, Tegalalang, and Gianyar, Bali, which must be protected as one of the most valuable biological resources for the Hindu community in Bali. White cattle require conservation efforts, with morphometric characterization and genetics providing the first phases of an animal conservation program. The purpose of this study was to identify the genetic diversity of Taro white cattle based on allele frequency, heterozygosity, and fixation index (FIT) using different microsatellite DNA loci so that it can be used as a study and reference in determining policies for the conservation of Taro white cattle in Indonesia, particularly in Bali. This cross-sectional observational study utilized *ETH 185*, *INRA 035*, and *INRA 037* microsatellite DNA markers. Polymerase chain reaction amplified a total of 22 Taro white cattle blood samples with an average of 4.33 alleles; the average observed heterozygosity and expected heterozygosity were 0.288 and 0.637, respectively, with an average fixation index value of 0.55. In conclusion, there is a significant deviation from the Hardy-Weinberg equilibrium with the likelihood of inbreeding, as indicated by the Hardy-Weinberg balance. The microsatellite loci used in this study can be further used to evaluate the genetic diversity of Taro white cattle.

Keywords: Conservation, Genetic diversity, Microsatellite DNA, Taro white cattle

INTRODUCTION

In the woodlands of Taro Village, Tegallalang District, Gianyar Regency, a very small cattle population is known as white cows native to Taro Village. The uniqueness of these white cattle is that they are holy, sacred, and respected by the community. The cattle may only be utilized in holy sites, including Merkur (Ngasti Widana), Tri Buana, and Eka Dasa Rudra. The extremely small population of Taro cattle will result in inbreeding. This can result in a loss of genetic diversity and a decline in environmental adaptability. Referring to the population risk threshold, white cattle are classified as critical (critical breed). According to FAO (2007), a population of less than 100 individuals is thought to be in an area critical to extinction. Provided that left uncontrolled, the amount of biological wealth not cared for will increase, resulting in the loss of genetic wealth. To prevent biodiversity loss in Indonesia, particularly the white cattle as germplasm and since they play a crucial role in religious events for the Hindu community in Bali, it is necessary to conserve white cattle.

This conservation initiative is linked to the Provincial Government of Bali's commitment and program to accelerate the achievement of the Millennium Development Goals (MDGs) in accordance with the Bali MDGs Roadmap, one of the eight Millennium Development Goals being environmental sustainability (Tanner et al., 2020). Therefore, support for the preservation of white cattle is essential. Reduced biological resource loss is one of the actions centered on conserving the environment (Allendorf, 2017). In conservation efforts, it is essential to understand the population's features; genetic characterization is a crucial stage in animal conservation projects. The genetic features can be used as a guide for determining the distinctness of the animal race (Abdelmanova et al., 2020). Microsatellite deoxyribonucleic acid (DNA) is now commonly used to determine genetic distances through molecular analysis (Moniruzzaman et al., 2015). Since it is not affected by the environment, microsatellites are ideally suited for identifying an order (Abdelkader et al., 2018). Microsatellites are valuable for genome mapping due to their very random distribution within the genome (Aguirre-Liguori et al., 2020). Microsatellites are currently the most prevalent genetic markers utilized in molecular testing (Bora et al., 2023).

To cite this paper: Heryani LGSS, Susari NNW, Yusmalinda NLA, and Merdana IM (2024). Identification of Genetic Diversity of Taro White Cattle Using Microsatellite DNA Markers. *World Vet. J.*, 14(3): 417-423. DOI: https://dx.doi.org/10.54203/scil.2024.wvj49

Microsatellites exhibit significant rates of polymorphism and mutation (Glazko et al., 2023). Variation in the number of repeat units accounts for the high microsatellite polymorphism (Bhargava and Fuentes, 2010). Primarily due to the existence of a slippage replication mechanism, mutations occur (Richard, 2021). The rapid mutation rate of microsatellite DNA is mainly attributable to variations in the number of repeating bases added or removed relative to variations in the base sequence (Lei et al., 2021). The reported microsatellite mutation rate is roughly 10-3 to 10-6 (Zhang et al., 2003; Svishcheva et al., 2020). Therefore, the present study aimed to identify the genetic diversity of Taro White cattle using microsatellite DNA loci.

MATERIALS AND METHODS

The present study was a cross-sectional observational study that involved collecting primary data and descriptive analysis of microsatellite DNA polymorphisms to establish the genetic profile of Taro White cattle to use in conservation efforts (Figure 1). A total of 22 adult White Taro cattle (8 males and 14 females) aged 2-8 years and weighing 200-350 kg were investigated in the present study. Three milliliters of blood samples were taken aseptically through the jugular vein and then charged with three distinct microsatellite DNA markers, *ETH 185*, *INRA 035*, and *INRA 037*, which are found on chromosome 17 (*ETH 185*), chromosome 16 (*INRA 035*), and chromosome 10 (*INRA 037*), respectively. Food and Agriculture Organization-International Society for Animal Genetics (FAO-ISAG) suggested that microsatellite DNA markers successfully extracted from diverse breeds of cattle in Indonesia were used to select the primers in the following order (FAO, 2011).

a. *ETH 185*: TGCATGGACAGAGCAGCCTGGC
   GCACCCCAACGAAAGCTCCCA
b. *INRA 035*: TTGTGCTTTATGACACTATCCG
   ATCCTTTGCAGCCTCCACATTG
c. *INRA 037*: GATCCTGCTTATATTTAACCAC
   AAAATTCCATGGAGAGAGAAAC

Figure 1. Taro White Cattle in Taro village, Tegalalang district, Gianyar Regency, Bali, Indonesia

The amplification using the applied biosystem thermal cycler was performed for 35 cycles, with the first cycle at 95°C for five minutes, the next 33 cycles (denaturation at 94°C for thirty seconds, annealing at 58°C for eighty seconds, extension at 72°C for ninety seconds), and the last cycle was elongation at 72°C for five minutes.

Analysis of genetic polymorphism

Allele frequency

The frequency of each allele at each microsatellite locus was estimated using the following formula (Chen et al., 2016).

$$X1 = \frac{(2N1.1 + N1.2)}{2N}$$

X1 is the allele frequency of a first locus, N1.1 is the number of individuals that are homozygous for allele 1, N1.2 is the number of individuals that are homozygous for allele 1, and N is the total number of individuals.

Heterozygosity

The level of heterozygosity was computed via the following formula.

$$H = \frac{2\,N\,(1 - \sum X1^2)}{2N - 1}$$

H is locus heterozygosity, X is allele frequency, and N is the number of individuals.

The microsatellite Toolkit V.3.1 program was used to calculate allele frequencies, allele counts, observed and anticipated heterozygosities (Ho and He), and total heterozygosity (Ht) (Meglécz et al., 2014). To determine random mating in the local population, Hardy-Weinberg equilibrium analysis used the fixation index (FIT), and to determine the deviation of the FIT value from zero (inbreeding occurred), it was evaluated with one degree of freedom, as indicated in the following formula.

$$F_{IT} = 1 - \frac{H_o}{H_T}\; X^2 = n\, {F_{IT}}^2$$

Ethical approval

The current study has received approval from the Animal Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, Indonesia, with Number B/70/UN14.2.9/PT.01.04/2024, and was carried out at the Biomedical Laboratory of the same institution from January to March 2024.

Data analysis

The observed number of alleles (na), allele frequency, observed Ho, expected He, and fixation index value or the inbreeding coefficient (F) from each locus of microsatellite DNA were analyzed using GenAlex, version 6.5 (Genetic analysis in Excel 6.5 version) (Peakall and Smouse, 2012). The GENEPOP, version 4.7.x was used to generate the P-value of Hardy-Weinberg equilibrium (HWE) and the P-value of Linkage disequilibrium (LD) (Rousset, 2008). Hardy-Weinberg disequilibrium is a model for predicting genotype and allele frequencies in a non-evolving population (Abramovs et al., 2020). Linkage disequilibrium is a measure of non-random association between segments of DNA (alleles) at different positions on the chromosome (loci) in a population (Slatkin, 2008). Loci are considered independent or in linkage disequilibrium when the association frequency of different alleles is higher than expected. Loci are said to be randomly associated when the association frequency of different alleles is lower than expected and lower than expected if the loci were associated randomly (Slatkin, 2008). The p-value significant deviation from the HWE test was $p < 0.05$. The p-value significance associated randomly with the LD test was $p < 0.05$.

RESULTS AND DISCUSSION

The results indicated that the three markers had been amplified successfully at the microsatellite locus of Taro cattle. All samples had 13 alleles, with the number of alleles at each location ranging from 3 at the *INRA 037* locus to 5 at the *ETH 185* and *INRA 035* loci, with an average allele diversity of 4.33. The average allele frequency was 0.231. The range of allele diversity was from 110 bp (*INRA 035*) to 250 bp (*ETH 185*, Table 1).

The *INRA 035* locus contained five alleles with the following product sizes: 110 bp, 114 bp, 118 bp, 120 bp, and 128 bp. The frequency of the most dominant 110 bp allele was 0.364%. The mean observed Ho and anticipated He were 0.18 and 0.74, respectively, and the fixation index was 0.755. The *INRA 037* locus had three distinct alleles with 144 bp, 148 bp, and 150 bp product length. The frequency of the most dominant 150-bp allele was 0.614. Mean observed Ho and anticipated He were 0.318 and 0.505, respectively, with a fixation index of 0.370.

The average fixation index (F) or inbreeding coefficient was 0.526, which means close to one. The *ETH 185*, *INRA 035*, and *INRA 037* locus suggested the likelihood of inbreeding in the Taro cattle population ($p < 0.05$, Table 2).

The p-value linkage disequilibrium test from three loci *ETH 185*, *INRA 035*, and *INRA 037* were 0.071, 0.114, and 0.194, respectively. These three loci that were analyzed stated that the loci were independent (Table 3, $p > 0.05$).

Table 1. The number, size, and frequency of alleles of locus *ETH 185*, *INRA 035*, and *INRA 037* in Taro white cattle

Breed	Locus	Number of alleles	Allele size (bp)	Allele frequency
Taro White cattle	*ETH 185*	5	230	0.523
			236	0.114
			240	0.068
			248	0.159
			250	0.136
Taro White cattle	*INRA 035*	5	110	0.364
			114	**0.023**
			118	0.182
			120	0.227
			128	0.205
Taro White cattle	*INRA 037*	3	144	0.045
			148	0.341
			150	**0.614**
Total allele		13		
Average Allele diversity		4.33		
Average allele frequency				0.231

Table 2. Observed heterozygosity, expected heterozygosity, fixation index, and Hardy-Weinberg equilibrium analysis among three microsatellite loci in adult Taro White Cattle

Locus	Number of alleles	Allele size	Ho	He	F	p-value*	HWE
ETH 185	5	230-250	0.364	0.665	0.453	0.0000	*
INRA 035	5	110-128	0.182	0.741	0.755	0.0000	*
INRA 037	3	144-150	0.318	0.505	0.370	0.0367	*
Average	4.33		0.288	0.637	0.526		

Ho: Observed heterozygosity, He: Expected heterozygosity, F: Fixation index, HWE: Hardy Weinberg equilibrium. *p less than 0.05 is Significant

Table 3. Linkage disequilibrium test among three microsatellite loci in adult Taro White Cattle

Pop	Loci 1	Loci 2	p-value*	LD
Taro Village	*ETH 185*	*INRA 035*	0.071	NS
Taro Village	*ETH 185*	*INRA 037*	0.114	NS
Taro Village	*INRA 035*	*INRA 037*	0.194	NS

Pop: Population, LD: Linkage disequilibrium, NS: Not significant; *p less than 0.05 is Significant

DISCUSSION

The development of several molecular markers (DNA markers) has made it possible to identify the genetic changes in a cross and their relationship to changes in cattle's quantitative and qualitative traits (Glazko et al., 2023). In addition, molecular markers can be utilized to distinguish between cattle breeds, particularly in connection with attempts to conserve and maintain the breed's purity (Yaro et al., 2017). Microsatellite markers can be used to examine the genetic diversity of a population and determine the genetic relationship between two populations (Machmoum et al., 2020).

The average heterozygosity within a breed quantifies its genetic variety (Abdelmanova et al., 2020). Heterozygosity is a parameter that can be used to determine a population's genetic diversity (Bora et al., 2023). The heterozygosity value runs from zero to one; provided that the value is close to zero, the heterozygosity is low, which can threaten the survival of a population or species (Misrianti et al., 2022). In case the value is close to one, the heterozygosity is high; the higher the heterozygosity value of a population, the greater the frequency of inbreeding (Alvarez et al., 2011). Low genetic variability can result in animal populations with high inbreeding coefficients (White et al., 2021). Additionally, a high level of inbreeding in specific animal groups might reduce hybrid vigor (Sharma et al., 2020).

At the *INRA 035* gene, five alleles with various sizes ranging from 110 to 128 bp were identified. The allele size with the highest frequency was 110 bp, with a frequency of 0.364. In contrast to the research of Kuantan 1 cattle, Kuantan 2 cattle, Pesisir cattle, and Madura cattle at Riau province, Indonesia, the number of alleles and allele sizes observed in the current study at the *INRA 035* locus are 17, 11, 5, and 98-120 bp, respectively (Misrianti et al., 2022). Indicative of the genetic variability of a population, the number of alleles is proportional to sample size. Increasing the number of populations and samples can increase the number of detectable alleles (Allendorf, 2017). A large number of alleles indicates greater genetic diversity (Sheriff and Alemayehu, 2017).

The Ho, the fraction of Ho in a population, and He, and the proportion of heterozygous loci per individual are the parameters most frequently employed to evaluate genetic diversity in a population (Toro et al., 2009; Yaro et al., 2017). The current study observed that the Ho was less than the expected He, indicating that the Taro cattle population was genetically homogeneous. The Ho values ranged from 0.182 to 0.364 with an average value of 0.288, whereas expected He values ranged from 0.505 to 0.741 with an average value of 0.637. Small sample sizes, inbreeding, and a lack of migration of new genetic material can all contribute to low genetic diversity (Cañón et al., 2006; Peixoto et al., 2021). The *ETH 185* and *INRA 037* have greater heterozygosity compared to *INRA 035*. The difference between the observed Ho and the predicted He can be utilized as a measure of genotype imbalance in the Taro cattle population, indicating that an acceptable selection activity has been carried out and that random mating has not occurred (Tambasco et al., 2003; Labroo et al., 2021). According to Allendorf (2017), a population is considered to be in equilibrium if the genotype and allele frequency remain constant from generation to generation due to random mating in a large population. The Hardy-Weinberg equilibrium model is based on many key assumptions that must be met for a population to be in genetic equilibrium. These key assumptions include no mutation, no gene flow, a high population size, random mating, and no natural selection (Cayuela et al., 2018). Inbreeding is a common cause of non-random mating, as it increases homozygosity across all genes (Alvarez et al., 2011; Abramovs et al., 2020).

According to the heterozygosity value, the *ETH 185* microsatellite locus with a Ho value of 0.364 was the most informative in this study. In contrast, the *INRA 035* microsatellite locus with a Ho value of 0.182 was the least informative locus, following the statement of Sharma et al. (2009), which asserts that a Ho value below 0.5 implies a low gene variation in a population. The average Ho of Taro cattle in this study was 0.288, indicating a very low level of variation in Taro cattle. A deviation from the Hardy-Weinberg equilibrium is shown by a significant difference between the Ho mean (0.288) and expected He (0.637) for the microsatellite loci employed in this study and the low Ho value. Tree loci have been analyzed, and the deviation of HWE with significant p-values was shown, indicating that inbreeding has occurred. The linkage disequilibrium test from three loci that have been analyzed was not significant, stating that the loci are independent. The meaning-independent loci used in this study did not cause deviations in the HWE test (Slatkin, 2008). The inbreeding coefficient or the fixation index (F) values also supported the deviation HWE results with a fixation index (F) value close to 1, which indicates low heterozygosity. The homozygosity of Taro cattle from the *ETH 185*, *INRA 035*, and *INRA 037* loci was greater than anticipated. According to (Alvarez et al., 2011), the reported genetic effects of inbreeding in a small population of animals can decrease heterozygosity, or gene variety, and increase the frequency of recessive disorders. According to Kardos (2021), inbreeding has negative effects on a small population, including a decrease in diversity and their ability to evolve or adapt to the environment. The population decrease of animals can also be attributed to habitat fragmentation, which disturbs gene flow and increases genetic drift and inbreeding (Bora et al., 2023).

CONCLUSION

According to the obtained results of the study, the average number of alleles was 4.33, with allele sizes at loci *ETH 185* (230-250), *INRA 035* (110-128), and *INRA 037* (144-150). The *INRA 037* locus had the highest allele frequency at 150 bp, while the INRA35 locus had the lowest frequency at 114 bp. *ETH 185* was the most informative microsatellite locus, whereas *INRA 035* was the least informative. In the present study, the Taro cattle population had low heterozygosity, confirmed by observed Ho, which was less than He. The loci did not cause low genetic diversity in the study, but it was caused by inbreeding. Inbreeding has caused low genetic diversity, as confirmed by the fixation index (F) or the inbreeding coefficient value close to one. The significant p-value of the HWE test also indicated that inbreeding occurred in this Taro cattle population. Further research is recommended to develop a breeding program that can avoid inbreeding and prevent loss of genetic diversity.

DECLARATIONS

Funding

This study is funded by the flagship research program of the Faculty of Veterinary Medicine, Udayana University with grant No.: B/78.239/UN14.4.A/PT.01.03/2023.

Availability of data and materials

The presented data is available and can be reasonably requested by correspondence.

Acknowledgments

The authors would like to thank the Dean of the Faculty of Veterinary Medicine, the Head of the Institute for Research and Community Service, and the Chancellor of Udayana University for supporting facilities and in-kind funding.

Authors' contribution

Luh Gde Sri Surya Heryani conceptualized and designed the research. Ni Nyoman Werdi Susari and I Made Merdana conducted research, collected samples, and literacy. Ni Luh Astria Yusmalinda carried out laboratory examinations. Luh Gde Sri Surya Heryani and Ni Nyoman Werdi Susari wrote the paper. All authors contributed proportionally and confirmed this article.

Competing interests

All authors state no conflict of interest in publishing this study.

Ethical considerations

Ethical issues, such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct, have been checked by all the authors before publication in this journal.

REFERENCES

Abdelkader AA, Ata N, Benyoucef MT, Djaout A, Azzi N, Yilmaz O, Cemal İ, and Gaouar SBS (2018). New genetic identification and characterisation of 12 Algerian sheep breeds by microsatellite markers. Italian Journal of Animal Science, 17(1): 38-48. DOI: https://www.doi.org/10.1080/1828051X.2017.1335182

Abdelmanova AS, Kharzinova VR, Volkova VV, Mishina AI, Dotsev AV, Sermyagin AA, Boronetskaya OI, Petrikeeva LV, Chinarov RY, and Brem G (2020). Genetic diversity of historical and modern populations of Russian cattle breeds revealed by microsatellite analysis. Genes, 11(8): 940. DOI: https://www.doi.org/10.3390/genes11080940

Abramovs N, Brass A, and Tassabehji M (2020). Hardy-weinberg equilibrium in the large scale genomic sequencing era. Frontiers in Genetics, 11: 210. DOI: https://wwwdoi.org/10.3389/fgene.2020.00210

Aguirre-Liguori JA, Luna-Sánchez JA, Gasca-Pineda J, and Eguiarte LE (2020). Evaluation of the minimum sampling design for population genomic and microsatellite studies: An analysis based on wild maize. Frontiers in Genetics, 11: 870. DOI: https://www.doi.org/10.3389/fgene.2020.00870

Allendorf FW (2017). Genetics and the conservation of natural populations: Allozymes to genomes. Molecular Ecology, 26(2): 420-430. DOI: https://www.doi.org/10.1111/mec.13948

Alvarez G, Quinteiro C, and Ceballos FC (2011). Inbreeding and genetic disorder. In: K. Ikehara (Editor), Advances in the study of genetic disorders. IntechOpen, pp. 21-44. DOI: https://www.doi.org/10.5772/18373

Bhargava A and Fuentes F (2010). Mutational dynamics of microsatellites. Molecular Biotechnology, 44: 250-266. DOI: https://www.doi.org/10.1007/s12033-009-9230-4

Bora SK, Tessema TS, Girmay G, and Jin X (2023). Genetic diversity and population structure of selected Ethiopian indigenous cattle breeds using microsatellite markers. Genetics Research, 2023: e28. DOI: https://www.doi.org/10.1155/2023/1106755

Cañón J, García D, García-Atance M, Obexer-Ruff G, Lenstra J, Ajmone-Marsan P, Dunner S, and Consortium E (2006). Geographical partitioning of goat diversity in Europe and the Middle East. Animal Genetics, 37(4): 327-334. DOI: https://www.doi.org/10.1111/j.1365-2052.2006.01461.x

Cayuela H, Rougemont Q, Prunier JG, Moore JS, Clobert J, Besnard A, and Bernatchez L (2018). Demographic and genetic approaches to study dispersal in wild animal populations: A methodological review. Molecular Ecology, 27(20): 3976-4010. DOI: https://www.doi.org/10.1111/mec.14848

Chen YS, Su YC, and Pan W (2016). Effect of spatial constraints on hardy-weinberg equilibrium. Scientific Reports, 6(1): 19297. DOI: https://www.doi.org/10.1038/srep19297

Food and agriculture organization (FAO) (2007). The state of the world's animal genetic resources for food and agriculture. In: B. Rischkowsky, D. Pilling (Editors), Commission on genetic resources for food and agriculture. Rome, pp. 37-38. Available at: https://www.fao.org/4/a1250e/a1250e.pdf

Food and agriculture organization (FAO) (2011). Molecular genetic characterization of animal genetic resources. FAO animal production and health guidelines, No. 9. Rome, pp. 67-69. Available at: https://www.fao.org/4/i2413e/i2413e00.pdf

Glazko V, Kosovsky GY, Glazko T, and Fedorova L (2023). DNA markers and microsatellite code. Sel'skokhozyaistvennaya Biologiya, 58(2): 223-248. DOI: https://www.doi.org/10.15389/agrobiology.2023.2.223eng

Kardos M (2021). Conservation genetics. Current Biology, 31(19): R1185-R1190. DOI: https://www.doi.org/10.1016/j.cub.2021.08.047

Labroo MR, Studer AJ, and Rutkoski JE (2021). Heterosis and hybrid crop breeding: A multidisciplinary review. Frontiers in Genetics, 12: 643761. DOI: https://www.doi.org/10.3389/fgene.2021.643761

Lei Y, Zhou Y, Price M, and Song Z (2021). Genome-wide characterization of microsatellite DNA in fishes: Survey and analysis of their abundance and frequency in genome-specific regions. BMC Genomics, 22(1): 421. DOI: https://www.doi.org/10.1186/s12864-021-07752-6

Machmoum M, Boujenane I, Azelhak R, Badaoui B, Petit D, and Piro M (2020). Genetic diversity and population structure of Arabian horse populations using microsatellite markers. Journal of Equine Veterinary Science, 93: 103200. DOI: https://www.doi.org/10.1016/j.jevs.2020.103200

Meglécz E, Pech N, Gilles A, Dubut V, Hingamp P, Trilles A, Grenier R, and Martin JF (2014). Qdd version 3.1: A user-friendly computer program for microsatellite selection and primer design revisited: Experimental validation of variables determining genotyping success rate. Molecular Ecology Resources, 14(6): 1302-1313. DOI: https://www.doi.org/10.1111/1755-0998.12271

Misrianti R, Wijaya S, Sumantri C, and Jakaria J (2022). Genetic diversity analysis and determination of specific alleles of Kuantan cattle using microsatellite markers. Tropical Animal Science Journal, 45(2): 134-140. DOI: https://www.doi.org/10.5398/tasj.2022.45.2.134

Moniruzzaman M, Khatun R, Yaakob Z, Khan M, and Mintoo A (2015). Development of microsatellites: A powerful genetic marker. The Agriculturists, 13(1): 152-172. DOI: https://www.doi.org/10.3329/agric.v13i1.26559

Peakall R and Smouse PE (2012). Genalex 6.5: Genetic analysis in Excel. Population genetic software for teaching and research an update. Bioinformatics, 28: 2537-2539. DOI: https://www.doi.org/10.1093/bioinformatics/bts460

Peixoto MGC, Carvalho MRS, Egito AA, Steinberg RS, Bruneli FÂT, Machado MA, Santos FC, Rosse IC, and Fonseca PAS (2021). Genetic diversity and population genetic structure of a guzerá (*Bos indicus*) meta-population. Animals, 11(4): 1125. DOI: https://www.doi.org/10.3390/ani11041125

Richard GF (2021). The startling role of mismatch repair in trinucleotide repeat expansions. Cells, 10(5): 1019. DOI: https://www.doi.org/10.3390/cells10051019

Rousset F (2008). Genepop'007: A complete re-implementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8(1): 103-106. DOI: https://www.doi.org/10.1111/j.1471-8286.2007.01931.x

Sharma R, Ahlawat S, Sharma H, Bhagat R, Singh P, and Tantia M (2020). Identification of a new potential native Indian cattle breed by population differentiation based on microsatellite markers. Molecular Biology Reports, 47: 6429-6434. DOI: https://www.doi.org/10.1007/s11033-020-05639-5

Sharma R, Pandey A, Singh Y, Prakash B, Mishra B, Kathiravan P, Singh P, and Singh G (2009). Evaluation of genetic variation and phylogenetic relationship among north Indian cattle breeds. Asian-Australasian Journal of Animal Sciences, 22(1): 13-19. DOI: https://www.doi.org/10.5713/ajas.2009.70047

Sheriff O and Alemayehu K (2017). Genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs. Asian Journal of Agriculture, 1(1): 46-51. DOI: https://www.doi.org/10.13057/asianjagric/g010109

Slatkin M (2008). Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. Nature Reviews Genetics, 9(6): 477-485. DOI: https://www.doi.org/10.1038/nrg2361

Svishcheva G, Babayan O, Lkhasaranov B, Tsendsuren A, Abdurasulov A, and Stolpovsky Y (2020). Microsatellite diversity and phylogenetic relationships among east Eurasian bos taurus breeds with an emphasis on rare and ancient local cattle. Animals, 10(9): 1493. DOI: https://www.doi.org/10.3390/ani10091493

Tambasco D, Paz C, Tambasco-Studart M, Pereira A, Alencar M, Freitas A, Coutinho L, Packer I, and Regitano LdA (2003). Candidate genes for growth traits in beef cattle crosses bos taurus × bos indicus. Journal of Animal Breeding and Genetics, 120(1): 51-56. DOI: https://www.doi.org/10.1046/j.1439-0388.2003.00371.x

Tanner C, Bicchieri M, Nijhoff P, and Daley E (2020). A review of land tenure issues and options for the future-FAO Indonesia report. FAO., Jakarta, pp. 54-56. DOI: https://www.doi.org/10.4060/cb0429en

Toro MA, Fernández J, and Caballero A (2009). Molecular characterization of breeds and its use in conservation. Livestock Science, 120(3): 174-195. DOI: https://www.doi.org/10.1016/j.livsci.2008.07.003

White KS, Levi T, Breen J, Britt M, Meröndun J, Martchenko D, Shakeri YN, Porter B, and Shafer AB (2021). Integrating genetic data and demographic modeling to facilitate conservation of small, isolated mountain goat populations. The Journal of Wildlife Management, 85(2): 271-282. DOI: https://www.doi.org/10.1002/jwmg.21978

Yaro M, Munyard KA, Stear MJ, and Groth DM (2017). Molecular identification of livestock breeds: A tool for modern conservation biology. Biological Reviews, 92(2): 993-1010. DOI: https://www.doi.org/10.1111/brv.12265

Zhang W, Wang Y, Long J, Girton J, Johansen J, and Johansen KM (2003). A developmentally regulated splice variant from the complexlola locus encoding multiple different zinc finger domain proteins interacts with the chromosomal kinase jil-1. Journal of Biological Chemistry, 278(13): 11696-11704. DOI: https://www.doi.org/10.1074/jbc.M213269200

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 424-434.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj50
PII: S232245682400050-14

Microorganisms' Growth Inhibition in Poultry Meat Using *Bacillus* spp.

Olena. V. Kolchyk[1], Iryna V. Borovuk[2], Andriy I. Buzun[1], Tetiana V. Illarionova[3], and Nadiia M. Zazharska[4*]

[1]*National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", 83 Pushkinska Str., Kharkiv, 61023, Ukraine*
[2]*State enterprise "Dnipropetrovsk Regional State Scientific and Technical Center of Standardization, Metrology and Certificati on", 23 Barrikadna Str., Dnipro, 49044, Ukraine*
[3]*Limited Liability Company "Sirion", 18 Akinfeeva Str., Dnipro, 49027, Ukraine*
[4]*Dnipro State Agrarian and Economic University, Faculty of Veterinary Medicine, Yefremov Str., 25, Dnipro, 49000, Ukraine*

*Corresponding author's Email: zazharskayan@gmail.com

ORIGINAL ARTICLE
Received: July 24, 2024
Revised: August 29, 2024
Accepted: September 13, 2024
Published: September 30, 2024

ABSTRACT

Meat processing enterprises are currently seeking ways to improve the efficiency of their operations. This study aimed to assess the presence of harmful microorganisms in poultry meat treated with a probiotic complex of *Bacillus* spp. bacteria during storage. Of the 2,516 meat samples collected from broiler chickens across six poultry processing enterprises in the Dnipropetrovsk region over three years, 1,845 samples tested positive for pathogens. *Listeria* spp. were isolated in 52.7% of meat samples, S. aureus in 28.7%, *P. aeruginosa* – in 6.9%, *E. coli* in 4.2%, and *Salmonella* spp. in 7.5%. The next stage of the study was the infection of 10 samples of poultry meat with pathogens of test cultures (*Escherichia coli UNCSM* - 007, *Pseudomonas aeruginosa UNCSM* - 012, *Staphylococcus aureus UNCSM* - 017, *Listeria ivanovii UNCSM* - 042, *Salmonella* Enteritidis *UNCSM* - 081), followed by aerosol treatment with a probiotic complex of *Bacillus* spp. (1.5×10^8 in ml (0.5 Mac Farland) administered at a dose of 1 ml per sample with daily registration of colony growth. Following pathogen contamination and a single aerosol treatment with the probiotic complex of *Bacillus* spp., the growth *of E. coli* and *S. aureus* was already suppressed on the second day of meat storage. The probiotic complex of *Bacillus* spp. was able to displace *Salmonella* Enteritidis on the third day and *P. aeruginosa* on day 4, but the growth of *L. ivanovii* could be observed only on day 5. The probiotic complex of *Bacillus* spp. formed visible biofilms from the five strains of microorganisms and remained viable for five days, forming a dense biofilm with a high accumulation rate of 4.73 D620. A distinctly noticeable ability to form microbial biofilms within three days was observed in planktonic forms of *L. ivanovii* up to 2.88 D620, followed by *P. aeruginosa* at 2.28 D620. Low biofilm density was observed for *Salmonella* Enteritidis (1.77 D620) and *S. aureus* (1.76 D620). The probiotic complex of bacteria of the genus *Bacillus* spp. shows potential for use in meat processing plants to prevent the growth of harmful microbial biofilms on meat products stored under refrigeration.

Keywords: Antagonistic activity, Biofilm formation, Microbial biofilm, Pathogen, Probiotic complex of *Bacillus* spp.

INTRODUCTION

Currently, much attention is directed to the safety of food products of animal origin. To meet international quality and safety standards, these products must be free from toxic substances, as well as pathogenic and opportunistic microorganisms. It is crucial to note that poultry meat may contain harmful microorganisms, which can lead to severe food poisoning and even death in humans. Therefore, the initial processing of poultry carcasses (thawing, pinching, burning,) plays a significant role in ensuring the quality of the meat. It is especially important to avoid contamination of the carcasses with pathogenic and opportunistic microflora. Additionally, cross-contamination of meat products during primary processing may result in a higher concentration of harmful microorganisms, which can pose a risk to human health (Bridier et al., 2015; Markowiak and Śliżewska, 2018; Zazharskyi et al., 2023). The microbiota found on the surfaces of food processing plants are often diverse and include foodborne pathogens as well as food spoilage bacteria. Predominant genera in meat processing plants include *Pseudomonas*, *Acinetobacter*, *Staphylococcus*, and *Serratia* (Fegan and Jenson, 2018). One of the pathogens regularly encountered in such environments is *Listeria monocytogenes*, which causes the life-threatening disease listeriosis. Therefore, it is important to maintain viability at low temperatures. Moreover, it should be noted that their pathogenic prevalence is not reduced by chilling the meat. Increased resistance to inhibitory agents is associated with the survival of microorganisms, which is often linked to the development of biofuels (Wang et al., 2015; Puga et al., 2016). One of the pathogens regularly living in such media is *Listeria monocytogenes*. This means that illness due to listeriosis poses a more serious threat to human health (D'Ostuni et al., 2016; Fan et al., 2020; Borovuk and Zazharska, 2022).

To cite this paper: Kolchyk OV, Borovuk IV, Buzun AI, Illarionova TV, and Zazharska NM. (2024). Microorganisms' Growth Inhibition in Poultry Meat Using *Bacillus* spp. *World Vet. J.*, 14(3): 424-434. DOI: https://dx.doi.org/10.54203/scil.2024.wvj50

Biofilms of *L. monocytogenes* on food contact surfaces have been identified as an important pathway for pathogenic persistence and subsequent product contamination (Nowak et al., 2017; Pažin et al., 2018; Zazharskyi et al., 2019). The formation of *Listeria* biofilms in combination with *Pseudomonas*, *Bacillus cereus*, *Escherichia coli O157:H7*, *Salmonella* spp., *Pseudomonas* spp. *i Staphylococcus aureus* bacteria contribute to the preservation and maintenance of the bacterial population, thereby showing resistance to antimicrobial drugs, ultraviolet radiation, drying, and disinfectants (Akinbobola et al., 2017; Wang et al., 2018; Zazharskyi et al., 2020). Microbial cells are capable of detaching from the biofilm and spreading, making the biofilm a potential source of contamination in the production of meat products (Flemming and Wingender, 2010; Abdullahi et al., 2016; Lin et al., 2017). Thus, contamination of food production environments with biofilms of pathogenic bacteria and the development of effective methods to remove these biofilms from meat products, production surfaces, and equipment is a significant challenge. The use of spore-probiotic strains of *Bacillus*, which can reduce microbial contamination during the storage of meat products in processing plants, is highly relevant. Previous studies have laid the foundation for this research by identifying the antagonistic properties of probiotic preparations in destroying microbial biofilms in animal feed, which is crucial for preventing microbial contamination in the human food chain. It was established that the probiotic complex of bacteria of the genus *Bacillus* during continuous exposure to feed samples could displace biofilms of almost all types of isolated feed microflora (Kolchyk et al., 2022). To improve the sanitation and hygiene of poultry meat and extend its shelf life while ensuring safe consumption, it is imperative to explore new methods that are safe for the environment. Therefore, this study aimed to evaluate the presence of harmful microorganisms in poultry meat that had been treated with a probiotic complex of *Bacillus* spp. bacteria during the storage process.

MATERIAL AND METHODS

Ethical approval

The experiment was approved by the Bioethics Committee of the National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine. During the research on animals, manipulations were carried out in accordance with the existing documents regulating the organization of work with the use of animals in experiments and adherence to the principles of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (WorldLII, 1986).

Study conditions

The research was carried out at the Laboratory of Swine Diseases at the National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine (Kharkiv, Ukraine) and the Dnipropetrovsk Regional State Laboratory of the State Service of Ukraine on Food Safety and Consumer Protection (Dnipro, Ukraine).

Sampling for laboratory research

Microbiological studies were performed on 2516 meat samples from broiler chickens obtained from six poultry processing enterprises in the Dnipropetrovsk region over three years. Immediately after slaughter, meat samples (chest and thigh muscles) were taken from each carcass, weighing 25g, and examined for the presence of *Listeria, Pseudomonas, Staphylococcus aureus, Escherichia coli, and Salmonella* spp. Pathogen's detection was carried out following current regulatory documents. Specifically, they were tested for *Escherichia coli* according to ISO/IEC 17025 (2017), *Pseudomonas* spp. – ISO 13720 (2010), *Staphylococcus aureus* – ISO 11290-1 (2017), *Salmonella* spp. – ISO 6579-1 (2017), and -ISO 6888-1 (2021), *Listeria* spp. The selection of meat samples from broiler chickens was conducted in accordance with ISO/TS 17728 (2015). Sample preparation for microbiological studies adhered to ISO 6887-2 (2014), 6887-3 (2017), and ISO 6887-2 (2017).

The identification of pathogenic microorganisms involved several main stages: Preliminary enrichment, selective enrichment, sowing on selective media, and confirmation of suspicious colonies. For preliminary enrichment of *Listeria* spp., the meat sample was minced and mixed with the Half Fraser broth (HiMedia, India) in a 1:10 ratio and incubated at (30.0 ± 0.5) °C for 24 hours. Then, 0.1 ml was transferred to Complete Fraser broth (HiMedia, India.) and incubated at a temperature of (37.0 ± 0.5) °C for 48 hours. It was sown on selective agar media (Agar Listeria) according to Ottaviani and Agosti (ALOA, HiMedia, India) and Polymyxin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol (PALCAM, HiMedia, India). Plates were incubated at a temperature of 37.0 ± 0.5 °C for 48 hours. After incubation, colonies of Listeria spp., which had typical morphology (blue-green colonies with or without a black center on ALOA or gray-green with a black halo on PALCAM) were assessed.

For the isolation of *Salmonella* spp, the meat sample was ground and mixed with buffered peptone water (BPW) in a ratio of 1:10, and incubated at a temperature of 37.0 ± 0.5 °C for 24 hours. Two selective media were used for

additional enrichment: soy peptone broth Rappaport-Vassiliadis (RVS, HiMedia, India), and tetrathionate broth (TT, Farmactiv, Ukraine). While the RVS broth was incubated at 41.5 ± 0.5 °C for 24 hours, the TT broth was incubated at 37.0 ± 0.5 °C for 24 hours. The samples were then plated on selective agar media: Xylose, lysine, and desocholate agar (XLD, HiMedia, India), and hexanoate-lysine agar (HE, HiMedia, India). The plates were incubated at a temperature of 37.0 ± 0.5 °C for 48 hours. After incubation, the plates were examined and suspicious colonies were counted. Typical Salmonella colonies on XLD agar displayed black centres with a red border, while on HE agar, blue-green colonies with black centres were observed.

For the isolation of *S. aureus*, 10 g of meat cut was crushed and homogenized in 90 ml of a phosphate buffer solution corresponding to a dilution of 1:10. The sample was sown on the surface of Beard-Parker agar (Ukraine), enriched with egg yolk and potassium tellurite, and using a micropipette, it was evenly distributed with a spatula. It was then incubated at a temperature of 37.0 ± 0.5 °C for 24 hours. After incubation, colonies with typical appearance of coagulase-positive staphylococci were counted. Typical colonies on Beard-Parker agar were black or gray-black with a distinct zone of lecithinase activity (opalescent zone).

The identification of *Pseudomonas* spp. was carried out using a meat sample weighing 10 g, which was homogenized in 90 ml of a phosphate buffer solution diluted 1:10. A 0.1 ml aliquot of the homogenized sample was applied to the surface of Cetrimide, Fucidin, and Cephaloridine (CFC, Merck, Germany) agar with a micropipette and evenly distributed using a sterile spatula. The plates were incubated at a temperature of 25.0 ± 0.5 °C for 24 hours. After incubation, the agar was examined and colonies with a typical appearance of *Pseudomonas* spp. (round, smooth, opalescent colonies) were counted.

