In the Name of God

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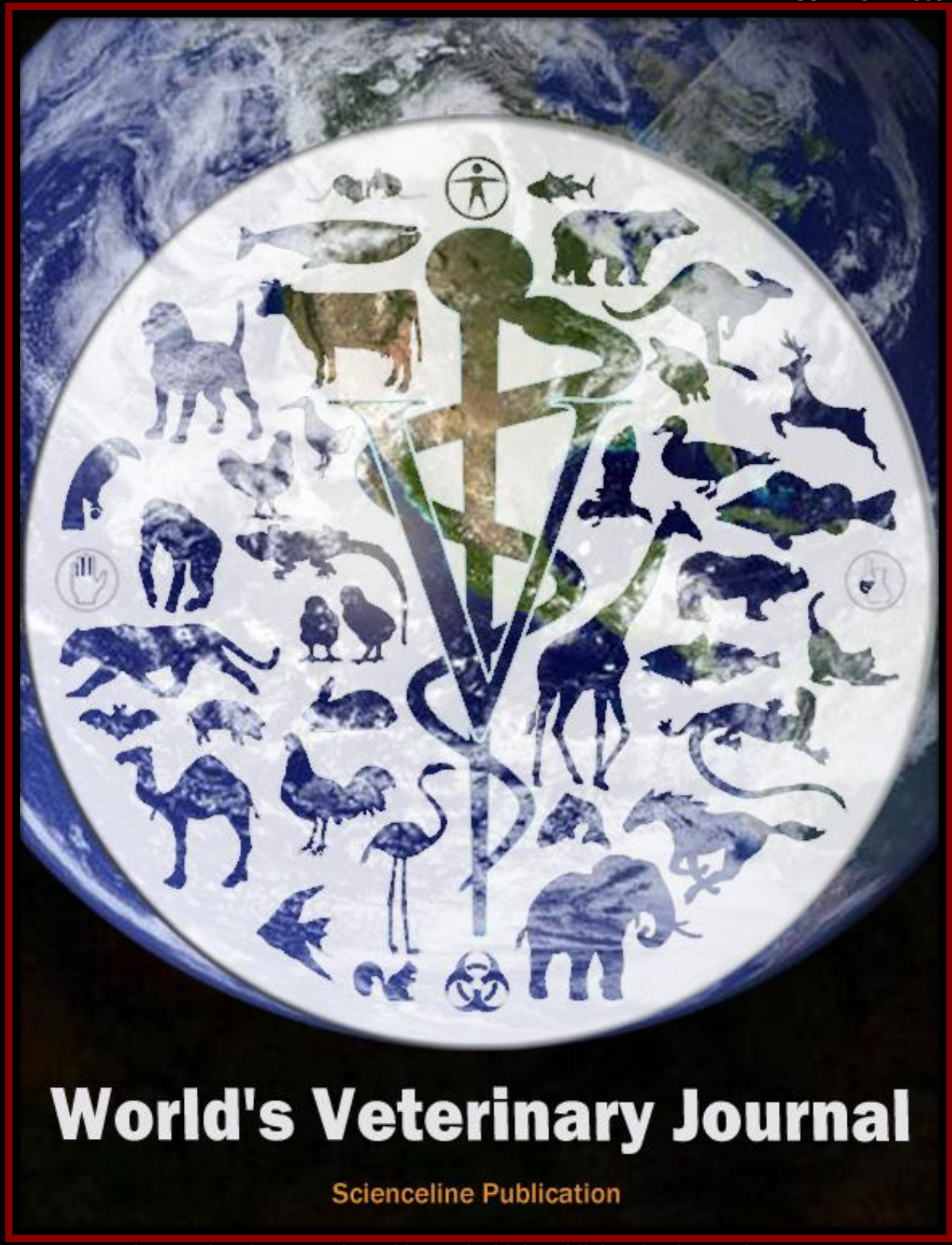

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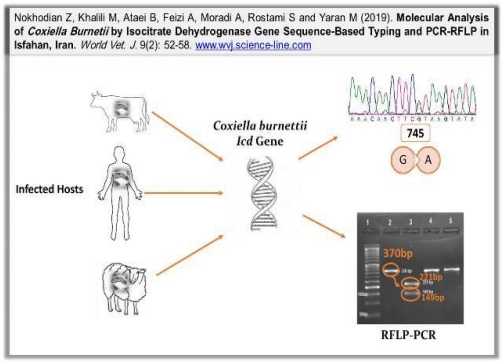
Research Paper



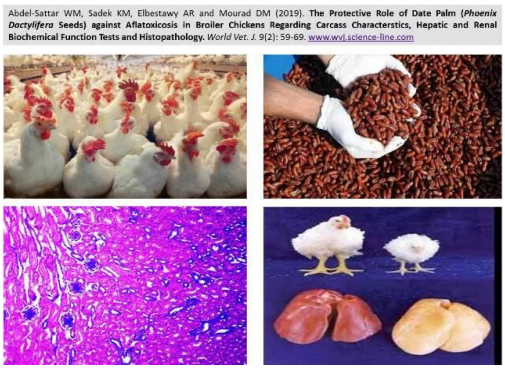
Molecular Analysis of *Coxiella Burnetii* by Isocitrate Dehydrogenase Gene Sequence-Based Typing and PCR-RFLP in Isfahan, Iran.

Nokhodian Z, Khalili M, Ataei B, Feizi A, Moradi A, Rostami S and Yaran M.
World Vet. J. 9(2): 52-58, 2019; pii:S232245681900008-9

ABSTRACT
In the recent years, considerable advances have been made in the detection and genotyping of *Coxiella burnetii*, the causative agent of Q fever. The selection of appropriate genotyping method has enabled description of the clonal diversity of *C. burnetii* around the word. Since, in the place of study, *C. burnetii* genotyping has not been done, the *icd* gene Restriction fragment length polymorphism (RFLP) and sequence-based typing for differentiation between the genomic detected *C. burnetii* from the various sources and compared the two methods is used. In a observational study, a total of 15 genomic positive cases of *C. burnetii* infection from different sources in Isfahan province (Central Iran) were enrolled and underwent two genotyping methods: the *icd* gene PCR-RFLP and *icd* gene sequence-based typing. The degree of similarity between the *icd* gene sequences was high (98.3-100%). In compare with *C. burnetii* Nine Mile *icd* gene sequence, the nucleotide sequences were different at 11 positions, which resulted in 7 differences in the amino acid sequences. After digesting the 370 bp amplified *icd* gene fragments all the samples indicated only one band of 370bp, while amplified *C. burnetii* Nine Mile strain *icd* gene were digested into two bands with sizes of 221bp and 149bp. The results of two genotyping methods matched together. Used methods in present study were cheaper and easier than new methods and they can used for detection of acute and chronic phases of infection.
Keywords: *Coxiella burnetii*, Isocitrate dehydrogenase, Iran, Restriction fragment length polymorphism, Sequence-based typing

[Full text-PDF]

Research Paper



The Protective Role of Date Palm (*Phoenix Dactylifera* Seeds) against Aflatoxicosis in Broiler Chickens Regarding Carcass Characterstics, Hepatic and Renal Biochemical Function Tests and Histopathology.

Abdel-Sattar WM, Sadek KM, Elbestawy AR and Mourad DM.
World Vet. J. 9(2): 59-69, 2019; pii:S232245681900009-9

ABSTRACT
Harmful effects caused by aflatoxin (AF) directed researchers towards to find out new strategies for its control and detoxification increasing the safety of poultry feed. The aim of the present work was to study the protective role of date pits (*Phoenix dactylifera*) seeds against aflatoxicosis regarding carcass traits, biochemical function tests and histopathology of both liver and kidney in broiler chickens. 210 one-day old Arbor Acres broiler chicks were allotted into 7 equal groups as the first control (G1) supplemented by the basal diet, G2 had the basal diet with date pits supplementation 2%, G3 fed on the basal diet with date pits 4%, G4 was fed a basal diet containing 100µg aflatoxin/kg (100 ppb). G5 fed on a basal diet containing Hydrated Sodium Calcium Aluminum Silicates (HSCAS) 0.3% plus aflatoxin, (G6) fed a basal diet containing date pits 2% plus aflatoxin and finally G7 fed a basal diet containing date pits 4% plus aflatoxin. The aflatoxin supplemented to the broiler ration from first day to the end of experiment at 35 days. Aflatoxins supplementation significantly increased relative liver and small intestine weight, affect liver and kidney biochemical function tests and induced histopathological changes as fatty degeneration of hepatocytes, and interstitial nephritis with mononuclear cell infiltrations in both liver and kidney, respectively. However, addition of date pits (2% and 4%) and HSCAS (0.3%) to broiler's diet partially ameliorated these harmful effects of aflatoxins, indicating their protective effect against aflatoxicosis and this protection is dose-related. Addition of date palm seed (2% and 4%) gave a better results regarding carcass traits, biochemical parameters and histopathological examination of liver and kidney, finally concluding

that date palm seed powder could be used as an effective feed additive to control aflatoxicosis in poultry with avoiding harmful effect of chemical mycotoxin binders (HSCAS).

Keywords: Aflatoxins, Broilers, Biochemical traits, Carcass characteristics, Date palm, Histopathological changes.

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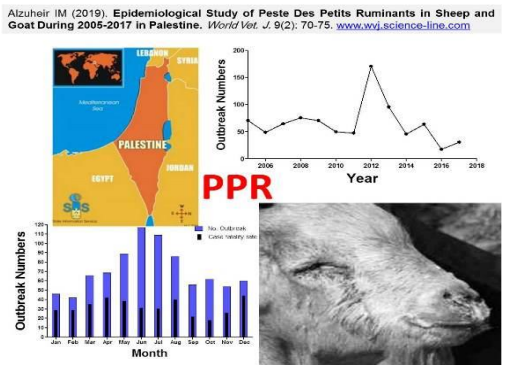
Research Paper



Epidemiological Study of Peste Des Petits Ruminants in Sheep and Goat During 2005-2017 in Palestine.

Alzuheir IM.
World Vet. J. 9(2): 70-75, 2019; pii:S232245681900010-9

ABSTRACT
The objective of this study was to analyze the epidemiological occurrence of Peste des petits ruminants in sheep and goat in Palestine during 2005-2017. Data were collected from the annual agricultural census released by the Palestinian central bureau of statistics and the reports of world organization for animal health, submitted by the general directorate of veterinary services and animal health between 2005 and 2017. The study indicated that Peste des petits ruminants is enzootic in Palestine, reported in each year of the study period. The incidence rate ranged from 1.78 to 14.36% with an average of 6.39% per year and per 104 animals. The average morbidity, morbidity and case fatality rate were 8.89%, 2.89%, and 33.57% respectively. Temporal analysis obtained that Peste des petits ruminants is more epizootic in the dry season between April and August with a significant peak on June. The Peste des petits ruminants vaccination rate in Palestine was low and not well organized, ranged from 0.77-34.39% with an average rate of 9%. The appropriate data recording, improving owner awareness, expand the use of the Peste des petits ruminants vaccine and a systematic disease monitoring program are required to control the spread of the disease.

Keywords: Epidemiology, Goat, Palestine, Peste des petits ruminants, Sheep

[Full text-PDF]

Research Paper

The Influence of Hairline Crack Eggs on Hatchery Parameters and Chicks Performance.

Jabbar A, Hameed A, Yousaf A, Riaz A and Ditta YA.
World Vet. J. 9(2): 76-83, 2019; pii:S232245681900011-9

ABSTRACT
The The purpose of study was to evaluate the influence of hairline crack eggs on hatchery parameters and later life of chicks. The study was conducted from October to December 2018 at Chakri hatchery Salman Poultry Pvt. Ltd Pakistan to evaluate the outcomes of hairline crack eggs. The shell of the eggs is essential in providing the shape of an egg and ensuring the safe packaging. The defects like breakage of this packaging increase the risk of microbial contamination. In this experiment, the crack eggs like hairline crack eggs were detected by Sanovo STAALKAT Alpha 125 Machine number JB 11786. The eggs were collected from eighteen different breeder farms. Each group contained (n= 50,000) eggs. The hairline crack eggs were compared with normal eggs for hatchability, candling, putrification/blasting and dead in shell. Significant difference was found for hatchability, candling, blasting/putrification and dead in the shell for normal and hairline crack eggs. The highest hatchability (49.07 ± 0.51b) and lowest candling (9.98 ± 0.064a) for hairline crack eggs were found for AP27 due to young age and good quality eggshell. The lowest hatchability was found for SP117 which is the oldest flock having thin egg shells. The blasting/putrification and dead in the shell were significantly higher for hairline crack eggs as compared normal eggs of same flocks. The highest blasting of hairline crack eggs was found for SSF6 f, SSF1. The dead in the shell was found highest for SSF6; SSF1 for the hairline crack eggs while lowest blasting was found for AP27 due to young age with good quality eggshell. On simple hatch debris analysis, the highest 1stweek mortality, infertile, contaminated eggs and 3rd week mortality were found for hairline crack eggs as compared to normal eggs for SSF5 flock. The water loss, chick yield and culling chicks percentage were also significantly better for normal eggs compared to hairline crack eggs. The hairline crack eggs of young flocks were better than old flocks due to a better quality of eggs shell. The chicks from normal eggs were also significantly better than chicks from hairline crack eggs in terms of mortality, feed intake, weight gain and FCR. The hairline crack eggs are the source of contamination. Such kinds of eggs should not be used for incubation.

Keywords: Candling, Dead in shell, Hairline crack, Hatchability, Water loss

[Full text-PDF]

Research Paper

Potency of Sansevieria masoniana Extract against Antimicrobial Resistant Bacteria Isolated from Faeces of Pet – Reptile.

Kurnianto A, Puspitasari, Widyaningrum LY, Widiyono I and Prakoso YA.
World Vet. J. 9(2): 84-89, 2019; pii:S232245681900012-9

ABSTRACT
Reptile plays an essential role in human life and act as a reservoir of pathogenic bacteria. It became necessary because of some bacteria resistant against several antibiotics. This study aimed to evaluate the potency of *Sansevieria masoniana* (SM) leaf extract against isolated bacteria from the faeces of pet-reptile. A total of 129 fresh faecal samples were collected from the reptile communities in Surabaya on February 2018 until January 2019. The faeces obtained from 72 snakes, 43 lizards and 14 tortoises. The isolation was conducted using the Micro ID system. All the isolated bacteria were tested against several antibiotics using disc diffusion method, and SM extract using minimum inhibitory concentration test. The isolated bacteria were *Aeromonas hydrophila* (44.96%), *Bacillus sp* (32.55%), *Enterobacter cloacae* (40.31%), *Enterococcus sp* (82.17%), *Escherichia coli* (96.89%), *Proteus sp* (76.74%), *Pseudomonas sp* (48.83%), *Salmonella enteritidis* (55.03%), and *Salmonella enterica arizonae* (53.48%). Those isolated bacteria indicated various resistance patterns against several commercial antibiotics. The minimum concentration of SM extracts that potential to inhibit the colonisation of both resistant and susceptible isolated bacteria was 62.5 mg/mL. This study proved that SM extract potential to inhibit the colonisation of the isolated bacteria from faeces of pet-reptile, even though, several of those isolates resistant against several commercial antibiotics.

Keywords: Antibiotic, Pet – reptile, Reservoir, Resistance, Sansevieria masoniana.

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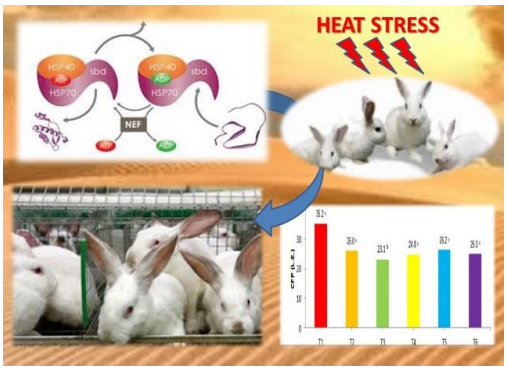
Research Paper



Effect of Early Heat Shock Exposure on Physiological Responses and Reproduction of Rabbits under Hot Desert Conditions.

Sakr OG, Mousa BH, Emam KRS, Morsy AS and Ahmed NA.
World Vet. J. 9(2): 90-101, 2019; pii:S232245681900013-9

ABSTRACT
This study aimed to apply early heat shock exposure programs for releasing HSP70 gene expression to improve production of rabbits reared under hot desert conditions at Egypt. 120 Hi-Plus rabbits, one-day old were randomly divided into six equal treatments (20 rabbits/ treatment), namely T1, T2, T3, T4, T5 and T6. T1 served as control. The rabbits of second, third, fourth, fifth and sixth treatments were exposed to heat shock (36±1 °C for 3 hours from 12:00 - 15:00 for three successive days). Rabbits of T2, T3, T4, T5 and T6 were exposed to heat shock at 3, 25, 60, 3+25 and 3+25+60 days of age, respectively. HSP70 expression and tri-iodothyronine hormone in the rabbits of T2, T3, T4, T5 and T6 were significantly increased. Rectal and fur temperatures, respiration rate, alanine transaminase, corticosterone hormone levels and overall mortality rate significantly decreased in the rabbits exposed to heat shock programs. Red blood cells count, packed cell volume and hemoglobin concentration increased in the rabbits of T2, T3 and T4. Total protein and globulin concentrations increased in the rabbits of T5 when compared to the rabbits of T1, T2 and T6. However, rabbits of T2 and T4 showed an increase in total antioxidant capacity when compared to the rabbits of T1. Conception rate was higher in the does of T5 than that in T3, T4 and T6. Litter traits, productive efficiency index, feed conversion and cost of feeding improved in the rabbits exposed to heat shock programs. In conclusion, applying heat shock exposure programs of rabbits especially T3 treatment, might increase HSP70 gene expression, this led to enhance immunity responses and production under severe heat stress conditions.

Keywords: Heat stress, HSP70, Physiological responses, Productive and reproductive performance, Rabbits

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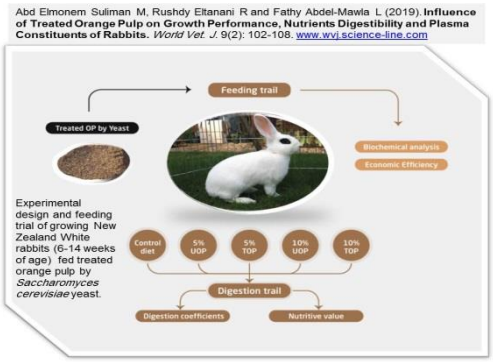
Research Paper



Influence of Treated Orange Pulp on Growth Performance, Nutrients Digestibility and Plasma Constituents of Rabbits.

Abd Elmonem Suliman M, Rushdy Eltanani R and Fathy Abdel-Mawla L.
World Vet. J. 9(2): 102-108, 2019;
pii:S232245681900014-9

ABSTRACT
The current study investigated the effect of replacement of Untreated Orange Pulp (UOP) and Treated Orange Pulp (TOP) protein by basal diet protein on growth performance, digestion coefficients, some blood constitute of rabbits and economic efficiency of growing rabbit diets. Sixty cross bread (New Zealand White, NZW X California), six weeks of age with live body weight ranging from 729.20 to 738.30g were divided to five experimental groups. The experimental diets were T1, control diet without OP; T2, 5%UOP; T3, 5% TOP; T4, 10%UOP and T5, 10%TOP. The results indicated that TOP by *Saccharomyces cerevisiae* yeast increased content of the crude protein (%) and digestible energy (Kcal/kg). The best Final Body Weight (FBW, g), Body Weight Gain (BWG, g/R/day) and feed conversion ratio recorded in 5%TOP group. Digestion coefficient of Crude Protein and Digestible Crude Protein (DCP%) were significantly ($P< 0.05$) increased in rabbits fed low replacement level of OP (5% UOP and 5%TOP). Total lipid of plasma was significantly differences ($P< 0.05$) in groups fed experimental diets compared to control group. Liver function was significantly affected by experimental diets, yeast treatment and replacement level of OP. Best economic efficiency observed with 10%UOP followed by 5%TOP. It was concluded that rabbit group fed 5%TOP recorded a better performance, best digestibility for CP%, DCP and economic efficiency. *Saccharomyces cerevisiae* yeast treatment didn't effect on digestibility and nutritive value of growing rabbits.

Key words: Digestibility, Economic, Growing rabbits, Performance, Plasma, Yeast.

[Full text-PDF]

Research Paper

Effect of Zeolite Dietary Supplementation on Physiological Responses and Production of Laying Hens Drinking Saline Well Water in South Sinai.

Emam KRS, Toraih HM, Hassan AM, El-Far AA, Morsy AS and Ahmed NA.
World Vet. J. 9(2): 109-122, 2019;
pii:S232245681900015-9

ABSTRACT
This study conducted to investigate the effects of dietary zeolite on egg production, egg quality and blood constituents of hens under drinking saline well water. 180 hens were randomly divided into six equal groups (30 hens / group). 1st group (T), hens drank tap water and fed basal diet. The 2nd group (T1), hens drank tap water and fed diet containing 2 % zeolite. The 3rd group (T2), hens drank tap water and fed diet containing 4 % zeolite. 4th group (S), hens drank saline well water and fed basal diet. 5th group (S1), hens drank saline well water and fed diet containing 2 % zeolite. 6th group (S2), hens drank saline well water and fed diet containing 4 % zeolite. Red blood cells and hemoglobin were significant lower in the hens of S compared to other treatments. Hens of S group showed significant decrease in total protein, globulin, glucose and total antioxidant capacity concentrations as compared to the hens of T and T2 groups. Alanine transaminase, aspartic transaminase and creatinine were significantly increased in the hens of S group compared to other treatments. Aldosterone hormone was significantly decreased in the hens of S compared to them in T, T1 and T2 groups. Egg weight significantly increased in the hens of S2 compared with hens in T and S groups. Egg number and egg mass were significant increase in the T1, T2 and S2 compared to hens in T, S and S1 groups. Hens of T1, T2 and S2 groups had significantly improved feed conversion compared to hens of S group. Hens of S group had significantly decreased shell thickness compared to other treatments. In conclusion, under drinking saline well water, addition of zeolite to laying hens' diets at levels 4 % might improve productive performance and eggshell quality.

Keywords: Hematological parameters, Laying hens, Productive performance, Saline water, Zeolite.

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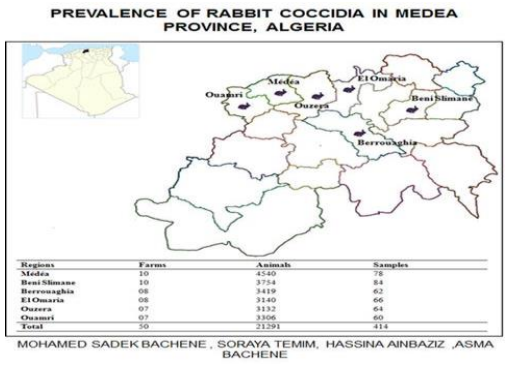
Research Paper



Prevalence of Rabbit Coccidia in Medea Province, Algeria.

Bachene MS, Temim S, Ainbaziz H and Bachene A.
World Vet. J. 9(2): 123-128, 2019;
pii:S232245681900016-9

ABSTRACT
Coccidiosis has an economic impact for poultry and livestock. The current study examined the prevalence of *Eimeria* infections in domestic rabbits in Medea province, North of Algeria. A total of 414 faecal samples were collected from 50 farms in six regions of the province. Each faecal sample was subjected to oocyst counting and isolation. The *Eimeria* species from samples containing isolated and sporulated oocysts were morphologically identified microscopically. The overall prevalence of coccidial infections was 47.6% (197/414). Weaners had the highest prevalence (77%, 77/100, P< 0.0001), followed by growing rabbits (46.8%, 30/64) and the adult rabbits showed the lowest prevalence (36%, 18/50). In breeding rabbits, females were more infected with a prevalence of 40% (P< 0.0001). Eleven rabbit *Eimeria's* species were present and identified from oocyst positive samples. *Eimeria magna* and *Eimeria media* were the most prevalent species (47.6% and 47.3%). Sulfonamides showed a better protection against rabbit coccidiosis than colistin and trimethoprim association (P< 0.0001, prevalence of 23.3% vs. 65.3% respectively). These results indicated that the prevalence of coccidiosis is high among the rabbit population in Medea province, North of Algeria. As a conclusion, it seems that the epidemiological situation of rabbit coccidiosis in Medea province must be taken into consideration in order to minimize the economic losses caused by this parasitosis.

Keywords: *Eimeria*, *Oryctolagus cuniculus*, Rabbit, Sulfonamides

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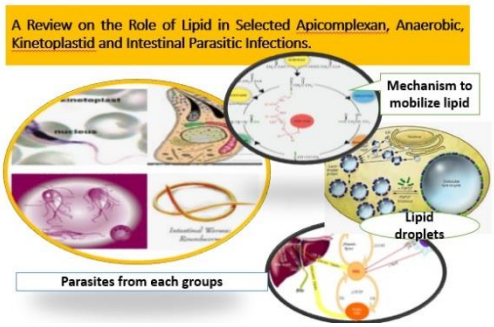
Review



A Review on the Role of Lipid in Selected Apicomplexan, Anaerobic, Kinetoplastid and Intestinal Parasitic Infections.

Yesuf M and Kenubih A.
World Vet. J. 9(2): 129-134, 2019;
pii:S232245681900017-9

ABSTRACT
Lipids are a diverse class of biomolecules that play a major role as energy source, membrane components and cellular signaling molecules. Because of the variation in modes of life, different parasites can partly or fully utilized significant amount lipids during infection. The aims of this paper were to provide an overview to the role of lipids in selected apicomplexan, anaerobic, kinetoplastid and intestinal parasitic infections. Lipid particles are fundamentally engaged in host-pathogen interactions like cell signaling and immunity. As a sources of eicosanoid production, they are involved in different aspects of innate signaling and antigen presentation for the host organism. For the pathogen, lipid droplets also employed to facilitate attachment, empowering pathogenesis and used to subvert host metabolism as ways of immune evasion. The apicomplexan parasites utilized lipid particles for various purpose including changing permeability and fragility of host cells, support the insertion of parasite into the host cell membrane, and promote growth, invasion and optimal replication of the organism. In anaerobic groups of parasites, the lipid plays a considerable role as growth promoter, increasing virulence, facilitate encystation and vesicle formation as well as initiation of immune system and maturation of dendritic cells. Kinetoplastid also engaged in the uptake of essential lipid particles to produce more complex lipids, develop protective mechanisms against host innate and adaptive immunity and support pathogen survival. The lipid bodies also utilized by the intestinal parasites for disease pathogenesis, differentiation and survival of larvae in the host tissue. This review showed that the different in vivo and in vitro studies indicated that lipids have different role in different stage of the parasites infection. The associations between parasites and the lipids were observed during the attachment, invasion and other stages of parasitic infection. So far, evidences in lipid profile alteration related to different parasitic infection suggested that parasites are able to remodel/metabolize host lipids during the overall pathogenesis of parasitic infection.

Keywords: Infection, Lipid, Parasitic, Role

[Full text-PDF]

Research Paper

Productive and Reproductive Performance and Metabolic Profile of Barki Ewes Supplemented with Two Forms of Probiotics as Feed Additives.

El-Hawy AS, El-Bassiony MF, Abo Bakr S, Gawish HA, Badawy MT and Gado HM.
World Vet. J. 9(2):135-145, 2019;
pii:S232245681900018-9

ABSTRACT
Present study aimed to evaluate the impacts of probiotic mixtures as a biological feed additive on the reproductive and productive performance of Barki ewes under desert conditions. A total number of 100 Barki ewes were randomly assigned and divided into five equal groups (20 each) to evaluate the effect of different levels and forms of biological additives mixtures on Barki ewes productivity. The first mixture of probiotic added as liquid forms (Mixture Probiotic Liquid, MPL), while the second added as powder forms (Mixture Probiotic Powder, MPP). The two forms of enzymes used at two levels (6 and 10ml or g/h/d). The two additives formed of exogenous enzymes and obtained through an anaerobic fermentation process of *Ruminococcus flavefaciens*. The results indicated that feed intake was higher in MPL and MPP treated groups compared to control group. During pregnancy and lactation stages, MPL and MPP groups recorded significantly increase in ewes body weight. The conception and lambing rates were tended to differ between groups, but the number of lambs born alive was significantly higher in MPP groups [19 lambs for group 2 (G2) and 18 lambs for group 3 (G3)] followed by MPL groups (16 and 18 lambs for group 4 (G4) and group 5 (G5), respectively), while the control group recorded 18 lambs. The mortality rate from birth to weaning decreased ($P<0.05$) in treated groups with 5%, 5%, 0% and 5% for G2, G3, G4 and G5, respectively, while the mortality rate increased ($P<0.05$) by 11% in control group. The milk yield tended to increase in MPP then MPL groups. The birth and weaning weights as well as average daily gain increased ($P<0.05$) in MPL and MPP groups. Thyroid hormones T3 and T4 concentrations increased ($P<0.05$) with enzymes mixtures supplementations. In conclusion, under the semi-arid conditions, supplementation of exogenous enzyme preparations of MPL and MPP to sheep rations, may improve weaning weight and daily gain of lambs as much as live body weight and milk production of ewes.

Keywords: Biological additives, Productive performance, Reproduction, Milk, Barki sheep

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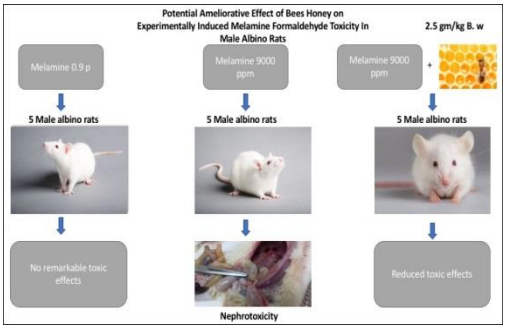
Research Paper

Potential Ameliorative Effect of Bee Honey on Experimentally Induced Melamine Formaldehyde Toxicity in Male Rats.



Hamouda AF, Amin AAE, Ibrahim SS, and Mahmoud MA.
World Vet. J. 9(2): 146-157, 2019;
pii:S232245681900019-9

ABSTRACT
Melamine is considered as one of urea derivatives. Recently it is added to feed stuffs for industrial purposes (falsely elevate its protein contents), however addition of melamine resulted in marked oxidative stress and toxic effect on different body organs, especially the nephrotoxicity and urolithiasis. Therefore, this work is designed to explore the beneficial effect of bee's honey to alleviate the harmful effect induced by melamine toxicity and to show the histological changes on male albino rats. In this work seven animal groups (five rats for each), group 1; negative control, while groups 2, 4, 6 received melamine-formaldehyde orally at dose 0.9, 90, 9000 ppm, respectively while groups 3, 5, 7 received the same melamine dose beside bee's honey (dose of 2.5 gm/kg body weight (B. w) for 45 days. Results declared that melamine treated rats showed marked oxidative, biochemical, hematological changes as well as pathological alterations in vital assets especially liver and urinary system. As distension of the urinary bladder, crystals deposition and stone formation were detected with variable degrees in all groups treated only with melamine. Microscopically, various pathological changes in kidneys, liver, lung, heart and intestine were also demonstrated. The severity of these changes varied from mild to severe changes depending upon the dose of melamine. Interestingly, rats treated with melamine plus the bee's honey showed mild changes in comparison to the only melamine treated rats. These findings assured that, marked antioxidant and ameliorative effect of bee's honey successfully reduced the noxious effect of melamine on different body organs.

Keywords: Melamine, Vital assets toxicity, Bee's honey, White albino rats

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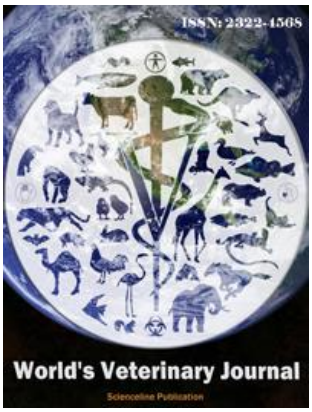
Archive



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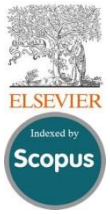

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Molecular Analysis of *Coxiella Burnetii* by Isocitrate Dehydrogenase Gene Sequence-Based Typing and PCR-RFLP in Isfahan, Iran

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ABSTRACT

In the recent years, considerable advances have been made in the detection and genotyping of *Coxiella burnetii*, the causative agent of Q fever. The selection of appropriate genotyping method has enabled description of the clonal diversity of *C. burnetii* around the word. Since, in the place of study, *C. burnetii* genotyping has not been done, the *icd* gene Restriction fragment length polymorphism (RFLP) and sequence-based typing for differentiation between the genomic detected *C. burnetii* from the various sources and compared the two methods is used. In a observational study, a total of 15 genomic positive cases of *C. burnetii* infection from different sources in Isfahan province (Central Iran) were enrolled and underwent two genotyping methods: the *icd* gene PCR-RFLP and *icd* gene sequence-based typing. The degree of similarity between the *icd* gene sequences was high (98.3-100%). In compare with *C. burnetii* Nine Mile *icd* gene sequence, the nucleotide sequences were different at 11 positions, which resulted in 7 differences in the amino acid sequences. After digesting the 370 bp amplified *icd* gene fragments all the samples indicated only one band of 370bp, while amplified *C. burnetii* Nine Mile strain *icd* gene were digested into two bands with sizes of 221bp and 149bp. The results of two genotyping methods matched together. Used methods in present study were cheaper and easier than new methods and they can used for detection of acute and chronic phases of infection.

Key words: *Coxiella burnetii*, Isocitrate dehydrogenase, Iran, Restriction fragment length polymorphism, Sequence-based typing

INTRODUCTION

Coxiella burnetii, the causative agent of Q fever in humans and animals, is a gram negative highly infectious coccobacillus, with an infectious dose of less than ten organisms (Massung et al., 2012). This bacterium has been found in all parts of the world except New Zealand and Antarctica (Sidi-Boumedine and Rousset, 2011; Prevention, 2013). The main reservoirs of human infection are ruminant such as cattle, goats and sheep (Capin et al., 2013). Humans are accidentally infected by *C. burnetii*. A common rout of transmission of *C. burnetii* to humans occurs through inhalation of contaminated aerosols arising from the infected animal body fluids. However, human infection has also occurred via the following ways: placental transmission to the fetus (Raoult and Stein 1994), blood transfusions (Pantanowitz et al., 2002) and consumption of raw milk (Signs et al., 2012). *C. burnetii* is also a potential bioterrorism agent and belongs to the category B of CDC –list (Massung et al., 2012). In human Q fever presents in two forms: acute and chronic. Acute disease often manifests as self-limiting febrile flu-like illness, pneumonia and hepatitis (Prevention, 2013). Whereas, chronic Q fever is a serious condition that present in forms of endocarditis, vascular infections and bone and joint infections (Million and Raoult, 2015). Even Q fever infection was seen as coinfection inside Scrub Typhus (Jeong et al., 2019).

Because Q fever is considered as a zoonotic disease, the human infection epidemiology relates to the circulation of the bacterium in animal reservoirs (Eldin et al., 2017). On the other hand, differentiation between the *C. burnetii* isolates is important in diagnostic and epidemiological research, due to the vast extent of Q fever infection and multiple hosts of *C. burnetii*. Genotyping can be a key tool for understanding and follow up the epidemiology of the Q fever and by using that it can be find the animal source of human infection (Eldin et al., 2017). Furthermore, in a study by Van Nguyen et al.

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(1999) was suggested that the differences at the molecular level between the strains of *C. burnetii* may be responsible for acute or chronic forms of Q fever (Van Nguyen and Hirai, 1999). The *C. burnetii icd* gene encoding isocitrate dehydrogenase is an acid-induced and housekeeping gene that may be associated with the ability of the bacterium to replicate in the acidic environment of the phagolysosomes (Van Nguyen and Hirai, 1999; Van Nguyen et al., 1999).

Several genotyping methods have been developed for differentiating *C. burnetii* isolates. One of these techniques is Restriction fragment length polymorphism (RFLP) analysis of genomic DNA and PCR-RFLP of specific genes (Eldin, et al., 2017). In the present study, we used the *icd* gene PCR-RFLP and sequence-based typing for differentiation between the genomic detected *C. burnetii* from the various sources. In addition, in this survey we compared the obtained *icd* gene sequences with some *C. burnetii* strains *icd* gene sequences submitted in GenBank and we found the relationship between the isolates based on the *icd* gene sequence.

MATRIALS AND METHODS

Ethical approval

Written informed consent was obtained from all individuals and farm owners and the study protocol was approved by the ethics committee of Isfahan University of medical sciences (No. 194033).

Bacteria

In a series of cross-sectional studies conducted by Isfahan Infectious Diseases and Tropical Medicine Research Center (Grant No. 293390 to 293393) on May to June 2015 in Isfahan province, Iran, 34 genomic positive cases of *C. burnetii* infection from different sources (Human whole blood, animal whole blood including: Sheep and Cow and Bulk Tank Milk (BTM) from dairy Cows) were detected. The present study was performed in the following of the mentioned studies with grant no. 194033. We randomly selected 15 cases from 34 positive cases that the original sources and other characteristics of them are shown in table 1.

C. burnetii icd gene nested PCR

Bacterial DNA was extracted from human and animal (Sheep and Cow) whole blood and BTM from dairy cows samples using the YTA Genomic DNA Extraction mini kit (Yekta Tajhiz Azma Co., Tehran., Iran) according to the manufacturer's instructions. Oligonucleotide primers used in this survey are presented in Table 2. First of all, we amplified a 400 bp fragment using *icd*1-F and *icd*2-R primers and then a 370 bp fragment using *icdN*-F and *icdN*-R primers (Van Nguyen and Hirai, 1999). PCR reaction was performed in a 25 µl mixture containing: 1X PCR buffer, 1.5 mM $MgCl_2$, 200 mM of each deoxynucleotide triphosphate (dNTP), 1U of Taq DNA polymerase (SinaClon Bioscience Co., Tehran., Iran), 0.2 mM of each primer (Bioneer Co., Daejeon., Korea) and 5 µl genomic DNA for first step of PCR and 1 µl PCR product for second step of PCR. PCR conditions were programmed in T100^{TM} Thermal Cycler (Bio-Rad, USA) for both steps as follows: Initial denaturation at 94°C for 3 min; followed by 35 cycles at 94°C for 60 s, 58°C for 45 s and 72°C for 45s and final extension at 72°C for 10 min. PCR products of second step were separated with electrophoresis on 2% agarose gel (SinaClon Bioscience Co., Tehran., Iran) and after staining with ethidium bromide, visualized under UV gel documentation system. Genomic DNA of *C. burnetii* Nine Mile strain was used as a positive control.

Sequencing of the *icd* gene fragments

For DNA sequencing, amplified products of second step of PCR underwent bidirectional Sanger sequencing using the ABI 3730 XL DNA analyzer (Applied Biosystems, USA) by Bioneer Co., Korea. The obtained sequences were blasted against the nucleotide database of the National Center for Biotechnology Information (NCBI, 2019). Then, the obtained sequences were aligned against the *icd* gene sequences of *C. burnetii* Nine Mile and 5 other *C. burnetii* strains using the Clustal W v2.0 software. The *icd* gene sequences accession numbers of used *C. burnetii* strains are as follows: AF069035 (Nine Mile, Type strain for acute Q fever), AF146291 (Bangui strain isolated from acute Q fever), AF146285 (TK-1 strain isolated from acute Q fever), AF146294 (Priscilla, Type strain for chronic Q fever), NC_011527.1 (CbuG_Q212 strain isolated from chronic Q fever) and CP001020.1 (CbuK_Q154 strain isolated from chronic Q fever). Phylogenetic tree was constructed by MEGA Version 6.0 (Koichiro Tamura, 2013) and Neighbor-Joining method (Saitou and Nei, 1987).

PCR-restriction fragment length polymorphism (PCR-RFLP) analysis

The *icd* gene nested-PCR products were digested with the FastDigest *Bsh1236* I restriction enzyme (Thermofisher Scientific., USA) as described by the manufactures. This enzyme recognizes CG↓CG site. Briefly, in a 30 µl reaction containing: 2 µL of appropriate 10X buffer, 1 µL of *Bsh1236* I enzyme and 17 µL distilled water, 10 µL of PCR products was added and incubated at 37°C for 5 minutes. Then, microtubes were incubated at 80°C for 10 minutes to deactivate

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the enzyme. Digested products were separated by electrophoresis on 2% agarose gel containing 0.1 µl/ml ethidium bromide and were visualized using an UV gel documentation system. Genomic DNA of *C. burnetii* Nine Mile strain was used as the control.

Table 1. Characteristics of the evaluated genomic positive cases of *C. burnetii* in this study

Samples	**Characteristics**					
Origin: Human	**Age (years)**	**Sex**	**Occupation**	**Length of employment (years)**	**Consumption of unpasteurized milk**	
CH3	30	Male	Butcher	9	No	
DH4	43	Male	Farmer	15	Yes	
EH5	48	Male	Slaughterer	30	Yes	
Origin: BTM	**Reproductive disorders in the herd**	**Types of cattle herds**	**Contact With other ruminant species**	**Contamination with ticks in the herd**		
AM1	No	Traditional	No	No		
IM9	Yes	Traditional	No	No		
TM20	Yes	Traditional	Yes	No		
VM22	No	Traditional	No	No		
WM23	No	Commercial	No	No		
XM24	No	Traditional	No	No		
Origin: Ruminants	**Type**	**Age (mounts)**	**Sex**	**Strain**	**Reproductive disorders**	**Contamination with ticks**
FA6	Sheep	36	Female	Mixed	No	Yes
GA7	Sheep	40	Male	Afshari	No	No
HA8	Cow	54	Female	Holstein Friesians	No	No
JA10	Cow	50	Female	Holstein Friesians	No	No
RA18	Sheep	40	Female	Afshari	No	No

Table 2. Primers sequences used in the study

Name of primers	**Sequences (5'-3')**	**Amplicon size**	**References**
***icd*1-F**	CGGAGTCTCTTAGTGATGACGGA	400 bp	(Van Nguyen and Hirai, 1999)
***icd*2-R**	GCCTTCTTTAGAAACCGGTTTAA		
***icd*N-F**	GGAGTTAACCGGAGTATCCA	370 bp	
***icd*N-R**	ATTGAGCGAACGTATGCCAC		

RESULTS

Fifteen obtained partial *icd* gene sequences were aligned and compared with six *C. burnetii icd* gene sequences derived from GeneBank. The degree of similarity between the nucleotide sequences of present study was high (98.3-100%). In compare with *C. burnetii* Nine Mile *icd* gene sequence (GenBank accession No.: AF069035.1), presentnucleotide sequences were different at 11 positions, which resulted in seven differences in the amino acid sequences (Table 3). Point mutation was the cause of all changes and other type of mutations were not seen. Among our sequences there was a common mutation at position 745 according to the *C. burnetii* Nine Mile *icd* gene sequence. In six samples just one point mutation (745 G→A) were seen. However, in seven samples were seen two different point mutations and in other two samples were observed three other point mutations. Figure 1 showed the phylogenetic tree constructed based on the *icd* gene sequences obtained in this study and *C. burnetii icd* gene sequences from six mentioned strains.

After digesting the 370 bp *C. burnetii* Nine Mile strain *icd* gene amplified with primers *icd* N-F and *icd* N-R, two bands were produced with size of 221bp and 149bp on the agarose gel. In contrast, amplified fragments from all the samples indicated only one band of 370bp. Numbers of the PCR-RFLP patterns of C. *burnetii* samples are presented in figure 2.

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Table 3. observed mutations in the samples compared with C. *burnetii* Nine Mile strain

Sample No.	Mutations		Gene Bank accession no.
	Nucleotide changes	Amino acid changes	
AM1	745 G→A	Ala53→Thr	KY962668.1
CH3	745 G→A/ 866 A→G	Ala53→Thr /Asp93→Ser	KY962669.1
DH4	745 G→A/ 800 A→G	Ala53→Thr /Lys71→ Arg	KY962670.1
EH5	745 G→A/ 690 T→A/ 861 A→G	Ala53→Thr	KY962671.1
FA6	745 G→A/ 883 A→T	Ala53→Thr/ Thr99→ Ser	KY962672.1
GA7	745 G→A	Ala53→Thr	KY962673.1
HA8	745 G→A/ 811 G→A	Ala53→Thr /Glu75→ Lys	KY962674.1
IM9	745 G→A/ 849 A→T	Ala53→Thr	KY962675.1
JA10	745 G→A	Ala53→Thr /Lys87→Asp	KY962680.1
RA18	745 G→A/ 934 A→C	Ala53→Thr	KY962681.1
VM22	745 G→A	Ala53→Thr	KY962676.1
WM23	745 G→A/ 750 C→T	Ala53→Thr	KY962677.1
XM24	745 G→A/ 866 A→G/902A→G	Ala53→Thr /Asp93→Ser/Glu105→Gly	KY962678.1
ZA25	745 G→A	Ala53→Thr	KY962679.1
TM20	745 G→A	Ala53→Thr	KY962682.1

No: Number

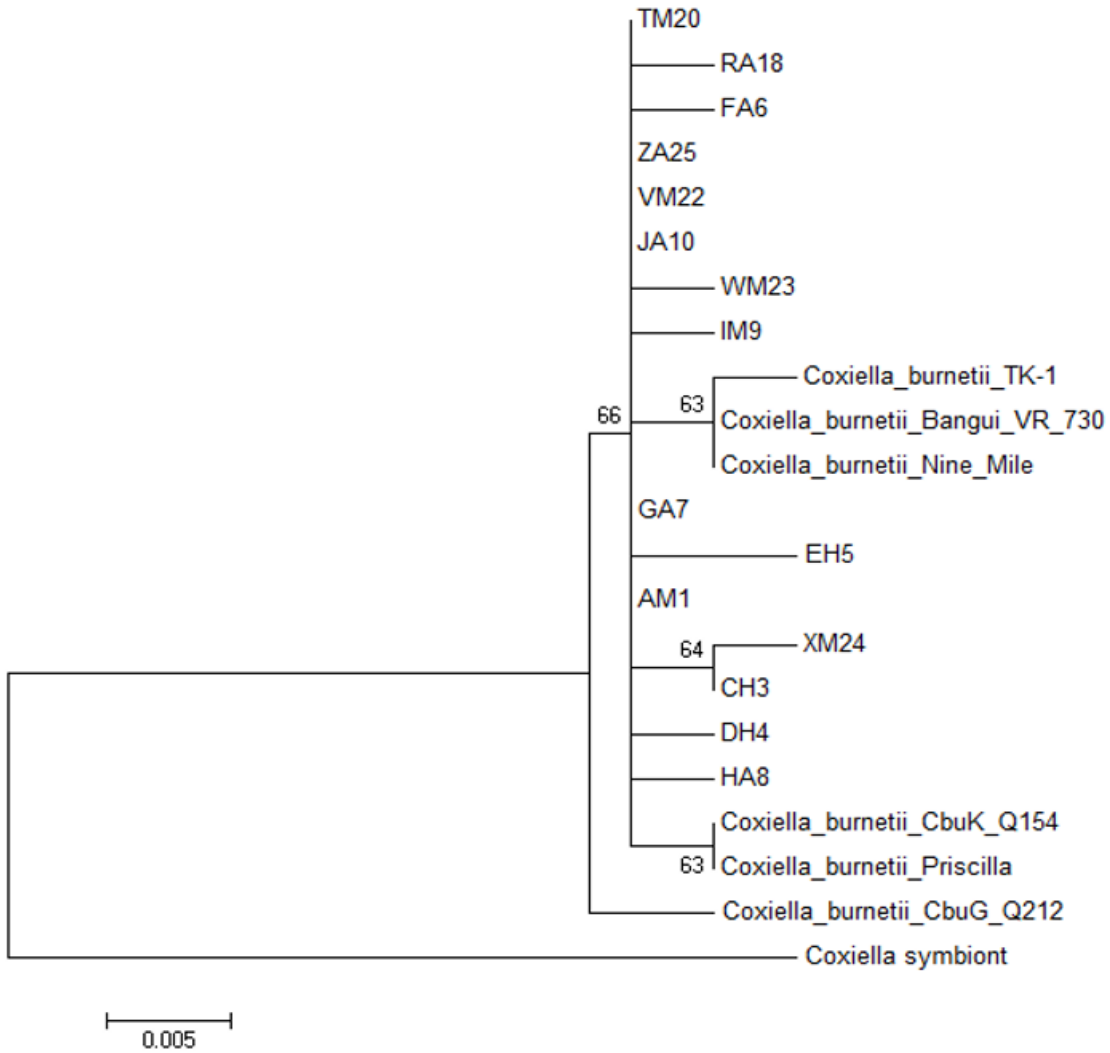

Figure 1. Phylogenetic tree constructed based on the icd gene sequences. The phylogenetic tree was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the p-distance method and were in the units of the number of base differences per site. The tree was rooted using *C. symbiont* as the out group. The support of each branch, as determined from 1000 bootstrap samples, is indicated by percentages at each nods.

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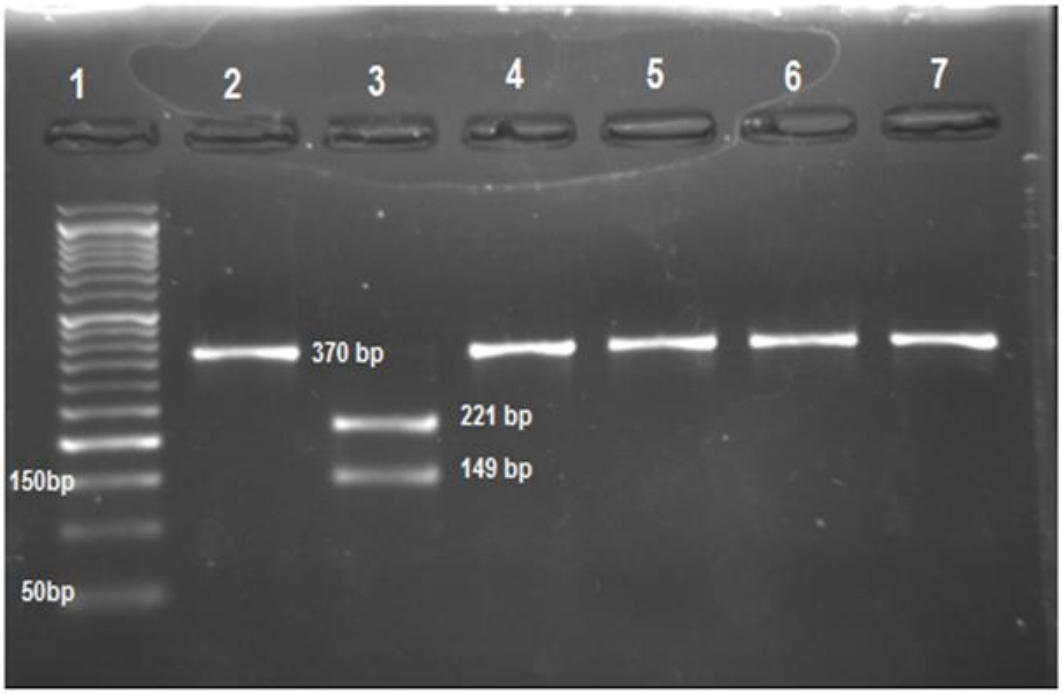

Figure 2. PCR-RFLP patterns of the icd gene from numbers of *C. burnetii* samples. Lanes: 1, 50bp DNA ladder; 2, undigested nested-PCR product from C. burnetii Nine Mile strain; 3, Bsh1236 I –digested nested-PCR products of C. burnetii Nine Mile strain; 4-7, Bsh1236 I –digested nested-PCR products of samples

DISCUSSION

In the recent years, the different epidemiological profile of *C. burnetii* infections was reported in Iran. In the seroprevalence studies Q fever infection rates varied from 7.8% to 68% in the different sources (animals and high-risk population) (Khalili and Sakhaee, 2009; Aflatoonian et al., 2014; Azizzadeh et al., 2014; Esmaeili et al., 2014; Esmaeili et al., 2017, Khalili et al., 2014; Nokhodian et al., 2017). In the molecular studies the prevalence rate of *C. burnetii* DNA was reported from 0% to 48.15% in various samples (animals, human and ticks) by amplification of different genes included *IS1111*, *16S rRNA* and *Com1* (Rahimi et al., 2010; Dehkordi 2011; Jamshidi et al., 2014; Khademi et al., 2014; Nokhodian et al., 2016). Unfortunately, until now, genotyping studies have not been done in Iran and according to the relative high prevalence of *C. burnetii* infection in this area, because of tremendous importance, to find the relationship between the isolates and the sources of the infection. In this study, for the first time in Iran, the number of isolates with the type strains of *C. burnetii* using the two simple and fast genotyping methods were compared. Based on the sequencing, it found that all the isolates had a common point mutation at nucleotide position 745 in the *icd* gene fragments. This point mutation also was seen in *icd* gene sequences of Priscilla (Type strain for chronic Q fever), CbuG_Q212 and CbuK_Q154 strains that isolated from chronic Q fever. However, this point mutation was not seen in *icd* gene sequences of Nine Mile strain (Type strain for acute Q fever), Bangui and TK-1 strains that isolated from acute Q fever.

On the other hand, in the Nine Mile strain *icd* gene amplified sequence there is one restriction site for *Bsh1236* I restriction enzyme (position 744 to 747) and as it revealed in figure 2; Nine Mile strain nested-PCR product digested and two band were observed in agarose gel. However, none of samples were digested by restriction enzyme, so only one band was observed. Since the common point mutation in the isolates located at nucleotide position 745 and this site is located in restriction site, the results of both methods are consistent. Van Nguyan and Hirai (1999) studied the *icd* gene profile of 19 *C. burnetii* isolates. Based on the gene sequences, they divided the isolates into three groups included one group originated from acute Q fever and two groups originated from chronic Q fever. Similar to the results they found a common point mutation at position 745 in isolates originated from chronic Q fever (Van Nguyen and Hirai, 1999). In another study by Ando et al., using *icd* gene PCR-RFLP and sequencing analysis, 49 of 72 isolates had a completely identical nucleotide sequences and these isolates, same as 6 the isolates, had only one point mutation in position 745 (G→A). They called these isolates "Japanese-specific" isolates and they claimed that the *icd* sequences of these isolates were not similar to other chronic *C. burnetii* strains (Andoh et al., 2004). However, in this study, all 6 identical nucleotide sequences were same as the chronic isolates submitted in GeneBank. Unfortunately, in the present study, no records about the clinical manifestation have seen (acute or chronic) of sources of isolates. However, according to these

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results, the isolates could be originated from the chronic or persistent focalized infections.Evaluating more *C. burnetii* isolates from different geographical area of Iran along with complete clinical information using these two methods is recommended in resent study. According to the primary studies, the initial steps of Citric Acid Cycle (CAC) pathway revealed the least conservation and changes in the genes encoding initial enzymes can effective on bacterial adaptations to different environments (Huynen et al., 1999). Since the isocitrate dehydrogenase enzyme is a member of the initial steps of CAC enzymes, mutations in the *icd* gene may be related to the *C. burnetii* pathogenicity and virulence. Therefore, it is suggestted more attention and study on the role of the isocitrate dehydrogenase enzyme in the different *C. burnetii* strains in the various environments.