E. coli was isolated from a meat sample weighing 10 g, which was homogenized in 90 ml of a phosphate buffer solution with a dilution of 1:10. The sample was plated on Endo and Levin agar (Farmaktiv, Ukraine), and incubated at a temperature of 37.0 ± 0.5 °C for 24 hours. After incubation, the agar was examined and the *E. coli* colonies on Endo had a characteristic metallic sheen, while on Levin agar, they appeared black with a metallic sheen.

According to the results, 1845 samples tested positive for pathogens out of 2516 research samples. *Listeria* spp. was isolated in 52.7% of the samples, *S. aureus* in 28.7%, *P. aeruginosa* in 6.9%, *E. coli* – 4.2%, and *Salmonella* spp. in 7.5% of the broiler chicken meat samples.

The pathogenicity of the isolated microorganisms (*Listeria* spp., *P. aeruginosa*, *S. aureus*, *E. coli*, *Salmonella* spp.) was determined using the Dosis Certa Lettalis (DCL) on 70 white outbred mice (n=10), weighing 18-20 g and aged 8-9 weeks, kept in a vivarium for a month. The animals were adapted to the conditions of detention for 15 days. Six experimental groups and one control group, each consisting of 10 animals of the same age, were formed. While mice from the six experimental groups were injected intraperitoneally with a certain type of bacteria in a dose of 0.5 ml (at a concentration of 4.0 MacFarland) isolated from meat samples, mice in the control group were injected with 0.5 ml of physiological solution. The behavioural reactions and the physiological state of mice were monitored for 10 days. In five experimental groups, 100% mortality of the animals was recorded on the second to third day of observation, indicating the pathogenicity of the field isolates. No deaths occurred in the control group (Schlegel, 1987).

Scheme of the experiment

At a poultry processing enterprise, immediately after the slaughter of broiler chickens, meat samples (muscles of the chest and thigh) were taken from 20 broiler chickens: Ten of the samples were stored on moist napkins treated with a probiotic complex of *Bacillus* spp., while the other ten samples were stored on untreated napkins (control) within 5 days. Throughout the experiment, the meat samples were kept at a temperature of + 4°C without being covered with food film. Microbial contamination of the napkins was examined when processed and unprocessed meat samples were stored on them for five days after storage. Additionally, an organoleptic assessment of broiler chicken meat was carried out on a 9-point scale with sensorial specifications (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely) (Moradi et al., 2019).

The next phase of the study was the contamination of 10 poultry meat samples with the test cultures of pathogens (*Escherichia coli UNCSM* – 007, *Pseudomonas aeruginosa UNCSM* – 012, *Staphylococcus aureus UNCSM* – 017, *Listeria ivanovii UNCSM* – 042, *Salmonella* Enteritidis *UNCSM* – 081), followed by aerosol treatment with a probiotic complex of *Bacillus* spp. The test cultures *Escherichia coli UNCSM* – 007, *Pseudomonas aeruginosa UNCSM* – 012, *Staphylococcus aureus UNCSM* – 017, *Listeria ivanovii UNCSM* – 042, and *Salmonella* Enteritidis *UNCSM* – 081 were purchased from the State Scientific Control Institute of Biotechnology and Strains of Microorganisms of Ukraine and used to contaminate the 10 poultry meat samples and the polymer base.

First, a suspension was prepared from five test cultures including *Escherichia coli UNCSM* – 007, *Pseudomonas aeruginosa UNCSM* – 012, *Staphylococcus aureus UNCSM* – 017, *Listeria ivanovii UNCSM* – 042, and *Salmonella*

Enteritidis *UNCSM* – 081. To create this suspension, 0.1 ml of each of the five reference test cultures was inoculated onto a liquid differential nutrient medium and incubated at a temperature of 30.0 to 37.0°C. After cultivation, 1.0 ml of each pathogen was added to the test tube and adjusted with phosphate buffer to the density of the suspension according to the MacFarland test standard of 0.5 units. Following this, 100 ml of sterile water and 10 ml of a daily test culture were added to sterile glass jars with a volume of 500 ml. Next, ten samples of poultry meat, each weighing 100 g, were immersed in this solution containing five pathogens, with a microbial body concentration of 1.5×10^8 /cm^3 (0.5 MacFarland) for 1 minute, according to its improved methodology. The samples were subsequently dried for 10 minutes at room temperature (20.0 ± 2.0) °C. Following the contamination of the poultry meat samples, a one-time aerosol treatment of each sample of meat products was carried out using a probiotic complex of *Bacillus* spp. bacteria. The probiotic complex contained the five strains of *Bacillus subtilis UNCSM - 020, Bacillus licheniformis UNCSM - 033, Bacillus amyloliquefaciens ALB 65, Bacillus pumilus UNCSM – 026,* and *Bacillus subtilis variant mesentericus UNCSM – 031* all purchased from the State Scientific Control Institute of Biotechnology and Strains of Microorganisms of Ukraine. For the aerosol treatment of poultry meat and moisture-retaining napkins, a probiotic complex of bacteria of the genus *Bacillus* with a concentration of microbial bodies of 1.5×10^8 in each cm^3 (0.5 MacFarland) was used. Samples of treated and untreated meat were stored for five days at a temperature of +4 °C and organoleptic studies were performed daily, assessing the appearance of the samples (appearance, texture, smell, and juiciness), total microbial contamination levels, and the presence of pathogenic microflora growth using methods specified by ISO 6887-2 (2017).

The next stage aimed to investigate how the probiotic complex of bacteria belonging to the *Bacillus* spp. genus affects the pathogenic microflora of poultry meat. For this purpose, a comparative analysis of the probiotic complex's *Bacillus* spp. film formation dynamics and the multispecies biofilm of pathogenic microorganisms *(E. coli UNCSM* – 007, *P aeruginosa UNCSM* – 012, *S. aureus UNCSM* – 017, *L. ivanovii UNCSM* – 042, *S.* Enteritidis *UNCSM* – 081*)* on poultry meat was conducted. The formation of biofilms of microorganisms was studied by determining the ability of isolates of microbial associations and individual types of microorganisms to adhere to the surface of a 24-well polystyrene plates according to the method outlined by O'Toole and Kolter (1998). To obtain biofilms, 24-well plates were used, into which the nutrient medium was poured, and 1 ml of each strain (*Bacillus subtilis UNCSM - 020, B. licheniformis UNCSM - 033, B. amyloliquefaciens ALB 65, B. pumilus UNCSM – 026, B. subtilis variant mesentericus UNCSM – 031)* and the probiotic complex of the genus *Bacillus* spp., as well as 10 CFU of a mixture of five pathogenic bacteria were added separately. A separate plate was used for each microorganism. Cultivation was carried out at a temperature of 37.0 ± 0.5 ^{0}C for 5 days.

After culturing the bacteria, the medium with planktonic cells was carefully collected from the wells of the plate. To remove the remaining planktonic cells, the wells with biofilms were washed for 2 minutes with sterile phosphate-buffered saline (pH 7.2-7.4) in a volume of 5 ml, and the buffer was completely removed. Then, 4 ml of filtered 0.1% gentian violet solution was added to each well of the 24-well plate. The biofilms were incubated with the dye for 15 minutes at room temperature (20.0 ± 2.0 °C). The dye was removed from the well, and unbound dye was thoroughly washed off with buffer. The plates were inverted onto filter paper and dried. A 95% ethanol solution (4 ml) was added to each well for dye elution. The solvent was collected and placed in clean flat-bottomed plates, and the optical density was measured at a wavelength of 620 nm. During the evaluation of the density of biofilms, the film-forming microorganisms served as the experimental groups, while a nutrient-differential diagnostic medium for the cultivation of biofilms was used as the control.

The probiotic complex of bacteria of the genus *Bacillus* spp. has been introduced in veterinary medicine for various applications, including aerosol disinfection, cleaning of water supply systems, and the stimulation and regulation of digestive processes (Alrubay et al., 2020; Valeris-Chacin et al., 2021). Similar commercial preparations of probiotic bacilli are widely used for these purposes in veterinary medicine. Therefore, the probiotic complex of Bacillus bacteria was used to treat poultry meat samples and to influence the pathogenic microflora. It has antimicrobial activity associated with the ability to synthesize antibiotic-like substances with a wide spectrum of action, thereby suppressing pathogenic and opportunistic bacteria, as well as fungal flora, and stimulating the protective functions of animals (Wu et al., 2018). The protective functions of the animal body present a promising area for utilizing this probiotic complex in extending the long-term storage of poultry carcasses.

Statistical analysis

The results of the study were statistically analyzed using Microsoft Excel 2016 (for Windows XP). The probability of the extracted results was assessed using the Student's criterion. Differences in data were considered significant at $p < 0.05$.

RESULTS

In ten meat samples of broiler chickens during the three-day storage period on an untreated napkin, no organoleptic signs of meat spoilage were detected and no pathogenic microphores were isolated. They were evaluated for 7 points on the 3rd day of storage. However, on the 4th and 5th days, the growth of *E. coli* (7.20 and 12.30 CFU/g), an unpleasant smell, the decomposition of tissues, and the appearance of dark spots on the surface were observed in the meat samples, which amounted to 2 points (Table 1). When storing meat products on a napkin treated with a probiotic complex of Bacillus spp. bacteria, signs of spoilage and pathogenic microorganisms were not detected within 5 days. The meat samples were rated at 8 points, which indicated inhibition of microflora growth.

Twenty-four hours after contamination with pathogens of poultry meat followed by aerosol treatment with a probiotic complex of *Bacillus* spp., the growth of pathogenic agents including *L. ivanovii, S. enteritidis, E. coli, P. aeruginosa,* and *S. aureus* was noted (Table 2). Complete suppression of the growth of *E. coli* and *S. aureus* microorganisms by the probiotic complex *Bacillus* spp. was already observed on the second day of meat storage. While the probiotic complex of bacteria of the genus *Bacillus* spp. displaced *S.* Enteritidis on the third day by 57.9% and P. *aeruginosa* on the fourth day by 65.4%, the growth of *Listeria spp*. was observed until the fifth day (6.7 CFU/g). Therefore, *L. ivanovii* can form a biofilm in monoculture, and in a consortium with microorganisms *S. enteritidis, E. coli, P. aeruginosa*, and *S. aureus*, which also have a species-specific ability to form a film, the biofilm-forming properties are strengthened and form a biofilm resistant to the action of disinfectants.

Bacterial contamination of meat treated with probiotic *Bacillus* spp. was lower on each day of storage compared to untreated control samples ($p < 0.05$). The probiotic complex of bacteria of the genus *Bacillus* spp. caused inhibition of the growth activity of the studied pathogenic microorganisms, which can form biofilms of different densities due to their high intensity of film formation and antagonistic effect. In this case, the antagonistic activity of *Bacillus* spp. manifested itself later in *L. ivanovii* and *P. aeruginosa* on days 4 and 5 due to the formation of joint dense biofilms by them.

The count of mesophilic aerobic and facultatively anaerobic microorganisms in poultry meat treated with a probiotic complex of *Bacillus* spp. was 18.2% lower after a day, 32.7% on the third day, and 27.8% on the fifth day compared to the value of pure meat in the control (Figures 1 and 2).

Table 1. Determination of the level of microbial contamination after aerosol treatment with a probiotic complex of bacteria of the genus *Bacillus* spp. in the meat of broiler chickens

Meat storage period (day)	Meat with untreated napkin		Meat with a napkin treated with the probiotic *Bacillus* spp.	
	Contamination (CFU/g)	Quality (points)	Contamination (CFU/g)	Quality (points)
1	Growth is absent	9	Growth is absent	9
2	Growth is absent	7	Growth is absent	8
3	Growth is absent	7	Growth is absent	8
4	7.20±0.20* E. coli	*3*	Growth is absent	8
5	12.30±0.26* E. coli	*2*	Growth is absent	8

*The difference in the values of the indicators is reliable at $p < 0.05$ relative to the corresponding indicators

Table 2. Experimental infection of poultry meat with test strains of pathogens followed by treatment with a probiotic complex of *Bacillus* spp.

Meat storage period, (day)	Bacterial contamination						
	Control pure meat, (CFU/g)	Meat, aerosol treated with probiotic *Bacillus* spp., (CFU/g)	Meat, treated with probiotic *Bacillus* spp., isolated pathogenic microorganisms, (CFU/g)				
			L. ivanovii *UNCSM* – 042	***S.* Enteritidis** *UNCSM* – 081	***E. coli*** *UNCSM* – 007	***P. aeruginosa*** *UNCSM* – 012	***S. aureus*** *UNCSM* – 017
1	111.10 ± 0.59	90.10 ± 0.55*	33.50 ± 0.58 *L. ivanovii*	*27.30* ± 0.26 *S. enteritidis*	17.20 ± 0.20 *E. coli*	26.20 ± 0.36 *P. aeruginosa*	14.10 ± 0.35 *S. aureus*
2	559.60 ± 0.45	322.20 ± 0.70*	24.60 ± 0.37* *L. ivanovii*	*11.50* ± 0.22* *S. enteritidis*	solid growth *Bacillus* spp.	24.20 ± 0.25* *P. aeruginosa*	solid growth *Bacillus* spp.
3	981.50 ± 0.60	656.0 ± 2.67*	16.10 ± 0.53* *L. ivanovii*	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	9.00 ± 0.26 *P. aeruginosa*	solid growth *Bacillus* spp.
4	1481.00 ± 0.65	953.00 ± 3.67*	6.70 ± 0.26* *L. ivanovii.*	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.
5	1801.9 ± 0.59	1312,00 ± 4.42*	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	solid growth Bacillus spp.	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.

• The difference in the values of the indicators is reliable at $p < 0.05$ relative to the corresponding indicators (between the values of columns 2 and 3);
*- the difference in the values of the indicators is reliable at $p < 0.05$ relative to the corresponding indicator of first day of storage

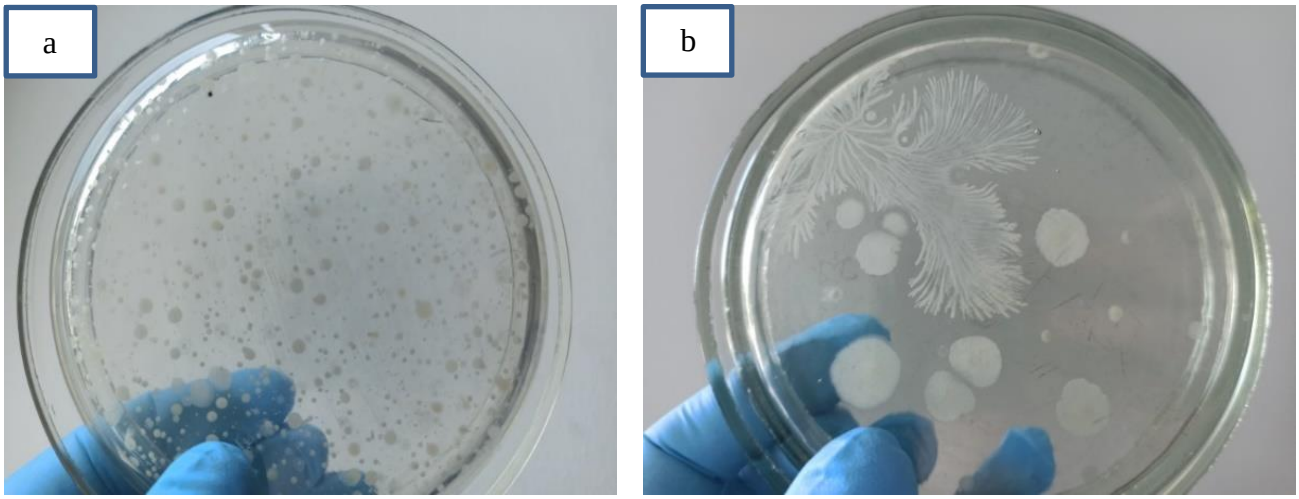


Figure 1. A swab from poultry meat on the third day after infection. **a**: Not treated with a probiotic complex of bacteria of the genus *Bacillus* spp., **b**: Treated with a probiotic complex of bacteria of the genus *Bacillus* spp.

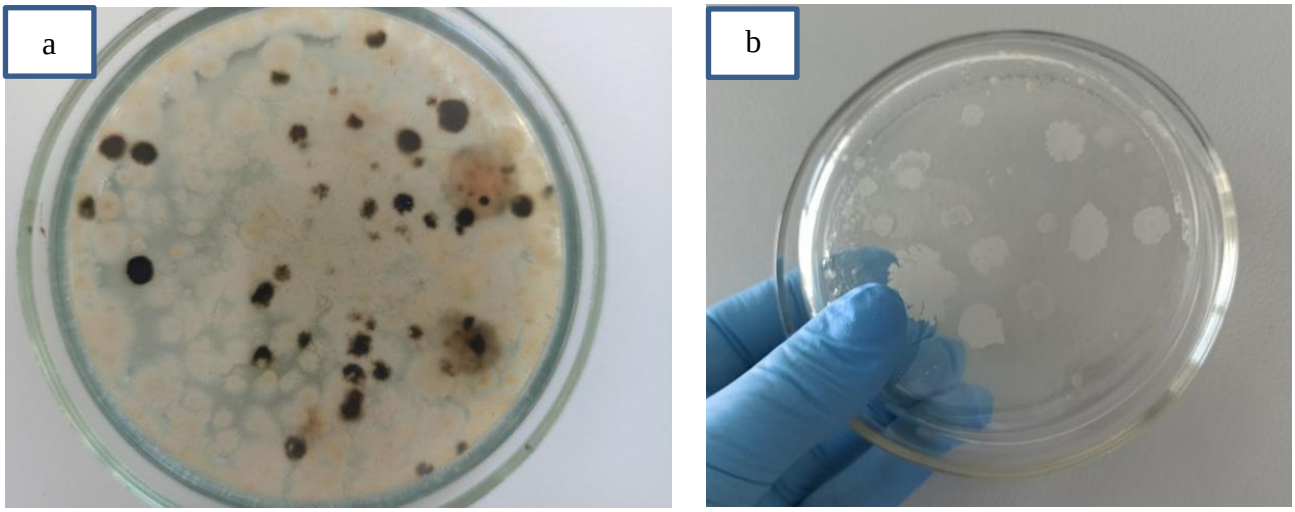


Figure 2. A swab from poultry meat on the fifth day after infection. **a:** Not treated with a probiotic complex of bacteria of the genus *Bacillus* spp., **b**: Treated with a probiotic complex of bacteria of the genus *Bacillus* spp.

According to the studies conducted, it has been established that each strain of the *Bacillus* spp. probiotic bacteria complex exhibits a significant biofilm formation ($p < 0.05$) intensity during a 5-day cultivation period. *Bacillus subtilis* formed a biofilm in only 24 hours, with an optical density reaching 3.25 ± 0.16 $D_{620,}$ which increased by 19.7% within 5 days (Figure 3). The growth intensity of biofilms of *B. pumilus, B. amyloliquefaciens, B. subtilis variant mesentericus,* and *B. licheniformis* also increased by the fifth day, and the density of biofilms was recorded at the level of 3.74 to 3.97 D_{620}, representing increases of 15.7 and 24.5% compared to the initial values. The difference between the accumulation of microbial biofilms among the five strains was noted to be insignificant ($p < 0.05$).

On the first day of cultivation, probiotic cells of the genus *Bacillus* spp. transit from a planktonic to a stationary state for the formation of a microbial biofilm. As they develop, stationary cells stick to each other and the surface, releasing an extracellular matrix that has properties contributing to the survival of the five strains of bacilli in the biofilm. The probiotic complex of bacteria of the genus *Bacillus* spp. developed visible biofilms from the five strains of microorganisms. The concentration of bacteria of the genus *Bacillus* spp. maintained its viability for 5 days and accumulated at a high rate, thereby forming a pronounced dense biofilm at - 4.73 D_{620}.

After conducting a series of experiments aimed at assessing the intensity of biofilm formation in pathogenic microorganisms that contaminate meat products, the following results were obtained (Figure 4). A pronounced ability to form microbial biofilms within 3 days was noted in planktonic forms of *Listeria* spp. (1.78 - 2.88 D620) and P. *aeruginosa* (1.95-2.28-2.28 D620), while low biofilm densities were observed in *S.* Enteritidis (1.38-1.77), *S. aureus* (1.33-1.76), and *E. coli* (1.49-1.91 D620). At the same time, in all planktonic forms of bacteria, a decrease in the density of biofilms was recorded from day 4 to day 5 by 4.2% in *Listeria* spp., 6.9% in *P. aeruginosa*, 6.8% in *S. enteritidis*, 14.8% in *S. aureus*, and 7.3% in *E. coli* compared to the values on day 3.

Planktonic forms of the above-mentioned microorganisms join each other within 4 hours to form strong microcolonies. During the next 12 hours, the number of planktonic forms decreases and mature colonies of a bacterial film with an optical density of 2.96 D_{620} are formed. The intensity of film formation of a multispecies biofilm reaches a maximum moderate density at the level of 3.24 D_{620} on the third day of cultivation, and then dispersion of planktonic forms into the nutrient medium occurred on day 5, resulting in a decrease of biofilm density by 11.5%.

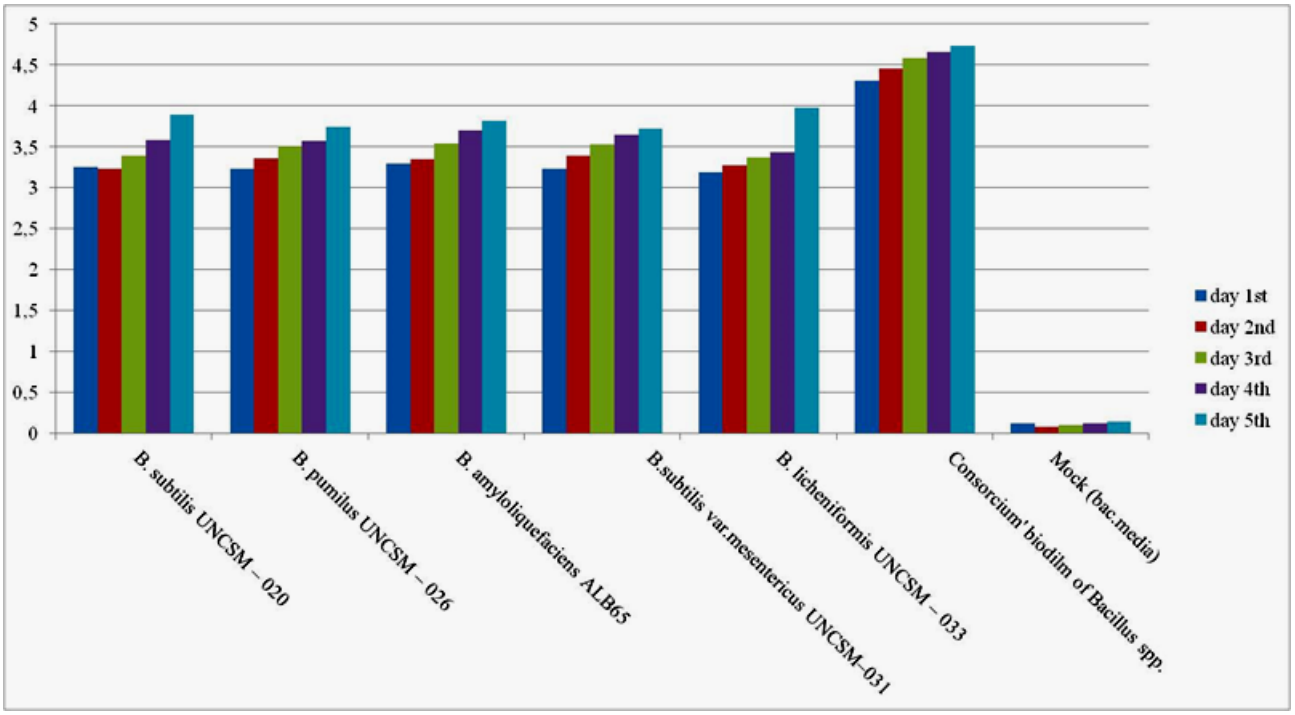


Figure 3. The intensity of biofilm formation of 5 strains of the probiotics complex *Bacillus* spp. on polystyrene plates during 5 days (n = 9, $p < 0.05$). Note: The optical density of biofilms up to $D_{620} < 2.0$ is low; $2.0 \leq D_{620} \leq 4.0$ – moderate; $D_{620} > 4x$ OD – expressed

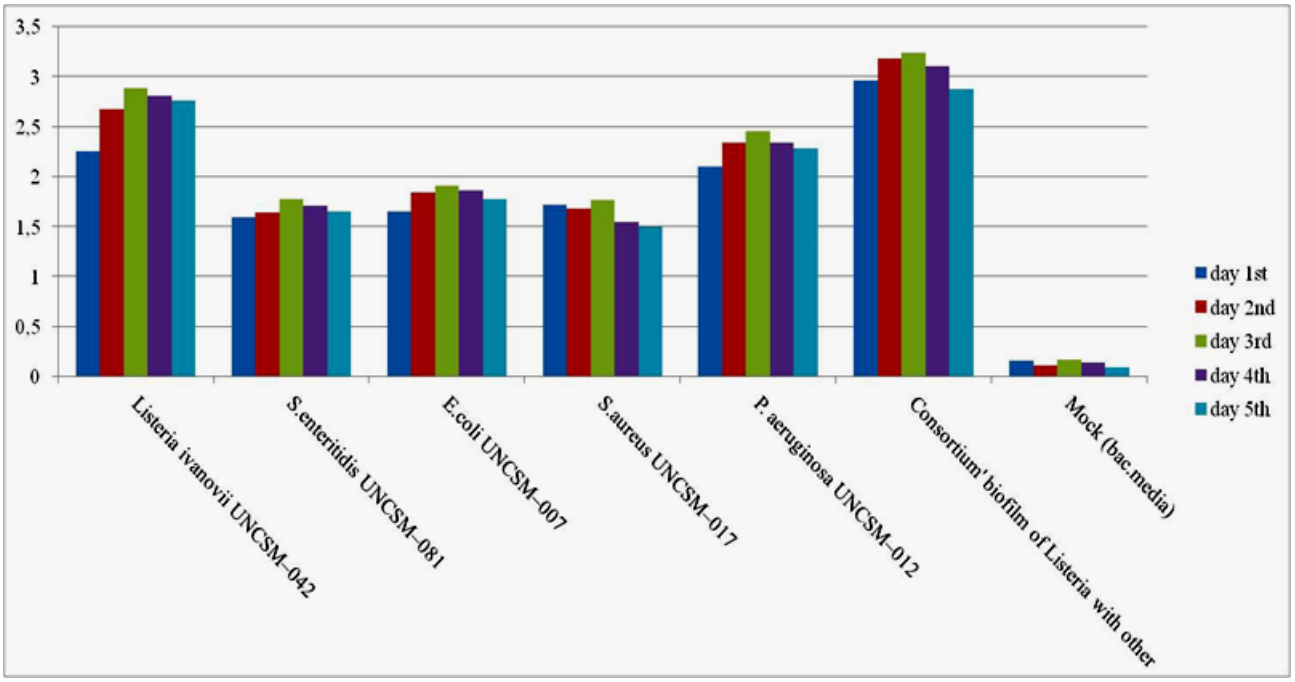


Figure 4. The intensity of biofilm formation of pathogenic microorganisms on polystyrene plates during 5 days (n = 7, $p < 0.01$). Note: The optical density of biofilms up to $D_{620} < 2.0$ is low; $2.0 \leq D_{620} \leq 4.0$ – moderate; $D_{620} > 4x$ OD– pronounced

DISCUSSION

The results of the present study indicated that *L. ivanovii* on poultry meat has the ability to adhere with the subsequent formation of biofilms of varying intensity, both in planktonic form and in association with pathogenic microorganisms *S. enteritidis*, *E. coli*, *S. aureus*, and *P. aeruginosa*, which interact as part of a mixed biofilm with one another, demonstrating a mutualistic relationship. The formation of a mixed biofilm was accompanied by the formation of an exopolysaccharide matrix by *L. ivanovii*, which holds the entire colony of pathogenic microorganisms capable of forming microbial biofilms on the surface of meat products, contributing to their preservation and maintenance.

According to the literature, interactions with other bacteria in biofilms explain the persistence of pathogens such as *L. monocytogenes* in food production environments (Gilbert et al., 2002). In multispecies biofilms, *L. monocytogenes* can interact directly with one or more other species in the biofilm (Elias and Banin, 2012; Jamal et al., 2018). *L. monocytogenes* can grow and survive in multispecies biofilms (*Psychromonas*, *Shewanella*, *Yersinia*, and *Lactobacillus*) formed from bacteria belonging to the background microbiota isolated in meat processing plants. In addition, it has been shown (Puga et al., 2018) that *L. monocytogenes* can colonize biofilms formed by *P. fluorescens* in co-culture experiments, which leads to an increase in bacterial cell density in vitro.

It is known that spore-forming bacteria of the genus *Bacillus* spp. can secrete bacteriocins and mycotoxins (antibiotic-like substances) and exhibit a sufficiently pronounced antibacterial effect against both opportunistic and pathogenic microflora including *Listeria*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Milyan et al, 2014; AlGburi et al., 2017).

Probiotic bacteria of the genus *Bacillus* spp. are characterized by their antimicrobial properties, which allow them to destroy (lyse) certain bonds in the peptidoglycan structure of the cell walls of a mixed biofilm (Khochamita et al., 2015; Petrova and Sauer, 2016; Kolchyk et al., 2020). It is known that spore-forming bacteria of the genus *Bacillus* spp. synthesize stable biofilms on various surfaces and show lytic sensitivity even in *Listeria* spp. and *P. aeruginosa*, suggesting high resistance to antimicrobial drugs due to their ability to form biofilms (Branda et al., 2004; Irkitova et al., 2018; Kolchyk et al., 2022).

The mechanism of the antagonistic activity of bacteria *Bacillus* spp. consists in the synthesis of non- polypeptide antibiotics (surfactin, bacillisin, bacitracin) and ribosomal synthesized peptides (subtilin, ericin S, mersacidin), which are synthesized by a bacterial cell in the stationary phase of growth (Cutting, 2011; Khardziani et al., 2017; Kotowicz et al., 2019). Different strains of *Bacillus* bacteria secrete a different set of antimicrobial substances, thereby exerting an inhibitory effect on a wide range of gram-positive and gram-negative bacteria (Baruzzi et al., 2011). In the mechanism of the selective antagonistic action of bacteria from the genus *Bacillus*, bacilli play a significant role in reducing the antilysozyme and adhesive activity of pathogenic bacteria, which determine the persistent properties of these microorganisms and their ability to parasitize intracellularly (Sorokulova, 2013; Rusaleyev et al., 2019; Chechet et al., 2022).

The obtained experimental results are consistent with the existing literature on the use of a probiotic complex of bacteria of the genus *Bacillus* spp. consisting of the five strains including *B. subtilis UNCSM* - 020, *B. licheniformis UNCSM* - 033, *B. amyloliquefaciens ALB* 65, *B. pumilus UNCSM* - 026, and *B. subtilis variant mesentericus UNCSM* - 031, which complement each other in the spectrum of antagonistic activity, as well as enzyme and amino acid production.

After the action of the probiotic complex of bacteria of the genus *Bacillus* spp. on the microbial biofilm of pathogenic microorganisms, *E. coli* and *S. aureus* cells were lysed to a greater extent (on the second day), and *S. enteritidis*, *P. aeruginosa*, and *Listeria* spp. (on days 3 and 4). This led to the destruction of the mixed biofilm and the dispersal of microorganisms, occurring due to a decrease in the number of essential substances for the body, after which they switched to the planktonic state with damage to their cell walls.

It should be noted that *Bacillus* spp. bacteria are capable of forming their biofilms, a type of cooperative existence in natural conditions. Bacilli form long chains of immobile cells that adhere to each other and the surface, secreting an extracellular matrix that provides rigidity and forms a stable biofilm (Wilking et al., 2012; Patel and DuPont, 2015). Spore-forming bacteria of the genus *Bacillus* spp. synthesize stable biofilms on various surfaces and show lytic sensitivity even in *Listeria* spp. and *P. aeruginosa*, which indicated high resistance to antimicrobial drugs due to the ability to form biofilms (Branda et al., 2004; Irkitova et al., 2018; Kolchyk et al., 2022).

Colonies of each strain of the probiotic complex of bacteria of the genus *Bacillus* spp. formed biofilms of moderate density: *B. subtilis* at the level of 3.89 D_{620}, *B. pumilus* at 3.72 D_{620}, *B. subtilis var. mesentericus* at 3.72 D_{620}, *B. amyloliquefaciens* at 3.81 D_{620}, and *B. licheniformis* at 3.97 D_{620}. In contrast, a consortium of the five strains synthesized a dense biofilm at 4.73 $D_{620,}$ with maximum preservation on day 5 of cultivation or intrageneric coaggregation.

As the biofilm expanded and matured, the production of the extracellular matrix continued, leading to the formation of folds (wrinkles) in the bacilli biofilms. These folds contributed to the formation of a complex network of channels in the biofilm, which facilitated the circulation of fluid and the distribution of nutrients. In this context, the biofilm of the probiotic complex of bacteria of the genus *Bacillus* spp. exhibited strong adhesion to the surface of meat products and co-aggregated pathogenic bacteria that multiplied and synthesized their biofilm. This process led to the dispersion of *L. ivanovii*, *S. enteritidis*, *E. coli*, *S. aureus*, and *P. aeruginosa* microorganisms and disrupted the growth mechanism of the microbial biofilm.

The obtained results are consistent with those in the literature in that the expression of *B. subtilis* biofilm genes is induced during co-culture with other members of the *Bacillus* genus (Shank et al., 2011; Bleich et al., 2015). *B.*

amyloliquefaciens ANT1 and its culture supernatant exhibit antibacterial activity against *B. cereus ATCC* 17778, *P. aeruginosa ATCC* 15442, *Aeromonas hydrophila ATCC* 7966 and *Aspergillus niger ATCC* 9642, all of which can form biofilms (Elias and Banin, 2012; Song et al., 2016).

Furthermore, bacteriocin BaCf3, isolated from *Bacillus amyloliquefaciens* BTSS3, can reduce biofilms by up to 80% even at low concentrations against strong food-borne biofilm producers such as *S. typhimurium, C. perfringens, E. faecalis,* and *P. aeruginosa* (Bindiya et al, 2019).

CONCLUSION

The results of the study showed that the probiotic complex of bacteria of the genus *Bacillus* spp completely inhibited the growth of pathogens *E. coli, S. aureus, S. enteritidis,* and *P. aeruginosa* during 2-4 days. However, the growth of *L. ivanovii, Listeria* spp. was observed up to day 5 after treatment, thereby reducing the number of pathogenic bacteria by about 11.5%. The probiotic complex containing *Bacillus* spp. exhibits antagonistic properties against pathogenic microflora and is capable of forming a biofilm, which facilitates the elimination of foodborne pathogens existing in the mixed biofilm on the surface of meat products. The broad specificity of bactericidal action and the high antagonistic activity of the probiotic complex of bacteria of the genus *Bacillus* spp. (*B. subtilis, B. licheniformis, B. amyloliquefaciens, B. pumilus,* and *B. subtilis variant. mesentericus*) against a consortium of pathogens (*L. ivanovii, S. enteritidis, E. coli, S. aureus,* and *P. aeruginosa)* opens prospects for practical application in meat processing and the storage of meat products. Based on the obtained results, it is possible to plan the development of spore-forming probiotic preparations for the neutralization of pathogenic microflora in meat products at meat processing enterprises in the future.

DECLARATIONS

Funding

No funding was received for conducting this study.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contribution

Andriy Buzun and Tetiana Illarionova conceived and designed the experiment, and Olena Kolchyk and Iryna Borovuk conducted bacteriological studies and analyzed the obtained results. Olena Kolchyk, Andriy Buzun, and Nadiia Zazharska interpreted and wrote the article, edited and reviewed the manuscript. All authors have read and approved the final draft of the manuscript for publication in the journal.

Competing interests

The authors have declared that no competing interests exist.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

REFERENCES

Abdullahi UF, Igwenagu E, Mu'azu A, Aliyu S, and Umar MI (2016). Intrigues of biofilm: A perspective in veterinary medicine. Veterinary World, 9(1): 12-18. DOI: https://www.doi.org/10.14202/vetworld.2016.12-18

Akinbobola AB, Sherrya L, Mckay WG, Ramage G, and Williams C (2017). Tolerance of *Pseudomonas aeruginosa* in *in-vitro* biofilms to high-level peracetic acid disinfection. Journal of Hospital Infection, 97(2): 162-168. DOI: https://www.doi.org/10.1016/j.jhin.2017.06.024

Alrubaye AAK, Ekesi NS, Hasan A, Koltes DA, Wideman RF Jr, and Rhoads DD (2020). Chondronecrosis with osteomyelitis in broilers: further defining a bacterial challenge model using standard litter flooring and protection with probiotics. Poultry Science, 99(12): 6474-6480. DOI: https://www.doi.org/10.1016/j.psj.2020.08.067

AlGburi A, Volski A, Cugini C, Walsh EM, Chistyakov VA, Mazanko MS, Bren AB, Dicks LMT, and Chikindas ML (2016). Safety properties and probiotic potential of Bacillus 68 subtilis KATMIRA1933 and Bacillus amyloliquefaciens B-1895. Advances in Microbiology, 6(6): 432-452. DOI: https://www.doi.org/10.4236/aim.2016.66043

Baruzzi F, Quintieri L, Morea M, and Caputo L (2011). Antimicrobial compounds produced by Bacillus spp. and applications in food. In: A. Méndez-Vilas (Editor), Science against microbial pathogens: Communicating current research and technological advances, pp. 1102-1111. Available at: https://bdigital.ufp.pt/bitstream/10284/9889/1/Metals_BookChapter_AFVinha_2011.pdf

Bindiya ES, Tina KJ, Sasidharan RS, and Bhat SG (2019). BaCf3: highly thermostable bacteriocin from *Bacillus amyloliquefaciens* BTSS3 antagonistic on food-borne pathogens. 3 Biotech, 9(4): 136. DOI: https://www.doi.org/10.1007/s13205-019-1639-2

Bleich R, Watrous JD, Dorrestein PC, Bowers AA, and Shank EA (2015). Thiopeptide antibiotics stimulate biofilm formation in *Bacillus subtilis*. Proceedings of the National Academy of Sciences, 112(10): 3086-3091. DOI: https://www.doi.org/10.1073/pnas.1414272112

Borovuk I and Zazharska N (2022). Evaluation of broiler meat in experimental listeriosis. Journal of Advanced Veterinary and Animal Research, 9(1): 155. DOI: https://www.doi.org/10.5455/javar.2022.i580

Branda SS, González-Pastor JE, Dervyn E, Ehrlich SD, Losick R, and Kolter R (2004). Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. Journal of Bacteriology, 186(12): 3970-3979. DOI: https://www.doi.org/10.1128/jb.186.12.3970-3979.2004

Bridier A, Sanchez-Vizuete P, Guilbaud M, Piard JC, Naitali M, and Briandet R (2015). Biofilm-associated persistence of food-borne pathogens. Food Microbiology, 45: 167-178. DOI: https://www.doi.org/10.1016/j.fm.2014.04.015.