Currently, the new genotyping methods were developed such as multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA), Multi-spacer Sequence Typing (MST), and Single Nucleotide Polymorphism (SNP) genotyping (Eldin et al., 2017). Since it is better to determine the phase of the disease (Acute or chronic infection) and type of the genotype of the bacterium in the epidemiological studies and to decide for treatment of the infection, so use of the RFLP method, that is cheaper and easier than sequenced-based methods, can be helpful.

DECLARATION

Acknowledgements

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Competing interests

All authors have no conflict of interest.

Consent to publish

All authors gave their informed consent prior to their inclusion in the study.

Authors' contributions

ZN, MK, BA and AF conceived and designed the project. ZN, MK and SR Managed activities to annotate (produce metadata), scrub data and maintain research data for initial use and later re-use. MY Verified whether as a part of the activity or separate, of the overall replication of results and other research outputs. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

Aflatoonian MR, Khalili M, Sami M and Abiri Z (2014). The frequency of IgM anti-*Coxiella burnetii* (Q fever) antibodies among slaughterhouse workers in kerman city, 2012. Journal of Kerman University of Medical Sciences, 21 (5): 368-375.

Andoh M, Nagaoka H, Yamaguchi T, Fukushi H and Hirai K (2004). Comparison of japanese isolates of *Coxiella burnetii* by PCR-RFLP and sequence analysis. Microbiology and immunology, 48 (12): 971-975. PMID: 15611614

Azizzadeh M, Taghavi Razavizadeh SAR and Mehrzad J (2014). Seroepidemiology of coxiellosis (Q fever) in sheep and goat populations in the northeast of iran. Iranian Journal of Veterinary Research, 15(1):1-6.

Capin GA, Emre Z, Canpolat S, Vatansever Y and Duzgun A (2013). Detection of *Coxiella burnetii* from ticks by polymerase chain reaction and restriction fragment length polymorphism. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 2013;60:263-268.

Dehkordi FS (2011). Prevalence study of *Coxiella burnetii* in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. American Journal of Animal and Veterinary Sciences, 6:180-186. DOI:https://doi.org/10.3844/ajavsp.2011.180.186

Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, Mege JL, Maurin M and Raoult D (2017). From Q fever to *Coxiella burnetii* infection: A paradigm change. Clinical Microbiology Reviews, 30 (1): 115-190. DOI:https://doi.org/10.1128/CMR.00045-16

Esmaeili S, Golzar F, Ayubi E , Naghili B and Mostafavi E (2017). Acute Q fever in febrile patients in northwestern of Iran. PLOS Neglected Tropical Diseases, 11(4):e0005535. DOI:http://doi.org/10.1371/journal.pntd.0005535

Esmaeili S, Bagheri Amiri F and Mostafavi E (2014). Seroprevalence survey of Q fever among sheep in northwestern iran. Vector-Borne and Zoonotic Diseases, 14 (3): 189-192. DOI: https://doi.org/10.1089/vbz.2013.1382

Huynen MA, Dandekar T and Bork P (1999). Variation and evolution of the citric-acid cycle: A genomic perspective. Trends in Microbiology, 7 (7): 281-291. PMID: 10390638

Jamshidi A, Razmyar J and Borji S (2014). Identification of *Coxiella burnetii* by touch-down PCR assay in unpasteurized milk and dairy products in north-east of iran. Iranian Journal of Veterinary Medicine, 8 (1): 15-19.

Jeong HJ, Choi S, Lee J, Gill B, Lee KM, Lee YS, Kim CM, Yun NR and Kim DM (2019). Case Report: Scrub Typhus and Q Fever Coinfection. American Journal of Tropical Medicine and Hygiene, ahead of print. DOI:https://doi.org/10.4269/ajtmh.18-0092

Khademi P, Mahzounieh MR, Kahrizsangi AE and Shdravan E (2014). Genomic detection of *Coxiella burnetii* in goat milk samples in animal farms khorramabad township, iran. Pajoohandeh Journal, 19 (3): 162-168.

Khalili M, Mosavi M, Diali HG and Mirza HN (2014). Serologic survey for *Coxiella burnetii* phase II antibodies among slaughterhouse workers in kerman, southeast of iran. Asian Pacific Journal of Tropical Biomedicine, 4 (Suppl 1): S209-212. DOI:https://doi.org/10.12980/APJTB.4.2014C1268

Khalili M and Sakhaee E (2009). An update on a serologic survey of Qfever in domestic animals in iran. American Journal of Tropical Medicine and Hygiene, 80 (6): 1031-1032. PMID: 19478271

Koichiro T, Stecher G, Peterson D, Filipski A and Kumar S (2013). Mega6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30 (12): 5. DOI:https://doi.org/10.1093/molbev/mst197

Massung RF, Cutler SJ and Frangoulidis D (2012). Molecular typing of *Coxiella burnetii* (Q fever). Advances in Experimental Medicine and Biology, 984: 381-396. DOI:https://doi.org/10.1007/978-94-007-4315-1_19

Million M and Raoult D (2015). Recent advances in the study of Q fever epidemiology, diagnosis and management. Journal of Infectio, 71 Suppl 1: S2-9. DOI:https://doi.org/10.1016/j.jinf.2015.04.024

National Center for Biotechnology Information (NCBI) (2019). availabe at http://www.ncbi.nlm.nih.gov/BLAST

Nokhodian Z, Ataei B, Moradi A, Yaran M, Hoseini SG, Feizi A and Sherkat R (2017). Seroprevalence and risk factors of *Coxiella burnetii* infection among high-risk population in center of iran, a neglected health problem. Acta Tropica, 169: 107-111. DOI:https://doi.org/10.1016/j.actatropica.2017.02.002

Nokhodian Z, Feizi A, Moradi A, Yaran M, Hoseini SG, Ataei B and Hosseini M (2016). Detection and risk factors of *Coxiella burnetii* infection in dairy cattle based on bulk tank milk samples in center of iran. Preventive Veterinary Medicine, 134: 139-144. DOI:https://doi.org/10.1016/j.prevetmed.2016.10.003

Pantanowitz L, Telford SR and Cannon ME (2002). Tick-borne diseases in transfusion medicine. Transfusion Medicine, 12 (2): 85-106. PMID: 11982962

Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J and Kersh GJ (2013). Diagnosis and management of q fever — united states, 2013: Recommendations from cdc and the q fever working group. Morbidity and mortality weekly report, 62 (3): 32. PMID: 23535757

Rahimi E, Doosti A, Ameri M, Kabiri E and Sharifian B (2010). Detection of *Coxiella burnetii* by nested pcr in bulk milk samples from dairy bovine, ovine, and caprine herds in iran. Zoonoses and public health, 57 (7-8): e38-e41. DOI:https://doi.org/10.1111/j.1863-2378.2009.01289.x

Raoult D and Stein A (1994). Q fever during pregnancy -- a risk for women, fetuses, and obstetricians. New England Journal of Medicine, 330 (5): 371-371. DOI: https://doi.org/10.1056/NEJM199402033300519

Saitou N and Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4 (4): 406-425. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sidi-Boumedine K and Rousset E (2011). Molecular epidemiology of q fever: A review of *Coxiella burnetii* genotyping methods and main achievements.

Signs KA, Stobierski MG and Gandhi TN (2012). Q fever cluster among raw milk drinkers in michigan, 2011. Clinical Infectious Diseases, 55 (10): 1387-1389. DOI: https://doi.org/10.1093/cid/cis690

Van Nguyen S and Hirai K (1999). Differentiation of *Coxiella burnetii* isolates by sequence determination and PCR-restriction fragment length polymorphism analysis of isocitrate dehydrogenase gene. FEMS Microbiology Letter, 180 (2): 249-254. DOI:https://doi.org/10.1111/j.1574-6968.1999.tb08803.x

Van Nguyen S, To H, Yamaguchi T, Fukushi H and Hirai K (1999). Molecular cloning of an immunogenic and acid-induced isocitrate dehydrogenase gene from *Coxiella burnetii*. FEMS Microbiololgy Letter, 175 (1): 101-106. DOI:https://doi.org/10.1111/j.1574-6968.1999.tb13607.x

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The Protective Role of Date Palm (*Phoenix Dactylifera* Seeds) against Aflatoxicosis in Broiler Chickens Regarding Carcass Characterstics, Hepatic and Renal Biochemical Function Tests and Histopathology

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ABSTRACT

Harmful effects caused by aflatoxin (AF) directed researchers towards to find out new strategies for its control and detoxification increasing the safety of poultry feed. The aim of the present work was to study the protective role of date pits (*Phoenix dactylifera*) seeds against aflatoxicosis regarding carcass traits, biochemical function tests and histopathology of both liver and kidney in broiler chickens. 210 one-day old Arbor Acres broiler chicks were allotted into 7 equal groups as the first control (G1) supplemented by the basal diet, G2 had the basal diet with date pits supplementation 2%, G3 fed on the basal diet with date pits 4%, G4 was fed a basal diet containing 100µg aflatoxin/kg (100 ppb). G5 fed on a basal diet containing Hydrated Sodium Calcium Aluminum Silicates (HSCAS) 0.3% plus aflatoxin, (G6) fed a basal diet containing date pits 2% plus aflatoxin and finally G7 fed a basal diet containing date pits 4% plus aflatoxin. The aflatoxin supplemented to the broiler ration from first day to the end of experiment at 35 days. Aflatoxins supplementation significantly increased relative liver and small intestine weight, affect liver and kidney biochemical function tests and induced histopathological changes as fatty degeneration of hepatocytes, and interstitial nephritis with mononuclear cell infiltrations in both liver and kidney, respectively. However, addition of date pits (2% and 4%) and HSCAS (0.3%) to broiler's diet partially ameliorated these harmful effects of aflatoxins, indicating their protective effect against aflatoxicosis and this protection is dose-related. Addition of date palm seed (2% and 4%) gave a better results regarding carcass traits, biochemical parameters and histopathological examination of liver and kidney, finally concluding that date palm seed powder could be used as an effective feed additive to control aflatoxicosis in poultry with avoiding harmful effect of chemical mycotoxin binders (HSCAS).

Key words: Aflatoxins, Broilers, Biochemical traits, Carcass characteristics, Date palm, Histopathological changes.

INTRODUCTION

Aflatoxins are belonging to a heterologous group of fungal secondary metabolites called mycotoxins that adversely affecting human and animal health. Aflatoxins have been most commonly produced by strains of *Aspergillus flavus*, *A. parasiticus*, and *A. nominus*. Also, many other *Aspergilli* including *Emericella* teleomorphs have aflatoxigenic capabilities. They are named according to their blue or green fluorescence under UV light, there are four primary aflatoxins: AFB1, AFB2, AFG1, and AFG2. AFB1 is the most hepatotoxic, mutagenic, and prevalent worldwide (Rawal et al., 2010).

In poultry, consumption of AFB1 cause huge economic losses by retarding bird growth, increasing feed efficiency, damage to internal organs as liver and kidney, increased leg problems, increased the incidence of secondary infections leading to increased mortalities and carcass condemnation (Bintvihok and Kositcharoenkul, 2006). Additionally, it causes immuno-suppression and changes in relative organs weight (Kubena et al., 1993). Aflatoxins cause a wide range of metabolic changes in poultry associated with reduced digestive enzyme activities, and immunosuppression (Edds and Bortell, 1983).

Inorganic absorbents, such as hydrated sodium calcium aluminosilicates (HSCAS), sodium bentonite, zeolites or super-activated charcoal, can bind the aflatoxin before its absorption in the gastrointestinal tract and are shown to effectively reduce aflatoxicosis (Edrington et al., 1996), and addition of dietary chemicals such as ammonium hydroxide, calcium hydroxide, hydrogen peroxide, sodium hydroxide, and sodium hypochlorite is a stratgey to detoxify ingested aflatoxin (Jalili et al., 2011), all of which can reduce AFB1 concentrations through hydrolysis and can produce a degraded form with reduced or no toxicity. Although, most of these chemicals are often expensive and can lead to decrease the nutrient value of feed components.

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Phoenix dactylifera (date palm seeds) is a useful traditional medicinal plant which belongs to the family *Arecaceae* (Sirisena et al., 2015). The genus *Phoenix* contained 14 species including *P. dactylifera* that were cultivated in the Middle East for at least 6000 years (Copley et al., 2001). The global production of date fruits could be approximately 7 million tons in 2010. Egypt, Iran and Saudi Arabia were being the main producing countries, with a production of 1.13 million ton, 1 million ton and 983,000 ton, respectively (FAO, 2010).

Date palm seeds (DPS) are a by-product of many date manufacturing processes, such as for date syrup and date confectionery. This by-product is having a high-fiber source. DPS can be mostly discarded or used as a form of roughage for domestic farm animals (Golshan Tafti et al., 2017). Addition of DPS powder to animal, poultry and fish feed has been demonstrated to enhance growth, improve feed efficiency and meat palatability (Al-Farsi and Lee, 2011). Glucomannan commonly has the ability to biologically inactivates multiple mycotoxins and glucomannan type A is the main component of the cell walls of palm kernels, which in this case acts as a food reserve and disappears during germination (Navid, 2007).

The nutritional value of DPS may introduce a source of healthy feed for animals and poultry nutrition and an alternative agent to reduce the cost of feed additive chemical mycotoxin binders used for aflatoxin decontamination in poultry feed and many studies have been carried out on date seeds in Egypt focusing mainly on their chemical composition but, lacking the effects of these seeds on aflatoxicosis in poultry farms. Therefore, the use of *P. dactylifera* to ameliorate the harmful effects of aflatoxicosis in broiler have been investigated in the current experiment through evaluation of broiler carcass traits, hepatic and renal biochemical function tests and histopathology in comparison to HSCAS (0.3%) supplementation in feed.

MATERIALS AND METHODS

Production of aflatoxin

Aflatoxin production was done using *Aspergillus parasiticus* NRRL - 2999 pure culture (National Research Centre, Cairo, Egypt) via fermentation of rice (Shotwell et al., 1966). The rice powder was incorporated into the basal diet at an estimated 100 µg/kg (100 ppb) entire the experimental period (35 days).

Feed additives (Mycotoxin binders)

Phoenix dactylifera seeds collection and preparation

It was purchased from a local date palm factory; washed, air-dried and finally grinded into a coarse powder and used as 2% and 4% mixed with basal diet according to each treatment.

Hydrated sodium and calcium aluminum Silicates (HSCAS)

A commercial mycotoxin adsorbent (Toxi-Mold Plus®) obtained from Egyco-Vet Company was mixed with basal diet through a dose of 3 kg per ton for their bird groups.

Experimental design

Two hundred and ten 1-day-old unsexed Arbor Acres broiler chicks were purchased from a local commercial hatchery (NASCO Egypt, Alexandria) and randomly allocated into 7 equal groups at the first day of age. Each one group was subdivided into three replicates (10 birds per replicate) and floor reared. Feed (starter feed from 1st to 21st days and grower feed from 22nd to 35th days) and water were supplied *ad-libitum* for 35 days of age and optimum managemental factors were applied regarding ventilation, temperature, lighting and litter management all over the experimental period.

The diet composition was formulated according to the recommendation of National Research Council Nutrient Requirements for Arbor Acres broiler chickens NRC (1994) without any feed additives rather than the compounds under study. A basal diet and 6 treatment diets were used in 7 groups as follows: the first group (G1) fed on a commercial broiler diets without supplement (control); G2 (DPS2%) fed on the basal diet with date pits 2% supplementation, G3 (DPS4%) fed on the basal diet with date palm 4%, G4 (AF) had aflatoxin as 100µg /kg, G5 (AF+HSCAS) fed a basal diet containing HSCAS 0.3% plus aflatoxin, G6 (AF+DPS2%) had a basal diet containing date palm 2% plus aflatoxin, and finally G7 (AF+DPS4%) had a basal diet containing date pits 4% plus aflatoxin. Also, the basal diets were tested for possible residual AF before start feeding and mixing the toxic dose and there were no detectable levels present. A recorded daily observation for health problems and mortality were carried out all over 35 days of age.

Ethical approval

The present study is affirmed by the Ethics of Animal Experiments Committee, Damanhour University, Egypt.

Carcass characteristics

At the end of experiment, five chickens in each group were randomly chosen, weighed, and humanely euthanized by cervical dislocation. After slaughtering of birds; abdominal fat, liver, pancreas, gizzard, heart, proventriculus, small

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intestine and lymphoid organs (bursa of Fabricius, thymus and spleen) were removed, weighed immediately and then; calculated as a percentage of carcass weight and finally, dressing percentage was calculated.

Biochemical function tests

The Blood samples were collected from wing vein by venous puncture at 2nd, 3rd, 4th and 5th weeks by using a sterile syringe in clean dry non-coated tubes. Each blood sample was left to coagulate at room temperature and centrifuged at 3000 rpm for 5 minutes and clear serum was collected individually in Eppendorf tubes. The collected sera were subjected to determination of ALT, AST, GGT, ALP, bilirubin, uric acid, creatinine, total cholesterol, triacylglycerol, LDL and HDL following the instructions enclosed in the manufactured kits produced by Biodiagnostic Company, Egypt.

Histopathological examination of the liver and kidney

At the end of experiment on day 35, three chickens from each group were euthanized, liver and kidney samples were obtained and submitted for histopathology to evaluate lesions and abnormalities. Fixation of samples was applied in 10 % buffered formalin solution for one week. Blocks and staining were carried out according to **(**Drury and Wallington, 1980).

Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA), with Duncan's multiple range tests for significant between means ($P \leq 0.05$) by SPSS.20® (IBM Cooperation, Armonk, NY, USA).

RESULTS

Carcass traits in chicks aged 35 days of old

The data illustrated in table 1 represented that dressing percentage of broilers are non-significantly ($P \geq 0.05$) affected in all treated groups in relation to negative control (G1), despite chicks received DPS2% (G2) have the highest dressing % and chicks received AF alone (G4) have the lowest dressing % when compared to the control (G1) (61.2±0.01 and 50.3±0.01 versus 58.3±0.01, respectively).

There were non-significant differences ($P \geq 0.05$) among the experimental groups in most measured percentages of relative weights of organs, except only small intestine and liver. The percentage of relative weight of small intestine of broilers was decreased significantly ($P \leq 0.05$) in G4 (AF) and non-significantly ($P \geq 0.05$) in G5 and G6 when compared to control, while it was increased significantly ($P \leq 0.05$) in G7 and non-significantly ($P \geq 0.05$) in G5 and G6 in relation to group treated with AF alone (G4). The relative weight % of liver of broilers was increased significantly ($P \leq 0.05$) in G4 (AF), G5 and G6, and increased non-significantly ($P \geq 0.05$) in G2, G3 and G7 when compared to control. On the other hand, the liver's relative weight decreased significantly ($P \leq 0.05$) in G7 and non-significantly ($P \geq 0.05$) in G5 and G6 in relation to group treated with AF alone (G4) despite, there are no significant difference ($P \geq 0.05$) between G5 and G6.

Also, the data are shown in tables 1 and table 2 indicated that relative weight % of gizzard, abdominal fat, heart, proventriculus, pancreas, bursa of Fabricius, thymus and spleen showed no significant difference among different experimental groups. Data also showed an enhancement in immune organ weights; bursa of Fabricius, thymus and spleen weight showed a numerical increase in G2 and G3, while showed a numerical decrease in G4 (AF) and in all aflatoxicated and treated groups (G5, G6 and G7) when compared to control. Also, they showed a numerical increase in G5, G6 and G7 in relation to group treated with AF alone (G4).

Determination of biochemical changes

The data obtained in table 3 revealed that ALT, AST, GGT, ALP and bilirubin were non-significantly ($P \geq 0.05$) affected at 2, 3, 4 and 5 weeks among different experimental groups. But at 5th week, liver enzymes; ALT, AST, GGT and ALP showed a numerical decrease in G2 and G3, while a numerical increase in G4 (AF) and in aflatoxicated and treated groups (G5, G6 and G7) when compared to control. Also, ALT, AST, GGT and ALP enzymes showed a numerical decrease in G5, G6 and G7 in relation to group treated with AF alone (G4). The present results in table 4 imply that creatinine, uric acid, total cholesterol, triacylglycerol, HDL-C and LDL-C were non-significantly ($P \geq 0.05$) affected at 2, 3, 4 and 5 weeks among different experimental groups. But at 5th week, renal functions (creatinine and uric acid), and lipid profile (total cholesterol, triacylglycerol, HDL-C and LDL-C) showed a numerical decrease in G2 and G3, while a numerical increase in G4 (AF) and in aflatoxicated and treated groups (G5, G6 and G7) when compared to control. Also, creatinine, uric acid, total cholesterol, triacylglycerol, HDL-C and LDL-C showed a numerical decrease in G5, G6 and G7 in relation to group treated with AF alone (G4).

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Table 1. Experimental results regarding dressing percentage and average relative weights (%) for small intestine, liver, gizzard and abdominal fat of all broiler groups at fifth week

Group/ n= 30	Dressing %	Small intestine	Liver	Gizzard	Abdominal fat
G1 (basal diet/ gm)	58.3±0.01^{a}	15.5±0.6^{ab}	3.29±0.8^{c}	4.72±1.1^{a}	1.32±0.04^{a}
G2 (2% DPS)	61.2±0.01^{a}	15.9±0.9^{ab}	4.3±0.4^{bc}	5.51±0.4^{a}	1.86±0.06^{a}
G3 (4% DPS)	59.6±0.01^{a}	16.6±0.9^{a}	4.2±0.2^{bc}	5.61±0.3^{a}	1.53±0.4^{a}
G4 (AF) µg/kg feed	50.3±0.01^{a}	11.1±1.9^{c}	5.21±0.4^{a}	4.10±0.2^{a}	1.31±0.1^{a}
G5 (AF+HSCAS)	55.4±0.01^{a}	13.9±0.9^{bc}	4.8±0.5^{ab}	4.21±0.1^{a}	1.14±0.12^{a}
G6 (AF+2% DPS)	57.2±0.01^{a}	13.9±0.6^{bc}	4.7±0.3^{ab}	4.42±0.3^{a}	1.33±0.13^{a}
G7 (AF+4% DPS)	53.9±0.01^{a}	15±0.3^{ab}	4.4±0.3^{bc}	4.51±0.2^{a}	1.2±0.1^{a}

G: group. DPS: date pits (2 or 4%). AF: aflatoxin (100 µg/kg feed). HSCAS: hydrated sodium calcium aluminosilicate (0.3%). Means within the same column under the same category carry different superscripts are significantly different (P≤0.05). n= number of chicks/ group. gm: gram. µg/kg: microgram/ kilogram. Values are expressed as means ± SE.

Table 2. Experimental results regarding average relative weights (%) for heart, proventriculus, pancreas and lymphoid organs (bursa of Fabricius, thymus and spleen) of all broiler groups at fifth week

Group/ n= 30	Heart	Proventriculus	Pancreas	Bursa	Thymus	Spleen
G1 (basal diet/ gm)	0.9±0.15^{a}	0.65±0.1^{a}	0.62±0.1^{a}	0.22±0.01^{a}	0.23±0.05^{a}	0.17±0.04^{a}
G2 (2%DPS)	1.0±0.08^{a}	0.7±0.05^{a}	0.6±0.04^{a}	0.24±0.0^{a}	0.3±0.02^{a}	0.23±0.02^{a}
G3 (4%DPS)	1.0±0.06^{a}	0.7±0.04^{a}	0.7±0.06^{a}	0.24±0.01^{a}	0.25±0.03^{a}	0.19±0.02^{a}
G4 (AF) µg/kg	1.1±0.05^{a}	0.61±0.1^{a}	0.5±0.04^{a}	0.15±0.0^{a}	0.1±0.03^{a}	0.12±0.02^{a}
G5 (AF+HSCAS)	1.0±0.04^{a}	0.6±0.07^{a}	0.6±0.06^{a}	0.18±0.0^{a}	0.16±0.02^{a}	0.14±0.01^{a}
G6 (AF+2%DPS)	1.0±0.06^{a}	0.7±0.04^{a}	0.6±0.02^{a}	0.19±0.01^{a}	0.19±0.04^{a}	0.16±0.03^{a}
G7 (AF+4%DPS)	0.9±0.07^{a}	0.7±0.03^{a}	0.7±0.03^{a}	0.21±0.0^{a}	0.17±0.02^{a}	0.15±0.02^{a}

G: group. DPS: date pits (2 or 4%). AF: aflatoxin (100 µg/kg feed). HSCAS: hydrated sodium calcium aluminosilicate (0.3%). Means within the same column under the same category carry different superscripts are significantly different (P≤0.05). Values are expressed as means ± SE. n= number of chicks/ group. gm: gram; µg/kg: microgram/ kilogram.

Table 3. Experimental results regarding ALT (U/l), AST (U/l), GGT (U/l), ALP (U/l) and bilirubin (mg/dl) for all broiler groups at second, third, fourth and fifth week

ITEM	Group	2nd Week	3rd Week	4th Week	5th Week
ALT (U/l)	G1 (basal diet)	9.0±1.3^{a}	8.9±1.0^{a}	9.20±1.2^{a}	9.4±1.0^{a}
	G2 (2%DPS)	9.0±1.4^{a}	8.7±1.6^{a}	8.60±1.5^{a}	8.0±1.8^{a}
	G3 (4%DPS)	8.9±1.8^{a}	8.8±1.1^{a}	8.75±1.7^{a}	8.4±2.3^{a}
	G4 (AF)	9.1±1.3^{a}	9.5±1.2^{a}	10.20±1^{a}	11.5±2.1^{a}
	G5 (AF+HSCAS)	9.0±2.3^{a}	9.0±1.3^{a}	9.50±2.4^{a}	9.8±1.8^{a}
	G6 (AF+2%DPS)	9.2±2.5^{a}	9.3±2.0^{a}	9.70±1.4^{a}	10±1.5^{a}
	G7 (AF+4%DPS)	9.1±1.0^{a}	9.4±1.9^{a}	9.98±1.8^{a}	10.8±1.2^{a}
AST (U/l)	G1 (basal diet)	188.5±11^{a}	188±39^{a}	187.6±20^{a}	194.4±24^{a}
	G2 (2%DPS)	188.2±24^{a}	186±12^{a}	182.6±13^{a}	169.4±14^{a}
	G3 (4%DPS)	189.0±28^{a}	187±19^{a}	184.6±10^{a}	175.8±11^{a}
	G4 (AF)	188.8±17^{a}	189±30^{a}	193.6±19^{a}	207.0±26^{a}
	G5 (AF+HSCAS)	189.8±33^{a}	189±19^{a}	192.6±16^{a}	195.2±25^{a}
	G6 (AF+2%DPS)	189.4±9^{a}	189±20^{a}	190.2±35^{a}	196.6±18^{a}
	G7 (AF+4%DPS)	188.8±22^{a}	189±23^{a}	193.6±10^{a}	202.6±13^{a}
GGT (U/l)	G1 (basal diet)	19.18±0.2^{a}	19.6±3.1^{a}	20.0±1.3^{a}	19.8±2.5^{a}
	G2 (2%DPS)	19.18±2.2^{a}	18.2±2.9^{a}	15.8±2.6^{a}	14.0±2.0^{a}
	G3 (4%DPS)	19.14±3.6^{a}	18.6±3.6^{a}	18.4±3.4^{a}	18.2±3.1^{a}
	G4 (AF)	19.16±2.0^{a}	20.8±2.8^{a}	22.6±3.6^{a}	24.8±3.2^{a}
	G5 (AF+HSCAS)	19.14±3.0^{a}	19.8±2.6^{a}	21.6±2.2^{a}	21.8±2.4^{a}
	G6 (AF+2%DPS)	19.12±2.8^{a}	19.6±3.0^{a}	20.6±2.6^{a}	21.6±3.6^{a}
	G7 (AF+4%DPS)	19.18±0.8^{a}	20.0±3.7^{a}	22.0±2.5^{a}	23.0±2.5^{a}
ALP (U/l)	G1 (basal diet)	5.8±1.06^{a}	6.0±0.94^{a}	7.0±1.20^{a}	7.4±1.74^{a}
	G2 (2%DPS)	5.8±0.96^{a}	5.6±0.97^{a}	5.3±1.09^{a}	4.8±1.59^{a}
	G3 (4%DPS)	5.8±1.15^{a}	5.7±1.09^{a}	5.4±1.37^{a}	5.0±1.4^{a}
	G4 (AF)	5.6±1.20^{a}	6.2±1.24^{a}	8.2±1.4^{a}	10±1.32^{a}
	G5 (AF+HSCAS)	5.8±1.59^{a}	6.1±1.06^{a}	7.4±1.24^{a}	8.4±1.44^{a}
	G6 (AF+2%DPS)	5.6±1.12^{a}	6.1±1.49^{a}	7.2±1.51^{a}	8.0±1.50^{a}
	G7 (AF+4%DPS)	5.8±1.01^{a}	5.83±1.57^{a}	7.9±1.36^{a}	8.8±1.19^{a}
Bilirubin (mg/dl)	G1 (basal diet)	0.14±0.01^{a}	0.14±0.02^{a}	0.15±0.02^{a}	0.15±0.02^{a}
	G2 (2%DPS)	0.14±0.02^{a}	0.14±0.01^{a}	0.14±0.01^{a}	0.13±0.02^{a}
	G3 (4%DPS)	0.14±0.01^{a}	0.14±0.02^{a}	0.14±0.02^{a}	0.14±0.01^{a}
	G4 (AF)	0.15±0.02^{a}	0.15±0.02^{a}	0.15±0.01^{a}	0.16±0.02^{a}
	G5 (AF+HSCAS)	0.14±0.02^{a}	0.14±0.01^{a}	0.15±0.02^{a}	0.15±0.01^{a}
	G6 (AF+2%DPS)	0.14±0.01^{a}	0.14±0.02^{a}	0.15±0.01^{a}	0.15±0.02^{a}
	G7 (AF+4%DPS)	0.15±0.02^{a}	0.15±0.02^{a}	0.15±0.01^{a}	0.15±0.02^{a}

G: group. DPS: date pits (2 or 4%). AF: aflatoxin (100 µg/kg feed). HSCAS: hydrated sodium calcium aluminosilicate (0.3%). ALT: Alanine amino transferase. AST: Aspartate transferase. GGT: Gamma glutamyl transferase. ALP: Alkaline phosphatase. U/l: units per liter. (mg /dl): milligrams per deciliter. Means within the same column under the same category carry different superscripts are significantly different (P≤0.05). Values are expressed as means ± SE.

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Table 4. Experimental results regarding serum creatinine (mg/dl), uric acid (mg/dl), total cholesterol (mg/dl), triacylglycerol (mg/dl), high density lipoprotein (mg/dl) and low density lipoprotein (mg/dl) for all broiler groups at second, third, fourth and fifth week

ITEM	Group	2nd Week	3rd Week	4th Week	5th Week
Creatinine (mg/dl)	G1 (basal diet)	0.20±0.02[a]	0.23±0.03[a]	0.24±0.06[a]	0.25±0.05[a]
	G2 (2%DPS)	0.21±0.04[a]	0.21±0.03[a]	0.19±0.04[a]	0.18±0.04[a]
	G3 (4%DPS)	0.26±0.06[a]	0.24±0.05[a]	0.20±0.04[a]	0.19±0.02[a]
	G4 (AF)	0.20±0.03[a]	0.25±0.04[a]	0.27±0.05[a]	0.30±0.04[a]
	G5 (AF+HSCAS)	0.22±0.05[a]	0.24±0.03[a]	0.25±0.05[a]	0.26±0.06[a]
	G6 (AF+2%DPS)	0.20±0.03[a]	0.24±0.05[a]	0.24±0.06[a]	0.27±0.07[a]
	G7 (AF+4%DPS)	0.21±0.02[a]	0.25±0.05[a]	0.26±0.03[a]	0.28±0.04[a]
Uric acid (mg/dl)	G1 (basal diet)	8.34±0.54[a]	8.50±0.48[a]	8.84±1.48[a]	8.92±0.99[a]
	G2 (2%DPS)	8.11±0.75[a]	8.06±0.64[a]	7.70±0.95[a]	7.00±1.52[a]
	G3 (4%DPS)	8.16±1.32[a]	8.04±0.49[a]	7.90±0.82[a]	7.74±0.57[a]
	G4 (AF)	8.46±0.70[a]	8.92±1.00[a]	9.40±1.07[a]	9.86±1.67[a]
	G5 (AF+HSCAS)	8.21±0.73[a]	8.64±1.19[a]	8.92±1.73[a]	9.42±1.0[a]
	G6 (AF+2%DPS)	8.36±0.46[a]	8.60±0.80[a]	8.82±1.49[a]	9.28±1.38[a]
	G7 (AF+4%DPS)	8.38±0.43[a]	8.86±0.71[a]	9.02±0.85[a]	9.64±0.38[a]
S.CH (mg/dl)	G1 (basal diet)	137±16.7[a]	136.6±11[a]	135.2±16[a]	134.6±13[a]
	G2 (2%DPS)	136±11.3[a]	135.4±17[a]	132.4±18[a]	125.2±13[a]
	G3 (4%DPS)	136.2±17[a]	135.8±22[a]	133.4±18[a]	128.2±4[a]
	G4 (AF)	137.5±17[a]	138.2±16[a]	142.8±15[a]	145.2±8[a]
	G5 (AF+HSCAS)	137±10.3[a]	137±19.1[a]	139±18.5[a]	140.4±13[a]
	G6 (AF+2%DPS)	137.1±16[a]	137.9±17[a]	139.5±17[a]	140±17.6[a]
	G7 (AF+4%DPS)	137.20±8[a]	138±11.8[a]	138.4±11[a]	142±9.79[a]
S.TG (mg/dl)	G1 (basal diet)	165.0±24[a]	166.6±25[a]	165.2±7[a]	165.2±26[a]
	G2 (2%DPS)	164.6±26[a]	163.4±26[a]	160±16.4[a]	158.2±17[a]
	G3 (4%DPS)	164.4±14[a]	164±20.9[a]	162.2±22[a]	159.8±19[a]
	G4 (AF)	165.6±15[a]	171.4±17[a]	173±23.7[a]	175.2±18[a]
	G5 (AF+HSCAS)	165±10.2[a]	167.2±15[a]	167.6±10[a]	169.8±28[a]
	G6 (AF+2%DPS)	165.6±18[a]	166.8±23[a]	167.2±12[a]	168.8±18[a]
	G7 (AF+4%DPS)	165.6±13[a]	166.6±19[a]	168.6±12[a]	171±16.2[a]
HDL-C (mg/dl)	G1 (basal diet)	77±7.28[a]	76.0±6.44[a]	74.0±8.22[a]	74.0±8.11[a]
	G2 (2%DPS)	76.0±5.11[a]	75.2±5.34[a]	72.0±5.44[a]	68.0±8.04[a]
	G3 (4%DPS)	76.75±6.1[a]	75.5±1.18[a]	72.9±5.84[a]	69.2±2.51[a]
	G4 (AF)	78.2±2.28[a]	76.3±1.98[a]	86.0±8.13[a]	85.4±4.05[a]
	G5 (AF+HSCAS)	79.3±3.58[a]	76.0±1.09[a]	76.8±8.08[a]	81.0±3.31[a]
	G6 (AF+2%DPS)	77.8±3.51[a]	76.1±5.1[a]	76.6±5.55[a]	79.3±4.28[a]
	G7 (AF+4%DPS)	78.0±4.98[a]	76.2±5.83[a]	77.3±4.84[a]	83.6±5.32[a]
LDL-C (mg/dl)	G1 (basal diet)	32.1±2.38[a]	32.3±2.61[a]	32.0±2.36[a]	31±1.97[a]
	G2 (2%DPS)	32.02±2.1[a]	31.9±1.0a	30.0±5.47[a]	28±3.96[a]
	G3 (4%DPS)	32.1±3.28[a]	32.0±1.3[a]	31.0±4.7[a]	29±2.81[a]
	G4 (AF)	32.3±2.7[a]	32.4±3.57[a]	34.5±2.24[a]	37±2.3[a]
	G5 (AF+HSCAS)	32.2±1.59[a]	32.3±3.43[a]	33±2.98[a]	34±2.94[a]
	G6 (AF+2%DPS)	32.2±1.68[a]	32.3±2.5[a]	32.7±4.51[a]	33.3±4.72[a]
	G7 (AF+4%DPS)	32.3±2.5[a]	32.4±3.35[a]	33.5±1.0[a]	35.5±2.34[a]

G: group. DPS: date pits (2 or 4%). AF: aflatoxin (100 µg/kg feed). HSCAS: hydrated sodium calcium aluminosilicate (0.3%). S.CH: Serum cholesterol. S.TG: Serum triglyceride. HDL-C: High density lipoproteins concentration. LDL-C: Low density lipoproteins concentration. (mg /dl): milligrams per deciliter. Means within the same column under the same category carry different superscripts are significantly different ($P \leq 0.05$). Values are expressed as means ± SE.

Histopathological examination of the liver and kidney

The examination of liver of birds in G1, G2 and G3 showed normal histological appearance and structure (Figures 1, 2 and 3 respectively). While the liver of birds of G4 exhibited hemorrhage replaced necrotic hepatocytes (Figure 4a) and focal hepatic necrosis with inflammatory cell infiltration and fatty degeneration of hepatocytes (Figure 4b). Moreover, the examined liver of birds in G5 showed moderate congestion of blood vessels (Figure 5) and in G6 showed mild activation of inflammatory cell infiltration (Figure 6) and finally in G7, moderate inflammatory cells infiltration was seen (Figure 7).

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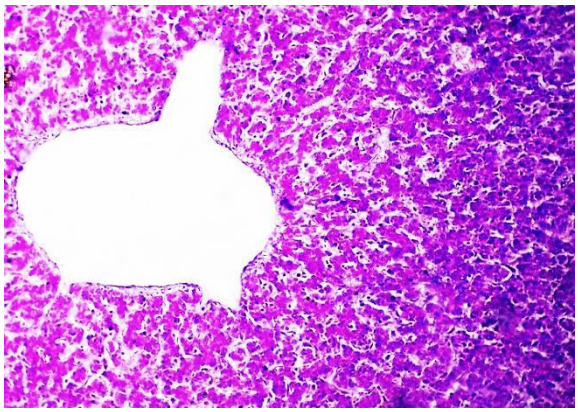

Figure 1. Liver of chicken of G1 (basal diet) at 35 days' old showing normal histological structure. H&E. (x160).

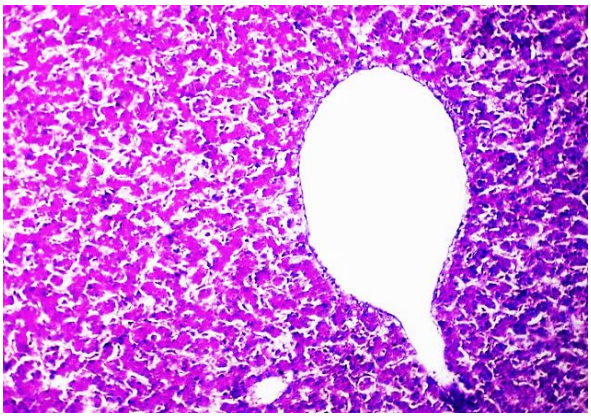

Figure 2. Liver of a chicken of G2 (2% date pits) at 35 days' old showing normal histological structure. H&E. (x160).

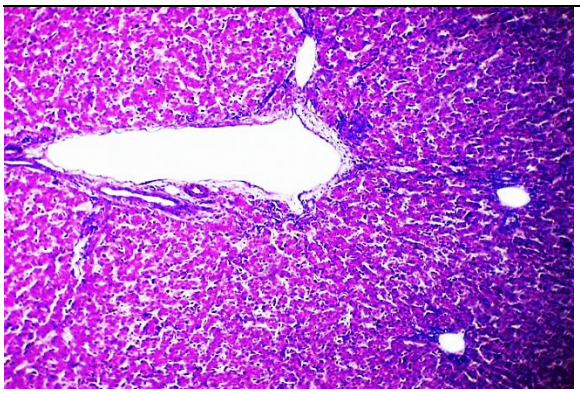

Figure 3. Liver of a chicken of G3 (4% date pits) at 35 days' old showing normal histological structure. H&E. (x160).

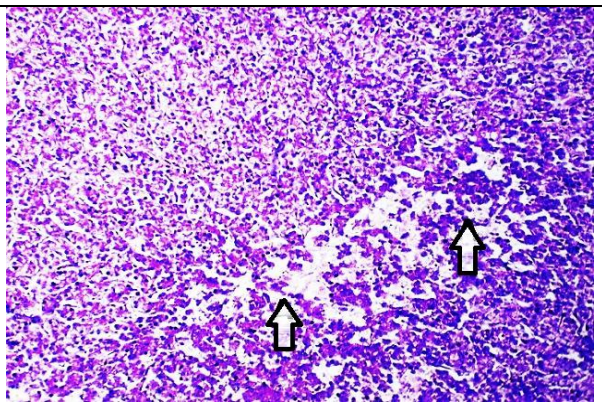

Figure 4a. Liver of a chicken of G4 (AFlatoxin) at 35 days' old showing hemorrhage replaced necrotic hepatocytes (arrows). H&E. (x160).

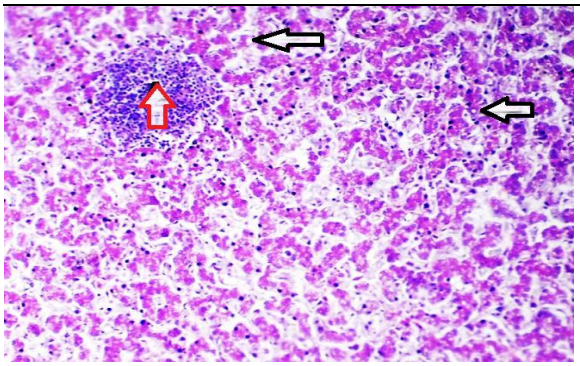

Figure 4b. Liver of a chicken of G4 (AFlatoxin) at 35 days' old showing focal hepatic necrosis with inflammatory cell infiltration (red arrow) and fatty degeneration of hepatocytes (black arrows). H&E. (x160).

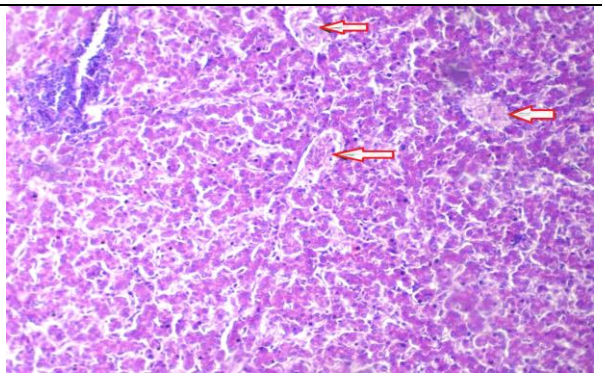

Figure 5. Liver of a chicken of G5 (AFlatoxin+ Hydrated sodium and calcium aluminum Silicates) at 35 days' old showing moderate congestion of blood vessels (arrows). H&E. (x160).

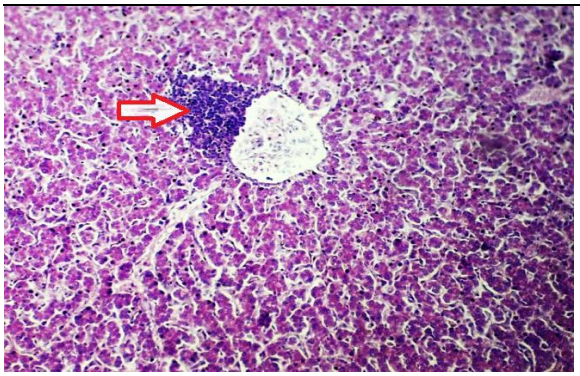

Figure 6. Liver of a chicken of G6 (AFlatoxin+2% date pits) at 35 days' old showing mild inflammatory cell infiltration (arrow). H&E. (x160).

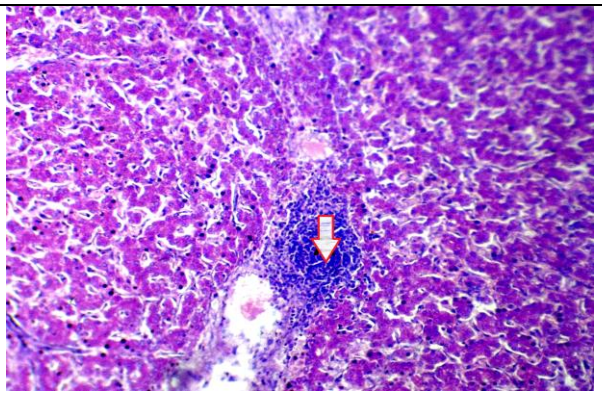

Figure 7. Liver of a chicken of G7 (AFlatoxin+4% date pits) at 35 days' old showing moderate inflammatory cells infiltration (arrow) in portal area. H&E. (x160).

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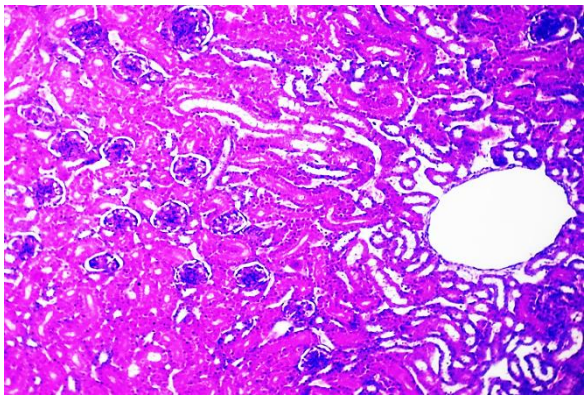

Figure 8. Kidney of a chicken of G1 (basal diet) at 35 days' old showing normal histological structure. H&E. (x160).

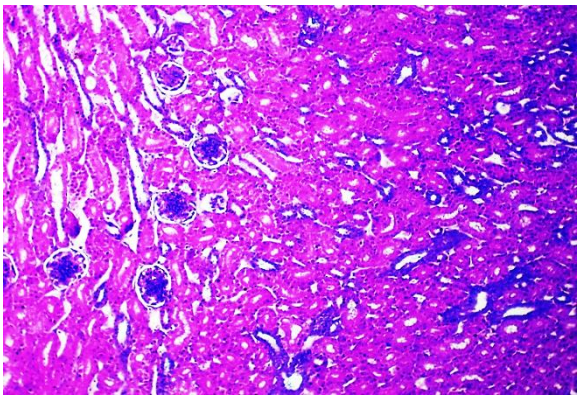

Figure 9. Kidney of a chicken of G2 (2% date pits) at 35 days' old showing normal histological structure. H&E. (x160).

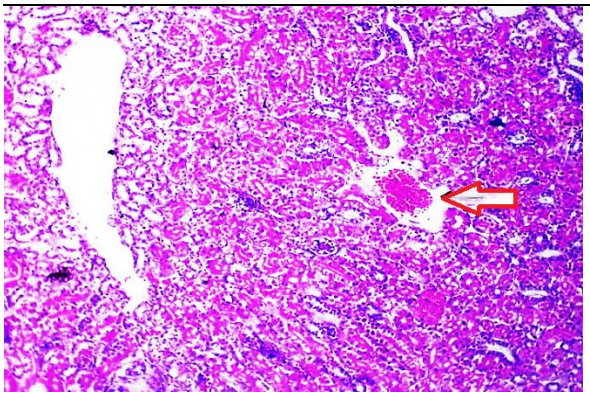

Figure 10. Kidney of a chicken of G3 (4% date pits) at 35 days' old showing normal histological structure with mild congestion of blood vessel (arrow). H&E. (x160).

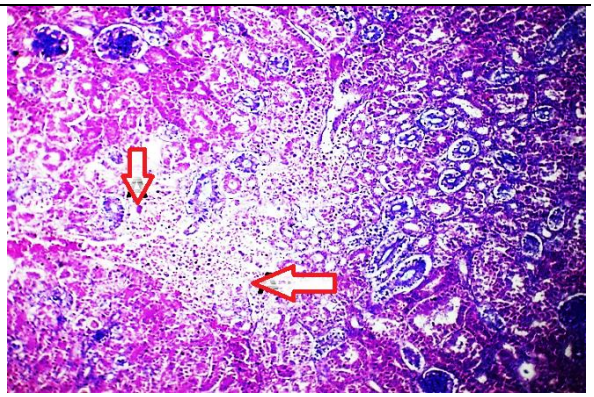

Figure 11a: Kidney of a chicken of G4 (AFlatoxin) at 35 days' old showing hemorrhage (arrow). H&E. (x160).

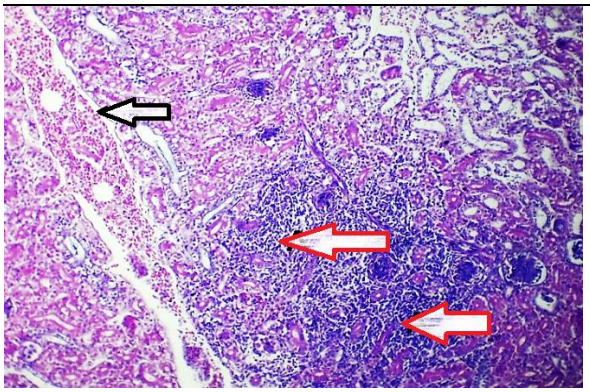

Figure 11b. Kidney of a chicken of G4 (AFlatoxin) at 35 days' old showing interstitial nephritis with mononuclear cell infiltrations (red arrow) and congestion of blood vessel (black arrow). H&E.

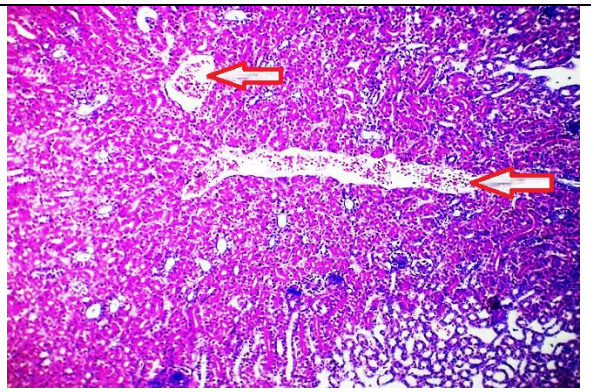

Figure 12. Kidney of a chicken of G5 (AFlatoxin+ Hydrated sodium and calcium aluminum Silicates) at 35 days' old showing mild to moderate congestion of blood vessel (arrows). H&E. (x160).

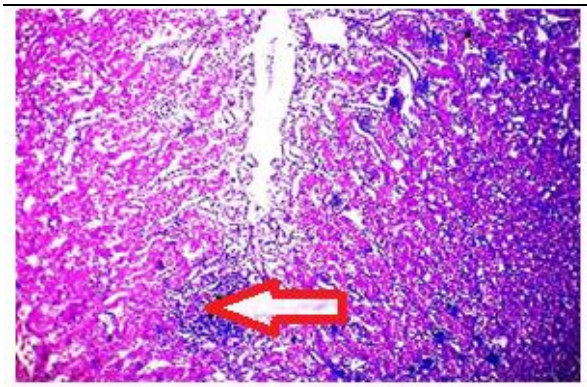

Figure 13. Kidney of a chicken of G6 (AFlatoxin+2% date pits) at 35 days' old showing focal interstitial nephritis with mononuclear cell infiltrations (arrow). H&E. (x160).

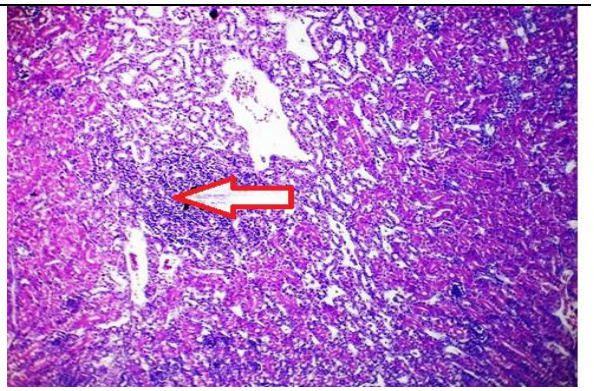

Figure 14. Kidney of a chicken of G7 (AFlatoxin+4% date pits) at 35 days' old showing focal interstitial nephritis with mononuclear cell infiltrations (arrow). H&E. (x160).

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The kidney of birds in G1 and G2 exhibited normal histological structure of glomeruli and renal tubule (Figures 8 and 9, respectively), while, in the sacrificed birds of G3 showed normal histological structure with mild congestion of blood vessel (Figure 10). The kidney of birds in G4 showed hemorrhage, where erythrocytes escape from the blood vessel beside interstitial nephritis with mononuclear cell infiltrations and congestion of blood vessel (Figures 11a and 11b respectively). Moreover, the kidney of the examined birds in G5 showed mild to moderate congestion of blood vessel (Figure 12), while, the microscopical findings of the kidney of birds in G6 and G7 showed focal interstitial nephritis with mononuclear cell infiltrations (Figures 13 and 14, respectively). Finally, no significant toxic microscopic lesions and normal histology were evident in liver and kidney sections of birds in G1 (control) or G2, G3 fed DPS 2% and 4% respectively and all these 3 groups had no mortalities.

DISCUSSION

Many feed additives had the ability to relieve aflatoxicosis but few researches are carried out on incorporation of date palm seed powder into the diet for poultry production. Therefore, this investigation provides some information to solve the problems of aflatoxicosis in poultry industry by introducing DPS in to the broiler's diets.

In this study, just the relative weight of both liver and small intestine of chickens showed significant differences. The liver's relative weight increased significantly in chickens fed Aflatoxin alone while decreased in chickens fed aflatoxin and treated with 2% and 4% date pits, which indicated that supplementation of date pits ameliorate the toxic effect of aflatoxin and utilized properly to improve growth and carcass traits. Bovo et al. (2015) reported that prolonged exposure to dietary AFB1 raised the relative weight of the liver. The relative liver weight is significantly increased by different levels of aflatoxin compared with any other organ, and this enhancement could due to an inhibition of lipid transport and lipid accumulation in the liver (Huff et al., 1986) or could be primarily attributed to necrosis, bile duct proliferation, fat infiltration, and enlarged liver cells (Yunus et al., 2011). Contraindicated results were noticed in previous reports, in which the liver weight of broilers did not change when the dietary AF level was lower as 90.2 µg/kg of diet but this might be due to the shorter duration of aflatoxin supplementation than our experiment (Fan et al., 2015).

The toxic effects of AFlatoxin on blood biochemical parameters were exhibited only at fifth week of ag in chickens fed diet containing aflatoxin alone where increased the concentrations of liver enzymes (ALT, AST, GGT and ALP), kidney functions (creatinine and uric acid) and lipid profile (total cholesterol, triacylglycerol, HDL-C and LDL-C). This may be due to the prolonged hepatic and renal damage and release of enzymes into the blood stream of chickens received AF resulting in chronic venous congestion with circulatory and degenerative changes in most of the body tissues (Jindal et al., 1994). The results matched with the previous findings in which aflatoxin treatment increased ALT, AST, GGT enzymes and served as markers of liver and kidney damage/dysfunction, which promoted the release of these aminotransferases from hepatocytes into the blood stream, indicating liver inflammation, lesions or obstruction of the biliary tract (Neeff et al., 2013; Uyar et al., 2016).

The numerical increase of kidney functions (creatinine and uric acid) in experimental birds that consumed diet contaminated with (100 ppb aflatoxin) at fifth week, as reported previously, may be due to the disturbed transportation function of epithelial cells in collecting tubules and diffuse impairment of proximal tubules' function (Hochleithner, 1994; Umar et al., 2012). Uric acid is the primary end product of protein metabolism in birds. It is synthesized in the liver and excreted through the kidney tubules. Increased blood uric acid and creatinine in broiler chickens receiving 50, 150 and 300 mg/kg aflatoxin in feed over 42 days were observed previously by George et al. (2006).

The addition of DPS to diet containing 100 ppb AF induced differences in serum parameters at 5th week of age; the liver enzymes (ALT, AST, GGT and ALP), kidney functions (creatinine and uric acid) and lipid profile (total cholesterol, triacylglycerol, HDL-C and LDL-C) showed a numerical decrease in G6 (DPS2%+AF) and G7 (DPS4%+AF) comparing with G4 treated with AF alone, indicating the hepatorenal protective activity of DPS against AF. The decrease in serum parameters may have been due to decreased release of tissue specific enzymes and other intracellular proteins which secondary to oxidative stress during metabolism. The mechanism by which DPS induces its protective activity is not clear but possibly due to the antioxidant effect and the content of vitamin C in DPS (0.137%) which may play a role in hepatoprotection (Burtis and Ashwood, 2001). Moreover, Vayalil (2002) reported that date fruit has antioxidant and antimutagenic activity and this implicated the presence of compounds with potent free-radical-scavenging activity.

The present results were according to the findings of Al-Ghasham et al. (2008) who reported the liver enzymes (ALT and AST) in AFB1 (50 µg/kg BW) treated group (half dose to our work) were significantly higher than in the control group, while in the AFB1 (50 µg/kg BW) and date group, the plasma levels of liver function enzymes (ALT and AST), creatinine and urea were significantly lower than the AFB1 group.

Histopathologically, supplementation of 100 ppb aflatoxin in G4 which had severe liver and kidney lesions as indicative for aflatoxicosis exhibited through hemorrhage and focal hepatic necrosis with inflammatory cell infiltration and fatty degeneration of hepatocytes which may be due to disturbance of oxidant/antioxidant balance system and kidney

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as congestion of renal blood vessels, interstitial nephritis with mononuclear cell infiltrations. Also, previous researches which recorded the toxic effect of aflatoxins in liver (Gholami-Ahangaran et al., 2016 and Tessari et al., 2006) and kidney (Mohamed and Mohamed, 2009). The inclusion of DPS to diet contains 100 ppb AF as in G6 and G7 reduced the severity of pathological changes. Although it was not only in liver but also in kidney and it seemed that the effect of mannanoligosacharides (MOS) in DPS on AF toxicity was not only related to the binding capacity with AF and also these MOS could prevent the colonization of opportunistic bacterial pathogens in the gastrointestinal tract (Olsen, 1995). Moreover, Al-Ghasham et al. (2008) reported that the liver and kidney of rats treated with AFB1 and Date, showed nearly normal and the mild changes were just vacuolation of hepatocytes, congestion and few mononuclear cells infiltration. The beneficial effect of date pits appeared in histopathology of both liver and kidney might be due to its antioxidant role.

Supplementation of HSCAS in the diet containing (100 ppb AF) in G5 showed a numerical decrease in liver enzymes (ALT, AST, GGT and ALP), kidney functions (creatinine and uric acid) and lipid profile (total cholesterol, triacylglycerol, HDL-C and LDL-C) compared with chickens receiving AF alone (G4) at 5th week. HSCAS have been demonstrated to be effective in binding AF molecules in the gastrointestinal tract, making them unavailable for adsorption and consequently alleviating aflatoxicosis (Phillips et al., 1990 and Kubena et al., 1990). Similar results, increased urea and creatinine as indices of impaired kidney function in aflatoxicosis were reported in chickens and rats, a partly reduction of the toxic effects on kidney function was achieved by adding mycosorbents to contaminated feeds (Yildirim et al., 2011).

However, the addition of HSCAS to diet contains (100 ppb of AF) in G5 couldn't fully improve the organ's histopathology as there were moderate congestion of hepatic blood vessels and mild to moderate congestion of renal blood vessels which indicated that HSCAS didn't completely protect broilers against aflatoxicosis but partially ameliorated its effect which may be attributed to the dose of HSCAS as mentioned previously by Neeff et al. (2013) who reported that HSCAS didn't completely protect broilers against aflatoxicosis, but was effective in reducing aflatoxin residues in liver and kidney of chicks fed 2.5 mg of AFB1/kg of diet during 0-21 days of age. Also, Phillips (1999) reported that the protective effect of HSCAS resulted from the rapid binding capacity of HSCAS to aflatoxins in the gastrointestinal tract of chickens, thus preventing its absorption and normal distribution to the liver. This effect could be increased through higher HSCAS dose supplemented in feed.

As the small intestine's relative weight decreased significantly in chickens fed Aflatoxin alone while increased in chickens fed aflatoxin and treated with 2% and 4% date pits, indicating that mannan-oligosaccharides in DPS increased the enteric development. These results agreed with Daneshyar et al. (2014) who reported the increased weight of the small intestine, due to decreased activity of AF in the intestines of chickens that received aflatoxicated diets (300 ppb) and supplemented with 4% date pits. In addition to, Yunus et al. (2011) who reported that dietary exposure to AFB1 can lower the unit weight (length/weight) of the duodenum and jejunum.

The absence of significant differences in dressing percentage and relative weight of gizzard, abdominal fat, heart, proventriculus, pancreas, Bursa of Fabricius (BF), thymus and spleen among different experimental groups agreed with the findings of researches on aflatoxin supplementation, Del Bianchi et al. (2005) who observed no differences in the relative weights of the analyzed organs (heart, BF, thymus, pancreas and proventriculus) among treatments in broilers fed AFB1 from 21 to 42 days of age. Fan et al. (2015) stated that no significant differences were observed for the weights of organs (heart, liver, spleen, BF and thymus) among all treatments in broilers fed mixed AFB1, B2, G1, G2 from 7 to 42 days of age. Moreover, on DPS supplementation, Zangiabadi and Torki (2010) reported that adding complete waste of 30% dates only significantly affected the relative gizzard weight but had no significant effect on the other body parts.

Finally, the safety of supplementation of DPS alone to the broiler's diet was recorded in G2 (2% DPS) and G3 (4% DPS) as it didn't have any drawback on the measured the biochemical parameters at 5th week These results are in accordance with those of earlier studies which investigated the effect of DPS and stated that there were no significant change in liver function (ALT and AST) and kidney function (Urea and creatinine) as well as metabolic markers (total cholesterol and triglycerides), indicating absence of any adverse effects of feeding date pits on hepatic and renal functions (Kamel et al., 2016).

CONCLUSION

Addition of date palm (2% and 4%) to broiler's diet ameliorated the hazardous effects of aflatoxins and this protection is dose-related as 2% supplementation gave better protection than the higher dose 4%. So, we advise to use date palm as a feed additive to control aflatoxicosis in poultry farms, avoiding the harmful chemical mycotoxin binders causing appreciable losses in nutritive value and palatability. Concomitantly, further studies on the combinations of date palm and other medicinal plants having protective effect against aflatoxicosis in poultry should be applied.

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DECLARATIONS

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Competing interests

With respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

Author's contribution

Dr. Disouky Mourad designed the experiment, article writing and revision, Dr. Kadry Saadek designed the experiment, manuscript writing, commenting and approval, Dr. Ahmed El-Bestawy helped in field study, collected data, laboratory analyses, statistical analysis, tabulation of experimental data and article writing; while, Dr. Ward Masoud helped in experiment application, statistical analysis, manuscript writing. All authors have read and approved the final manuscript.

REFERENCES

Al-Farsi MA and Lee CY (2011). Usage of date (Phoenix dactylifera L.) seeds in human health and animal feed. Nuts and seeds in health and disease prevention book, chapter 53, pp. 447-452. DOI:10.1016/B978-0-12-375688-6.10053-2.

Al-Ghasham, A., Ata H.S, El-Deep S, Meki AR and Shehada S (2008). Study of protective effect of date and Nigella sativa on aflatoxin B1 toxicity. International journal of health sciences, 2(2): .26-44. PMID:21475486.

Bintvihok A and Kositcharoenkul S (2006). Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon, 47 (1): 41–46. DOI: 10.1016 /J. toxicon 2005. 09. 009.

Bovo F, Franco LT, Kobashigawa E, Rottinghaus GE, Ledoux DR and Oliveira CAF (2015). Efficacy of beer fermentation residue containing Saccharomyces cerevisiae cells for ameliorating aflatoxicosis in broilers. Poultry science, 94(5): 934-942. DOI: 10.3382 /ps / pev 067.

Burtis CA and Ashwood ER (2001). Tietz's Fundamentals of Clinical Chemistry, Philadelphia: WB Saunders, Elsevier, 11830 westline industrial Drive St. louis, Missouri 63146. BN: 978-0-7216-3865-2. Library of congress control number, 2007921126.

Copley M S, Rose P J, Clapham A, Edwards D N, Horton M C and Evershed R P (2001). Detection of palm fruit lipids in archaeological pottery from Qasr Ibrim, Egyptian Nubia. Proceedings of the Royal Society of London B: Biological Sciences, 268(1467): 593-597. DOI: 10.1098/ rspb. 2000. 1394.

Daneshyar F, Afzali N and Farhangfar H (2014). Effects of different levels of date pits in broilers' feed contaminated with aflatoxin B 1 on broilers' performance and carcass characteristic. African Journal of Biotechnology, 13(1): 185-193. DOI:.10.5897/ AJB 2013. 13132.

Del Bianchi M, Oliveira C A F, Albuquerque R, Guerra J L and Correa B (2005). Effects of prolonged oral administration of aflatoxin B1 and fumonisin B1 in broiler chickens. Poultry Science, 84 (12):1835–1840. DOI: 10. 1093 / ps / 84. 12. 1835.

Drury RAB and Wallington EA (1980). Preparation and Fixation of Tissues in Carleton's Histological Technique, fifth ed. Oxford, Newyork, Oxford University Press.

Edds G T and Bortell R R (1983). Biological effects of aflatoxin in poultry. Southern cooperative series bulletin. Agris.fao.org., SIDALC.NET, G279, G56-61, 1983.

Edrington T, Sarr A, Kubena L, Harvey R and Phillips T (1996). (HSCAS), acidic HSCAS, and activated charcoal reduce urinary excretion of aflatoxin M 1 in turkey poults. Lack of effect by activated charcoal on aflatoxicosis. Toxicology letters, 89 (2): 115-122 PMID: 8960153. DOI: 10.1016 / 50378 – 4274 (96) 03795- 2.

Fan Y, Zhao L, Ji C, Li X, Jia R, Xi L, Zhang J and Ma Q (2015). Protective effects of bacillus subtilisansb060 on serum biochemistry, histopathological changes and antioxidant enzyme activities of broilers fed moldy peanut meal naturally contaminated with aflatoxins. Toxins, 7(8): .3330-3343. DOI: 10.3390 / toxins / 7083330

FAO (2010). Food and agriculture organization of the United Nations. Rome: Date Palm cultivation.

George A J, Balachandran C, Murali Manohar B, Dhinakar Raj G and Kirubakaran J J (2006). Pathological changes in broiler chicken exposed to sublethal doses of aflatoxin in broiler chicken. In: Compendium of invited papers and abstracts and souvenir, XIII Indian Association of Veterinary Pathologist Conference, 27–29, Chennai.

Gholami-Ahangaran M, Rangsaz N and Azizi S (2016). Evaluation of turmeric (Curcuma longa) effect on biochemical and pathological parameters of liver and kidney in chicken aflatoxicosis. Pharm biol, 54(5): 780-787. PMID: 26450181. DOI: 10.3109 / 13880209.2015.1080731.

Golshan Tafti A, Solaimani Dahdivan N and Yasini Ardakani SA (2017). Physicochemical properties and applications of date seed and its oil. International Food Research Journal, 24(4): 1399-1406.

Hochleithner M (1994). Biochemistries. In: Avian Medicine and Surgery: Principles and Applications. eds B. W. Ritchie, G. J. Harrison & L. R. Harrison, Wingers Publishing Inc., Lake Worth, FL: 223–245.

Huff W E, Kubena L F, Harvey R B, Corrier D E and Mollenhauer H H (1986). Progression of aflatoxicosis in broiler chickens. Poultry Science, 65 (10):1891–1899. DOI: 10.3382 / ps. 0651891. PMID: 3797371.

Jalili M, Jinap S and Son R (2011). The effect of chemical treatment on reduction of aflatoxins and ochratoxin A in black and white pepper during washing. Food Additives and Contaminants, 28(4): 485-493. DOI: 10.1080/19440049.2010.551300. PMID: 21416415.

Jindal N, Mahipal SK and Mahajan NK (1994). Effect of hydrated sodium calcium aluminosilicate on prevention of aflatoxicosisin broilers. Indian Journal of Animal Science,63: 649-652.

Kamel ER, Manaa E and Farid AS (2016). The Effects of Dietary Date Pit on the Productive and Economic Efficiency of Japanese Quail. Alexandria Journal for Veterinary Sciences, 51(2): 211-221. DOI: 10.5455/ ajvs.233838.

Kubena LF, Harvey RB, Huff WE, Corrier DE, Phillips TD and Rottinghaus GE (1990). Efficacy of hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poultry Science, 69 (7):1078-1086. DOI:10.3382/ ps.0691078. PMID: 2235827.

Mohamed M.A and Mohamed M H (2009). Hemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. Veterinaria Italiana, 45 (2): 323–337. PMID: 20391383.

Navid Shad BJSA (2007). Animal Nutrition (translated). (Sixth edition. Haghshenas publications). 702-700.

Neeff D V, Ledoux D R, Rottinghaus G E, Bermudez A J, Dakovic A, Murarolli R A and Oliveira C A F. 2013. In vitro and in vivo efficacy of a hydrated sodium calcium aluminosilicate to bind and reduce aflatoxin residues in tissues of broiler chicks fed aflatoxin B1. Poultry Science, 92 (1):131–137. DOI: 10.3382/ ps.2012-02510. PMID: 23243239.

NRC (1994). National Research Council: Nutrient requirement of Poultry. National Academy Press, Washington, DC. Pp. DOI: 10.17226/ 2114.

Olsen R (1995). Mannanoligosaccharides: Experience in commercial turkey production. Biotechnology in the Feed Industry. TP Lyons and KA Jacques. ed. Nottingham University Press, Loughborough, Leics, UK, 389-392.

Phillips T D (1999). Dietary clay in the chemoprevention of aflatoxin-induced disease. Toxicological sciences: an official journal of the Society of Toxicology, 52 (2):118–126. DOI: 10.1093/ toxsci/ 52. Suppl-1.118. PMID: 10630600.

Phillips T D, Clement B A, Kubena L F and Harvey R B (1990). Detection and detoxification of aflatoxins: Prevention of aflatoxicosis and aflatoxin residues with hydrated sodium calcium aluminosilicate. Veterinary and Human Toxicology, 32: 15-19. PMID: 1965459.

Rawal S, Kim JE and Coulombe R (2010). Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. Research in Veterinary Science, 89 (3): 325–331. DOI: 10.1016/ J. rvsc.2010.04.011. PMID: 20462619.

Shotwell OL, Hesseltine CW, Stubblefield RD and Sorenson WG (1966). Production of aflatoxin on rice. Journal of Applied Microbiology, 14(3): 425-428. PMID: 5970829.

Sirisena S, Ng K and Ajlouni S (2015). The Emerging Australian Date Palm Industry: Date Fruit Nutritional and Bioactive Compounds and Valuable Processing By-Products. Comprehensive Reviews in Food Science and Food Safety, 14(6): 813-823. DOI: 10.1111/ 1541-4337.12162.

Tessari ENC, Oliveira CAF, Cardoso ALSP, Ledoux DR and Rottinghaus GE (2006). Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. British poultry science, 47(3): 357-364. DOI: 10.1080/ 00071660600756071. PMID: 16787861.

Umar S, Arshad A, Ahmad B and Arshad M (2012). Clinico biochemical and hematological changes in broilers induced by concurrent exposure to aflatoxin B1 and ochratoxin A. Journal of Public Health and Biological Sciences, 1: 79–85. DOI: 10.13140/ 2.1.2854.3681.

Uyar A, Yener Z and Dogan A (2016). Protective effects of Urtica dioica seed extract in aflatoxicosis: histopathological and biochemical findings. British poultry science, 57(2): 235-245. DOI:10.1080 / 00071668.2015.1129664. PMID: 26947348.

Vayalil PK (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera L. Arecaceae). Journal of Agricultural and Food Chemistry, 50(3): 610-7. PMID: 11804538. DOI: 10.1021/ jf 010716t.

Yildirim E, Yalchinkaya I, Kanbur M, Çnar M and Oruc E (2011). Effects of yeast glucomannan on performance, some biochemical parameters and pathological changes in experimental aflatoxicosis in broiler chickens. Révue de Médicine Vétérinaire, 162 (8): 413–420.

Yunus AW, Razzazi-Fazeli E and Bohm J (2011). Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: A review of history and contemporary issues. Toxins, 3 (6): 566–590. DOI: 10.3390/ toxins3060566.

Zangiabadi H and Torki M (2010). The effect of a Beta-mannanase-based enzyme on growth performance and humoral immune esponse of broiler chickens fed diets containing graded levels of whole dates. Tropical Animal Health Production, 42 (6): 1209-1217. DOI: 10.1007/ s11250-010-9550-1. PMID: 20383608.

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Epidemiological Study of Peste Des Petits Ruminants in Sheep and Goat During 2005-2017 in Palestine

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ABSTRACT

The objective of this study was to analyze the epidemiological occurrence of Peste des petits ruminants in sheep and goat in Palestine during 2005-2017. Data were collected from the annual agricultural census released by the Palestinian central bureau of statistics and the reports of world organization for animal health, submitted by the general directorate of veterinary services and animal health between 2005 and 2017. The study indicated that Peste des petits ruminants is enzootic in Palestine, reported in each year of the study period. The incidence rate ranged from 1.78 to 14.36% with an average of 6.39% per year and per 104 animals. The average morbidity, morbidity and case fatality rate were 8.89%, 2.89%, and 33.57% respectively. Temporal analysis obtained that Peste des petits ruminants is more epizootic in the dry season between April and August with a significant peak on June. The Peste des petits ruminants vaccination rate in Palestine was low and not well organized, ranged from 0.77-34.39% with an average rate of 9%. The appropriate data recording, improving owner awareness, expand the use of the Peste des petits ruminants vaccine and a systematic disease monitoring program are required to control the spread of the disease.

Keywords: Epidemiology, Goat, Palestine, Peste des petits ruminants, Sheep

INTRODUCTION

Peste Des Petits Ruminants (PPR) is a contagious acute viral disease of sheep and goats, characterized by fever, stomatitis, enteritis, pneumonia, and high morbidity and mortality rate (Kozat and Sepehrizadeh, 2017). The disease causes abortion in pregnant animals, increase neonatal mortality and exacerbate the economical losses (Jones et al., 2016). Sheep and goats are the usual targets, besides, cattle and other wild ruminants have been infected most often experimentally (Kumar et al., 2014). The causative agent of PPR belongs to the family *Paramyxoviridae*, genus *Morbillivirus* under order *Mononegavirales.* PPR virus exists as a single serotype but at the genetic level is divided into four distinct lineages (I-IV) based on the fusion (F) protein gene sequence (Banyard, 2010). The virus is present in the secretions of infected animals. Close contact between animals enhance inhalation of droplets that are released into the water, feed, and bedding from the affected animals are the main sources of infection. However, the virus does not survive for a long time outside the body of a host animal (Baron et al., 2016).

There is no effective drug for the treatment of the PPR disease. The control of the disease is limited by using antibacterial drugs to prevent secondary bacterial infection. It is also of importance that the animal should be vaccinated with the commercially available attenuated vaccine as the main control measure in endemic regions (Banyard et al., 2010). The disease occurs worldwide, commonly located in the developing country particularly Asia, the Middle East, and Africa (Özkul et al., 2002; Saeed et al., 2010; Banyard et al., 2014), where veterinary services and resources are inadequate to control or eradicate. Information on the occurrence, prevalence and characterization of PPR disease is available from a number of countries in which the disease has been reported such as Asian countries (Al-Dubaib, 2009; Zahur et al., 2009; Balamurugan et al., 2011). However, the pattern of PPR disease in Palestine has not been investigated before. Also, no systematic study of PPR infection has been done in small ruminants in Palestine. Therefore, the current study was performed to analyze the epidemiological situation of PPR in Palestine over a period of 12 years. This information would be necessary to understand the disease dynamics, mortality, incidence, and temporal distribution and other factors responsible for the persistence of infection in Palestine. In addition, it will help assess the risks, of the disease in order to formulate the appropriate preventative and reactive measures to decrease the infection or to eradicate the disease.

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MATERIALS AND METHODS

Sheep and goat livestock in Palestine 2005–2017

The available numbers of sheep and goat populations in Palestine between 2005 and 2017 were collected from different sources, the primary source was the annual agricultural census released by the Palestinian central bureau of statistics conducted in Palestine (PCBS, 2010). The numbers of sheep and goat populations for the year 2017 has not published yet.

Peste des petits ruminants vaccination, annual and temporal trends

The quantitative data on PPR outbreaks, cases, deaths and vaccinations were collected for the period from 2005 to 2017. Data was extracted from the published annual reports on the world animal health information system of the period between 2005 and 2017 (Jebara et al., 2012).

Data analysis

The frequency of the outbreaks, incidence rate, incidence rate upon exposed, mortality rate, case fatality and vaccination rate of PPR diseases considered in each month of the year according to standard methods (Thrusfield, 2018). These epidemiological parameters were calculated according to the following formulae:

Incidence rate per 10^4 animals = number of cases per year/ total population of sheep and goat during that year × 10^4, infectious mortality rate = number of deaths /number of susceptible animals during the outbreak at the same year ×100, mortality rate per 10^4 animals = number of deaths /total population of sheep and goat during the same year × 10^4, case fatality rate = number of deaths /number of cases, vaccination rate = total number of vaccinated animals / average population of sheep during that year ×100. The data collected were analyzed using Microsoft office excel (2007) and figures were performed using GraphPad Prism 5 software (GraphPad Software Incorporatio, San Diego, USA).

RESULTS

Cumulative profiles

The retrospective quantitative data of PPR for the past 13 years in Palestine are shown in the table 1 included the following information: An average annual sheep population was 1.18 million. An average immunization coverage rate was 9%. A total of 856 outbreaks, 8972 cases, and 2942 deaths were indicated. The average incidence rate was 6.4% per year and per 10^4 animals. The average morbidity rate was 8.9%. The average morbidity rate was 2.9%. The average case fatality rate was 33.6%. Data for numbers of vaccinated animals during years 2005-2011 and 2013 were not reported.

Peste des petits ruminants annual trends

The finding obtained that outbreaks occurred in all years (Figure 1), with the highest number occurring in 2012 (n=312), followed by 2013 (n=96). The lowest number reported in 2017 (n=31) and 2014 (n=46). The increase in the number of outbreaks during 2012–2013 is interesting (Figure 1). The highest incidence rate per 10000 animals was observed in 2005 (14.36%), followed by 2013 (14.08%) and 2015 (8.31%), while the lowest incidence rate occurred in 2016 (1.78%). Morbidity rate ranged from 3.50% in 2007 to 14.63% in 2005. The highest mortality rate per 10000 animals occurred in 2015 (4.94%), followed by 2012 (4.30%). The lowest mortality rate was observed in 2008 (1.31%) (Table 1). The highest case fatality occurred in 2013 (48.25%) followed by 2008, and the lowest occurred in 2015.

Temporal distribution

Data for various parameters were collated by month for the period from January 2005 to December 2017. During this period, the disease occurred in all months of the year, but the highest number of outbreaks occurred on June (Table 2 and Figure 2). The highest numbers of reported outbreaks occurred in summer season between May and August (Figure 2). The lowest outbreaks and the lowest mortality occurred on November and October (Table 2). The greatest mortality rate was reported on November. The disease trend tends to be more severe on December and April with the highest case fatality rate (Table 2).

Trends in vaccine utilization

The total utilization of the Peste des petits ruminants vaccine was compared across different years. There is some limitation about the numbers of vaccinated animals in 2012 and 2014-2017 years. The vaccine used is a live attenuated vaccine. The total of immunization coverage was 845762 animals with an average vaccination rate of 9.0%. The annual utilization data showed that the highest number of doses (250228 doses) utilized with a vaccination rate of 21.31% achieved in 2005, whereas in 2012 only 34,769 doses were utilized, and the vaccination rate was 4.08% (Figure 3). With

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regards to vaccination rate, the highest vaccination percentage was achieved in 2011 (21.54%), followed by 2005, 2010 and 2006. The lowest vaccination percentage was in 2013 (4.08%) (Table 1). The vaccination rate was not available for the 2012, 2015-2017 years.

Table 1. Annual statistics of Peste des petits ruminants in sheep and goat in Palestine (2005-2017)

Year	Total No. Outbreaks	No. Susceptible	No. Cases	No. Deaths	Morbidity rate (%)	Mortality rate (%)	Incidence rate/10^4	Case fatality rate (%)	Vaccination rate (%)
2005	71	11523	1686	359	14.63	3.12	14.36	21.29	4.21
2006	49	7946	349	124	4.39	1.56	2.96	35.53	6.99
2007	65	9251	324	132	3.50	1.43	2.98	40.74	3.37
2008	76	11070	494	145	4.46	1.31	5.21	29.35	6.08
2009	71	8730	441	170	5.05	1.95	3.77	38.55	7.67
2010	50	6794	664	180	9.77	2.65	3.65	27.11	10.35
2011	48	6412	692	175	10.79	2.73	7.12	25.29	34.39
2012	171	9599	1054	413	10.98	4.30	5.26	39.18	NA
2013	96	15441	1200	579	7.77	3.75	14.08	48.25	0.77
2014	46	9395	690	200	7.34	2.13	7.29	28.99	NA
2015	64	4923	785	243	15.95	4.94	8.31	30.96	NA
2016	18	1892	200	75	10.57	3.96	1.78	37.50	NA
2017	31	3791	393	147	10.37	3.88	NA	37.40	NA

No: Number, NA: data not available

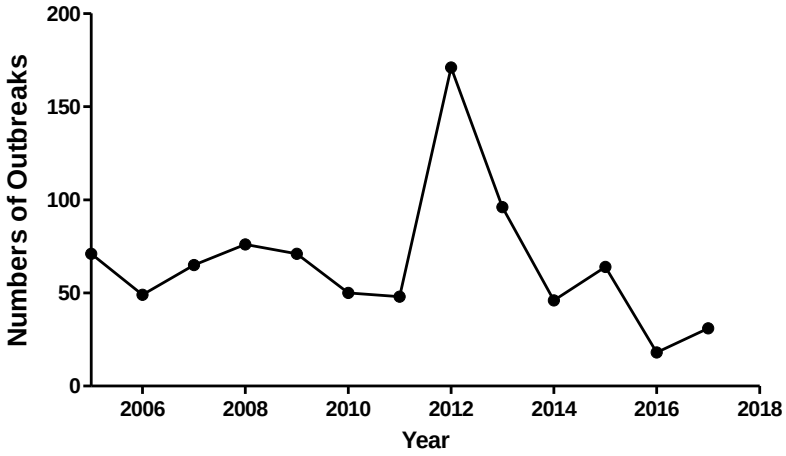

Figure 1. Numbers of Peste des petits ruminants outbreaks in sheep and goat in Palestine during 2005-2017.

Table 2. Seasonality of PPR in sheep and goat in Palestine (January 2005 to December 2016)

Month	No Outbreak	No. Susceptible	No. Case	No. Deaths	Morbidity rate (%)	Mortality rate (%)	Case fatality rate (%)
January	46.0	5165	397	115	7.7	2.2	29.0
February	42.0	5018	517	149	10.3	3.0	28.8
March	66.0	9318	923	325	9.9	3.5	35.2
April	69.0	10913	765	322	7.0	3.0	42.1
May	89.0	13208	1104	424	8.4	3.2	38.4
June	117.0	15835	1320	409	8.3	2.6	31.0
July	109.0	16665	1765	534	10.6	3.2	30.3
August	86.0	8972	594	238	6.6	2.7	40.1
September	56.0	5929	589	129	9.9	2.2	21.9
October	62.0	4589	414	75	9.0	1.6	18.1
November	54.0	5518	201	52	3.6	0.9	25.9
December	60.0	5637	383	170	6.8	3.0	44.4

No: Number

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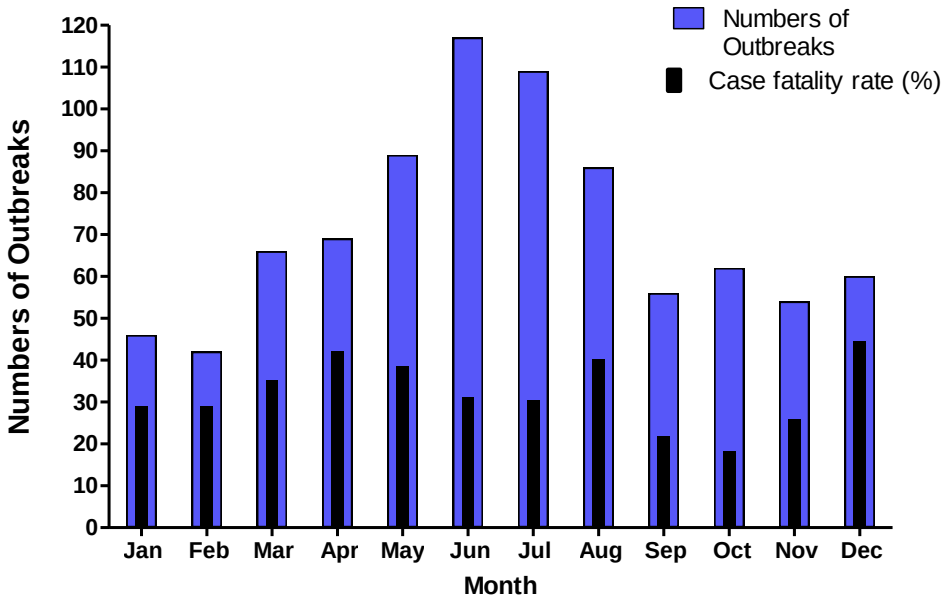

Figure 2. Seasonality of Peste des petits ruminants in sheep and goat in Palestine (January 2005 to December 2017)

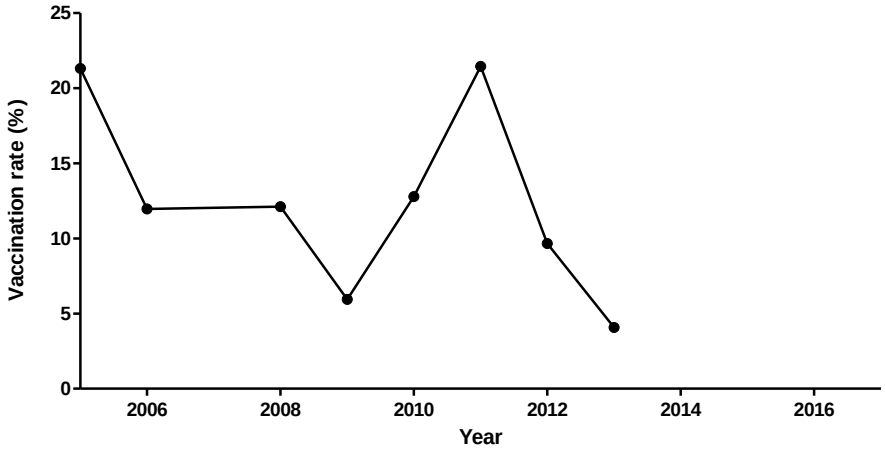

Figure 3. Vaccination rate of Peste des petits ruminants in sheep and goat in Palestine during 2005-2017

DISCUSSION

PPR is an extremely contagious viral disease of sheep and goats. Due to the massive economic impacts of PPR in Palestine, it is essential to perform the analysis of epidemiological data of this disease. Understanding the disease epidemiology is necessary for effective control in the aim to the eradication of the disease. The present study was performed to provide epidemiological information in Palestine about the available confirmed PPR outbreaks among sheep and goats during 2005 and 2017.

The first report of PPR in the country was in 1993 (Parida et al., 2015). Since a large number of outbreaks have occurred and documented, these documentations were based on the confirmation of the World Organization for Animal Health (OIE) (Jebara et al., 2012). Indeed, other outbreaks, deaths and other data based on clinical observations were not accurately recorded due to inadequate animal disease diagnosis, reporting, and surveillance systems. The findings indicated that PPR in Palestine is present in an enzootic form with a low morbidity and mortality rate, however, the prevalence rate needs to be investigated by a national surveillance campaign. There is a limitation of reports regarding the incidence of PPR from other countries, most of the studies described PPR prevalence in a selected population at a certain time. For example, in Saudi Arabia, the prevalence rate was 2.3% in a total of 1035 serum samples from sheep and goat in 2004 (Adel et al., 2004). In 2010 in Pakistan, about 10% out of 2723 samples tested by ELISA were positive (Munir et al., 2010). In a survey performed by Al-Majali et al. (2008), a high prevalence rate was detected in sheep (29%, n= 929) and goat (49%, n= 400) tested in Northern Jordan in 2007 (Al-Majali et al., 2008). Outbreaks also most frequently being characterized in Iraq with high morbidity and low mortality rates (Banyard et al., 2010), while 22.4%

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out of 1607 samples were positive in Turkey in 2002 (Özkul et al., 2002). These studies confirmed that PPR is enzootic in this region of the world. In the enzootic area, the mortality rates may be as low as 20% with outbreaks occurring at a regular interval (Dhar et al., 2002). These similar estimates confirmed that PPR is a regional problem and countries in the Middle East. The variations in PPR incidence between these countries are mostly related to the different density of the flocks in the studied area, animal husbandry systems, flock size, vaccinations, humidity, rainfall, temperature and the technique used for detection of the disease (Sharma et al., 2015; Ma et al., 2017). The disease in Palestine is circulating quietly during the season. The findings revealed that the PPR outbreaks numbers increase in the dry season from April to August with a peak in June. The disease then expresses itself in epizootic outbreaks that appear with a seasonal frequency. These results are in agreement with other studies (Singh et al., 2004; Folitse et al., 2017), who observed higher prevalence in the dry season. This increase could be related to animal movement, animals flocked from one place to another that favor the spread of the virus (Dhar et al., 2002). Sheep and goats from different places are brought into close contact during spring and summer seasons at the marketplaces, this could also promote PPR virus transmission (Das et al., 2007). In contrast other studies reported that PPR is mostly epizootic during the wet season (Balamurugan et al., 2011), this could be due to the limited availability of feed during this period of time and close confinement of the animals in farm buildings, as well as to the increased introduction of susceptible young animals in the flocks rather than a seasonal surge in viral activity (Balamurugan et al., 2011; Ban-Bo et al., 2014). The findings revealed that PPR mortality rate range from 0.9 to 3.5%, in agreement with other studies that the mortality rate in endemic areas may be as low as 20%, with outbreaks occurring at a regular interval (Dhar et al., 2002). PPR tends to be more severe in the change in weather such as the late dry season and the onset of the rainy season (hot and humid) or dry, cold periods (Dhar et al., 2002). A nation-wide program vaccination against PPR has been practiced in Palestine to control the disease. Currently, the available PPR vaccine is a live attenuated prepared from a reference strain Nig. 75/1 from Jordan bio-industries center (JOVAC, Jordan). The PPR vaccination in Palestine is not well organized, the findings indicated that the rate of vaccinated animals is too low and will not lead to effective containment and control of PPR. It is important to note that the Israeli measures defy access to perform veterinary services of animals in Palestine (MoA, 2015). The difficulties to reach animals holdings near the Israeli Settlements and the “closed military zone” by Israeli military barriers also contribute to the low vaccination rate (PCBS, 2011). The restriction of vaccine administration by the veterinary service at the ministry of agriculture as well as the unawareness of owners about the benefits of vaccination could be probable explanations of this low rate of vaccination.

CONCLUSION

In conclusion, based on present findings, PPR is enzootic in sheep and goat in Palestine. Outbreaks occurred during the year; more epizootic with low morbidity and mortality, and sever in wet cold seasons. Proper and effective record keeping is essential for tracking and evaluation of the disease status in Palestine. A sero-surveillance plan should be taken up particularly in the unvaccinated areas to estimate the prevalence. Besides, a vaccination campaign must be applied at the level of the state to control the disease followed by a systematic monitoring program should be initiated to assess the efficacy of the vaccination campaign.

DECLARATIONS

Competing interests

The author has declared that no competing interest exists.

Consent to publish

The author gives the consent for information and images concerning the article to be published in the World's Veterinary Journal.

REFERENCES

Adel AA, Abu-Elzein E, Al-Naeem and AM and Amin M (2004). Serosurveillance for peste des petits ruminants (ppr) and rinderpest antibodies in naturally exposed saudi sheep and goats. Veterinarski Arhiv, 74 (6): 459-465. Accessed via: http://vetarhiv.vef.unizg.hr/papers/2004-74-6-8.pdf

Al-Dubaib M (2009). Peste des petitis ruminants morbillivirus infection in lambs and young goats at qassim region, saudi arabia. Tropical animal health and production. 41 (2): 217-220. DOI: 10.1007/s11250-008-9178-6

Al-Majali AM, Hussain NO and Amarin NM and Majok AA (2008). Seroprevalence of, and risk factors for, peste des petits ruminants in sheep and goats in northern jordan. Preventive Veterinary Medecine, 85 (1-2): 1-8. DOI: 10.1016/j.prevetmed.2008.01.002

Balamurugan V, Saravanan P, Sen A, Rajak K, Bhanuprakash V, Krishnamoorthy P and Singh RK (2011). Sero-epidemiological study of peste des petits ruminants in sheep and goats in india between 2003 and 2009. Revue Scientifique et Technique-OIE, 30 (3): 889. DOI: 10.20506/rst.30.3.2087

Ban-Bo B, Mian-Oudanang K, Mopate L and Nakour N and Brahim G (2014). Knowledge of diseases and animal control methods at the grand kanem in chad. Journal of Research in Biology, 4: 1387-1396. Accessed via: http://jresearchbiology.com/documents/RA0455.pdf

Banyard AC and Wang ZandParida S (2014). Peste des petits ruminants virus, eastern asia. Emerging Infectious Dissease, 20 (12): 2176-2178. DOI: 10.1128/JVI. 02448-05 3.

Banyard AC, Parida S, Batten C, Oura C, Kwiatek O and Libeau G (2010). Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. Journal of general virology, 91(12):2885-2897. DOI 10.1099/vir.0.025841-0

Baron MD, Diallo A, Lancelot R and Libeau G (2016). Peste des petits ruminants virus. Advances in virus research, 95(1) 1-42. DOI: 10.1016/bs.aivir.2016.02.001.

Das KK and Shil NKandIslam MR (2007). Sero-epidemiological investigation on peste des petits ruminants in black bengal goats. Bangladesh Journal of Microbiology, 24 (2): 143-145. DOI: 10.3329/bjm.v24i2.1260

Dhar P, Sreenivasa BP, Barrett T, Corteyn M, Singh RP and Bandyopadhyay SK (2002). Recent epidemiology of peste des petits ruminants virus (PPRV). Veterinary microbiology, 88(2):153-9. DOI: 10.1016/S0378-1135(02)00102-5

Folitse R, Amemor E, Rejoice EN and Emikpe B and Tasiame W (2017). Pattern of peste des petits ruminants (ppr) distribution in ghana (2005-2013). Bulgarian Journal of Veterinary Medicine, 20 (1):51-57. DOI: 10.15547/bjvm.958

Jebara KB, Cáceres P, Berlingieri F and Weber-Vintzel L (2012). Ten years' work on the world organisation for animal health (OIE) worldwide animal disease notification system. Preventive veterinary medicine, 107(3-4):149-59. DOI: https://doi.org/10.1016/j.prevetmed.2012.08.008

Jones BA, Rich KM, Mariner JC, Anderson J, Jeggo M, Thevasagayam S, Cai Y, Peters AR and Roeder P (2016). The economic impact of eradicating peste des petits ruminants: a benefit-cost analysis. PLoS One, 11(2):1-18. DOI | DOI:10.1371/journal.pone.0149982

Kozat S and Sepehrizadeh E (2017). Peste Des Petit Ruminants. Journal of Istanbul Veterinary Sciences, 25;1(2):47–56. DOI:10.30704/http-www-jivs-net.319937

Kumar N, Maherchandani S, Kashyap S, Singh S, Sharma S, Chaubey K and Ly H (2014). Peste des petits ruminants virus infection of small ruminants: a comprehensive review. Viruses, 6(6):2287-327. DOI: 10.3390/v6062287.

Ma J, Jianhua X, Han L, Xiang G, Hao C and Hongbin W (2017). Spatiotemporal pattern of peste des petits ruminants and its relationship with meteorological factors in china. Preventive Veterinary Medecine, 147: 194-198. DOI: 10.1016/j.prevetmed.2017.09.009.

Ministry of Agriculture, State of Palestine (MoA) (2015). Livestock Sector Strategy 2015-2019. Accessed via: http://extwprlegs1.fao.org/docs/pdf/pal165804.pdf

Munir M, Siddique M, Shehzad A, Zohari SandStahl K (2010). Seroprevalence of antibodies to peste des petits ruminants at various governmental livestock farms of punjab, pakistan. Asian Journal of Epidemiology. 3 (3): 183-191. DOI: 10.3923/aje.2008.82.90

Özkul A, Akca Y, Alkan F, Barrett T, Karaoglu T, Dagalp SB, Anderson J, Yesilbag K, Cokcaliskan C, Gencay A, Burgu I (2002). Prevalence, distribution, and host range of peste des petits ruminants virus, Turkey. Emerging infectious diseases. 8 (7): 709. DOI: 10.3201/eid0807.010471

Saeed IK, Ali YH, Khalafalla AI, Rahman-Mahasin E (2010). Current situation of peste des petits ruminants (ppr) in the sudan. Tropical animal health and production. 42 (1): 89. DOI: 10.1007/s11250-009-9389-5.

Sharma KK, Kshirsagar DP, Kalyani IH, Patel DR, Vihol PD, Patel JM (2015). Diagnosis of peste des petits ruminants infection in small ruminants through in-house developed indirect ELISA: Practical considerations. Veterinary world. 8 (4): 443-448. DOI: 10.14202/vetworld.2015.443-448

Singh RP, Saravanan P, Sreenivasa BP, Singh RK, Bandyopadhyay SK (2004). Prevalence and distribution of peste des petits ruminants virus infection in small ruminants in India. Revue Scientifique et Technique-OIE, 23(3):807-19. Accessed via: https://pdfs.semanticscholar.org/963f/3b0c4a7b8668f8c7d5984b954225922bd909.pdf

The Palestinian Central Bureau of Statistics (PCBS) (2011). Agricultural census 2010, final results. Palestinian central bureau of statistics, Ramallah, Palestine. Accessed via: http://www.pcbs.gov.ps/Downloads/book1818.pdf

Parida S, Muniraju M, Mahapatra M, Muthuchelvan D, Buczkowski H and Banyard AC (2015). Peste des petits ruminants. Veterinary microbiology, 181(1-2):90-106. DOI: 10.1016/j.vetmic.2015.08.009

Thrusfield M (2018). Veterinary epidemiology, 4th Edition. John Wiley and Sons. pp 67-80.

Zahur A, Ullah A, Irshad H, Farooq M, Hussain M and Jahangir M Epidem (2009). Epidemiological investigations of a peste des petits ruminants (ppr) outbreak in afghan sheep in pakistan. Pakistan Veterinary Journal, 29 (4): 174-178. Accessed via: http://www.pvj.com.pk/pdf-files/29_4/174-178.pdf

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The Influence of Hairline Crack Eggs on Hatchery Parameters and Performance of Chicks

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ABSTRACT

The purpose of study was to evaluate the influence of hairline crack eggs on hatchery parameters and later life of chicks. The study was conducted from October to December 2018 at Chakri hatchery Salman Poultry Pvt. Ltd Pakistan to evaluate the outcomes of hairline crack eggs. The shell of the eggs is essential in providing the shape of an egg and ensuring the safe packaging. The defects like breakage of this packaging increase the risk of microbial contamination. In this experiment, the crack eggs like hairline crack eggs were detected by Sanovo STAALKAT Alpha 125 Machine number JB 11786. The eggs were collected from eighteen different breeder farms. Each group contained (n=50,000) eggs. The hairline crack eggs were compared with normal eggs for hatchability, candling, putrification/blasting and dead in shell. Significant difference was found for hatchability, candling, blasting/putrification and dead in the shell for normal and hairline crack eggs. The highest hatchability (49.07±0.51) and lowest candling (9.98±0.064) for hairline crack eggs were found for AP27 flock due to young age and good quality eggshell. The lowest hatchability was found for SP117 flock which was the oldest flock having thin egg shells. The blasting/putrification and dead in the shell were significantly higher for hairline crack eggs as compared to normal eggs of same flocks. The lowest blasting was found for AP27 flock. On a simple hatch debris analysis, the highest mortality, infertile, contaminated eggs were for first and third weeks. The higher mortality were found for hairline crack eggs as compared to the normal eggs for SSF5 flock. The water loss, chick yield and culling chicks percentage were also significantly better for normal eggs compared to hairline crack eggs. The hairline crack eggs of young flocks were better than old flocks due to a better quality of eggs shell. The chicks from normal eggs were also significantly better than chicks from hairline crack eggs in terms of mortality, feed intake, weight gain and FCR. The hairline crack eggs are the source of contamination. Present study recommended that hairline crack eggs do not use for incubation.

Key words: Candling, Dead in shell, Hairline crack, Hatchability, Water loss

INTRODUCTION

Fertility and hatchability are major determinants for profitability in a hatchery enterprise. A healthy chicken starts from a good quality intact breeder's egg. The egg is a multifunctional biomineral complex consists of highly structured calcium carbonate shell act as a barrier for growing embryo to prevent microbial invasion and provide mechanical strength (Wellman et al., 2008). The eggs should appear smooth and free of cracks by the naked eye. The damage of eggshell may occur at any level occasionally including transportation and while eggs settings. Some eggshell breakage may be complete crack, star crack, pimples, pinholes, sandpaper, leathery appearance and hairline cracks, account for approximately 0.5% to 6% of total eggs production (King'ori et al., 2012). Many scientists developed different techniques to identify the crack eggs (Wei et al., 2015) developed a magnetostrictive transducer technique to identify crack eggs. The swept vibration signals from 1 to 14 kHz created in the computer by software. Then it was amplified by circuit board to drive the magnetostrictive transducer. The collision between the egg and magnetostrictive transducer generated sounds. The computer recorded the acoustic signals at 44 kHz sampling rate through the microphone. The sampled acoustic signals contain rich information about the quality of the eggs. By analyzing the acoustic signals we can identify the egg is intact or not.

Another technique described by Xie and He (2016) which can be used for eggshellcolour and shell strength detection is hyperspectral imaging technique which can produce spectral information as well as spatial information for objectives at the same time. A spatial hyperspectral cube can be generated when one sample was scanned by the hyperspectral imaging camera. The hyperspectral cube (hyperspectral image) contains a series of images covering the whole wavelength, and each pixel for one image has both spectral and spatial information. Because of this feature, it can

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be used to detect external characteristics, such as fruit defect, colour and sugar beet disease and internal information, such as moisture content, other chemical indexes and eggshellcolour and shell strength.

The eggshell is protection for growing embryo to the external environment as well as microbial contamination. The breakage of eggshell provides an ideal rout entrance for penetrating microbes. Van den Brand et al. (2016) found that fertility and hatchability of clean eggs were higher than the floor or dirty eggs. Through the crack, shell microbes penetrate and cause embryonic mortality at any stage of incubation. Jabbar and Ditta (2017a) conducted an experiment to know the effect of floor eggs and found that floor eggs are a source of low hatchability, improper water loss, high candling and dead in shell. The floor eggs are a source of contamination. Salahi et al. (2011) also found that hairline cracks eggs become a source of low hatchability, high candling, high dead in shell and embryonic mortality as compared to star crack or intact shell eggs. Moreover, chick length, yolk-free body mass, breast and liver weight were significantly decreased than normal eggs. The contamination was higher for hairline crack eggs than that of star crack eggs. The aim of study was to evaluate the outcome from hairline crack eggs in term of hatchery parameters and its effects on later life of chicks.

MATERIALS AND METHODS

Ethical approval

This experiment was performed considering to all animal rights (Society for Protection and Care of Animals. University of veterinary and Animal Sciences, Pakistan)

Site selection

The experiment was performed at Salman Poultry (Pvt) Limited, Chakri Hatchery Rawalpindi which is situated 5 km from the Chakri interchange on the motorway (M2) Pakistan The hatchery is facilitated with latest Heating Ventilation and Air Conditioning (HVAC) automation, having ISO (International standard organization) 1900-2000 certified. This hatchery is one of the largest chicks producing hatchery of south Asia, which is producing 6.5-7 million high quality chicks through the single stage incubation system (Avida G4, Chick Master USA).

Selection of eggs

Each experimental group was consisting of n=50,000 eggs. SP 117-AI B, C, D, E, F (Salman Poultry Flock number 117 with artificial insemination (team B, C, D, E, F), Salman Poultry Flock number 5 (SSF5), Salman Poultry Flock number 6 (SSF6), Salman Poultry Flock number 1 with artificial insemination team C (SSF-R1-AI-C), Salman Poultry Flock number 2 with artificial insemination team A (SSF-R2-AI-A), Salman Poultry Flock number 2 with artificial insemination team B (SSF-R2-AI-B) and Salman Poultry Flock number 3 with artificial insemination team D (SSF-R3-AI-D). Arslan Poultry flock number 27 (AP).

Crack eggs detection

The crack eggs detection was performed through STAALKAT Alpha 125 Machine number JB 11786. This machine has multiple functions including eggs grading on the basis of weight, crack eggs detection through sound, leaker and dirty eggs detection. The machine converts digital signals into multiple crack categories. The crack eggs detection was performed through this machine for each flock.

Table 1. Percentage of hairline crack eggs with respect to flock age

Flocks	Hairline Crack (%)	Flock Age (weeks)
Salman Poultry Flock 117-2	0.5	60
Salman Poultry Flock 117-1-AI-B	1.18	60
Salman Poultry Flock 117-1-AI-C	1.17	60
Salman Poultry Flock 117-1-AI-D	1.18	60
Salman Poultry Flock 117-1-AI-E	1.17	60
Salman Poultry Flock 117-1-AI-F	1.17	60
Salman Sadiq Flock 1-R1-AI-C	0.8	46
Salman Sadiq Flock1-R2-AI-A	0.75	46
Salman Sadiq Flock1-R2-AI-B	0.79	46
Salman Sadiq Flock1-R3-AI-D	0.65	46
Salman Sadiq Flock-5	0.41	41
Salman Sadiq Flock-6	0.4	40
Salman Sadiq Flock 2-Ross1	0.2	31
Salman Sadiq Flock 2-Ross2	0.21	30
Salman Sadiq Flock 8	0.21	30
Salman Sadiq Flock 2-Ross3	0.20	29
Arslan Poultry Flock 27-Ross2	0.20	29
Arslan Poultry flock 27-Ross1	0.19	28

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Weight of eggs

Before setting the egg's weight of each individual group was calculated by setting eggs into one setter tray then applying the formula,

$$\text{Egg weight: } \frac{\text{Full tray weight at Setting- Weight of empty tray}}{\text{Total No of eggs in tray}}$$

Eggs fumigation

Before the weighing, the trial eggs were fumigated with 20 g $KMnO_4$ and 40ml formalin (40%) and 40 ml of water for 100ft^3 areas for 15 minutes through automatic fumigation process provided by Chick Master.

Incubation programme

Standard incubation profiles recommended by chick master were selected on the basis of breeder's age. Pre-heating was performed for all experimental groups following automatically the incubation stage profile (Recommended by Chicks Master USA).

Setter hall and hatcher hall

Environmental conditions in setter hall were at 75^0F temperatures and 40% relative humidity; whereas in the hatcher hall temperature was at 75^0F and relative humidity had been increased up to 60%. The positive pressure in setter and hatcher hall was 15 Pascal and 10 Pascal respectively, while negative pressure inside setter and hatcher plenum was -25 Pascal during the course study.

Candling

Fertility of eggs was performed through candling then shifted to hatchers for next 50 hrs. These entire incubation stage programs have been recommended by chick master USA.