Cutting SM (2011). Bacillus probiotics. Food Microbiology, 28(2): 214-220. DOI: https://www.doi.org/10.1016/j.fm.2010.03.007

Chechet OM, Kovalenko VL, Horbatyuk OI, Gaidei OS, Kravtsova OL, Andriyashchuk VO, Musiets IV, and Ordynska DO (2022). Study *in vitro* of antagonistic activity of *Bacillus* isolates and selection of promising probiotic strains. Scientific and Technical Bulletin of State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology, 23(1): 219-227. DOI: https://www.doi.org/10.36359/scivp.2022-23-1.2

D'Ostuni V, Tristezza M, De Giorgi MG, Rampino P, Grieco F, and Perrotta C (2016). Occurrence of *Listeria monocytogenes* and *Salmonella* spp. in meat processed products from industrial plants in Southern Italy. Food Control, 62: 104-109. DOI: https://www.doi.org/10.1016/j.foodcont.2015.10.025

Elias S and Banin E (2012). Multi-species biofilms: Living with friendly neighbours. FEMS Microbiology Reviews, 36(5): 990-1004. DOI: https://www.doi.org/10.1111/j.1574-6976.2012.00325.x

Fan Y, Qiao J, Lu Z, Fen Z, Tao Y, Lv F, Zhao H, Zhang C, and Bie X (2020). Influence of different factors on biofilm formation of *Listeria monocytogenes* and the regulation of cheY gene. Food Research International, 137: 109405. DOI: https://www.doi.org/10.1016/j.foodres.2020.109405

Fegan N and Jenson I (2018). The role of meat in foodborne disease: Is there a coming revolution in risk assessment and management?. Meat Science, 144: 22-29. DOI: https://www.doi.org/10.1016/j.meatsci.2018.04.018

Flemming HC and Wingender J (2010). The biofilm matrix. Nature Reviews Microbiology, 8(9): 623-633. DOI: https://www.doi.org/10.1038/nrmicro2415

Gilbert P, Maira-Litran T, McBain AJ, Rickard AH, and Whyte FW (2002). The physiology and collective recalcitrance of microbial biofilm communities. Advances in Microbial Physiology, 46: 203-256. DOI: https://www.doi.org/10.1016/s0065-2911(02)46005-5

Irkitova AN, Grebenshchikova AV, and Matsyura AV (2018). Antagonistic activity of *Bacillus subtilis* strains isolated from various sources. Ukrainian Journal of Ecology, 8(2): 354-364. DOI: https://www.doi.org/10.15421/2018_354

ISO 11290-1 (2017). Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method.

ISO 13720 (2010). Meat and meat products — Enumeration of presumptive *Pseudomonas* spp.

ISO/TS 17728 (2015). Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples.

ISO 6579-1 (2017). Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1: Detection of *Salmonella* spp.

ISO 6887-2 (2017). Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2: Specific rules for the preparation of meat and meat products.

ISO 6887-3 (2017). Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 3: Specific rules for the preparation of fish and fishery products.

ISO 6888-1 (2021). Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Method using Baird-Parker agar medium.

ISO/IEC 17025 (2017). General requirements for the competence of testing and calibration laboratories.

Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, and Kamil MA (2018). Bacterial biofilm and associated infections. Journal of the Chinese Medical Association, 81(1): 7-11. DOI: https://www.doi.org/10.1016/j.jcma.2017.07.012

Kolchyk OV, Buzun AI, Paliy AP, Nalivayko LI, Chekan OM, Grebenik NP, Bondarenko IV, Rebenko HI, Kushnir VY, and Todorov NI (2020). Biofilms of pathogenic bacteria in pig industry. Ukrainian Journal of Ecology, 10(4): 202-209. DOI: https://www.doi.org/10.15421/2020_189

Kolchyk O, Illarionova T, Buzun A, Paliy A, and Palii A (2022). Influence of probiotic microorganisms on microbial biofilms in feeds. Scientific Horizons, 25(1): 41-50. DOI: https://www.doi.org/10.48077/scihor.25(1).2022.41-50

Khardziani T, Sokhadze K, Kachlishvili E, Chistyakov V, and Elisashvili V (2017). Optimization of enhanced probiotic spores production in submerged cultivation of *Bacillus amyloliquefaciens* B-1895. Journal of Microbiology, Biotechnology and Food Sciences, 7(2): 132-136. DOI: https://www.doi.org/10.15414/jmbfs.2017.7.2.132-136

Kotowicz N, Bhardwaj RK, Ferreira WT, Hong HA, Olender A, Ramirez J, and Cutting SM (2019). Safety and probiotic evaluation of two *Bacillus* strains producing antioxidant compounds. Beneficial Microbes, 10(7): 759-772. DOI: https://www.doi.org/10.3920/bm2019.0040

Khochamita N, Siripornadulsila S, Sukonb P, and Siripornadulsil W (2015). Antibacterial activity and genotypic–phenotypic characteristics of bacteriocin-producing *Bacillus subtilis* KKU213: Potential as a probiotic strain. Microbiological Research, 170 (1): 36-50. DOI: https://www.doi.org/10.1016/j.micres.2014.09.004

Lin S, Yang L, Chen G, Li B, Chen D, Li L, and Xu Z (2017). Pathogenic features and characteristics of food borne pathogens biofilm: Biomass, viability and matrix. Microbial Pathogenesis, 111: 285-291. DOI: https://www.doi.org/10.1016/j.micpath.2017.08.005

Milyan VO, Harhota MA, and Nechipurenko OO (2014). Research of probiotic properties of *Bacillus* spp. 1.1. and *B. amyloliquefaciens* УКМ B-5113 strains. ScienceRise, 5(1): 15-22. DOI: https://doi.org/10.15587/2313-8416.2014.32023

Markowiak P and Śliżewska K (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens, 10(1): 1-20. DOI: https://www.doi.org/10.1186/s13099-018-0250-0

Moradi M, Tajik H, Mardani K, and Ezati P (2019). Efficacy of lyophilized cell-free supernatant of *Lactobacillus salivarius* (Ls-BU2) on *Escherichia coli* and shelf life of ground beef. Veterinary Research Forum, 10(3): 193-198. DOI: https://www.doi.org/10.30466/vrf.2019.101419.2417

Nowak J, Cruz CD, Tempelaars M, Abee T, van Vliet AHM, Fletcher GC, Hedderley D, Palmer J, and Flint S (2017). Persistent *Listeria monocytogenes* strains isolated from mussel production facilities form more biofilm but are not linked to specific genetic markers. International Journal of Food Microbiology, 256: 45-53. DOI: https://www.doi.org/10.1016/j.ijfoodmicro.2017.05.024

O'Toole GA and Kolter R (1998). Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. Molecular Microbiology, 28(3): 449-461. DOI: https://www.doi.org/10.1046/j.1365-2958.1998.00797.x

Patel R and DuPont HL (2015). New Approaches for bacteriotherapy: prebiotics, new-generation probiotics, and synbiotics. Clinical Infectious Diseases, 60(suppl 2): S108-S121. DOI: https://www.doi.org/10.1093/cid/civ177

Pažin V, Jankuloski D, Kozačinski L, Dobranić V, Njari B, Cvrtila Ž, Lorenzo JM, and Zdolec N (2018). Tracing of *Listeria monocytogenes* contamination routes in fermented sausage production chain by pulsed-field gel electrophoresis typing. Foods, 7(12): 198. DOI: https://www.doi.org/10.3390/foods7120198

Petrova OE and Sauer K (2016). Escaping the biofilm in more than one way: Desorption, detachment or dispersion. Current Opinion in Microbiology, 30: 67-78. DOI: https://www.doi.org/10.1016/j.mib.2016.01.004

Puga CH, SanJose C, and Orgaz B (2016). Biofilm development at low temperatures enhances *Listeria monocytogenes* resistance to chitosan. Food Control, 65: 143-151. DOI: https://www.doi.org/10.1016/j.foodcont.2016.01.012

Puga CH, Dahdouh E, SanJose C, and Orgaz B (2018). *Listeria monocytogenes* colonizes *Pseudomonas fluorescens* biofilms and induces matrix over-production. Frontiers in Microbiology, 9: 1706. DOI: https://www.doi.org/10.3389/fmicb.2018.01706

Rusaleyev VS, Pruntova OV, and Vasilyev DA (2019). Cultural morphological and biochemical characteristics of *Bacillus subtilis* strains. Veterinary Science Today, 1: 58-62. DOI: https://www.doi.org/10.29326/2304-196X-2019-1-28-58-62

Shank EA, Klepac-Ceraj V, Collado-Torres L, Powers GE, Losick R, and Kolter R (2011). Interspecies interactions that result in *Bacillus subtilis* forming biofilms are mediated mainly by members of its own genus. Proceedings of the National Academy of Sciences, 108(48): E1236-1243. DOI: https://www.doi.org/10.1073/pnas.1103630108

Schlegel G (1987). General microbiology: Translated from German. Moscow, pp. 329-336

Song B, Wang Y, Wang G, Liu GL, Li W, and Yan F (2016). The lipopeptide 6-2 produced by Bacillus amyloliquefaciens anti-CA has potent activity against the biofilm-forming organisms. Marine Pollution Bulletin, 108(1-2): 62-69. DOI: https://www.doi.org/10.1016/j.marpolbul.2016.04.062

Sorokulova I (2013). Modern status and perspectives of bacillus bacteria as probiotics. Journal of Probiotics & Health, 1(4): e106. DOI: https://www.doi.org/10.4172/2329-8901.1000e106

Valeris-Chacin R, Pieters M, Hwang H, Johnson TJ, and Singer RS (2021). Association of broiler litter microbiome composition and *Campylobacter* isolation. Frontiers in Veterinary Science, 24(8): 654927. DOI: https://www.doi.org/10.3389/fvets.2021.654927

Wang H, Zhang X, Zhang Q, Ye K, Xu X, and Zhou G (2015). Comparison of microbial transfer rates from *Salmonella* spp. biofilm growth on stainless steel to selected processed and raw meat. Food Control, 50: 574-580. DOI: https://www.doi.org/10.1016/j.foodcont.2014.09.049

Wang H, Cai L, Li Y, Xu X, and Zhou G (2018). Biofilm formation by meat-borne Pseudomonas fluorescens on stainless steel and its resistance to disinfectants. Food Control, 91: 397-403. DOI: https://www.doi.org/10.1016/j.foodcont.2018.04.035.

Wilking JN, Zaburdaev V, De Volder M, Losick R, Brenner MP, and Weitz DA (2012). Liquid transport facilitated by channels in Bacillus subtilis biofilms. Proceedings of the National Academy of Sciences, 110(3): 848-852. DOI: https://doi.org/10.1073/pnas.1216376110

World legal information institute (WorldLII) (1986). Council of Europe treaty series - explanatory reports. Council of European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Available at: http://www.worldlii.org/int/other/treaties/COETSER/1986/1.html

Wu T, Zhang Y, Lv Y, Li P, Yi D, Wang L, Zhao D, Chen H, Gong J, and Hou Y (2018). Beneficial impact and molecular mechanism of Bacillus coagulans on piglets' intestine. International Journal of Molecular Sciences, 19(7): 2084. DOI: https://www.doi.org/10.3390/ijms19072084

Zazharskyi V, Parchenko M, Fotina T, Davydenko P, Kulishenko O, Zazharska N, and Borovik I (2019). Synthesis, structure, physicochemical properties and antibacterial activity of 1,2,4-triazoles-3-thiols and furan derivatives. Voprosy Khimii i Khimicheskoi Tekhnologii, 6: 74-82. DOI: https://www.doi.org/10.32434/0321-4095-2019-127-6-74-82

Zazharskyi V, Parchenko M, Parchenko V, Davydenko P, Kulishenko O, and Zazharska N (2020). Physicochemical properties of new S-derivatives of 5-(5-bromofuran-2-yl)-4-methyl-1,2,4-triazol-3-thiols. Voprosy Khimii i Khimicheskoi Tekhnologii, 6: 50-58. DOI: https://www.doi.org/10.32434/0321-4095-2020-133-6-50-58

Zazharskyi VV, Alifonova KV, Brygadyrenko VV, Zazharska NM, Goncharenko VP, and Solomon VV (2023). The ability of *Sitophilus oryzae* (Coleoptera, Curculionidae) to transmit Mycobacterium bovis: Morphology, cultural biochemical properties of the bacteria. Regulatory Mechanisms in Biosystems, 14(3): 476-486. DOI: https://www.doi.org/10.15421/10.15421/022368

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 435-448.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj51
PII: S2322456824000051-14

A Holistic Approach to Bovine Brucellosis: Serological Tests, Knowledge, Attitudes, and Practices Evaluation, and Risk Factor Identification on Dairy Farms of Ethiopia

Mastewal Birhan *

Department of Veterinary Pathobiology, College of Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia

*Corresponding author's Email: maste675@gmail.com

ORIGINAL ARTICLE
Received: July 27, 2024
Revised: August 24, 2024
Accepted: September 12, 2024
Published: September 30, 2024

ABSTRACT

Brucellosis, a contagious bacterial disease affecting animals globally presents a substantial zoonotic risk that is frequently underestimated, hinders animal trade, and endangers livestock and human health. The present study was conducted from November 2023 to June 2024 in Central and North Gondar Zone, Ethiopia. The current cross-sectional study aimed to evaluate the prevalence of brucellosis and to explore the related knowledge, attitudes, and practices within the specified region. A total of 384 serum samples were collected via random sampling from 20 dairy farms located in Ethiopia. Both local and cross-breed samples screened using the Rose Bengal Plate test and confirmed through an enzyme-linked immunosorbent assay. The seroprevalence of bovine brucellosis for both tests was 17.71% overall, with 9.62% for Central Gondar and 27.27% for North Gondar. Extensive farms exhibited notably higher odds of brucellosis compared to intensive farms, with unadjusted crude odds ratios of 3.01 and adjusted odds ratios of 2.37, respectively. Medium-sized herds also demonstrated increased odds in the multivariate analysis compared with small herds. Young respondents displayed the highest awareness levels, followed by adults and older individuals, with statistically significant differences observed across all categories. Regarding a semi-structured survey from 150 farmers on the association between sociodemographic data and knowledge, females exhibited higher awareness levels, with 117 (80.14%) responding positively. Young respondents showed a higher positive response rate of 58% compared to adults (52.7%) and the elderly (36.54%). In conclusion, these results emphasize the need for comprehensive strategies to address the factors influencing bovine brucellosis prevalence and respondent awareness.

Keywords: Brucellosis, Central Gondar, Dairy Farm, North Gondar, Seroprevalence

INTRODUCTION

Ethiopia is known as the residence of Africa's most extensive livestock numbers, due to the hosting of approximately 60.9 million cattle, 31.3 million sheep, and 32.7 million goats (Dinka and Ababa, 2011). The prevalence of animal diseases throughout any country significantly hindered the abundance of livestock and limiting their potential as reliable food sources. Despite playing a significant role in the country's economy, the productivity of each animal remained notably low, largely attributed to technical limitations and diseases such as brucellosis (Merga Sima et al., 2021). Bovine brucellosis, a globally infectious and contagious disease, constituted a significant economic burden on livestock industries in developing countries, impacting cattle production worldwide. Well-controlled in developed nations, it caused reproductive waste, economic losses, and barriers to international trade in developing countries. In dairy production, crossbreeding impeded the import of high-yielding breeds and improvements in milk production (Dahouk and Nöckler, 2011). The extensive presence of these factors rendered the disease not only endemic but also one of the foremost zoonotic threats to public health in the nation (Gashaw et al., 2022). Although bovine brucellosis significantly affected socioeconomic and zoonotic impacts, it received little attention (Bekele et al., 2002). Brucellosis, caused by *Brucella abortus* in sexually mature cattle in Africa, led to reproductive problems, such as abortion and retention of fetal membranes in cows and orchitis and epididymitis in bulls (McDermott and O'connor, 2002; Radostits et al., 2007). Brucellosis was first reported in the 1970s in Ethiopia and identified as an important livestock disease (Domenech, 1977). The infection transmitted via direct exposure to miscarried cows and their fetuses or indirectly through tainted items. It spread through polluted feed, water, and interactions with infected animals or tissues, endangering livestock handlers, vets, and lab staff. Although a dangerous sickness, no vaccine was available for human application, and the bacterium's intracellular behavior significantly hampered treatment efficacy (Qureshi et al., 2023). Addressing

To cite this paper: Birhan M (2024). A Holistic Approach to Bovine Brucellosis: Serological Tests, Knowledge, Attitudes, and Practices Evaluation, and Risk Factor Identification on Dairy Farms of Ethiopia. *World Vet. J.*, 14(3): 435-448. DOI: https://dx.doi.org/10.54203/scil.2024.wvj51

brucellosis was vital for sustaining Ethiopia's livestock industry and ensuring human well-being (Acha and Szyfres, 2001).

Crucial risk factors included the herd size, age, and gender of cattle, management practices, interactions with wild animals, environmental influences, and mixing of different species within a herd (Muma et al., 2007; Tsegaye et al., 2016). Effective management of *Brucella* in endemic regions involved vaccinating calves or heifers. Additionally, brucellosis control strategies encompassed isolating infected cattle and employing test-and-slaughter techniques (OIE, 2004).

Effective control measures encompassed surveillance, prevention of transmission, and management of the infection source, which involved practices like culling. Accurate diagnosis was pivotal and typically involved isolating and identifying *Brucella* from aborted materials, udder secretions, or tissues, alongside detecting specific antibodies using suitable serological techniques. Nevertheless, treating brucellosis was frequently challenging due to the bacterium's intracellular nature, often leading to recurrences within 3 to 6 months after halting early therapy (Khurana et al., 2021).

Brucellosis was transmitted to other cattle through direct or indirect interaction with diseased cattle or their discharges such as feeding pooled colostrum to newborn calves, and rarely, through sexual contact during artificial insemination. The disease could also spread through ingestion of contaminated feed and drinking water, as well as through birth products and uterine discharge. Mucosa/abrasions coming into contact with the fluid or tissues of aborted fetuses of diseased cattle could also serve as a source of disease in humans, especially through the ingestion of unpasteurized milk or milk products. Abattoir, farm, and laboratory workers, along with veterinarians, were recognized as at-risk groups for *Brucella* infection. Brucellosis could be eradicated through measures such as quarantining infected cattle, vaccination, and test-and-slaughter methods (Tulu, 2022). *Brucella* infection caused huge financial losses and community health concerns in many countries including Ethiopia (Tulu, 2022). This study aimed to evaluate the seroprevalence of bovine brucellosis, knowledge, attitudes, and practices (KAPs), and identify the risk factors associated with it in dairy cattle located in the North and Central Gondar Zones of Northwest Ethiopia.

MATERIALS AND METHODS

Ethical approval

The research project titled 'Major Zoonotic Reproductive Diseases and Production Problems of Current Dairy Cattle in the Milk Shade Area of Amhara Region, Ethiopia,' conducted at the University of Gondar, Ethiopia, on 12-Nov-2022, has undergone review by the institutional ethical review board of the University of Gondar for its ethical soundness. It was deemed ethically acceptable on 11/25/2022 with reference number VP/RTT/05/166/2022.

Study area

The current study took place in the North and Central Gondar Zone, located in the Amhara regional state of Ethiopia. This region is situated around 740 km north of Addis Ababa, the capital city of Ethiopia. The Amhara National Regional State falls within the tropical region, with climatic zones ranging from hot dry tropical to subtropical, temperate, and alpine (Teshome and Adamu, 2010). It is composed of 13 administrative zones and has an estimated population of 21,134,988. The geographical coordinates of the study area are approximately 12°4' North latitude and 27°2' East longitude, with altitudes ranging from 1800 to 2500 meters above sea level. The region experiences a bimodal rainfall pattern, with an average annual precipitation of 1000 mm. The short rainy season occurs in March, April, and May, while the long rainy season extends from June to September (Abdulkadir, 2019). The average yearly temperature in the location was 19.7°C. Agriculture in this area predominantly focuses on cereal crops and livestock rearing (Commission, 2008; CSA, 2011). The livestock count in the North and Central Gondar zones is estimated to comprise 2,771,701 cattle, 815,716 sheep, 1,251,867 goats, 27,248 horses, 9,695 mules, 376,841 donkeys, 3,628,832 poultry, and 227,463 beehives (CSA-Ethiopia, 2012, Figure 1).

Study population and source

A cross-sectional study was conducted on dairy farms in Ethiopia between November 2023 and June 2024. The study population consisted of cows of different breeds, encompassing indigenous, cross-breed, and exotic breeds, in the context of bovine brucellosis. The participants comprised both breeding females and replacement heifers, reared under varied management systems including intensive, semi-intensive, and extensive. To determine the occurrence of brucellosis in dairy cows, the first screening involved the Rose Bengal Plate Test, with further confirmation of positive cases through the enzyme-linked immunosorbent assay.

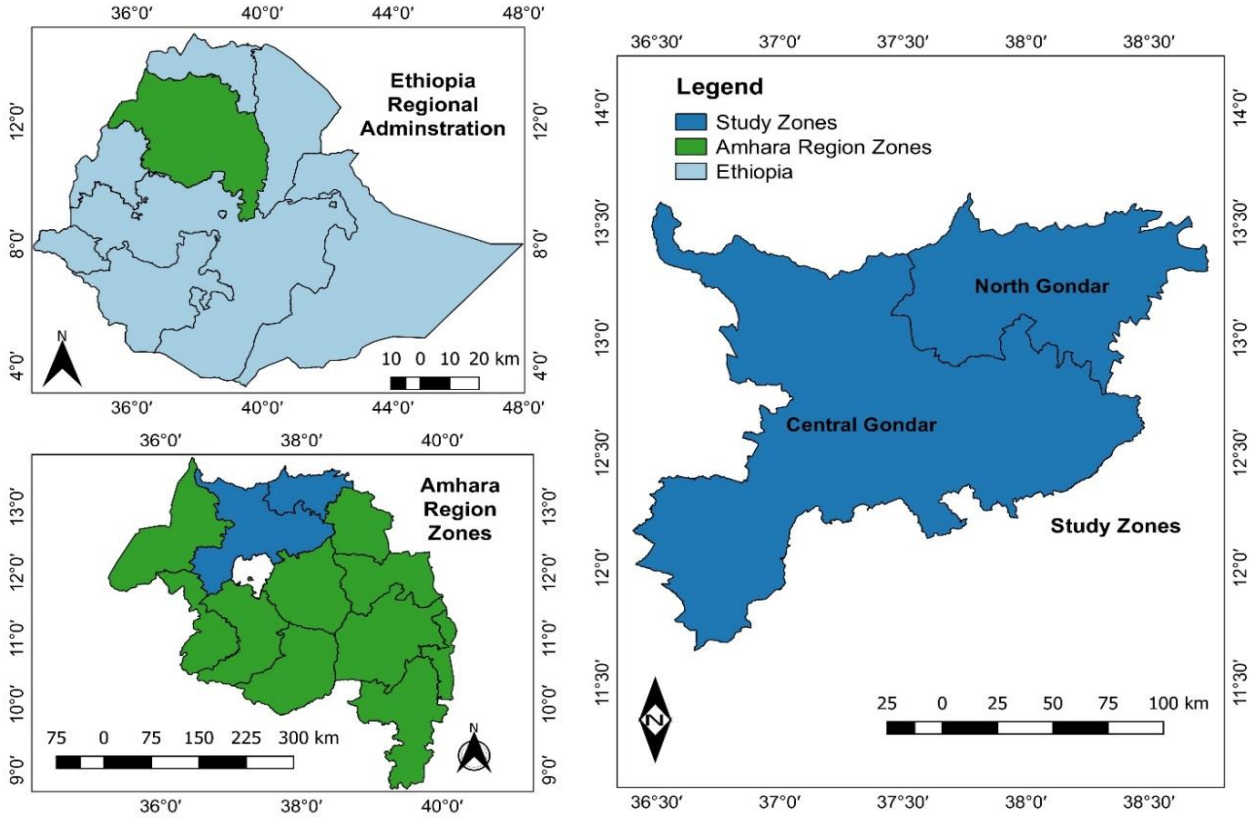


Figure 1. Map of the location of the study and dairy farms in Ethiopia

Sampling technique and sample size determination

The total number of animals needed was calculated using the formula by Thursfield (2015). The sample size was determined based on a 95% confidence level, an expected prevalence of 50%, and a desired absolute precision of 0.05.

The formula used is following:

n = ([1.96]^2 * Pexp * [1 - Pexp]) / d^2 = 384.

To enhance accuracy, the sample size was tripled and selected from various agroecological regions. Here, n represents the required sample size, Pexp signifies the expected prevalence, and d indicates the desired absolute precision.

Sample collection and processing

Experienced veterinary professionals collected information on the socio-demographic characteristics and risk factors for dairy cattle using a pretested structured questionnaire. To determine whether the language of the questions was understandable, the questionnaire was pretested on 150 dairy cattle owners in a pretest study. Pretested questionnaires were used, and the instrument's reliability and validity were confirmed in the pretest study. Around 10 mL of blood was drawn from the jugular vein of all chosen animal utilizing a standard vacutainer tube and needle. Each animal was then identified on the respective vacutainer tubes and left at room temperature overnight to facilitate clotting. The following day, the serum was separated from the clot into a different tube. These serum samples were preserved at -20°C in the Medical Microbiology Laboratory of the Faculty of Health Science at the University of Gondar until subjected to testing through the Rose Bengal Plate Agglutination Test (RBPT, Yohannes et al., 2012 a).

Rose Bengal Plate Agglutination Test and ELISA were employed as preliminary screening tests for serum samples to identify the presence of *Brucella* agglutinins. The OIE-recommended protocol was followed to screen for the presence of *Brucella* antibodies in the sampled sera. This test is generally recognized for its sensitivity, with a reported sensitivity of 97.9% (Dohoo et al., 1986). The test was conducted following the manufacturer's instructions. Prior to the test, the antigen and sera were equilibrated to room temperature. To initiate the test, 30 µL of serum was extracted from a glass slide using a micropipette. The Rose Bengal antigen bottle was adequately agitated to ensure a uniform suspension, and then one drop (30 µL) of the antigen was added. The antigen and serum were thoroughly mixed using a spreader, and the slide was rotated for 4 minutes. The test result was promptly interpreted immediately after 4 minutes rotation.

Questionnaire survey

A total of 150 semi-structured questionnaires were employed to gather data on the Knowledge, Attitudes, and Practices (KAP) concerning bovine brucellosis and the factors that contribute to the disease's prevalence in the research locations. The questionnaire consisted of three sections including part-I focused on gathering socio-demographic details about the participants; part-II aimed to evaluate factors that predispose to the disease; and part-III was dedicated to assessing the Knowledge, Attitudes, and Practices of animal owners regarding bovine brucellosis.

Statistical analysis

The data collected from field level and laboratory investigation were coded into appropriate variables and entered into a Microsoft Office Excel 2019 spreadsheet. The data were checked for errors of entry, coded, and then imported to STATA for descriptive and further analyses. All statistical analyses were performed using STATA version 14 software. Descriptive statistics involving frequency and percentage were used to determine the seroprevalence of the disease. Binary logistic regression analysis was used to identify potential risk factors associated with bovine brucellosis. First, univariable logistic regression analysis with the flock as a random effect was performed and potential risk factors (explanatory variables) with p values less than 0.25 were screened for the multivariable mixed-effect logistic regression. In statistical analysis, a p-value below 0.05 (at a significance level of 5%) was deemed to demonstrate statistical significance for both tests.

RESULTS

The prevalence study of bovine brucellosis on dairy farms explored multiple factors linked to the disease by analyzing 384 serum samples obtained from 20 farms. Among these samples, 68 (17.71%) tested positive for brucellosis based on RBPT and Indirect enzyme-linked immunosorbent assay (i-ELISA) tests. The result presented in Table 1 indicated that local breeds had a higher seroprevalence rate of 21.29% compared to exotic breeds at 13.74%. A difference in the seroprevalence rates was observed between intensive (8.82%) and extensive (22.58%) farm types. Adult cattle (>2 years) exhibited a higher seroprevalence rate of 24.85% compared to younger cattle (6 months to 2 years) at 12.33% (Table 1).

The result shown in Table 2 revealed that the seroprevalence of bovine brucellosis was higher in North Gondar (27.27%) compared to Central Gondar (9.62%). Additionally, dairy farms with poor calf management practices had a significantly higher seroprevalence rate (24.14%) compared to those with good management (7.89%). The seroprevalence rate of brucellosis for Livestock owners with a primary education level was higher (23.70%) compared to those with a secondary education or above (12.80%, Table 2).

Table 1. Seroprevalence and host risk factors for bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	Category	No. of Examined	No. of Positive	Prevalence (%)	(95% CI)
Breed type	Local	202	43	21.29	(0.48-0.58)
	Exotic	182	25	13.74	(0.42-0.52)
Farm type	Intensive	136	12	8.82	(0.31-0.40)
	Extensive	248	56	22.58	(0.60-0.69)
Herd size	Small	145	25	17.24	(0.33-0.43)
	Medium	142	32	22.54	(0.32-0.42)
	Large	97	11	11.34	(0.21-0.30)
Age	Young (6 month – 2 years)	219	27	12.33	(0.52-0.62)
	Adult (> years)	165	41	24.85	(0.38-0.48)
Vaccination	Vaccinated	164	18	10.98	(0.38-0.48)
	Non-vaccinated	220	50	22.73	(0.52-0.62)
Feeding status	Properly feed	144	16	11.11	(0.33-0.42)
	Non-properly feed	240	52	21.67	(0.58-0.67)
Breeding method	Natural mating	253	55	21.74	(0.61-0.71)
	AI	131	13	9.92	(0.30-0.39)

95% CI: 95% confidence interval. p values less than 0.05 were statistically significant; No. of examined: Number of examined; No. of positive: Number of positive; AI: Artificial Insemination

Table 2. Seroprevalence and environmental risk factors of bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	Category	No. of examined	No. of positive	Prevalence (%)	(95% CI)
Zone	Central Gondar	208	20	9.62	(0.49-0.59)
	North Gondar	176	48	27.27	(0.41-0.51)
Ventilation	Well	184	39	21.20	(0.43-0.53)
	Poor	200	29	14.50	(0.47-0.57)
Calf Management	Good	152	12	7.89	(0.35-0.45)
	Poor	232	56	24.14	(0.55-0.65)
Disposal After Birth	Yes	86	12	13.95	(0.18-0.27)
	No	298	56	18.79	(0.73-0.82)
Educational level	Primary	173	41	23.70	(0.40-0.50)
	Secondary and above	211	27	12.80	(0.50-0.60)
Space	Adequate	192	37	19.27	(0.45-0.55)
	Confined	192	31	16.15	(0.45-0.55)
House sanitation	Good	126	8	6.35	(0.28-0.38)
	Poor	258	60	23.26	(0.62-0.72)

No. of examined: Number of examined; No. of positive: Number of positive

In the multivariable analysis, a statistically significant association was observed between the seroprevalence of brucellosis with zone and herd size ($p < 0.05$, Table 3). The findings presented in Table 4 revealed a statistically significant association ($p < 0.05$) between the prevalence of brucellosis and both house sanitation and education level. The likelihood of brucellosis was 6.15 times higher (95% CI: 2.77, 13.68) in households with poor sanitation compared to those with good sanitation (Table 4).

Table 3. Univariate and multivariate mixed-effect logistic regression analysis of host factors for bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	Category	Univariable		Multivariable	
		COR (95% CI)	p value	AOR (95%CI)	p value
Zone	Central Gondar	Reference			
	North Gondar	3.53(2.00-6.22)	0.00*	4.48(2.42-8.27)	0.00*
Breed type	Exotic	Reference			
	Local	0.59(0.34-1.01)	0.06		
Farm type	Intensive	Reference			
	Extensive	3.01(1.55-5.85)	0.00*		
Herd size	Small	Reference			
	Medium	1.40(0.78-2.50)	0.26	2.37(1.24-4.51)	0.01*
	Large	0.61(0.29-1.31)	0.21		
Ventilation	Well	Reference			
	Poor	0.63(0.37-1.07)	0.09		
Age	Young	Reference			
	Adult	2.35(1.38-4.02)	0.00*		
Vaccination	Vaccinated	Reference			
	Non-Vaccinated	2.39(1.33-4.27)	0.00*		
Feeding status	Properly Feed	Reference			
	Non-Properly Feed	2.21(1.21-4.05)	0.01*		
Breeding method	AI	Reference			
	Natural	0.40(0.21-0.710	0.01*		

COR: Crude odds ratio; AOR: Adjusted odds ratio; 95% CI: 95% confidence interval. p values less than 0.05 were statistically significant; No. of examined: Number of examined; No. of positive: Number of Positive; AI: Artificial Insemination

Table 4. Univariable and multivariable mixed-effect logistic regression analysis of environmental risk factors for bovine brucellosis in dairy farms of Ethiopia between November 2023 and June 2024

Variables	Category	Univariable		Multivariable	
		COR (95% CI)	p value	AOR (95%CI)	p value
Space	Adequate	Reference			
	Confined	0.81(0.48-1.36)	0.42		
House sanitation	Good	Reference			
	Poor	4.47(2.07-9.67)	0.00*	6.15(2.77-13.68)	0.00*
Calf Management	Good	Reference			
	Poor	3.71(1.92-7.20)	0.01		
Disposal after birth	Yes	Reference			
	No	1.43(0.73-2.80)	0.30		
Educational level	Primary	Reference			
	Secondary and above	0.47(0.28-0.81)	0.01*	0.33(0.19-0.58)	0.00*

COR: Crude odds ratio, AOR: Adjusted odds ratio; Referenence: In the provided result table, the term Reference: Serves as a point of comparison for the other categories within each variable. It is the baseline or default category against which the other categories are compared to determine the association between those categories and the outcome of interest.

An Area Under the Curve (AUC, Figure 2) value of 0.7084 indicated the overall performance of the model in distinguishing between the positive and negative classes. This suggested that the model had a moderate level of discrimination ability. Raising the probability cutoff beyond 0.5 in Figure 3 generally elevated the bar for categorizing an observation as positive.

The survey depicted in Table 5 revealed a predominance of female respondents (80%) compared to males (20%). Among the respondents, the 'adult' age group (35-52 years) constituted the largest segment at 50.67%, followed by the 'young' category (18-35 years) at 34.67% and the 'old' category (52 and above) at 14.67% (Table 5). In Table 6, the majority of respondents (62.67%) disagreed with being concerned about the impact of bovine brucellosis on cattle health and productivity. A significant portion (31.33%) neither agreed nor disagreed, while only 6.00% agreed. A considerable number of respondents (46.00%) agreed with having awareness regarding the symptoms and transmission of bovine brucellosis. 42.00% disagreed, and 12.00% neither agreed nor disagreed.

In Table 7, a majority of respondents (61.33%) correctly identified that the primary causative agent of bovine brucellosis was a bacterium called *Brucella*, while 38.67% answered incorrectly. A significant portion of respondents (68.67%) correctly identified that bovine brucellosis is typically transmitted among cattle through contact with infected reproductive fluids or tissues, with 31.33% answering incorrectly. A majority of respondents (61.33%) incorrectly identified common symptoms of bovine brucellosis in cattle as fever, abortion, and decreased milk production, while 38.67% answered correctly.

Among females, 57.53% acknowledged transmission among cattle, while 42.47% did not. In comparison, males exhibited a higher affirmative response rate, with 75% acknowledging transmission. This gender-based divergence, although present, did not exhibit significant statistical variance ($\chi^2 = 0.49$, $P = 0.49$). When considering age groups, distinct patterns in knowledge levels were evident. Among the younger participants, 76% were aware of common symptoms, while 84% recognized the impact on cattle productivity. These figures contrasted with the responses from adult participants, where 91.67% acknowledged the zoonotic transmission and 93.75% understood its impact on cattle productivity. The statistical analysis underscored significant differences between the age groups, particularly concerning the common symptoms and impacts of bovine brucellosis ($\chi^2 = 20.48$, $p < 0.05$ and $\chi^2 = 7.80$, $P = 0.02$, respectively, Table 8).

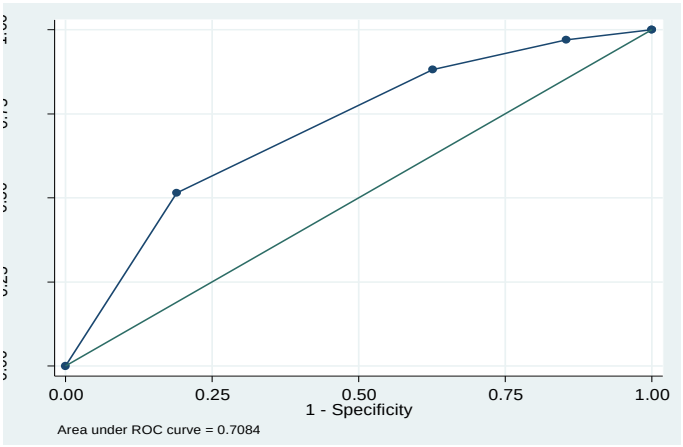


Figure 2. The area under the receiver operating characteristic (ROC) curve the performance of diagnostic tests and the predictive accuracy of various factors in the context of bovine brucellosis management and control strategies

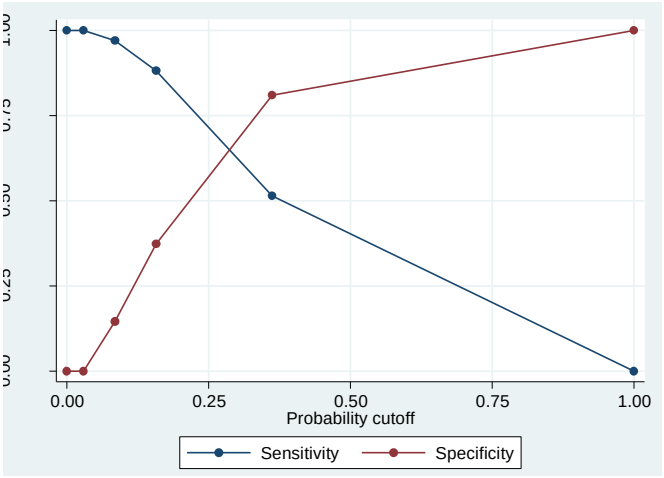


Figure 3. Illustration explaining the relationship between setting a probability cutoff in classification models and its impact on model sensitivity

Table 5. Socio-demographic characteristics of respondents (n = 150) in Ethiopia between November 2023 and June 2024

Variables	Category	Frequency (%)
Gender	Male	30(20)
	Female	120(80)
Age	Young (18-35)	52(34.67)
	Adult (35-52)	76(50.67)
	Old (52 and above)	22(14.67)
Education Level	No formal education	74(49.33)
	Primary school	66(44.00)
	Secondary school and above	10(6.67)
Occupation	Farmer	56(37.33)
	Veterinary professional	8(5.33)
	Agricultural worker	27(18.00)
	Student	59(39.33)
Herd Size	Small	64(42.67)
	Medium	59(39.33)
	Large	27(18.00)
Experience with Livestock	Novice (less than 1 year)	59(39.33)
	Intermediate (1-5 years)	64(42.67)
	Experienced (5+ years)	27(18.00)
Access to Veterinary Services	Easily accessible	27(18.00)
	Somewhat accessible	59(39.33)
	Not accessible	64(42.67)
Body Condition	Good	33(22)
	Poor	117(78)

Table 6. Respondents' attitude toward bovine brucellosis (n = 150) in Ethiopia between November 2023 and June 2024

Attitude queries	Response	Frequency (%)
Q1. How concerned are you about the impact of bovine brucellosis on cattle health and productivity?	Agree	9(6.00)
	Neither agree nor disagree	47(31.33)
	Disagree	94(62.67)
Q2. What is your level of awareness regarding the symptoms and transmission of bovine brucellosis?	Agree	69(46.00)
	Neither agree nor disagree	18(12.00)
	Disagree	63(42.00)
Q3. Do you believe that bovine brucellosis poses a significant threat to public health?	Agree	104(69.33)
	Neither agree nor disagree	36(24.00)
	Disagree	10(6.67)
Q3. In your opinion, what are the main challenges in preventing and controlling bovine brucellosis in cattle populations?	Agree	112(74.67)
	Neither agree nor disagree	20(13.33)
	Disagree	18(12.00)
Q4. How likely are you to seek veterinary assistance or report suspected cases of bovine brucellosis in your cattle?	Agree	69(46.00)
	Neither agree nor disagree	18(12.00)
	Disagree	63(42.00)
Q5. Are you satisfied with the current level of government support and policies aimed at preventing and controlling bovine brucellosis?	Agree	17(11.33)
	Neither agree nor disagree	34(22.67)
	Disagree	99(66.00)

Table 7. Respondents' knowledge level on bovine brucellosis (n = 150) in Ethiopia between November 2023 and June 2024

Knowledge queries	Response	Frequency (%)
Q1. Is the primary causative agent of bovine brucellosis a bacterium called *Brucella?*	Yes	92(61.33)
	No	58(38.67)
Q2. Is bovine brucellosis typically transmitted among cattle through contact with infected reproductive fluids or tissues?	Yes	103(68.67)
	No	47(31.33)
Q3. Are common symptoms of bovine brucellosis in cattle fever, abortion, and decreased milk production?	Yes	58(38.67)
	No	92(61.33)
Q4. Can bovine brucellosis be transmitted from cattle to humans, causing a zoonotic infection?	Yes	43(28.67)
	No	107(71.33)
Q5. If you answered "Yes" to the previous question, which of the following are clinical signs of Bovine brucellosis?	Abortion	23(15.33)
	Decreased milk production	53(35.33)
	Joint swelling	74(49.33)
Q6. Does bovine brucellosis impact cattle productivity and reproduction by causing abortion, reduced fertility, and decreased milk production?	Yes	116(77.33)
	No	34(22.67)
Q7. Do potential economic consequences of bovine brucellosis outbreaks in a cattle herd include loss of productivity, treatment costs, and trade restrictions?	Yes	114(76.00)
	No	36(24.00)
Q8. Can biosecurity measures such as quarantine, disinfection, and testing help prevent the spread of bovine brucellosis within a cattle herd?	Yes	143(95.33)
	No	7(4.67)
Q9. If you answered "Yes" to the previous question, which of the following factors were associated with the occurrence of Bovine brucellosis in your flock?	Contact with infected animals	136(90.67)
	Contaminated feed or water sources	3(2.00)
	Poor biosecurity practices	11(7.33)

Table 8. Association between demographic variables with knowledge level of participants in bovine brucelosis in Ethiopia between November 2023 and June 2024

Variable	Characteristic	Response									
		Transmission among cattle		Common symptoms		Zoonotic transmission		Availability of vaccines		Impact on cattle productivity	
		Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Gender	Female	84(57.53)	62(42.47)	117(80.14)	29(19.86)	55(37.67)	91(62.33)	50(34.25)	146(65.75)	127(86.99)	19(13.01)
	Male	3(75)	1(25)	1(25)	3(75)	2(50)	2(50)	1(25)	3(75)	3(75)	1(25)
	Statistics	χ^2= 0.49 P=0.49		χ^2=7.05 P=0.00		χ^2=0.25 P=0.61		χ^2=0.15 P= 0.70		χ^2=0.48 P=0.49	
Age	Young	29(58)	21(42)	38(76)	12(24)	11(22)	39(78)	8(16)	42(84)	42(84)	8(16)
	Adult	39(52.7)	9(18.75)	44(91.67)	4(8.33)	16(33.33)	32(66.67)	27(43.75)	21(43.75)	45(93.75)	3(6.25)
	Old	19(36.54)	33(63.46)	36(69.23)	16(30.77)	30(57.69)	22(42.31)	16(30.77)	36(69.23)	43(82.69)	9(17.3)
	Statistics	χ^2=20.48 P=0.000		χ^2=7.80 P=0.02		χ^2=14.44 P=0.00		χ^2=18.05 P=0.00		χ^2=3.10 P= 0.21	
Education	No education	39(52.7)	35(47.3)	61(82.43)	13(17.57)	25(33.78)	49(66.22)	23(31.08)	51(68.92)	64(86.49)	10(13.51)
	Primary school	40(60.61)	26(39.39)	50(75.76)	16(24.24)	26(39.39)	40(60.61)	24(36.36)	42(63.64)	57(86.36)	9(13.64
	2nd and above	8(80)	2(20)	7(70)	3(30)	6(60)	4(40)	4(60)	6(60)	9(90)	1(10)
	Statistics	χ^2=3.02 P=0.22		χ^2=1.41 P=0.49		χ^2=2.67 P=0.26		χ^2=0.61 P= 0.74		χ^2=0.10 P=0.95	
BCS	Good	24(72.73)	9(27.27)	20(60.61)	13(39.39)	13(39.39)	20(60.61)	11(33.33)	22(66.67)	28(84.85)	5(15.15)
	Poor	63(53.85)	54(46.15)	98(83.76)	19(16.24)	44(37.61)	73(62.39)	44(36.36)	77(63.64)	102(87.18)	15(12.82)
	Statistics	χ^2=3.77 P=0.05		χ^2=8.22 P=0.00		χ^2=0.04 P=0.85		χ^2=0.01 P=0.93		χ^2=0.12 P=0.73	
Season	Dry	60(60)	40(40)	81(76.42)	25(23.58)	44(41.51)	62(58.49)	34(32.08)	72(67.92)	91(85.85)	15(14.15)
	Wet	27(61.36)	17(38.42)	37(84.07)	7(15.91)	13(29.55)	31(70.45)	17(38.36)	27(61.64)	39(88.64)	5(11.36)
	Statistics	χ^2=0.29 P=0.59		χ^2=1.09 P=0.29		χ^2=1.89 P=0.17		χ^2=0.59 P=0.44		χ^2=0.20 P=0.65	
Access	Accessible	19(57.58)	14(42.42)	27(81.82)	6(18.18)	7(21.21)	26(78.79)	12(36.36)	21(63.64)	25(75.76)	8(24.24)
	Somewhat	33(58.93)	23(41.07)	46(82.14)	10(17.86)	27(48.21)	29(51.79)	16(28.57)	40(71.43)	52(92.86)	4(7.14)
	Not accessible	35(57.38)	26(42.62)	45(73.77)	16(26.23)	23(37.7)	38(62.3)	23(3770)	38(62.3)	53(86.89)	8(13.11)
	Statistics	χ^2=0.03 P=0.98		χ^2=1.47 P=0.48		χ^2=6.42 P=0.04		χ^2=1.19 P=0.55		χ^2=5.26 P=0.03	

χ^2: chi-squared; P: p-value; BCS: Body Condition Score

To cite this paper: Birhan M (2024). A Holistic Approach to Bovine Brucellosis: Serological Tests, Knowledge, Attitudes, and Practices Evaluation, and Risk Factor Identification on Dairy Farms of Ethiopia. *World Vet. J.*, 14(3): 435-448. DOI: https://dx.doi.org/10.54203/scil.2024.wvj51

DISCUSSION

Brucellosis, a zoonotic disease prevalent in many developing regions, inflicted significant losses on the livestock industry and small-scale livestock keepers. This disease, capable of transmission from animals to humans, posed a substantial public health risk (Franc et al., 2018). The present research revealed that the combined prevalence of *Brucella* antibodies, as detected through both RBPT and ELISA tests, stood at 27.27% in North Gondar and 9.62% in Central Gondar areas within the Amhara region. The overall prevalence of bovine brucellosis in dairy farms across the Central and North Gondar zones of the Amhara region was calculated to be 17.71%.

The current investigation unveiled a heightened prevalence compared to certain preceding studies. This overall seroprevalence of 17.71% exceeded figures documented in previous research conducted in Ethiopia, such as 0.4% in the Oromia Special Zone encircling Addis Ababa by Bifo et al. (2020), 3% in Bishoftu Town, Oromia by Waktole et al. (2018), and 0.6% in selected cities of the Central Highlands of Ethiopia by Getahun et al. (2023). Nielsen (2018) reported an overall prevalence of 0.14% in the North Gondar Zone, contrasting with the higher prevalence found in the current study.

The detection of *Brucella* antibodies relied solely on the Complement Fixation Test, revealing a prevalence of 5.7% in selected districts of the Afar National Regional State, Ethiopia as documented by Negash and Dubie (2021). Individual animal-level prevalence of 0.06% and herd-level prevalence of 0.8% were reported through c-ELISA in dairy farms within Addis Ababa by Edao et al. (2018). Asmare et al. (2013) reported an overall farm-level prevalence of 10.6% in Ethiopia. Additionally, Mekonnen et al. (2010) found a prevalence of 4.9% in Western Tigray, while Mussie et al. (2007) reported 4.63% in the Bahir Dar milk shed, similar with the current study's results.

Degefu et al. (2011) reported prevalence of 1.38% in the Jigjiga Zone, which was lower that current study results. Alehegn et al. (2017) found 4.9% around Gondar Town, and Yayeh (2003) found 0.14% in North Gondar Zone. Notably, the current found prevalence of 17.71% which was higher the previous reports of Molla (1989) at 8.2% in the Arsi area and Yohannes et al. (2012b) at 8.1% in and around Addis Ababa. Factors contributing to this increase may include geographical location and specific demographics of the studied population. Differences in farming practices, animal husbandry methods, and biosecurity measures among regions or populations could contribute to varying levels of *Brucella* infection (Wolff et al., 2017). Utilizing more accurate or sensitive diagnostic techniques in the current study may have led to a higher detection rate compared to studies that relied on less sensitive methods. Changes in these herd management practices, breeding practices, herd size; presence of wildlife, geographical location, feed and water quality, presence of aborted fetuses, human factors, lack of vaccination, climate conditions over time could potentially impact the spread and high prevalence of the disease.

Similar to present findings, Bekele et al. (2002) documented a prevalence of 18.4% in selected farms and ranches in South Eastern Ethiopia. In the Arsi region, Molla (1989) reported a prevalence of 16.8%, while Taye (1991) identified 14.2% at Abernosaranch. Surrounding Addis Ababa, Gebremariam (1985) found an 18.4% prevalence in dairy farms. Taye (1991) and Yirgu (1991) observed a prevalence of 19.5% in East of Ethiopia. Moreover, urban and peri-urban dairy farms displayed a prevalence of 16.9%. Taken together, these results indicate a consistent prevalence pattern across different regions and types of dairy farms in Ethiopia. In contrast, the current findings demonstrate a lower prevalence compared to earlier studies. For example, Reshid (1993) documented a prevalence of 38.7% in and around Addis Ababa. Mekonnen et al. (2010) observed a prevalence of 24.1% in Western Tigray, while Corbel (2006) noted a 33% prevalence in commercial and breeding farms, with Asmare et al. (2013) reporting a prevalence of 20.0%. When comparing the current research outcomes from Ethiopia with studies primarily conducted in African nations, the present results indicate a higher prevalence compared to previous findings across Africa. In South Africa, Kolo et al. (2019) documented a prevalence of 5.5% in animals that were tested via RBPT. Subsequent confirmation through I-ELISA revealed an overall animal-level prevalence of 1.20% in Bangladesh, as reported by Hassan et al. (2014). In Caquetá state, Motta-Delgado et al. (2020) identified a prevalence of 3.23%. Furthermore, Nahar and Ahmed (2009) reported a prevalence of 4.5% in the Mymensingh District in Bangladesh. Conversely, the current seroprevalence results in Ethiopia exhibit a lower prevalence compared to other African countries. For instance, Angara et al. (2004) found a prevalence of 24.9% in the Kuku Dairy Scheme in Sudan, with c-ELISA as a confirmatory test subsequent to RBPT screening. Maiga et al. (1995) noted a prevalence of 19.7% in Mal, while Kabagambe et al. (1988) identified a prevalence of 25.7% in Rwanda. Understanding these variations can offer valuable insights for developing targeted interventions and control measures to effectively manage disease prevalence across diverse geographical regions (Jagapur et al., 2013).

The current findings held significant importance in identifying various associated risk factors. Dairy farms situated in North Gondar showed notably higher odds of bovine brucellosis compared to those in Central Gondar. In the univariate analysis, the odds ratio (COR) was recorded at 3.53 (95% CI 2.00-6.22), which increased to 4.48 (95% CI 2.42-8.27) during the multivariate analysis after accounting for other factors. The examination revealed that extensively categorized farms demonstrated significantly higher odds of bovine brucellosis compared to intensively managed farms in both univariate (COR = 3.01, 95% CI 1.55-5.85) and multivariate (AOR = 2.37, 95% CI 1.24-4.51) analyses, emphasizing the impact of farm management practices on disease prevalence.

Herd size also played a role, with medium-sized herds showing increased odds in the multivariate analysis (AOR = 2.37, 95% CI 1.24-4.51) compared to small herds. Factors such as age (2.35, 95% CI 1.38-4.02), vaccinations (2.39, 95% CI 1.33-4.27), feeding status (2.21, 95% CI 1.21-4.05), and breeding methods (0.40, 95% CI 0.21-0.71) all displayed

To cite this paper: Birhan M (2024). A Holistic Approach to Bovine Brucellosis: Serological Tests, Knowledge, Attitudes, and Practices Evaluation, and Risk Factor Identification on Dairy Farms of Ethiopia. *World Vet. J.*, 14(3): 435-448. DOI: https://dx.doi.org/10.54203/scil.2024.wvj51

significant associations with bovine brucellosis in the analyses, highlighting their importance in disease transmission and control. Animals in natural mating may exhibit a higher seropositivity for *Brucella* infection compared to those animals contributed in artificial insemination, likely due to close confinement and exposure to diseased animals.

In the study that examined awareness levels of brucellosis across different demographic factors, several key findings emerged. Females displayed higher awareness across all categories compared to males, particularly excelling in understanding common symptoms, zoonotic transmission, vaccine availability, and the impact on cattle productivity. Age groups were significantly linked to awareness levels regarding transmission among cattle, common symptoms, zoonotic transmission, vaccine availability, and the impact on cattle productivity. In terms of education, individuals with primary school education demonstrated the highest awareness levels, followed by those with no education and secondary education, with no significant associations identified between education levels and awareness levels. Cattle with poor body condition scores exhibited higher awareness, with significant associations in understanding common symptoms and vaccine availability. Awareness levels remained consistent throughout the year, with no significant differences between dry and wet seasons. Access to information did not notably impact awareness levels, except for a marginally significant effect on understanding zoonotic transmission and cattle productivity impacts. These findings collectively highlight the necessity for targeted educational interventions to enhance brucellosis awareness, particularly among males, older individuals, and those with lower educational attainment.

Furthermore, cattle situated in environments with inadequate house sanitation exhibited significantly higher odds of contracting bovine brucellosis compared to those in areas with acceptable house sanitation. After conducting a multivariable analysis with adjustments for other factors, the odds ratio increased to 6.15, highlighting a strong and significant correlation between the quality of house sanitation and the prevalence of bovine brucellosis. A similar trend was observed in calf management practices concerning bovine brucellosis. Cattle under inadequate calf management displayed notably higher odds of contracting the disease compared to those under proper management practices. The impact of educational attainment on bovine brucellosis prevalence was also examined. In the univariable analysis, it was noted that livestock owners with a secondary education or higher exhibited significantly reduced odds of being affected by bovine brucellosis compared to those with a primary education level. Following a multivariable analysis considering other variables, the odds ratio decreased further to 0.33, indicating a robust inverse relationship between higher educational levels and the prevalence of bovine brucellosis. This association was also reflected in the relatively good hygienic conditions on the farms and the practices implemented to dispose of aborted materials to prevent animal contact (Getahun et al., 2023).

There were no significant differences in transmission rates between females and males. Females displayed significantly higher rates of common symptoms compared to males. No significant variance was detected in zoonotic transmission between females and males. Vaccine availability remained consistent across genders. Females had a greater impact on cattle productivity compared to males. Notably, there was a significant difference in transmission rates among different age groups, with younger individuals exhibiting higher rates. Young individuals also displayed more common symptoms compared to adults and older individuals. A significant distinction was observed in zoonotic transmission among age groups, with younger individuals having a higher potential. Vaccination availability varied significantly among age groups, with young individuals having a more substantial impact on cattle productivity compared to adults and older individuals. No significant differences were noted based on education levels ($\chi^2 = 3.02$, $P = 0.22$). Common symptoms did not show significant differences based on education levels. Zoonotic transmission did not present a clear pattern based on education levels. Vaccine availability appeared consistent across education levels. The impact on cattle productivity varied slightly based on education levels, but these differences were not statistically significant. There was a notable difference in transmission rates between individuals with good and poor BCS. Individuals with poor BCS exhibited significantly more common symptoms. No significant variation in zoonotic transmission was evident based on BCS. Vaccine availability remained consistent across BCS categories. Individuals with poor BCS had a higher impact on cattle productivity. There were no significant differences in transmission rates based on the season. However, the dry season showed slightly higher common symptoms than rainy season. No clear pattern emerged based on the season for zoonotic transmission. Vaccine availability appeared consistent across seasons. The dry season had a slightly higher impact on cattle productivity, however these differences were not statistically significant. No significant differences in transmission rates were observed based on accessibility. There was no clear pattern based on accessibility levels.

CONCLUSION

The data presented in this study indicated a significant association between gender and knowledge of common symptoms related to bovine brucellosis, specifically fever, abortion, and decreased milk production in cattle. The questionnaire survey conducted in this study revealed a notable association between gender and awareness of bovine brucellosis symptoms. 80.14% of females were informed compared to 25% of males. Concerning age, 58% of young respondents, 52.7% of adults, and 36.54% of older individuals were knowledgeable about brucellosis transmission,

indicating a strong correlation. In Ethiopia, brucellosis presented a substantial public health concern, notably in Central Gondar (9.62%) and North Gondar (27.27%), with an overall seroprevalence of 17.71%, highlighting a high prevalence of bovine brucellosis antibodies. Future research on bovine brucellosis could explore genetic susceptibility factors in cattle breeds for resistant breeding, develop advanced diagnostic tools, analyze transmission dynamics, adopt a One Health approach, assess vaccine efficacy, investigate antimicrobial resistance, evaluate economic impacts, analyze farm biosecurity, explore zoonotic risks, and consider the effects of climate change to enhance prevention and management of the disease.

DECLARATIONS

Competing of interests

There are no conflicts of interest.

Availability of data and materials

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethical consideration

The animal study was reviewed and approved by Institutional Review Board of University of Gondar. Written informed consent was obtained from the owners for the participation of their animals in this study.

Authors' contributions

Mastewal Birhan was responsible for the conception of the study and prepared the initial draft of the manuscript. Mastewal Birhan collected the blood samples and questionnaire data, analyzed the data, and supervised the study's conduct. Mastewal Birhan has read, revised, improved, and approved the final manuscript. Mastewal Birhan approved the submitted version of the manuscript.

Funding

This work is part of a mega research project funded by the University of Gondar, the office of the vice president for research and community service (Budget code: VPRCS 6223, 2019/20- 2020/21) to SI as PI and the co-authors as co-I of the project. We declare that the funding body did not contribute to the execution of any part of the study, interpretation of the results, and write-up of this article.

Acknowledgments

We are grateful to laboratory technicians at UoG, CVMAS, and NVI, animal owners, and district veterinarians for their invaluable contribution to the success of the study.

REFERENCES

Abdulkadir M (2019). Seroprevalence of brucellosis in livestock and human in Asayita and Mille districts of Afar region, Ethiopia. Journal of Biology, Agriculture and Healthcare, pp. 1-22. DOI: https://www.doi.org/10.21203/rs.3.rs-3078324/v1

Acha PN and Szyfres B (2001). Zoonoses and communicable diseases common to man and animals, 3rd Edition. Bacterioses and mycoses. Scientific and Technical Publication., USA, pp. 1-309. Available at: https://www3.paho.org/hq/dmdocuments/2012/Acha-Zoonoses-Eng.pdf

Al Dahouk S and Nöckler K (2011). Implications of laboratory diagnosis on brucellosis therapy. Expert Review of Anti-Infective Therapy, 9(7): 833-845. DOI: https://www.doi.org/10.1586/eri.11.55

Alehegn E, Tesfaye S, and Chane M (2017). Seroprevalence of Bovine Brucellosis and its risk factors in cattle in and around Gondar Town, North West Gondar, Ethiopia. Journal of Advanced Dairy Research, 4(4): 1000166. DOI: https://www.doi.org/10.4172/2329-888X.1000166

Angara T, Ismail A, Agab H, and Saeed N (2004). Sero-prevalence of bovine brucellosis in Kuku Dairy Scheme, Khartoum North, Sudan. Journal of Veterinary Science & Animal Husbandry, 48(1): 2.

Asmare K, Sibhat B, Molla W, Ayelet G, Shiferaw J, Martin AD, Skjerve E, and Godfroid J (2013). The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. Acta Tropica, 126(3): 186-192. DOI: https://www.doi.org/10.1016/j.actatropica.2013.02.015

Bekele T, Zeleke,M, and Baars RMT (2002). Milk production performance of the one humped camel (*Camelus dromedarius*) under pastoral management in semi-arid eastern Ethiopia. Livestock Production Science, 76(1-2): 37-44. DOI: https://www.doi.org/10.1016/S0301-6226(01)00333-5

Bifo H, Gugsa G, Kifleyohannes T, Abebe E, and Ahmed M (2020). Sero-prevalence and associated risk factors of bovine brucellosis in Sendafa, Oromia Special Zone surrounding Addis Ababa, Ethiopia. PloS one, 15: e0238212. DOI: https://www.doi.org/10.1371/journal.pone.0238212

Corbel MJ (2006). Brucellosis in humans and animals. World Health Organization, pp. 1-89. Available at: https://iris.who.int/bitstream/handle/10665/43597/WHO_CDS_EPR_2006.7_eng.pdf?sequence=1

Central statistical agency (CSA) (2011). Ethiopia demographic and health survey: Preliminary report. Addis Ababa, Ethiopia. Available at: https://dhsprogram.com/pubs/pdf/fr255/fr255.pdf

Central statistical agency (CSA) (2012). International: Ethiopia demographic and health survey 2011. Central statistical agency of Ethiopia and ICF international Addis Ababa, Ethiopia and Calverton, Maryland, USA.

Degefu H, Mohamud M, Hailemelekot M, and Yohannes M (2011). Seroprevalence of bovine brucellosis in agro pastoral areas of Jijjiga zone of Somali National Regional State, Eastern Ethiopia. Ethiopian Veterinary Journal, 15(1): 37-47. DOI: https://www.doi.org/10.4314/evj.v15i1.67683

Desta KB (2002). Analyses of dairy cattle breeding practices in selected areas of Ethiopia, Köster Germany.

Dinka H and Ababa A (2011). A study on school factors that affect girls academic achievement in the general secondary schools of Addis Ababa (implications to EFA goals). Available at: https://aacaebc.wordpress.com/wp-content/uploads/2018/02/a-study-on-school-factors-that-affect-girls-academic.pdf

Dohoo IR, Wright P, Ruckerbauer G, Samagh B, Robertson F, and Forbes L (1986). A comparison of five serological tests for bovine brucellosis. Canadian Journal of Veterinary Research, 50: 485. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1255253/

Domenech J (1977). Enquête sérologique sur la brucellose du dromadaire en Ethiopie [Serological survey on camel brucellosis in Ethiopia]. Revue D'élevage et de Médecine Vétérinaire des Pays Tropicaux, 30(2): 141-142. DOI: https://www.doi.org/10.19182/remvt.8090

Edao BM, Hailegebreal G, Berg S, Zewude A, Zeleke Y, Sori T, Almaw G, Whatmore AM, Ameni G, and Wood JLN (2018). Brucellosis in the Addis Ababa dairy cattle: The myths and the realities. BMC Veterinary Research, 14(1): 396. DOI: https://www.doi.org/10.1186/s12917-018-1709-4

Franc K, Krecek R, Häsler B, and Arenas-Gamboa A (2018). Brucellosis remains a neglected disease in the developing world: A call for interdisciplinary action. BMC Public Health, 18: 125. Available at: https://link.springer.com/article/10.1186/s12889-017-5016-y

Gashaw AE, Fikru RG, and Gezahegne MK (2022). Bovine brucellosis and its public health significance in Ethiopia. Zoonoses, 2(1): 1-10. Available at: https://www.scienceopen.com/hosted-document?doi=10.15212/ZOONOSES-2022-0005

Gebremariam K (1985). Prevalence of brucellosis in four different farms around Addis Ababa. DVM. Thesis, FVM, Addis Ababa University, Ethiopia.

Getahun T, Urge B, and Mamo G (2023). Seroprevalence of bovine brucellosis in selected sites of central highland of Ethiopia. Vet Med, 14: 11-22. Available at: https://www.tandfonline.com/doi/full/10.2147/VMRR.S388970

Hassan AA, Uddin MB, Islam MR, Cho HS, and Hossain MM (2014). Serological prevalence of brucellosis of cattle in selected dairy farms in Bangladesh. Korean Journal of Veterinary Research, 54(4): 239-243. DOI: https://www.doi.org/10.14405/kjvr.2014.54.4.239

Jagapur RV, Rathore R, Karthik K, and Somavanshi R (2013). Seroprevalence studies of bovine brucellosis using indirectenzyme-linked immunosorbent assay (i-ELISA) at organized and unorganized farms in three different states of India. Veterinary World, 6(8): 550-553. Available at: https://www.veterinaryworld.org/Vol.6/August%20-%202013/Seroprevalence%20studies%20of%20bovine%20brucellosis%20using%20indirect-.pdf

Kabagambe J, Nshimiyimana A, Muberuka J, and Nykligira J (1988). Les maladies enzootiques des bovins au Bugesera [Enzootic diseases of cattle in Bugesera]. Bulletin Agricole du Rwanda, 21(3): 159-168.

Khurana SK, Sehrawat A, Tiwari R, Prasad M, Gulati B, Shabbir MZ, Chhabra R, Karthik K, Patel SK, Pathak M et al. (2021). Bovine brucellosis–A comprehensive review. Veterinary Quarterly, 41(1): 61-88. DOI: https://www.doi.org/10.1080/01652176.2020.1868616

Kolo FB, Adesiyun AA, Fasina FO, Katsande CT, Dogonyaro BB, Potts A, Matle I, Gelaw AK, and Van Heerden H (2019). Seroprevalence and characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa. Veterinary Medicine and Science, 5(4): 545-555. DOI: https://www.doi.org/10.1002/vms3.190

Maiga S, Niang M, and Touré I (1995). Enquête séroépidémiologique sur la brucellose bovine dans la ceinture laitière de Bamako, Mali [Seroepidemiological investigation into bovine brucellosis in the dairy belt of Bamako, Mali]. Livestock production and diseases in the tropics, livestock production and human welfare. Proceedings of the 8th International Conference of Institutions of Tropical Veterinary Medicine, Berlin, pp. 25-29.

McDermott CM and O'connor GC (2002). Managing radical innovation: An overview of emergent strategy issues. Journal of Product Innovation Management, 19(6): 424-438. DOI: https://www.doi.org/10.1111/1540-5885.1960424

Mekonnen H, Kalayou S, and Kyule M (2010). Serological survey of bovine brucellosis in Barka and Arado breeds (Bos indicus) of Western Tigray, Ethiopia. Preventive Veterinary Medicine, 94(1-2): 28-35. DOI: https://www.doi.org/10.1016/j.prevetmed.2009.12.001

Merga Sima D, Abdeta Ifa D, Merga AL, and Tola EH (2021). Seroprevalence of bovine brucellosis and associated risk factors in Western Ethiopia. Veterinary Medicine: Research and Reports, 12: 317-324. DOI: https://www.doi.org/10.2147/VMRR.S338930

Molla B (1989). Seroepidemiological survey of bovine brucellosis in Arsi region. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Motta-Delgado PA, Martínez-Tovar RA, Londoño-Giraldo M, Rojas-Vargas EP, and Herrera-Valencia W (2020). Sero-prevalence of brucellosis (*Brucella abortus*) in bovines from Caquetá state, Colombia. Ciencia y Agricultura, 17(1): 19-30. DOI: https://www.doi.org/10.19053/01228420.v17.n1.2020.9917

Muma JB, Samui KL, Oloya J, Munyeme M, and Skjerve E (2007). Risk factors for brucellosis in indigenous cattle reared in livestock–wildlife interface areas of Zambia. Preventive Veterinary Medicine, 80: 306-317. DOI: https://www.doi.org/10.1016/j.prevetmed.2007.03.003

Mussie H, Tesfu K, and Yilkal A (2007). Seroprevalence study of bovine brucellosis in Bahir dar milk shed, northwestern Amhara region. Ethiopian Veterinary Journal, 11(1): 42-49.

Nahar A and Ahmed M (2009). Sero-prevalence study of brucellosis in cattle and contact human in Mymensingh District. Bangladesh Journal of Veterinary Medicine, 7(1): 269-274. Available at: https://lamjol.info/index.php/BJVM/article/view/5071/4047

Negash W and Dubie T (2021). Study on seroprevalence and associated factors of bovine brucellosis in selected districts of Afar National Regional State, Afar, Ethiopia. Veterinary Medicine International, 2021: 8829860. DOI: https://www.doi.org/10.1155/2021/8829860

Nielsen K (2018). Animal brucellosis. CRC Press., Boca Raton. DOI: https://www.doi.org/10.1201/9781351069687

Office international des epizooties (OIE) (2004). Manual of diagnostic tests and vaccines for terrestrial animals.

Qureshi KA, Parvez A, Fahmy NA, Abdel Hady BH, Kumar S, Ganguly A, Atiya A, Elhassan GO, Alfadly SO, Parkkila S et al. (2023). Brucellosis: Epidemiology, pathogenesis, diagnosis and treatment–A comprehensive review. Annals of Medicine, 55(2): 2295398. DOI https://www.doi.org/10.1080/07853890.2023.2295398

Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007). Veterinary medicine. A text book of diseases of cattle, sheep, pigs, goats and horses, 10th Edition. Elsevier Saunders., London, pp. 963-985.

Reshid M, Fantaye M, Gebreab F, and Moldemeskel M (1993). Ovine helminthiasis at Bako. Available at: https://agris.fao.org/search/en/providers/122600/records/6471f4df2a40512c710ee22d

Taye Y (1991). Sero prevalence of bovine brucellosis at Abernosaranch. Ethiopia DVM.

Teshome A (2010). Impact of climate change on household water security and sustainable livelihoods in Shebel Berenta Woreda, East Gojjam Zone, Amhara Region, Ethiopia. Diss. Addis Ababa University, Ethiopia. Available at: http://repository.hoarec.org/home/handle/123456789/6

Thursfield S (2015). Medieval tailor's assistant: Common Garments 1100-1480. Crowood, pp. 96-102. Available at: https://www.archers-du-bailli.be/costume/documents/cos-doublet/patron-doublet-homme-MTA.pdfTsegaye Y, Kyule M, and Fikre lobago F (2016). Seroprevalence and risk factors for bovine brucellosis in Arsi Zone, Oromia Regional State, Ethiopia. American Scientific Research Journal for Engineering, Technology, and Sciences, 24(1): 16-25. Available at: https://core.ac.uk/download/pdf/235049969.pdf

Tulu D (2022). Bovine brucellosis: Epidemiology, public health implications, and status of brucellosis in Ethiopia. Veterinary Medicine: Research and Reports, 13: 21-30. DOI: https://www.doi.org/10.2147/VMRR.S347337

Waktole H, Geneti E, Ahmed W, Mammo G, and Abunna F (2018). Sero-prevalence and associated risk factors of bovine brucellosis in selected dairy farms in Bishoftu Town, Oromia, Ethiopia. International Journal of Microbiological Research, 9: 45-53. DOI: https://www.doi.org/10.5829/idosi.ijmr.2018.45.53

Wolff C, Boqvist S, Ståhl K, Masembe C, and Sternberg-Lewerin S (2017). Biosecurity aspects of cattle production in Western Uganda, and associations with seroprevalence of brucellosis, salmonellosis and bovine viral diarrhoea. BMC Veterinary Research, 13: 382. DOI: https://www.doi.org/10.1186/s12917-017-1306-y

Yayeh T (2003). A survey of bovine brucellosis in selected areas of North Gondar Zone, Ethiopia. DVM Thesis. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Yirgu T (1991). Sero-prevalence study of bovine brucellosis at Abernosa. DVM Thesis. Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.

Yohannes M, Gill JPS, Ghatak S, Singh DK, and Tolosa T (2012)a. Comparative evaluation of the Rose Bengal plate test, standard tube agglutination test and complement fixation test for the diagnosis of human brucellosis. Revue Scientifique et Technique - Office International des Epizooties, 31(3): 979-984. DOI: org/10.20506/rst.31.3.2175

Yohannes M, Mersha T, Degefu H, Tolosa T, and Woyesa M (2012b). Bovine brucellosis: Serological survey in Guto-Gida district, East Wollega zone, Ethiopia. Global Veterinaria, 8(2): 139-143. Available at: http://www.idosi.org/gv/GV8(2)12/8.pdf

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 449-460.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj52
PII: S232245682400052-14

Survey on Dermatological Disorders of Dogs during 2020-2022 in Rabat, Morocco

Abderrahmane Zahri[1]*, Mohammed Bouslikhane[2], Sara El Mazini[3,4], Meryem Lemrani[3], Ikhlass El Berbri[2], Mohammed Amine Abouelkaram[1], Thomas Balenghien[5,6], and Maria Bourquia[1]

[1]*Parasitology and Parasitic Diseases Unit, Department of Animal Pathology and Public Health, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco*
[2]*Microbiology, Immunology and Contagious Diseases Unit, Department of Animal Pathology and Public Health, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco*
[3]*Laboratory of Parasitology and Vector-Borne-Diseases, Pasteur Institute of Morocco, Casablanca 20360, Morocco*
[4]*Laboratory of Microbial Biotechnology and Bioactive Molecules, Faculty of Sciences and Technologies, Sidi Mohammed Ben Abdellah University, Fes 30000, Morocco*
[5]*CIRAD, UMR ASTRE, F-34398 Montpellier, France*
[6]*ASTRE, University of Montpellier, CIRAD, INRAE, Montpellier, France*

*Corresponding author's Email: zahriabderrahmane@iav.ac.ma

ORIGINAL ARTICLE
Received: July 26, 2024
Revised: August 21, 2024
Accepted: August 30, 2024
Published: September 30, 2024

ABSTRACT

Dermatology is an important specialty in veterinary medicine, focusing on the skin and its appendages. Therefore, the present study highlighted the percentage of skin disorders, as well as their associated risk factors, from cases received at the Parasitology-Dermatology clinic of the Hassan II Institute of Agronomy and Veterinary Medicine (IAV Hassan II), Rabat, Morocco for two years. A total of 1561 dogs (1450 dogs were in 28 different pure breeds and 111 dogs were mongrels, 805 males, and 756 females, with an average age of 6.5 years old) were presented at the University Veterinary Teaching Hospital (UVTH) of the IAV Hassan II from the end of October 2020 to the end of May 2022 (including vaccinations) and 125 dogs were assessed and 161 skin diseases were found (a few dogs had more than one skin disease). Dermatological examinations represented an average of 8.00% (125/1561) of all canine cases received at the University Veterinary Teaching Hospital. The most common clinical signs were pruritus, alopecia, erythema, onychogryphosis, and visible ectoparasites. Parasitic dermatoses were the most frequent, representing 44.10% of all dermatological cases, followed by allergic dermatoses (25.47%) and fungal skin infections (19.25%). Bacterial skin infections and dermatological manifestations of endocrine disorders were infrequent, representing 8.70% and 2.48%, respectively of all observed cases. Risk factors contributing to the occurrence of canine skin disorders included age and lifestyle for sarcoptic mange. An apparent predilection for the living environment was observed in the case of canine leishmaniosis, and an apparent predilection for sex regarding otodectic mange was also demonstrated. Similar results were found for the living environment and lifestyle concerning canine atopic dermatitis. Data reported herein fill gaps in knowledge of skin disorders and their associated risk factors in dogs in Morocco, demonstrating the dominance of skin diseases of zoonotic interest, including flea bite allergy dermatitis (FBAD), dermatophytosis, and canine leishmaniosis.

Keywords: Dog, Morocco, Rabat, Skin disease, Survey

INTRODUCTION

The skin, is an organ, with a crucial role in safeguarding against physical, chemical, and microbiological aggressions. Its sensory components facilitate the perception of heat, cold, pain, touch, and pressure (Hsu and Fuchs, 2022). Veterinary dermatology is gaining increasing attention, given its significant representation in routine examinations (Khoshnegah et al., 2013; Tawfik et al., 2020; Joshi, 2022; Alizadeh et al., 2024). Canine and feline skin disorders represent an important veterinary problem worldwide. A previous comprehensive study indicated that 21.40% of examinations in small animal veterinary practices in the United Kingdom (UK) were related to skin issues (Hill et al., 2006). Similarly, a study carried out at the Ferdowsi University of Mashhad in Iran reported that dermatological disorders affected 17.00% of diagnosed dogs (Khoshnegah et al., 2013). Canine skin disorders are prevalent in India, where the prevalence of infection ranges from 12.00% to 27.60% (Khurana et al., 2016; Kumar and Shekhar, 2020; Joshi, 2022). The origin of skin diseases has been evaluated in different studies to throw light on the clinical signs and cutaneous lesions in dogs. The most common sign in all dermatological examinations is pruritus (Bensignor et al., 2013; Bruet et al., 2022). Superficial pyoderma, canine leishmaniosis, flea infestation, flea bite allergy dermatitis (FBAD), and canine atopic dermatitis (CAD) were the most frequent diagnoses in Mashhad, Iran (Khoshnegah et al., 2013). In contrast, the majority of diagnoses in the UK were parasitic dermatoses and bacterial skin infections (Hill et al., 2006). Generally, the most known skin problems in dogs include sarcoptic mange, demodectic mange, allergic dermatoses, dermatophytosis, and bacterial skin infections

To cite this paper: Zahri A, Bouslikhane M, El Mazini S, Lemrani M, El Berbri I, Abouelkaram MA, Balenghien T, and Bourquia M (2024). Survey on Dermatological Disorders of Dogs during 2020-2022 in Rabat, Morocco. *World Vet. J.*, 14(3): 449-460. DOI: https://dx.doi.org/10.54203/scil.2024.wvj52

(Khoshnegah et al., 2013; Khurana et al., 2016; Tawfik et al., 2020; Kumar and Shekhar, 2022). These skin disorders were diagnosed by clinical examination followed by the appropriate complementary tests required to confirm clinical suspicions (Curtis, 2012; Beco et al., 2013; Gortel, 2013; Hillier et al., 2014; Moriello et al., 2017; Mueller et al., 2020; Miller et al, 2023).

The objective of the current study was to determine the percentage of canine skin conditions assessed and the associated risk factors at the Parasitology-Dermatology clinic of the Hassan II Institute of Agronomy and Veterinary Medicine (IAV Hassan II), Rabat, Morocco. The usefulness of the provided information should be of great interest to veterinary students, new veterinary graduates, and practicing veterinarians in the country.