Egg's weight loss

Before being transferred from setter to hatchers water loss e-g egg weight loss was measured for from each group individually after 456 hrs of incubation in setters by the given formula:

$$\text{Water Loss \%: } \frac{\text{Full tray weight at Setting- Full Tray Weight at Transfer} \times 100}{\text{Full tray weight at Setting- Empty Tray Wight}}$$

Chick yield

After hatch pulls out immediately, the chick's weight was measured through electrical weight balance to know the chick yield using following formula:

$$\text{Chick Yield \%: } \frac{\text{Weight of chick's} \times 100}{\text{Egg weight}}$$

Hatch window

Hatch window was done between the first chicks to last chick hatch out (Noiva et al., 2014). The range of hatch window was measured through the graph produced by Maestro software (Chick Master USA). The increase hatching process inside hatcher becomes a source of increase humidity which can be easily detected.

Chick grading

Grading of chicks was performed on the conveyor and automatic grading table while chicks counting and packing was performed through chick counter (KUHL-USA). Only stranded (shining eyes, soft legs and nose, healed naval and healthy chicks) were shifted to chick's box after counting, while underweight, weak, and unhealed naval chicks were removed as an international standard as described by Yousaf et al. (2017).

Hatch out analysis

Hatch out analysis was performed to investigate the reason of embryo's mortality inside the eggs as described by Jabbar and Ditta (2017a, b and c).

Poultry house

The chicks from normal (n=30000) and hairline crack eggs (n=30000) were shifted to broiler houses through environmental control vehicles $23C^0$ and 65% humidity to access the outcomes from the hairline crack eggs chicks in term of FCR, feed intake and chick's mortality

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Performance of chick

The Feed Concervation Ratio (FCR) was calculated by measuring the amount of feed consumed by birds divided to the chicks weight gain. Calculating the chicks mortality percentage was possible by counting the mortality of chicks for complete flock divided to total number of chicks and multiply by 100.

FCR (g/g) = feed intake (g)/weight gain (g)

Mortality (%) = Number of chicks died during complete flock/ Total number of chicks in flock × 100

Statistical analyses

All data were analyzed by using Statistical Analysis System package software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). All means were compared using t-test and results were presented as mean ± SEM (standard error of the mean). Results were considered significant if $P<0.05$.

RESULTS AND DISCUSSION

The eggs shell constitutes approximately 10% of the whole egg. The calcium participates 95-97% of eggs hellweight. The percentage of eggshell decreases with the increase of flock age. The percentage of eggshell was 10.65, 10.29 and 9.81% with 25, 47 and 61 weeks of age respectively. Eggshell percentages at the ages of 25, 47 and 61 weeks were10.65, 10.29 and 9.81%, respectively (Salahi et al., 2011). That's the reason the percentage of crack eggs increase with respect to flock age (Table 1). The flock SP117-1 and SP117-2 have the maximum percentage of hairline crack eggs followed by SSF1, SSF5 and SSF6. The flocks SSF2 and AP27 have a minimum percentage of hairline crack eggs due to young age. The eggshell is essential in providing the shape of an egg to assure the safe packaging. Samiullah et al. (2014) found that egg weight, shell weight and shell thickness decreases with flock age. The eggs internal quality albumin heights decreased with flock age. The amount of cuticle also varied with flock age. Due to change in eggshell quality with chicken age, the defects in eggshell like breakage increases and risk of microbial contamination to eggs increases. This microbial contamination becomes a source of embryonic mortality at any stage of incubation (Jabbar and Ditta, 2017a), becomes a source of low hatchability and high candling (Table 2). Significant difference ($P<0.05$) was found for hatchability, candling, blasting/putrification and dead in the shell for normal and hairline crack eggs. The highest hatchability (49.07±0.51) and lowest candling (9.98±0.064) for hairline crack eggs were found for AP27 due to young age and good quality eggshell. The lowest hatchability was found for SP117 which was the oldest flock having thin egg shells (Table 2).

The breakage of eggshell and shell membrane exposes the growing embryo to contamination. The contamination may cause embryonic mortality depending on the severity of infection at any stage of incubation (Barnett et al., 2004). The crack eggs are a good source of microbial contamination, result in putrification and blasting during transfer of eggs from setter machine to hatchers. The high blasting/purification and dead in the shell are mainly due to penetration of microbes through eggshell from eggs surface. The egg shell and shell membrane is a barrier to avoid such kind of contamination. The breakage of these barriers provides easy access for microbes to infect the embryo cause its death and enhances dead in shell. The blasting/putrification and dead in the shell were significantly ($P<0.05$) higher for hairline crack eggs as compared with normal eggs of same flocks. The highest blasting and dead in the shell of hairline crack eggs were indicated in table 2. The lowest blasting was found for AP27 due to young age and good quality eggshell (Table 2).

The percentage of third week embryonic mortality was higher for a hairline crack eggs up to 7.9% as compared to normal eggs (6.8%). The maximum quantity of contaminated eggs was found during simple hatch debris analysis for a hairline crack eggs (7.8%) as compared to normal eggs (2.3%). The effect of shell quality at second week of embryonic period on mortality and crack shell were the same for both kinds of eggs. The infertile eggs were also higher in hairline crack eggs (1.0%) as compared to normal eggs (0.6%). The term external pipes are used for such kind of chicks which can break both shell membrane and eggshell during the hatching process but unable to come out from eggs due to any reason. The external pips may be due to low humidity in hatchers near pipping, weak chicks due to the young age of breeders and infection which causes omphalitis. Amare et al. (2013) found that *Escherichia coli* (51.17%) was most prominent followed by *Staphylococcus* (23.53), Proteus microbes (22.94) and other bacteria like streptococcus and *Bacillus* species were most prominent for development of yolk sac infection. The infection may start at any stage of incubation depending upon severity form eggs surface to the growing embryo. Such kinds of infections increase if the barriers for growing embryo are broken like hairline crack eggs.

Chick yield and water loss are related to each other. The chick yield is a percentage of chick conversion from the egg. We found better chick yield and water loss for normal unbroken eggs as compared to a hairline crack eggs (Table 4). The water loss and chick yield are related to each other, water loss less than standard level (11-12%) becomes a source for ascites and early embryonic mortality during the brooding phase. The water loss more than standard causes

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low chick yield result in dehydration (Jabbar and Ditta, 2017b). The water loss, chick yield and culling chicks were significantly (P<0.05) better for normal eggs as compared to hairline crack eggs. The young flocks AP27 R1, AP27R2, SSF2R1, SSF2R2 and SSF8 were better even for hairline crack eggs compared to old flocks due to the better quality eggshell.

Simple hatch debris analysis was performed to access the percentage of infertile, early embryonic mortality, mid, late embryonic mortality, external pips, contaminated eggs, poor shell quality eggs and crack shell eggs for flock SSF5 normal eggs and hairline crack eggs (Jabbar and Ditta, 2017a-c).

The chicks from normal (n=30000) and hairline crack eggs (n=30000) were shifted to broiler houses through environmental control vehicles 23^0C and 65% humidity to access the outcomes from the hairline crack eggs chicks in term of FCR, feed intake and chick's mortality. The environmental conditions were kept the same for both kinds of chicks from brooding to raring to minimize any kind of stress (Table 5). The chicks from normal eggs were significantly (P<0.05) better feed intake, weight gain, FCR and mortality (Table 6).

Table 2. Hatchability and candling of hairline crack and normal eggs

Flocks	Hatchability		Candling/Infertile	
	Normal	Hairline	Hairline	Normal
Salman Poultry Flock 117-2	77.69±0.1^a	41.41±0.02^b	39.19±0.05^a	13.41±0.81^b
Salman Poultry Flock 117-1-AI-B	86.42±0.2^a	43.03±0.07^b	36.36±0.014^a	4.16±0.19^b
Salman Poultry Flock 117-1-AI-C	86.80±0.1^a	40.00±0.31^b	39.39±0.014^a	4.01±0.17^b
Salman Poultry Flock 117-1-AI-D	86.57±0.11^a	42.42±0.14^b	36.36±0.064^a	3.98±0.14^b
Salman Poultry Flock 117-1-AI-E	86.63±0.2^a	39.39±0.57^b	38.79±0.016^a	4.02±0.15^b
SP117-1-AI-F	86.95±0.2^a	45.45±0.14^b	33.33±0.025^a	3.90±0.17^b
Salman Sadiq Flock -5	72.59±0.1^a	42.42±0.15^b	34.55±0.047^a	13.46±0.61^b
Salman Sadiq Flock F-6	77.54±0.3^a	44.24±0.34^b	33.3±0.019^a	9.86±0.31^b
Salman Sadiq Flock 1-R1-AI-C	82.00±0.09^a	40.91±0.92^b	36.67±0.071^a	6.21±0.61^b
SSF1-R2-AI-A	80.71±0.31^a	40.40±0.84^b	35.96±0.064^a	7.02±0.43^b
Salman Sadiq Flock 1-R2-AI-B	82.30±0.04^a	41.52±0.47^b	35.15±0.017^a	5.07±0.13^b
Salman Sadiq Flock 1-R3-AI-D	81.07±0.02^a	43.33±0.24^b	34.85±0.027^a	6.94±0.98^b
Arslan Poultry 27-R1	93.61±0.07^a	49.07±0.51^b	9.98±0.064^a	4.18±0.61^b

$^{a-b}$ denote difference for hatchability and candling of normal and hairline crack eggs within rows (P< 0.05)

Table 3. Putrification of dead in shell of hairline crack and normal eggs

Flocks	Blasting/Putrification		Dead in Shell	
	Hairline	Normal	Hairline	Normal
Salman Poultry Flock 117-2	12.1±0.64^a	5.14±0.48^b	19.39±0.61^a	8.90±0.25^b
Salman Poultry Flock 117-1-AI-B	15.15±0.31^a	5±0.71^b	20.61±0.24^a	9.42±0.34^b
Salman Poultry Flock 117-1-AI-C	10.30±0.61^a	1.5±0.61^b	20.61±0.61^a	9.18±0.24^b
Salman Poultry Flock 117-1-AI-D	9.09±0.95^a	1.5±0.21^b	21.21±0.14^a	9.45±0.21^b
Salman Poultry Flock 117-1-AI-E	7.57±0.97^a	1.5±0.64^b	21.82±0.31^a	9.35±0.36^b
Salman Poultry Flock 117-1-AI-F	7.27±0.68^a	1.5±0.34^b	21.21±0.15^a	9.15±0.36^b
Salman Sadiq Flock -5	10.3±0.16^a	1.5±0.81^b	23.03±0.17^a	12.95±0.24^b
Salman Sadiq Flock -6	13.63±0.46^a	1.5±0.15^b	22.42±0.34^a	12.60±0.64^b
Salman Sadiq Flock 1-Ross1-AI-C	10.3±0.57^a	1.5±0.93^b	22.42±0.31^a	11.79±0.37^b
Salman Sadiq Flock 1-Ross2-AI-A	11.71±0.94^a	1.5±0.65^b	23.01±0.27^a	12.27±0.27^b
Salman Sadiq Flock 1-Ross2-AI-B	9.09±0.39^a	1.5±0.87^b	23.01±0.61^a	12.63±0.61^b
Salman Sadiq Flock 1-R3-AI-D	10.57±0.68^a	1±0.51^b	21.82±0.34^a	11.99±0.91^b
Arslan Poultry 27-R1	5.12±0.97^a	0.97±0.81^b	12.75±0.31^a	2.09±0.64^b

$^{a-b}$ denote difference of blasting and dead in shell of hair line crack eggs and normal eggs within rows (P<0.005)

Table 4. Effect of hairline crack eggs on chick yield, water loss and culling chicks

Flocks	Chick Yield (%)		Water loss (%)		Culling (%)	
	Normal Eggs	Hairline crack	Normal Eggs	Hairline crack	Normal Eggs	Hairline crack
Salman Poultry Flock 117-2	69±0.1^a	66±0.21^b	12.1±065^a	14.3±0.24^b	1.21±0.34^a	2.1±0.34^b
Salman Poultry Flock 117-1-AI-B	69±0.02^a	67±0.014^b	12.4±0.31^a	14.65±0.5^b	1.2±0.42^a	2.01±0.61^b
Salman Poultry Flock 117-1-AI-C	68±0.01^a	66±0.051^b	12.3±0.32^a	13.98±0.34^b	1.02±0.31^a	2.3±0.12^b
Salman Poultry Flock 117-1-AI-D	69±0.21^a	67±0.01^b	11.87±0.6^a	14.9±0.37^b	1.1±0.32^a	2.14±0.24^b
Salman Poultry Flock 117-1-AI-E	69±0.21^a	65±0.02^b	11.91±0.6^a	14.57±0.7^b	1.3±0.91^a	2.15±0.51^b
Salman Poultry Flock 117-1-AI-F	68±0.03^a	66±0.01^b	11.54±0.6^a	14.87±0.24^b	1.3±0.34^a	2.31±0.64^b
Salman Sadiq Flock -R1-AI-C	69±0.04^a	67±0.031^b	12.6±0.6^a	14.56±0.94^b	1.2±0.51^a	2.14±0.46^b
Salman Sadiq Flock 1-R2-AI-A	69±0.03^a	65±0.014^b	12.14±0.7^a	15.34±0.1^b	1.2±0.51^a	2.64±0.64^b
Salman Sadiq Flock 1-R2-AI-B	69±0.01^a	66±0.015^b	12.3±0.7^a	16.2±0.9^b	1.2±0.44^a	3.14±0.67^b
Salman Sadiq Flock 1-R3-AI-D	68±0.02^a	65±0.01^b	11.89±0.6^a	16.65±0.93^b	1.2±0.94^a	3.1±0.51^b

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Salman Sadiq Flock -5	69±0.02[a]	65±0.01[b]	11.68±0.7[a]	15.98±0.6[b]	1.3±0.71[a]	3.1±0.58[b]
Salman Sadiq Flock -6	69±0.02[a]	65±0.02[b]	12.67±0.3[a]	16.74±0.6[b]	1.31±0.64[a]	3.12±0.64[b]
Arslan Poultry flock 27-R2	69±0.03[a]	67±0.03[b]	11.39±0.6[a]	12.69±0.9[b]	0.98±0.81[a]	1.89±0.57[b]
Arslan Poultry 27 flock-R1	68±0.20[a]	66±0.01[b]	11.24±0.2[a]	12.51±0.5[b]	0.97±0.36[a]	1.84±0.96[b]
Salman Sadiq Flock 2-R1	69±0.01[a]	67±0.04[b]	11.68±0.2[a]	13.14±0.6[b]	0.98±0.89[a]	1.89±0.58[b]
Salman Sadiq Flock 2-R2	69±0.04[a]	66±0.03[b]	12.67±0.5[a]	12.69±0.5[b]	0.97±0.48[a]	1.84±0.74[b]
Salman Sadiq Flock F8	68±0.21[a]	67±0.20[b]	11.39±0.5[a]	13.51±0.35[b]	0.98±0.54[a]	1.89±0.54[b]

[a-b] denotes difference of chick yield, water loss and culling chicks of hairline and normal eggs (P<0.005)

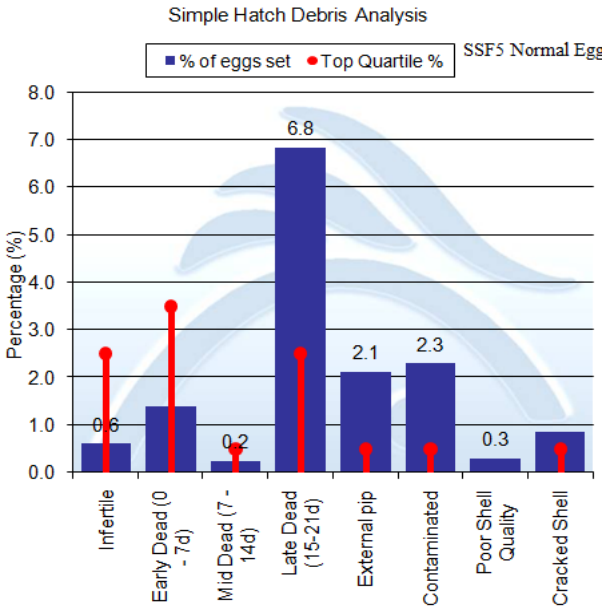

Figure 1. Dead in shell analysis of normal eggs at Chakri hatchery Rawalpindi, Pakistan

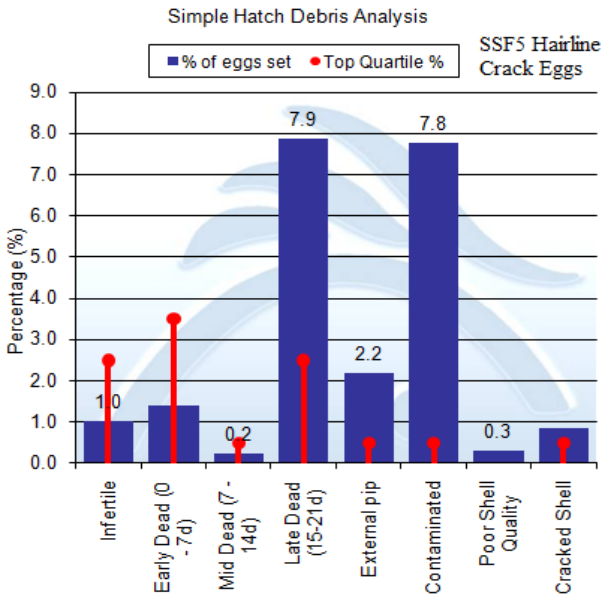

Figure 2. Dead in shell analysis of hairline crack eggs at Chakri hatchery Rawalpindi, Pakistan

Table 5. The temperature, humidity and ventilation of poultry house

Parameters	First week	Second week	Third week	Fourth week	Fifth week
Temperature (^{0}F)	95-86	86-83	83-77	77-75	75
Humidity (%)	65	65	65	65	65
Ventilation (m^3/ hour/ bird)	0.07	0.25	0.4	0.59	0.87

Table 6. The effect of hairline crack eggs on chick's mortality and performance traits

Parameters	Normal eggs chicks	Hairline crack egg chicks
Mortality (%)	1.86±0.06[a]	3.17±0.31[b]
Weight gain (g)	2200±0.14[a]	1855±0.071[b]
Feed intake (g)	3255±0.21[a]	3310±0.091[b]
Feed conversion ratio (%)	1.82±0.038[a]	1.57±0.048[b]

[a-b] denote significant difference for mortality, weight gain, feed intake and FCR of chicks from normal and hairline crack eggs (P < 0.05)

CONCLUSION

The eggshell and shell membrane are barriers for growing embryo provide safe packaging and avoid contamination. The damages to these barriers negatively affected the hatchery parameters and quality of chicks in present study. The chicks of hairline crack eggs become a source of economic loss in term of FCR and feed intake.

DECLARATIONS

Author's contribution

Dr. A. Jabbar and Dr. Abdul Hameed were responsible for designing the experiment and gathering the data along the result analysis under the supervision of Dr. A. Riaz and Dr.Yasir Allah Ditta. Finally Dr. Adnan Yousaf wrote the article and checked the statistical application.

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Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship and/or publication of this research.

REFERENCES

Salahi A, Moosanezhad Khabisi M and Mousavi SN (2011). Effects of the length (size) of eggshell cracks on hatchability and chick qualityof broiler breeder flocks. European symposium on the quality of poultry meat, p.52.

Amare A, Amin AM, ShiferawA, Nazir S and Negussie H (2013). Yolk Sac Infection (Omphalitis) in Kombolcha Poultry Farm, Ethiopia American-Eurasian. Journal of Scientific Research 8 (1): 10-14, 2013. Doi: 10.5829/idosi.aejsr.2013.8.1.65108

Barnett DM, Kumpula BL, Petryk R, Robinson L, Renema RA and Robinson FE (2004). Hatchability and Early Chick Growth Potential of Broiler Breeder Eggs with Hairline Cracks. Poultry Science Association, 65-70. Doi: 10.1093/japr/13.1.65

Jabbar A and Ditta YA (2017a). Effect of Floor Eggs on Hatchability, Candling, Water Loss, Chick Yield, Chick Weight and Dead in Shell. Journal of World`s Poultry Research, 7(4): 154-158.

Jabbar A and Ditta YA (2017b). Incubation Duration of Broiler Breeder Egg and Post Hatch Performance. Worlds Veterinary Journal, 7(3): 83-88. Doi: http://dx.doi.org/10.5455/wvj.20170897

Jabbar A and Ditta YA (2017c). Effect of Broiler Breeders Age on Hatchability, Candling, Water Loss, Chick Yield and Dead in Shell. World's Veterinary Journal, 7(2): 40-46.

Khabisi MM, Salahi A and Mousavi SN (2011). The influence of eggshell crack types on hatchability and chick quality. European Symposium on poultry nutrition Poster presentation 3-4 November. Available at: http://journals.tubitak.gov.tr/veterinary/issues/vet-12-36-3/vet-36-3-11-1103-20.pdf

King'ori AM (2012). Egg quality defects: Types, causes and occurrence: a review. Journal of Animal Production Advances; 2:350-7.

NoivaRute M, MenezesAntónio C and Peleteiro Maria C (2014). Influence of temperature and humidity manipulation on chicken embryonic development. BMC Veterinary Research, 10:234. Doi: 10.1371/journal.pone.0103040

Samiullah Roberts JR and Chousalkar KK (2014). Effect of production system and flock ageon egg quality and total bacterial loadin commercial laying hens. Journal of Applied Poultry Research, 23:59–70. Doi:http://dx.doi.org/ 10.3382/japr.2013-00805

Van den Brand H, Sosef MP, Lourens A, and van Harn J (2016).Effects of floor eggs on hatchability and later life performance in broiler Chickens. Poultry Science, 95:1025–1032. Doi: http://dx.doi.org/10.3382/ps/pew008

Wellman Labadie O, Picman J and Hincke MT (2008). Antimicrobial activity of the Anseriform outer eggshell and cuticle. Comparative Biochemistry Physiology B: Biochemistry and Molecular Biology, 149: 640-649. Doi: https://doi.org/10.1016/j.cbpb.2008.01.001

Wei LU, Chengyu ZHANG, HuiLuo, Weimin DING and Lin WANG (2015). Micro-Cracks Detection of Eggshells Based on a Magnetostrictive Transducer. Sensors & Transducers, 186: 49-54.

Xie C and He Y (2016). External characteristic determination of eggs and cracked eggs identification using spectral signature. Scientific Reports, 6:21130. Doi: 10.1038/srep21130

Yousaf A, Jabbar A and Ditta YA (2017). Effect of pre-warming on broiler breeder eggs hatchability and post-hatch performance. Journal of Animal Health and Production, 5(1): 1-4. Doi: http://dx.doi.org/10.14737/journal.jahp/2017/5.1.1.4

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Potency of *Sansevieria masoniana* Extract against Antimicrobial Resistant Bacteria Isolated from Faeces of Pet – Reptile

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ABSTRACT

Reptile plays an essential role in human life and act as a reservoir of pathogenic bacteria. It became necessary because of some bacteria resistant against several antibiotics. This study aimed to evaluate the potency of *Sansevieria masoniana* (SM) leaf extract against isolated bacteria from the faeces of pet-reptile. A total of 129 fresh faecal samples were collected from the reptile communities in Surabaya on February 2018 until January 2019. The faeces obtained from 72 snakes, 43 lizards and 14 tortoises. The isolation was conducted using the Micro ID system. All the isolated bacteria were tested against several antibiotics using disc diffusion method, and SM extract using minimum inhibitory concentration test. The isolated bacteria were *Aeromonas hydrophila* (44.96%), *Bacillus sp* (32.55%), *Enterobacter cloacae* (40.31%), *Enterococcus sp* (82.17%), *Escherichia coli* (96.89%), *Proteus sp* (76.74%), *Pseudomonas sp* (48.83%), *Salmonella enteritidis* (55.03%), and *Salmonella enterica arizonae* (53.48%). Those isolated bacteria indicated various resistance patterns against several commercial antibiotics. The minimum concentration of SM extracts that potential to inhibit the colonisation of both resistant and susceptible isolated bacteria was 62.5 mg/mL. This study proved that SM extract potential to inhibit the colonisation of the isolated bacteria from faeces of pet-reptile, even though, several of those isolates resistant against several commercial antibiotics.

Key words: Antibiotic, Pet – reptile, Reservoir, Resistance, *Sansevieria masoniana*.

INTRODUCTION

In recent years, reptile becomes one of the favourite domesticated animals in the urban area (Williams and Jackson, 2016). It indicated by the increasing number of reptile collector around the world (Pasmans et al., 2017). Reptile carriers various pathogenic bacteria that its antimicrobial resistance patterns unclearly understood. It plays an essential role in human life and its implication for public health. Reptile can act as reservoirs of Salmonella or other bacteria, and potentially pathogenic for human (Zancolli et al., 2015). Moreover, resistant bacteria have high pathogenicity and, it may increase the mortality during infection in both human and animal. Commonly, the bacteria transmits from reptile to human by direct contact (such handling) and indirect contact (ingestion of contaminated foods or consumption of reptile product) (Ebani, 2017). The best way to prevent and overcome the resistance is known by using herbal medicine, such as *Sansevieria* sp. Several species of *Sansevieria* had potential effect against degenerative and infectious disease, such Ehrlich ascites carcinoma (Haldar et al., 2010); and anti-ulcerative activity due to its saponin, flavonoid, glycoside, alkaloid, terpenoid, tannin, and anthraquinone content (Ighodaro et al., 2017). Another previous study reports the potential role of SM on the infected wound (Prakoso et al., 2018). It was necessary to elucidate the species of bacteria that potentially transmitted via the faecal-oral from pet-reptile and its resistance pattern against commercial antibiotics. Moreover, this study aimed to analyse the potency of *Sansevieria masoniana* (SM) against isolated bacteria from pet reptile.

MATERIALS AND METHODS

Ethical approval

Not applicable as the samples were collected from the faeces without any direct contact with the pet-reptile.

Sample collection

A total of 129 fresh faecal samples were collected from the reptile communities in Surabaya on February 2018 until January 2019. All the owners were interviewed about the sex, age, feeding and nursing of the reptile before sample collection. That data were used to compare with the results of a bacterial examination. The samples were classified into three categories that were the snake, lizard, and tortoise. Total 72-snakes faeces contanied 2 *Boa constrictor* (BC), 10

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Morelia viridis (MV), 3 *Python molurus* (PM), and 57 *Python reticulatus* (PR). 43-lizards included 4 *Iguana iguana* (II), 15 *Pogona vitticeps* (PV), and 24 *Varanus salvator* (VS). 14 tortoises faeces contanied 6 *Centrochelys sulcata* (CS) and 8 *Geochelone elegans* (GE) used in this study. All the faeces were taken with an aseptic procedure and then stored in a sterile plastic. All samples were collected and transported to the Laboratory of Bacteriology, Faculty of Health, University of Muhammadiyah Sidoarjo, East Java, Indonesia for bacterial isolation and identification.

Isolation and identification

The isolation of bacteria were conducted following the standard laboratory procedure. The isolates were reacted using Micro-ID system by utilising 15 biochemical tests and incubated at 37° C for 24-hours. The biochemical test included Voges-Proskauer, nitrate broth, phenylalanine deaminase, H_2S producing, indole, decarboxylase ornithine, decarboxylase lysine, malonate, urease, esculin, Ortho-Nitro Phenyl-β-Galactoside (ONPG), arabinose, adonitol, inositol, and sorbitol. The results of the biochemical reaction were scored and recorded on data sheets. Five digits octal number was calculated and identified using Micro-ID identification manual.

***Sansevieria masoniana* leaf extraction and phytochemical screening**

SM fresh leaves were obtained from the herbal store in Sidoarjo, East Java, Indonesia. It sliced and dried at 80° C for an hour and extracted using the 70% ethanol (Prakoso and Kurniasih, 2018). The crude extract was filtered using Whatman paper and stored at 4°C inside the refrigerator. Qualitative phytochemical screening was performed using standard methods against several constituents such as alkaloid, anthraquinone, flavonoid, glycoside, phenol, saponin, tannin, and terpenoid.

Disc diffusion test and minimum inhibitory concentration

The isolated bacteria were transferred into the broth media and incubated at 37° C until reaching the turbidity of 0.5 Mc Farland. It was inoculated on the muller hinton agar surface and waited until the inoculum infiltrates the media. Several commercial antibiotic discs (ampicillin 10 µg; chloramphenicol 30 µg; ciprofloxacin 5 µg; penicillin 10 IU; streptomycin 10 µg; and tetracycline 30 µg) and incubated at 37° C for 24-hours. The inhibition zone was measured using a calliper and classified as Susceptible (S), Intermediate (I), and Resistant (R) (Adesiyun et al., 2007). Prior the Minimum Inhibitory Concentration **(**MIC), the extract was diluted into a stock solution using the equation below (Andrews, 2001):

Weight (W) of extract (mg) = (1000/ potency (µg/mL)) × volume (mL) × concentration of solution with multiple of 1000 (mg/L)

The MIC was conducted by adding the 100 µL extract's stock solution on the two rows of well and move 50 µL to the other well until it reaches zero concentration. Following the extract, add 100 µL bacterial suspension to every well that contains the stock solution, and cover using lid then incubated at 37 C for 24-hours. The lowest concentration that invisible the bacterial growth indicated as the potential concentration and it reported in (mg/mL).

Analysis data

The prevalence of the isolated bacteria and its resistance against commercial antibiotics were measured using the formulae below: Prevalence (P) = [Positive Sample (Ps)/Total Samples (TS)] × 100

Prevalence of the isolated bacteria and/ or its resistance against commercial antibiotics is the purpose of the prevalence word in above formulae. This study contains large variables. The relation between each variable was analysed using the multivariate analysis. It was applied to elucidate the risk factors. The potency of SM extract was analysed using the Kruskal Wallis and Man Whitney U test (SPSS, Version 16) with a probability value at level of $P < 0.05$.

RESULTS

Prevalence of bacteria

Based on the isolation and identification the highest prevalence of isolated bacteria from faeces was *Escherichia coli* (EC) and it followed by *Enterococcus sp.* (ES), *Proteus sp.* (PTS), *Salmonella enteritidis* (SE), *Salmonella enterica arizonae* (SEA), *Pseudomonas sp.* (PS), *Aeromonas hydrophila* (AH), *Enterobacter cloacae* (ENC), and *Bacillus sp* (BS). Those result assumed that reptile was a natural reservoir for the several pathogenic bacteria such as *Salmonella*. It proved by the prevalence of both *Salmonella* (SE and SEA) in the fourth and fifth rank. EC was the highest one because it commonly found on the lower digestive system together with ENC, ES and PTS. AH was isolated from the faeces, and it suspected due to water contamination during the faecal excreted by the pet-reptile (Table 1). Another reason, it caused by food contamination. Further, a total of 685-isolates (58 of AH, 42 of BS, 52 of ENC, 106 of ES, 125 of EC, 99 of PTS, 63 of PS, 71 of SE, and 69 of SEA) collected in this study. The resistance pattern of all isolated bacteria examined against several commercial antibiotics.

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Table 1. Prevalence of isolated bacteria from faeces of pet-reptile in Surabaya, Indonesia on February 2018 until January 2019

Pet-reptile species	N	Total of the positive sample								
		AH	BS	ENC	ES	EC	PTS	PS	SE	SEA
BC	2	2	1	2	2	2	2	2	0	1
MV	10	2	4	2	3	10	10	5	7	7
PM	3	3	1	2	3	3	3	3	1	2
PR	57	32	14	18	47	57	53	23	34	30
II	4	0	0	3	4	4	2	1	3	2
PV	15	2	5	6	15	15	8	9	7	8
VS	24	12	12	16	18	24	15	11	11	15
CS	6	2	2	1	6	4	3	3	4	1
GE	8	3	3	2	8	6	3	6	4	3
N	129	58	42	52	106	125	99	63	71	69
P (%)	100.00	44.96	32.55	40.31	82.17	96.89	76.74	48.83	55.03	53.48

N: Total sample, AH: *Aeromonas Hydrophila*, BS: *Bacillus Sp*, ENC: *Enterobacter Cloacae*, ES: *Enterococcus Sp*, EC: *Escherichia Coli*, PTS: *ProTeus Sp*, PS: *Pseudomonas Sp* SE: *Salmonella Enteritidis*, SEA: *Salmonella Enterica Arizonae*, BC: *Boa Constrictor*, MV: *Morelia Viridis*, PM: *Python Molurus*, PR: *Python Reticulatus*, II: *Iguana Iguana*, PV: *Pogona Vitticeps*, VS: *Varanus Salvator*, CS: *Centrochelys Sulcata*, GE: *Geochelone Elegans.*

Antimicrobial susceptibility

The isolated bacteria indicated thevaries resistance profile against tested commercial antibiotics. Mostly, the isolated bacteria exhibited high resistance profile to ampicillin, chloramphenicol, penicillin, streptomycin, and tetracycline. On the other hands, those bacteria susceptible to ciprofloxacin (6/9 species), except for PS (47.61 %) (Table 2). It suspected due to the contamination or residue on the pet-reptile feeds that was increasing the resistance of isolated bacteria.

Table 2. The resistance profile of isolated bacteria from pet reptile in Surabaya, Indonesia on February 2018 until January 2019

Bacteria species	N	Resistant isolate (%)					
		Amp	Chl	Cipr	Pnc	Strep	Tetra
AH	58	100.00	41.37	0	1.72	37.93	41.37
BS	42	30.95	4.76	0	73.80	7.14	21.42
ENC	52	75.00	57.69	19.23	61.53	48.07	28.84
ES	106	1.88	17.92	18.86	4.71	0	0
EC	125	10.40	4.80	0	3.20	7.20	2.40
PTS	99	41.41	38.38	0	49.49	58.58	34.34
PS	63	50.79	68.25	47.61	50.79	33.33	34.92
SE	71	56.33	78.87	0	29.57	100	63.38
SEA	69	55.07	73.91	0	0	81.15	49.27

N: total sample, Amp: ampicillin, Chl: chloramphenicol, Cipr: ciprofloxacin, Pnc: penicillin, Strep: streptomycin, Tetra: tetracycline, AH: *Aeromonas Hydrophila*, BS: *Bacillus Sp*, ENC: *Enterobacter loacae*, ES: *Enterococcus Sp*, EC: *Escherichia Coli*, PTS: *ProTeus Sp*, PS: *Pseudomonas Sp* SE: *Salmonella Enteritidis*, SEA: *Salmonella Enterica Arizonae*.

Risk factors

This study observed several predictor factors that associated with the prevalences of the antimicrobial resistance profile of isolated bacteria. Based on the finding, there were no consistent factors that influence the antimicrobial resistance profile. Several bacteria such as AH, BS, ENC, PTS, and PS indicated that age, cleaning, and feed's type was an influence on its resistance, however, there was no potential factor to ES, EC, and SEA (Table 3). Cleaning, age and type of feed had a significant and coefficient value which respectively contained coefficient = 0.99 for AH (P = 0.037); coefficient = -1.486 for PTS (P = 0.019) and coefficient = 0.93 for PS (P = 0.044) based on cleaning; coefficient = -0.602 for BS (P = 0.043) and coefficient = 0.48 for PS (P = 0.046) based on age and coefficient = -0.629 for AH (P = 0.033) and coefficient = -0.674 for ENC (P = 0.026) based on type of feeds. It proved that the mentioned factors partially influence the antimicrobial resistance profile among species of present study.

Qualitative phytochemical screening of SM extract

The preliminary study proved that SM extract contains several bioactive compounds such as alkaloid, anthraquinone, flavonoid, glycoside, phenol, saponin, tannin and terpenoid. The preliminary study proved that SM extract contains several bioactive compounds such as alkaloid (+), anthraquinone (+), flavonoid (+), glycoside (+), phenol (+), saponin (+), tannin (+) and terpenoid (+).

Minimum inhibitory concentration of *Sansevieria masoniana* extract against isolated bacteria

The SM extracts had different potency to inhibit bacterial colonisation. It could repress 100% of the bacterial growth of AH, BS, ENC, and ES in 125 mg/mL concentration. Surprisingly, the effective potential (100%) of the lower concentration (65 mg/mL) and high concentration (500 mg/mL) of SM extracts against both isolated *Salmonella* species

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were obtained in table 4. Based on the statistical results, the SM extract was significantly (P< 0.05) inhibiting all the bacterial colonisation from reptile's faeces with varying doses. The highest effective doses were 500 mg/ml and the lowest was 62.5 mg/ml (P< 0.05).

Table 3. Logistic regression analysis of antimicrobial resistances pattern of isolated bacteria

Bacteria species	N	Odds ratio of the predictor factors				
		Pet-reptile species	Sex	Age	Cleaning	Type of feed
AH	58	0.96	1.84	1.10	2.69*	0.53*
BS	42	0.95	1.51	0.54*	0.39	1.00
ENC	52	1.22	0.45	0.75	1.59	0.51*
ES	106	1.27	1.12	0.70	1.83	1.72
EC	125	0.89	0.50	1.12	0.65	0.76
PTS	99	0.77	1.00	1.40	0.22*	0.63
PS	63	1.00	0.86	1.61*	2.55*	1.71
SE	71	0.91	3.79*	0.80	0.71	1.02
SEA	69	0.95	1.22	0.89	0.74	0.69

N: total sample, AH: *Aeromonas Hydrophila*, BS: *Bacillus Sp*, ENC: *Enterobacter Cloacae*, ES: *Enterococcus Sp*, EC: *Escherichia Coli*, PTS: *ProTeus Sp*, PS: *Pseudomonas Sp* SE: *Salmonella Enteritidis*, SEA: *Salmonella Enterica Arizonae*, * the different superscript on the same column showed significance value (P<0.05)

Table 4. Minimum inhibitory concentration of *Sansevieria masoniana* extract (mg/mL) against isolated bacteria

Bacteria species	N	Percentage of susceptible isolates against several concentration of SM extract (%)									
		500	250	125	62.5	31.25	15.6	7.8	4	2	1
AH	58	100.00*	100.00*	100.00*	44.82	0	0	0	0	0	0
BS	42	100.00*	100.00*	100.00*	69.04*	0	0	0	0	0	0
ENC	52	100.00*	100.00*	100.00*	46.15	0	0	0	0	0	0
ES	106	100.00*	100.00*	100.00*	0	0	0	0	0	0	0
EC	125	100.00*	62.40*	0	0	0	0	0	0	0	0
PTS	99	42.42	0	0	0	0	0	0	0	0	0
PS	63	100.00*	100.00*	0	0	0	0	0	0	0	0
SE	71	100.00*	100.00*	100.00*	100.00*	0	0	0	0	0	0
SEA	69	100.00*	100.00*	100.00*	100.00*	85.50*	5.79	0	0	0	0

N: total sample, AH: *Aeromonas Hydrophila*, BS: *Bacillus Sp*, ENC: *Enterobacter Cloacae*, ES: *Enterococcus Sp*, EC: *Escherichia Coli*, PTS: *Pro Teus Sp*, PS: *Pseudomonas Sp* SE: *Salmonella Enteritidis*, SEA: *Salmonella Enterica Arizonae*, * the different superscript on the same column showed significance value (P<0.05),

DISCUSSION

The growing of pet-reptile owners increases the risk number of direct and indirect contact of humans with reptiles. It can promote the transmission of the pathogenic bacteria to human. Moreover, several pathogenic bacteria such as *Salmonella* isolated from the pet-reptile faeces proves that it potentially implicatesed for human health (Mughini-Gras et al., 2016). The previous study reported that all the excretion products of the pet-reptile harbour the pathogenic bacteria (Tomastikova et al., 2017). The high antimicrobial resistant elucidated that those bacteria increase its pathogenicity via generation of protective properties against antibiotics such as change of its membrane, produce an enzyme that inactivates the drugs, pump and neutralises the antimicrobials agents before it kills the bacteria, and decrease membrane permeability (Munita and Arias, 2016). The high resistance pattern in the isolated bacteria of pet-reptile could generate financial burden, severe infection and death.

The resistance profile that occurs in this study quite varies. From total six-commercial antibiotics, just ciprofloxacin indicatd the high susceptible pattern against isolated bacteria. It was because of ciprofloxacin is one of the semisynthetic fluoroquinolone derivates that have a broad-spectrum activity, high bioavailability and alsociprofloxacin had a DNA target (Conley et al., 2018). However, it restricted to use in animals production for the last 10-years (Jia et al., 2017). The utilisation of antibiotic in both livestock and poultry increases the risk of antibiotic's residue in food final product that potentially generates the bacterial resistant (Gouvea et al., 2015; Haag et al., 2016). It was similar to the results of present study that type of fed partially influenced on the bacterial resistant, although the other factors were not affected.

It was necessary to restrict the utilisation of synthetic antibiotic as therapy because of the high prevalence of bacterial resistant in animal and human. In recent years, the researchers observed the herbal as the antimicrobial agents, and this study utilises the SM extract (Prakoso et al., 2018). This study proved that SM potentially inhibited the bacterial colonisation *in vitro*. The effective concentration of the SM extract against several bacterial species was 62.5% at 125 mg/mL, even though the lower concentration (31.25 mg/mL) synergistically potential to more than 50% isolates of SEA. Similar to synthetic antibiotics, the SM extracts had various doses as an antimicrobial agent. Unfortunately, the SM

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extract indicated the low activity to depress the PTS colonisation *in vitro*. The SM extract had inhibited the bacterial colonisation because of its bioactive compound such as alkaloid. An alkaloid from the herbal extract potential to prevent the efflux pump system that generates the accumulation of alkaloid intracellularly and promotes the destruction of the bacterial cell (Mabhiza et al., 2016). The potential role of SM extracts increased by the anthraquinone. As the previous study reported, anthraquinone increases the aliphatic chain of the methoxy group that switch the lipophilicity of the compound and synergically upgrade its antimicrobial activity (Kemegne et al., 2017). Those mechanisms were similar to flavonoid (Wu et al., 2013), glycoside (Tagousop et al., 2018), and phenolic compound of herbal extract (Rodriguez-Perez et al., 2016). Saponin of the SM extract suspects played a prominent role in a proton-donating ability and can utilise as the oxidant inhibitors. Moreover, this role impairsed the membrane lipidic and cytoplasmic phase of bacteria (Akinpelu et al., 2014). Antimicrobial activity of the SM extract was supported by tannin and terpenoid. Tannin inhibits the enzyme production of bacteria (Redondo et al., 2014), and terpenoid forms a strong atomic interaction that both of those significant to destruct the bacterial cell's membrane (Daisy et al., 2008).

CONCLUSION

The prevalence of antimicrobial resistant to isolated bacteria from faeces of pet-reptile partially depends on several factors such as cleaning and type of feed. Moreover, this study proved that SM extract have potential to inhibit the colonisation of the isolated bacteria from faeces of pet-reptile, even though, several of those isolates resistant against several commercial antibiotics. Further study needs to observe the potency of SM extract against the other species of bacteria both *in vitro* and *in vivo*.

DECLARATIONS

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Competing interests

The authors declared that they had no conflict of interest.

Consent to publish

All the authors were aware of the fact and agreed to be so named. This study did not partially or totally published elsewhere.

Author's contribution

AK, P and YAP designed the research. LYW and YAP performed the research. YAP, AK, IW wrote the manuscript. YAP checked and edited the final form of composed article.

REFERENCES

Adesiyun A, Offiah N, Seepersadsingh N, Rodrio S, Lashley V and Musai L (2007). Antimicrobial Resistance of *Salmonella* spp. and *Escherichia coli* Isolated from table Eggs. Food Control, 18: 306-311. Doi:http://dx.doi.org/10.1016%2Fj.foodcont.2005.10.013.

Akinpelu BA, Igbeneghu OA, Awotunde AI, Iwalewa EO and Oyedapo OO (2014). Antioxidant and Antibacterial Activity of Saponin Fractions of *Erythropheleum suaveolens* (Guill. and Perri.) Stem Bark Extract. Scientific Research and Essays, 9: 826-833. Doi:http://dx.doi.org/10.5897/SRE2014.5844.

Andrews JM (2001). Determination of Minimum Inhibitory Concentrations. Journal of Antimicrobial Chemotherapy, 48: 5-16. Doi:https://doi.org/10.1093/jac/48.suppl_1.5.

Conley ZC, Bodine TJ, Chou A and Zechiedrich L (2018). Wicked: The Untold Story of Ciprofloxacin. PLoS Pathogens, 14, e1006805. Doi: https://dx.doi.org/10.1371/journal.ppat.1006805.

Daisy P, Mathew S, Suveena S and Rayan NA (2008). A Novel Terpenoid from *Elephantopus Scaber* – Antibacterial Activity on *Staphylococcus aureus*: a Substantiate Computational Approach. International Journal of Biomedical Science, 4: 196-203. Doi:https://www.ncbi.nlm.nih.gov/pubmed/23675090.

Ebani VV (2017). Domestic Reptiles as Source of Zoonotic Bacteria: A Mini Review. Asian Pacific Journal of Tropical Medicine, 10, 723-728. Doi: http://dx.doi.org/10.1016/j.apjtm.2017.07.020.

Gouvea R, Santos FF, Dos Aquino MHC, De Pereira VL and De A (2015). Fluoroquinolones in Industrial Poultry Production, Bacterial Resistance and Food Residues: a Review. Brazillian Journal of Poultry Science, 17: 1-10. Doi:http://dx.doi.org/10.1590/1516-635x17011-10.

Haag G, Marin GH and Errecalde J (2016). Quantification of Residual Enrofloxacin and Ciprofloxacin in Feathers of Broiler Chickens by High-Performance Liquid Chromatography-Fluorescence after Oral Administration of the Drugs. Journal of Advanced Pharmaceutical Technology and Research, 7: 2-5. Doi: https://dx.doi.org/10.4103%2F2231-4040.173265.

Haldar PK, Kar B, Bala A, Bhattacharya S and Mazumder UK (2010). Antitumor Activity of *Sansevieria roxbughiana* against Ehrlich Ascites Carcinoma in Mice. Pharmaceutical Biology, 48: 1337-1343. Doi: https://doi.org/10.3109/13880201003792592.

Ighodaro OM, Adeosun AM, Ojiko BF, Akorede AT and Williams OF (2017). Toxicity Status and Antiulcerative Potential of *Sansevieria trifasciata* Leaf Extract in Wistar Rats. Journal of Intercultural Ethnopharmacology, 6: 234-239. Doi:https://dx.doi.org/10.5455%2Fjice.20170421103553.

Jia S, Zhang XX, Miao Y, Zhao Y, Ye L, Bing L and Zhang T (2017). Fate of Antibiotic Resistance Genes and Their Associations with Bacterial Community in Livestock Breeding Wastewater and Its Receiving River Water. Water Research, 124: 259-268. Doi: https://dx.doi.org/10.1016/j.watres.2017.07.061.

Kemegne GA, Mkounga P, Ngang JJE, Kamdem, SLS and Nkengfack AE (2017). Antimicrobial Structure Activity Relationship of Five Anthraquinone of Emodine Type Isolated from *Vismia laurentii*. BMC Microbiology, 17: 41. Doi:https://dx.doi.org/10.1186%2Fs12866-017-0954-1.

Mabhiza D, Chitemerere T and Mukanganyama S (2016). Antibacterial Properties of Antibacterial Extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. International Journal of Medicinal Chemistry, 2016, 6304163. Doi: http://dx.doi.org/10.1155/2016/6304163.

Mughini-Gras L, Heck M and Van Pelt W (2016). Increase in Reptile-Associated Human Salmonellosis and Shift toward Adulthood in the Age Group at Risk, the Netherland, 1985 to 2014. Euro Surveillance, 21: 30324. Doi:https://dx.doi.org/10.2807%2F1560-7917.ES.2016.21.34.30324.

Munita JM and Arias CA (2016). Mechanism of ntibiotic Resistance. Microbiology Spectrum, 4: 1-37. Doi:https://dx.doi.org/10.1128%2Fmicrobiolspec.VMBF-0016-2015.

Pasmans F, Bogaerts S, Braeckman J, Cunningham AA, Hellebuyck T, Griffiths RA, Sparreboom M, Schmidt BR and Martel A (2017). Future of Keeping Pet Reptiles and Amphibians: Towards Integrating Animal Welfare, Human Health, and Environmental Sustainability. The Veterinary Record, 181: 264-264. Doi: http://dx.doi.org/10.1136/vr.104296corr1.

Prakoso YA and Kurniasih (2018). The effects of *Aloe vera* Cream on the Expression of CD4+ and CD8+ Lymphocytes in Skin Wound Healing. Journal of Tropical Medicine, 2018, 6218303. Doi: https://dx.doi.org/10.1155/2018/6218303.

Prakoso YA, Rini CS and Wirjaatmadja R (2018). Efficacy of *Aloe vera*, *Ananas comosusu*, and *Sansevieria masoniana* Cream on the Skin Wound Infected with MRSA. Advances in Pharmacological Science, 2018, 4670569. Doi:https://dx.doi.org/10.1155/2018/4670569.

Redondo LM, Chacana PA, Dominguez JE and Fernandez-Miyakawa ME (2014). Perspectives in the Use of Tannins as Alternative to Antimicrobial Growth Promoter Factors in Poultry. Frontiers in Microbiology, 5: 118. Doi:https://dx.doi.org/10.3389%2Ffmicb.2014.00118.

Rodriguez-Perez C, Quirantes-Pine R, Uberos J, Jimenez-Sanchez C, Pena A and Segua-Carretero A (2016). Antibacterial Activity of Phenolic Compounds from Cranberry (*Vaccinium macrocarpon*) against *Escherichia coli*. Food and Function, 7: 1564. Doi:http://dx.doi.org/10.1039/c5fo01441g.

Tagousop CN, Tamokou JDD, Ekom SE, Ngnokam D and Voutquenne-Nazabadioko L (2018). Antimicrobial Activity of Flavonoid Glycosides from *Graptophyllum grandulosum* and Their Mechanism of Antibacterial Action. BMC Complementary and Alternative Medicine, 18: 251. Doi: https://dx.doi.org/10.1186%2Fs12906-018-2321-7.

Tomastikova Z, Romero SB, Knotek Z and Karpiskova R (2017). Prevalence and Characteristics of *Salmonella* Species Isolated from Captive Reptiles in the Czech Republic. Veterinarni Medicina, 62: 456-469. Doi:https://dx.doi.org/doi:10.17221/44/2017-VETMED.

Williams DL and Jackson R (2016). Availability of Information on Reptile Health and Welfare from Stores Selling Reptiles. Open Journal of Veterinary Medicine, 6: 59-67. Doi:http://dx.doi.org/10.4236/ojvm.2016.63007.

Wu T, He M, Zang X, Zhou Y, Qui T, Pan S and Xu X (2013). A Structure-Activity Relationship Study of Flavonoida as Inhibitors of *E. coli* by Membrane Interaction Effect. Biochimica et Biophysica Acta (BBA) Biomembranes, 11: 2751-2756. Doi:https://dx.doi.org/10.1016/j.bbamem.2013.07.029.

Zancolli G, Mahsberg D, Sickel W and Keller A (2015). Reptiles as Reservoirs of Bacterial Infections: Real Threat or Methodological Bias?. Microbial Ecology, 70: 579-584. Doi: https://doi.org/10.1007/s00248-015-0618-3.

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Effect of Early Heat Shock Exposure on Physiological Responses and Reproduction of Rabbits under Hot Desert Conditions

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ABSTRACT

This study aimed to apply early heat shock exposure programs for releasing HSP70 gene expression to improve production of rabbits reared under hot desert conditions at Egypt. 120 Hi-Plus rabbits, one-day old were randomly divided into six equal treatments (20 rabbits/ treatment), namely T1, T2, T3, T4, T5 and T6. T1 served as control. The rabbits of second, third, fourth, fifth and sixth treatments were exposed to heat shock (36±1 °C for 3 hours from 12:00 - 15:00 for three successive days). Rabbits of T2, T3, T4, T5 and T6 were exposed to heat shock at 3, 25, 60, 3+25 and 3+25+60 days of age, respectively. HSP70 expression and tri-iodothyronine hormone in the rabbits of T2, T3, T4, T5 and T6 were significantly increased. Rectal and fur temperatures, respiration rate, alanine transaminase, corticosterone hormone levels and overall mortality rate significantly decreased in the rabbits exposed to heat shock programs. Red blood cells count, packed cell volume and hemoglobin concentration increased in the rabbits of T2, T3 and T4. Total protein and globulin concentrations increased in the rabbits of T5 when compared to the rabbits of T1, T2 and T6. However, rabbits of T2 and T4 showed an increase in total antioxidant capacity when compared to the rabbits of T1. Conception rate was higher in the does of T5 than that in T3, T4 and T6. Litter traits, productive efficiency index, feed conversion and cost of feeding improved in the rabbits exposed to heat shock programs. In conclusion, applying heat shock exposure programs of rabbits especially T3 treatment, might increase HSP70 gene expression, this led to enhance immunity responses and production under severe heat stress conditions.

Key words: Heat stress, HSP70, Physiological responses, Productive and reproductive performance, Rabbits

INTRODUCTION

In Egypt, rabbit production suffered from heat stress conditions which considered as one of the important environmental stressors challenging rabbit production especially in desert areas (Morsy et al., 2011 and Nagwa et al., 2012). Many authors reported the deleterious effects of heat stress on physiological responses, productive and reproductive performance of rabbits (Sakr, 2003; Abdel-Samee et al., 2005 and Morsy, 2007).

Exposure of rabbits to heat stress conditions led to modulate their physiological responses to dissipate the latent heat (Morsy, 2007). Marai et al. (1994 and 1996) and Lebas et al. (1997) reported that rabbit's heat production and losses vary to maintain body temperature by using three devices for heat loss; general body position, respiration rate and peripheral temperature (temperature of ear). Under severe heat stress, rabbits use strategies that include; depression in feed intake, efficiency of feed utilization, disturbances in water, protein, energy and mineral metabolism balances, enzymatic reactions, hormonal secretions, blood metabolites and depressed immune function (Nagwa et al., 2005; Morsy, 2007 and Morsy et al., 2011).