MATERIALS AND METHODS

Ethical approval

The study protocol was approved by the Hassan II Institute of Agronomy and Veterinary Medicine (IAV Hassan II), Rabat, Morocco, and the Moroccan Ministry of Agriculture. The handling of dogs has been conditioned by respect for animal welfare, which is in agreement with international ethical guidelines (European Union Directive 2010/63/EU and Animal Research, reporting of *In Vivo* experiments guidelines (ARRIVE)).

Study area

Rabat is the capital of the Kingdom of Morocco, which is located on the Atlantic side in the region of Rabat Salé - Kénitra. Situated precisely at 33° 58' 17.724" North 6° 50' 59.3268" West, within the estuarine zone of the Bouregreg River, with a Mediterranean climate, characterized by abundant rainfall in winter and a hot summer (Morocco's climate, 2023). The pet population in Morocco is estimated to be 2.65 million pets (GlobalPETS, 2023).

Case selection

From the end of October 2020 to the end of May 2022, 1561 dogs were presented to the UVTH of the IAV Hassan II in Rabat, Morocco. A total of 125 dogs (105 dogs were in 24 different pure breeds, 20 dogs were mongrels, 74 males, and 51 females, with an average age of 4.5 years old) were assessed for skin problems at the Parasitology-Dermatology Clinic of the IAV Hassan II (following the sanitary measures taken due to the pandemic of COVID-19, assessments were conducted three days a week). The diagnostic approach comprised three sequential steps, including, dermatological history, and clinical examination which aims to perform a physical examination, describe clinical signs (pruritus, alopecia, erythema, wounds, pustular eruptions, and ulcerative lesions), detect nail disorders (onychogryphosis), and identify visible ectoparasites (fleas, lice, and ticks), and complementary examinations. For this purpose, a printed dermatological form was meticulously developed and employed for each assessment (Appendix 1).

Dog's demographic information

Breed, sex, age, living environment, diet, and lifestyle of dogs with skin disorders were recorded by a vet to find statistical and/or epidemiological associations between these risk factors and the dermatoses.

Diagnostic methods

Based on the hypothesis of diagnosis following the clinical examination, the adequate and appropriate complementary examinations were chosen, which are divided into the following sections. Complementary examinations with immediate interpretation include combing, trichogram, otoscopic examination, Wood's lamp examination, ear swabbing, skin scraping, impression cytology and swab cytology of ear canal, smear of lymph node aspirates, and dermoscopy (Zanna et al., 2017).

Complementary examinations with deferred interpretation require more time to analyze their results and include, fungal culture used to diagnose dermatophytosis and Malasseziosis, blood tests to assess hematological and biochemical profiles (hypertriglyceridemia and hypercholesterolemia are related to canine hypothyroidism and Cushing's syndrome (Sieber-Ruckstuh et al., 2022) and thrombocytopenia is a common finding in canine leishmaniosis), and endocrine function tests (cortisol and thyroid hormone assays). Canine leishmaniosis was diagnosed using SNAP® *Leishmania* (Canine Leishmania Antibody test, IDEXX Laboratories, Inc. Westbrook, Maine, USA) by detecting antibodies produced against *Leishmania* (*L*) *infantum* (de Souza et al., 2019), which was also confirmed by nested PCR conditions based on the amplification of the kinetoplast DNA minicircles using two sets of primers including CSB2XF (CGAGTAGCAGAAACTCCCGTTCA), CSB1XR (ATTTTTCGCGATTTTCGCAGAACG), LiR (TCGCAGAACGCCCCT) and 13Z (ACTGGGGGTTGGTGTAAAATAG) (Noyes et al., 1998). The amplification reactions were carried out in the thermal cycler 2720 (Applied Biosystems, USA) following the thermal program, including 94°C for 5 minutes, 30 cycles (94°C for 30 seconds, 55°C for 60 seconds, and 72°C for 90 seconds), and 72°C for 5 minutes.

Statistical analysis

The descriptive statistical analysis was carried out using the statistical program Epi-Info 7.2.5.0 (CDC's National Center for Public Health Informatics, USA) and it is based on the study of the statistical and/or epidemiological associations between the dermatoses affecting dogs and the risk factors taken into consideration (breed, sex, age, living environment and lifestyle). Relative risk was quantified by estimating the odds ratio (OR) for the association between the selected risk factors and the corresponding outcome for each skin disease. Similarly, the confidence intervals for each OR were estimated with the calculation of the Chi-Square (χ^2) test. Statistical analysis was considered significant When the p-value < 0.05.

RESULTS

Demographic information

A total of 125 dogs were assessed with 161 dermatoses (dogs can be diagnosed with one or more skin disorders at the same time). Skin disorders represented 8.00% of all assessed dogs, of which 59.20% (74 cases) were males and 40.80% (51 cases) were females with an age ranging between 1 to 192 months. Most cases were of purebred dogs (84.00%) as opposed to mongrel dogs (16.00%). At the same time, the majority of dogs had a group lifestyle (76.00%) and lived outdoors (60.00%). Risk factors contributing to the occurrence of canine skin disorders included age (OR = 3.50; p = 0.02) and lifestyle (OR = 6.23; p = 0.002) for sarcoptic mange. Additionally, an apparent predilection for the living environment (OR = 6.50; p = 0.007) was observed in the case of canine leishmaniosis, and an apparent predilection for sex regarding otodectic mange (OR = 4.27; p = 0.04). Dogs who lived indoors were 5.26 times more likely to have CAD (OR = 5.26; p = 0.02) compared to dogs who lived outdoors. Additionally, dogs living alone were 10.07 times more likely to have CAD (OR = 10.07; p = 0.009) than those living in a group. Furthermore, there were epidemiological associations only in the case of different dermatoses and risk factors taken into consideration including mongrel dogs living in groups that were more exposed respectively to canine leishmaniosis (OR for mongrel dogs= 3.32; p = 0.08 - OR for lifestyle= 6.02; p = 0.08) and demodicosis (OR = 2.13; p = 0.39 - OR = 2.88; p = 0.06). Males and young dogs were more predisposed to dermatophytosis (OR = 2.14; p = 0.16, OR = 2.90; p = 0.25) and CAD was linked to young age (OR = 3.62; p = 0.36), while a solitary lifestyle without congeners favors exposure to food allergy (OR = 5.06; p = 0.21) and FBAD (OR = 2.72; p = 0.06).

Description of dermatological condition

The dermatoses diagnosed were divided according to their etiologies. Parasitic dermatoses were the most frequent conditions, occupying first place with a percentage of 44.10% (71/161), followed by allergic dermatoses (25.47%, 41/161) and fungal skin infections (19.25%, 31/161). Bacterial skin infections and dermatologic manifestations of endocrine disorders were infrequent, representing 8.70% (14/161) and 2.48% (4/161), respectively in all observed dogs (Figure 1). The ten most frequent dermatological disorders diagnosed in dogs were, FBAD (20 out of 161, 12.42%), dermatophytosis due to *Microsporum canis* (19 out of 161, 11.80%), canine leishmaniosis caused by *Leishmania infantum* (18 out of 161, 11.18%, Figure 2A), sarcoptic mange caused by *Sarcoptes scabiei* (15 out of 161, 9.32%, Figure 2B), demodicosis caused by *Demodex canis* (15 out of 161, 9.32%, Figure 2C), otodectic mange caused by *Otodectes cynotis* (13 out of 161, 8.07%, Figure 2D), CAD (12 out of 161, 7.45%), Malasseziosis caused by *Malassezia pachydermatis* (12 out of 161, 7.45%, Figure 2E), flea infestation caused by *Ctenocephalides felis* (10 out of 161, 6.21%, Figure 2F), food allergy (7 out of 161, 4.35%), and canine pyoderma (7 out of 161, 4.35%). In contrast, only one case of Cushing's syndrome, eosinophilic furunculosis and pododermatitis was diagnosed in the present study.

Breed-wise distribution

The percentage of skin disorders was the highest in common breed dogs (16.00%; 20/125), followed by German Shepherd (12.00%; 15/125); Maltese (11.20%; 14/125); French Bulldog (7.20%; 9/125); Poodle and Labrador Retriever (6.40%; 8/125; each); Rottweiler (5.60%; 7/125); Belgian shepherd (4.80%; 6/125); Chihuahua and Siberian Husky (4.00%; 5/125; each); Cane Corso, Chow Chow, Dalmatian, Jacque Russel Terrier and English Pointer (2.40%; 3/125; each); Brittany spaniel, Pekingese and Dachshund (1.60%; 2/125; each); Akita Inu, White Swiss Shepherd, Pug, Pyrenean Mountain Dog, American Pit Bull Terrier, Great Dane and American Staffordshire Terrier (0.80%; 1/125; each).

Sex-wise distribution

The percentage of skin disorders was higher in males (59.20%; 74/125) compared with females (40.80%; 51/125, Figure 3**)**.

Age-wise distribution

Dermatological disorders were most common in dogs under two years of age (50.40%; 63/125) compared to dogs between two and five years of age (26.40%; 33/125) and dogs over five years of age (23.20%; 29/125, Figure 4).

Living environment and lifestyle-wises distribution

Skin disorders were more common in dogs living outside (60.00%; 75/125) in comparison with those living indoors (40.00%; 50/125). In contrast, the majority of cases lived in groups (76.00%; 95/125) compared to those living alone (24.00%; 30/125, Figure 5).

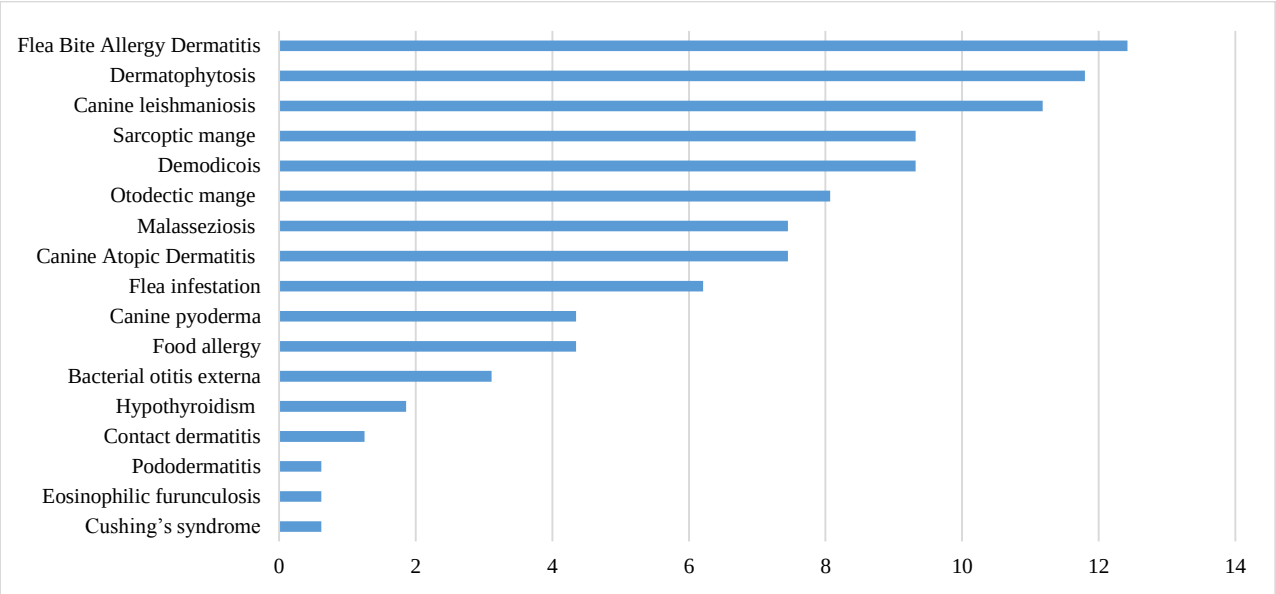


Figure 1. Percentage of dermatoses diagnosed in dogs located in Rabat, Morocco during 2020-2022

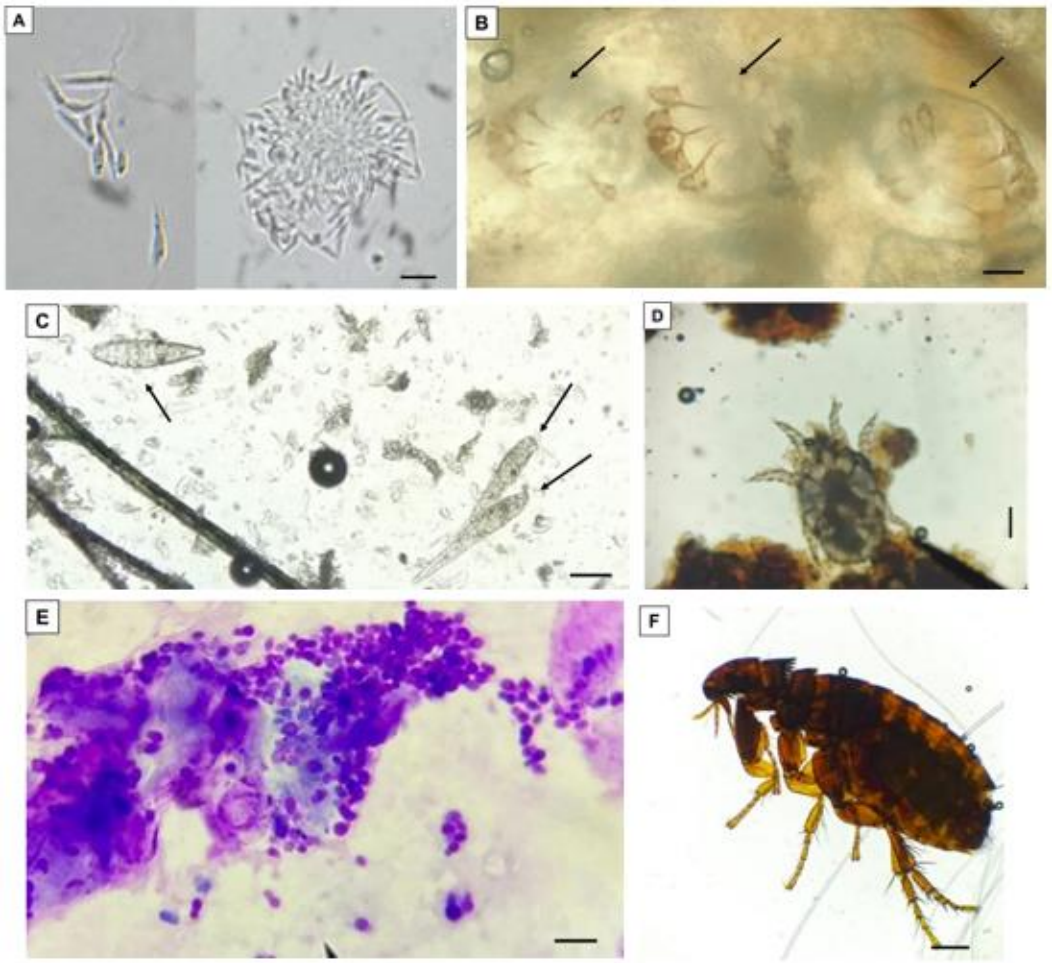


Figure 2. Etiological agents causing skin disorders in dogs located in Rabat, Morocco during 2020-2022. **A:** *Leishmania infantum* promastigotes isolated in Roswell Park Memorial Institute (RPMI) - 1640 culture medium after in vitro culture in the Novy-MacNeal-Nicolle (NNN) medium from a naturally infected English pointer and observed under an optical microscope (magnification 200x). Scale-bar: 10 µm, **B:** Adult mites of *Sarcoptes scabiei* (arrows) isolated from a common breed dog and morphologically identified under an optical microscope (magnification 400x). Scale-bar: 450 µm, **C**: Optical microscopy observation (magnification 400x) of adult mites of *Demodex canis* (arrows) in a Rottweiler puppy. Scale-bar: 150 µm, **D**: Adult mites of *Otodectes cynotis* examined in the earwax of a Chow-chow using an optical microscope (magnification 400x). Scale-bar: 250 µm, **E**: Cytological examination of the skin (by impression) in a Siberian Husky: The presence of numerous free yeasts of *Malassezia pachydermatis* (magnification 1000x) adhering to keratinocytes (rapid staining). Scale-bar: 20 µm, and **F**: Microscopic examination of *Ctenocephalides felis* collected in a German Shepherd after mounting between slide and coverslip through an optical microscope (magnification 400x). Scale-bar: 1mm

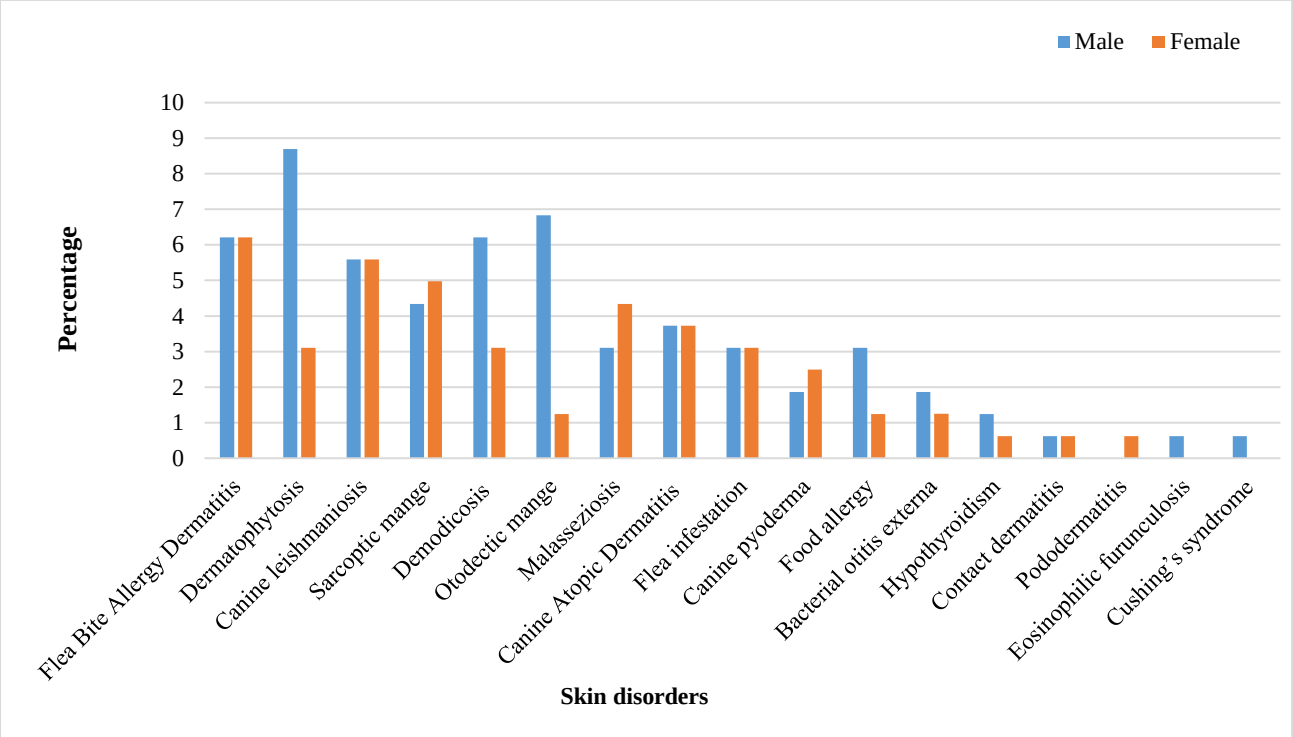


Figure 3. Sex-wise percentage of skin disorders in dogs located in Rabat, Morocco during 2020-2022

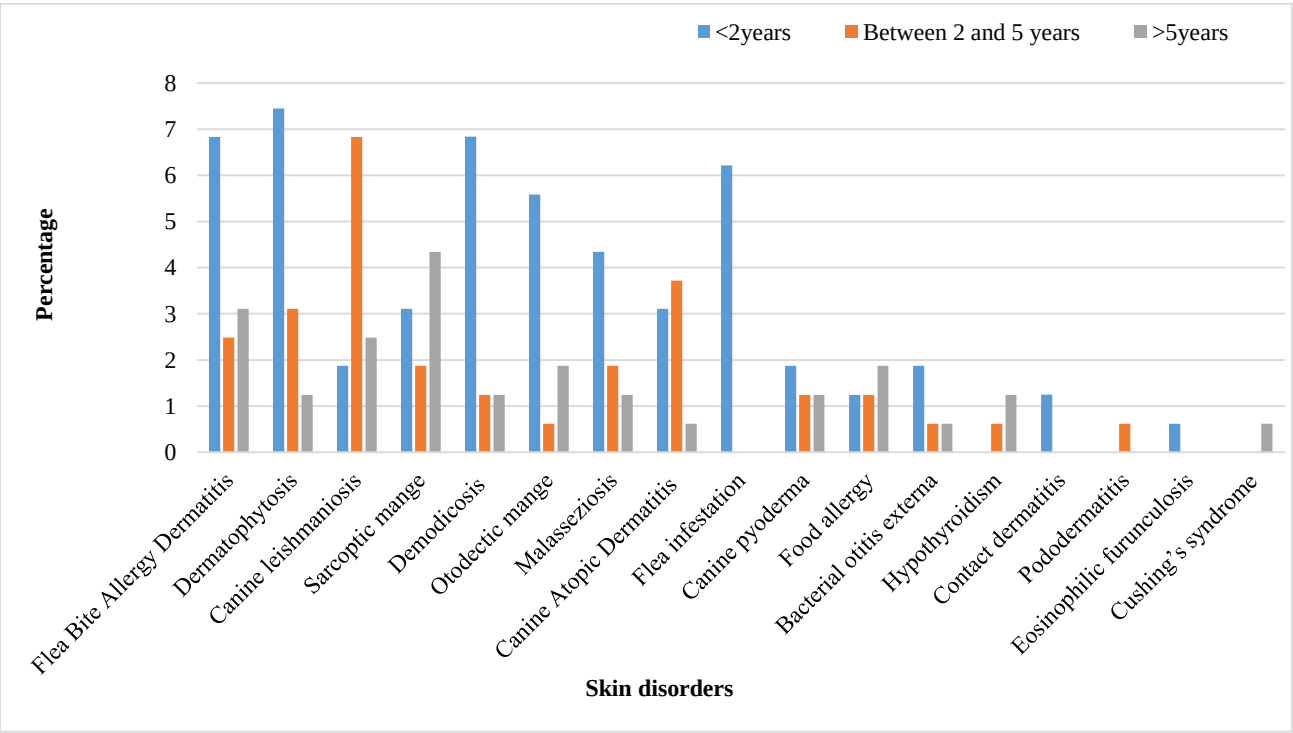


Figure 4. Age-wise percentage of dermatological disorders in dogs located in Rabat, Morocco during 2020-2022

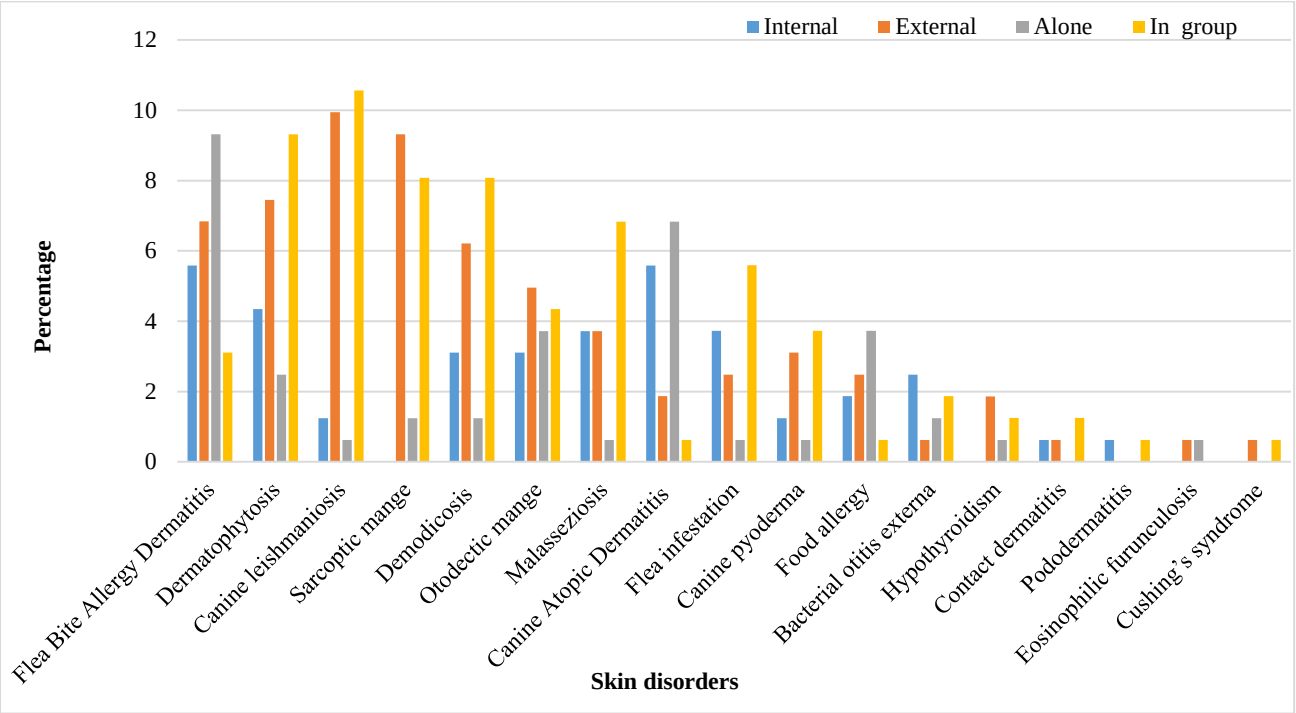


Figure 5. Living environment and lifestyle-wises percentage of dermatological disorders in dogs located in Rabat, Morocco during 2020-2022

DISCUSSION

According to the clinical study, 161 dermatoses in 125 dogs were assembled, since several dogs were affected by more than one skin disorder at the same time, this is the case for the mongrel dogs that presented simultaneously parasitic dermatoses (sarcoptic and *otodectic* mange) and fungal skin infections (dermatophytosis). Dermatology evaluations made up 8.00% of the total examinations conducted at the UVTH of the IAV Hassan II, a figure slightly below the 12.06% reported in a study by Thapa and Sarkar (2018). In other studies, the prevalence of skin disorders has been estimated between 17.00% and 27.26% (Khoshnegah et al., 2013; Khurana et al., 2016; Kumar and Shekhar, 2020). These percentage differences were due to the large number of dogs received at the UVTH of the IAV Hassan II for vaccination that were taken into account in the present study, which was not the case in previous studies.

Parasitic dermatoses represent the most frequent dermatological disorders with a percentage of 44.10%, which is in agreement with the study of Khurana et al. (2016), with a marked predominance of canine leishmaniosis, sarcoptic mange, and demodicosis. In the current study, 18 cases (11.18%) of canine leishmaniosis caused by *L. infantum* were diagnosed, and the same percentage was reported in Iran (Khoshnegah et al., 2013).

Veterinarians in Morocco struggle with the accurate diagnosis of canine leishmaniasis, primarily due to the expensive supplementary tests required to confirm the presence of the disease. Eight cases were from Rabat (the capital of Morocco and Rabat-Salé-Kénitra region) and almost all of them never left the city, four cases were from the rural commune of Sidi-Yahya-Zaër (Rabat-Salé-Kénitra region), two cases were from the city of Mehdia (Rabat-Salé-Kénitra region), and the remaining four cases were from the cities of Sala Al Jadida (Rabat-Salé-Kénitra region), Ain El-Aouda (Rabat-Salé-Kénitra region), Benslimane (Casablanca-Settat region), and Casablanca (Casablanca-Settat region). It can be assumed that there has been a change in the epidemiology profile of leishmaniosis by *L. infantum*, which is generally confined to pre-arid, semi-arid, humid, and sub-humid bioclimatic stages (Hakkour et al., 2019). Additionally, the possible urbanization of canine leishmaniosis should be seen as a wake-up call, as visceral leishmaniasis caused by *L. infantum* is a major public health problem due to its zoonotic aspect, with dogs as the main reservoir of the disease (Saridomichelakis, 2009). In light of the above results, mongrel dogs were more exposed to canine leishmaniosis than purebred dogs. Knowing that racial predisposition has been confirmed for some breeds such as German Shepherds and Rottweiler (Saridomichelakis, 2009). However, it is generally accepted that mongrel and autochthonous breeds are resistant to parasite exposure, even if they live in endemic areas (Solano-Gallego et al., 2000).

It should be noted that the age of affected animals varies from one to seven years, and based on the knowledge of Briffod (2011), it seems that dogs under three years old with those over eight years old are the most susceptible to developing the disease characterized by a long incubation period of up to four years (Müller et al., 2022). In terms of

gender, there were nine male cases and nine female cases. However, male dogs are typically favored over females for hunting and guarding, which could increase their risk of being bitten by sandflies (Rombolà et al., 2021).

Regarding the living environment and lifestyle, there were epidemiological and statistical associations between the above-mentioned risk factors and positive cases who lived outside and did not follow a regular program of prevention against external parasites, which is an important risk factor making the dogs exposed to sandflies and promoting infection (Ribeiro et al., 2018).

Regarding scabies, the reported percentage in other studies (2.82% in India and 5.06% in Iran) is lower than that found in the present study (9.32%; Khoshnegah et al., 2013; Anikar et al., 2021). In the current study, it was noticed that adult dogs are more predisposed to be affected by this dermatosis compared to young dogs although, it is observed in dogs of all ages and it is well-known in dogs under one year old (Chen et al., 2014). Only a single owner was impacted by sarcoptic mange, displaying scabies pimples on her thigh and feet. These observations are in line with Bandi and Saikumar's (2013); observations, which confirm that sarcoptic mange is a pruritic and highly contagious dermatosis in animals and humans.

For demodicosis, the results of the clinical study revealed a percentage of 9.32%, which is in agreement with Khurana et al. (2016), where it ranked second after sarcoptic mange. In addition, the study of Hill et al. (2006) identified very few cases of canine demodicosis, which presented less than 1% of dermatology examinations. Similarly, cases of demodicosis remain very limited (five cases (0.94%)) in the study of Khoshnegah et al. (2013) while the number of cases of demodicosis in the study of Shchelkanov et al. (2020) was around 409 cases (15.90%). Common breed dogs showed significant susceptibility to this skin disorder, although racial predisposition is not a risk factor to be taken into consideration (Shchelkanov et al., 2020).

Dogs living in groups were more exposed to demodectic mange than those living alone. In this regard, the overpopulation of *Demodex canis*, may be linked mainly to puppy nursing, along with other stress factors such as estrus, parturition, and endoparasites (Gortel, 2006). Juvenile demodicosis was the most common in the present study. It could be due to the immune deficiency against *D. canis* in the assessed cases, which conforms with Hugnet et al. (2001). For otodectic mange (8.07%), the most affected cases were young dogs, which is mainly due to their low immunity (Taenzler et al., 2017). Males were more exposed to infestation by *Otodectes cynotis* than females, which is capable of leaving the ear canal and spreading to other parts of the body (Sotiraki et al., 2001). However, Rodriguez-vivas et al. (2003), reported that both sexes have equal sensitivity to the mites responsible for the disease, and there is no characteristic sexual preference. It was also noted that the majority of affected dogs lived with cats who often go outdoors, which may be a factor favoring transmission. This agrees with the findings of Souza et al. (2008), which confirmed that dogs living outdoors were more exposed to *O. cynotis* infestation, and direct contact with infected animals remains the most common mode of transmission for these mites.

Finally, flea infestation accompanied or not by FBAD, is classified as the fifth dominant parasitic dermatosis in the current clinical study (6.21%), which is in agreement with Khoshnegah et al. (2013), suggesting that owners are not sufficiently aware of the need to improve hygiene conditions, particularly environmental conditions as well as the means of prevention that can reduce its frequency. It remains to be pointed out that cat fleas are the most identified under microscopy, which is reported by a previous study confirming that *Ctenocephalides felis* is the most widespread flea in dogs (Linardi and Santos, 2012). Therefore, it can be inferred that the cohabitation of dogs with cats increases the risk of flea exposure, as cats with an external lifestyle are rarely treated.

Allergic dermatoses occupied the second position (25.47%) in the current study. Flea bite allergy dermatitis (FBAD, 12.42%) and canine allergy dermatitis (CAD, 7.45%) represent the most common allergic dermatoses encountered in the present study also by several studies (Hill et al., 2006; Dávila et al., 2018; Drechsler et al., 2024). In contrast with these findings, few cases of these two dermatoses have been diagnosed by Lund et al. (1999). For flea bite allergy dermatitis (FBAD), intense pruritus was the most common clinical sign in all cases examined. This agrees with the data reported by Bruet et al. (2012), mentioning that this pruritus is essentially located in the dorso-lumbar region, accompanied by chewing, and characterized by high intensity and frequency. No breed predispositions or those linked to age, sex, or living environment have been statistically or epidemiologically proven in the case of this dermatosis. However, it was found that dogs living alone were more exposed to the risk of this skin disorder. For canine allergy dermatitis (CAD), despite the absence of an association between this dermatosis and the breeds affected in this study, the risk of exposure remains very high in West Highland white terriers (Jaeger et al., 2010). Young dogs were significantly more susceptible to this dermatosis than adults. This data agrees with the findings of previous studies (Griffin and DeBoer, 2001; Hensel et al, 2015). Similarly, predilections have been observed for the living environment and lifestyle of affected dogs in the present survey, which leads us to believe that house dust mites are incriminated in the pathogenesis of CAD in this case, especially, *Dermatophagoides farina* (Bensignor and Carlotti, 2002). Regarding food allergy, the percentage obtained in this study (4.35%) was lower than those reported in studies carried out in France (6.00%) and the UK (7.60%) by

Carlotti et al. (1990) and Chesney (2002) respectively. At the same time, it was higher compared to other studies, which estimated that food hypersensitivity accounted for 1.70% of all diagnosed canine dermatoses (Wilhelm and Favrot, 2005). In agreement with Carlotti et al. (1990), no breed, sex, age, or living environment predispositions were demonstrated for this dermatosis in the present study. On the contrary, young age seems like a risk factor for this skin disease (Rosser, 1993). Similarly, Labradors present a non-negligible risk for this dermatosis (Chesney, 2002). Furthermore, it was shown in the present study that dogs living alone were more exposed than those living in groups. Contact dermatitis remains very rare in this study (2/125; 1.25%), representing 1.89% and 0.86% of all allergic dermatoses in previous studies carried out in Iran and UK respectively (Hill et al., 2006; Khoshnegah et al., 2013). No risk factor studied could be linked to this dermatosis, due to the absence of a significant number of cases. However, Olivry et al. (1990) confirmed that Danish German Shepherd dogs are more likely to be affected than other breeds without predisposition to sex and age. The acute onset of lesions is more suggestive of an irritant contact dermatitis, which is observed in the first case (scrotal dermatitis representing an irritant reaction). On the other hand, according to Olivry et al. (1990), a chronic evolution after a long period of exposure is compatible with contact dermatitis, which is noticed in the second case.

Superficial cutaneous mycoses represented 19.25% of the dermatoses diagnosed during the current clinical survey. This finding differs from those of other studies (Hill et al., 2006; Khoshnegah et al., 2013). The percentage of dermatophytosis (11.80%) obtained in the present study was lower than that mentioned by other documented studies in Italy and Iraq (Cafarchia et al., 2004; Jarjees and Issa, 2022). It may be explained by the climate, which is a predisposing factor, and the prevalence of dermatophytes has recently seen a marked increase, especially in warmer regions (Moriello et al., 2017). Indeed, Morocco has recently experienced significant climatic changes, which could contribute to the creation of favorable conditions for the development of spores, constituting the main infectious form of dermatophytosis. It has to be emphasized that dogs and cats are the main reservoirs for zoophilic species such as *Microsporum canis*, ensuring their spread to humans (Cafarchia et al., 2004). The majority of the cases assessed showed a greenish fluorescence under the Wood's lamp, which confirms that the infections concerned are those due to *M. canis* caused by contact with an infected animal, notably cats. However, non-fluorescent lesions could be associated with either, infections related to strains of *M. canis* which do not fluoresce under the Wood's lamp and *Microsporum gypseum* that is caused by contact with contaminated soil, which can also infect humans (Tobeigei et al., 2023. In the current study, young male dogs were more predisposed to dermatophyte infection. These findings are in line with those documented by Jarjees and Issa (2022), who have reported that infection is favored by the immune deficiency associated with young age, and the particular composition of sebum in males, which increases the risk of exposure. Interestingly, Cafarchia et al. (2004) reported a high prevalence of dermatophytosis due to *M. canis*, statistically proven in Yorkshire terriers compared to other breeds, and linked to differences in skin defenses. For Malasseziosis, unlike other studies (Crespo et al., 2002; Berlanda et al., 2022), the percentage of *Malassezia* yeasts was too low (7.45%) in the present study. *Malassezia pachydermatis* is the most frequent species associated with dogs and is responsible for *Malassezia* dermatitis and otitis externa, when there is the proliferation of these commensal organisms acting as opportunistic pathogens (Guillot and Bond, 2020). As far as clinical diagnosis, it is well-recognized that it is based on the presence of pruritus accompanied by skin lesions (Bond, 2010). The results of this survey, as well as those of another similar study, confirmed the absence of any correlation between the sex, and age of animals affected by *M. pachydermatis* (Crespo et al., 2002). Although, it seems that breeds characterized by skin folds are more predisposed to *Malassezia* overgrowth (Bond et al., 2020).

Regarding bacterial skin infections, the overall percentage of these dermatoses in the present study was significantly lower (8.70%) compared to those reported in Canada, Iran, and the UK (Scott and Paradis, 1990; Hill et al., 2006; Khoshnegah et al., 2013). It is also worth noting that no association has been proven in the present study between bacterial dermatoses and risk factors likely to cause it. It may be explained by the limited number of cases received during the study period. Canine pyoderma was the most common, and the causative agent of this skin condition most frequently isolated from dogs is *Staphylococcus pseudintermedius*. Bacterial otitis externa ranked second after canine pyoderma. According to Korbelik et al. (2019) and Nuttal (2023), Otitis externa is a disease characterized by a high prevalence (up to 20.00%) and it's a recurrent affection that's harder to manage in dogs, caused by a combination of autoimmune, endocrine and parasitic disorders. Only one case of pododermatitis has been encountered in a three-year-old English pointer dog living outdoors, with a foreign body (spikelet). Breathnach et al. (2005), confirmed that the causes of pododermatitis in dogs are multiple, and are mainly due to trauma and reactions to foreign bodies. Similarly, only one case of eosinophilic furunculosis was diagnosed in the current study in a one-year-old pit bull who still frequents the outpatient setting. This is an uncommon skin disorder that affects young dogs living outdoors and develops characteristic lesions (ulcers and pustules) on the face, trunk, and legs (Curtis et al., 1995).

Finally, three cases of hypothyroidism were diagnosed, which is the most common endocrinopathy with skin manifestations in dogs (Ferguson, 1994). These dogs are aged over six years, which is accurately reported by O'Neill et

al. (2022), who confirm that hypothyroidism is an endocrine disorder of adult dogs, and the average age at diagnosis is seven years (Scott-Moncrieff, 2007). The cases concerned were two males and one spayed female who has an increased risk of developing this endocrinopathy compared to sexually intact animals according to Scott-Moncrieff (2007). In the present study, a single case of iatrogenic Cushing's syndrome was diagnosed in a six-year-old dachshund. The anamnesis revealed that this dog has atopic dermatitis, and has been treated with corticosteroid drugs for years. Indeed, long-term administration of corticosteroid drugs is one of the main causes of Cushing's syndrome according to Bennaim et al. (2019).

CONCLUSION

In summary, the overall prevalence of skin disorders was 8.00%. The results of the present study highlighted the predominance of FBAD (12.42%), followed by dermatophytosis (11.80%), and canine leishmaniosis (11.18%) in dogs examined at the Parasitology-Dermatology clinic. There was an apparent breed, sex, age, living environment, and lifestyle predilections for skin diseases diagnosed herein. The zoonotic aspect of these dermatoses requires a high level of awareness from veterinarians and owners, who are involved in control and prevention strategies. In light of these data, it is recommended to conduct further studies over a more extended period, covering various Moroccan regions, to assess the prevalence of large-scale skin disorders and identify the associated risk factors.

DECLARATIONS

Authors' contributions

Abderrahmane Zahri designed and drafted the manuscript. Mohammed Bouslikhane contributed to the design and validation of the statistical analysis. Meryem Lemrani and Sara El Mazini confirmed canine leishmaniosis by k-DNA nested PCR. Ikhlass El Berbri provided advice throughout the study. Mohammed Amin Abouelkaram helped to examine the cases received at the Parasitology-Dermatology clinic. Thomas Balenghien reviewed and edited the manuscript. Maria Bourquia supervised the study from the outset, providing valuable comments and suggestions to improve the quality of the manuscript. The final draft of the manuscript was approved by all authors before submission.

Acknowledgments

The authors thank Saad El Airbati (Cotts Equine Hospital, United Kingdom) and Andreia Wendt (University of Bern, Switzerland) for their help in revising the manuscript.

Availability of data and materials

All data related to this study are available upon request to the corresponding author.

Funding

This study was not supported by any grant.

Competing interests

No conflicts of interest are to be declared by the authors of this study.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time.

REFERENCES

Alizadeh A, Sadr S, Azizzadeh M, and Khoshnegah J (2024). Feline dermatoses at Ferdowsi university of Mashhad (Iran): 154 cases (2009-2020). Veterinary Dermatology, 35(4): 450-452. DOI: https://www.doi.org/10.1111/vde.13244

Anikar MJ, Bhadesiya CM, Chaudhary GR, Patel TP, Patil DB, Dadawala AI, and Makwana PP (2021). Incidence of dermatological disorders in dogs at Leo Animal and Bird Clinic, Vastral, Ahmedabad (Gujarat). International Journal of Advanced Research in Biological Sciences, 8(3): 1-7. Available at: https://www.ijarbs.com/pdfcopy/2021/mar2021/ijarbs1.pdf

Bandi KM and Saikumar C (2013). Sarcoptic mange: A zoonotic ectoparasitic skin disease. Journal of Clinical and Diagnostic Research, 7(1): 156-157. DOI: https://www.doi.org/10.7860/JCDR/2012/4839.2694

Beco L, Guaguère E, Méndez CL, Noli C, Nuttall T, and Vroom M (2013). Suggested guidelines for using systemic antimicrobials in bacterial skin infections (1): Diagnosis based on clinical presentation, cytology and culture. Veterinary Record, 172(3): 72-78. DOI: https://www.doi.org/10.1136/vr.101069

Berlanda M, Valente C, Guglielmini C, Danesi P, Contiero B, and Poser H (2022). *Malassezia* overgrowth in dogs in northern Italy: Frequency, body distribution, clinical signs and effects of pharmacologic treatments. Veterinaria Italiana, 58(1): 103-109. DOI: https://www.doi.org/10.12834/VetIt.2124.12936.1

Bennaim M, Shiel RE, and Mooney CT (2019). Diagnosis of spontaneous hyperadrenocorticism in dogs. Part 1: Pathophysiology, aetiology, clinical and clinicopathological features. Veterinary Journal, 252: 105342. DOI: https://www.doi.org/10.1016/j.tvjl.2019.105342

Bensignor E and Carlotti DN (2002). Sensitivity patterns to house dust mites and forage mites in atopic dogs: 150 Cases. Veterinary Dermatology, 13(1): 37-42. DOI: https://www.doi.org/10.1046/j.0959-4493.2001.00270.x

Bensignor E, Marignac Crosaz O, and Cavana P (2013). Pruritus in dogs. Veterinary Dermatology, 24(2): 292. DOI: https://www.doi.org/10.1111/vde.12005

Bond R (2010). Superficial veterinary mycoses. Clinics in Dermatology, 28(2): 226-236. DOI: https://www.doi.org/10.1016/j.clindermatol.2009.12.012

Bond R, Morris DO, Guillot J, Bensignor EJ, Robson D, Masson KV, Kano R, and Hill PB (2020). Biology, diagnosis and treatment of *Malassezia* dermatitis in dogs and cats: Clinical consensus guidelines of the world association for veterinary dermatology. Veterinary Dermatology, 31(1): 73-77. DOI: https://www.doi.org/10.1111/vde.12834

Breathnach RM, Baker KP, Quinn PJ, McGeady TA, Aherne CM, and Jones BR (2005). Clinical, immunological and histopathological findings in a subpopulation of dogs with pododermatitis. Veterinary Dermatology, 16(6): 364-372. DOI: https://www.doi.org/10.1111/j.1365-3164.2005.00471.x

Briffod C (2011). Revue actuelle en matière de leihmaniose canine [Current review of canine leishmaniosis]. Veterinary medicine thesis, Toulouse Paul-Sabatier University, Toulouse, France. pp. 50-51. Available at: https://oatao.univ-toulouse.fr/4968/1/briffod_4968.pdf

Bruet V, Bourdeau PJ, Roussel A, Imparato L, and Desfontis JC (2012). Characterization of pruritus in canine atopic dermatitis, flea bite hypersensitivity and flea infestation and its role in diagnosis. Veterinary Dermatology, 23(6): 487-493. DOI: https://www.doi.org/10.1111/j.1365-3164.2012.01092.x

Brue V, Mosca M, Briand A, Bourdeau P, Pin D, Cochet-Faivre N, and Cadiergues MC (2022). Clinical guidelines for the use of antipruritic drugs in the control of the most frequent pruritic skin diseases in dogs. Veterinary Sciences, 9(4): 149. DOI: https://www.doi.org/10.3390/vetsci9040149

Cafarchia C, Romito D, Sasanelli M, Lia R, Capelli G, and Otranto D (2004). The epidemiology of canine and feline dermatophytoses in southern Italy. Mycoses, 47(11-12): 508-513. DOI: https://www.doi.org/10.1111/j.1439-0507.2004.01055.x

Carlotti DN, Remy I, and Prost C (1990). Food allergy in dogs and cats. A review and report of 43 cases. Veterinary Dermatology, 1(2): 55-62. DOI: https://www.doi.org/10.1111/j.1365-3164.1990.tb00080.x

Chen YZ, Liu GH, Song HQ, Lin RQ, Weng YB, and Zhu XQ (2014). Prevalence of *Sarcoptes scabiei* infection in pet dogs in southern China. ScientificWorldJournal, 2014(1-3): 718590. DOI: https://www.doi.org/10.1155/2014/718590

Chesney CJ (2002). Food sensitivity in the dog: A quantitative study. Journal of Small Animal Practice, 43(5): 203-207. DOI: https://www.doi.org/10.1111/j.1748-5827.2002.tb00058.x

Crespo MJ, Abarca ML, and Cabañes FJ (2002). Occurrence of *Malassezia* spp. in the external ear canals of dogs and cats with and without otitis externa. Medical Mycology, 40(2): 115-121. DOI: https://www.doi.org/10.1080/mmy.40.2.115.121

Curtis CF, Bond R, Blunden AS, Thomson DG, McNeil PE, and Whitbread TW (1995). Canine eosinophilic folliculitis and furunculosis in three cases. Journal of Small Animal Practice, 36(3): 119-123. DOI: https://www.doi.org/10.1111/j.1748-5827.1995.tb02850.x

Curtis CF (2012). Canine sarcoptic mange (sarcoptic acariasis, canine scabies). Companion Animal, 17(8): 32-36. DOI: https://www.doi.org/10.1111/j.2044-3862.2012.00222.x

Dávila I, Domínguez-Ortega J, Navarro-Pulido A, Alonso A, Antolín-Amerigo D, González-Mancebo E, Martín-García C, Núñez-Acevedo B, Prior N et al. (2018). Consensus document on dog and cat allergy. Allergy, 73(6): 1206-1222. DOI: https://www.doi.org/10.1111/all.13391

de Souza CF, Silva VL, and Labarthe N (2019). Evaluation of DPP® and SNAP® Rapid Tests for diagnosis of *Leishmania infantum* canine infections. Revista da Sociedade Brasileira de Medicina Tropical, 52: e20190154. DOI: https://www.doi.org/10.1590/0037-8682-0154-2019

Drechsler Y, Dong C, Clark DE, and Kaur G (2024). Canine atopic dermatitis: Prevalence, impact, and management strategies. Veterinary Medicine: Research and Reports, 15: 15-29. DOI: https://www.doi.org/10.2147/VMRR.S412570

Ferguson DC (1994). Update on diagnosis of canine hypothyroidism. Veterinary Clinics of North America: Small Animal Practice, 24(3): 515-539. DOI: https://www.doi.org/10.1016/S0195-5616(94)50057-3

GlobalPETS (2023). The pet industry in Morocco. Available at: https://globalpetindustry.com/article/pet-industry-morocco#:~:text=For%202023%2C%20the%20pet%20population,%25)%20and%20dogs%20(37%25)

Gortel K (2006). Update on canine demodicosis. Veterinary Clinics of North America: Small Animal Practice, 36(1): 229-241. DOI: https://www.doi.org/10.1016/j.cvsm.2005.09.003

Gortel K (2013). Recognizing pyoderma: More difficult than it may seem. Veterinary Clinics of North America: Small Animal Practice, 43(1): 1-18. DOI: https://www.doi.org/10.1016/j.cvsm.2012.09.004

Griffin CE and DeBoer DJ (2001). The ACVD task force on canine atopic dermatitis (XIV): Clinical manifestations of canine atopic dermatitis. Veterinary Immunology and Immunopathology, 81(3-4): 255-269. DOI: https://www.doi.org/10.1016/S0165-2427(01)00346-4

Guillot J and Bond R (2020). *Malassezia* yeasts in veterinary dermatology: An updated overview. Frontiers in Cellular and Infection Microbiology, 10: 79. DOI: https://www.doi.org/10.3389/fcimb.2020.00079

Hakkour M, El Alem MM, Hmamouch A, Delouane B, Fellah H, Faiza S, Rhalem A, Habbari K, and Sadak A (2019). Leishmaniasis in Northern Morocco: Predominance of *Leishmania infantum* compared *to Leishmania tropica*. BioMed Research International, 2019(506): 1-14. DOI: https://www.doi.org/10.1155/2019/5327287

Hensel P, Santoro D, Favrot C, Hill P, and Griffin C (2015). Canine atopic dermatitis: Detailed guidelines for diagnosis and allergen identification. BMC Veterinary Research, 11: 196. DOI: https://www.doi.org/10.1186/s12917-015-0515-5

Hill PB, Lo A, Eden CAN, Huntley S, Morey V, Ramsey S, Richardson C, Smith DJ, Sutton C, Taylor MD et al. (2006). Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice. Veterinary Record, 158(16): 533-539. DOI: https://www.doi.org/10.1136/vr.158.16.533

Hillier A, Lloyd DH, Weese JS, Blondeau JM, Boothe D, Breitschwerdt E, Guardabassi L, Papich MG, Rankin S, Turnidge JD et al. (2014). Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial guidelines working group of the

international society for companion animal infectious diseases). Veterinary Dermatology, 25(3): 163-e43. DOI: https://www.doi.org/10.1111/vde.12118

Hsu YC and Fuchs E (2022). Building and maintaining the skin. Cold Spring Harbor Perspectives in Biology, 14(7): 1-32. DOI: https://www.doi.org/10.1101/cshperspect.a040840

Hugnet C, Bruchon-Hugnet C, Royer H, and Bourdoiseau G (2001). Efficacy of 1.25% amitraz solution in the treatment of generalized demodicosis (eight cases) and sarcoptic mange (five cases) in dogs. Veterinary Dermatology, 12(2): 89-92. DOI: https://www.doi.org/10.1046/j.1365-3164.2001.00231.x

Jaeger K, Linek M, Power HT, Bettenay SV, Zabel S, Rosychuk RAW, and Mueller RS (2010). Breed and site predispositions of dogs with atopic dermatitis: A comparison of five locations in three continents. Veterinary Dermatology, 21(1): 118-122. DOI: https://www.doi.org/10.1111/j.1365-3164.2009.00845.x

Jarjees KI and Issa NA (2022). First study on molecular epidemiology of dermatophytosis in cats, dogs, and their companions in the Kurdistan region of Iraq. Veterinary World, 15(12): 2971-2978. DOI: https://www.doi.org/10.14202/vetworld.2022.2971-2978

Joshi M (2022). Dermatological disorders in dogs in Udaipur district and their nutritional management. Indian Journal of Canine Practice, 14(2): 161-162. Available at: http://www.indianjournalofcaninepractice.com/december2022/IJCP-December-2022-Vol-14-iss-2-pp161-162.pdf

Khoshnegah J, Movassaghi AR, and Rad M (2013). Survey of dermatological conditions in a population of domestic dogs in Mashhad, northeast of Iran (2007-2011). Veterinary Research Forum, 4(2): 99-103. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4313009/

Khurana R, Kumar T, Agnihotri D, and Sindhu N (2016). Dermatological disorders in canines- A detailed epidemiological study. Haryana Veterinarian, 55(1): 97-99. Available at: https://www.luvas.edu.in/haryana-veterinarian/download/harvet2016/24.pdf

Korbelik J, Singh A, Rousseau J, and Weese JS (2019). Characterization of the otic bacterial microbiota in dogs with otitis externa compared to healthy individuals. Veterinary Dermatology, 30(3): 228-270. DOI: https://www.doi.org/10.1111/vde.12734

Kumar P and Shekhar S (2020). Occurrence of dermatological disorders and haemato-biochemical alteration, treatment of demodicosis in dogs. Journal of Entomology and Zoology Studies, 8(2): 1256-132. Available at: https://www.entomoljournal.com/archives/2020/vol8issue2S/PartD/S-8-2-33-765.pdf

Linardi PM and Santos JLC (2012). *Ctenocephalides felis felis* vs. *Ctenocephalides canis* (Siphonaptera: Pulicidae): Some issues in correctly identify these species. Revista Brasileira de Parasitologia Veterinária, 21(4): 345-354. DOI: https://www.doi.org/10.1590/S1984-29612012000400002

Lund EM, Armstrong PJ, Kirk CA, Kolar LM, and Klausner JS (1999). Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. Journal of the American Veterinary Medical Association, 214(9): 1336-1341. DOI: https://www.doi.org/10.2460/javma.1999.214.09.1336

Miller J, Simpson A, Bloom P, Diesel A, Friedeck A, Paterson T, Wisecup M, and Yu CH (2023). 2023 AAHA management of allergic skin diseases in dogs and cats guidelines. Journal of the American Animal Hospital Association, 59(6): 255-284. DOI: https://www.doi.org/10.5326/JAAHA-MS-7396

Moriello KA, Coyner K, Paterson S, and Mignon B (2017). Diagnosis and treatment of dermatophytosis in dogs and cats: Clinical consensus guidelines of the world association for veterinary dermatology. Veterinary Dermatology, 28(3): 266-268. DOI: https://www.doi.org/10.1111/vde.12440

Morocco's climate (2023). Caractéristiques climatiques générales du Maroc [Morocco's general climatic characteristics], pp. 1-33. Available at: https://www.marocmeteo.ma/sites/default/files/climat_report/pdfs/Maroc_Etat_Climat_2022.pdf

Mueller RS, Rosenkrantz W, Bensignor E, Karaś-Tęcza J, Paterson T, and Shipstone MA (2020). Diagnosis and treatment of demodicosis in dogs and cats: Clinical consensus guidelines of the world association for veterinary dermatology. Veterinary Dermatology, 31(1): 5-27. DOI: https://www.doi.org/10.1128/JCM.36.10.2877-2881.1998

Müller A, Montoya A, Escacena C, de la Cruz M, Junco A, Iriso A, Marino E, Fúster F, and Miró G (2022). *Leishmania infantum* infection serosurveillance in stray dogs inhabiting the Madrid community: 2007-2018. Parasites and Vectors, 15(1): 96. DOI: https://www.doi.org/10.1186/s13071-022-05226-6

Noyes HA, Reyburn H, Bailey JW, and Smith D (1998). A nested-PCR-based Schizodeme method for identifying *Leishmania* kinetoplast minicircle classes directly from clinical samples and its application to the study of the epidemiology of *Leishmania tropica* in Pakistan. Journal of Clinical Microbiology, 36(10): 2877-2881. DOI: https://www.doi.org/10.1128/JCM.36.10.2877-2881.1998

Nuttall T (2023). Managing recurrent otitis externa in dogs: What have we learned and what can we do better?. Journal of the American Veterinary Medical Association, 261(S1): S10-S22. DOI: https://www.doi.org/10.2460/javma.23.01.0002

Olivry T, Prélaud P, Héripret D, and Atlee BA (1990). Allergic contact dermatitis in the dog: Principles and diagnosis. Veterinary Clinics of North America: Small Animal Practice, 20(6): 1443-1456. DOI: https://www.doi.org/10.1016/S0195-5616(90)50154-0

Ormerod EJ, Edney ATB, Foster SJ, and Whyham MC (2005). Therapeutic applications of the human-companion animal bond. Veterinary Record, 157(22): 689-691. DOI: https://www.doi.org/10.1136/vr.157.22.689

O'Neill DG, Khoo JSP, Brodbelt DC, Church DB, Pegram C, and Geddes RF (2022). Frequency, breed predispositions and other demographic risk factors for diagnosis of hypothyroidism in dogs under primary veterinary care in the UK. Canine Medicine and Genetics, 9(1): 11. DOI: https://www.doi.org/10.1186/s40575-022-00123-8

Ribeiro RR, Michalik MSM, Da Silva ME, Dos Santos CCP, Frézard FJG, and Da Silva SM (2018). Canine leishmaniasis: An overview of the current status and strategies for control. BioMed Research International, 2018(6): 1-12. DOI: https://www.doi.org/10.1155/2018/3296893

Rodriguez-Vivas RI, Ortega-Pacheco A, Rosado-Aguilar JA, and Bolio GME (2003). Factors affecting the prevalence of mange-mite infestations in stray dogs of Yucatán, Mexico. Veterinary Parasitology, 115(1): 61-65. DOI: https://www.doi.org/10.1016/S0304-4017(03)00189-4

Rombolà P, Barlozzari G, Carvelli A, Scarpulla M, Iacoponi F, and Macrì G (2021). Seroprevalence and risk factors associated with exposure to Leishmania infantum in dogs, in an endemic Mediterranean region. PLoS One, 16(1): e0244923. DOI: https://www.doi.org/10.1371/journal.pone.0244923

Rosser Jr EJ (1993). Diagnosis of food allergy in dogs. Journal of the American Veterinary Medical Association, 203(2): 259-262. DOI: https://www.doi.org/10.2460/javma.1993.203.02.259

Saridomichelakis MN (2009). Advances in the pathogenesis of canine leishmaniosis: Epidemiologic and diagnostic implications. Veterinary Dermatology, 20(5-6): 471-489. DOI: https://www.doi.org/10.1111/j.1365-3164.2009.00823.x

Scott DW and Paradis M (1990). A survey of canine and feline skin disorders seen in a university practice: Small animal clinic, University of Montréal, Saint-Hyacinthe, Québec (1987-1988). Canadian Veterinary Journal, 31(12): 830-835. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1480900/

Scott-Moncrieff JC (2007). Clinical signs and concurrent diseases of hypothyroidism in dogs and cats. Veterinary Clinics of North America: Small Animal Practice, 37(4): 709-722. DOI: https://www.doi.org/10.1016/j.cvsm.2007.03.003

Shchelkanov MY, Moskvina TVT, Kim EM, Derunov DA, and Galkina IV (2020). The prevalence and risk factors of canine demodicosis: A retrospective long-term study of 409 cases. Tropical Biomedicine, 37(3): 778-782. DOI: https://www.doi.org/10.47665/tb.37.3.778

Sieber-Ruckstuh NS, Tham WK, Baumgartner F, Selva JJ, Markus R Wenk MR, Burla B, and Boretti FS (2022). Serum lipidome signatures of dogs with different endocrinopathies associated with hyperlipidemia. Metabolites, 12(4): 306. DOI: https://www.doi.org/10.3390/metabo12040306

Solano-Gallego L, Llull J, Ramos G, Riera C, Arboix M, Alberola J, and Ferrer L (2000). The Ibizian hound presents a predominantly cellular immune response against natural Leishmania infection. Veterinary Parasitology, 90(1-2): 37-45. DOI: https://www.doi.org/10.1016/S0304-4017(00)00223-5

Sotiraki ST, Koutinas AF, Leontides LS, Adamama-Moraitou KK, and Himonas CA (2001). Factors affecting the frequency of ear canal and face infestations by *Otodectes cynotis* in the cat. Veterinary Parasitology, 96(4): 309-315. DOI: https://www.doi.org/10.1016/S0304-4017(01)00383-1

Souza CP, Ramadinha RR, Scott FB, and Pereira MJS (2008). Factors associated with the prevalence of *Otodectes cynotis* in an ambulatory population of dogs. Pesquisa Veterinária Brasileira, 28(8): 375-378. DOI: https://www.doi.org/10.1590/S0100-736X2008000800005

Taenzler J, De Vos C, Roepke RKA, Frénais R, and Heckeroth AR (2017). Efficacy of fluralaner against *Otodectes cynotis* infestations in dogs and cats. Parasites and Vectors, 10(1): 30. DOI: https://www.doi.org/10.1186/s13071-016-1954-y

Tawfik MF, Oda SS, and Khafaga AF (2020). Pathological study of skin disorders in dogs and cats at Alexandria governorate, Egypt. Alexandria Journal of Veterinary Sciences, 65(1): 66-75. Available at: https://www.bibliomed.org/mnsfulltext/31/31-1584540185.pdf?1719592442

Thapa G and Sarkar S (2018). Occurrence of canine skin disorder and its haematobiochemical alterations. International Journal of Current Microbiology and Applied Sciences, 7(12): 184-195. Available at: https://www.ijcmas.com/7-12-2018/Geetanjali%20Thapa%20and%20Samar%20Sarkar.pdf

Tobeigei FH, Joseph MR, Al-Hakami A, and Hamid ME (2023). *Microsporum gypseum* infection among two related families with a zoonotic aspect: A prospective case series. Cureus, 15(12): e51402. DOI: https://www.doi.org/10.7759/cureus.51402

Wilhelm S and Favrot C (2005). Futtermittelhypersensitivitäts-Dermatitis beim Hund: Möglichkeiten der Diagnose [Food hypersensitivity dermatitis in the dog: diagnostic possibilities]. Schweizer Archiv für Tierheilkunde, 147(4): 165-171. DOI: https://www.doi.org/10.1024/0036-7281.147.4.165

Zanna G, Roccabianca P, Zini E, Legnani S, Scarampella F, Arrighi S, and Tosti A (2017). The usefulness of dermoscopy in canine pattern alopecia: A descriptive study. Veterinary Dermatology, 28(1): 161-e34. DOI: https://www.doi.org/10.1111/vde.12359

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 461-466.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj53
PII: S232245682400053-14

How to Design a Biomonitoring Study – A Practical Guide for Veterinary Professionals under a One Health Approach

Catarina Jota Baptista[1,2]*, Fernanda Seixas[3,4], José M. Gonzalo-Orden[5], and Paula A. Oliveira[2,3]

[1]*Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, Almada, Portugal*
[2]*Centro de Investigação das Tecnologias Agroambientais e Biológicas (CITAB/Inov4Agro), Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal*
[3]*Departamento de Ciências Veterinárias, Escola de Ciências Agrárias e Veterinárias (ECAV), UTAD, Vila Real, Portugal*
[4]*Animal and Veterinary Research Center (CECAV/Al4Animals), UTAD, Vila Real, Portugal*
[5]*Instituto de Biomedicina (IBIOMED), Universidad de León, León, España*

*Corresponding author's Email: catabap@hotmail.com

MINI REVIEW
Received: July 23, 2024
Revised: August 27, 2024
Accepted: September 09, 2024
Published: September 25, 2024

ABSTRACT

Currently, veterinarians can see their daily practice and medical tasks as constant opportunities for passive surveillance of One Health threats, such as infectious zoonotic diseases and chemical pollution effects on living beings. The present study aimed to provide a practical guide to designing a biomonitoring study during veterinary clinical practice without time-consuming procedures or significant costs. The constant access to several species' specimens provides the necessary samples to perform a biomonitoring study of environmental pollutants at the regional or national level. Generally, most health professionals know what to do (or where to find information) to report a disease outbreak. However, a summarized background to perform a biomonitoring study of a chemical hazard is missing. The authors of the current study provided a flow chart with the main steps to conduct a biomonitoring study in different fields of veterinary medicine. Thus, a biomonitoring study might give veterinarians (as other health professionals) a positive contribution to the clinical cases' resolution, while improving the general knowledge about the impact of environmental contamination on animals and human health.

Keywords: Contamination, Guideline, Monitoring, One Health, Pollution

INTRODUCTION

The One Health concept assumes that human health is deeply connected with animal and environmental health, as three main branches. Although it is not a new term, One Health principles have become more and more relevant and justified during the past few years due to several factors. These include the human population growth, the global movement of people, animals, and resources, the emergence and reemergence of new infectious diseases, natural habitat loss, and climate change, all affecting the three mentioned branches of One Health (Sleeman et al., 2019; Wilcox and Steele, 2021; CDC, 2022).

The One Health approach has continuously grown. Veterinarians can see their daily tasks as constant opportunities for passive surveillance of One Health threats, such as infectious diseases, antibiotic resistance, or environmental pollution. For instance, the practitioners have been detecting and reporting new disease outbreaks or pathogens in various hosts (companion animals, livestock, and wildlife). In the same way, they may verify the resistance of an isolated agent to antimicrobial treatment or chronic exposure to an environmental pollutant by pets and their owners (Kelly et al., 2017; Collignon and McEwen, 2019; Mackenzie and Jeggo, 2019; Sleeman et al., 2019).

The application of the One Health principles by veterinary professionals can provide information of considerable importance in pollution surveillance and ecotoxicology. Animals and humans often depend on the same environment and ecosystem, sharing potential chemical and physical hazards, such as organic and inorganic pollutants or radiation. Patterns of bioaccumulation and health effects of several xenobiotics (pesticides, drugs, inorganic compounds) are frequently shared by species from different taxa (including humans), just like other aspects of comparative medicine (Buttke, 2011). A substance's carcinogenic, hepatotoxic, or nephrotoxic potentials are often known and expected in several host species. The constant access to several species' specimens allows each veterinarian to perform a biomonitoring study of environmental pollutants in their operating region. Generally, veterinarians are trained to report a new outbreak or perform a study of disease prevalence, or at least they can find standardized procedures for these purposes (Buttke, 2011; Hryhorczuk et al., 2018). Notwithstanding, they may not have the autonomy to perform a biomonitoring study of a chemical hazard.

Therefore, the current study aimed to provide a practical guide to veterinary professionals from different fields, including wildlife) to design a biomonitoring study during their clinical practice without specific material or time-consuming procedures.

To cite this paper: Jota Baptista C, Seixas F, Gonzalo-Orden JM, and Oliveira PA (2024). How to Design a Biomonitoring Study – A Practical Guide for Veterinary Professionals under a One Health Approach. *World Vet. J.*, 14(3): 461-463. DOI: https://dx.doi.org/10.54203/scil.2024.wvj53

DEVELOPING A BIOMONITORING STUDY

Step 1 – Defining a problem/xenobiotic

At first, as in many other research studies, veterinarians must define priorities for biomonitoring contaminants considering their location and the animal species they usually work with them. For instance, if veterinarians have been diagnosing and monitoring respiratory clinical cases in companion animals, this group of cases may represent an opportunity to assess the effects of indoor air pollution, a problem with repercussions for humans and companion animals' health (Lin et al., 2018). Moreover, food-producing animal veterinarians can perform biomonitoring studies to evaluate the composition of the pasture in terms of toxic plants (Green et al., 2023), heavy metals (Afzal and Mahreen, 2024) or mycotoxins (Gallo et al., 2015; Muñoz-Solano and González-Peñas, 2023), if a considerable number of cases in a short-time period, and with a compatible clinical presentation, has been registered. These are just some examples.

Step 2 – Choosing a bioindicator

Some veterinarians may work with more than one species or animal population daily. Before starting to implement their research project and data collection, researchers should do some literature review regarding the pathophysiology, exposure effects, and metabolism of the chosen xenobiotic because species-specific enzymes or metabolic pathways can influence the obtained results and prevent the authors from developing correct and practical conclusions. As an illustration, dogs (and other canines) can adequately metabolize and eliminate some persistent organic pollutants (POPs), such as organochlorine pesticides and polychlorinated biphenyls, which humans (and other mammals) accumulate throughout life (Shore et al. 2001; Ruiz-Suárez et al., 2015). In this case, despite sharing the same environment as the owners, dogs are not suitable sentinels of pollution. In contrast, in indoor environments, cats have been pointed out as better bioindicators of some POPs, such as polychlorinated biphenyls or polybrominated diphenyl ethers (Ma et al., 2022). Therefore, a companion animal veterinarian who intends to measure and study the chronic effects of POPs, must choose cats as sentinels instead of dogs.

Some aspects of the species or population may represent advantages for its use as a bioindicator (Talmage and Walton, 1991), as what happens in hedgehogs or shrews (Sánchez-Chardi, et al., 2009; Jota Baptista et al., 2023). For instance, living in a restricted region, representing local pollution, living long enough to show effects from the exposure, and for a comparison between periods or age groups, or presenting an adequate dose-effect relationship (Jota Baptista et al. 2022). On the other hand, some xenobiotics (as some POPs or metals) are frequently accumulated through food chains, which usually makes carnivores better bioindicators than the herbivores they prey, considering the same ecosystem. However, there is no total consensus or a fixed list of species that can or cannot be used as bioindicators. For instance, wild boars have been widely used, mainly because they are a game species (and, therefore, a possible source of contaminants to consumers). Nevertheless, their broad distribution and frequent migrations in most countries lead to a poor definition of the pollution source (Tataruch and Kierdoft, 2003).

Step 3 – Choosing samples

This step highly depends on what xenobiotic will be analyzed (Step 1), the species involved (Step 2), and also on the study's goals. All these aspects should be decided first, before choosing a sample type.

Furthermore, at this stage, vets may decide if the study will involve the use of invasive samples (such as blood, liver, or kidney biopsies, and other internal tissues) or non-invasive samples (such as hair, feathers, feces, or parasites). If the study is designed to include only alive individuals (instead of including dead animals), aspects of welfare and restraint may conditionate the use of invasive samples (Jota Baptista et al., 2022). Nevertheless, invasive samples are crucial to understanding some toxicological aspects, such as tissue bioaccumulation and distribution in the organism. One practical use of this information is to evaluate which internal tissues of game species and livestock usually accumulate toxic substances or drugs, and which are more frequently consumed, as some muscles compared to bones or offal (Jukna et al., 2006; Jota Baptista et al., 2024). Some POPs are often searched in the adipose tissue of marine mammals through skin biopsies (Baini et al. 2020); and lead is frequently measured in bones from recently-died animals or even specimens preserved in museums (Martiniaková et al., 2011; Lanocha et al., 2013; Baini et al., 2020).

Moreover, it is necessary to think about the objectives of the study. If the goal is to evaluate long-term exposure to a substance, some tissues may be better accumulators than others, such as bones for cadmium or lead (González-Reimers et al., 2023), or hair for mercury (Treu et al., 2018). On the other hand, blood is a good option if it is intended to clinically monitor patients through recovery time and evaluate their response (Jota Baptista et al., 2022). Finally, authors may intend to use biomarkers of effect (evaluate or measure the cellular and molecular consequences of exposure to a particular substance). In that case, the liver and kidney are organs that usually suffer changes due to chronic or acute exposure to toxic compounds and are usually used for measure catalase, superoxide dismutase, and lipid peroxidation, among other biochemical biomarkers of effect (García-Niño and Pedraza-Chaverri, 2014; Hampel et al., 2016). Nevertheless, it is crucial to highlight that this depends significantly on the xenobiotic and host, and it is always advised to review the literature before starting the work.

Step 4 – Sending the samples to the laboratory

Since the primary goal of this article is to provide brief guidelines to veterinary professionals to perform a biomonitoring study, a detailed explanation of the various methods available for the detection and quantification of xenobiotics goes far beyond the scope. It is, however, essential to select a good and experienced laboratory that is familiar with preparing the samples and dealing with different organic matrices. Deciding whether you want to detect or

quantify a substance is also essential. For instance, detecting the substance may provide enough evidence to make practical conclusions if the goal is to study fecal antibiotic residues (Li et al., 2013). In contrast, measuring zinc or other elements in cattle to evaluate their actual bioavailability in a pasture may require a quantitative toxicological analysis (Rigueiro-Rodríguez et al., 2012).

Most toxicological analyses do not need special requirements and materials to collect and preserve the samples. Dinis-Oliveira et al. (2016) have published detailed guidelines for collecting each biological sample for toxicological analysis. In most cases, it is recommended to send frozen samples, collected without external contamination to proper sterile containers (such as dry tubes or plastic bags; without preservatives) and correctly identified. Notwithstanding, sampling guidelines should be checked for each xenobiotic and biological sample.

Whatever the method is used (gas chromatography, liquid chromatography, atomic absorption spectrophotometry, or induced-coupled plasma mass spectrophotometry), the laboratory must ensure suitable quality-control methods (the use of duplicates, blanks, and suitable reference matrices, Caballero-Casero et al. (2021), and enough sample must be provided by the clinician.

Step 5 – Results' interpretation

Interpreting results might be the most challenging part of a biomonitoring study, especially if the authors intend to compare exposure levels with effects. There is a lack of scientific literature on the effects of each relevant hazardous substance in each animal taxa and what to define as gold standard biomarkers for most relevant xenobiotics (Varea et al. 2020; Desforges et al., 2022). This may implicate an extensive literature search (references of similar studies in humans) and an exercise of presenting a hypothesis for further research (Jota Baptista et al., 2023). Authors should be aware that most lesions or biomarkers that may be associated with hepatotoxic, nephrotoxic, carcinogenic, and neurotoxic, among others, substances are not pathognomonic of a specific compound. Age, sex, chronic diseases, food habits, or environmental conditions may strongly influence the effect of xenobiotics in animals and provoke distinct individual or populational effects (Ortiz et al. 2022). Notwithstanding, each local study will represent scientific evidence and help build detailed and long-term surveys and metanalysis by the research institutions. A small and practical guideline for implementing biomonitoring studies for veterinarians is provided as a summary of this article (Figure 1).

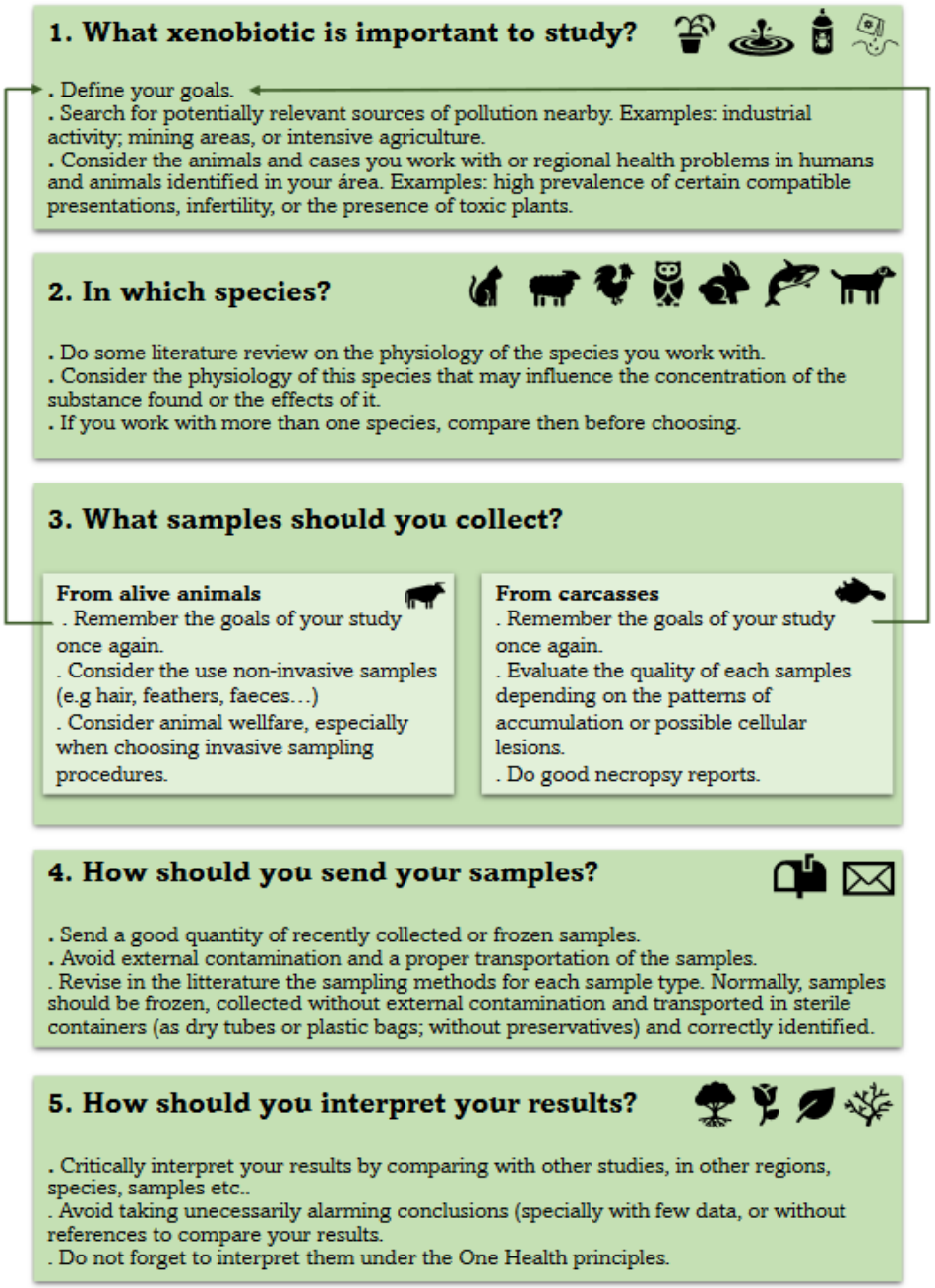


Figure 1. Guidelines for a quick implementation of a biomonitoring study by veterinarians

CONCLUSIONS

Veterinarians should never forget their crucial part in One Health. Therefore, each biomonitoring study should provide a concise interpretation of the results under a One Health approach. After all, as mentioned above, environmental contamination *sensu lato* is a global problem with remarkable One Health repercussions. Thus, veterinarians could analyse their daily practice, considering the species they work with and the xenobiotics of interest in their geographic region, and try to implement a biomonitoring study that might give a positive comprehension of their clinical cases, as well as improve the general knowledge about the impact of environmental contamination in animals and humans' health. The authors of the present study certainly believe these guidelines may also be applied to environmental researchers, biologists, or other health professionals (rather than veterinarians).