Heat stress conditions accompanied with a peak of mortality rate in rabbits. Many authors reported 25-75 % increase of mortality rate in rabbits reared under hot weather conditions of Egypt and may be attributed this higher percentage to inability of regulate body temperature under heat stress conditions (Nagwa et al., 2005; Morsy, 2007 and Morsy et al., 2011). Currently, heat shock exposure programs during earlier age are alternative practice to increase thermo-tolerance and acclimate rabbits to heat stress conditions which led to minimize heat-related mortality, enhance productive and reproductive performance, feed conversion and enhance immune responses (Yalcin et al., 2001; De Basilio et al., 2003; Rahimi, 2005; Hanan, 2006; Faisal et al., 2008; El-Badry et al., 2009; Star et al., 2009; Zulkifli et al., 2009; El-Moniary et al., 2010; Nagwa et al., 2012; Emam, 2013; Morsy, 2013 and Morsy, 2018). Heat Shock Proteins (HSP's) have been noticed in every cell type and tissues. Exposure of rabbits to heat stress conditions during growth

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period led to induce HSP70. Members of HSP70 protein family act as chaperone, which assists in the folding, transport and assembly of protein in cytoplasm, mitochondria and endoplasmic reticulum or appears to play a critical role in protecting cells against the adverse effects of hyperthermia, helps newly synthesized proteins fold (Morimoto et al., 1990). Therefore, this study aimed to release the HSP70 gene expression by applying heat shock exposure programs at early ages and investigate its effects on physiological responses, productive and reproductive performance of Hi-Plus rabbits under hot desert conditions of South Sinai, Egypt during production period.

MATERIALS AND METHODS

Experimental region

The present study was carried out in South Sinai research station, located at Ras Suder that belongs to the desert research center, ministry of agriculture and land reclamation, Egypt. The experiment started at January 2016 up to August 2016. Laboratory work was carried out in the laboratories compound of desert research center.

Ethical approval

This experiment was performed according to all ethics and animal rights (desert research center, Egypt). As much as this work had considering all rules and regulations in conformity with the European union directive for the protection of experimental animals (2010/63/EU).

Experimental animals and feeding and management

The experimental rabbits were kept under the same managerial, hygienic conditions and examined clinically safe and free from internal and external parasites. Rabbits were vaccinned to keep them healthy. At production period, rabbits were individually housed in standard dimensions (50×60×40 cm) wired metallic cages attached with nest box (40×30×27 cm) for kindling and nursing. Cages were equipped with feeding hoppers. The rabbits fed, ad-libitum, a commercial concentrate pelleted diet containing 18.0% crude protein, 16.0% crude fiber, 2.5% fat, 0.6% minerals mixture and 2600 kcal/kg digestible energy according to NRC (1994). Fresh water was available all day through nipples drinker system.

Experimental design

120 one-day old of Hi-Plus rabbits were randomly divided into six equal treatments (20 rabbits/treatment). The first treatment (T1) served as control (non-exposure to heat shock program). The rabbits of second, third, fourth, fifth and sixth treatments were exposed to heat shock program (36±1 °C for 3 hours from 12:00 to 15:00 for three successive days). The second treatment (T2) exposed at three days of age, the third treatment (T3) exposed at 25 days of age, the fourth treatment (T4) exposed at 60 days of age, the fifth treatment (T5) was exposed at 3 and 25 days of age and the sixth treatment (T6) exposed at 3, 25 and 60 days of age. After the end of heat shock exposure all treatments returned to be reared under natural conditions. During productive period, all treatments reared under heat stress conditions.

Ambient temperature and relative humidity

Table 1 indicated monthly indoor climatic conditions recorded during the experimental period using electronic digital thermo-hygrometer (Model 303, China). The relationship between ambient temperature and relative humidity was termed as Temperature-Humidity Index (THI) and calculated according to Marai et al. (2001).

$$THI = db°C - [(0.31 - 0.31 \times RH) \times (db°C - 14.4)]$$

Where, db°C = dry bulb temperature in centigrade and RH = relative humidity %. The THI values were classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

Table 1. Indoor ambient temperature, relative humidity and temperature-humidity index throughout experimental period under conditions of South Sinai, Egypt

Months	Minimum AT (0C)	Maximum AT(0C)	Minimum RH (%)	Maximum RH (%)	Minimum THI	Maximum THI
January	9.3±0.54	18.1±0.38	34.2±1.24	58.3±2.30	10.3±0.54	17.6±0.82
February	10.6±0.88	17.9±0.66	31.2±1.09	57.2±1.11	11.4±0.57	17.4±0.63
March	12.2±0.50	21.1±0.55	33.1±1.53	53.6±0.99	12.7±0.53	20.1±0.33
April	13.4±0.87	26.0±1.11	31.0±1.88	43.9±1.65	13.6±0.87	24.0±1.1
May	19.4±0.63	30.2±0.44	27.2±2.11	46.5±1.32	18.3±0.52	27.6±0.38
June	24.1±0.24	33.9±0.48	25.9±1.22	41.6±1.43	21.9±0.22	30.4±0.35
July	23.8±0.81	35.6±0.78	25.3±1.98	42.1±2.10	21.6±0.55	31.8±0.66
August	25.1±0.61	35.9±0.71	29.8±1.65	49.6±1.60	22.8±0.37	32.5±0.81

AT= ambient temperature, RH= relative humidity, THI= temperature humidity index

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Thermo-respiratory responses

Thermo-respiratory responses of rabbits were randomly measured on 10 rabbits / treatment during production period. The measurements were monthly recorded at 2:00 pm. Rectal Temperature (RT) was measured by inserting a clinical thermometer two cm in the rectum for one minute. Temperatures of Skin (ST), Fur Temperatures (FT) and Ear Temperatures (ET) were recorded using digital tele-thermometer Digitemp D2000/D2010 (Morsy et al., 2011). Respiration rate (breaths/min) was determined by counting the frequency of flank movements/minute (Nagwa et al., 2012).

Blood samples

Monthly blood samples were taken from the marginal ear vein into EDTA treated tubes (five rabbits/treatment). Hemoglobin (Hb) concentration, Red Blood Cells (RBC's) count and Packed Cell Volume (PCV%) were determined immediately by the coulter (HA-VET, Clinding, Belgium). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated by the following equations: MCH (in pico gram, pg) = (Hb content g/dl × 10) / RBCs in million. MCHC (%) = (Hb content × 100) / PCV %. MCV (femtoliter, fl) = (PCV % × 10)/ RBCs in million. The rest of the blood was centrifuged for 15 minutes at 3500 rpm to collect plasma before being stored at -70^{o} Celesius for determination of HSP70, blood hormones (progesterone, corticosterone and tri-iodothyronine) and blood metabolites (total protein, albumin, glucose, cholesterol, alanine transaminase, aspartic transaminase and total antioxidant capacity). Blood metabolites were determined calorimetrically by using commercial kits. Concentration of HSP70 was determined by ELISA kit of Uscn Life Science Inc. Wuhan, Chain. Specificity of this assay has high sensitivity and excellent specificity for detection of gallinaceous HSP70. Concentrations of tri-iodothyronine, corticosterone and progesterone hormones were determined by ELISA kits of Monobind Inc. Lake Forest, USA.

Reproductive and productive performance

Conception rate (%), number of services per conception were calculated. Gestation period (day) and litter traits were recorded. Milk yield of the doe was estimated by deprivation of the litters from suckling for 24 hours. The litters were weighed before and after suckling and the increase in litters' weight was used as the doe milk yield. Stillbirth (%), pre-weaning mortality (%) and overall of mortality rate (%) were recorded.

Daily feed intake (g) was measured (offered diet–residual diet), total feed intake (Kg)= daily feed intake×123 days. Productive efficiency index (kg, live weight) = litter size at weaning× number of parities × total weaning weight (kg), cost of feed for producing one Kg live weight of rabbit = feed conversion × price of one kg feed and feed conversion of doe = total feed intake (kg) of doe / productive efficiency index (kg, live weight) of doe were calculated.

Average Daily Gain (ADG) and Relative Growth Rate (RGR) were calculated throughout the suckling period (28 days). ADG = (weaning weight – birth weight) /28 days. RGR = [(weaning weight – birth weight)/ 0.5× (weaning weight +birth weight)] × 100.

Statistical analysis

Data was analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004). The model was as follows: $Y_{ij}= \mu + T_i + e_{ij}$

Y_{ij}= Any observations of i^{th} rabbit within j^{th} treatment

μ = Overall mean

T_i = Effect of i^{th} treatment, (i: 1-6)

e_{ij} = Standard error

All statements of significance are based a probability of less than 0.05. Significant differences among means were tested using Duncan multiple range test (Duncan, 1955). Mortality rate of does was analyzed by Chi square analysis.

RESULTS AND DISCUSSION

Heat shock protein 70

Early heat exposure program significantly (P< 0.05) increased HSP70 expression (Figure 1) in the rabbits of T2, T3, T4, T5 and T6 by (27.5-42.5 %) as compared to the rabbits of T1 (control group). This result exhibited that early heat exposure program may increase HSP70 expression and might suggesting the proteins involved in the stress caused by heat shock exposure in the rabbits (Maak et al., 2003; Hanan, 2006; Nagwa et al., 2012; Emam, 2013 and Morsy, 2018). Release of HSP70 during heat stress conditions may play an important role in protecting stressed cells and reversing disorders caused by stress through acts as a molecular chaperone by binding to other cellular proteins, assisting intracellular transport and folding into the proper secondary structures and thus preventing aggregation of protein during

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stress (Chirico et al., 1988 and Li and Werb, 1982) and hence it may positively return on rabbit's performance (Abd El-Kafy et al., 2008).

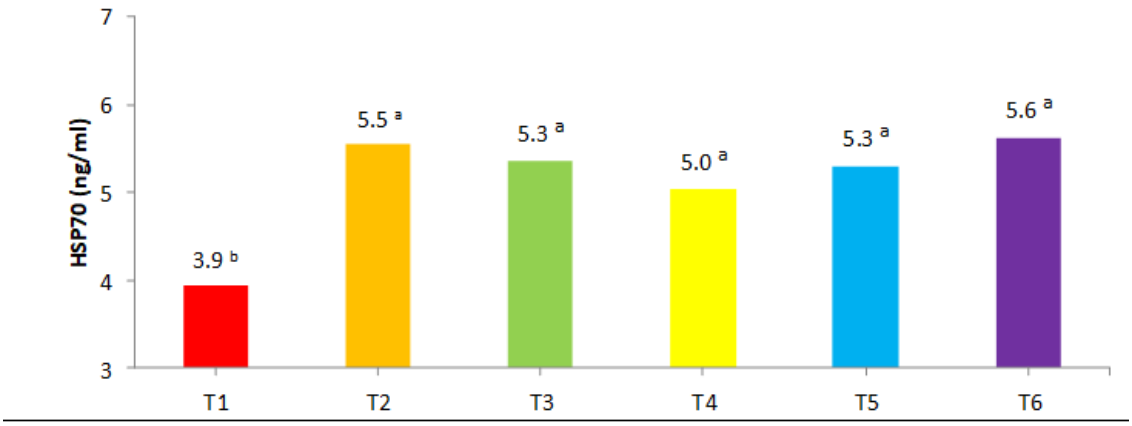

Figure 1. Effect of heat shock programs on formation of heat shock proteins 70 in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, HSP70 = heat shock protein 70, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

Thermo-respiratory responses

RT decreased (P< 0.05) in the rabbits of T3, T5 and T6 by about 0.63, 0.57 and 0.75 ^{o}C, respectively as compared with control group (Table 2). Indeed, FT and RR significantly (P< 0.05) decreased in the rabbits exposed to heat shock programs (T2, T3, T4, T5 and T6) by about 1.1 – 1.5 ^{o}C and 8.7 – 16.7 %, respectively as compared to the rabbits in T1. However, no significant (P> 0.05) differences were detected between treatments in ST and ET.

These results advice that early heat exposure and release HSP70 may enhance thermo-tolerance of rabbits exposed to heat stress at later age (Nagwa et al., 2012 and Morsy, 2013) that enable rabbits to cope any exposure to unexpected heat waves at later ages. Also, the HSP70 may play a critical role in cellular homeostasis during development of thermo-tolerance (Hahn and Li, 1990; Yahav and Hurwitz, 1996; Zhou et al., 1996; Yahav and Mc-Murtry, 2001; Morsy, 2013 and 2018). On the other hand, Abdel-Kafy et al. (2008) reported that the reduction of the RT in the rabbits exposed to heat shock program (34^{o}C) may be due to the fact that rabbits were able to maintain constant RT during heat exposure by low metabolic rate when previously acclimated to high temperature. These results agree with the results of Faisal et al. (2008); El-Badry et al. (2009); Star et al. (2009); Zulkifli et al. 2009 and El-Moniary et al. (2010), they used heat shock exposure programs to acclimate birds to heat stress and enhance some physiological responses of birds.

Table 2. Effect of heat shock programs on thermo-respiratory responses of Hi-Plus rabbits in South Sinai, Egypt.

Items	T1	T2	T3	T4	T5	T6	±SE
Rectal temperature (^{o}C)	39.5^{a}	39.2^{abc}	38.9^{c}	39.4^{ab}	39.0^{bc}	38.8^{c}	0.13
Fur temperature (^{o}C)	34.5^{a}	33.3^{b}	33.3^{b}	33.4^{b}	33.2^{b}	33.0^{b}	0.32
Skin temperature (^{o}C)	36.5	35.7	35.5	35.7	35.5	35.8	0.32
Ear temperature (^{o}C)	37.8	37.5	37.5	37.4	37.5	37.2	0.22
Respiration rate (breath/min.)	185.3^{a}	169.2^{b}	165.1^{bc}	154.2^{c}	162.5^{bc}	163.7^{bc}	3.8

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error, $^{a, b, c}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

Hematological parameters

WBC's count decreased (P< 0.05) in the rabbits of T2, T3 and T4 by 29.9, 37.6 and 25.1%, respectively as compared to the rabbits of T1 (Table 3). However, RBC's count and PCV increased (P<0.05) in the rabbits of T2 (by 15.5 and 19.8%, respectively), T3 (by 12.2 and 15.2%, respectively) and T4 (by 15.0 and 19.4%, respectively) when compared to the rabbits of T1. In addition, Hb concentration increased (P<0.05) in the rabbits of T2, T3, T4 and T6 by 14.6, 13.4, 14.8 and 15.1%, respectively as compared to the rabbits of T1. However, MCH increased (P<0.05) in the rabbits of T6 by (10.9%) compared with control group (Table 3). There are insignificant (P<0.05) effects of treatments on MCV and MCHC. Enhancement in hematological parameters might attribute to enhancement in the thermo-respiratory responses of rabbits exposed to heat shock exposure (Morsy, 2013). On the other hand, Nagwa et al. (2012); Emam (2013) and Morsy (2018) demonstrated that exposure of hens to heat stress conditions might impair the synthesis of blood cells. Yahav et al. (1997a) reported that hemoglobin increased in acclimated chickens compared with un-acclimated chickens.

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Table 3. Effect of heat shock programs on hematological parameters of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
WBC's (×10^3/mm^3)	10.2^a	7.1^{bc}	6.4^c	7.6^{bc}	9.9^{ab}	8.2^{abc}	0.66
RBC's (×10^6/mm^3)	4.8^b	5.6^a	5.4^a	5.5^a	5.0^{ab}	5.0^{ab}	0.22
Hb (g/dl)	9.9^b	11.4^a	11.3^a	11.4^a	10.7^{ab}	11.4^a	0.36
PCV (%)	31.0^b	37.1^a	35.7^a	37.0^a	33.3^{ab}	33.0^{ab}	1.7
MCV (fl)	64.2	66.8	66.3	66.4	65.9	65.4	1.9
MCH (pg)	20.7^b	21.1^{ab}	21.6^{ab}	20.9^{ab}	21.3^{ab}	23.0^a	0.64
MCHC (%)	33.1	31.9	32.6	31.9	32.8	35.4	1.2

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively ±SE = standard error, WBC's = white blood cells, RBC's = red blood cells, Hb =hemoglobin, PCV= packed cell volume, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration, $^{a, b, c}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

Metabolites parameters

Total protein and globulin concentrations increased (P< 0.05) in the rabbits of T5 group when compared to the rabbits of T1, T2 and T6 groups (Table 4). Cholesterol concentration significantly (P< 0.05) decreased in the rabbits of T6 group as compared to the rabbits of T4 group. Alanine transaminase concentration decreased (P< 0.05) in the rabbits of T2, T3, T4, T5 and T6 groups (11.9, 12.5, 14.4, 17.1 and 17.7 %, respectively) as compared to the rabbits of T1 (control group). This increase of liver enzyme in the rabbits of T1 might indicate that rabbits were not capable to defeat the heat stress conditions and hence led to adverse effect on liver function (Faisal et al., 2008). Meanwhile, using early heat shock programs and release HSP70 might cause the reductions in the severity of histopathological degeneration in the liver which may occur resulting from exposure of rabbits to severe heat stress (Okolie and Ivoanya, 2003).

However, rabbits of T2 and T4 showed increase (P< 0.05) in Total Antioxidant Capacity (TAC) (70.0 and 73.3%, respectively) when compared to the rabbits of control group (Figure 2). The significant (P< 0.05) increase of TAC and globulin concentrations used as an indicator of immune responses and source of antibodies production (El-Kaiaty and Hassan, 2004; Morsy, 2018). These results agree with findings of Mashaly et al. (2004) and Nagwa et al. (2012). The differences were not significant (P> 0.05) among groups in albumin, glucose and aspartic transaminase concentrations (Table 4). Although, aspartic transaminase concentration insignificantly (P> 0.05) decreased in the heat shock exposure treatments compared with control group.

Table 4. Effect of heat shock programs on biochemical parameters of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
TP (g/dl)	6.2^b	6.1^b	6.7^{ab}	6.7^{ab}	7.1^a	6.3^b	0.18
Albumin (g/dl)	4.1	3.9	4.1	4.1	4.1	4.05	0.11
Globulin (g/dl)	2.1^b	2.2^b	2.5^{ab}	2.5^{ab}	3.0^a	2.2^b	0.20
Glucose (mg/dl)	169.1	161.3	168.9	161.7	169.4	162.6	9.0
Chol (mg/dl)	62.5^{ab}	61.7^{ab}	58.3^{ab}	67.3^a	64.4^{ab}	53.2^b	5.5
ALT (i.u./l)	9.6^a	8.5^b	8.4^b	8.2^b	8.0^b	7.9^b	0.27
AST (i.u./l)	20.6	19.6	20.1	20.0	20.8	18.3	0.85

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error. TP = total protein, Chol = cholesterol, ALT =alanine transaminase, AST=aspartate transaminase, $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

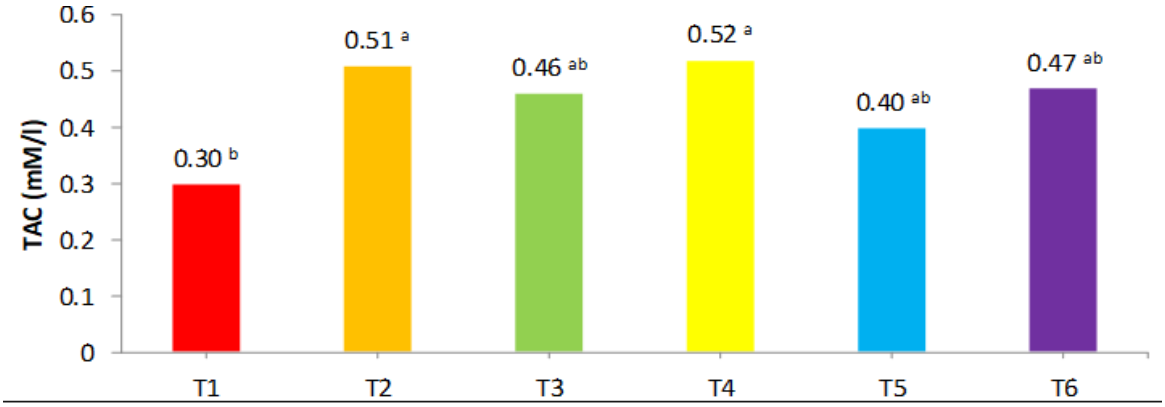

Figure 2. Effect of heat shock programs on total antioxidant capacity in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, TAC = total antioxidant capacity, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

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Hormonal profile

Tri-iodothyronine (T_3) hormone increased (P<0.05) in the rabbits of T2, T3, T4, T5 and T6 (7.8, 9.4, 7.5, 6.8 and 7.5 % respectively) as compared to the rabbits of T1 group (Figure 3). T_3 hormone plays an important role in regulating metabolism and thermogenesis (Tao et al., 2006). Concentration of T3 hormone is highly correlated to decrease of feed intake and heat stress conditions (Yahav et al. 1995). Hence, exposure of rabbits to heat stress conditions caused decrease of T3 level and decreased heat production and sustain homoeothermic (Uni et al., 2001; Attia et al., 2016). These results were in agreement with finding of Moraes et al. (2003); Nagwa et al. (2012) and Morsy (2018). However, the increase in T3 hormone of the rabbits exposed to heat shock programs may be explained the release of HSP70 which might play a role for maintaining metabolic rate and/or reducing the harmful effects of stress (Yahav and Hurwitz, 1996; Zhou et al., 1996; Yahav and Plavnik, 1999; Yahav and Mc-Murtry, 2001). However, Corticosterone (Cor) hormone decreased (P< 0.05) in the rabbits of T3, T4 T5 and T6 (62.1, 55.3, 52.8 and 60.7%, respectively) when compared to the rabbits of control group (Figure 4). In opposite, corticosteroid secretion increases as a response to heat stress (Morsy, 2018). The Cor hormone considered a more effective biological indicator of sever heat stress response (Siegel, 1995). However, lower Cor hormone level in the group of heat shock exposure programs submitted that the rabbits success to habituate to the heat stress. These results agree with the results of Star et al. (2009); Nagwa et al. (2012) and Morsy (2013). On the other hand, there was no significant (P> 0.05) difference among groups on progesterone hormone, although it insignificantly (P> 0.05) increased by 12.8 and 19.7% in T3 and T6 groups respectively as compared to the rabbits in T1 group (Figure 5).

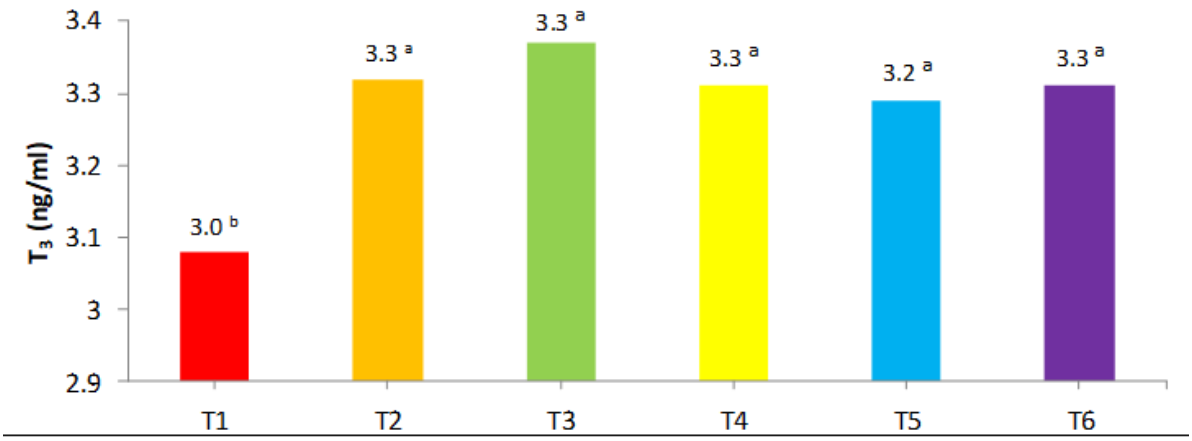

Figure 3. Effect of heat shock programs on tri-iodothyronine hormone in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, T_3 = tri-iodothyronine hormone, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

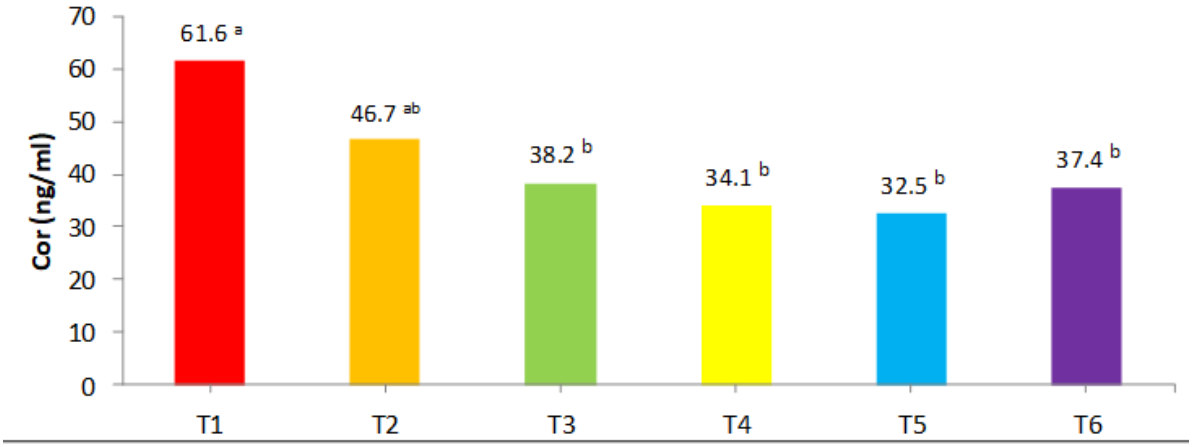

Figure 4. Effect of heat shock programs on corticosterone hormone level in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, Cor = corticosterone hormone, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

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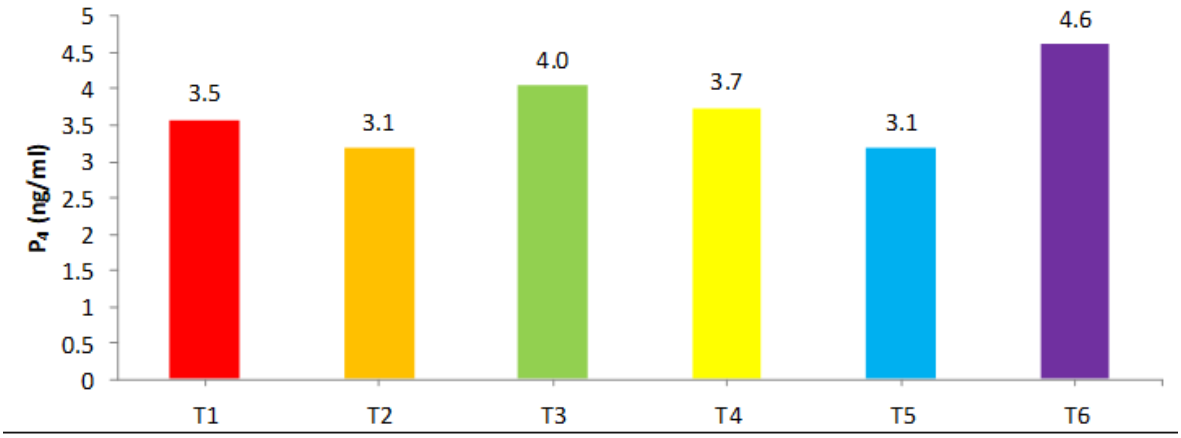

Figure 5. Effect of heat shock programs on progesterone hormone level in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, P_4 = progesterone hormone.

Body weight

Final body weight and body weight changes were significantly (P< 0.05) increased in the does of T2, T3, T4, T5 and T6 groups as compared to the does of control group (Table 5). This increase in final body weight and body weight changes in the does exposed to heat shock exposure during growth period (T2, T3, T4, T5 and T6) might attributed to enhance thermo-tolerance of doe rabbits that would face severe heat stress in advanced or later ages and may be reversed on increased body weight during exposure to heat stress conditions (Faisal et al., 2008; El-Badry et al., 2009; Star et al., 2009; Zulkifli et al., 2009; El-Moniary et al., 2010; Nagwa et al., 2012; Morsy, 2013 and Morsy, 2018).

Table 5. Effect of heat shock programs on doe body weight and body weight changes of Hi-Plus rabbits in South Sinai, Egypt.

Items	T1	T2	T3	T4	T5	T6	±SE
IBW (g)	3104.0	3115.3	3179.3	3194.5	3156.4	3168.1	66.2
FBW (g)	3308.0^{b}	3622.1^{a}	3678.1^{a}	3716.2^{a}	3583.5^{a}	3649.3^{a}	107.3
BWC (g)	204.0^{b}	506.7^{a}	498.7^{a}	521.6^{a}	427.1^{a}	481.2^{a}	67.1

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, IBW = initial body weight, FBW = final body weight, BWC = body weight change. ±SE = standard error. $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

Reproductive performance

Conception rate was higher (P<0.05) in the does of T5 than that of T3, T4 and T6 groups (Table 6). Furthermore, does in T5 and T1 had insignificant more parities compared to the other treatments. However, number of services/conception was lower (P<0.05) in the does of T5 as compared to the does of T3, T4 and T6 groups. Gestation length significantly (P<0.05) increased in the does of T3 by 2.8% when compared to the does of T1 and insignificantly (P> 0.05) increased in the does of T2, T4, T5 and T6 group by 1.9, 0.63, 1.9 and 1.6%, respectively as compared to the does of T1 (Table 6). These results might attribute to that rabbits exposed to heat shock programs during growth period led to increase gestation period through heat stress conditions. However, release HSP70 which may be suggested to regulate female fertility, enhancing ovulation-inducing and maintaining integrity of the fetal membranes and reducing the frequency of birth defects (Abd El-Kafy, 2006). On the other hand, milk yield significantly (P<0.05) increased in the does of T4 and T6 by 26.4 and 34.5%, respectively as compared to the does of T5 (Figure 6). This increase related to the increasing litter size in both of T4 and T6. These results showed that there is irreversible relationship between milk yield and conception rate. The lower hormonal levels in the suckling rabbits (estrogen hormone) and higher prolactin may reveal poorer ovarian activity, which could result in reducing reproductive efficiency. Our observations confirm the existence of a partial antagonism between lactation and reproduction in rabbits (Marongiu and Dimauro, 2013).

Table 6. Effect of heat shock programs on reproductive performance of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
Number of parity	2.1	1.5	1.7	1.6	2.0	1.6	0.19
CR (%)	51.1^{ab}	52.3^{ab}	45.5^{b}	42.2^{b}	62.3^{a}	45.5^{b}	5.4
NSC	1.9^{ab}	2.1^{ab}	2.4^{a}	2.4^{a}	1.9^{b}	2.3^{a}	0.19
GL (day)	31.4^{b}	32.0^{ab}	32.3^{a}	31.6^{ab}	32.0^{ab}	31.9^{ab}	0.21

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error. GL= gestation length, CR= conception rate, NSC= number of services per conception, $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05)

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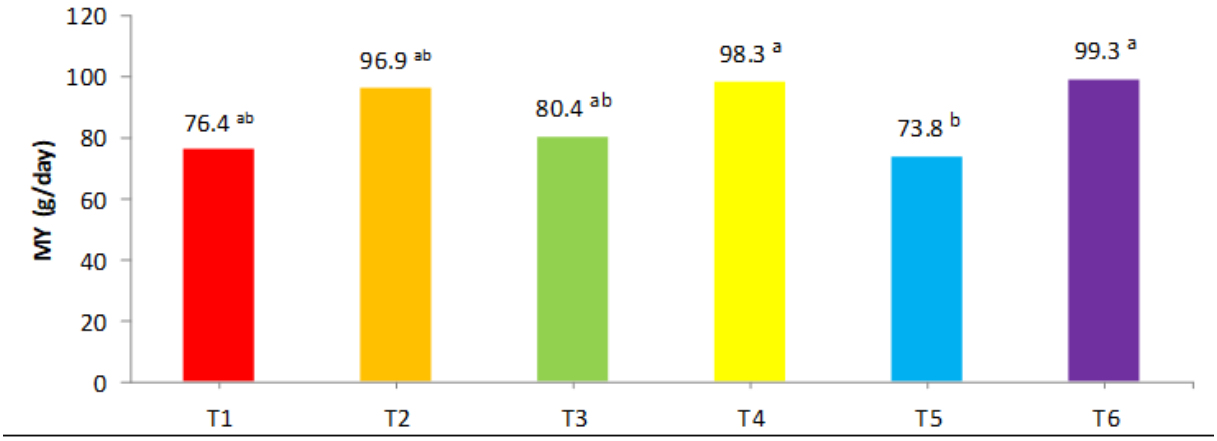

Figure 6. Effect of heat shock programs on milk yield in Hi-Plus doe rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, MY = milk yield, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

Litter traits

Viable Litter Size at Birth (VLSB) significantly increased in the does of T6 by about 47.0 and 48.0% as compared to those in T5 and T1, respectively (Table 7). However, there were insignificantly (P>0.05) increased VLSB in the does of T2, T3 and T4 about 32, 25 and 24%, respectively as compared to the does in T1 group. In addition, Litter Size at Weaning (LSW) increased (P< 0.05) in the does of T6, T4, T3 and T2 about 64, 70, 57 and 60%, respectively as compared to the rabbits of T1 group. However, there was insignificantly (P>0.05) increased of LSW in the does of T5 about 25.0% as compared to the does in T1 group. Litter Weight at Birth (LWB) increased (P<0.05) in the does of T2, T4 and T6 groups (43.0, 28.0 and 49.0%, respectively) as compared to the rabbits of control group (T1). Meanwhile, Litter Weight at Weaning (LWW) increased (P<0.05) in the does of T2, T3, T4, T5 and T6 groups as compared to the rabbits in control group (T1). These results indicated that heat shock exposure programs did not have negative effect on litter size and weight at birth which attribute the higher litter size at birth to a role of IGF-1 in protection embryos of mammalian exposed to stress; where the effects of heat shock on total cell number and development to the blastocyst stage (Jousan and Hansern, 2004 and Abd-El Kafy, 2006). In addition, LWW increased (P<0.05) in the does of heat shock programs groups as compared to the does of control group. Exposure of doe rabbits to heat shock exposure at early age led to increase thermo-tolerance and acclimate rabbits at severe heat stress which led to decrease heat-related mortality, improve productive performance and enhance immunity responses. These results agree with the results of Abd El-Kafy (2006); Nagwa et al. (2012) and Morsy (2018).

Table 7. Effect of heat shock programs on litter traits and mortality rate of Hi-Plus rabbits under South Sinai, Egypt conditions

Items	T1	T2	T3	T4	T5	T6	±SE
TLSB	6.4	8.2	7.2	7.8	6.2	8.7	0.80
VLSB	5.6^{b}	7.5^{ab}	7.1^{ab}	7.0^{ab}	5.7^{b}	8.3^{a}	0.76
LSW	3.6^{b}	5.8^{a}	5.7^{a}	6.2^{a}	4.5^{ab}	6.0^{a}	0.62
LWB (g)	268.3^{b}	384.3^{a}	312.7^{ab}	342.2^{a}	306.4^{ab}	400.8^{a}	29.9
LWW (g)	1595.3^{c}	2940.0^{a}	2831.1^{ab}	2795.5^{ab}	2248.5^{b}	2709.7^{ab}	201.7
MRB-W (N)	2.0	1.6	1.3	0.75	1.1	2.3	0.57
MRB-W (%)	35.7^{a}	18.3^{b}	13.3^{b}	9.6^{b}	17.6^{b}	28.1^{ab}	6.7
Stillbirth (n)	0.78	0.75	0.13	0.88	0.57	0.38	0.35
Stillbirth (%)	11.8	7.6	2.5	9.5	5.1	4.3	4.2
Overall MRB-W (%)	47.6^{a}	26.0^{b}	15.8^{b}	19.1^{b}	22.8^{b}	32.5^{ab}	8.8

T1 = control, T2, T3, T4, T5 and T6= rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days+25 days of age and at 3 days+25 days+2 months of age, respectively, ±SE = standard error, TLSB = total litter size at birth, VLSB =viable litter size at birth, LSW = litter size at weaning, LWB= litter weight at birth, LWW= litter weight at weaning, MRB-W = mortality rate from birth to weaning, $^{a, b, c}$ Means bearing different superscripts within the same row are significantly different (P< 0.05)

Mortality rate

Results of table 7 declared that Mortality Rate from Birth to Weaning (MRB-W) and overall MRB-W were lower (P<0.05) in does of T2, T3, T4 and T5 groups than control group (T1). These results suggested that release HSP70 synthesis (Figure 1) may protect doe immune function against some forms of stress as reported by Ciavarra and Simone (1990 a and b) and Abd El-Kafy (2006). On the other hand, Yahav et al. (1997 a and b) and Nagwa et al. (2012) reported that early heat shock exposures led to decrease mortality rate and this may be due to enhance thermo- tolerance and immunity. However, Figure 7 demonstrated that heat shock programs led to decrease (P<0.05) mortality in doe Hi-Plus rabbits and this might be due to enhance thermos-tolerance mechanism. Indeed, increasing mortality rate in control group

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(T1) may be attributed to inability to regulate body temperature and respiration rate under heat stress conditions as shown in table 2. These results agree with the results of Yahav et al. (1997a); Nagwa et al. (2005) and Abd El-Kafy (2006).

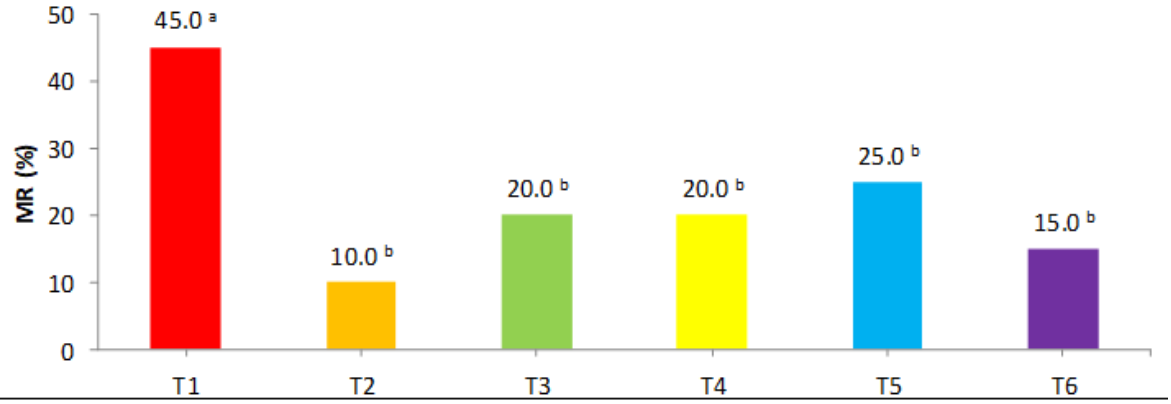

Figure 7. Effect of heat shock programs on doe mortality rate in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, MR = mortality rate, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

Offspring's traits

Results in table 8 revealed that early heat shock exposure programs of does had no significant (P>0.05) effect on offspring growth performance, though, does of T2, T3 and T5 had higher bunny weight at weaning and bunny daily gain as compared to does of T1.

Economic indicators

Productive efficiency index and feed conversion were improved (P< 0.05) in the does of T2 (28.8 and 26.1%), T3 (44.3 and 34.3%), T4 (41.5 and 29.5%), T5 (28.8 and 25.6%) and T6 (33.2 and 29.0%), respectively when compared to the does of T1 group (Table 9). However, no significant (P>0.05) differences between treatments in feed intake. Indeed, cost of feeding for producing one kg live weight under desert hot conditions decreased (P<0.05) in the does of T2, T3, T4, T5 and T6 it was 9.2, 12.1, 10.4, 8.9 and 10.2 L.E., respectively as compared to the does of T1 group (Figure 8). This improvement in productive traits might be attributed to application of heat exposure programs (Franco-Jimenez et al., 2007; Emam, 2013 and Morsy, 2018). Heat shock exposure programs at early ages might enhance thermo-tolerance of doe rabbits for facing severe heat stress in later ages (Faisal et al., 2008; El-Badry et al., 2009; Zulkifli et al., 2009; El-Moniary et al., 2010; Nagwa et al., 2012 and Morsy, 2013). So, increase of HSP70 expression might suggesting that the proteins involved in the stress caused by heat shock exposure in the does that play important role in protecting stressed cells and reversing disorders caused by stress (Li and Werb, 1982; Emam, 2013) and hence it reflected on improvement of productive performance of doe rabbits (Abd El-Kafy, 2006).

Table 8. Effect of heat shock programs on offspring's of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
BWB (g)	48.5	52.7	46.9	49.7	56.9	48.5	3.3
BWW (g)	484.1	521.2	545.7	460.1	531.7	490.7	51.4
BDGB-W (g)	15.5	16.7	17.8	14.6	16.9	15.7	1.8
GR (%)	159.8	161.1	166.9	160.4	159.8	162.7	3.4

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error, BWB = bunny weight at birth, BWW = bunny weight at weaning, BDGB-W = bunny daily gain from birth to weaning, GR = growth rate.

Table 9. Effect of heat shock programs on economic indicators of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
Daily FI (g)	145.3	148.6	148.7	148.0	148.4	146.5	1.4
TFI (kg)	17.8	18.2	18.3	18.2	18.2	18.0	0.17
EI (kg live weight)	3.2^{b}	4.1^{a}	4.6^{a}	4.5^{a}	4.1^{a}	4.3^{a}	0.43
FC	6.4^{a}	4.7^{b}	4.2^{b}	4.5^{b}	4.7^{b}	4.5^{b}	0.64

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error, DFI = daily feed intake, TFI = total feed intake, EI = efficiency index, FC = feed conversion, $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05)

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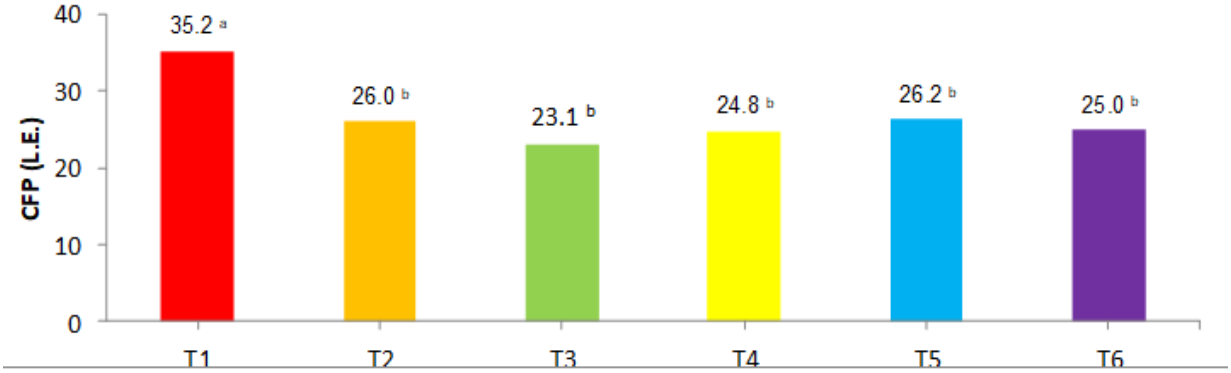

Figure 8. Effect of heat shock programs on cost of feeding for producing one kilogram of live weight in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, CFP = cost of feeding for producing one kg live weight, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

CONCLUSION

In conclusion, applying heat shock exposure programs for doe rabbits especially T3 treatment (rabbits were exposed to heat shock exposure at 25 days of age) might increase HSP70 gene expression and enhance acclimation of doe rabbits, this led to enhance immunity responses, reproductive performance and production under severe heat stress conditions.

DECLARATIONS

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Competing interests

The authors declare that they have no conflict of interests with respect to the research, authorship, and/or publications of this article.

Consent to publish

All the authors approved and agreed to publish the manuscript and declared that this work has not been previously published elsewhere.

Author's contribution

Dr. Nagwa Abde El-Hady Ahmed designed the experiment, article writing and revision, Dr .Ali Saber Morsy designed the experiment, laboratory analyses, statistical analysis, tabulation of experimental data, manuscript writing, commenting and approval, Dr. Osama Glal Sakr helped in statistical analysis, tabulation of experimental data and article writing; Dr .Khamis Refay Sayed Emam designed the experiment, tabulation of experimental data, manuscript writing, commenting and approval; while, Mr .Baliegh Hamdy Mohammed Mousa helped in field study, collected data, laboratory analyses, manuscript writing. All authors have read and approved the final manuscript.

REFERENCES

Abd El-Kafy EM (2006). Effect of heat exposure and feed restriction as bio-stimulation method for enhancing rabbit productivity. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Giza, Egypt, p. 95.

Abd El-Kafy EM, Ali WA, Hoda AS and Azoz AA (2008). Effect of short heat exposure, balanced feed restriction and acetic acid supplement at post weaning on growth and thermoregulation in growing rabbits during hot season. 9th World Rabbit Congress, June 10,13, Verona, Italy.

Abd El-Samee AM, Ali AM, Mousa MR and Abd El-Ghaffar MA (2005). Productivity and reproductive of heat stressed rabbits as influenced by nutritional treatments under Sinai conditions. Proceedings of the 4th International Conference on Rabbit Production in Hot Climate, (RPHC'05), Sharm El-Sheikh, Egypt, pp: 265-371.

Attia YA, Abd El-Hamid AE, Abedalla AA, Berika MA, Al-Harthi MA, Kucuk O, Sahin K and Abou-Shehema BM (2016). Laying performance, digestibility and plasma hormones in laying hens exposed to chronic heat stress as affected by betaine, vitamin C, and/or vitamin E supplementation. Springer Plus, 5: 1619: 2-12. DOI:10.1186/s40064-016-3304-0

Chirico WJ, Waters MG and Blobel G (1988). 70K Heat shock related protein stimulate protein translocation into microsomes. Nature, 332: 805-810. DOI: 10.1038/332805a0

Ciavarra RP and Simeone A (1990a). T Lymphocyte stress response. i- induction of heat shock protein synthesis at febrile temperature is correlated with enhanced resistance to hyperthermic stress but not to heavy metal toxicity or dexamethasone-induced immunosuppression. Cell Immunol, 129(2): 363-376. DOI: 10.1016/0008-8749(90)90212-A

Ciavarra RP and Simeone A (1990b). T lymphocyte stress response. ii. protection of translation and DNA replication against some forms of stress by prior hypothermic stress. Cell Immunol, 131(1):11-26. DOI: https://doi.org/10.1016/0008-8749(90)90231-F

De Basilio V, Requena F, Leon A, Vilarin OM and Picard M (2003). Early age thermal conditioning immediately reduces body temperature of broiler chicks in a tropical environment. Poultry Science, 82:1235-1241. DOI: https://doi.org/10.1093/ps/82.8.1235

Duncan DB (1955). Multiple range and multiple F-test. Biometrics, 11: 1-42.

El-Badry AS, Hassanane MM, Ahmed ES and El-Kholy KH (2009). Effect of early-age acclimation on some physiological, immunological responses and chromosomal aberrations in Muscovy ducks during exposure to heat stress. Global Journal of Biotechnology and Biochemistry, 4(2):152-159.

DOI: https://www.researchgate.net/publication/237614195

El-Kaiaty AM and Hassan MS (2004). Some physiological and immunological parameters in the female of local chicken strains. Egyptian Poultry Science, 24: 901- 916.

El-Moniary MM, Hemid AA, El-Wardany I, Gehad AE and Gouda A (2010). The effect of early age heat conditioning and some feeding programs for heat-stressed broiler chicks on: 1 - productive performance. World Journal of Agriculture Science, 6(6): 689-695.

DOI: https://pdfs.semanticscholar.org/18e8/70a844cc0d42a067265ad9fb31830cd13696.pdf

Emam, KR (2013). Using bio-stimulation to alleviate heat stress of chickens under desert conditions. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Giza Egypt, p. 85.

Faisal BA, Abdel–Fattah SA, El–Hommosany YM and Maie FM (2008). Immunocompetence, hepatic heat shock protein 70 and physiological responses to feed restriction and heat stress in two body weight lines of Japanese quail. International Journal Poultry Science, 7 (2): 174-183. DOI: 10.3923/ijps.2008.174.183

Franco-Jimenez DJ, Scheideler SE, Kittok RJ, Brown-Brandl TM, Robeson LR, Taira H and Beck MM (2007). Differential effects of heat stress in three strains of laying hens. Journal of Applied Poultry Research, 16(4): 628-634. DOI: 10.3382/japr.2005-00088

Hahn GM and Li GC (1990). Thermotolerance, thermo resistance, and thermo sensitization. in: stress proteins in biology and medicine. (Eds. Morimoto, R.I.; Tissieres, A. and Geogopoulos, C.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 79-100.

Hanan SM (2006). Some physiological and immunological measurements in local breed of chickens after heat stress. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Egypt, 189 p.

Jousan FD and Hansen PJ (2004). Insulin-like Growth Factor-I as a Survival Factor for the Bovine Preimplantation Embryo Exposed to Heat Shock. Biology of Reproduction, 71(5): 1665-1670. DOI: https://doi.org/10.1095/biolreprod.104.032102

Lebas FP, Coudert H and Thebault RG (1997). The rabbit husbandry, health and production. Food and Agriculture Organization of the United Nations, FAO, Rome, Italy.

Li GC and Werb Z (1982). Correlation between synthesis of heat-shock proteins and development of thermotolerance in Chinese hamster fibroblasts. Proceedings of the National Academy of Science, 79: 3218-3222. DOI: 10.1073/pnas.79.10.3218

Maak S, Melesse A, Schmidt R, Schneider F and Von Lengerken G (2003). Effect of long-term heat exposure on peripheral concentrations of heat shock protein 70 (hsp70) and hormones in laying hens with different genotypes. British Poultry Science, 44:133-138. DOI: 10.1080/0007166031000085319

Marai IF, Habeeb AA, El-Sayed GA and Nessem MZ (1994). Growth performance and physiological response of New Zealand White and Californian rabbit under hot summer conditions of Egypt. 1st International Conference Rabbit Prod. In Hot Climates, 6-8 September, 1994, Cairo, Egypt, pp. 619-625. DOI: 10.1023/A:1012772311177

Marai, IF, Ayyat MS, Gabr HA and Abdel-Monem UM (1996). Effect of summer heat stress and its amelioration on production Performance of New Zealand White Adult Female and Male Under Egyptian Conditions. 6th World Rabbit Congress, 2, 197-208.

Marai IF, Ayyat MS and Abdel-Monem UM (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. Journal Tropical Animal Health Production, 33:1-12. DOI: https://doi.org/10.1023/A:1012772311177

Marongiu ML and Dimauro C (2013). Preliminary study on factors influencing rabbit doe reproductive efficiency: effect of parity, day of mating, and suckling on ovarian status and estrogen levels at day 6 of pregnancy. Canadian Journal of Veterinary Research, Volume 77, Number 2, April 2013, pp. 126-130. DOI: http://agris.fao.org/agris-search/search.do?recordID=US201500107171

Mashaly MM, Hendricks GL, Kalama MA, Gehad AE, Abbas AO and Patterson PH (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Science, 83:889-894. DOI: 10.1093/ps/83.6.889

Moraes VM, Malheiros RD, Bruggeman V, Collin A, Tona K, Van Onagbesan OM, Buyse J, Decuypere E, Macari M and Van AS (2003). Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. Journal of Thermal Biology, 28(2): 133-140. DOI: 10.1016/S0306-4565(02)00049-9

Morimoto R I, Tissieres A and Georo Poulos C (1990). Stress protein in biology and medicine. Cold spring Harper, New York. DOI:10.1126/science.572-a

Morsy AS (2001). Reproductive and productive performance of rabbits under different housing systems. Master Science, Thesis, Faculty of agricultural, Cairo University, Giza, Egypt.

To cite this paper: Sakr OG, Mousa BH, Emam KRS, Morsy AS and Ahmed NA (2019). Effect of Early Heat Shock Exposure on Physiological Responses and Reproduction of Rabbits under Hot Desert Conditions. *World Vet. J.* 9(2): 90-101. www.wvj.science-line.com

Morsy AS (2007). Enhancing rabbit performance reared in newly reclaimed areas by alleviating the environmental stress. PhD Thesis Animal Production Department, Faculty of Agriculture, Cairo University.

Morsy AS (2013). Effect of heat shock exposure on the physiological responses and semen quality of male chickens under heat stress conditions. Egyptian Poultry Science, 33, (1): 143-161.

Morsy AS (2018). Hematological parameters and productive performance of heat-stressed laying hens as influenced by early heat shock program, sodium bicarbonate and/or vitamin C supplementation. research Journal Animal and Veterinary Science, 10(2): 1-8.

Morsy AS, Amal MH and Shaker YM (2011). Monitoring of physiological responses of Gabali rabbits under semi-arid conditions of Egypt. The 4th Egyptian Conference on Rabbit Science, Faculty of agricultural, Cairo University, 29 October, 2011, 34-50.

Nagwa AA, Amal, MH, Mehaisen GM and Emam KR (2012). Effect of using heat shock programs on thermoregulation responses and performance of laying hens under desert conditions. Egyptian Poultry Science, 32 (4): 777-790.

Nagwa AA, Amina F, Shafie MM and Azoz AA (1994). Effect of post-partum mating interval of rabbits on progesterone level through pregnancy. Egyptian Journal Animal Production, 31 (supplement): 463 – 473.

Nagwa, AA, El-Tantawy SM, Khadr, Amina F and El-Badry AS (2004). Effect of early heat exposure and feeding system on physiological responses and productive performance of ducting under heat stress conditions. Egyptian Journal Animal Production, 41 (Supplement): 485-499.

Nagwa AA, Ismail AM and Abdel-Kafy EM (2005). Effect of heat exposure and feed restriction on performance of pregnant rabbit-does with emphasis on heat shock protein detection. The 2nd Inter. Conference Veterinary Research Division NRC Cairo, Egypt, 27-37.

National Research Council (1994). Nutrient Requirements of Poultry. 9th edition. National Academic Press. Washington, DC. Availabe at: Chttps://www.scirp.org/(S(oyulxb452alnt1aej1nfow45)) /reference/ReferencesPapers.aspx? ReferenceID=1955728.

Okolie NP and Ivoanya CU (2003). Some histologic and biochemical evidence for mitigation of cyanide-induced tissue lesions by antioxidant vitamin administration in rabbits. Food and Chemical Toxicology, 41 (4): 463-469.

DOI: https://doi.org/10.1016/S0278-6915(02)00224-7

Rahimi G (2005). Effect of heat shock at early phase on glucose and calcium regulation axis in broiler chickens. International Journal Poultry Science, 4(10): 790-794. DOI: .3923/ijps.2005.790.794"10.3923/ijps.2005.790.794

Sakr OG (2003). Effect of ambient temperature on the behavior and the reproductive performance of rabbits. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Giza, Egypt.