DECLARATIONS

Funding

This study was supported by National Funds by FCT—Portuguese Foundation for Science and Technology, under the PhD scholarship 202104520.BD, and also under the projects 10.54499/UIDB/04033/2020 (CITAB researchers, CJB and PAO), 10.54499/UIDB/04585/2020 (CiiEM research, CJB); and UIDB/CVT/00772/2020 and LA/P/0059/2020 (CECAV/Al4 Animals researcher, FS)

Availability of data and materials

The data of the current study are available by reasonable request.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues have been checked by the authors before submission.

Authors' contributions

The authors responsible for the conceptualization of the article were, Catarina Jota Baptista, Fernanda Seixas, José M. Gonzalo-Orden, and Paula A. Oliveira. Catarina Jota Baptista conducted the data collection and provided the original draft of the manuscript, while Fernanda Seixas, José M. Gonzalo-Orden, and Paula A. Oliveira revised it. All authors agreed with the final version of the manuscript.

Competing of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

Afzal A and Mahreen N (2024). Emerging insights into the impacts of heavy metals exposure on health, reproductive and productive performance of livestock. Frontiers in Pharmacology, 15: 1375137. DOI: https://www.doi.org/10.3389/fphar.2024.1375137

Baini M, Panti C, Fossi MC, Tepsich P, Jiménez B, Coomber F, Bartalini A, Muñoz-Arnanz J, Moulins A, and Rosso M (2020). First assessment of POPs and cytochrome P450 expression in Cuvier's beaked whales (*Ziphius cavirostris*) skin biopsies from the Mediterranean Sea. Scientific Reports, 10: 21891. DOI: https://www.doi.org/10.1038/s41598-020-78962-3

Buttke DE (2011). Toxicology, environmental health, and the One Health concept. Journal of Medical Toxicology, 7(4): 329-332. DOI: https://www.doi.org/10.1007/s13181-011-0172-4

Caballero-Casero N, Belova L, Vervliet P, Antignac JP, Casta A, Debrauwe L, Esteban López ML, Huber C, Klanova J, Krauss M et al. (2021). Towards harmonized criteria in quality assurance and quality control of suspect and non-target LC-HRMS analytical workflows for screening of emerging contaminants in human biomonitoring. TrAC Trends in Analytical Chemistry, 136: 116201. DOI: https://www.doi.org/10.1016/j.trac.2021.116201

Centers of disease control and prevention (CDC) (2022). One Health basics. One Health, CDC. Available at: https://www.cdc.gov/onehealth/basics/index.html

Collignon PJ and McEwen SA (2019). One health– Its importance in helping to better control antimicrobial resistance. Tropical Medicine and Infectious Disease, 4(1): 22. DOI: https://www.doi.org/10.3390/tropicalmed4010022

Desforges JP, Weijs L, Hickie B, and Gergs A (2022). Models as much-needed tools in ecotoxicology: Integrative approaches to cross barriers. Archives of Environmental Contamination and Toxicology, 83: 295-298. Available at: https://link.springer.com/article/10.1007/s00244-022-00964-1

Dinis-Oliveira RJ, Vieira DN, and Magalhães T (2016). Guidelines for collection of biological samples for clinical and forensic toxicological analysis. Forensic Science Research, 1(1): 42-51. DOI: https://www.doi.org/10.1080/20961790.2016.1271098

Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, and Nielsen KF (2015). Review on mycotoxin issues in ruminants: Occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. Toxins, 7: 3057-3111. DOI: https://www.doi.org/10.3390/toxins7083057

García-Niño WR and Pedraza-Chaverrí J (2014). Protective effect of curcumin against heavy metals-induced liver damage. Food and Chemical Toxicology, 69: 182-201. DOI: https://www.doi.org/10.1016/j.fct.2014.04.016

González-Reimers E, Velasco-Vázquez J, Arnay-de-la-Rosa M, Alberto-Barroso V, Galindo-Martín L, and Santolaria-Fernández F (2003). Bone cadmium and lead in prehistoric inhabitants and domestic animals from Gran Canaria. Science of the Total Environment, 301: 97-103. DOI: https://www.doi.org/10.1016/S0048-9697(02)00299-1

Green BT, Welch KD, Lee ST, Stonecipher CA, Gardner DR, Stegelmeier BL, Davis TZ, and Cook D (2023). Biomarkers and their potential for detecting livestock plant poisonings in Western North America. Frontiers in Veterinary Science, 10: 1104702. DOI: https://www.doi.org/10.3389/FVETS.2023.1104702

Hampel M, Blasco J, and Martín Díaz ML (2016). Biomarkers and effects. Marine Ecotoxicology. Chapter 5, pp. 121-165. DOI: https://www.doi.org/10.1016/B978-0-12-803371-5.00005-9

Hryhorczuk D, Beasley VR, Poppenga RH, and Durrani T (2018). One toxicology, One Health, one planet. In: J. A. Herrmann, Y. J. Johnson-Walker, (Editors). Beyond One Health: From recognition to results, pp. 117-154. DOI: https://www.doi.org/10.1002/9781119194521.CH5

Jota Baptista C, Seixas F, Gonzalo-Orden JM, and Oliveira PA (2022). Biomonitoring metals and metalloids in wild mammals: Invasive versus non-invasive sampling. Environmental Science and Pollution Research International, 29: 18398-18407. DOI: https://www.doi.org/10.1007/S11356-022-18658-5

Jota Baptista C, Seixas F, Gonzalo-Orden JM, Patinha C, Pato P, Ferreira da Silva E, Casero M, Brazio E, Brandão R, Costa D et al. (2023). High levels of heavy metal(loid)s related to biliary hyperplasia in hedgehogs (*Erinaceus europaeus*). Animals, 13: 1359. DOI: https://www.doi.org/10.3390/ANI13081359

Jota Baptista C, Seixas F, Gonzalo-Orden JM, Patinha C, Pato P, da Silva EF, Merino-Goyenechea LJ, and Oliveira PA (2024). Heavy metals and metalloids in wild boars (*Sus scrofa*) – A silent but serious public health hazard. Veterinary Research Communications, 48: 1015-1023. DOI: https://www.doi.org/10.1007/s11259-023-10272-1

Jukna Č, Jukna V, and Šiugždaitė J (2006). Determination of heavy metals in viscera and muscle of cattle. Bulgarian Journal of Veterinary Medicine, 9(1): 35-41. Available at: https://lsmu.lt/cris/entities/publication/1b6d0fe1-16e6-42db-b2e0-514701f4844b

Kelly TR, Karesh WB, Johnson CK, Gilardi KVK, Anthony SJ, Goldstein T, Olson SH, Machalaba C, Mazet JAK, Aguirre A et al. (2017). One health proof of concept: Bringing a transdisciplinary approach to surveillance for zoonotic viruses at the human-wild animal interface. Preventive Veterinary Medicine, 137: 112-118. DOI: https://www.doi.org/10.1016/j.prevetmed.2016.11.023

Lanocha N, Kalisinska E, Kosik-Bogacka DI, Budis H, Sokolowski S, and Bohatyrewicz A (2013). Comparison of metal concentrations in bones of long-living mammals. Biological Trace Element Research, 152: 195-203. DOI: https://www.doi.org/10.1007/s12011-013-9615-x

Li YX, Zhang XL, Li W, Lu XF, Liu B, and Wang J (2013). The residues and environmental risks of multiple veterinary antibiotics in animal faeces. Environmental Monitoring and Assessment, 185: 2211-2220. DOI: https://www.doi.org/10.1007/S10661-012-2702-1

Lin CH, Lo PY, Wu HD, Chang C, and Wang LC (2018). Association between indoor air pollution and respiratory disease in companion dogs and cats. Journal of Veterinary Internal Medicine, 32: 1259-1267. DOI: https://www.doi.org/10.1111/JVIM.15143

Ma T, Wu P, Ding Z, Wang T, and Luo Y (2022). Pet cats, the better sentinels for indoor organic pollutants. Frontiers in Environmental Science, 10: 1023818. DOI: https://www.doi.org/10.3389/FENVS.2022.1023818

Mackenzie JS and Jeggo M (2019). The One health approach-Why is it so important?. Tropical Medicine and Infectious Disease, 4(2): 88. DOI: https://www.doi.org/10.3390/TROPICALMED4020088

Martiniaková M, Omelka R, Jančová A, Stawarz R, and G Formicki (2011). Concentrations of Selected heavy metals in bones and femoral bone structure of bank (*Myodes glareolus*) and common (*Microtus arvalis*) voles from different polluted biotopes in Slovakia. Archives of Environmental Contamination and Toxicology, 60: 524-532. DOI: https://www.doi.org/10.1007/s00244-010-9545-y

Muñoz-Solano B and González-Peñas E (2023). Mycotoxins and seafood: Occurrence and legislation. Marine Drugs, 21: 253. DOI: https://www.doi.org/10.3390/md21040253

Ortiz P, Torres-Sánchez A , López-Moreno A, Cerk K, Ruiz-Moreno Á, Monteoliva-Sánchez M, Ampatzoglou A, Aguilera M, and Gruszecka-Kosowska A (2022). Impact of cumulative environmental and dietary xenobiotics on human microbiota: Risk assessment for One Health. Journal of Xenobiotics, 12: 56-63. DOI: https://www.doi.org/10.3390/JOX12010006

Rigueiro-Rodríguez A, Mosquera-Losada MR, and Ferreiro-Domínguez N (2012). Pasture and soil zinc evolution in forest and agriculture soils of Northwest Spain three years after fertilization with sewage sludge. Agriculture, Ecosystems & Environment, 150: 111-120. DOI: https://www.doi.org/10.1016/j.agee.2012.01.018

Ruiz-Suárez N, Rial C, Boada LD, Henríquez-Hernández LA, Valeron PF, Camacho M, Zumbado M, Almeida González M, Lara P, and Luzardo OP (2015). Are pet dogs good sentinels of human exposure to environmental polycyclic aromatic hydrocarbons, organochlorine pesticides, and polychlorinated biphenyls?. Journal of Applied Animal Research, 44: 135-145. DOI: https://www.doi.org/10.1080/09712119.2015.1021808

Sánchez-Chardi A, Ribeiro CAO, and Nadal J (2009). Metals in liver and kidneys and the effects of chronic exposure to pyrite mine pollution in the shrew Crocidura russula inhabiting the protected wetland of Doñana. Chemosphere, 76(3): 387-394. DOI: https://www.doi.org/10.1016/j.chemosphere.2009.03.036

Shore RF, Casulli A, Bologov V, Wienburg CL, Afsar A, and Toyne P (2001). Organochlorine pesticide, polychlorinated biphenyl and heavy metal concentrations in wolves (Canis lupus L. 1758) from north-west Russia. Science of The Total Environment, 280: 45-54. DOI: https://www.doi.org/10.1016/S0048-9697(01)00802-6

Sleeman JM, Richgels KLD, White CL, and Stephen C (2019). One Health: A perspective from wildlife and environmental health sectors. Scientific and Technical Review, 38: 91-98. DOI: https://www.doi.org/10.20506/RST.38.1.2944

Talmage SS and Walton BT (1991). Small mammals as monitors of environmental contaminants. In: G. W. Ware (Editor), Reviews of environmental contamination and toxicology. Reviews of environmental contamination and toxicology. Springer., New York, NY, pp. 47-145. DOI: https://www.doi.org/10.1007/978-1-4612-3078-6_2

Tataruch F and Kierdorf H (2003). Mammals as biomonitors. In: B. A. Markert, A. M. Breure, H. G. Zechmeister (Editors), Trace metals and other contaminants in the environment. Elsevier., Oxford, UK, pp. 737-772. DOI: https://www.doi.org/10.1016/S0927-5215(03)80150-9

Treu G, Krone O, Unnsteinsdóttir ER, Greenwood AD, and Czirják GÁ (2018). Correlations between hair and tissue mercury concentrations in Icelandic arctic foxes (*Vulpes lagopus*). Science of the Total Environment, 619-620: 1589-1598. DOI: https://www.doi.org/10.1016/j.scitotenv.2017.10.143

Varea R, Piovano S, and Ferreira M (2020). Knowledge gaps in ecotoxicology studies of marine environments in Pacific Island countries and territories- A systematic review. Marine Pollution Bulletin, 156: 111264. DOI: https://www.doi.org/10.1016/j.marpolbul.2020.111264

Wilcox BA and Steele JA (2021). One Health and emerging zoonotic diseases. In: S. R. Quah, W. C. Cockerham (Editors), Handbook of global health. Springer., Cham, pp. 2099-2147. DOI: https://www.doi.org/10.1007/978-3-030-45009-0_88

2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 467-475
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj54
PII: S232245682400054-14

Atrial Natriuretic Peptide and Cardiovascular Diseases in Dogs and Cats

José Leandro Pereira do Carmo[1]*, Gliére Silmara Leite Soares[2], Lucas da Silva Vieira[1], and Daniela Oliveira[1]

[1]*Federal University of Agreste of Pernambuco, Garanhuns, 55292-278, Brazil*
[2]*Federal University of Piauí, Bom Jesus, 64900-000, Brazil*

*Corresponding author's Email: joseleandrojct111@gmail.com

REVIEW ARTICLE
Received: June 28, 2024
Revised: July 25, 2024
Accepted: August 26, 2024
Published: September 30, 2024

ABSTRACT

The term biomarker encompasses various biological indicators that objectively reflect a patient's medical status with precision and reproducibility. These indicators range from basic measurements like pulse and blood pressure to more intricate laboratory tests. Cardiac markers are crucial for accurate and prompt diagnosis of heart diseases in animals. Given the challenge of diagnosing cardiac diseases in small animals due to nonspecific clinical signs, cardiac markers provide quantitative indicators of biological processes. These markers include cardiac troponins for myocardial injury, natriuretic peptides for myocardial function, lipoproteins for serum homeostasis, and markers for inflammation of the cardiovascular system. Among natriuretic peptides, atrial natriuretic peptide (ANP) has emerged as a significant tool in diagnosing and monitoring cardiac diseases. ANP, primarily synthesized in cardiac atria, regulates salt and fluid excretion, counteracts vasoconstriction, and inhibits the renin-angiotensin-aldosterone system, contributing to the maintenance of cardiovascular homeostasis. Additionally, it functions as a biomarker for ventricular hypertrophy and congestive heart failure (CHF) in animals. Furthermore, it protects against hypertension and cardiac remodeling by demonstrating antagonism to the same system. This review addresses the definition of biomarkers within the context of molecular biology, elucidates their multifaceted functions in the animal organism in light of integrative physiology, and explores the pathologies correlated with ANP, with an emphasis on its etiopathogenesis and clinical manifestations.

Keywords: Biomarker, Canine, Feline, Heart physiology

INTRODUCTION

According to Barbosa (2022), biomarkers play a crucial role in both the diagnosis and the prognosis of animal pathologies, providing a valuable tool for the clinical evaluation of these conditions. In 1998, the Biomarkers Definitions Working Group of the National Institutes of Health established the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Strimbu and Tavel, 2010).

Cardiac markers are used to enhance the accuracy and speed of diagnosing heart disease in animals, thereby allowing for early prognosis and therapeutic interventions (Yonezawa et al., 2010). These cardiac markers are quantifiable indices of biological processes executed by a specific organ or system, as noted by Oyama (2015). In the medical field, cardiac markers are categorized into four groups, each associated with distinct clinical conditions. The first category relates to myocardial injury or necrosis, typically indicated by cardiac troponins. The second category pertains to myocardial function represented by natriuretic peptides. The third involves serum lipoprotein homeostasis including high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The fourth category, concerns the inflammation of the cardiovascular system (Yonezawa, 2010). In the contemporary scientific landscape, natriuretic peptides (NPs) have been recognized as pivotal instruments in the diagnostic evaluation and therapeutic monitoring of cardiac pathologies. Empirical evidence indicates that the quantification of N-terminal pro-brain natriuretic peptide (NT-proBNP) in the serum and plasma of canine and feline species serves as the sole biomarker facilitating the identification and monitoring of congestive phenomena as well as indirectly, assessing myocardial function in small animals (Lima and Ferreira, 2017). ANP has demonstrated clinical utility in various scenarios. For instance, in patients with heart failure, ANP levels are often elevated as a compensatory mechanism to counterbalance volume overload and increased cardiac workload (Felker et al., 2017). ANP also shows promise in managing acute kidney injury due to its ability to dilate renal vasculature and increase renal blood flow, underscoring the importance of biomarkers in identifying subclinical acute kidney injury before the onset of renal failure (Pinto, 2023). These examples highlight the clinical relevance and potential therapeutic applications of natriuretic peptides in various medical conditions (Felker et al., 2017).

To cite this paper: do Carmo JLP, Soares GSL, Vieira LS, and Oliveira D (2024). Atrial Natriuretic Peptide and Cardiovascular Diseases in Dogs and Cats. *World Vet. J.*, 14(3): 467-475. DOI: https://dx.doi.org/10.54203/scil.2024.wvj54

When evaluating patients with comorbidities in veterinary medicine using cardiac markers, it is crucial to consider species differences, the impact of concurrent medical conditions, and the complexity of interpreting results to ensures an accurate and comprehensive assessment of the animal's cardiac health. Baseline values for cardiac markers can vary among animal species, implying that normal reference ranges can vary between them (Mendes et al., 2019). The presence of comorbidities, such as renal, hepatic, or endocrine diseases, can alter cardiac marker levels, resulting in falsely elevated or reduced values, such as kidney failure, which can affect BNP levels in dogs and cats (Mendes et al., 2019) and heart failure, in which the evaluation of plasma levels of ANP and its molecular precursors has proven fundamental for diagnosis in small animals (Freire et al., 2024).

In this context, the present review aims to address cardiac markers by analyzing cardiovascular physiology, with a particular focus on atrial natriuretic peptide (ANP). It explores ANP's functions in canine and feline organisms, its non-physiological behavior, and its applications in veterinary medicine.

METHODOLOGY

This study was conducted through a bibliographic review, with emphasis on literature available on the internet relevant to ANP. A total of 78 academic articles were examined using databases such as Scopus, Web of Science, Google Scholar, SciELO, PubMed, and journals provided by CAPES. The inclusion criteria for articles in the review were the presence of specific keywords, the origin of the research sources, and the analysis of the titles and summaries of the articles. On the other hand, the exclusion criteria were the irrelevance of the content to the topic of the study, the type of publication, and the date of publication. The strategies for finding appropriate articles included the identification of keywords related to ANP (Biomarker, Canine, Feline, Heart physiology), applying publication date and type filters, analyzing titles and abstracts, and cross-referencing. The research was carried out systematically and comprehensively to ensure the consideration of all the relevant literature.

BIOMARKER OF MYOCARDIAL FUNCTION: NATRIURETIC PEPTIDE

Natriuretic peptides have been studied for several decades to understand their role in various physiologically important processes (Rao et al., 2021). Initially, they were recognized for regulating salt excretion and blood pressure volume (Maack, 2006). Atrial Natriuretic Peptide is a peptide consisting of 28 amino acids with a cysteine-cysteine disulfide bridge (Volpe, 2014). Primarily found in the cardiac atria and stored in secretory granules, ANP is a significant marker of ventricular hypertrophy and congestive heart failure (Maack, 2006). This peptide, the most important among circulating natriuretic peptides, plays a crucial role in the regulation of renal function and plasma volume under normal conditions (Maack, 2006; Richards, 2007). The secretion of ANP is directly stimulated by atrial stretch or pressure, as occurs during volume expansion and hypertension (Richards, 2007). In the vasculature, ANP counteracts vasoconstriction, and in systemic capillaries, it increases permeability, facilitating fluid transfer from the intravascular compartment to the interstitial space, a phenomenon known as the “third spacing effect” (Sisson, 2004). Additionally, ANP inhibits the renin-angiotensin-aldosterone system, neutralizes sympathetic effects, and interferes with the synthesis and effects of other hormones or paracrine/autocrine substances that regulate blood pressure and volume, such as endothelin and vasopressin (Gonsalez et al., 2018). This combined action of ANP aims to maintain blood pressure and blood volume within normal physiological ranges, contributing to cardiovascular homeostasis (Gonzalez et al., 2018).

Atrial natriuretic peptide and its N-terminal pro-ANP cleavage product (Figure 1) were the first natriuretic peptides to be studied (Srisawasdi et al., 2010). ANP is one of the primary natriuretic hormones produced and secreted by the cardiac muscle in response to stress or stretching of cardiomyocytes caused by volume overload, hypoxia, and myocardial hypertrophy (Oyama, 2015; Ruaux et al., 2015; Alkhawan et al., 2016; Pelander et al., 2017). It is worth noting that all natriuretic peptides (NPs) are synthesized as pre-pro-hormones (Samad et al., 2023) with long peptide sequences called proANP and proBNP, and stored in granules attached to the membranes of atrial and ventricular tissues, respectively (Del Ry et al., 2014; Cahill et al., 2015; Harris et al., 2017). The physiological effects of the active fractions of natriuretic peptides stimulate, through the ANP receptor, diuresis and natriuresis, increasing the glomerular filtration rate and inhibiting the tubular transport of sodium through the renal collecting duct. These receptors are present in the lungs, heart, kidneys, adrenal glands, blood vessels, and the central nervous system (Cahill et al., 2015; Hezzel et al., 2015; Harris et al., 2017). Various factors can affect ANP concentrations in dogs and cats. Among these, the age of the animal is an important factor, as changes in cardiac function with aging can lead to an increase in ANP levels. Additionally, the breed of the animal can influence plasma ANP levels. Furthermore, concomitant diseases, such as cardiac lesions and renal insufficiency, can also affect ANP levels (Cruz et al., 2017). There are several testing techniques for the measurement of ANP, including immunoassays such as the enzyme-linked immunosorbent assay

(ELISA) and the radioimmunoassay (RIA). ELISA is frequently used for its high sensitivity and specificity, while RIA, although also highly sensitive, involves the use of radioisotopes (Hezzell et al., 2015).

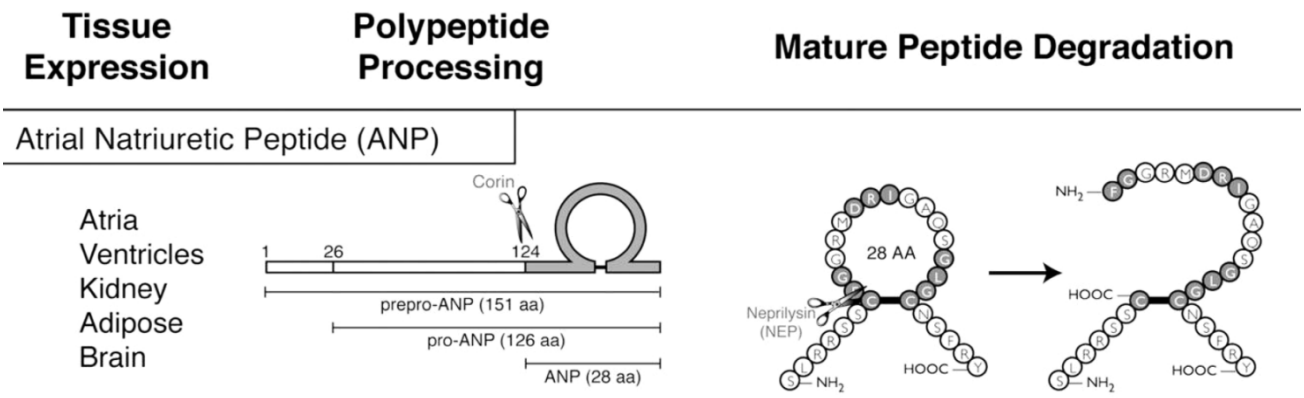


Figure 1. Atrial natriuretic peptide cleavage. (Source: Potter et al., 2009).

Figure 1 illustrates the structure of the pre-prohormone for ANP on the left. The final amino acid sequence and the structure of the mature peptide, along with the main degradation product, are shown on the right, with the cleavage site indicated by scissors. Initially, the ANP gene, located on chromosome 1 in humans, produces a 151-amino acid hormone known as pre-pro-ANP. Then, 25 amino acids are removed from the signal sequence, converting the chain into pro-ANP, which is transported to the Golgi complex. Before secretion, pro-ANP is cleaved again, possibly by membrane proteins, resulting in a functional chain of ANP with 28 amino acids (Potter et al., 2009).

Pro-atrial natriuretic peptide (proANP) is primarily produced by atrial myocytes and, to a lesser extent, by ventricular myocytes (Potter et al., 2009). When the myocardium is subjected to stress or stretch, (such as in response to volume overload, the production of proANP increases, especially in ventricular myocytes (Mahendram et al., 2022). Atrial wall distension or pressure stimulates the release of ANP, which acts as a protective mechanism of the body against cardiovascular volume overload and helps regulate the renal function (Del Ry et al., 2014; Kanno et al., 2016). ANP is valuable for assessing cardiac function, particularly in volume overload situations (Rao et al., 2021). However, its sensitivity and specificity can be influenced by factors such as age, sex, heart rhythm, and renal function. Concurrent diseases like heart failure, renal disease, or hypertension can also affect ANP levels (Kanno et al., 2016; Mahendran et al., 2022). Monitoring variations in ANP levels over time and comparing them to species-specific reference ranges can aid in evaluating cardiovascular health and making therapeutic decisions in dogs and cats (Kanno et al., 2016). Cardiac troponins (Tnl and TnT) are highly sensitive and specific for myocardial infarction although their levels can increase in various conditions such as heart failure, pulmonary embolism, septicemia, and renal failure (Mahendran et al., 2022). Brain natriuretic peptide (BNP) is another biomarker, particularly sensitive and specific for heart failure. Additionally, it is useful in distinguishing between cardiac and pulmonary dyspnea (Barbosa, 2022). Each of these three biomarkers has its distinctive advantages in clinical practice: Whereas BNP assesses volume overload, cardiac troponins detect myocardial muscle damage, and BNP evaluates heart failure and distinguishes dyspnea. Clearly, the choice of a biomarker depends on the specific clinical conditions of the patient (Hori et al., 2020).

ANTAGONISM OF ATRIAL NATRIURETIC PEPTIDE HORMONE TO THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

ANP plays a crucial role in regulating blood pressure, exerting an antagonistic relationship with the renin-angiotensin-aldosterone system (RAAS) (Araújo and Ramos, 2022). The RAAS comprises interrelated elements including renin, an enzymatically active hormone, angiotensin peptides with various activities, aldosterone, and a mineralocorticoid (Martyniak and Tomasik, 2022). ANP, circulating through the system, increases natriuresis and diuresis, acting specifically on renal tubules (Araújo and Ramos, 2022). Currently, this hormone is recognized as a potent suppressor of the RAAS (Maack, 2006). Juxtaglomerular cells are responsible for renin production, a key component of the RAAS, which functions to promote salt reabsorption and elevate blood pressure (Rao et al., 2021). Maack (2006) underscores the role of ANP as a physiological equilibrium to salt-retention mechanisms that developed as mammals made the transition from aquatic to terrestrial habitats. ANP secretion adds endocrine characteristics to the heart, obtained by cardiomyocytes, especially in response to atrial volume distension, resulting in atrial cavity expansion and subsequent release of ANP into the systemic circulation (Araújo et al., 2021). Furthermore, it has been observed that ANP presents

relevant antagonism to the RAAS, which can attenuate the physiological mechanism triggered by the release of renin in the kidneys. This release triggers a series of reactions to control blood pressure (Atlas et al., 1986). Additionally, ANP acts as a hypotensive agent, counteracting the effects of angiotensin II and aldosterone, which lead to the increase in systemic blood pressure (Atlas et al., 1986; Atlas and Maack, 1992; Maack, 1996). The stimulating role of angiotensin II in aldosterone release by the adrenal glands is notable, as it increases sodium and water reabsorption, thereby elevating blood pressure and restoring homeostasis (de Oliveira et al., 2019). In molecular terms, ANP inhibits the activity of juxtaglomerular cells in the kidneys responsible for producing renin, an enzyme that catalyzes the conversion of angiotensinogen to angiotensin I (Schettini et al., 2022). Thus, by inhibiting renin release, ANP reduces the production of angiotensin I, which is further converted into angiotensin II by the angiotensin-converting enzyme (ACE), stimulating aldosterone release (Simões et al., 2006). Moereover, aldosterone promotes sodium and water reabsorption in the kidneys and increases blood volume and blood pressure (Wagner, 2014). ANP inhibits aldosterone release and reduces sodium and water reabsorption, leading to decreased blood volume and blood pressure (Freire et al., 2024). These molecular mechanisms enable ANP to regulate blood pressure and fluid balance in the body, counteracting the effects of the RAAS (Bekele, 2023). From a pharmacological standpoint, the use of mineralocorticoid receptor antagonists (MRAs) is a pharmacological intervention that modulates the activity of the RAAS in managing cardiac diseases in animals (Spencer et al., 2020). Spironolactone is the only MRA licensed for veterinary use which is utilized in the treatment of congestive heart failure caused by valvular regurgitation in dogs. Additionally, the combination of spironolactone with benazepril is a therapeutic option for this condition.

CARDIORENAL SYNDROME AND ITS RELATIONSHIP WITH ATRIAL NATRIURETIC PEPTIDE

The cardiorenal syndrome (CRS) in humans is described as 'conditions involving the heart and kidneys where dysfunction, whether acute or chronic, in one of these organs can cause corresponding acute or chronic dysfunction in the other' (Gavazza et al., 2020). This interaction between the two systems involves various mechanisms related to volume regulation and dysfunction (Pouchelon et al., 2015; Athwani et al., 2017; Smyth et al., 2017). Martinelli et al. (2016) highlighted that there are currently no specific biomarkers for cardiorenal syndrome. Assessment of renal function or damage is always performed for the renal part, while atrial natriuretic peptide and troponin I are used for the cardiac component (Martinelli et al., 2016).

ARTERIAL HYPERTENSION

Systemic arterial hypertension (SAH) is a pathological condition of significant relevance in the clinical management of dogs and cats, given that this condition is defined by the continuous increase in systolic or diastolic blood pressure, which can result in harmful effects, primarily affecting the kidneys, heart, eyes, and the central nervous system (Pellegrino et al., 2010). According to Ware (2006), conditions associated with SAH may include renal diseases, cardiac diseases, hyperadrenocorticism, and hyperthyroidism. Blood pressure (BP) is determined by cardiac output and peripheral vascular resistance; therefore, an increase in cardiac output, peripheral vascular resistance, or both will bring about elevated levels of BP (Athavale and Lewis, 2009).

Blood pressure is controlled by nervous, endocrine, cardiovascular, and renal mechanisms. Thus, conditions that impact these mechanisms can lead to changes in blood pressure, such as hypotension and hypertension (Nogueira and Poggiani, 2013). Hall (2011) and Nogueira and Poggiani (2013) described the function of ANP as inhibiting sodium reabsorption, promoting glomerular hyperfiltration, and halting renin release, acting on renal arterioles, venules, and tubular epithelium. Natriuretic peptides, particularly ANP and BNP, have emerged as effective biomarkers for left ventricular dysfunction and are increasingly utilized as biomarkers in pulmonary arterial hypertension (PAH) and right ventricular (RV) dysfunction (Nogueira and Poggiani, 2013). Notably, ANP and BNP are upregulated in response to hypoxia and exert cardioprotective effects (McMahon and Bryan, 2017). In recent years, the recognition of the importance of systemic hypertension in dogs and cats has led to fundamental changes in understanding the pathophysiology and management of various diseases, including chronic kidney disease and arterial hypertension (Brown et al., 2022).

In humans, pulmonary hypertension has been associated with increased levels of cardiac troponin and ANP (Rolph & Cavanaugh, 2022). While studies have evaluated cardiac biomarkers in dogs with pulmonary hypertension, reports on cats with this condition are, thus far, limited (da Silva et al., 2012; Rolph and Cavanaugh, 2022). Although cardiac biomarkers have been rigorously assessed for their use in heart pathologies, many are influenced by various respiratory or systemic diseases (Smith et al., 2015). Several cardiac biomarkers show elevated levels in cases of pulmonary

hypertension (PH). However, distinguishing between congestive heart failure (CHF), PH, or a combination of both is challenging when relying solely on biomarkers (Smith et al., 2015).

CHRONIC KIDNEY DISEASE

Chronic Kidney Disease (CKD) is a metabolic disease characterized by progressive and irreversible loss of renal function due to a decrease in the number of functional nephrons (Tavares, 2021). According to De Nicola et al. (1997), high concentrations of ANP are observed in patients with CKD, a condition characterized by irreversible kidney lesions that progress over months to years. Although more common in elderly dogs, CKD can occur at any age (McGrotty, 2008; Polzin, 2011; Bartges, 2012). As nephrons are lost, there is a decrease in the glomerular filtration rate (GFR), resulting in azotemia due to increased concentration of nitrogenous compounds in the blood. Additionally, substances that are typically reabsorbed by the renal tubules, such as proteins, may be excreted, leading to proteinuria. Therefore, ANP is recognized as a biomarker of renal injury (Polzin, 2011; Bartges, 2012).

Cruz et al. (2017) reported elevated levels of natriuretic peptides in hypertensive cats with CKD, indicating that the release of ANP by cardiomyocytes may increase in response to arterial hypertension, myocardial hypoxia, and ischemia. CKD is a frequently diagnosed condition in dogs and cats. Studies suggest that its prevalence ranges from 0.5% to 7% in dogs and from 1.6% to 20% in cats (Lund et al., 1999; Polzin, 2011). These statistics highlight CKD as one of the most common pathologies in feline species (Polzin, 2011). The disease, which can arise from various causes, is characterized by progressive and irreversible loss of renal function, leading to a range of health complications in affected animals (Lund et al., 1999; Polzin, 2011). Although there is no breed or age predilection (Polzin, 2011), it is known that morbidity and mortality are more prevalent in older dogs and cats (Polzin, 2011).

In CKD, there is often an observed increase in plasma levels of natriuretic peptides (Suda et al., 1988). Although CKD is commonly associated with changes in cardiac hemodynamics, the mechanisms underlying the rise in plasma concentration of natriuretic peptides in patients with renal dysfunction remain undetermined (Tsutamoto et al., 2006). According to Polzin (2011) and Courand and Lantelme (2014), several studies have established that CKD is a leading cause of death in dogs.

CONGESTIVE HEART FAILURE

Congestive heart failure (CHF) occurs when the heart is no longer able to supply the necessary amount of blood to the body (Queiroz et al., 2022). Changes in cardiac muscle or valves result in a chronic decrease in cardiac output and activation of compensatory mechanisms, such as the renin-angiotensin system (RAS) and the sympathetic nervous system (Knight, 1992). These mechanisms induce tachycardia, peripheral vasoconstriction, and retention of sodium and water, leading to an increase in blood pressure and cardiac output, which optimizes tissue perfusion (Awan and Mason, 1996; Camacho, 1996). However, the prolonged activation of these compensatory mechanisms leads to the evolution of the clinical signs observed in CHF, including congestion, ascites, pleural effusion, pulmonary and limb edema, as well as symptoms such as fatigue, exercise intolerance, dyspnea, tachycardia, gallop rhythm, murmurs, and arrhythmias (Calvert, 1991; Camacho, 1996; De Morais, 2000).

ANP is an important biomarker for the diagnosis and monitoring of CHF in dogs and other mammals. It is secreted by cardiac cells in response to stress and volume overload, leading to an increase in plasma concentration in dogs with CHF (Hori et al., 2012). Measurement of ANP levels can be valuable in diagnosing CHF in dogs, especially when clinical signs are nonspecific or when there is uncertainty about the presence of the disease (Hori et al., 2012; Oyama, 2015; Lima and Ferreira, 2017). Kanno et al.'s (2016) study involving 16 healthy dogs and 51 untreated dogs, with some having right-sided heart failure (RHF), demonstrated that plasma levels of ANP in dogs with RHF were significantly higher than those in both the healthy control group and those without RHF.

The connection between ANP and CHF in cats has been the subject of a study by Mendes et al. (2019), which aimed to understand the role of ANP in cardiac conditions in cats. In cases of CHF in cats, there may be an increase in ANP levels, which acts as a compensatory mechanism to regulate fluid balance and blood pressure. Research suggests that plasma ANP levels may vary in cats with cardiac conditions, including congestive heart failure (CHF), where ANP levels fluctuate depending on the severity of heart failure and the stage of the disease (François et al., 2002). However, Khaki et al. (2022) report that ANP is not an effective parameter for diagnosing the subclinical stages of myxomatous mitral valve disease (MMVD). They suggest that serum BNP levels are more accurate across all stages of DMVM.

CONCLUSION

The use of biomarkers has become increasingly common in veterinary medicine, as they not only provide an efficient means of monitoring animal health and identifying diseases in their early stages but are also particularly useful for the diagnosis and monitoring of cardiac, renal, and metabolic diseases. The serum concentration of ANP is useful for diagnosing cardiovascular diseases in dogs and cats. Its crucial function in regulating blood volume and blood pressure makes it a valuable indicator of the animal's cardiovascular health. In the case of chronic kidney disease, it is believed that ANP may play a role in regulating renal function. It is of paramount importance for veterinary medicine to conduct further studies on ANP, given that ANP establishes itself as an indispensable tool for the care and treatment of cardiovascular diseases in dogs and cats. However, there is still much to be discovered about the role of ANP in different animal health conditions and diseases. Thus, additional research in this area can contribute to improving diagnoses and treatments in veterinary medicine.

DECLARATIONS

Authors' contributions

Formal analysis and investigation were carried out by all authors, and the original draft of the manuscript was collectively prepared by them. Additionally, all authors participated in the review and editing process. All authors checked and approved the final version of the manuscript for publication in the present journal.

Funding

No funding was received for conducting this study.

Availability of data and materials

All data generated during the research are relevant and included in this published article.

Competing interests

The authors did not indicate any conflicts of interest.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

REFERENCES

Alkhawan H, El-Hunjul M, Nguyen J, Desai R, Syed U, and Vittorio T (2016). Natriuretic peptide hormones in congestive heart failure: Challenges, clinical interpretation and review of studies. Acta Cardiologica, 71(4): 417-424. DOI: https://www.doi.org/10.1080/AC.71.4.3159694

Araújo FG, Zanzarini TJ, Mendes ECB, Gonçalves JBB, and Ramos RR (2021). Antagonism of atrial natriuretic peptide hormone to the renin-angiotensin-aldosterone system. Anais do fórum de iniciação científica do Unifunec, 12(12). DOI: https://www.doi.org/10.24980/aficf.v12i12

Araújo FG and Ramos RR (2022). Atrial natriuretic peptide antagonist to the renin-angiotensin-aldosterone system and effects on systemic blood pressure regulation. Jornal de Química Medicinal Moderna, 10: 13-22. DOI: https://www.doi.org/10.12970/2308-8044.2022.10.02

Athavale D and Lewis MA (2009). Physiology and treatment of hypertension. Paediatrics and Child Health, 19(1): 9-14. DOI: http://www.doi.org/10.1016/j.paed.2008.09.015

Athwani V, Bhargava M, Chanchlani R, and Mehta AJ (2017). Incidence and outcome of acute cardiorenal syndrome in hospitalized children. Indian Journal of Pediatrics, 84(6): 420-424. DOI: http://www.doi.org/10.1007/s12098-017-2307-3

Atlas SA and Maack T (1992). Atrial natriuretic factor. In: E. E. Windhager (Editor), Handbook of physiology: Renal physiology. Oxford University Press., New York, pp. 1577-1673. DOI: https://www.doi.org/10.1093/med/9780199592548.003.0034_update_001

Atlas SA, Volpe M, Sosa RE, Laragh JH, Camargo MJ, and Maack T (1986). Effects of atrial natriuretic factor on blood pressure and the renin-angiotensin-aldosterone system. Federation Proceedings, 45(7): 2115-21. Available at: https://pubmed.ncbi.nlm.nih.gov/2940120/

Awan NA and Mason DT (1996). Direct selective blockade of the vascular angiotensin II receptors in therapy for hypertension and severe congestive heart failure. American Heart Journal, 131(1): 177-185. DOI: https://www.doi.org/10.1016/S0002-8703(96)90067-8

Barbosa GVM (2022). Renal biomarkers in acute kidney injury in small animals. Faculty of Veterinary Medicine and Animal Science, Universidade Estadual Paulista, Brazil. Available at: https://repositorio.unesp.br/server/api/core/bitstreams/a19c1ad6-624a-46b7-9614-ef1e9e262ffe/content

Bartges JW (2012). Chronic kidney disease in dogs and cats. Veterinary Clinics of North America: Small Animal Practice, 42(4): 669-692. DOI: https://www.doi.org/10.1016/j.cvsm.2012.04.008

Bekele AT (2023). Natriuretic peptide receptors (NPRs) as a potential target for the treatment of heart failure. Relatórios Atuais de Insuficiência Cardíaca, 20(5): 429-440. DOI: https://www.doi.org/10.1007/s11897-023-00628-8

Brown SA (2022). Systemic hypertension. IRIS, International Renal Society. Available at: http://www.iris-kidney.com/education/hypertension.html

Cahill RJ, Pigeon K, Strong-Townsend MI, Drexel JP, Clark GH, and Buch JS (2015). Analytical validation of a second-generation immunoassay for

the quantification of N-terminal pro–B-type natriuretic peptide in canine blood. Journal of Veterinary Diagnostic Investigation, 27(1): 61-67. DOI: http://www.doi.org/10.1177/1040638714562826

Calvert CA (1991). Effect of medical therapy on survival of patients with dilated cardiomyopathy. Veterinary Clinics of North America: Small Animal Practice, 21(5): 919-930. DOI: https://www.doi.org/10.1016/S0195-5616(91)50103-0

Camacho AA (1996). Clinical study, morphometric and quantitative characterization of atrial ganglion neurons in the intercaval band of dogs with idiopathic congestive dilated cardiomyopathy. Tese (*Livre Docência*) - Faculdade de Ciências Agrárias e Veterinárias, Thesis, São Paulo State University, Brazil.

Courand P and Lantelme P (2014). Significance, prognostic value and management of heart rate in hypertension. Archives of Cardiovascular Disease, 107(1): 48-57. DOI: https://www.doi.org/10.1016/j.acvd.2013.11.003

Cruz FACS, Torres MM, Poletto D, Mendonça AJ, Guimaraes LD, Almeida ABPF, Silva FG, and Sousa VR (2017). Evaluation of natriuretic peptide in chronic kidney disease dogs. Pesquisa Veterinária Brasileira, 37(11): 1322-1326. DOI: https://www.doi.org/10.1590/S0100-736X2017001100020

da Silva AC, Oberlender G, Muzzi RAL, Muzzi LAL, and Coelho MR (2012). Pulmonary hypertension in dogs and cats: Literature review. Revista Científica Eletrônica de Medicina Veterinária, 10(19): 1-13. Available at: https://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/vYNDV5Ck63NFrFd_2013-6-24-15-7-6.pdf

De Morais HA (2000). Pathophysiology of heart failure and clinical evaluation of cardiac function. In: S. J. Ettinger and E. C. Feldman (Editors), Textbook of veterinary internal medicine. W.B. Saunders., Philadelphia. pp. 692-712. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20002212143

De Nicola L, Bellizzi V, Cianciaruso B, Minutolo R, Colucci G, Balletta M, Fuiano G, and Conte G (1997). Pathophysiological role and diuretic efficacy of atrial natriuretic peptide in renal patients. Journal of the American Society of Nephrology, 8(3): 445-55. DOI: https://www.doi.org/10.1681/ASN.V83445

de Oliveira MM, Furtado LLA, De Abreu CB, Schulien T, Pinto AMBG, De Oliveira LED, Coelho MR, and Lázaro MRA (2019). Cardiorenal syndrome in dogs: The search for control measures. Veterinária e Zootecnia, 26: 1-7. DOI: https://www.doi.org/10.35172/rvz.2019.v26.383

Del Ry S, Cabiati M, and Clerico A (2014). Natriuretic peptide system and the heart. Cardiovascular Issues in Endocrinology, 43: 134-143. DOI: https://www.doi.org/10.1159/000360597

François LMG, Kalil RAK, Pereira JB, Sant'anna JRM, and Nesralla IA (2002). Relationship of atrial pressures with atrial natriuretic peptide and its effects on diuresis and natriuresis during cardiac surgery with cardiopulmonary bypass. Brazilian Journal of Cardiovascular Surgery, 17(3): 258-265. DOI: https://www.doi.org/10.1590/S0102-76382002000300011

Felker GM, Anstrom KJ, Adams KF, Ezekowitz JA, Fiuzat M, Houston-Miller N, Januzzi JL Jr, Mark DB, Piña IL, Passmore G et al. (2017). Effect of natriuretic peptide-guided therapy on hospitalization or cardiovascular mortality in high-risk patients with heart failure and reduced ejection fraction: A randomized clinical trial. Journal of American Medical Association, 318(8): 713-720. DOI: https://www.doi.org/10.1001/jama.2017.10565

Freire Neto APL, Barrera CL, Castro GA, Lamella GML, Gama HC, Dragone LG, Martiniano RKG, Xisto RAN, and Oliveira R (2024). Emerging insights into the role of cardiac hormones in cardiovascular disease. Epitaya E-Books, 1(78): 699-722. DOI: https://www.doi.org/10.47879/ed.ep.2024479p699

Gavazza A, Fruganti A, Turinelli V, Marchegiani A, Spaterna A, Tesei B, Rossi G, and Cerquetella M (2020). Canine traditional laboratory tests and cardiac biomarkers. Frontiers in Veterinary Science, 7: 320. DOI: https://www.doi.org/10.3389/fvets.2020.00320

Gonsalez SR, Ferrão FM, Souza AM, Lowe J, and Morcillo LSL (2018) Inappropriate activity of local renin-angiotensin-aldosterone system during high salt intake: Impact on the cardio-renal axis. Brazilian Journal of Nephrology, 40(2): 170-178. DOI: https://www.doi.org/10.1590/2175-8239-JBN-3661

Hall JE (2011). Textbook of medical physiology. Elsevier Saunders., Rio de Janeiro, pp. 1151. Available at: B2n.ir/p95682

Harris AN, Estrada AH, Gallagher AE, Winter B, Lamb KE, Bohannon M, Hanscom J, and Mainville CA (2017). Biologic variability of N-terminal pro-brain natriuretic peptide in adult healthy cats. Journal of Feline Medicine and Surgery, 19(2): 216-223. DOI: https://www.doi.org/10.1177/1098612X15623825

Hezzell MJ, Boswood A, Lotter N, and Elliott J (2015). The effects of storage conditions on measurements of canine N-terminal pro-B-type natriuretic peptide. Journal of Veterinary Cardiology, 17(1): 34-41. DOI: https://www.doi.org/10.1177/1098612X15623825

Hori Y, Uchide T, Saitoh R, Thoei D, Uchida M, Yoshioka K, Chikazawa S, and Hoshi F (2012). Diagnostic utility of NT-proBNP and ANP in a canine model of chronic embolic pulmonary hypertension. Veterinary Journal, 194: 215-221. DOI: https://www.doi.org/10.1016/j.tvjl.2012.03.022

Hori Y, Iguchi M, Hirakawa A, Kamiya Z, Yamano S, Ibaragi T, Isayama N, Yamashita Y, Iwasa N, Inaba H et al. (2020). Evaluation of atrial natriuretic peptide and cardiac troponin I concentrations for assessment of disease severity in dogs with naturally occurring mitral valve disease. Journal of the American Veterinary Medical Association, 256(3): 340-348. DOI: https://www.doi.org/10.2460/javma.256.3.340

Kanno N, Hori Y, Hidaka Y, Chikazawa S, Kanai K, Hoshi F, and Itoh N (2016). Plasma atrial natriuretic peptide and N-terminal pro B-type natriuretic peptide concentrations in dogs with right-sided congestive heart failure. Journal of Veterinary Medical Science, 78(4): 535-542. DOI: http://www.doi.org/10.1292/jvms.15-0435

Khaki Z, Nooshirvani P, Shirani D, and Masoudifard M (2022). Diagnostic value of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and their correlation with lipoproteins in dogs with myxomatous mitral valve disease. BMC Veterinary Research, 18(1): 448. DOI: https://www.doi.org/10.1186/s12917-022-03548-2

Knight DH (1992). Fisiopatologia da insuficiência cardíaca [Pathophysiology of heart failure]. In: S. J. Ettingr (Editor), Tratado de medicina interna veterinária [Textbook of veterinary internal medicine]. Manole., São Paulo, pp. 944-969. Available at: https://repository.ufrpe.br/bitstream/123456789/2016/1/tcc_veruskadinardelimasilva.pdf

Lima GV and Ferreira FS (2017). N-terminal-pro brain natriuretic peptides in dogs and cats: A technical and clinical review. Veterinary World, 10(9): 1072-1082. DOI: https://www.doi.org/10.14202/vetworld.2017.1072-1082

Lund E, Armstrong PJ, Kirk CA, Kolar LM, and Klausner JS (1999). Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. Journal of the American Veterinary Medical Association, 214(9): 1336-1341. Available at: https://pubmed.ncbi.nlm.nih.gov/10319174/

Maack T (2006). The broad homeostatic role of natriuretic peptides. Arquivos Brasileiros de Endocrinologia e Metabologia, 50(2): 198-207. DOI: http://www.doi.org/10.1590/S0004-27302006000200006

Mahendran K, Thakur N, Chethan GE, Choudhary SS, Dey S, Saxena AC, Mahajan S, and Kavitha K (2022). Comparative assessment of troponin t, atrial natriuretic peptide, b-type natriuretic peptide and echocardiography in the diagnosis of cardiac and renal disorders in canine. Indian Journal of Animal Research, 56(2): 234-238. Available at: https://www.indianjournals.com/ijor.aspx?target=ijor:ijar1&volume=56&issue=2&article=017

Martyniak A and Tomasik PJ (2022). A new perspective on the renin-angiotensin system. Diagnostics, 13(1): 16. DOI: https://www.doi.org/10.3390/diagnostics13010016

Martinelli E, Locatelli C, Bassis S, Crosara S, Paltrinieri S, Scarpa P, Spalla I, Zanaboni AM, Quintavalla C, and Brambilla P (2016). Preliminary investigation of cardiovascular-renal disorders in dogs with chronic mitral valve disease. Journal of Veterinary Internal Medicine, 30(5): 1612-1618. DOI: http://www.doi.org/10.1111/jvim.14524

Mcgrotty Y (2008). Diagnosis and management of chronic kidney disease in dogs and cats. Comparative Animal Practice, 30(9): 502-507. DOI: https://www.doi.org/10.1136/inpract.30.9.502

McMahon TJ and Bryan NS (2017). Biomarkers in pulmonary vascular disease: Gauging response to therapy. American Journal of Cardiology, 120(8S): S89-S95. DOI: https://www.doi.org/10.1016/j.amjcard.2017.06.014

Mendes T, Sousa PR, Moura R, and Carvalho R (2019). Cardiac biomarkers and their clinical applicability in dogs and cats. Enciclopédia Biosfera, 16(29): 412-436. Available at: https://conhecer.org.br/ojs/index.php/biosfera/article/view/191

Nogueira TQ and Poggiani SSC (2013). Systemic hypertension associated with chronic kidney disease, literature review. Pubvet, 7(1): 1484. DOI: https://www.doi.org/10.22256/pubvet.v7n1.1484

Oyama MA (2015). Using cardiac biomarkers in veterinary practice. Clinics in Laboratory Medicine, 35(3): 555-566. DOI: http://www.doi.org/10.1016/j.cll.2015.05.005

Pelander L, Häggström J, Ley CJ, and Ljungvall I (2017). Cardiac troponin I and amino-terminal pro B-Type natriuretic peptide in dogs with stable chronic kidney disease. Journal Veterinary Internal Medicine, 31(3): 805-813. DOI: http://www.doi.org/10.1111/jvim.14703

Pellegrino A, Petrus LC, Yamaki FL, Santos ALF, and Larsson MHMA (2010). Blood pressure values of clinically healthy Golden Retriever dogs. Biblioteca Digital da Produção Intelectual-Bdpi, 47(4): 307-314. DOI: https://www.doi.org/10.11606/issn.1678-4456.bjvras.2010.26830

Pinto KRD (2023). Intensive nephrology from the perspective of translational research: Narrative review at the frontier of knowledge. Práticas e Cuidado: Revista De Saúde Coletiva, 4: e16525. Available at: https://www.revistas.uneb.br/index.php/saudecoletiva/article/view/16525

Polzin DJ (2011). Chronic kidney disease in small animals. Veterinary Clinics of North America: Small Animal Practice, 41(1): 15-30. DOI: https://www.doi.org/10.1016/j.cvsm.2010.09.004

Potter LR, Yoder AR, Flora DR, Antos LK, and Dickey DM (2009). Natriuretic peptides: Their structures, receptors, physiologic functions and therapeutic applications. In: H. H. H. W. Schmidt, F. Hofmann, and J. P. Stasch (Editors), cGMP: Generators, effectors and therapeutic implications. Handbook of experimental pharmacology. Springer., Berlin, Heidelberg, pp. 341-366. DOI: http://www.doi.org/10.1007/978-3-540-68964-5_15

Pouchelon JL, Atkins CE, Bussadori C, Oyama MA, Valden SL, Bonagura JD, Chetbul V, Cowgill LD, Elliot J, Francey T et al. (2015). Cardiovascular-renal axis disorders in the domestic dog and cat: A veterinary consensus statement. Journal of Small Animal Practice, 56(9): 537-552. DOI: http://www.doi.org/10.1111/jsap.12387

Queiroz SA, Maingué AP, and Luz PE (2022). Congestive heart failure in dogs: Therapeutic update– Literature review. Revista Terra e Cultura: Cadernos de Ensino e Pesquisa, 38(75): 139-152. Available at: http://periodicos.unifil.br/index.php/Revistateste/article/view/2490

Rao S, Pena C, Shurmur S, and Nugent K (2021). Atrial natriuretic peptide: Structure, function, and physiological effects: A narrative review. Current Cardiology Reviews, 17(6): e051121191003. DOI: https://www.doi.org/10.2174/1573403X17666210202102210

Richards AM (2007). Natriuretic peptides. Update on peptide release, bioactivity, and clinical use. Hypertension, 50(1): 25-30. Available at: https://hyper.ahajournals.org/cgi/reprint/50/1/25

Rolph KE and Cavanaugh SM (2022). Feline pulmonary hypertension: Are we overlooking an important comorbidity?. Journal of Feline Medicine and Surgery, 24(12): e636-46 DOI: https://www.doi.org/10.1177/1098612X221127233

Ruaux C, Scollan K, Suchodolski JS, Steiner JM, and Sisson D (2015). Biologic variability in NT-pro BNP and cardiac troponin-I in healthy dogs and dogs with mitral valve degeneration. Veterinary Clinical Pathology, 44(3): 420-430. DOI: http://www.doi.org/10.1111/vcp.12268

Samad M, Malempati S, and Restini CBA (2023). Natriuretic peptides as biomarkers: Narrative review and considerations in cardiovascular and respiratory dysfunctions. Yale Journal of Biology and Medicine, 96(1): 137-149. DOI: https://www.doi/10.59249/NCST6937

Schettini IVG, Faria DV, Nogueira LS, Otoni A, Silva ACS, and Rios DRA (2022). Renin angiotensin system molecules and chemokine (C-C motif) ligand 2 (CCL2) in chronic kidney disease patients. Brazilian Journal of Nephrology, 44(1): 19-25. DOI: https://www.doi.org/10.1590/2175-8239-JBN-2021-0030

Simões ACS, Pinheiro SV, Pereira RM, Ferreira AJ, and Santos RA (2006). The therapeutic potential of Angiotensin-(1 -7) as a novel renin-angiotensin system mediator. Mini reviews in medicinal chemistry, 6(5): 603-609. DOI: https://www.doi.org/10.2174/138955706776876203

Sisson DD (2004). Avaliação neuroendócrina de doença cardíaca [Neuroendocrine assessment of heart disease]. Veterinary Clinics: Small Animal Practice, 34(5): 1105-1126. Available at: https://www.vetsmall.theclinics.com/article/S0195-5616(04)00062-2/abstract

Smith KF, Quinn RL, and Rahilly LJ (2015). Biomarkers for differentiation of causes of respiratory distress in dogs and cats: Part 1–Cardiac diseases and pulmonary hypertension. Journal of Veterinary Emergency and Critical Care, 25(3): 311-329. DOI: https://www.doi.org/10.1111/vec.12318

Smyth A, Ronco C, and Garovic VD (2017). Preeclampsia: A cardiorrenal syndrome in pregnancy. Current Hypertension Reports, 19(2): 15. DOI: http://www.doi.org/10.1007/s11906-017-0714-5

Spencer S, Wheeler-Jones C, and Elliott J (2020). Aldosterone and the mineralocorticoid receptor in renal injury: A potencial therapeutic target in feline chronic kidney disease. Journal of Veterinary Pharmacology and Therapeutics, 43(3): 243-267. DOI: https://www.doi.org/10.1111/jvp.12848

Srisawasdi P, Vanavanan S, Charoenpanichkit C, and Kroll MH (2010). The effect of renal dysfunction on BNP, NT-proBNP, and their ratio. American Journal of Clinical Pathology, 133(1): 14-23. DOI: https://www.doi.org/10.1309/AJCP60HTPGIGFCNK

Strimbu K and Tavel JA (2010). What are Biomarkers?. Current Opinion in HIV and AIDS, 5(6): 463-466. DOI: https://www.doi.org/10.1097/coh.0b013e32833ed177

Suda S, Weidmann P, Saxenhofer H, Cottier C, Shaw S, and Ferrier C (1988). Atrial natriuretic factor in mild to moderate chronic renal failure. Hypertension, 11: 483-490. DOI: https://www.doi.org/10.1161/01.HYP.11.5.483

Tavares APDS, Santos CGDS, Tzanno-Martins C, Barros Neto J, Silva AMMD, Lotaif, L, and Souza JVL (2021). Kidney supportive care: An update of the current state of the art of palliative care in CKD patients. Jornal Brasileiro de Nefrologia, 43(1): 74-87. DOI: https://www.doi.org/10.1590/2175-8239-JBN-2020-0017

Tsutamoto T, Wada A, Sakai H, Ishikawa C, Tanaka T, and Hayashi M (2006). Relationship between renal function and plasma brain natriuretic peptide in patients with heart failure. Journal of the American College of Cardiology, 47(3): 582-586. DOI: https://www.doi.org/10.1016/j.jacc.2005.10.038

Volpe M, Rubattu S, and Burnett J Jr (2014). Natriuretic peptides in cardiovascular diseases: Current use and perspectives. European Heart Journal, 35(7): 419-425. DOI: https://www.doi.org/10.1093/eurheartj/eht466

Wagner CA (2014). Effect of mineralocorticoids on acid-base balance. Nephron Physiology, 128(1-2): 26-34. DOI: https://www.doi.org/10.1159/000368266

Ware WL (2006). Systemic arterial hypertension. In: R. W. Nelson and C. G. Couto (Editors), Small animal internal medicine. Elsevier., Rio de Janeiro, pp. 1360-1363. Available at: https://vetbooks.ir/small-animal-internal-medicine-6th-edition/

Yonezawa LA, Silveira VF, Machado LP, and Kohayagawa A (2010). Cardiac markers in veterinary medicine. Ciência Rural, 40(1): 222-230. DOI: https://www.doi.org/10.1590/S0103-84782009005000227

Instructions for Authors

Manuscript as Original Research Paper, Short Communication, Case Reports and Review or Mini-Review are invited for rapid peer-review publishing in the *World's Veterinary Journal* (WVJ). Considered subject areas include: Behavior; environment and welfare; animal reproduction and production; parasitology, endocrinology, microbiology, immunology, pathology, pharmacology, epidemiology, molecular biology, immunogenetics, surgery, radiology, ophthalmology, dermatology, chronic disease, anatomy, and non-surgical pathology issues of small to large animals, cardiology and oncology are sub-specialties of veterinary internal medicine. ... view full aims and scope

WVJ EndNote Style
Manuscript Template (.doc)
Sample Articles
Declaration form
Publication Ethics

Submission

The manuscript and other correspondence should be submit online preferentially. Please embed all figures and tables in the manuscript to become one single file for submission. Once submission is complete, the system will generate a manuscript ID and password sent to author's contact emails**:** editor@wvj.science-line.com. All manuscripts must be checked (by English native speaker) and submitted in English for evaluation (in totally confidential and impartial way).

Supplementary information

The online submission form allows supplementary information to be submitted together with the main manuscript file and covering letter. If you have more than one supplementary files, you can submit the extra ones by email after the initial submission. Author guidelines are specific for each journal. Our Word template can assist you by modifying your page layout, text formatting, headings, title page, image placement, and citations/references such that they agree with the guidelines of journal. If you believe your article is fully edited per journal style, please use our Word template before submission.
Supplementary materials may include figures, tables, methods, videos, and other materials. They are available online linked to the original published article. Supplementary tables and figures should be labeled with a "S", e.g. "Table S1" and "Figure S1". The maximum file size for supplementary materials is 10MB each. Please keep the files as small possible to avoid the frustrations experienced by readers with downloading large files.

Submission to the Journal is on the understanding that

1.The article has not been previously published in any other form and is not under consideration for publication elsewhere;
2.All authors have approved the submission and have obtained permission for publish work.
3.Researchers have proper regard for conservation and animal welfare considerations (see IAVE-Author Guidelines on Animal Ethics and Welfare). Attention is drawn to the '*Guidelines for the Treatment of Animals in Research and Teaching*'. Any possible adverse consequences of the work for populations or individual organisms must be weighed against the possible gains in knowledge and its practical applications. If the approval of an ethics committee is required, please provide the name of the committee and the approval number obtained.

Ethics Committee Approval

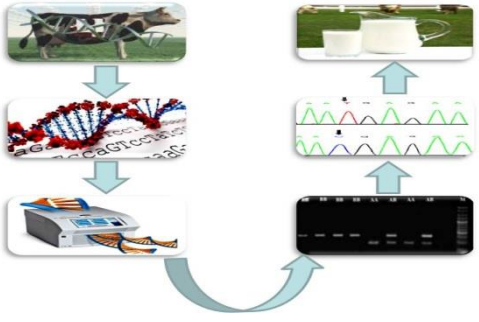
Experimental research involving human or animals should have been approved by author's institutional review board or ethics committee. This information can be mentioned in the manuscript including the name of the board/committee that gave the approval. Investigations involving humans will have been performed in accordance with the principles of *Declaration of Helsinki*. And the use of animals in experiments will have observed the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee. If the manuscript contains photos or parts of photos of patients, informed consent from each patient should be obtained. Patient's identities and privacy should be carefully protected in the manuscript.

Competing Interests

Competing interests that might interfere with the objective presentation of the research findings contained in the manuscript should be declared in a paragraph heading "Competing interests" (after Acknowledgment section and before References). Examples of competing interests are ownership of stock in a company, commercial grants, board membership, etc. If there is no competing interest, please use the statement "The authors have declared that no competing interest exists.

Graphical Abstract

Authors should provide a graphical abstract (a beautifully designed feature figure) to represent the paper aiming to catch the attention and interest of readers. Graphical abstract will be published online in the table of content. The graphical abstract should be colored, and kept within an area of 12 cm (width) x 6 cm (height) or with similar format. Image should have a minimum resolution of 300 dpi and line art 1200dpi. **Note:** Height of the image should be no more than the width. Please avoid putting too much information into the graphical abstract as it occupies only a small space. Authors can provide the graphical abstract in the format of PDF, Word, PowerPoint, jpg, or png, after a manuscript is accepted for publication. If you have decided to provide a Professional Graphical Abstract, please click **here**.



Presentation of the article

Main Format

First page of the manuscripts must be properly identified by the title and the name(s) of the author(s). It should be typed in Times New Roman (font sizes: 17pt in capitalization for the title, 10pt for the section headings in the body of the text and the main text, 9pt for References, double spaced, in A4 format with 2cm margins. All pages and lines of the main text should be numbered consecutively throughout the manuscript. The manuscript must be saved in a .doc format, (not .docx files). Abbreviations in the article title are not allowed.

Manuscripts should be arranged in the following order:

a. TITLE (brief, attractive and targeted)
b. Name(s) and Affiliation(s) of author(s) (including post code) and corresponding E-mail
c. ABSTRACT
d. Key words (separate by semicolons; or comma,)
e. Abbreviations (used in the manuscript)
f. INTRODUCTION
g. MATERIALS AND METHODS
h. RESULTS
i. DISCUSSION
j. CONCLUSION
k. DECLARATIONS
1. REFERENCES
m. Tables
n. Figure captions
o. Figures

Results and Discussion can be presented jointly if preferred.
Discussion and Conclusion can be presented jointly if preferred.

Article Sections Format

Title should be a brief phrase describing the contents of the paper. The first letter of each word in title should use upper case. The Title Page should include the author(s)'s full names and affiliations, the name of the corresponding author along with phone and e-mail information. Present address (es) of author(s) should appear as a footnote.

Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The abstract should be 150 to 300 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and Methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the author(s)'s experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section.

Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusion can be presented jointly if preferred.

Declarations section

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph forms or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or PowerPoint before pasting in the Microsoft Word manuscript file. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

Declarations section - Please include declarations heading

Please ensure that the sections:
-Ethics (and consent to participate)
-Authors' contributions
-Competing interests
-Availability of data and materials
are included at the end of your manuscript in a Declarations section.

Authors' Contributions

For manuscripts with more than one author, WVJ require an Authors' Contributions section to be placed after the Competing Interests section.
An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
We suggest the following format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.
For authors that equally participated in a study please write 'All/Both authors contributed equally to this work.'
Contributors who do not meet the criteria for authorship should be listed in an acknowledgements section.

Competing Interests

Competing interests that might interfere with the objective presentation of the research findings contained in the manuscript should be declared in a paragraph heading "Competing interests" (after Acknowledgment section and before References). Examples of competing interests are ownership of stock in a company, commercial grants, board membership, etc. If there is no competing interest, please use the statement "The authors declare that they have no competing interests.".
World's Veterinary Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.
It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Change in authorship

We do not allow any change in authorship after provisional acceptance. We cannot allow any addition, deletion or change in sequence of author name. We have this policy to prevent the fraud.

Acknowledgements

We strongly encourage you to include an Acknowledgements section between the Authors' contributions section and Reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include their source(s) of funding. Please also acknowledge anyone who contributed materials essential for the study.
Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please list the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section. Authors must describe the role of the funding body, if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Data Deposition

Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article. In computational studies where the sequence information is unacceptable for inclusion in databases because of lack of experimental validation, the sequences must be published as an additional file with the article.

References

1. A WVJ reference style for **EndNote** may be found **here**.
2. All references to publications made in the text should be presented in a list with their full bibliographical description.
3. In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's surname should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.
4. References in the text should be arranged chronologically (e.g. Kelebeni, 1983; Usman and Smith, 1992 and Agindotan et al., 2003). The list of references should be arranged alphabetically on author's surnames, and chronologically per author. If an author's name in the list is also mentioned with co-authors, the following order should be used: Publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1992a, l992b, etc.
5. Names of authors and title of journals, published in non-latin alphabets should be transliterated in English.
6. A sample of standard reference is "1th Author surname A, 2th Author surname B, 3th Author surname C. 2013. Article title should be regular and 7 pt . *World Vet. J.,* Add No. of Volume (Issue No.): 00-00."
7. The color of references in the text of article is dark blue. Example: (Preziosi et al., 2002; Mills et al., 2015).
8. At least 35% of the references of any submitted manuscript (for all types of article) should include scientific results published in the last five years.

-Examples (at the text- blue highlighted)

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001).

--Examples (at References section)

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. Journal of Dairy Science, 83: 1635-1647. DOI: XXX

Kareem SK (2001). Response of albino rats to dietary level of mango cake. Journal of Agricultural Research and Development. pp 31-38. DOI: XXX

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology. 7: 3535-3539. DOI: XX

b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (Pampus argentens euphrasen) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. Asian Fisheries Society Manila, Philippine 13: 191-199. Link

c) For edited symposia, special issues, etc., published in a journal:

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27. Link

d) For books:

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. Link

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603. Link

e) Books, containing sections written by different authors:

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg). Link

In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text.

Nomenclature and Abbreviations

Nomenclature should follow that given in NCBI web page and Chemical Abstracts. Standard abbreviations are preferable. If a new abbreviation is used, it should be defined at its first usage. Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".
E.g. ANN: artificial neural network; CFS: closed form solution...

Abbreviations of units should conform to those shown below:

Decilitre	dl	**Kilogram**	kg
Milligram	mg	**hours**	h
Micrometer	mm	**Minutes**	min
Molar	mol/L	**Mililitre**	ml
Percent	%		

Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (The Royal Society of Medicine London 1977).
Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication".

Formulae, numbers and symbols

1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter 0, and between one (1) and the letter I.
2. Describe all symbols immediately after the equation in which they are first used.
3. For simple fractions, use the solidus (/), e.g. 10 /38.
4. Equations should be presented into parentheses on the right-hand side, in tandem.
5. Levels of statistical significance which can be used without further explanations are *P < 0.05, **P < 0.01, and ***P < 0.001
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. In chemical formulae, valence of ions should be given, e.g. Ca2+ and CO32-, not as Ca++ or CO3.
8. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
9. Greek letters should be explained in the margins with their names as follows: Aα - alpha, Bβ - beta, Γγ - gamma, Δδ - delta, Eε - epsilon, Zζ - zeta, Hη - eta, Θθ - theta, Iι - iota, Kκ - kappa, Λλ - lambda, Mμ - mu, Nν - nu, Ξξ - xi, Oo - omicron, Ππ - pi, Pρ - rho, Σσ - sigma, Tτ - tau, Yυ - ipsilon, Φφ - phi, Xχ - chi, Ψψ - psi, Ωω - omega.

Review/Decisions/Processing

Firstly, all manuscripts will be checked by one of the plagiarism finding tools (iThenticate, PlagScan and or Docol©c). A double-blind reviewing model is used by WVJ for non-plagiarized papers. The manuscript is edited and reviewed by the English language editor and at least 2 reviewers (1 external and 1 internal) selected by section editor of WVJ respectively. Also, a reviewer result form is filled by reviewer to guide authors. Possible decisions are: accept as is, minor revision, major revision, or reject. See sample of evaluation form. The estimated time from submission to first decision is 5.4 weeks and the estimated time from submission to final decision is 6.9 weeks. The estimated time for final publication of accepted manuscript is 6 weeks

To submit a revision please sign in here, fill out the form, and mark "Revised" attach the revision (MS word) and submit when completed. After review and editing the article, a final formatted proof is sent to the corresponding author once again to apply all suggested corrections during the article process. The editor who received the final revisions from the corresponding authors shall not be hold responsible for any mistakes shown in the final publication. Manuscripts with significant results are typically reviewed and published at the highest priority.

Plagiarism: There is a zero-tolerance policy towards plagiarism (including self-plagiarism) in our journals. Manuscripts are screened for plagiarism by one of the plagiarism finding tools (iThenticate, PlagScan and or Docol©c), before or during publication, and if found they will be rejected at any stage of processing. See sample of Docol©c-Report.

Declaration

After manuscript accepted for publication, a declaration form will be sent to the corresponding author who that is responsible to coauthors' agreements to publication of submitted work in WVJ after any amendments arising from the peer review.

Date of issue

The journal will be issued on 25th of March, June, September and December, each year.

Publication charges

No peer-reviewing charges are required. However, the publication costs are covered through article processing charges (APCs). There is a modest APC of 150 Euro(€) editor fee for the processing of each primary accepted paper (1000-4000 words) to encourage high-quality submissions. APCs are only charged for articles that pass the pre-publication checks and are published. A surcharge will be placed on any article that is over 4000 words in length to cover the considerable additional processing costs. Payment can be made by credit card, bank transfer, money order or check. Instruction for payment is sent during publication process as soon as manuscript is accepted. Meanwhile, this journal encourages the academic institutions in low-income countries to publish high quality scientific results, free of charges.

WORD COUNT	PRICE*
1000-4000 words	€150
over 4000 words	€230

* The prices are valid until 30th December 2024.

The Waiver policy

The publication fee will be waived for invited authors, authors of hot papers, and corresponding authors who are editorial board members of the *World's Veterinary Journal* (WVJ). The Journal will consider requests to waive the fee for cases of financial hardship (for high quality manuscripts and upon acceptance for publication). Requests for waiver of the submission fee must be submitted via individual cover letter by the corresponding author and cosigned by an appropriate institutional official to verify that no institutional or grant funds are available for the payment of the fee. Letters including the manuscript title and manuscript ID number should be sent to: editor.wvj@gmail.com. It is expected that waiver requests will be processed and authors will be notified within one business day.

Submission Preparation Checklist

- Authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to the following guidelines.
- The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
- The submission file is in Microsoft Word, RTF, or PDF document file format.
- Where available, URLs for the references have been provided.
- The text is single-spaced; uses a 12-point font; and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
- The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

Scienceline Publication, Ltd.
Editorial Office:
Ömer Nasuhi Bilmen Road, Dönmez Apart., G Block, No:1/6, Yakutiye, Erzurum/25100, Turkey
Homepage: www.science-line.com ; Email: administrator@science-line.com
Phone: +90 538-7708824 (Turkey)

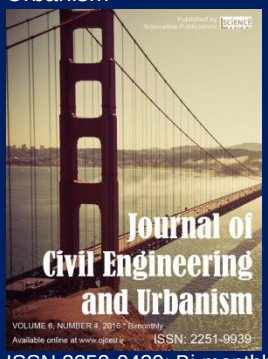
SCIENCELINE PUBLISHING CORPORATION

Scienceline Publication Ltd is a limited liability non-profit non-stock corporation incorporated in Turkey (Company No. 0757086921600001). Scienceline journals that concurrently belong to many societies, universities and research institutes, publishes internationally peer-reviewed open access articles and believe in sharing of new scientific knowledge and vital research in the fields of life and natural sciences, animal sciences, engineering, art, linguistic, management, social and economic sciences all over the world. Scienceline journals include:

Online Journal of Animal and Feed Research

ISSN 2228-7701; Bi-monthly
View Journal | Editorial Board
Email: editors@ojafr.ir
Submit Online >>

Journal of Civil Engineering and Urbanism

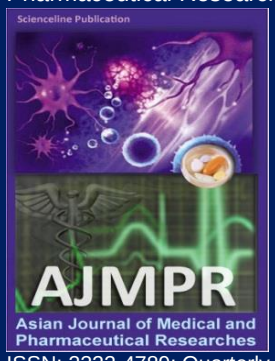


ISSN 2252-0430; Bi-monthly
View Journal | Editorial Board
Email: ojceu@ojceu.ir
Submit Online >>

Journal of Life Sciences and Biomedicine

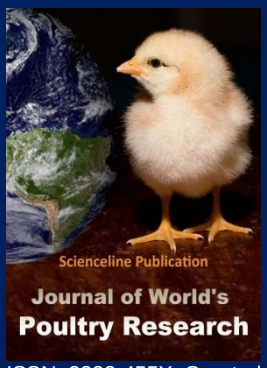
ISSN: 2251-9939; Bi-monthly
View Journal | Editorial Board
Email: editors@jlsb.science-line.com
Submit Online >>

Asian Journal of Medical and Pharmaceutical Researches



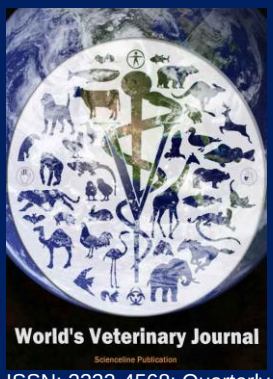
ISSN: 2322-4789; Quarterly
View Journal | Editorial Board
Email: editor@ajmpr.science-line.com
Submit Online >>

Journal of World's Poultry Research



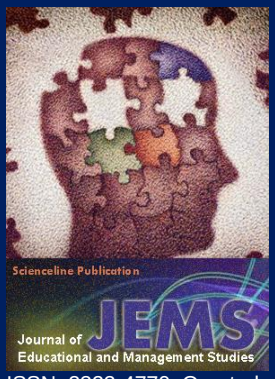
ISSN: 2322-455X; Quarterly
View Journal | Editorial Board
Email: editor@jwpr.science-line.com
Submit Online >>

World's Veterinary Journal



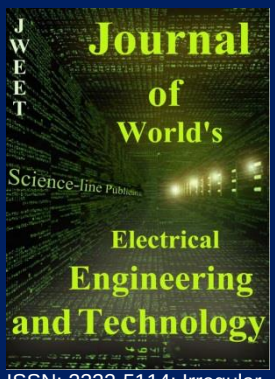
ISSN: 2322-4568; Quarterly
View Journal | Editorial Board
Email: editor@wvj.science-line.com
Submit Online >>

Journal of Educational and Management Studies



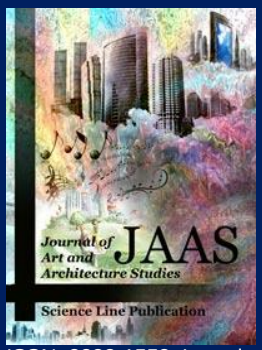
ISSN: 2322-4770; Quarterly
View Journal | Editorial Board
Email: info@jems.science-line.com
Submit Online >>

Journal of World's Electrical Engineering and Technology



ISSN: 2322-5114; Irregular
View Journal | Editorial Board
Email: editor@jweet.science-line.com
Submit Online >>

Journal of Art and Architecture Studies



ISSN: 2383-1553; Irregular
View Journal | Editorial Board
Email: jaas@science-line.com
Submit Online >>

Asian Journal of Social and Economic Sciences

ISSN: 2383-0948; Quarterly
View Journal | Editorial Board
Email: ajses@science-line.com
Submit Online >>

Journal of Applied Business and Finance Researches

ISSN: 2382-9907; Quarterly
View Journal | Editorial Board
Email: jabfr@science-line.com
Submit Online >>

Scientific Journal of Mechanical and Industrial Engineering

ISSN: 2383-0980; Quarterly
View Journal | Editorial Board
Email: sjmie@science-line.com
Submit Online >>

Scienceline is a non-profit organisation inspired by research funders and led by scholars. Our mission is to help researchers accelerate discovery and innovation by operating a platform for research communication that encourages and recognises the most responsible behaviours in science.

Scienceline Publications, Ltd is a limited liability non-profit non-stock corporation registered in the State of Erzurum, Turkey, with company number 0757086921600001, and branch number 18677/25379 at the address: Scienceline Publications, Ltd., Ömer Nasuhi Bilmen Road, Dönmez Apart., G1/6, Yakutiye, Erzurum 25100, Turkey