SAS Institute (2004). SAS User's Guide: Statistics. Release 9.1. SAS Institute Incorporated., Cary, North Carolina.

Siegel HS (1995). Stress, strains and resistance. British Poultry Science, 36: 3-22. DOI: https://doi.org/10.1080/00071669508417748

Star L, Juul-Madsen HR, Decuypere E, Nieuwland MB, de Vries Reilingh G, van den Brand H, Kemp B and Parmentier H K (2009). Effect of early life thermal conditioning and immune challenge on thermotolerance and humoral immune competence in adult laying hens. Poultry Science, 88: 2253-2261.doi: 10.3382/ps.2008-00373

Tao X, Zhang ZY, Dong H, Zhang H and Xin H (2006). Responses of thyroid hormones of market-size broilers to thermoneutral constant and warm cyclic temperatures. Poultry Science, 85: 1520-1528. DOI: 10.1093/ps/85.9.1520

Uni Z, Gal-Garger O, Geyra A, Sklan D and Yahav S (2001). Changes in growth and function of chick small intestine epithelium duo to early thermal conditioning. Poultry Science, 80: 438–445. DOI: https://doi.org/10.1093/ps/80.4.438

Yahav S and Hurwitz S (1996). Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poultry Science, 75:402-406. DOI: https://doi.org/10.3382/ps.0750402

Yahav S and McMurtry JP (2001). Thermotolerance acquisition in broiler chickens by temperature conditioning early in life-the effect of timing and ambient temperature. Poultry Science, 80: 1662–1666. DOI: 10.1093/ps/80.12.1662

Yahav S and Plavnik I (1999). Effect of early-age thermal conditioning and food restriction on performance and thermotolerance of male broiler chickens. British Poultry Science, 40:120– 126. DOI: 10.1080/00071669987944

Yahav S, Goldfeld S, Plavnik I and Hurwitz S (1995). Physiological response of chickens and turkey to relative humidity during exposure to high ambient temperature. Journal biology thermodynamics, 20: 245–253. DOI: 10.1016/0306-4565(94)00046-L

Yahav S, Shamai A, Haberfeld A, Horev G, Hurwitz S and Einat M (1997a). Induction of thermo-tolerance in chickens by temperature conditioning: heat shock protein expression. Annals of the New York Academy of Sciences, 15: 628-636. DOI: 10.1111/j.1749-6632. 1997.tb51757.x

Yahav S, Shamay A, Horev G, Barilan D, Genina O and Friedman-Einat M (1997b). effect of acquisition of improved thermotolerance on the induction of heat shock proteins in broiler chickens. Poultry Science, 76:1428- 1434.

DOI: 10.1093/ps/76.10.1428

Yalcin S, Zkan OS, Turkmut L and Siegel PB (2001). Response to heat stress in commercial and local broiler stocks. 1. Performance traits. British Poultry Science, 42:149-152. DOI: 10.1080/00071660120048375

Zhou WT, Fujita M, Ito T and Yamamoto S (1996). Effects of intermittent short-term acclimation to 32oc on some thermoregulatory responses and standing behavior of laying hens exposed acutely to 36 °c. Japanese Poultry Science, 33: 221- 229.

Zulkifli I, Al-Aqil A, Omar AR, Sazili AQ and Rajion MA (2009). Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. Poultry Science, 88: 471–476. DOI: 10.3382/ps.2008-00287

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Influence of Treated Orange Pulp on Growth Performance, Nutrients Digestibility and Plasma Constituents of Rabbits

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ABSTRACT

The current study investigated the effect of replacement of Untreated Orange Pulp (UOP) and Treated Orange Pulp (TOP) protein by basal diet protein on growth performance, digestion coefficients, some blood constitute of rabbits and economic efficiency of growing rabbit diets. Sixty cross bread (New Zealand White, NZW X California), six weeks of age with live body weight ranging from 729.20 to 738.30g were divided to five experimental groups. The experimental diets were T1, control diet without OP; T2, 5%UOP; T3, 5% TOP; T4, 10%UOP and T5, 10%TOP. The results indicated that TOP by *Saccharomyces cerevisiae* yeast increased content of the crude protein (%) and digestible energy (Kcal/kg). The best Final Body Weight (FBW, g), Body Weight Gain (BWG, g/R/day) and feed conversion ratio recorded in 5%TOP group. Digestion coefficient of Crude Protein and Digestible Crude Protein (DCP%) were significantly ($P<0.05$) increased in rabbits fed low replacement level of OP (5% UOP and 5%TOP). Total lipid of plasma was significantly differences ($P<0.05$) in groups fed experimental diets compared to control group. Liver function was significantly affected by experimental diets, yeast treatment and replacement level of OP. Best economic efficiency observed with 10%UOP followed by 5%TOP. It was concluded that rabbit group fed 5%TOP recorded a better performance, best digestibility for CP%, DCP and economic efficiency. *Saccharomyces cerevisiae* yeast treatment didn't effect on digestibility and nutritive value of growing rabbits.

Key words: Digestibility, Economic, Growing rabbits, Performance, Plasma, Yeast.

INTRODUCTION

Nutrition accounts for 70% of the total cost of rabbit or animal production (Oyawoye and Nelson, 1999; Spring, 2013). Incorporation of fruits and vegetable wastes in animal feeds improved palatability of diet and consequently increased the feed intake in addition to decrease feed cost (Chaudry et al., 2004 and Alnaimy et al., 2017). Sun dried orange peel meal and citrus pulp has been used as untraditional calorie and protein source in broiler diets (Oluremi et al., 2006; De Bals et al., 2018). Hon et al. (2009) concluded that sweet orange pulp meal could be utilized up to 20% of growing rabbit diets without any adverse effects on performance. Wang et al. (2017) found that using citrus pulp in geese diets less than 12% had no negative effects on growth performance and carcass traits.

Probiotics are live microorganisms used in animal diets as feed supplementation to enhance of the intestinal microbial balance (Fortun-Lamothe and Boullier, 2007). The use of probiotics in farm animal diets is based on the concept that the balance of intestinal microorganisms in healthy animals increases resistance to diseases, and it is necessary for efficient digestion and maximum absorption of nutrients. Some characteristics of probiotics are reported by Strompfova et al. (2006) which included the ability to reduce antibiotic use, high index of safety and natural or alternative therapies. The *Saccharomyces cerevisiae* is a probiotic and a possible using in animal diets because of its availability, safety and cheapness. Its cells contain a lot of proteins, carbohydrates, lipids, vitamins and minerals (Reddy et al., 2006). *S. cerevisiae* is a valuable and qualitative growth promoter for feeding livestock (Falcão-e-Cunha et al., 2007) and positive effects on Japanese quails performance (Nikpiran et al., 2013). So, present study aimed to investigate the effect of replacement of Untreated Orange Pulp (UOP) and Treated Orange Pulp (TOP) protein by basal diet protein on performance, digestion coefficients of nutrients, blood constitute of growing rabbits and economic efficiency of rabbit diets.

MATERIALS AND METHODS

The experiment was conducted in Borg El-Arab station following to Animal Production Research Institute (APRI), agricultural research center, ministry of agriculture and land reclamation, Egypt. The laboratories works were carried out at laboratories of

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utilization of by-products research department, APRI, Giza, Egypt. Dried orange pulp and other ingredients obtained from by-product of food industries and locally market. The dried yeast purchased from three pyramids Alexandria yeast Company, Alexandria, Egypt. Feed mixing and pelleting processes were carried out at Nobaria manufactory, Nobaria station following to APRI, Egypt.

Ethical approval

This study was carried out after obtaining the ethical approval of the APRI, Egypt (Code No 12-2-16).

Yeast treatment and experimental diets

Orange Pulp (OP) was mixed with water at 1:2 ratio (1pulp:2 water) to supply relative humidity of 85%. The measured pH value was 3.6. The optimum pH for yeast activity is between 5 and 6. Therefore, 6.4% bicarbonate was added to mixture to increase the pH value. Then added 4% dried yeast (*S. cerevisiae*) according to Dadvar et al. (2014). Five experimental diets were formulated, control diet without dried orange pulp and the other experimental diets were replacement of two levels of UOP or TOP (*S.cerevisiae*) protein by control diet protein. The experimental diets were divided into basal diet without OP as a control group (T_1), while 5% UOP (T_2), 5% TOP (T_3), 10% UOP (T_4) and 10% TOP (T_5) were different levels of UOP and TOP by *S. cerevisiae* yeast. The diets and fresh water were supplied *ad libitum.* The experimental period lasted for eight weeks from 6 to 14 weeks of age. All experimental diets (Table 1) were formulated to be isonitrogenous and isocaloric, to meet all the essential nutrients requirements of growing rabbits (Lebas, 2004).

Table 1. Ingredients and chemical composition of experimental diets in growing rabbits

Ingredients	Control diet	Replacement Level of OP protein (%)			
		5% UOP	5% TOP	10% UOP	10% TOP
Soybean meal (44% crude protein)	16.30	16.30	16.30	16.30	16.30
Yellow corn	13.80	11.8	11.35	9.75	10.90
Barley	13.00	13.00	13.00	13.00	13.00
Wheat bran	16.85	16.85	16.85	16.85	16.85
Clover hay	34.00	27.97	30.00	22.00	23.99
Untreated orange pulp (UOP)	00.00	8.03	00.00	16.05	00.00
Treated orange pulp (TOP)	00.00	00.00	6.45	00.00	12.91
Dl- methionine	0.20	0.20	0.20	0.20	0.20
Di calcium phosphate	2.00	2.00	2.00	2.00	2.00
Sodium chloride (NaCl)	0.35	0.35	0.35	0.35	0.35
Vitamin and mineral primix[1]	0.30	0.30	0.30	0.30	0.30
Anti coccidia and fungi	0.20	0.20	0.20	0.20	0.20
Molasses	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00
Chemical analysis (DM basis)					
DM%	82.94	82.96	82.37	82.98	82.98
OM%	85.76	85.23	85.38	84.71	84.88
CP%	17.49	17.30	17.55	17.12	17.51
CF%	13.88	13.28	13.60	12.70	12.74
EE%	2.17	2.29	2.25	2.45	2.37
NFE%	55.43	55.64	55.14	55.83	55.59
Ash%	5.09	5.53	5.53	5.98	5.86
DE kcal/kg[2]	2615.42	2628.44	2610.37	2640.38	2642.64
Price of feed (L.E./ton)	4927.18	4721.67	4756.58	4516.00	4601.73

[1]Each kg of vitamins and minerals mixture contains: Vit. A: 2.000.000 IU, Vit.B1: 0.33g, Vit.B2: 1.0g, Vit.D3: 150.000 IU, Vit E: 8.33g, Vit. K: 0.33 g, Pantothenic acid: 3.33g, Nicotinic acid: 30.00g, Vit. B6: 2.00g, Vit. B12: 1.7 mg, Folic acid: 0.83g, Biotin: 33 mg, Cu: 0.5g, choline choloride: 200mg, Mn: 5.0g, Fe: 12.5g, Mg: 66.7mg, Co: 1.33 mg, Se: 16.6 mg, Zn: 11.7 Iodine: 16.6 mg and Anti-oxidant: 10.0g, [2]DE= Digestible energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF%)] according to Cheeke, (1987), UOP: untreated orange pulp, TOP: treated orange pulp.

Experimental animals and housing

Sixty cross breed (New Zealand White, NZW X California), six weeks of age with live body weight ranging from 729.20 to 738.30 g were divided to five experimental groups (12 rabbits in each). All rabbits were kept under the same managerial and hygienic conditions and housed in metal battery cages supplied with separated feeders. All rabbits were kept under veterinary control and vaccinated against diseases; SERVAC RHDV oil vaccine and SERVAC Formalized polyvalent Rabbit Pasteurellosis Vaccine. All vaccines had purchased from Veterinary Serum and Vaccine Research Institute, Elabasia, Cairo, Egypt.

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Productive performance

Final body weight, daily feed intake (FI, g/rabbit/day) and daily body weight gain (BWG, g/rabbit/day) were recorded weekly, feed conversion ratio (FCR) was calculated accordingly as g feed / g gain over an experimental period.

Digestion coefficients and nutritive value

Fifteen rabbits were used in digestion trial and divided to five groups (three replicates/each). Rabbits were housed in individual metabolism cages (56 × 38 × 28 cm, L × W × H). Feces were collected daily before the morning meal and weighed fresh and dried at 60 °C for 24 h in an air drying oven (Perenz et al., 1995). Samples of UOP and TOP, diets and feces were prepared to determine moisture, ash, nitrogen, ether extract and crude fiber according to AOAC (2000). Data of quantities and chemical analysis of feed and feces were used to nutrients digestibility and nutritive value calculation for each dietary treatment (Fekete, 1985). Digestible energy (DE, Kcal/Kg diet) was calculated as follow: Total Digestible Nutrient (TDN) × 44.3 according to Schneider and Flatt (1975).

Biochemical analysis

Blood samples of all 15 rabbits were collected (3 rabbits/treatment randomly selected) into dry clean centrifuge tubes and centrifuged at 3000 rpm for 20 minutes, then, samples were transferred and stored in deep freezer at -20°C till the time of analyses. Blood chemical analyses were carried out for determination of plasma total lipids (Frings and Dunn, 1970), triglycerides, cholesterol, creatinine and urea nitrogen (Young, 2000), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were determined by Henry (1964). All kits had purchased from Bio-dignostic, diagnostic and research reagents company, Dokki, Giza, Egypt. All plasma biochemical analyses were determined spectrophotometrically at 546 nm using colorimetric kits.

Economic efficiency

The Economic Efficiency (EEF) was calculated according to the following equation:

EEF (%) = (Net revenue/ total feed cost) × 100. Whereas, Net revenue =Selling price/rabbit- total feed cost/rabbit.

The price of ingredients and selling of one kg live weight of rabbits as the same price in the local market at the time of experiment (October, 2018). Price of one kg live body weight was 50 LE (1 LE = 17.32 $).

Statistical analysis

Data were analyzed using general linear model procedure of SAS software (SAS, 2004) by using model: $Yij = \mu + L_i + S_j + (LS)_{ij} + e_{ij}$ Where, Y_{ij} = an observation; μ = overall mean; L_i = effect of OP substitution levels; S_j= the effect of *S.cerevisiae* yeast treatment, $(LS)_{ij}$= effect of interaction between OP substitution levels with yeast treatment and e_{ij} = random error. Duncan's multiple range test (Duncan, 1955) was performed to detected significant differences among means. Significant level was acceptable at $P \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition of UOP and TOP

Table 2 is summarized the chemical composition of OP before and after treated by *S. cerevisiae* yeast. Percentage of the Dry Matter (DM), Organic Matter (OM), Crude Fiber (CF), Ether Extract (EE) and Nitrogen Free Extract (NFE) content decreased in TOP when compared to UOP. In contrast, Crude Protein (CP%) (13.17 vs. 10.59%), ash and Digestible Energy (DE, kcal/kg) were higher in TOP than UOP. The obtained results are in agreement with Dadvar et al. (2015) reported treated lemon pulp with *S. cerevisiae* yeast up to 4% increased CP% content of lemon pulp. Also, treated castor meal by *Penicillium funiculosm* caused increase in CP%, DE, Kcal/kg and ash% content while, decreased in DM%, OM%, CF% and NFE% (Suliman et al., 2015). The biological treatment is used for increasing the nutritional value of many by-products because they had significant concentrations of simple carbohydrates such as mono and disaccharides (Villas-Boas et al., 2002). Moreover, increasing ash content attributed to the growth or degradation of organic matter by microorganism. While, reducing CF and NFE contents were due to microorganisms which depend on carbohydrates consumption as energy sources for growth, the microbial protein formation, and multiplication (Abdel-Aziz et al., 2015; Suliman et al., 2015).

Table 2. Chemical composition of untreated and treated orange pulp by *Saccharomyces cerevisiae* yeast

Items	DM%	OM%	CP%	CF%	EE%	Ash%	NFE%	DEkcal/kg*
Untreated orange pulp (UOP)	87.84	88.50	10.59	15.53	3.87	11.50	58.52	2442.77
Treated orange pulp (TOP)	78.95	87.93	13.17	14.83	3.67	12.07	56.25	2465.3

DM: dry matter, OM: orange matter, CP: crude protein, CF: crude fiber. EE: ether extract, NFE: nitrogen free extract. *Digestible energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF%)] according to Cheeke, (1987)

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Growth performance

Influence of several levels of UOP or TOP on growing rabbits performance is shown in table 3. The FBW (g) of growing rabbits didn't significantly effect by the yeast treatment, replacement level of OP and their interaction. Rabbits fed 10% UOP were significantly ($p<0.05$) decreased in daily BWG when compared with other groups while, group fed 5% UOP did not affected significantly. The BWG was significantly ($p<0.05$) affected by yeast treatment while, OP different levels did not appear any significant effect. The rabbit groups fed 5% UOP, TOP and 10% TOP significantly ($p<0.05$) consumed more daily feed than control and 10%UOP groups. Moreover, effect of the yeast treatment and OP replacement levels consumed significantly ($p<0.05$) FI. FCR significantly ($p<0.05$) improved in 5% TOP group compared to other groups except control and 10% TOP groups that weren't significantly different. The effects of yeast treatment and OP replacement levels did not cause significant differences. The results were in partial agreement with findings of Suliman and Ameen, (2018) who observed no significantly difference in FBW, BWG, FI and FCR among experimental rabbits groups fed lemon pulp treated by *S.cerevisiae* yeast. The FI increased probably due to increase in palatability of dietary orange pulp. This result conformed by Rizal et al. (2010) were found that adding up to 20% of juice wastes mixture (carrot, apple, mango, orange, melon and tree tomato) in broiler diets, increased the amount of feed consumption. The improvement of FI by yeast treatment confirmed by Shehu et al. (2014) who found intake of growing rabbits increased ($p<0.05$) in rabbits fed diets supplemented 60g/kg *S. cerevisiae* yeast. On the contrary, FI, BWG and FCR of growing rabbits didn't affect by the dietary citrus pulp (Lu et al., 2018) and yeast supplementation (Belhassen et al., 2016). The DM intake of growing rabbits recorded did not significantly affected by yeast supplementation (Khanna et al., 2014).

Table 3. Growth performance of rabbits (6-14 weeks of age) fed on different levels of orange pulp untreated and treated by *Saccharomyces cerevisiae* yeast

Items	Control diet	Replacement Level of OP protein (%)				P-value		
		5%		10%				
		Untreated	Treated	Untreated	Treated	L × Y effect	Yeast effect	Level effect
IBW (g)	730.8±83.78	738.3±90.07	731.7±86.25	738.3±88.93	729.2±84.07	1.00	0.94	0.99
FBW (g)	2063.3±64.14	1978.3±117	2125.8±155.44	1865.0±93.28	2040.0±123.28	0.57	0.27	0.61
BWG (g/R/day)	23.38^{a}±1.16	21.75^{ab}±0.97	24.46^{a}±1.40	19.77^{b}±0.29	23.00^{a}±0.69	0.02	0.03	0.21
FI (g/R/day)	95.89^{b}±1.92	104.11^{a}±2.04	103.30^{a}±2.21	95.92^{b}±1.06	101.92^{a}±1.53	0.004	0.04	0.005
FCR (Feed:gain)*	4.14^{b}±0.14	4.84^{a}±0.26	4.28^{b}±0.21	4.86^{a}±0.07	4.45^{ab}±0.14	0.02	0.19	0.10

[a and b] means (mean± SE) within the same row with common letter are not significantly different ($P>0.05$). *Feed conversion ratio (FCR): g feed/g gain. OP: Orange Pulp, L x Y effect: statistical effect of interaction between level of orange pulp and yeast treatment effect on measurement parameters, Yeast effect: statistical effect of yeast only on measurement parameters, Level effect: statistical effect of replacement levels of OP only on measurement parameters.

Digestion coefficient of nutrients and nutritive value

Table 4 indicated the effect of several experimental diets on nutrients digestion coefficients and nutritive value of growing rabbits. The percentages of DM and OM did not have significant differences among all experimental groups except the group fed 5% UOP that was significantly ($P<0.05$) increased. While, the yeast treatment and OP replacement levels did not significantly effect on DM% and OM% values. The CP% was significantly ($p<0.05$) increased in rabbit fed 5% UOP and TOP diets when compared to other rabbits. The replacement levels of OP were significantly ($p<0.0001$) effect on CP% digestibility, while the yeast treatment did not cause a significant change. No significant differences were recorded in digestion coefficient of CF among different groups except group fed 5%UOP diet which was significantly ($p<0.05$) increased. The replacement levels of OP revealed significantly ($p< 0.02$) effect on CF digestibility but no significant effect due to yeast treatment was seen. The EE and NFE digestibility did not cause significantly differences among all rabbit groups. So, the yeast treatment and levels of replacement of OP didn't significantly effect on digestibility of EE and NFE. Santos et al. (2014) found that the inclusion of citrus pulp in the daily feed of lactating cow improved the apparent digestibility of DM, CP, EE and non-fibrous carbohydrates. However, rabbits fed diet supplemented with 60g *S. cerevisiae*/kg had higher ($p<0.05$) digestibility of nutrients than those fed diets supplemented with 20, 40 and 80g/kg (Shehu et al., 2014).

The highest significant value ($p<0.05$) of Digestible Crude Protein (DCP%) recoded in rabbits fed 5% UOP and TOP diets compared to control,10% UOP and 10% TOP groups. However, the group fed 10% TOP recorded the lowest significant ($p<0.05$) value of DCP when compared to all rabbits fed other diets. Yeast treatment didn't cause significantly difference in DCP% but the OP replacement levels had significant ($p<0.05$) effect on DCP%. The differences in values of TDN and DE were not significantly

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among all experimental rabbits. Also, the yeast treatment and replacement levels of OP didn't affect in TDN and DE values. Generally, feeding dietary OP treated by yeast significantly ($p<0.05$) improved some digestion coefficients of nutrients and DCP%. This improvement in digestion of nutrients reported by Ibrahim et al. (2011) who found that digestion coefficients of nutrients improved in rabbits fed 20% and 60% OP. In present study, the yeast treatment did not effect in all nutrients digestibility and nutritive value however the replacement levels of OP recoded significantly ($p<0.0001$) differences in digestion coefficient of CP% and DCP%.

Table 4. Digestion coefficients and nutritive value of growing rabbits (6-14 weeks of age) fed on different levels of orange pulp untreated and treated by by *Saccharomyces cerevisiae* yeast

Items		Control diet	Replacement level of OP protein (%)				P-value		
			5%		10%				
			Untreated	Treated	Untreated	Treated	L ×Y effect	Yeast effect	Level effect
Digestion coefficients (%)	DM	59.54^{b}±0.614	64.85^{a}±1.65	59.69^{b}±0.36	58.44^{b}±2.06	59.25^{b}±0.84	0.03	0.37	0.12
	OM	64.73^{b}±0.43	69.79^{a}±1.44	65.87^{b}±0.29	65.55^{b}±1.79	65.56^{b}±0.66	0.04	0.46	0.12
	CP	62.50^{b}±0.48	70.12^{a}±1.43	70.34^{a}±0.25	61.08^{b}±2.04	60.37^{b}±1.53	0.001	0.77	<0.0001
	CF	31.49^{b}±0.67	41.76^{a}±2.89	33.05^{b}±0.53	30.76^{b}±3.48	25.59^{b}±1.77	0.006	0.11	0.02
	EE	71.20±3.58	72.66±3.78	74.69±2.21	74.66±3.01	75.88±2.24	0.81	0.35	0.51
	NFE	71.89±4.29	75.86±3.19	71.78±3.30	72.93±5.25	74.94±5.15	0.94	0.95	0.90
Nutritive value	DCP %	10.93^{b}±0.08	12.13^{a}±0.24	12.34^{a}±0.04	10.19^{c}±0.34	8.82^{d}±0.19	<0.0001	0.51	<0.0001
	TDN %	58.63±2.41	63.67±2.18	60.20±2.01	58.93±3.86	57.79±3.04	0.61	0.58	0.38
	DE Kcal/kg	2597.50±106.79	2820.40±96.58	2667.00±89.39	2610.40±171.21	2560.10±134.87	0.61	0.58	0.38

$^{a \text{ and } b}$ means (mean± SE) within the same row with common letter are not significantly different ($p>0.05$). OP: orange pulp, DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fiber, EE: ether extract, NFE: nitrogen free extract, DCP: digestible crude protein, TDN: total digestible nutrients, DE: digestible energy=TDN x 44.3 (Schneider and Flatt, 1975): L x Y effect: statistical effect of interaction between level of orange pulp and yeast treatment effect on measurement parameters, Yeast effect: statistical effect of yeast only on measurement parameters, Level effect: statistical effect of replacement levels of OP only on measurement parameters.

Plasma parameters

Some plasma parameters of growing rabbits fed diets contained levels of UOP or TOP are presented in table 5. All values of blood biochemistry were within the physiological ranges. Total Lipids (TL) of rabbits plasma blood was significantly ($p<0.05$) increased among all rabbits fed four experimental diets when compared to those fed control diet. Two levels of OP caused significant ($p<0.05$) changes on TL but the yeast treatment did not change significantly the TL. No significant differences were recorded among different experimental groups in plasma cholesterol and triglyceride. Also, the yeast treatment and levels of OP did not obtained a significant ($p>0.05$) effect on plasma cholesterol and triglyceride values except yeast treatment that caused significant ($p<0.05$) difference in plasma triglyceride. Both AST and ALT had significantly ($p<0.05$) differences among all experimental groups except group fed 5%UOP diet did not cause significant different level of ALT when compared to control group. Yeast treatment and replacement levels of OP were significantly ($p<0.05$) affect the liver function. The plasma creatinine didn't effect by OP replacement level, yeast treatment or their interaction. However, plasma urea had significantly ($p<0.05$) changes. These results confirmed by Solomon et al. (2015) that observed significant differences in AST and ALT levels in rabbit blood when feed diets containing 25, 50 and 75% fresh citrus lemon juice. Suliman and Ameen (2018) who found that total lipids, cholesterol, triglycerides, AST, ALT and urea had significant ($p<0.05$) difference among all rabbit groups fed lemon pulp treated by yeast when compared to control group. However, Jingzhi et al. (2018) found that AST did not significantly change in rabbits at 21% citrus pulp. Generally, the experimental diets were significantly different ($p<0.05$) in some blood parameters among rabbit fed dietary containing 5% and 10% OP treated by *S. cerevisiae* yeast. Also, yeast treatment and replacement levels of OP caused significant effect on some blood parameters of growing rabbits.

Economic efficiency

The effect of test diets on profit and economic efficiency is presented in table 6. It is clear that experimental rabbit group fed 10% TOP diet had lowest total feed cost/rabbit followed by 10% UOP and control group. While, better economic efficiency observed with rabbits fed 10% UOP followed by 5% TOP when compared to the rabbit groups fed other experimental diets include control group.

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Table 5. Blood constituents of growing rabbits (6-14 weeks of age) fed on different levels of orange pulp untreated and treated by by *Saccharomyces cerevisiae* yeast

Items	Control diet	Replacement Level of OP protein (%)				P-value		
		5%		10%				
		Untreated	Treated	Untreated	Treated	L × Y effect	Yeast effect	Level effect
Total lipid (mg/dl)	1.36^{e}±0.005	2.465^{a}±0.03	2.340^{b}±0.017	2.225^{c}±0.02	2.075^{d}±0.037	<.0001	0.38	<.0001
Cholesterol (mg/dl)	130.87±16.83	175.33±35.97	138.67±1.45	121.00±13.05	134.00±1.154	0.35	0.74	0.29
Triglyceride mg/dl)	280.63±32.24	273.00±51.25	196.00±20.428	271.00±43.15	166.00±15.874	0.13	0.006	0.49
AST (U/l)	38.76^{b}±1.657	58.01^{a}±3.15	18.70^{c}±2.44	13.26^{cd}±1.64	10.12^{d}±1.085	<.0001	0.01	0.01
ALT(U/l)	32.91^{a}±1.74	32.75^{a}±1.46	15.73^{b}±1.89	20.59^{b}±5.95	17.11^{b}±1.70	0.004	0.004	0.007
Creatinine (mg/dl)	1.58±0.48	1.20±0.06	1.63±0.025	1.09±0.25	1.58±0.075	0.44	0.17	0.75
Urea (mg/dl)	53.66^{c}±3.425	79.77^{bc}±7.95	113.27^{a}±15.54	76.87^{bc}±1.09	86.13^{b}±0.669	0.005	0.008	0.01

[a, b and c] means (mean± SE) within the same row with common letter are not significantly different (p>0.05). OP: Orange Pulp, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, L x Y effect: statistical effect of interaction between level of orange pulp and yeast treatment effect on measurement parameters, Yeast effect: statistical effect of yeast only on measurement parameters, Level effect: statistical effect of replacement levels of Orange Pulp only on measurement parameters.

Table 6. Economic efficiency of experimental diets of growing rabbits on October, 2018 in Egypt

Items	Control	5% UOP	5% TOP	10% UOP	10% TOP
Total body weight gain (kg)	1.33	1.24	1.39	1.31	1.12
Price of 1 kg body weight (LE*)	50	50	50	50	50
Selling price/rabbit (L.E.) (A)	66.63	62	69.71	65.54	56.33
Total feed intake (kg)	5.46	5.93	5.89	5.81	5.47
Price of 1 kg feed (LE)	4.93	4.72	4.76	4.52	4.60
Total feed cost/rabbit (LE) (B)	26.93	28.02	28.01	26.23	25.16
Net revenue (LE)[1]	39.69	33.98	41.70	39.31	31.17
Economical efficiency[2]	147.39	121.28	148.88	149.83	123.90

UOP: untreated orange pulp, TOP: treated orange pulp, Net revenue[1]: A - B, Economical efficiency (%)[2]: (Net revenue / B) × 100. *LE: 17.32$.

CONCLUSION

Treated orange pulp by *S. cerevisiae* yeast increased the CP% and DE kcal/kg content. Replacing TOP protein by basal diet protein in growing rabbit diets at 5% improved performance and achieved the best economic efficiency. Digestion coefficient of CP% and DCP% increased with 5% OP groups. However, yeast treatment didn't effect on digestible nutrients.

DECLARATIONS

Author's contributions

Dr. Marwa A. Suliman designed the work, collaborated the chemical analyses and drafted the manuscript. Dr Reham R. Eltanani performed the statistical analysis, tabulation of the experimental data and helped in blood analyses. Dr. Lamiaa Fathy Abdel-Mawla performed the practical part of the experiment and collaborated the chemical analyses.

Competing interests

The authors have declared that no competing interest exists.

REFERENCES

Abdel-Aziz NA, Abdelfattah ZMS, El-Adawy MM, Camacho LM, Kholif A E, Elghandour Mona MY and Borhami BE (2015). Biological Treatments and Feeding Sugarcane Bagasse in Agriculture Animals–An Overview. Journal of Integrative Agriculture, 14: 534-543. DOI:10.1016/S2095-3119(14)60829-7

Alnaimy A, Gad AE, Mustafa MM, Atta MAA and Basuony HAM (2017). Using of citrus by-products in farm animals feeding. Open Access Journal of Science, 1: 58-67. DOI: 10.15406/oajs.2017.01.00014

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AOAC (2000). Association of official Analytical chemists. Official Methods of Analysis. 14th Edition. Washington, D.C.

Belhassen T, Bonai A, Gerencsér ZS, Matics ZS, Tuboly T, Bergaoui R and Kovacs M (2016). Effect of diet supplementation with live yeast saccharomyces cerevisiae on growth performance, caecal ecosystem and health of growing rabbits. World Rabbit Science, 24: 191-200. DOI: https://doi.org/10.4995/wrs.2016.3991

Cheeke P R (1987). Rabbit Feeding and Nutrition. Academic Press, Orlando, Florida USA, pp. 376.Chaudry MA, Badshan A and Bibi N (2004). Citrus waste utilization in poultry rations. European Poultry Science, 68: 206-210.

Dadvar P, Dayani O, Mehdipour M and Morovat M (2014). Determination of physical characteristics, chemical composition and digestion coefficients of treated lemon pulp with Saccharomyces cerevisiae in goat diet. Journal of Animal Physiology and Animal Nutrition, 99:107-113. DOI: 10.1111/jpn.12204

De Blas JC, Ferrer P, Rodrigue CA, Cerisuelo A, Garcia RP, Calvet S and Farias C (2018). Nutritive value of citrus co-products in rabbit feeding. World Rabbit Science, 26: 7-14. DOI:10.4995/wrs.2018.7699

Duncan DB (1955). Multiple Range and Multiple F-Test. Biometrics, 11:1-42.

Falcão-e-Cunha L, Castro-Solla L,Maertens L, Marounek M,Pinheiro V,Freire J and Mourão JL (2007). Alternatives to antibiotic growth promoters in rabbit feeding: A review. World Rabbit Science, 15: 127-140. DOI: 10.4995/wrs.2007.597

Fekete S(1985). Rabbit feeds and feeding with special regard to tropical condition. Journal of Applied Rabbit Research, 8:167-173.

Frings CS and Dunn RT (1970). A colorimetric method for determination of lipid based on sulpha-phosphovanillin reaction. American Journal of Clinical Pathology, 53:89-91. DOI: 10.1093/ajcp/53.1.89

Fortun-Lamothe Land Boullier S (2007). A review on the interactions between gut microflora and digestive mucosal immunity: Possible ways to improve the health of rabbits. Livestock Science, 107:1-18. DOI: 10.1016/j.livsci.2006.09.005

Henry RJ (1964). Clinical Chemistry, Principles and Technics. Harper row Publishers New York, pp. 350.

Hon FM, Oluremi OI and Anugwa FOI (2009). Effect of dried sweet orange (Citrus sinensis) fruit pulp meal on the growth performance of rabbits. Pakistan Journal Nutrition, 8: 1150-1155.

Ibrahim MR, El-Banna HM, Omara II and Suliman MA (2011). Evaluation of nutritive value of some citrus pulp as feedstuffs in rabbit diets. Pakistan Journal of Nutrition, 10:667-674. DOI:10.3923/pjn.2011.667.674

Jingzhi L, Long X, He Z, Shen Y, Yang Y, Pan Y, Zhang J and Li H (2018). Effect of dietary inclusion of dried citrus pulp on growth performance, carcass characteristics, blood metabolites and hepatic antioxidant status of rabbits. Journal of Applied Animal Research, 46: 529-533. DOI: 10.1080/09712119.2017.1355806

Khanna S, Gulati HK,Verma AK,Sihag SS,Sharma DP and Kapoor PK (2014). Effect of yeast supplementation and alternative housing systems on performance of rabbits. Haryana Veterinary, 53:23-27.

Lebas F (2004). Reflections on rabbit nutrition with special emphasis on feed ingredients utilization. In: Becerril, C.M. and Pro, A. (Editors) Proceedings of the 8th World Rabbit Congress, Puebla. Colegio de Postgraduados, Montecillo, Spain, pp. 686-736.

Lu J, Xianghua L, Zhifei H, Yingchun S, Yanhong Y, YuanqingP, Jiahua Zand Hongjun Li (2018). Effect of dietary inclusion of dried citrus pulp on growth performance, carcass characteristics, blood metabolites and hepatic antioxidant status of rabbits. Journal Applied Animal Research, 46: 529–533. DOI.org/10.1080/09712119.2017.1355806

Nikpiran H, Vahdatpour T, Babazadeh D and Vahdatpour S (2013). Effects of saccharomyces cerevisiae, thepax and their combination on blood enzymes and performance of japanese quails (coturnix japonica). Journal of Animal and Plant Science, 23: 369-375.

Oluremi OI A, OjighenVO and Ejembi EH (2006). The nutritive potentials of sweet orange (Citrus sinensis) rind in broiler production. International Journal of Poultry Science, 5: 613-617. DOI: 10.3923/ijps.2006.613.617

Oyawoye EO and Nelson FS (1999). Optimum levels of inclusion of rice offal in the diet of young cockerels. In Proceedings of the 26th Annual Conference of the Nigeria Society of Animal Production, Ilorin, Nigeria, 99: 263-264

Perenz JM, Lebas F, Gidenne T, Mertens L, Xiccato G, Parigi-Bini R, Dalle ZA, CossuME, Carazzolo A, Villamide MJ, et al. (1995). European reference method for in vivo determination of diet digestibility in rabbits. World Rabbit Science, 3: 41-43.

Redd VR, Rama SVand Panda AK (2006). Yeast: Multifunctional feed supplement for poultry. Poultry International Journal. 1:16-20.

Rizal Y, Mahata ME, Andriani M and Wu G (2010). Utilization of juice wastes as replacement in broiler diet. International Journal of Poultry Science, 9: 886-889. DOI: 10.3923/ijps.2010.886.889

Santos GT, Lima LS, Schogor ALB, Romero JV, De Marchi FE, Grande PA, Santos NW, Santos FS and Kazama R (2014). Citrus pulp as a dietary source of antioxidants for lactating Holstein cows fed highly polyunsaturated fatty acid diets. Asia Astralasian Journal of of Animal Sciences, 27:1104-1113. DOI: 10.5713/ajas.2013.13836

SAS, (2004). User's guide. Statistic. SAS Inst. Cary, N.C. Releigh.

Schneider BH and Flatt WP (1975). The Evaluation of Feed Through Digestibility Experiments. University of Georgia Press Athens, Georgia, USA, p.423.

Shehu BM, Ayo JO, Ayanwale BA, Jiya EZ and Tsado DN (2014). Growth performance and nutrient digestibility of weaned rabbits fed diets supplemented with varying levels of baker's yeast (Saccharomyces cerevisiae). International Journal of Agricultureand Rural Development, 17: 1619-1627.

Spring P (2013). The challenge of cost effective poultry and animal nutrition: optimizing existing and applying novel concepts. Lohman Information, 48: 38-46.

Solomon IP, Oyebadejo SA, Ebenso IE and Otoho EA (2015). Biochemical Effect of Citrus lemon Juice on the Liver of Growing Rabbits (Oryctolagus cuniculus). Scholars Journal of Agriculture and Veterinary Sciences, 2: 356-361.

Strompfova VM, Marcinakova M, Simonva B, Bogovic M and Laukova A (2006). Application of potential probiotic Lactobacillus fermentum AD1 strain in healthy dogs. Anaerobe, 12: 75-79. DOI:10.1016/j.anaerobe.2005.12.001

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Suliman MA and Ameen HF (2018). The impact of treated lemon pulp with yeast on growth performance, nutritive value, total anti-oxidant enzyme and immune response of growing rabbits. Egyptian Journal of Rabbit Science, 28: 23- 37.

Suliman MA, Salama WA, Matari RIM and El-Shora MA (2015). Productive performance of New Zealand white growing rabbits fed on diets containing different levels of treated castor meal. Egyptian Journal utrition and Feeds, 18 (Special Issue): 361-369.

Villas-Boas SG, Esposito E and MitchellDA (2002). Microbial conversion of lignocellulosic residues for production of animal feeds. Animal Feed Science and Technology, 98:1-2.

Wang C, Gao GL, Huang JX, Zhang KS, Zhong H, Wang HW, Su J, Xie M and Wang QG (2017). Nutritive value of dry citrus pulp and its effect on performance in geese from 35 to 70 days of age. Journal Applied of Poultry Research, 26: 253-259.

Young DS (2000). Effects of drugs on clinical laboratory tests, 5th Edition. Washington D.C. AACC press, pp. 3122-3131.

To cite this paper: Abd Elmonem Suliman M, Rushdy Eltanani R and Fathy Abdel-Mawla L (2019). Influence of Treated Orange Pulp on Growth Performance, Nutrients Digestibility and Plasma Constituents of Rabbits. *World Vet. J.* 9(2): 102-108. www.wvj.science-line.com

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Effect of Zeolite Dietary Supplementation on Physiological Responses and Production of Laying Hens Drinking Saline Well Water in South Sinai

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ABSTRACT
This study conducted to investigate the effects of dietary zeolite on egg production, egg quality and blood constituents of hens under drinking saline well water. 180 hens were randomly divided into six equal groups (30 hens / group). 1st group (T), hens drank tap water and fed basal diet. The 2nd group (T1), hens drank tap water and fed diet containing 2 % zeolite. The 3rd group (T2), hens drank tap water and fed diet containing 4 % zeolite. 4th group (S), hens drank saline well water and fed basal diet. 5th group (S1), hens drank saline well water and fed diet containing 2 % zeolite. 6th group (S2), hens drank saline well water and fed diet containing 4 % zeolite. Red blood cells and hemoglobin were significant lower in the hens of S compared to other treatments. Hens of S group showed significant decrease in total protein, globulin, glucose and total antioxidant capacity concentrations as compared to the hens of T and T2 groups. Alanine transaminase, aspartic transaminase and creatinine were significantly increased in the hens of S group compared to other treatments. Aldosterone hormone was significantly decreased in the hens of S compared to them in T, T1 and T2 groups. Egg weight significantly increased in the hens of S2 compared with hens in T and S groups. Egg number and egg mass were significant increase in the T1, T2 and S2 compared to hens in T, S and S1 groups. Hens of T1, T2 and S2 groups had significantly improved feed conversion compared to hens of S group. Hens of S group had significantly decreased shell thickness compared to other treatments. In conclusion, under drinking saline well water, addition of zeolite to laying hens' diets at levels 4 % might improve productive performance and eggshell quality.

Key words: Hematological parameters, Laying hens, Productive performance, Saline water, Zeolite.

INTRODUCTION

Water is important nutrient for livestock and represents between 55 % and 75 % of the weight of a chicken, 65 % of the egg, about 70 % is inside the cells and 30 % is in fluid surrounding the cells and in blood (Ahmed and Abdel-Rahman, 2004). Quality of ground water depends upon the naturally occurring inclusions such as cations, anions and heavy metals. Scarcity of tap water in such desert areas generated a competition between human and animals. Hence, it is essential to use saline well water as possible supplies for animal's drinking water. In Egypt, saline well water is the main factor determining the suitability of particular water source for poultry and one of the major factors affecting water quality is the amount of Total Dissolved Salts (TDS) in the water. Drinking high amount of TDS may cause harmful effects resulting in poor performance, illness or even death (Morsy et al., 2012 and Amal, 2013). Poultry in desert and newly reclaimed areas probably dependent on drinking saline well water with varying degrees of salinity and such water often contains high concentrations of dissolved minerals salts (Morsy et al., 2016, 2017 and 2018). These high concentrations of minerals can show toxic and ascites and hence cause reduction in laying performance (Julian, 1987). Morsy et al. (2012) reported that high level of minerals in saline water may contribute to the production of defective shells in eggs particularly reduced shell thickness and shell calcium, increased the number of damaged eggs and increased plasma calcium and phosphorus of laying hens. So, cause an economic problem for poultry industry. Blood constituents are changed by drinking saline water (Morsy, 2018; Amal, 2013; Morsy et al., 2012).

In recent years, there are evidences in the literature that using of zeolite (Clinoptilolite) has encouraging effects on the poultry performance (Basha et al., 2016; Wawrzyniak et al., 2017; Morsy, 2018). Zeolite-natural and modified, because of their specific structure, are excellent adsorbed and thus can diminish the harmful effect of heavy metals. Clinoptilolites, due to its structural stability under high temperatures and acidity, are the most widely used zeolite in animal studies. The important research data indicated the positive influence of the in-feed inclusion of clinoptilolite on animal health. Zeolite is an excellent "trapper" of waste products and heavy metals because of its chemical composition

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and specific lattice structure (Pavelic et al., 2001). These minerals are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations (Na, K and/or Ca cations). Zeolite is porous material, able to adsorb molecules of appropriate cross-sectional diameter and to exchange their constituent cations without major change of their structure. Thus, zeolite appears to posse two important properties: adsorption and ion-exchange. The exploitation of these properties underlies the use of zeolite in a wide range of industrial and agricultural applications and particularly in animal nutrition (Beltcheva et al., 2015).

Dietary zeolite improves feed efficiency and egg production in laying hens (Samara, 2003). Moreover, Pavelic and Hadzija (2003) suggested that natural zeolite may have a beneficial effect against aflatoxicosis and other health disorders. Zeolite clinoptilolite is able to adsorb damaging toxins that can potentially reduce the performance of animals (Oğuz and Kurtoğlu, 2000), affect gut morphology, decrease pH, and lower pathogenic bacteria counts, which suggests that intestinal health improvement (Wu et al., 2013). Also, zeolite, being the adsorbents, eliminates a number of toxic substances (heavy metal salts, nitrates, nitrites, mycotoxins, radionuclides, metabolism products) from the organism.

At present, use of natural zeolite develops by utilizing features of ion-exchange, water and gas absorption (Binats et al., 2014). Beneficial effects of zeolite may also be attributed to the silicon, aluminum or sodium content which can influence calcium-metabolism, thus improving calcium and phosphorus utilization (Leach et al., 1990; Watkins and Southern, 1991). It has been demonstrated in a number of studies that the inclusion of zeolites in diets improves weight gains and feed conversion in broilers (Fethiere et al., 1994) and their physical properties (Tserveni-Gousi et al., 1997). Addition of natural zeolite to broiler diet led to promote of chicken performance (Nikolakakis et al., 2013) and improve body weight gain and feed conversion ratio (Debeic, 1994). Boyer (2000) reported that clinoptilolite supplementation led to a significant increase in feed efficiency and reducing the toxic effects of surplus ammonium ions. In contrast, some researchers found that using of zeolite had no effect on poultry production (Evans and Farrell, 1993).

Therefore, this study was to investigate the effects of dietary zeolite (Clinoptiolite) on egg production, egg quality and blood constituents of Golden Montazah hens under drinking saline well water (3398 ppm total dissolved solids) in South Sinai.

MATERIALS AND METHODS

Experimental region

The present study was conducted in the South Sinai Research Station, located at Ras Suder that belongs to the Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt. The experiment started on June 2015 up to August 2015.

Ethical approval

This experiment was performed according to all ethics and animal rights (Desert Research Center). As much as this work had considering all rules and regulations in conformity with the European union directive for the protection of experimental animals (2010/63/EU).

Experimental animals, feeding and management

A total number of 180 hens (22- weeks old and body weight of 1629.72 g ± 23.54) were randomly divided into six equal groups (30 hens / group). The 1st group (T), hens drank tap water (containing 265 ppm TDS) and fed basal diet. The 2nd group (T1), hens drank tap water and fed diet containing 2 % zeolite. The 3rd group (T2), hens drank tap water and fed diet containing 4 % zeolite. The 4th group (S), hens drank saline well water (containing 3398 ppm TDS) and fed basal diet. The 5th group (S1), hens drank saline well water and fed diet containing 2 % zeolite. The 6th group (S2), hens drank saline well water and fed diet containing 4 % zeolite. According to Emam et al. (2019), chemical analysis of tap and saline well water was presented in Table 1. Chemical composition of the zeolite (Clinoptilolite) was shown in Table 2.

Experimental procedures

Birds until 34 weeks of age (end of experiment) were housed in wire cages, supplied with clean fresh water and fed ad-libitum on recommended standard rations according to NRC (1994) as shown in Table 3. At the age of 18 weeks the natural day length was artificially increased from 14 h to 16 h/day in peak of egg production (30 weeks) and then it maintained constant until the end of experiment. Birds were kept under the same managerial and hygienic conditions. Birds were healthy and examined against diseases and treated with antibiotics and vaccine

Ambient temperature and relative humidity

Indoor climatic conditions (ambient temperature, relative humidity and temperature-humidity index recorded during the experimental period (Figure 1). Temperature Humidity Index (THI) calculated according to Marai et al. (2001).

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Table 1. Chemical analysis of tap water and saline well water under conditions of South Sinai, Egypt

Parameters	TW	SW
Total dissolved solids (mg/l)	265.0	3398.0
Electric conductivity (µS/m)	512.0	5540.0
pH	6.9	7.6
Sodium (mg/l)	30.0	640.0
Potassium (mg/l)	4.0	8.0
Calcium (mg/l)	46.0	302.7
Magnesium (mg/l)	10.7	160.3
Carbonate (mg/l)	0.0	15.0
Bicarbonate (mg/l)	125.0	115.9
Sulphate (mg/l)	52.4	800.0
Chloride (mg/l)	59.1	1414.0

TW= tap water (265 ppm total dissolved solids); SW= saline well water (3398 ppm total dissolved solids)

Table 2. Chemical composition of the zeolite (Clinoptilolite) used in the experiment

Cations	%	Major elements (oxides)	%	Other elements	Trace
K_2O	3.266	SiO_2	62.22	Cl	0.025
CaO	3.583	Al_2O_3	11.096	BaO	0.085
Na_2O	0.780	Na_2O	0.780	P_2O_5	0.033
		MgO	0.599	ZnO	0.025
		CaO	3.583	SrO	0.047
		Fe_2O_3	4.033	PbO	0.002
		K_2O	3.266		
		TiO_2	0.339		
		ZrO_2	0.112		

Table 3. Composition and calculated analysis of the experimental diets

Ingredients	Basal diet	Zeolite (2 %)	Zeolite (4 %)
Zeolite	0.0	2.00	4.00
Yellow corn	57.00	55.00	53.00
Soybean meal (44 % CP)	29.00	29.00	29.00
Wheat bran	2.84	2.84	2.84
Limestone ground	7.60	7.60	7.60
Dicalcium phosphate	1.50	1.50	1.50
Vitamins and minerals premix*	0.40	0.40	0.40
Oil	1.30	2.00	2.80
Salt	0.30	0.30	0.30
Dl- methionine	0.06	0.06	0.06
Total	100	100	100
Calculated values			
Crude protein	18.05	17.88	17.71
Crude fiber	3.32	3.32	3.32
Ether extract	2.81	2.81	2.81
Ash	2.37	2.37	2.37
Metabolizable energy (kcal/kg)	2763.6	2763.2	2763.7
Calcium (%)	3.31	3.31	3.31
Available phosphorus (%)	0.40	0.40	0.40

* Each 2.5 kg Vitamins and minerals premix comprises (per ton of feed), Vit. A 10000000 IU, Vit.D_3 2000000 IU, Vit. E I0g, Vit.K_3 1000 mg, Vit. B_1 1000 mg, Vit.B_2 5000mg, Vit.B_6 1.5g, Vit. B_{12} 10 mg, Pantothenic acid 10g, Niacin 30g, Folic acid 1g, Biotin 50 mg, Iron 30g, Manganese 70g, Choline chlorite 10g, Iodine 300 mg, Copper 4g, Zinc 50g and Selenium 100 mg.

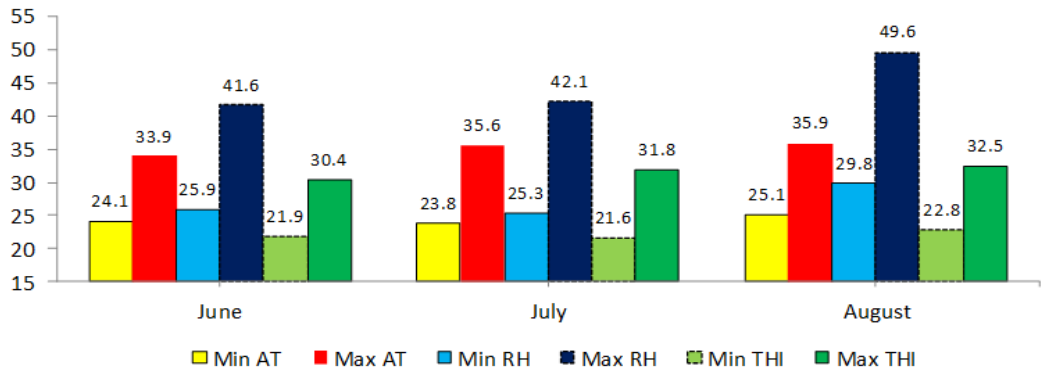

Figure 1. Indoor ambient temperature, relative humidity and temperature-humidity index throughout experimental period. AT (˚C) = ambient temperature, RH (%) = relative humidity, THI = temperature-humidity index, THI= db°C – [(0.31 – 0.31 × RH) × (db°C – 14.4)]. Where, db°C = dry bulb temperature in centigrade, The THI values were classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

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Blood samples and hormonal assay

Blood samples were monthly collected from wing vein from all hens into two tubes (anticoagulant EDTA treated and non-EDTA tubes). Samples treated with anticoagulant EDTA used for determination of red blood cells (RBC's), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), mean corpuscular hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) by the coulter (HA-VET, Clinding – Belgium). Plasma was collected by centrifugation for 15 minutes at 3000 rpm and it stored at –20 °C until determination of hormones (aldosterone, tri-iodothyronine, progesterone and corticosterone) by ELISA method using commercial kits and blood metabolites (total protein, albumin, glucose, cholesterol, alanine and aspartic transaminase, creatinine and total antioxidant capacity) using commercial kits. Globulin was calculated by the difference between total protein and albumin. Other samples (non-EDTA tubes) used to collected serum by centrifugation for 15 minutes at 3000 rpm and it stored at –20°C until determination blood minerals (calcium, phosphorus, sodium and potassium) by calorimetrically using commercial kits.

Productive performance

All experimental hens were used to evaluate the productive performance. Body weight was recorded at initial body weight (22 weeks) and at final body weight (34 weeks). Egg number and egg weight were daily recorded for 90 days (egg production period). Egg mass was calculated by multiplying average egg weight by egg number. Feed and water intake were recorded. Feed conversion was calculated as follows: feed conversion=total feed intake/ total egg mass. Mortality rate was daily recorded for each group from one day to end of the experiment.

Thirty eggs were randomly collected from each treatment (180 eggs) to measure egg quality traits according to Stino et al. (1982) and El –Wardany et al. (1994). These traits included that: Egg shape index = egg width/egg length×100 using Vernier Caliper for measurements. Egg shell weight was recorded by digital balance to nearest 0.1 gram. Shell (%) = shell weight/egg weight × 100. Egg shell thickness measured with membrane in mm (average of the broad, narrow ends and equator areas of egg). Yolk weight was recorded by digital balance to nearest 0.1 gram. Yolk (%) = yolk weight / egg weight × 100. Yolk index calculated as yolk height / yolk diameter × 100. Albumen weight was calculated by subtracting yolk and shell weight from total egg weight. Albumen (%) = albumen weight / egg weight × 100. Yolk/Albumen ratio = yolk weight / albumen weight × 100.

Statistical analysis

Data were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004) according to following model:

$Y_{ij}=\mu + Tr_i + e_{ij}$

Where, Y_{ij}= observations; μ = overall mean; Tr_i = effect of i^{th} group (i: 1-6); e_{ij}= experimental error.

Duncan's New Multiple Range Test (Duncan, 1955) separated differences among treatment means.

RESULTS AND DISCUSSION

Hematological parameters

Red blood cells (RBC's) count and hemoglobin (Hb) concentration were lower ($P < 0.05$) in the hens of S (hens drank saline well water) as compared to the hens in the other treatments (Table 4). On the other hand, MCV increased ($P<0.05$) in the hens of S group when compared to the hens of T, T1, T2 treatment groups. Meanwhile, MCHC decreased ($P<0.05$) in the hens of S group as compared to the hens of T, T1, T2 and S1 groups. Nevertheless, no significant differences between the treatment's groups in PCV and MCH was seen. Hematological parameters have been considered important as indicator for the healthy birds, so hens drank saline well water might suffer from drastic physiological changes to preserve the consistence and body stability (Morsy et al., 2012).

These results agree with the results of Morsy et al. (2012); Emam et al. (2017) and Morsy (2018). They reported that the negatively disturbances in hematological parameters as a result of drank saline well water might be due to increased water intake that may be caused hemodilution and increased total body water. In addition, salt intake caused varying degrees of anhydremia resulting in an elevation of specific gravity and hematocrit value in the blood (Amal, 2013). On the other hand, the enhancement in hematological parameters of hens fed 2 and 4 % zeolite (T1, T2, S1 and S2) may suggest that the principal role of zeolite supplemental in reducing the deleterious effects of saline stress by decreased a number of toxic substances such as heavy metal salts, nitrates, nitrites, mycotoxins, radionuclides and metabolism products (Morsy, 2018). Also, zeolites, being ion-exchangers, participate in certain biochemical transformations, normalize the homeostasis of animals and increase the nutrient conversion, so it may be reversible in increase of RBC's and Hb values (Andronikashvili et al., 2009).

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Table 4. Effect of zeolite supplementation on hematological parameters of laying hens drinking saline well water

Traits	T	T1	T2	S	S1	S2	±SE
RBC's (× 10^6/mm^3)	2.97^a	3.15^a	3.11^a	2.60^b	2.90^{ab}	2.96^a	0.14
Hb (g/dl)	11.83^a	12.17^a	12.63^a	10.73^b	11.96^a	11.63^{ab}	0.61
PCV (%)	33.29	34.43	35.35	33.08	34.17	34.80	1.89
MCV (fl)	111.40^b	110.21^b	111.74^b	130.03^a	118.29^{ab}	118.08^{ab}	6.00
MCH (pg)	39.67	38.99	39.96	41.99	41.25	39.46	1.54
MCHC (%)	35.61^a	35.35^a	35.76^a	32.77^b	35.14^a	33.65^{ab}	0.79

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1 = hens drank tap water and fed diet containing 2 % zeolite, T2 = hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1 = hens drank saline well water and fed diet containing 2 % zeolite, S2 = hens drank saline well water and fed diet containing 4 % zeolite, ±SE = standard error, RBC's = red blood cells, Hb = hemoglobin, PCV = packed cell volume, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P<0.05).

Metabolites

Hens drank saline well water (S group) showed decrease (P<0.05) in total protein, globulin and glucose concentrations by 25.07, 57.87 and 11.95 %, respectively as compared to the hens drank tap water (T group) and by 26.65, 57.08 and 14.26 %, respectively as compared to the hens of T2 which drank tap water + 4 % zeolite (Table 5).

These results revealed that the decrease in some metabolites might be due to the effect of salt stress. Drinking saline well water might reduce hepatic synthesis of RNA, which in turn depressed the incorporation of amino acids for protein synthesis (Tata and Widnell, 1966). Also, the depression in total protein could be considered as a physiological accommodation to prevent excessive passage of fluids into the interstitial tissue due to the level of salinity (Abdel Samee and El-Masry, 1992; Amal, 2013; Emam et al., 2017). Meanwhile, it's worthy to reveal that biochemical parameters values of zeolite groups had analogous with control group. Valpotic et al. (2016) reported that zeolite improved the absorption of immunoglobulins, total proteins and some microelements, especially iron and copper in cow and cocks (Ipek et al., 2012 and Morsy, 2018). Moreover, Macháček et al. (2010) demonstrated that differences in glucose and total protein as a result of zeolite (clinoptilolite) administration in feed of laying hen (at level of 2% and 4%) were not significantly effective as compared to control ones and remained at the range of reference values (Lotfollahian et al., 2004; Eleroglu et al., 2011 and Safaeikatouli et al., 2011). Also, Nik-Khan (2002) reported that useing 1 g zeolite/ kg body weight/ daily had the positive effects on increasing serum immunoglobulins.

The decrease in glucose level could be interpreted as a consequence of the increased transport of glucose and salt through membranes into tissue cells (Karadjole et al., 1999). However, drank saline well water led to expose the body to a metabolic stress and elevation in energy requirements to maintain the sodium/potassium gradient and hence decreased concentration of blood glucose (Guyton and Hall, 2006; Amal, 2013; Morsy, 2018). However, cholesterol concentration significantly decreased in the hens of S group as compared to the hens of other treatments. These results agree with the results of Miles and Henry (2007); Safaeikatouli et al. (2011); Amal (2013) and Morsy (2018) that confirmed protein and fat metabolism were negatively affected as a result of drinking saline water and additional of zeolite in diets but did not have adverse effects on cholesterol concentration. On the other hand, no significant effect was observed among treatments on albumin concentration. Hens drank saline well water obtained decrease (P<0.05) in total antioxidant capacity (TAC) concentrations (44.87 %) as compared to the hens that drank tap water (37.68 %) and the hens of T2. However, hens of S1 and S2 groups showed insignificant increment in TAC as compared to the hens of S group (Figure 2).

Table 5. Effect of zeolite supplementation on biochemical parameters of laying hens drinking saline well water

Traits	T	T1	T2	S	S1	S2	±SE
Total protein (g/dl)	6.50^a	5.67^{ab}	6.64^a	4.87^b	5.70^{ab}	6.08^{ab}	0.39
Albumin (g/dl)	3.76	3.19	3.96	3.72	3.44	3.94	0.24
Globulin (g/dl)	2.73^a	2.48^{ab}	2.68^a	1.15^b	2.25^{ab}	2.14^{ab}	0.39
Glucose (g/dl)	267.26^a	260.66^a	274.47^a	235.32^b	254.14^{ab}	255.81^{ab}	10.30
Cholesterol (g/dl)	75.07^a	81.04^a	83.20^a	58.01^b	72.79^a	78.92^a	5.93

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1 = hens drank tap water and fed diet containing 2 % zeolite, T2 = hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1 = hens drank saline well water and fed diet containing 2 % zeolite, S2 = hens drank saline well water and fed diet containing 4 % zeolite, ±SE = standard error, $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

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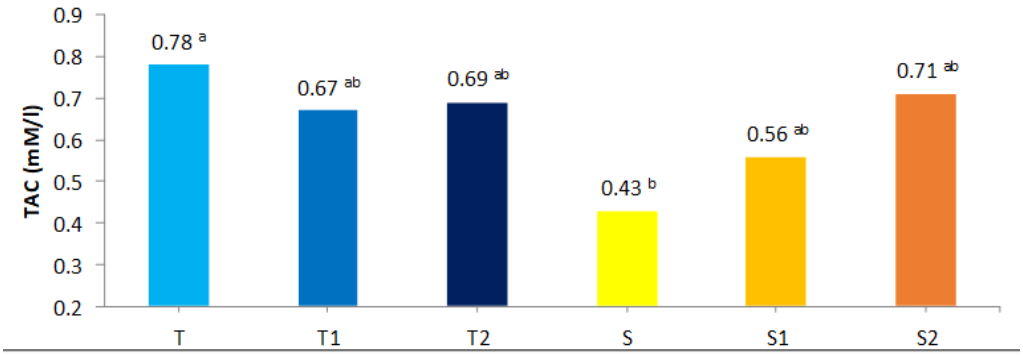

Figure 2. Effect of zeolite supplementation on total antioxidant capacity of laying hens drinking saline well water

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1= hens drank tap water and fed diet containing 2 % zeolite, T2= hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1= hens drank saline well water and fed diet containing 2 % zeolite, S2= hens drank saline well water and fed diet containing 4 % zeolite, TAC= total antioxidant capacity, [a, b] Means bearing different superscripts are significantly different (P< 0.05).

Liver and kidney functions

Alanine transaminase (ALT) and aspartic transaminase (AST) were increased (P<0.05) in the hens of S (hens drank saline well water) as compared to the hens in other treatments. Meanwhile, creatinine level significantly increased in the hens of S as compared to the hens of T, T2, T2 and S1. However, ALT was increased (P<0.05) in the hens of S1 and S2 as compared to the hens of T, T1 and T2. However, no significant differences between the hens drank saline well water plus zeolite (S1 and S2) and the hens drank tap water only (T) or drank tap water plus zeolite (T1 and T2) in AST and creatinine concentrations (Table 6). These results indicating that increased salt load might cause harmful effects on the liver and kidney functions and in turn the animals health (Amal, 2013 and Morsy et al., 2012 and 2016 and Morsy, 2018). On the other hand, zeolite (Clinoptiolite) might be reduced lipid peroxidation and normalized the liver functions in the animals drinking saline water and/or may be safe supplements even though more histological studies are needed to prove it (Tukmechi et al., 2011; Sheikhzadeh et al., 2017; Morsy, 2018).

Table 6. Effect of zeolite supplementation on liver and kidney functions parameters of laying hens drinking saline well water

Traits	T	T1	T2	S	S1	S2	±SE
ALT (i.u./l)	38.23^{c}	40.31^{c}	39.60^{c}	67.96^{a}	53.62^{b}	55.23^{b}	1.49
AST (i.u/l)	82.75^{b}	84.85^{b}	88.18^{b}	97.80^{a}	88.79^{b}	83.56^{b}	2.60
Creatinine (mg/dl)	0.41^{b}	0.39^{b}	0.35^{b}	0.60^{a}	0.39^{b}	0.44^{ab}	0.09

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1 = hens drank tap water and fed diet containing 2 % zeolite, T2 = hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1 = hens drank saline well water and fed diet containing 2 % zeolite, S2 = hens drank saline well water and fed diet containing 4 % zeolite, ±SE = standard error, ALT = alanine transaminase, AST= aspartic transaminase, [a, b] Means bearing different superscripts within the same row are significantly different (P< 0.05).

Mineral parameters

Hens of drank saline well water had higher (P<0.05) calcium, sodium, sodium/potassium ratio and sodium/ (sodium + potassium) index concentrations by 35.79, 15.29, 69.20 and 1.02 %, respectively as compared to the hens drank tap water. Meanwhile, no significant differences between the hens S1 and S2 groups and the hens of T, T1 and T2 groups in calcium, sodium, sodium/potassium ratio and sodium/ (sodium + potassium) index was seen (Table 7).

In contrary trend, potassium level decreased (P<0.05) in the hens of S group as compared with the hens of other treatments. However, no significant differences between the hens of S1 and S2 groups and the hens of T, T1 and T2 groups in potassium level was seen. Minerals in body fluids and tissues as electrolytes, alarmed with the maintenance of osmotic pressure, acid–base balance, membrane permeability and tissue irritability (Milne, 1996; Underwood and Suttle, 1999). So, the higher levels of electrolytes might be explained as hens drank saline well water (Table 1) containing higher concentration of these minerals as compared to the hens drank tap water and it appears quite logical that the high level of electrolytes in drinking water entailed the increased levels in the serum (Karadjole et al., 1999; Morsy et al., 2012; Amal, 2013; Morsy, 2018). In addition, increased calcium level in the hens of T3 group might resulted in increasing rate of its reabsorption and so, its blood level with insignificantly decreased phosphorus level due to the reciprocal reverse relationship as increased blood calcium level resulted in increasing parathyroid hormone secretion which inhibits the reabsorption of phosphorus in renal tubules (Morsy et al., 2012 and Amal, 2013). Indeed, the negative relationship between serum sodium and potassium levels might protect the body against hyperkalemia and muscle irritability (Ahmed and Abdel-Rahman, 2004; Abbas et al., 2008; Morsy et al., 2012 and 2016 and Amal, 2013). Likewise, the increase in the sodium/potassium ratio and sodium/(sodium+potassium) index in the hens of T3 group might be due to increase the rate of glomerular filtration in the kidney for such electrolytes due to the drinking saline

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well water and thus a direct increase in the blood (Amal, 2003; Morsy et al., 2012; Amal, 2013; Morsy, 2018). On the other hand, the results of blood mineral parameters indicated no significant differences among the hens drank saline well water and received zeolite (S1 and S2 groups) obtaing that zeolite might be being ion-exchangers, participates in certain biochemical transformations, including the transport, activation and prolongation of enzyme and hormone action, maintain ion balance in terms of electrolyte system and normalize the homeostasis of hens (Andronikashvili et al., 2009; Colella, 2011). Roland et al. (1985) hypothesized that the beneficial effect of zeolite on bone quality may be related to its high affinity for calcium and phosphorus and its ion-exchange capability. These results agree with the results of Frost et al. (1992); Ward et al. (1993) and Eleroglu et al. (2011) that reported zeolite have a positively effect on the balance of serum calcium and phosphorus levels and regulate electrolyte balance in birds.

Table 7. Effect of zeolite supplementation on blood minerals of laying hens drinking saline well water

Traits	T	T1	T2	S	S1	S2	±SE
Ca (mg/dl)	9.61^{b}	9.26^{b}	10.46^{ab}	13.05^{a}	10.09^{ab}	9.63^{b}	0.98
P (mg/dl)	3.27	3.39	3.22	3.10	3.28	3.31	0.13
Na (mmol/l)	151.13^{b}	144.54^{b}	142.10^{b}	174.24^{a}	139.66^{b}	135.90^{b}	5.49
K (mmol/l)	4.05^{a}	4.57^{a}	4.47^{a}	2.76^{b}	4.18^{a}	4.22^{a}	0.44
Na/K ratio	37.31^{b}	31.62^{b}	31.79^{b}	63.13^{a}	33.41^{b}	32.20^{b}	7.01
Na/(Na+K) index	0.974^{b}	0.969^{b}	0.969^{b}	0.984^{a}	0.971^{b}	0.970^{b}	0.002

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1 = hens drank tap water and fed diet containing 2 % zeolite, T2 = hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1 = hens drank saline well water and fed diet containing 2 % zeolite, S2 = hens drank saline well water and fed diet containing 4 % zeolite, ±SE = standard error, Ca = calcium, P = phosphorus, Na = sodium, K = potassium, $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

Hormonal parameters

Figure 3 showed that aldosterone hormone significantly decreased (P<0.05) in the hens of S group by 23.16, 24.57 and 21.26 % as compared to the T, T1 and T2 groups, respectively. Meanwhile, no significant differences were observed among the hens of S1, S2, T2, T1 and T groups. This decreased in aldosterone hormone in the hens of S group could be owing to the physiological effects of salt stress retention and excretion by reducing the plasma aldosterone concentration in nearly 50 % of control values (Amal, 2003 and Morsy, 2018). On the other hand, no significant differences among treatments were observed in tri-iodothyronine and progesterone hormones (Figures 4 and 5). However, progesterone hormone was insignificantly lower in the hens of S group when compared to the other treatments.

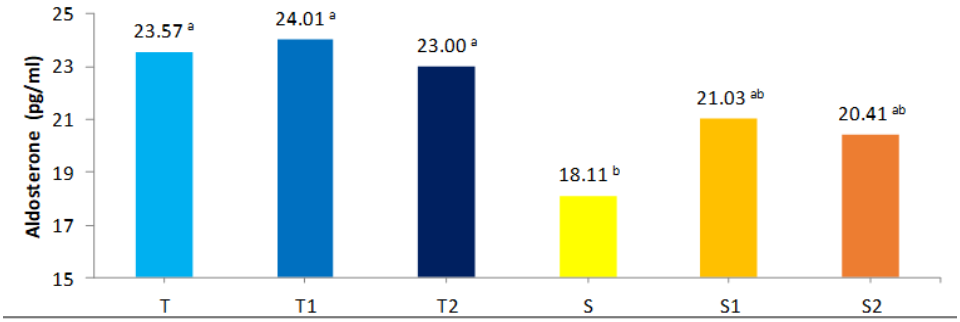

Figure 3. Effect of zeolite supplementation on aldosterone hormone of laying hens drinking saline well water. T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1= hens drank tap water and fed diet containing 2 % zeolite, T2= hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1= hens drank saline well water and fed diet containing 2 % zeolite, S2= hens drank saline well water and fed diet containing 4 % zeolite, $^{a, b}$ Means bearing different superscripts are significantly different (P< 0.05).

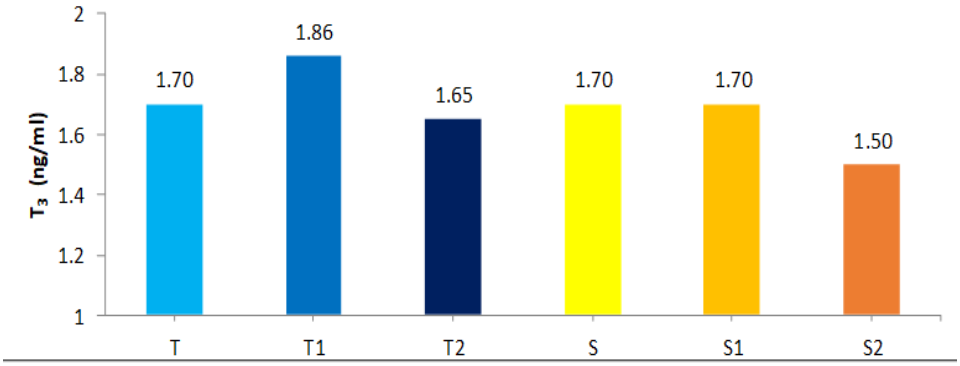

Figure 4. Effect of zeolite supplementation on tri-iodothyronine hormone of laying hens drinking saline well water

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1= hens drank tap water and fed diet containing 2 % zeolite, T2= hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1= hens drank saline well water and fed diet containing 2 % zeolite, S2= hens drank saline well water and fed diet containing 4 % zeolite, T_3= tri-iodothyronine hormone

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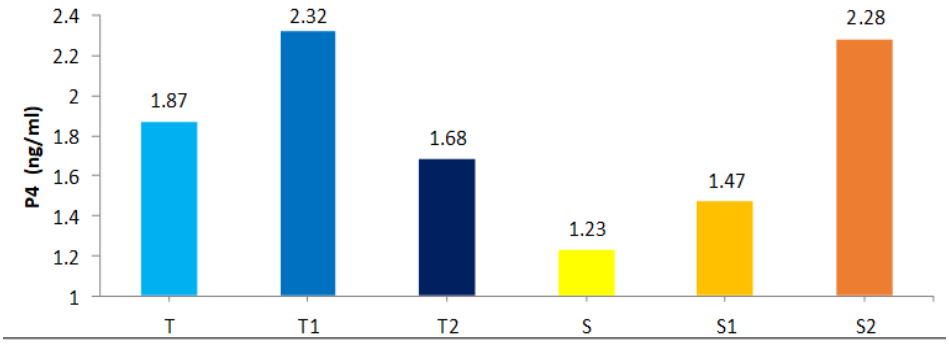

Figure 5. Effect of zeolite supplementation on progesterone hormone of laying hens drinking saline well water

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1= hens drank tap water and fed diet containing 2 % zeolite, T2= hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1= hens drank saline well water and fed diet containing 2 % zeolite, S2= hens drank saline well water and fed diet containing 4 % zeolite, P_4= progesterone hormone.

Body weight

The Final Body Weight (FBW) and Body Weight Changes (BWC) were insignificantly increased in the hens of S group as compared to the hens in T, T1 and T2 groups, respectively (Table 8). However, hens of S2 group showed insignificantly increased in FBW and BWC when compared to the hens in other treatments. This increase in final body weight and body weight changes might correlated to increase water intake in the hens of S, S1 and S2 groups. Olver (1989) reported no significant dietary effects between treatments which were observed with respect to body weight.

Table 8. Effect of zeolite supplementation on body weight of laying hens drinking saline well water

Traits	T	T1	T2	S	S1	S2	±SE
IBW (g)	1629.53	1629.26	1629.93	1629.76	1630.76	1629.14	61.40
FBW (g)	1919.23	1939.60	1956.92	1970.91	1995.33	2014.35	77.55
BWC (g)	289.69	310.33	301.28	341.41	337.83	385.21	55.07

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1 = hens drank tap water and fed diet containing 2 % zeolite, T2 = hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1 = hens drank saline well water and fed diet containing 2 % zeolite, S2 = hens drank saline well water and fed diet containing 4 % zeolite, ±SE = standard error, IBW = initial body weight, FBW = final body weight, BWC = body weight change.

Productive performance

Table 9 showed that there was significant ($P< 0.05$) increases in the value of egg weight in the hens of S2 group as compared to the hens in T and S groups. On the other hand, egg number and egg mass increased ($P<0.05$) in the hens of T1, T2 and S2 groups as compared to the hens of T, S and S1 groups. However, no significant differences were observed among treatments in feed intake. These results agree with the results of Miazzo et al. (2005) and Salari et al. (2006). These results declared that the hens of T1, T2 and S2 groups had significantly improvement values in feed conversion by 25.05, 24.84 and 25.25 %, respectively as compared to the hens of S group hens drank saline well water. On the other hand, no significant differences among the hens of S1, S2, T2, T1 and S groups in feed conversion was seen.

Moreover, water intake was significantly increased in the hens of S and S2 groups when compared to the hens of T, T1 and T2 groups (Figure 6). There has been little attempt to measure whether zeolite has any effect on water intake (Emam et al. 2019). Mumpton and Fishman (1977) reported that water intake was reduced when zeolite was included in the diet of broiler chickens. Many authors described the adverse effect of drinking saline well water on productive performance (Ahmed and Abdel-Rahman, 2004; Morsy et al., 2012). Drinking saline well water might increase the need for water used in the excretion of the most anions and cations through increasing water output and so, the hens increases its water intake (Ahmed and Abdel-Rahman, 2004) and/or the increase in water intake depends on kidney function; the concentration ability of kidney determines the increase in water intake that is necessary to excrete any added salt (Potter, 1968). On the other hand, delayed feed conversion in the hens of S (drank saline well water) group may be attributed to its lowest egg mass as compared to that of other groups.

Improvement in productive performance may attribute to zeolites (clinoptilolite) supplementation by having a number of beneficial effects they provide including better utilization of feed nutrients and positive effects on intestinal microflora and the mechanism of digestion and protection of animals against harmful effects of mycotoxins, stimulation of liver detoxification processes, elimination of heavy metals and radioactive elements (Macháček et al., 2010). Because of their properties, in-feed zeolites participate in many biochemical processes including high cation exchange capacity, adsorption, catalysis and dehydration-rehydration (Macháček et al., 2010). These results agree with the results of Tserveni-Gousi et al. (1997); Nys (1999) and Fendri (2012) that reported the inclusion of zeolites in animal diets improved weight gains and feed conversion, egg production and egg shell quality in laying hens, and had a positive

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effect on egg weight and the physical properties. In addition, positive significant dietary effects of clinoptilolite feeding were noticed with the number of eggs laid per hen, egg weight, shell thickness and efficiency of feed utilization (Miles et al., 1986; Olver, 1989; Fethiere et al., 1990; Roland et al., 1991).

Mortality rate during the experiment increased (P<0.05) in the hens drinking saline well water when compared to the hens in T1, T2 and S2 groups (Figure 7). However, no significant differences among the hens of S1, S2 and T groups was seen. There were positive effects of zeolite on decreased mortality rate in hens and enhancing prevention of some diseases and improving the health status by reduced colony counts in the gut microflora of the proximal and distal gut and described reduced mortality in broilers and layers (Olver, 1983 and Papaioannou et al., 2005). Indeed, zeolites are used as effective adsorbents of toxic agents, particularly aflatoxins from the feeds (Parlat et al., 1999; Phillips, 1999; Ortatatli and Oguz, 2001; Rizzi et al., 2003). However, they indicated that zeolites may have other effects when included in animal diets (Desheng et al., 2005). Similarly, Pavelic et al. (2002) and Ipek et al. (2012) reported that reactive oxygen species concentration decreased in the liver of mice fed zeolite supplementation as antioxidant. This reducing effect might be associated with adhesion-adsorption, ion-exchange and cation binding properties of clinoptilolite.

Table 9. Effect of zeolite supplementation on productive performance of laying hens drinking saline well water

Traits	T	T1	T2	S	S1	S2	±SE
Egg weight (g)	44.16^{b}	46.41^{ab}	46.53^{ab}	43.52^{b}	45.51^{ab}	47.82^{a}	1.08
Egg number	54.80^{b}	60.59^{a}	63.53^{a}	52.67^{b}	55.45^{b}	60.37^{a}	1.34
DFE (g)	106.41	105.39	110.88	114.50	106.23	107.78	5.49
TFE (kg)	10.64	10.53	11.08	11.45	10.62	10.77	0.54
Egg mass (g)	2415.22^{b}	2811.98^{a}	2956.05^{a}	2291.49^{b}	2523.52^{b}	2886.89^{a}	83.00
Feed conversion	4.40^{ab}	3.74^{b}	3.75^{b}	4.99^{a}	4.20^{ab}	3.73^{b}	0.26

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1 = hens drank tap water and fed diet containing 2 % zeolite, T2 = hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1 = hens drank saline well water and fed diet containing 2 % zeolite, S2 = hens drank saline well water and fed diet containing 4 % zeolite, ±SE = standard error, DFE = daily feed intake, TFE = total feed intake, $^{a, b, c}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

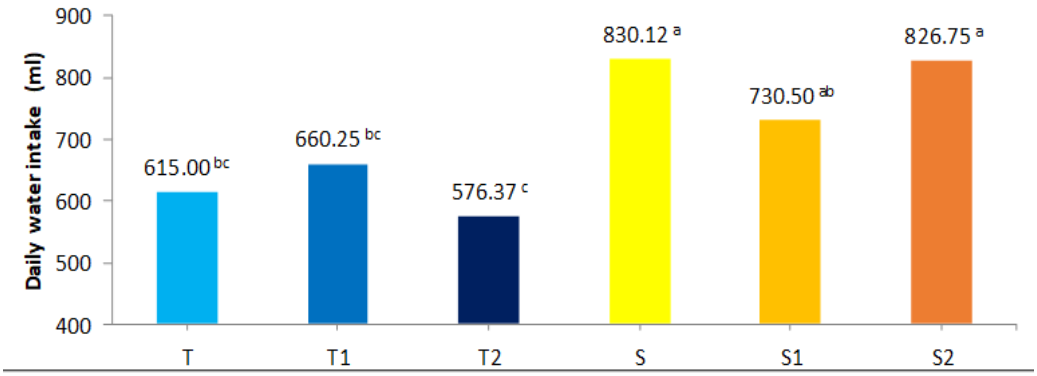

Figure 6. Effect of zeolite supplementation on daily water intake of laying hens drinking saline well water

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1= hens drank tap water and fed diet containing 2 % zeolite, T2= hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1= hens drank saline well water and fed diet containing 2 % zeolite, S2= hens drank saline well water and fed diet containing 4 % zeolite, $^{a, b, c}$ Means bearing different superscripts are significantly different (P< 0.05).

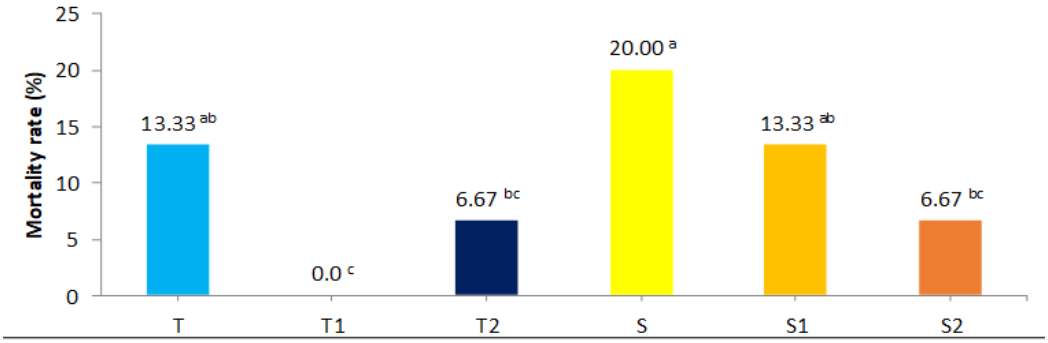

Figure 7. Effect of zeolite supplementation on mortality rate of laying hens drinking saline well water

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1= hens drank tap water and fed diet containing 2 % zeolite, T2= hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1= hens drank saline well water and fed diet containing 2 % zeolite, S2= hens drank saline well water and fed diet containing 4 % zeolite, $^{a, b, c}$ Means bearing different superscripts are significantly different (P< 0.05).

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Egg quality

Hens drank saline well water had decreased (P<0.05) in shell thickness by 6.50, 11.61, 6.28, 7.39 and 12.01 % as compared to the hens of T, T1, T2, S1 and S2 groups, respectively. However, hens of T, T1, T2, S1 and S2 groups caused insignificantly differences in shell thickness (Figure 8). On the other hand, shell weight increased (P<0.05) in the hens of S2 group as compared to the hens in T, T2, S and S1 groups. Moreover, shell (%) was significantly increased in the hens of S2 when compared to the hens of S. Also, albumen weight was significantly increased in the hens of T1 and S2 when compared to the hens of T and S (Table 10). However, yolk weight was increased (P<0.05) in the hens of S2 as compared to the hens in drank tap water (T).

Table 10. Effect of zeolite supplementation on egg quality of laying hens drinking saline well water

Traits	T	T1	T2	S	S1	S2	±SE
Egg weight (g)	43.63^{c}	46.81^{ab}	46.38^{ab}	44.83^{bc}	45.61^{bc}	48.71^{a}	0.74
Egg shape index (%)	74.65	76.95	76.60	74.97	75.23	77.32	0.86
Shell weight (g)	4.52^{c}	4.87^{ab}	4.61^{bc}	4.42^{c}	4.66^{bc}	5.13^{a}	0.10
Shell (%)	10.39^{ab}	10.40^{ab}	9.95^{ab}	9.90^{b}	10.23^{ab}	10.55^{a}	0.17
Albumen (%)	58.90	60.42	60.15	59.18	60.07	59.57	0.63
Albumen weight (g)	25.73^{c}	28.30^{a}	27.95^{ab}	26.39^{bc}	27.61^{ab}	29.00^{a}	0.58
Yolk (%)	30.69	29.17	29.91	30.90	29.70	29.87	0.60
Yolk/Albumen (%)	52.29	48.50	49.96	53.49	49.68	50.33	1.84
Yolk weight (g)	13.38^{b}	13.64^{ab}	13.86^{ab}	13.85^{ab}	13.56^{ab}	14.56^{a}	0.32
Egg yolk index (%)	34.34	33.86	33.77	34.52	33.95	33.24	0.61

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1 = hens drank tap water and fed diet containing 2 % zeolite, T2 = hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1 = hens drank saline well water and fed diet containing 2 % zeolite, S2 = hens drank saline well water and fed diet containing 4 % zeolite, ±SE = standard error, $^{a, b, c}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

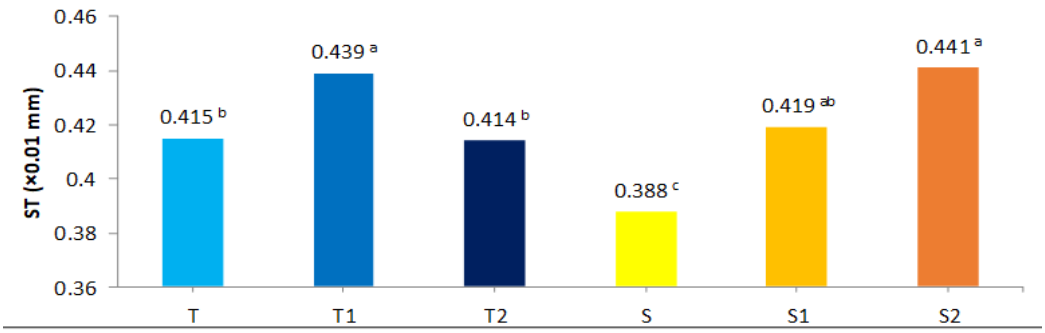

Figure 8. Effect of zeolite supplementation on shell thickness of laying hens drinking saline well water

T= hens drank tap water (265 ppm total dissolved solids) and fed basal diet, T1= hens drank tap water and fed diet containing 2 % zeolite, T2= hens drank tap water and fed diet containing 4 % zeolite, S= hens drank saline well water (3398 ppm total dissolved solids) and fed basal diet, S1= hens drank saline well water and fed diet containing 2 % zeolite, S2= hens drank saline well water and fed diet containing 4 % zeolite, ST= shell thickness, $^{a, b, c}$ Means bearing different superscripts are significantly different (P< 0.05).

These results are agreeing with the results of Yoselewitz et al. (1988); Shafey (1993); Damron (1998); Pourreza and Edriss (1998) and Morsy et al. (2012) that reported sodium and chloride ions contribute to the production of defective shells in eggs from hens receiving NaCl supplements in the drinking water. The primary metabolic lesion associated with the poor egg shell quality which results from the intake of saline well water appears to be related to the supply of bicarbonate rather than calcium to the lumen of the shell gland for eggshell formation. A reduced activity of carbonic anhydrase in the shell gland mucosa is the particular importance. Several studies have been made on the effect of NaCl in drinking water, and the results indicated a reduction of calcium-binding protein (Balnave and Zhang, 2003) and the carbonic anhydrase enzyme which is important for shell formation (Balnave, 1993). This might have a negative impact on the egg shell quality. Poor shell quality was related to a reduced supply of bicarbonate, rather than with an effect on calcium in the lumen of the shell gland. However, the adverse effect of drinking saline well water on shell quality of hens may lead to an increase in egg breakage, then great economic losses in egg production (Balnave et al., 1989). On the other hand, temperature plays a role in aggravating the detrimental effects of saline water on egg shell quality since in the current and in the previous studies hens were exposed to severe heat stress.

This experiment clearly demonstrated that egg shell thickness was significantly decreased for hens drank saline well water without dietary zeolite supplementation. Similar trends were observed in other egg quality traits (Samara, 2003). However, adding zeolite to the diet of poultry improved the process of egg quality and shells strength without deleterious effects on the contents of the egg itself (Olver, 1997; Öztürk et al., 1998; Kermanshahi et al., 2011; Fendri et

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al., 2012). It has been reported that, feeding laying hens with zeolite improves eggshell quality (Roland et al., 1985; Rabon et al., 1991). The benefits effects of zeolite might depend on its concentration, the aluminum and phosphorus content of the aluminosilicate (Kermanshahi et al., 2011). On the other hand, natural zeolite a clinoptilolite-bearing rock material, were found to increase egg weight and albumin weight when it was incorporated in the hen's diet (Elliot and Edwards, 1991; Yannakopoylps et al., 1998).

CONCLUSION

Supplementation with zeolite (clinoptilolite) at level of 4% to the diets of hens reared under hot desert conditions and drinking saline well water could improve productive performance and eggshell quality characteristics.

DECLARATIONS

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Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

Consent to publish

All the authors approved and agreed to publish the manuscript and declared that this work has not been previously published elsewhere.

Author's contribution

Dr. Nagwa Abde El-Hady Ahmed designed the experiment, wrote and revised the article, Ahmed Abd-Elatif El-Far, wrote and revised the article, Dr. Ali Saber Morsy designed the experiment, laboratory analyses, statistical analysis, tabulation of experimental data, manuscript writing, commenting and approval, Dr. Amal Mohamed Hasan helped in statistical analysis, tabulation of experimental data and article writing; Dr. Khamis Refaay Said Emam designed the experiment, tabulation of experimental data, manuscript writing, commenting and approval; while, Mr. Hossam El-Din Mohamed Ibrahim helped in field study, collected data, laboratory analyses, manuscript writing. All authors have read and approved the final manuscript.

REFERENCES

Abbas TEE, Elzubeir EA and Arabbi OH (2008). Drinking water quality and its effects on broiler chick's performance during winter season. International Journal of Poultry Science, 7(5): 433-436. DOI: 10.3923/ijps.2008.433.436.

Abdel-Samee AM and El-Masry KA (1992). Effect of drinking natural saline well water on some productive and reproductive performance of California and New-Zealand White rabbits maintained under North Sinai conditions. Egypt Journal Rabbit Science, 2(1): 1-11. Available at: http://www.aun.edu.eg/env_enc/env%20mar/env%20oct/125-136.PDF.

Ahmed MM and Abdel-Rahman MA (2004). Effect of drinking natural saline ground water on growth performance behavior and some blood parameters in rabbits reared in new reclaimed lands of arid areas in Assiut Governorate. Assiut University Bulletin Environment Research, 7 (2): 125-135.

Andronikashvili TK, Pagava T, Kurashvili and Eprikashvili L (2009). Possibility of application of natural zeolites for medicinal purposes. Bulletin of the Georgian National Academy of Sciences, 3(2): 158-167. Available at: https://pdfs.semanticscholar.org/1c4e/2b6651c07ac5df53074bc7cc6fe64fb0e937.pdf.

Amal MH (2003). Some physiological and productive effects of promoting growth in rabbits drinking natural saline water. Ph.D. Thesis, Faculty of Agriculture. Cairo, University.

Amal MH (2013). Semen quality and hematology parameters of White leghorn cocks drinking saline water under hot desert conditions. Egypt. Poultry Science, 33(1): 163-179. DOI: https://epsj.journals.ekb.eg/article_5383_53f9a45e6ea07f8eb3d0434d51b89e34.pdf.

Assad FM, Nassar AM, Hussein N and Abdelmageed SM (1997). Effect of saline water on some water on some biochemical parameters in sheep. Egyptian Journal Applied Science, 12: 1-13. DOI: 10.1292/jvms.17-0596

Balnave D (1993). Influence of saline drinking water on eggshell quality and formation. World Poultry Science Journal, 49: 109-119. DOI:org/10.1079/WPS19930009.

Balnave D and Zhang (2003). "Research Note: Responses of laying hens on saline drinking water to dietary supplementation with various zinc compounds", Poultry Science, 72: 603-606. DOI:https://doi.org/10.3382/ps.0720603

Balnave D, Yoselewitz I and Dixon RJ (1989). Physiological changes associated with the production of defective egg-shells by hens receiving sodium chloride in the drinking water. British Journal Nutrition, 61(1): 35 - 43. DOI:https://doi.org/10.1079/BJN19890090.

Basha HA, Goma AA, Taha AE and Abou Elkhair R (2016). Effect of different forms of natural zeolite (clinoptilolite) on productive performance and behavioral patterns of broiler chickens. International Journal of Agriculture Science and Veterinary Medicine, 4(4): 1-11. DOI: https://www.researchgate.net/publication/315676631

Beltcheva MR, Metcheva M, Topashka-Ancheva N, Popov S, Teodorova JA, Heredia-Rojas AO, Rodríguez-de la Fuente and Rodríguez-Flores LE (2015). Zeolites versus lead toxicity. Journal of Bioequivalence & Bioavailability, 7(1): 12-29.

Bintaş EM, Bozkurt K, Küçükyılmaz R, Konak M, Çınar H, Akşit K, Seyrek and Çatl AL (2014). Efficacy of supplemental natural zeolite in broiler chickens subjected to dietary calcium deficiency. Italian Journal of Animal Science, 13: 3141. https://doi.org/10.4081/ijas.2014.3141.

Boyer J (2000). "Zeolite in Animal Nutrition", Undergraduate Seminar, http://animsci. agrenv.mcgi l l .ca/ courses/ugradsem/00_01/Boyer_Sci.html

Colella C (2011). A critical reconsideration of biomedical and veterinary applications of natural zeolites. Clay Miner, 46: 295-309. https://doi.org/10.1180/claymin.2011.046.2.295.

Damron BL (1998). Sodium chloride concentration in drinking water and eggshell quality. Poultry Science, 77: 1488-1491. https://doi.org/10.1093/ps/77.10.1488

Debeic M (1994) "Influence of Clinoptiloliteson Chicken Growth", Poultry. Abstract, Vol. 21, pp. 9-309.

Desheng Q, Fan L, Yanhu Y and Niya Z (2005) Adsorption of aflatoxin B-1 on montmorillonite. Poultry Science, 84: 959-961. DOI: 10.1093/ps/84.6.959

Duncan DB (1955). Multiple range and multiple F-test. Biometrics, 11: 1-42.

Elliot MA and Edwards HM (1991). Comparison on the effects of synthetic and natural zeolite on laying hen and broiler chicken performance. Poultry Science, 70: 2115–2130. DOI:10.3382/ps.0702115

Eleroğlu HH and Yalçın AY (2011). Dietary effects of Ca-zeolite supplementation on some blood and tibial bone characteristics of broilers. South African Journal Animal Science, 41: 319-330.DOI: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0375-15892011000400001

El-Wardany AM, Goher LM and Enab AA (1994). Effect of breed, laying period and selection for egg weight on egg Quality in two local breed of chickens. Egypt Poultry Science, 14: 23-49.

Emam KRS, Abdel-dayem AA and Abd El-Galil K (2019). Effect of zeolite supplementation on productive performance and blood constituents of broiler chickens under drinking saline well water conditions. Egyptian Poultry Science Journal, 39(1). http://www.epsj.journals.ekb.eg.

Emam KRS, Amal MH, Morsy AS and Abd El-Galil K (2017). Semen quality and hematological parameters of Sina cocks fed Atripex nummularia leaves meal under desert conditions. Egyptian Poultry Science, 37(IV): 1097-1111.

Evans M, Farrell DJ and Farrell DJ (1993). Are there economic benefits to adding zeolites to poultry diets';' Recent advances in animal nutrition in Australia, University New England, Armidale, Australia, 303-316. DOI: http://livestocklibrary.com.au/handle/1234/19714

Fendri I, Khannous L, Mallek Z, Traore AI, Gharsallah N and Gdoura R (2012). Influence of Zeolite on fatty acid composition and egg quality in Tunisian Laying Hens. Lipids in health and disease, 11(1): 71. Available at:http://www.lipidworld.com/content/11/1/71

Fethiere R, Miles RD and Harms RH (1994). The utilization of sodium in sodium zeolite A by broilers. Poultry Science, 73: 118-121. DOI:10.3382/ps.0730118

Fethiere R, Miles RD and Harms RH (1990). Influence of synthetic sodium aluminosilicate on laying hens fed different phosphorus levels. Poultry Science, 69: 2195–2198. DOI:10.3382/ps.0692195

Frost TJ, Roland DA, Barnes DG and Laurent SM (1992). The effect of sodium zeolite A and cholecalciferol on plasma levels of 1,25–dihydroxycholecalciferol, calcium and phosphorus in commercial leghorns. Poultry Science, 71: 886–893.

Guyton AC and Hall JE (2006). Textbook of Medical Physiology. 11th. WB Sounders Company, Philadelphia.

Ipek HM, Avci N, Aydilek and Yerturk M (2012). The effect of zeolite on oxidant/ antioxidant status in healthy dairy cows. Acta Veterinary Brno, 81: 043-047. DOI: https://doi.org/10.2754/avb201281010043

Julian RJ (1987). The effect of increased sodium in drinking water on right ventricular hypertrophy, right ventricular failure and ascites in broiler chickens. Avian Pathology, 16: 61-71. DOI.org/10.1080/03079458708436353.

Karadjole I, Tofant A, Vucemilo M and Hadziosmanovic M (1999). Effect of saline drinking water on acid-base balance and electrolyte levels in laying hen blood. Veterinary Medicine Czech, 44: 49-52. http://agris.fao.org/agris-search/search.do?recordID=CZ1999000585

Kermanshahi H, Haji Agha Jani E, Hashemipour H and Pilevar M (2011). Efficacy of natural zeolite and pigments on yolk color and performance of laying hens. African Journal of Biotechnology 1, 10:3237–3242. DOI: 10.5897/AJB10.1713

Leach RM, Brenda S, Heinrichs BS and Burdette J (1990). Broiler chicks feed low calcium diets. 1. İnfluence of zeolite on growth rate and parameters of bone metabolism. Poultry Science, 69: 1539-1543. DOI:10.3382/ps.0691539

Lotfollahian HF, Shariatmadari M, Shivazad and Mirhadi SA (2004). Study on the effects of two kinds of natural zeolite in diets on blood biochemical parameters, relative weight of body organs and broilers performance. Pajouhesh and Sazandegi, 64: 18-34. https://www.sid.ir/En/Journal/ViewPaper.aspx?ID=113672

Macháček MV, Večerek, Mas N, Suchý P, Straková E, Šerman V and Herzig I (2010). Effect of the feed additive Clinoptilolite (ZeoFeed) on nutrient metabolism and production performance of laying hens. Acta Veterinary Brno, 79: 29-34. DOI:https://doi.org/10.2754/avb201079S9S029

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Marai IFM, Ayyat MS and Abdel-Monem UM (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. Journal of Tropical Animal Health Production, 33, 1-12. https://www.ncbi.nlm.nih.gov/pubmed/11770200

Miazzo RD, Peralta MF, Magnoli C, Salvano M, Ferrero S, Chiacchiera SM, Carvalho ECQ, Rosa CAR and Dalcero A (2005). Efficacy of Sodium Bentonite as a Detoxifier of Broiler Feed Contaminated with Aflatoxin and Fumonisin. Poultry Science, 84: 1-8. DOI:10.1093/ps/84.1.1

Miles RD and Henry PR (2007). Safety of improved Milbond-TX® when fed in broilers diets at greater than recommended levels. Animal Feed Science Technology, 138: 309-317. DOI: 10.1016/j.anifeedsci.2007.04.008

Miles RD, Harms RH and Laurent SM (1986). Influence of sodium zeolite A (EthacalR) on laying hen performance. Nutrition Reproductive International, 34: 1097–1103.

Milne DB (1996). Trace elements. In: Tietz Fundamentals of clinical chemistry. Burtis CA and Ashood ER (4th Edition.), 485-496.

Morsy AS, Mona MH and Amal MH (2012). Effect of natural saline drinking water on productive and physiological performance of laying hens under heat stress conditions. Egyptian Poultry Science, 32(III): 561-578.

Morsy AS, Manal MH, Gad- El-Moula, Dooa O, Hassan MS and Nagwa AA (2016). Blood picture, metabolites and minerals of rabbits as influenced by drinking saline water in Egypt. Global Journal of Advanced Research, 3(11): 1008-1017. DOI: http://gjar.org/publishpaper/vol3issue11/d628r62.pdf

Morsy AS, Manal MH, Gad- El-Moula, Dooa O, Hassan MS and Nagwa AA (2017). Effect of alternated drinkingsaline well water on the reproductive performance of rabbits. Egyptian Journal of Rabbit Science, 27 (1): 131- 148.

Morsy AS (2018). Effect of zeolite (Clinoptilolite) as a salinity stress alleviator on semen quality and hemato- biochemical parameters of Montazah cocks under South Sinai conditions. Research Journal of Animal and Veterinary Sciences, 10(2): pages 9-17.

Mumpton FA and Fishman PH (1977). The application of natural zeolites in animal science and aquaculture. Journal of Animal Science, 45 (5): 1188–1203. DOI: https://doi.org/10.2527/jas1977.4551188x

National Research Council (NRC) (1994). Nutrient Requirements of Poultry. 9th edition. National Academic Press. Washington, D.C, 3(1): 101-101. Available at:https://www.scirp.org/(S(oyulxb452alnt1aej1nfow45))/reference/ReferencesPapers.aspx?ReferenceID=1955728.

Nik-Khan A and Sadeghi AA (2002). Natural Clinoptilolite-Tuff Effects on Health Homo-Immuno Parameters in Newborn Calves, Zeolite '02, 6th International Conference, Occurrence, Properties and Utilization of Natural Zeolites, pp: 253. https://www.researchgate.net/publication/288958500

Nikolakakis (2013). Effect of natural zeolite (clinoptilolite) on the performance and litter quality of broiler chickens. Turkish Journal of Veterinary and Animal Sciences, 37: 682-686. Available at:http://www.journals.tubitak.gov.tr/veterinary/

Nys Y (1999). Nutritional factors affecting eggshell quality. Czech Journal Animal Science, 44: 135-143. Available at: http://agris.fao.org/agris-search/search.do?recordID=CZ1999000678

Oğuz H and Kurtoğlu V (2000). Effects of clinoptilolite on performance of broiler chickens during experimental aflotoxicocis. British Poultry Science, 41: 512-517. DOI:10.1080/713654953

Olver MD (1997). Effect of feeding clinoptilolite (zeolite) on the performance of three strains of laying hens, British Poultry Science, 38, 220-22. DOI:10.1080/00071669708417973

Olver MD (1989). Effect of feeding clinoptilolite (zeolite) to three strains of laying hens. British Poultry Science, 36: 115–121. DOI:10.1080/00071668908417130

Ortatatli M and Oguz H (2001). Ameliorative effects of dietary clinoptiloliteon pathological changes in broiler chickens during aflatoxicosis. Research Veterinary Science, 71: 59-66. DOI: 10.1053/rvsc.2001.0487

Öztürk E, Erener G and Sarica M (1998). Influence of Natural Zeolite on Performance of Laying Hens and Egg Quality. Tr Journal Agricultuer Forest, 22:623–628. http://www.zeocat.es/docs/animalegglayinghens.pdf

Papaioannou D, Katsoulos PD, Panousis N and Karatzias H (2005). The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. Microporous and Mesoporous Material, 84: 161-170. DOI:https://doi.org/10.1016/j.micromeso.2005.05.030

Parlat SS, Yildiz AOH and Oguz H (1999). Effect of clinoptilolite on fattening performance of Japanese quail (Coturinix coturnix japonica) during experimental aflatoxicosis. British Poultry Science, 40: 495-500.

Pavelic K, Katic M, Serko V, Marotti T, Bosnjak B, Balog T, Stojkovic R, Radacic M, Kolic M and Poljak-Blazi M (2002). Immunostimulatory effect of natural clinoptilolite as a possible mechanism of its antimetastatic ability. Journal of Cancer Researsh Clinical Oncology, 128: 37-44. DOI: 10.1007/s00432-001-0301-6

Pavelic K and Hadzija M (2003). Medical applications of zeolites. In: Auerbach SM Carrado KA Dutta PK (eds.) Handbook of Zeolite Science and Technology, Marcel Dekker Inc., New York-Basel, pp: 1143-1174.

Pavelić KM, Hadžija L, Bedrica J, Pavelić I, Dikić M, Katić M, Kralj MH, Bosnar S, Kapitanović M, Poljak-Blazi, et al. (2001). Natural zeolite clinoptilolite new adjuvant in anticancer therapy. Journal of Molecular Medicine, 78: 708- 720.

Phillips TD (1999). Dietary clay in the chemoprevention of aflatoxin induced disease. Toxicology Science, 52: 118-126.

Potter BJ (1968). The influence of previous salt ingestion on the renal function of sheep subjected to intravenous hypertonic saline. Journal of Physiology, 194: 435-455. https://physoc.onlinelibrary.wiley.com/doi/pdf/10.1113/jphysiol.1968.sp008417

Pourreza J and Edriss MA (1998). Effect of Saline Drinking Water on Egg Shell Quality of Leghornand Native Hens. British Poultry Science, 35: 755-762.

Rabon HW, Roland DA SR, Bryant M, Barns DG and Laurent SM (1991). Influence of sodium zeolite A with and without pullet-sized limestone or oyster shell on eggshell quality, Poultry Science, 70, 1943-1947. DOI:10.3382/ps.0701943

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Rizzi L, Simioli M, Roncada P and Zaghini A (2003). Aflatoxin B1 and clinoptilolite in feed for laying hens: effects on egg quality, mycotoxin residues in livers, and hepatic mixed-function oxygenase activities. Journal of Food Protection, 66: 860-865. Available at: https://www.ncbi.nlm.nih.gov/pubmed/12747697

Roland DA and Barnes DG and Laurent SM (1991). Influence of sodium aluminosilicate, hydroxy–sodalite, carnegicite, aluminum sulfate and aluminum phosphate on performance of commercial leghorns. Poultry Science, 70: 805–811. DOI:10.3382/ps.0700805

Roland DA, Laurent SM and Orloff HD (1985). Shell quality as influenced by zeolite with high ion–exchange capability. Poultry Science, 64: 1177–1187. DOI: 10.3382/ps.0641177

Safaeikatouli MY, Jafariahangari and Baharlouei A (2011). An evaluation on the effects of dietary kaolin and zeolite on broilers blood parameters, T4, TSH and growth hormones. Pakistani Journal of Nutrition, 10: 233-237. DOI: 10.3923/pjn.2011.233.237

Salari S, Kermanshahi H and Nasirimoghaddam H (2006). Effect of Sodium Bentonite and comparison of pellet vs mash on performance of broiler chickens. International Journal of Poultry Science, 5: 31-34. DOI: 10.3923/ijps.2006.31.34

Samara M (2003). Effect of feeding natural zeolite on performance of laying hens drinking saline water. An-Najah University Journal Research, (Master Science, Thesis), 17(2): 184-189. Available at: http://hdl.handle.net/20.500.11888/2055

SAS Institute (2004). SAS User's Guide: Statistics. Release 9.1. SAS Institute Incorporated., Cary, North Carolina.

Shafey TM (1993). Calcium tolerance of growing chickens: effect of ratio of dietary calcium to available phosphorus. Journal widget, 49, Issue 01. DOI:https://doi.org/10.1079/WPS19930002

Sheikhzadeh NM, Kouchaki M, Mehregan H, Tayefi-Nasrabadi B, Divband M, Khataminan AK, Oushani and Shabanzadeh S (2017). Influence of nanochitosan/ zeolite composite on growth performance, digestive enzymes and serum biochemical parameters in Rainbow trout (Oncorhynchus mykiss). Aquaculture Research, 48: 5955-5964. DOI:https://doi.org/10.1111/are.13418

Stino FKR, Goher NE, Kamar GAR and Hanash NA (1982). The effect of breed and housing system on the egg quality of White Baladi and Fayoumi hens in the subtropics. Egyptian Journal Animal Production, 23: 191-198.

Tata JR and Widnell CC (1966). Ribonucleic and synthesis during the early action of hormone. Biochemistry, 98: 604. Available at: https://www.ncbi.nlm.nih.gov/pubmed/5941352

Tserveni-Gousi AS, Yannakopoulos AL, Katsaounis NK, Filippidis A and Kassoli-Fournaraki A (1997) Some interior egg characteristics as influenced by addition of Greek clinoptilolitic rock material in the hen diet. Archive of fur Geflugelkunde, 61: 291-296. Available at: https://www.european-poultry-science.com/artikel.dll/1997-61-291-296_NTAwMzcxMg.PDF

Tukmechi A, Rahmati Andarani HR, Manaffar R and Sheikhzadeh N (2011). Dietary administration of beta-mercapto-ethanol treated Saccharomyces cerevisiae enhanced the growth, innate immune response and disease resistance of the Rainbow trout, Oncorhynchus mykiss. Fish and Shellfish Immunology, 30: 923-928. DOI:10.1016/j.fsi.2011.01.016

Underwood EJ and Suttle NF (1999). The mineral nutrition of livestock. 3rd Edition. Chapter 1. New York, USA, 614.

Valpotić HS, Terzic S, Vince M, Samardzija R, Turk G, Lackovic B, Habrun D, Djuricic M, Sadikovic and Valpotic I (2016). In-feed supplementation of a clinoptilolite favorably modulates intestinal and systemic immunity and some production parameters in weaned pigs. Veterinary Medicine, 61: 317-327. https://www.semanticscholar.org/paper/In-feed-supplementation-of-clinoptilolite-modulates-Valpoti%C4%87-Terzi%C4%87/adef6b75248393980ecc4a6c81e86326c7ff9bc1

Ward TL, Watkins KL and Southern LL (1993). Interactive effect of sodium zeolite A and Eimeria acervulina infection on growth and tissue minerals in chicks. Poultry Science, 72(11): 2172-2175.

Watkins KL, Southern LL (1991). Effect of dietary sodium zeolites A and graded levels of calcium on growth, plasma and tibia characteristics of chicks. Poultry Science, 70: 2295–2303. DOI:10.3382/ps.0711048

Wawrzyniak AM, Kapica D, Stępień-Pyśniak R, Szewerniak A, Olejarska and Jarosz V (2017). Effect of feeding transcarpathian zeolite on gastrointestinal morphology and function in broiler chickens. Brazilian Journal of Poultry Science, 19(4): 737-746. DOI:http://dx.doi.org/10.1590/1806-9061-2016-0360

Wu QJ, Wang LC, Zhou YM, Zhang JF and Wang T (2013). Effects of clinoptilolite and modified clinoptilolite on the growth performance, intestinal microflora, and gut parameters of broilers. Poultry Science, 92: 684-692. DOI:10.3382/ps.2012-02308

Yannakopoylps AL, Tserveni-Gousi AS and Christaki E (1998). Effect of natural zeolite on yolk: albumine ratio in hen eggs. British Poultry Science, 39: 506 – 510.

Yoselewitz I, Balnave D and Dixon RJ (1988). Factors influencing the production of defective egg shell by laying hens receiving sodium chloride in drinking water. Nutrition Reports International, 38: 697-703.

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Prevalence of Rabbit Coccidia in Medea Province, Algeria

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ABSTRACT

Coccidiosis has an economic impact for poultry and livestock. The current study examined the prevalence of *Eimeria* infections in domestic rabbits in Medea province, North of Algeria. A total of 414 faecal samples were collected from 50 farms in six regions of the province. Each faecal sample was subjected to oocyst counting and isolation. The *Eimeria* species from samples containing isolated and sporulated oocysts were morphologically identified microscopically. The overall prevalence of coccidial infections was 47.6% (197/414). Weaners had the highest prevalence (77%, 77/100, p<0.0001), followed by growing rabbits (46.8%, 30/64) and the adult rabbits showed the lowest prevalence (36 %, 18/50). In breeding rabbits, females were more infected with a prevalence of 40% (p<0.0001). Eleven rabbit *Eimeria's* species were present and identified from oocyst positive samples. *Eimeria magna* and *Eimeria media* were the most prevalent species (47.6% and 47.3%). Sulfonamides showed a better protection against rabbit coccidiosis than colistin and trimethoprim association (p< 0.0001, prevalence of 23.3% vs. 65.3% respectively). These results indicated that the prevalence of coccidiosis is high among the rabbit population in Medea province, North of Algeria. As a conclusion, it seems that the epidemiological situation of rabbit coccidiosis in Medea province must be taken into consideration in order to minimize the economic losses caused by this parasitosis.

Key words: *Eimeria*, *Oryctolagus cuniculus*, Rabbit, Sulfonamides

INTRODUCTION

Coccidiosis is the major parasitosis in poultry and other domestic animals, including rabbits: Oryctolagus cuniculus (Pakandl, 2009; Geru et al., 2016). It is one of the most important infectious causes of digestive disorders in rabbits (Pakandl, 2009; Geru et al., 2016). This disease is caused by intercellular protozoa parasites of the genus *Eimeria* and can be responsible for significant mortality in domestic rabbits (Pakandl, 2009). The symptoms of the disease include anorexia, diarrhea, body weight loss, poor feed conversion and even death to weaning rabbits (Pakandl, 2009). Eleven distinct *Eimeria* species have been identified in rabbits. Among these species, ten colonise the intestinal tract, invading and destroying intestinal cells, and causing anaemia, electrolyte imbalance and poor absorption of nutrients (Pakandl, 2009). *Eimeria stiedae* infects the biliary ducts of the liver. Hepatic coccidiosis is most often subclinical, but it can be the cause of poor feed conversion (Al-Mathal, 2008; Pakandl, 2009). All domesticated rabbit breeds can be infected by coccidia, especially the younger animals between one and four months of age (Drouet-Viard et al., 1997a; González-Redondo et al., 2008; Bachene et al., 2014; Bachene et al., 2018). The identification of these coccidia is based on the morphological characteristics of the oocysts and the sporulation time (Coudert et al., 1995; Pakandl et al., 2008). The site of infection and clinical signs can also guide the identification of coccidian species (Pakandl et al., 2008). In Algeria, the epidemiological situation of rabbit coccidiosis is almost unknown, Henneb and Aissi (2013) reported the prevalence of coccidia in Eastern Algeria rabbit breeding: *Eimeria magna* (43%), *Eimeria stieda*e (23%), *Eimeria media* (19%), *Eimeria perforans* (9%), *Eimeria exigua* (3%) and *Eimeria coecicola* (3%) and Maziz-Bettahar et al. (2018) reported the prevalence of rabbit coccidial infection in three regions in the north of Algeria: *Eimeria magna* (42.5%), *Eimeria media* (17.6%) *and Eimeria irresidua* (14.9%).

The present study was undertaken in Medea, a Northern agricultural province of Algeria, first, to investigate the natural prevalence of coccidial infections in different rabbit farms according to age, sex as well as chemoprevention, and second, to identify *Eimeria* species present in these farms.

MATERIALS AND METHODS

Ethical approval

This work was approved by the scientific council of the Higher National Veterinary School of Algiers, Algeria.

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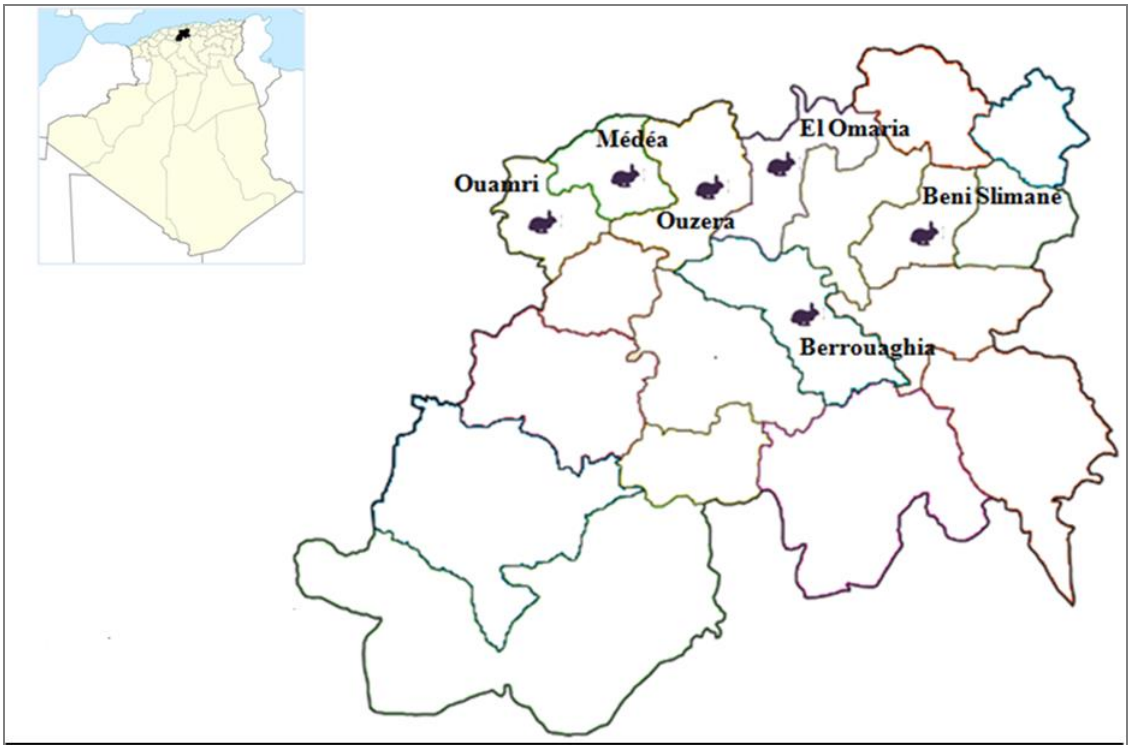
Study area and selection of rabbitories

The study focused on the rabbit populations in Medea province, a Northern agricultural province of Algeria (Figure 1). This province is located 36.26 latitude, 2.75 longitude and 910 meters above sea level. It has an average annual temperature of 14.4 °C and an average annual rainfall of 736 mm. A total of 414 faecal samples were collected randomly (random numbers table method) from apparently healthy animals of 50 rabbit breeding farms of six regions in this province (Figure 1). Faeces were collected from 21291 rabbits in order to test the presence of oocysts. These rabbits included 15923 weaners (1 to 3 months old), 782 growing rabbits (3 to 6 months old), 502 adult rabbits (older than 6 months) and 4084 breeding rabbits including 585 males and 3499 does.



Regions	Farms	Local rabbits	Fecal samples
Médéa	10	4540	78
Beni Slimane	10	3754	84
Berrouaghia	08	3419	62
El Omaria	08	3140	66
Ouzera	07	3132	64
Ouamri	07	3306	60
Total	50	21291	414

Figure 1. A map of Medea's province, Algeria showing the selected regions and the number of farms of local rabbits breed and fecal samples

Fecal sampling and parasitological analysis

From each class (weaners /growing/ adults/ breeding males/ breeding females), 500 g of fresh faecal pellets were collected as one sample. After homogenization, 300g of each sample were mixed in 1500 ml of water, and then 40g of the mixture were put into 60 ml of saturated magnesium sulfate ($MgSO_4$) solution (Coudert et al., 1995). The suspension was then emptied into a modified McMaster chamber to check the oocysts, and the Oocyst Per Gram (OPG) was calculated to estimate the degree of infection (Coudert et al., 1995). The limitation of detection value was set as 200 oocysts per gram faecal sample. Purified oocysts were sporulatedin a shaker and diluted into a 2.5 % potassium dichromate solution at 28 °C for seven days to ensure good aeration. Concentrated oocysts in each sample were identified based on their sizes and morphological characteristics using a microscope equipped with a camera connected to a computer. To ensure that species identification is valid, at least 100 sporulated oocysts from each species were observed and measured according to Coudert et al. (1995) method.

Statistical analysis

Prevalence of rabbit coccidiosis according to age, sex, chemoprevention, *Eimeria's* species and regions was calculated using SPSS (version 17 package) and analysed by Chi square test, and a value of $p<0.05$ was considered significant.

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RESULTS

Prevalence of coccidial infection in rabbits of Medea province

A total of 414 samples were collected and analysed. Overall, coccidian oocysts of *Eimeria* were found in 197of 414faecal samples (46.7 %) obtained from six regions of Medea Province, Algeria. The prevalence of coccidian oocysts in these regions ranged from 34.4% to 59.5 % (Table 2). Benislimane region had the highest prevalence (59.5 %) and Ouzera region had the lowest prevalence (34.4 %). Weanling rabbits were the most infected with a prevalence of 77%, followed by growing and adult rabbits with prevalence of 46.8% and 36% respectively (differences were statistically significant, P<0.0001). Concerning breeding rabbits, females were more infected with a prevalence of 40% (Table 1).

Eimeria's species identification results

The morphological identification of *Eimeria* oocysts revealed the presence of the eleven known species of rabbits' *Eimeria*. *E. magna* and *E. media* were the most prevalent species (47.6 % and 47.3%, p< 0.0001), followed in order by *E. stiedae*, *E. exigua*, *E. coecicola*, *E. flavescens*, *E.* perforans, *E. piriformis*, *E. vejdovskyi*, *E. irresidua* and *E. intestinalis* with prevalence of 43%, 36.2%, 21.7%, 20.8%, 20.5%, 17.9%, 12.8%, 11.1% and 8.7 %, respectively. (Table 3). *E. magna* and *E. media* were most prevalent in Bensilimane and Ouzera respectively (Table 4).

Prevalence of coccidial infection in rabbits according to chemo-prevention

All of the 50 farms used anticoccidials to their rabbits for coccidiosis prevention, but 11 farms did not provide us the kinds of anticoccidials that they had used (Table 5). Colistine+Trimethoprim association (COLISULTRIX) and Sulfaquinoxaline sodium + Sulfadiazine sodium association (COCCIDIOPAN) are the most frequently used drugs in these farms. In farms using Colistine+Trimethoprim association, oocysts were found in 65.3% (98/150) of faecal samples compared to 23.3% (41/176) for those using Sulfaquinoxaline sodium + Sulfadiazine sodium association (p<0.0001).

Table 1. Prevalence and intensity of coccidial infection in local rabbits breed according to age and sex in Medea province, Algeria

Rabbit classes	Examinated no	Positive no	Prevalence %	OPG	χ^2	P value
Weanling	100	77	77.0	$[9\times10^2 - 1.5\times10^6]$	56.4	<0.0001
Growing	64	30	46.8	$[1.2\times10^2 - 9\times10^5]$		
Adults	50	18	36.0	$[7\times10^2 - 3\times10^5]$		
Breeding male	100	32	32.0	$[8\times10^2 - 9\times10^4]$		
Breeding female	100	40	40.0	$[1.3\times10^2 - 1.2\times10^5]$		

Examined no: number of fecal samples analyzed, OPG: Oocyst per gram counting

Table 2. Prevalence and intensity of coccidial infection in local rabbits breed in different regions of Medea province, Algeria

Examined regions	Examinated no	Positive no	Prevalence %	OPG	χ^2	P value
Benislimane	84	50	59.5	$[8\times10^2 - 4.5\times10^4]$	15.3	P=0.0089
Medea	78	37	47.4	$[9\times10^2 - 1.1\times10^6]$		
Berouaghia	62	36	58.1	$[1\times10^3 - 1.5\times10^6]$		
El Omaria	66	24	36.4	$[7\times10^2 - 2.6\times10^4]$		
Ouzera	64	22	34.4	$[9\times10^2 - 2.6\times10^4]$		
Ouamri	60	28	46.7	$[9\times10^2 - 5.9\times10^4]$		

Examined no: number of fecal samples analyzed, OPG: Oocyst per gram counting

Table 3. Prevalence of faecal samples infected with coccidia species in local rabbits breed in Medea province, Algeria

Species	Positive no	Prevalence %	OPG	χ^2	P value
E. exigua	150	36.2	$[0 - 9.7\times10^4]$	650.7	<0.0001
E. perforans	85	20.5	$[0 - 4.6\times10^4]$		
E. piriformis	74	17.9	$[0 - 8.1\times10^3]$		
E. flavescens	86	20.8	$[0 - 1.4\times10^5]$		
E. irresidua	46	11.1	$[0 - 1.1\times10^4]$		
E. stiedae	178	43.0	$[0 - 4.2\times10^5]$		
E. intestinalis	36	8.7	$[0 - 5.2\times10^4]$		
E. media	196	47.3	$[0 - 4.3\times10^5]$		
E. vejdovskyi	53	12.8	$[0 - 1.5\times10^5]$		
E. coecicola	90	21.7	$[0 - 2.2\times10^4]$		
E. magna	197	47.6	$[2.5\times10^2 - 5.9\times10^5]$		

Examined no: number of fecal samples analyzed, OPG: Oocyst per gram counting

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Table 4. Percentage of faecal samples infected with coccidia species in local rabbits breed in Medea province, Algeria

Eimeria species	Medea (n=37)	Benislimane (n=50)	Berouaghia (n=36)	El Omaria (n=24)	Ouzera (n=22)	Ouamri (n=28)
E. exigua	7.16%	8.74%	4.58%	4.13%	8.82%	8.21%
E perforans	4.19%	0.98%	3.94%	5.17%	0.68%	2.64%
E. piriformis	3.11%	3.46%	2.56%	3.21%	2.32%	1.96%
E. flavescens	1.7%	3.58%	3.92%	3.21%	5.77%	4.11%
E. irresidua	1.03%	0.76%	0.67%	1.5%	2%	1.86%
E. stiedae	17.84%	14.04%	12.92%	7.29%	10.14%	18.82%
E. intestinalis	0.81%	2.46%	3.14%	0.75%	0.64%	0.79%
E. media	18.43%	18.44%	21.83%	24.21%	30.14%	18.32%
E. vejdovskyi	1.41%	0.76%	1.72%	1.63%	1.41%	1.04%
E. coecicola	2.59%	3.14%	3.72%	6.21%	2.95%	3.71%
E. magna	41.73%	43.64%	41%	42.71%	35.14%	38.54%

n: positive fecal samples

Table 5. Prevalence of local rabbits breed coccidial infection on farms according to the anticoccidial used in Medea province, Algeria

Anticoccidials	Examined no	Positive no	Prevalence %	OPG	χ^2	P value
Colistin+ trimethoprim	150	98	65.3	$[8\times10^2 - 1.5\times10^6]$	72.4	<0.0001
Sulfonamides	176	41	23.3	$[9\times10^2 - 1.7\times10^5]$		
Unknown	88	58	65.9	$[9\times10^2 - 5\times10^5]$		

Examined no: number of fecal samples analyzed, OPG: Oocyst per gram counting

DISCUSSION

Coccidiosis constitutes a major health problem in rabbit breeding affecting mainly young rabbits after weaning (Drouet-Viard et al., 1997b, Pakandl and Hlálsková, 2007). In the present study, the prevalence of coccidia infection in six regions of Medea Province was surveyed. Based on the analysis of 414 faecal samples collected from 50 rabbit farms, the overall infection rate was 47.6 %. Due to the importance of the disease there is a continuous use of coccidiostat sat farm level but in spite of this the prevalence of coccidiosis is still high. This may be explained by maternal transmission of coccidiosis to young rabbits (Henneb and Aissi, 2013; Papeschi et al., 2013). Furthermore, in rabbit breeding, therapy should concern not only the young rabbits but also the nursing females mainly during the week preceding weaning where the contamination from mother to young rabbits takes place (Pakandl and Hlálsková, 2007). The existence of all rabbits' *Eimeria* species was confirmed in these faecal samples. As reported previously, the natural infections with a single *Eimeria* species are rare (Abdel-Baki and Al-Quraishy, 2013; Jing et al., 2012). The prevalence of *E. magna* in Medea province was convergent to the reported by Henneb and Aissi (2013) in Eastern Algeria and Maziz-Bettahar et al. (2018) in Northern Algeria (47.6% versus 43% and 42.5% respectively) which could be due to poor hygiene noticed in Algeria's farms (Henneb and Aissi, 2013). Gonzalez-Redondo et al. (2008) confirmed that a fair control of hygienic conditions is sufficient to maintain a low level of coccidia and also Schlolaut et al. (2013) indicated that housing conditions could have an impact on health of rabbits. *E. magna* and *E. media* which are recognized as mildly pathogenic (Licois et al., 1995; Drouet-Viard et al., 1997a, Drouet-Viard et al., 1997b) were the most predominant in Medea followed by *E. stiedae* but OPG values for these samples were less than concerning clinical coccidiosis. This result indicated that sub-clinical coccidiosis is common in Medea province. In fact sub-clinically infected rabbits looked to be healthy in general, but have reduced feed conversion and growth performance, resulting in huge economic losses as reported by Jing et al. (2012), Okumu et al. (2014) and Yin et al.(2016). The prevalence of coccidian oocysts in weaner rabbits was higher than growing rabbits and adult rabbits, this could be due to lower resistance or incomplete immunity against coccidiosis in young rabbits compared to elder animals as described previously by Licois et al. (1995); Drouet-viard et al. (1997a,b); Pakandl et al. (2008); Pakandl (2009); El-Ghoneimy and El-Shahawy (2017) and Geru et al. (2017).

CONCLUSION

The present survey revealed that rabbit coccidiosis prevalence in Medea Province, Algeria, is not negligible. In fact, an overall infection rate of 47.6% has been registered. The study revealed that *E. magna* and *E. media* were the most predominant species and weanling rabbits were the most infected followed by growing and adult rabbits. Females were more infected than males and coccidiopan seems to be more effective than colisultrix. Knowledge about the prevalence of coccidiosis and current *Eimeria* species will help to evaluate the infection potential and control programs, and therefore minimizing the economic losses caused by this disease.

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DECLARATIONS

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Competing interests

The authors declare that they have no conflict of interests.

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Author's contributions

MSB proposed and conducted the study. MSB and AB drafted and revised the manuscript. ST, HA supervised the work. MSB and AB analyzed the data. All authors read and approved the final manuscript.

REFERENCES

Abdel-Baki AAS and Al-Quraishy S (2013). Prevalence of coccidia (*Eimeria* spp.) infection in domestic rabbits, *Oryctolagus cuniculus*, in Riyadh, Saudi Arabia. Pakistan Journal of Zoology, 45(5): 1329-1333.

Al-Mathal IM (2008). Hepatic Coccidiosis of the Domestic Rabbit *Oryctolagus cuniculus domesticus L*. in Saudi Arabia World Journal of Zoology, 3(1): 30-35.

Bachene MS, Maziz-Betahar S, Temim S, Aissi M and Ain Baziz H (2014). Evaluation of the pathogenicity of *Eimeria Magna* in the rabbit of local population *Oryctolagus cuniculus*. International Journal of Animal and Veterinary Sciences, 8(6): 3.

Bachene MS, Temim S, Ainbaziz H, Bachene A and Suo X (2018). A vaccination trial with a precocious line of Eimeria magna in Algerian local rabbits Oryctolagus cuniculus. Veterinary parasitology, 261: 73–76. DOI:10.1016/j.vetpar.2018.08.013

Coudert P, Licois D and Drouet-Viard F (1995).*Eimeria* species and strains of rabbits, In Biotechnology Guidelines on techniques in coccidiosis research. Eckert J Braun R, Shirley MW, Coudert P, Sc,Eds. Luxembourg: European commission, pp. 52-73.

Drouet-viard F, Coudert P, Licois P and Boivin M (1997a). Acquired protection of the rabbit (*Oryctolaguscuniculus*) against coccidiosis using a precocious line of *Eimeria magna*: effect of vaccine dose and age at vaccination. Veterinary Parasitology, 69: 197-201. DOI: https://doi.org/10.1016/S0304-4017(96)01133-8

Drouet-Viard F, Coudert P, Licois D and Boivin M (1997b). Vaccination against *Eimeria magna* coccidiosis using spray dispersion of precocious line oocysts in the nest box. Veterinary Parasitology, 70(1-3): 61-66. DOI.org/10.1016/S0304-4017(96)01134-X

El-Ghoneimy A and El-Shahawy I (2017). Evaluation of amprolium and toltrazuril efficacy in controlling natural intestinal rabbit coccidiosis. Iranian Journal of Veterinary Research, 18(3): 164-169. DOI : 10.22099/IJVR.2017.4217

Geru T, Wang Y, Li C, Gu X, Cui P, Fang S, Suo X and Liu X (2017). High pathogenicity and strong immunogenicity of a Chinese isolate of *Eimeria magna* Pérard, 1925. Parasitology International, 66(3): 207-209. DOI:10.1016/j.parint.2017.01.014

González-Redondo P, Finzi A, Negretti P and Micci M (2008) Incidence of coccidiosis in different rabbit keeping systems. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 60(5): 1267-1270. DOI:10.1590/S0102-09352008000500034

Henneb M and Aissi M (2013). Etude cinétique de l'excrétion oocystale chez la lapine et sa descendance et identification des différentes espèces de coccidies. 15 th day of research about rabbits, November 19 th – 20 th France: 221-224.

Jing F, Yin GW, LiuXY, Suo X and QinYH (2012). Large-scale survey of the prevalence of Eimeria infections in domestic rabbits in China. Parasitology Research, 110(4):1495-1500. DOI: 10.1007/s00436-011-2653-4

Licois D, Coudert P, Drouet-Viardand F and Boivin M (1995). *Eimeria magna*: pathogenicity, immunogenicity and selection of a precocious line. Veterinary Parasitology, 60: 27-35. DOI:https://doi.org/10.1016/0304-4017(94)00768-8

Maziz-Bettahar S, Aissi M, Ainbaziz H, Bachene MS, Zenia S and Ghisani F (2018). Prevalence of coccidian infection in rabbit farms in North Algeria. Veterinary World, 11(11): 1569-1573. DOI: 10.14202/vetworld.2018.1569-1573

Okumu PO, Gathumbi PK, KaranjaDN, Mande JD,Wanyoike MM, GachuiriCK, KiarieN, MwanzaRN and Borter DK (2014). Prevalence, pathology and risk factors for coccidiosis in domestic rabbits (*Oryctolaguscuniculus*) in selected regions in Kenya. Veterinary Quarterly, 34(4): 205-210.DOI.org/10.1080/01652176.2014.978044

Pakandl M and Hlálsková L (2007). Reproduction of *Eimeria flavescens* and *Eimeria intestinalis* in suckling rabbits. Parasitology Research, 101 (5): 1435-1437. DOI:10.1007/s00436-007-0646-0

Pakandl M, HlaskovaL, Poplstein M, ChromaM, Vodicka T, Salat J and Mucksova J (2008). Dependence of the immune response to coccidiosis on the age of rabbit suckling. Parasitology Research, 103(6): 1265–1271. DOI:10.1007/s00436-008-1123-0

Pakandl M (2009). Coccidia of rabbit: a review. *Folia* Parasitologica, 56(3): 153–166. DOI: 10.14411/fp.2009.019

Papeschi C, Fichi G and Perrucci S (2013). Oocyst excretion pattern of three intestinal *Eimeria* species in female rabbits. World Rabbit Science, 21 (2): 77-83. DOI: 10.4995/wrs.2013.1235

Schlolaut W, Hudson R and Rödel HG (2013). Impact of rearing management on health in domestic rabbits: A review. World Rabbit Science, 21(3): 145-159. DOI:10.4995/wrs.2013.1029

Yin G, Goraya MU, HuangJ, SuoX, Huang Z and Liu X (2016).Survey of coccidial infection of rabbits in Sichuan Province, Southwest China .SpringerPlus, 5:87. DOI: 10.1186/s40064-016-2586-6

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A Review on the Role of Lipid in Selected Apicomplexan, Anaerobic, Kinetoplastid and Intestinal Parasitic Infections

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ABSTRACT

Lipids are a diverse class of biomolecules that play a major role as energy source, membrane components and cellular signaling molecules. Because of the variation in modes of life, different parasites can partly or fully utilized significant amount lipids during infection. The aims of this paper were to provide an overview to the role of lipids in selected apicomplexan, anaerobic, kinetoplastid and intestinal parasitic infections. Lipid particles are fundamentally engaged in host-pathogen interactions like cell signaling and immunity. As a source of eicosanoid production, they are involved in different aspects of innate signaling and antigen presentation for the host organism. For the pathogen, lipid droplets also employed to facilitate attachment, empowering pathogenesis and used to subvert host metabolism as ways of immune evasion. The apicomplexan parasites utilized lipid particles for various purpose including changing permeability and fragility of host cells, support the insertion of parasite into the host cell membrane, and promote growth, invasion and optimal replication of the organism. In anaerobic groups of parasites, the lipid plays a considerable role as growth promoter, increasing virulence, facilitate encystation and vesicle formation as well as initiation of immune system and maturation of dendritic cells. Kinetoplastid also engaged in the uptake of essential lipid particles to produce more complex lipids, develop protective mechanisms against host innate and adaptive immunity and support pathogen survival. The lipid bodies also utilized by the intestinal parasites for disease pathogenesis, differentiation and survival of larvae in the host tissue. This review showed that the different in vivo and in vitro studies indicated that lipids have different role in different stage of the parasites infection. The associations between parasites and the lipids were observed during the attachment, invasion and other stages of parasitic infection. So far, evidences in lipid profile alteration related to different parasitic infection suggested that parasites are able to remodel/metabolize host lipids during the overall pathogenesis of parasitic infection.

Key words: Infection, Lipid, Parasitic, Role

INTRODUCTION

The burden of parasitic disease is enormous. It accounts 1 million deaths per year and 3 billion infections per year experienced worldwide (GBD, 2017). These parasite are residing in various organ and tissue like intestine, blood, liver, lungs, brain, muscles and lymphatic tissues and causing great impact on their habitants (Beaver et al., 2006).

Pathogenic microbes have evolved myriad strategies to evade the host cells and secure a protected environment within their hosts. Some avoid ingestion by the phagocytic cells designed to degrade them and some others promote their uptake and reside within safe compartments inside host cells. It is becoming increasingly apparent that the mechanism by which microbes enter cells can impact their intracellular survival (Shin and Gao, 2000). Their nutritional requirements and means of obtaining and utilizing the nutrients required for growth, motility and reproduction are also varied. Lipid is required for internalization of eukaryotic pathogens under such variable circumstances. It is present in tissues and plasma lipoprotein either as free cholesterol or combined with a long-chain fatty acid, as cholesteryl ester. It is synthesized in many tissues from acetyl-CoA and is ultimately eliminated from the body in the bile as cholesterol or bile salts (Maxfield, 2008).

Lipids are a diverse class of biomolecules that play a major role not only as membrane components, but also as cellular signaling molecules (Aswin et al., 2018). Chemically, lipids can be classified on basis of their head group (choline, ethanolamine, inositol and serine), their backbone (glycerol or sphingosine) or on basis of their lipid anchors (acyl and alkyl) (Zufferey and Mamoun, 2005). Lipid micro domains, including rafts and caveolae, have an important role in the organization of membrane proteins, in cell–cell contact and in numerous signaling processes. They are increasingly being implicated in the interactions between macrophages and parasites. Host cell lipid rafts and other lipid microdomains also serve as targets for disrupting host cell function and indeed, act as a portal of parasite entry (Rodriguez et al., 2006).

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The lipid raft of these micro domains are small, highly dynamic and enriched in cholesterol, glycosphingolipids and signaling phospholipids, which compartmentalize cellular processes (Beaver et al., 2006). These lipid rafts engaged in various activities and played considerable role in organism attachment, engulfing process and signal transduction of disease pathogenesis. During infection, many pathogens utilized host membrane micro domain dependent mechanisms to evade the host immune system (Rodriguez et al., 2006).

Lipid has a vital role in the overall invasion of pathogens. The interactions between lipid-raft and endosomal system is detrimental to pathogens residing within the cells compartment, avoiding of host's defense mechanism and for antimicrobial arsenal. The requirement of lipids in pathogens under such variable circumstances is poorly understood. Thus, the present review highlights the role of lipids and their metabolic products in certain parasitic infections.

General overview

Lipid rafts appear to have many functions, although their complete roles are not well understood. These functions included diverse processes as polarized secretion, membrane transport, transcytosis across epithelial monolayers and the generation of cell polarity (Vallochi et al., 2018). The importance of lipid rafts in signal transduction is highlighted by their enrichment for many signaling molecules such as receptor tyrosine kinases, mitogen-activated protein kinases, adenylyl cyclase and lipid signaling intermediates (Toledo et al., 2016). Although lipid rafts comprise only a small percentage of the cell surface area, their high concentration of signaling molecules makes them a natural target for microbes to communicate with the host cell. Lipid rafts are also known to undergo fission from the plasma membrane mediating a form of endocytosis (Parton et al., 2004). Microbial agents might favor interaction with lipid rafts as a potential way to enter host cells. While utilizing caveolae and other lipid micro domains as sites for microbial action, the biological consequence of this interaction is important for both hosts and pathogens (Rodriguez et al., 2006). The role of lipid rafts in different cell types has been numerous and their physiological significance for cell biology has recently become clear. These membrane regions play an important role in a variety of cellular functions including polarization, signal transduction, endocytosis, secretion, cell–cell and cell–pathogen adhesion (Jacobson, 2007). One of the most widely appreciated roles of lipid rafts is the recruitment and concentration of molecules involved in cellular signaling. The formation of a molecular cluster and their signal transduction machinery in membrane rafts leads to enhanced signaling efficiency (Triantafilou et al., 2002).

An interesting manner that allows pathogens to evade the immune system is through membrane microdomains (Borges et al., 2016). As signaling for the innate and adaptive immune responses initiated in rafts, some pathogens have evolved mechanisms to subvert this signaling by co-opting raft-associated pathways (Melo et al., 2006). Different pathogens can use the host-cell lipid rafts to secure their entrance and maintenance inside the target cells. The benefit provided by interaction with lipid rafts can vary from one pathogen to another (Borges et al., 2016). Parasites interacted with lipid rafts during the disease pathogenesis is indicated by parasitophorous vacuole membranes that contain host raft lipids and proteins. Furthermore, Glycosyl Pphosphatidyl Inositol (GPI)-anchored proteins, such as cluster of Differentiation 55 (CD55) and CD59 which are major inhibitors of membrane complements progressively depleted from the infected cell surface (Nolan et al., 2017).

Lipid role in certain parasitic infestation

Apicomplexan parasites

The presence of hypercholesterolemia and hypertriglyceridemia observed in both uncomplicated and complicated malaria indicated the interaction between lipid molecule and the pathogen (Sabrina et al., 2006; Ross et al., 2009). The amount of octadecenoic fatty acids and cholesterol in the host erythrocyte plasma membrane are highly linked with the lipid metabolism of the parasites to change permeability and fragility of the cells (Bansal et al., 2005).

The difference in concentration of serum High Density Lipoprotein (HDL) indicated reverse effect to the culture of *Plasmodium falciparum*. The low concentration of HDL promote the growth of whereas high concentration was found to be toxic to the organism (Vielemeyer et al., 2004). The plasmodium genome contains genes encoding enzymes for phospholipids metabolism allowing de novo synthesis of phosphatidylcholine via the Kennedy pathway and necessitating the uptake of the small choline molecule (Imrie et al., 2004). During malaria invasion, the host cell membrane rapidly expands around the parasite to form the Parasitophorous Vacuole Membrane (PVM) that support the insertion of parasite lipids into the host cell membrane (Wein.et al., 2018). In this phenomena, relative depletion of intra-membranous particles in the outer leaflet of the PVM is observed in malaria infection (Ross et al., 2009). Host cell cholesterol also implicated during the entry and replication of toxoplasma pathogens. The PVM surrounding *Toxoplasma gondii* utilized cholesterol during entry and invasion through a caveolae independent mechanism (Johnston et al., 2016). This parasite attachment and entrance is greatly impaired when the host cell plasma membrane cholesterol content is depleted (Nolan et al., 2017). *Toxoplasma gondii also* exploits host low density lipoprotein receptor-mediated by endocytosis for cholesterol acquisition and acyl-CoA, cholesterol acyl transferase and cholesterol esters to the optimal

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replication of the organism (Coppens et al., 2008). The successful replication of *T. gondii* within the Parasitophorous Vacoul (PV) requires considerable amounts of selected lipids for membrane biogenesis (Charron and Sibley, 2004). Eventhough *T. gondii* has the ability to synthesize phospholipids, it takes precursors of these lipids from the environment to construct more complex lipids (Gupta et al., 2005). In *Cryptosporidium*, the parasite is unable to de novo synthesize cholesterol, it relies on host cell-derived cholesterol. It is auxotroph for plasma Low Density Lipoprotein (LDL) and derives its cholesterol from the host cells. Moreover, the cholesterol incorporated by *Cryptosporidium* did not only originate from de novo synthesis of the host cell but also from micelles imported via transporter into the cell (Ehrenman et al., 2013).

Anaerobic parasites

In vitro study, the finding indicated that the cholesterol is a growth promoter for *Entamoeba histolytica* and the avirulent strain can be revived by adding cholesterol to culture media (Vallochi et al., 2018). Moreover, replacing bovine serum with a lipoprotein cholesterol solution and bovine serum albumin in pre-encystation and excystation media stimulates *Giardia lamblia* encystations and vesicle formation. This suggests that the parasites utilize cholesterol for their growth from infected individuals (Bansal et al., 2012). In *Entamoeba*, the disruption of cholesterol rich raft like membrane with the cholesterol binding agent (fillipin and methyl-β-cyclodextrin) inhibit several important virulence functions, fluid phase pinocytosis and adhesion to host cell monolayers (Laughlin et al., 2004). In Giardia, membrane biogenesis requires cholesterol. Because *Giardia* is unable to synthesize cholesterol, it obtains this from upper small intestine, which is rich in biliary and dietary cholesterol (Kaneda and Goutsu, 2013). In vitro, the addition of lipoprotein cholesterol solution in pre-encystation and excystation media of *G.1amblia* promote encystation of specific secretory vesicles formation. Cholesterol also regulate the receptor dependent signaling responsible encystations process (Kaul et al., 2011). The HDL in *Trichomonas* have important roles in parasite biology and disease pathogenesis (Gilbert et al., 2009). The addition of purified Lipophosphoglycan (LPG) mediated the adhesion of *Trichomonas* parasites to human vaginal epithelial cells in a dose-dependent manner. The Interleukin 8 (IL-8) is also triggered by LPG to promote inflammatory cell that initiate immune system and maturation of dendritic cell (Raina et al., 2006). Giardia possessing a limited capacity to synthesize lipid molecules depends on host lipids for its growth and differentiation. It has been suggested that most lipids and fatty acids are taken up by endocytic and non-endocytic pathways and used by for energy production and membrane/organelle biosynthesis of the organism (Yichoy et al., 2011).

Kinetoplastid parasite

Among kinetoplastid parasites, *Trypanosomes* bind and take up plasma LDL from host lipids. They require lipoproteins to multiply under axenic culture conditions (Johndrow et al., 2014). The other cholesterol like HDL, LDL, and *Trypanosome* Lytic Factor (TLF) were bound and taken up by a lipoprotein scavenger receptor as the parasite's major pathway mediating the uptake of essential lipids. The role of HDL, LDL and TLF1 also involved for attaching to the surface receptors of Trypanosomes (Green et al., 2003). The lipid particles as sites of prostaglandin E2 synthesis during chagas disease has grate role to the escape mechanism of the parasite against host immunity (Almeida et al., 2018). Lipid bodies (LBs) in *Trypanosoma cruzi* also act as dynamic organelles to respond to host interaction and a potent immune-modulatory that hinder innate and adaptive immunity and support the pathogen survival in its host (Toledo et al., 2016). Microscopic investigation on *Trypanosoma cruzi* indicated the brown fat tissue where lipid bodies are higher in number and smaller in size is the preferred localization to the organism. The parasites disrupt adipokines synthesis in this tissue and used as a site of reservoir (Comb et al., 2005). *Trypanosoma cruzi* parasite present in the adipose tissue biopsy of chronically infected human patients have further confirmed the finding that adipose tissue is the reservoir of chronic *T. cruzi* infection (Matos et al., 2011). In *Leishmania* infection the plasma membrane cholesterol is required for efficient attachment and internalization of the parasite in to macrophages (Rodriguez et al. 2017). Stage-specific binding of different lectins to distinct forms of the *Leishmania* parasite during its cell cycle demonstrates molecular changes in the glycocalyx. Changes in the major component of the promastigote glycocalyx such as in the glycosyl-phosphatidylinositol anchored Lipophosphoglycan (LPG), are important in the defense against lytic activities of the mammalian host. LPG also protects the procyclic promastigote against hydrolases in the midgut of the insect vector (Wilson and Pearson, 2004). Increased infectivity of the metacyclic promastigote is mediated by increased number free fatty acids, cholesterol, sphingomyelin and phosphatidylserine (Hana et al., 2017). In *Leishmania amazonensis* the proliferation of the parasite during cultivated in dilapidated fetal calf serum influenced by the presence or absence of LDL. The media supplemented with LDL cholesterol showed enhanced and sustainable parasite growth (Nuccia et al., 2012).

Intestinal worms

The cestode parasites utilized greater amount total lipid and phospholipid whereas the nematode takes more of neutral lipid and glycolipid by absorbing them from the intestinal fluids (Mondal et al., 2016). The trematode also

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engaged *in* taking up the lipid requirements from host rumen fluid which is required for their reproductive strategy (Ghosh and Misra, 2014). A low level of HDL cholesterol in hookworm, strongyloides and trichuris infected patients evidenced the involvement of lipid particles by the parasites to disease pathogenesis and larvae survive in the host tissue (Wiedermann et al., 2011). The decreased in total cholesterol, HDL and triglycerides observed in guinea pig affected by Ascaris evidenced the utilization of lipid by the parasite. The lipid particles are involving in enhancing larval survival, yield and growth of L4 stage of *Ascaris* (Biadun, 2005). The Phospholipid/cholesterol ratio in liver of golden hamsters infected with *Ancylostoma ceylanicum* showed significantly reduction due to structural and functional disturbance of the membrane by utilization of these biomolecules by the parasite and its hepatotoxic effects (Srivastava, 2004).

CONCLUSION

Lipids are a diverse class of biomolecules that play a major role as energy source, membrane components and cellular signaling molecules. Pathogenic microbes have evolved myriad strategies to evade the host cells to secure a protected environment and reside within safe compartments inside host cells. The interaction between parasites and the lipid contents were observed during penetration, invasion and at various developmental stage of parasitic infestation. The uptake of the lipid particles by the parasites employed for production of more complex lipids, developing protective mechanisms against host innate and adaptive immunity, support pathogen survival, differentiation of larvae stage and promote growth, invasion and optimal replication of the organism. But the mechanism of such interaction remains elusive. A comprehensive lipidomic analysis will be needed for identification and characterization of lipid molecules and enzymes involved in pathways for the proper understanding of the interaction between parasites and lipid molecules. Recent technologies in molecular biology and parasite genome have also be applied to identify the genes and enzymes in lipid metabolic pathways during parasitic infection.

DECLARATIONS

Competing interests

The authors declared that they have no competing interests.

Author contributions

All authors equally involved in searching literature review, write up the paper and critically analyze the core idea of the paper and reviewed the manuscript. Finally, all authors read and approved the final version of the manuscript.

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REFERENCES

Almeida PE, Toledo DAM, Rodrigues GSC and D'Avila H (2018). Lipid bodies as sites of prostaglandin E2 synthesis during chagas disease: impact in the parasite escape mechanism. Front Microbiology, 9:499. Doi:10.3389/fmicb.2018.00499.

Aswin T, Srivatsav MM and Shobhna K (2018). Small-Molecule Modulation of Lipid-Dependent Cellular Processes against Cancer: Fats on the Gunpoint. Biomedical Research International, 2018(1):1-17. Article ID 6437371, 17 pages, Doi:https://doi.org/10.1155/2018/6437371.

Bansal D, Bhat HS and Sehgal R (2005). Role of lipid in parasitic infection. Biomedical central, 4(10):342-349.

Bansal D, Bhatti HS and Sehgal R (2012). Altered lipid parameters in patients infected with Entamoeba histolytica, Entamoeba dispar and Giardia lamblia. British Journal of Biomedical Science, 5:13-15.

Beaver P, Jung R and Cupp E (1984). Clinical Parasitology, 9th edition. Lea and Febiger, Philadelphia, pp. 515-526.

Biadun W (2005). Studies of serum lipids in guinea pigs with larval Ascariasis. Wiad Parazytol, 36:15-26.

Borges TK, Alves ÉA, Vasconcelos HA, Carneiro FP, Nicola AM and Magalhães KG (2017). Differences in the modulation of reactive species, lipid bodies, cyclooxygenase-2, 5-lipoxygenase and PPAR-γ in cerebral malaria-susceptible and resistant mice. Immunobiology, 222:604–19. doi: 10.1016/j.imbio.2016.11.010.

Charron AJ and Sibley LD (2004). Molecular partitioning during host cell penetration by Toxoplasma gondii. Traffic, 5:855-867.

Combs TB, Mukherjee S and De Almeida CJ (2005). The adipocyte as an important target cell for Trypanosoma cruzi infection. Journal of Biological Chemistry, 280(25):4085-4094.

Coppens I, Baudhuin P, Opperdoes FR and Courtoy PJ (2008). Receptors for the Host Low Density Lipoproteins on the Hemoflagellate Trypanosoma brucei: Purification and Involvement in the Growth of the Parasite. Proceedings of the National Academy of Sciences of the United States of America, 85:753-757.

Ehrenman K, Wanyiri JW, Bhat N, Ward HD, and Coppens I (2013). Cryptosporidium parvum scavenges LDL-derived cholesterol and micellar cholesterol internalized into enterocytes. Cellular Microbiology, 15: 1182-1197.

Ghosh D and Misra KK (2014). Comparison of fatty acid contents of the neutral and phospholipids of the trematode *Paramphistomum cervi* and liver of its host, *Capra hircus*. Journal of Parasitic Diseases, 38:223–232. doi: 10.1007/s12639-012-0229-6.

Gilbert RO, Elia DH, Beach S, Klaessig H and Singh BN (2009). Cytopathogenic effect of Trichomonas vaginalis on human vaginal epithelial cells cultured in vitro. Infection and Immunity, 68:4200-4206.

Global Burden Diseases (GBD) (2017). Measuring progress from 1990 to 2017 and projecting attainment to 2030 of the health-related Sustainable Development Goals for 195 countries and territories: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet, 8 Nov 2018: 392.

Green HP, Del Pilar MPM, St Jean EN, Lugli EB and Raper J (2003). Evidence for a Trypanosoma brucei lipoprotein scavenger receptor. Journal of Biological Chemistry, 278:422-426.

Gupta N, Zahn MM, Coppens I, Joiner KA, and Voelker DR (2005). Selective disruption of phosphatidylcholine metabolism of the intracellular parasite Toxoplasma gondii arrests its growth. Journal of Biology and Chemistry, 280:1645-1653.

Hana B, Marion G, Philippe L, Isabelle D and Samira A (2017). Changes in Lipid and Fatty Acid Composition during Intramacrophagic Transformation of Leishmania donovani Complex Promastigotes into Amastigotes. Lipids, 52: 433-441.

Imrie H, Ferguson DJP, Carter M, Drain J, Schiflett A and Hajdur SL (2004). Light and electron microscopical observations of the effects of high-density lipoprotein on growth of Plasmodium falciparum in vitro. Parasitology, 128:577-584.

Jacobson K, Mouritsen OG and Anderson RG (2007). Lipid rafts: at a crossroad between cell biology and physics. Natural Cell Biology, 9:7-14.

Johndrow C, Nelson R, Tanowitz H, Weiss LM and Nagajyothi F (2014). Trypanosoma cruzi infection results in an increase in intracellular cholesterol. Microbes and Infection, 16:337–344. doi: 10.1016/j.micinf.2014.01.001.

Johnston AC, Piro A, Clough B, Siew M, Virreira Winter S, Coers J, and Frickel EM (2016). Human GBP1 does not localize to pathogen vacuoles but restricts Toxoplasma gondii. Cellular Microbiology, 18(8):1056–1064. doi:10.1111/cmi.12579

Kaneda Y and Goutsu T (2013). Lipid analysis of Giardia lamblia and its culture medium. Annals of Tropical Medicine and Parasitology, 82:83-90.

Laughlin RC, McGuan GC, Powell RR, Welter BH and Temesvari LA (2004). Involvement of raft-like plasma memebrane domains of Entamoeba histolytica in pinocytosis and adhesion. Infection and Immunity, 72:5349-5357.

Matos AV, Segatto M and Menezes Z (2011). Evidence for Trypanosoma cruzi in adipose tissue in human chronic Chagas disease. Microbes and Infection, 13 (12):1002-1005.

Maxfield F (2008). Plasma membrane microdomains. Current Opinion in Cell Biology, 14:483-487.

Melo RCN, Fabrino DL, Dias FF and Parreira GG (2006). Lipid bodies: structural markers of inflammatory macrophages in innate immunity. Inflammation Research, 55(8):342–8. doi:10.1007/s00011-006-5205-0

Mondal M, Kundu JK and Misra KK (2016). Variation in lipid and fatty acid uptake among nematode and cestode parasites and their host, domestic fowl: host-parasite interaction. Journal of parasitic diseases. official organ of the Indian Society for Parasitology, 40(4), 1494–1518. doi:10.1007/s12639-015-0718-5

Nolan SJ, Romano JD and Coppens I (2017). Host lipid droplets: an important source of lipids salvaged by the intracellular parasite Toxoplasma gondii. PLoS Pathogenis, 13: e1006362. doi: 10.1371/journal.ppat.1006362

Nuccia NT, Miria G, Jose R, Correa VV, Felipe B and Saraiva C (2012). LDL uptake by Leishmania amazonensis: Involvement of membrane lipid microdomains. Experimental Parasitology, 130:330-340.

Parton RG, Joggerst B and Simons K (2004). Regulated internalization of caveolae. Journal of Cell Biology, 128:1199-1215.

Raina NF, Radiana TT, Robert OG, Catherine EC, Gary RH and John J (2006). Trichomonas vaginalis Lipophosphoglycan triggers a selective upregulation of cytokines by human female reproductive tract epithelial cells. Infection and Community, 74 (10):5773-5779.

Rodriguez NE, Gaur UA and Wilson ME (2006). Role of caveolae in Leishmania Chagasi phagocytosis and intracellular survival in macrophages. Cell Microbiology, 8:1106-1120.

Rodríguez NE, Lockard RD, Turcotte EA, Araújo-Santos T, Bozza PT and Borges VM (2017). Lipid bodies accumulation in Leishmania infantum -infected C57BL/6 macrophages. Parasite Immunology, 39: e12443. doi:10.1111/pim.12443

Ross BM, Markus K, Kaylan S, Wadwat PS, and Rupert SU (2009). The role of lipids in Plasmodium falciparum invasion of erythrocytes: A coordinated biochemical and microscopic analysis. Journal of Cell Science, 102:527-532.

Sabrina S and Adrian BH (2006). Lipid biology of Apicomplexa: perspectives for new drug targets. Trends in Parasitology, 22:41-47.

Shin J, Gao Z and Abraham S. (2000). Involvement of cellular caveolae in bacterial entry into mast cells. Science, 289: 785-788.

Srivastava SC (2004). Liver plasma membrane-bound enzymes and lipids in golden hamsters infected with Ancylostoma ceylanicum. International Journal of Parasitology, 24:249-51.

Toledo DA, D'Avila H and Melo RC (2016). Host lipid bodies as platforms for intracellular survival of protozoan parasites. Front Immunology, 7:174. doi:10.3389/fimmu.2016.00174.

Toledo DAM, Roque NR, Teixeira L, MilánGarcés EA, Carneiro AB and Almeida MR, (2016). Lipid Body Organelles within the Parasite Trypanosoma cruzi: A Role for Intracellular Arachidonic Acid Metabolism. PLoS ONE, 11(8): e0160433. doi: 10.1371/journal.pone. 0160433.

Triantafilou M, Miyake K, Golenbock DTK and Triantafilou K (2002). Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide induced cell activation. Journal of Cell Science, 115:2603-2611.

Vallochi AL, Teixeira L, Oliveira K, Maya-Monteiro CM and Bozza PT (2018). Lipid Droplet, a Key Player in Host-Parasite Interactions. Frontiers in immunology, 9, 1022. doi:10.3389/fimmu.2018.01022

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Vielemeyer O, McIntosh MT, Joiner KA and Coppens I (2004). Neutral lipid synthesis and storage in the intraerythrocytic stages of Plasmodium falciparum. Molecular and Biochemical Parasitology, 135:197–209. doi:10.1016.

Wein S, Ghezal S, Bure C, Maynadier M, Perigaud C, Vial HJ, Lefebvre-Tournier I, Wengelnik K and Cerdan R (2018). Contribution of the precursors and interplay of the pathways in the phospholipid metabolism of the malaria parasite. Journal of Lipid Research 59, 1461- 1471

Wiedermann U, Stemberger H, Unfried E, Widhalm K, Kundli M and Altenriederer M (2011). Intestinal worm burden and serum cholesterol or lipid concentration in a Shipibo population (Peru). International Journal of Medical Microbiology, 275:279-286.

Wilson ME and Pearson RD (2004). Stage-Specific Variations in Lectin Binding to Leishmania donovani. Microbiology, 46:128-134.

Yichoy M, Durate TT, Deachatterjee A, Mendez TL, Aguilera KY and Roy D (2011). Lipid metabolism in Giardia: a post-genomic perspective. Parasitology, 38 (3) 267-78.

Zufferey R and Mamoun CB (2005). The initial step of glycerolipid metabolism in Leishmania major promastigotes involves a single glycerol-3-phosphate acyltransferase enzyme important for the synthesis of triacylglycerol but not essential for virulence. Molecular microbiology, 56:800-810.

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Productive and Reproductive Performance and Metabolic Profile of Barki Ewes Supplemented with Two Forms of Probiotics as Feed Additives

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ABSTRACT

Present study aimed to evaluate the impacts of probiotic mixtures as a biological feed additive on the reproductive and productive performance of Barki ewes under desert conditions. A total number of 100 Barki ewes were randomly assigned and divided into five equal groups (20 each) to evaluate the effect of different levels and forms of biological additives mixtures on Barki ewes productivity. The first mixture of probiotic added as liquid forms (Mixture Probiotic Liquid, MPL), while the second added as powder forms (Mixture Probiotic Powder, MPP). The two forms of enzymes used at two levels (6 and 10ml or g/h/d). The two additives formed of exogenous enzymes and obtained through an anaerobic fermentation process of *Ruminococcus flavefaciens*. The results indicated that feed intake was higher in MPL and MPP treated groups compared to control group. During pregnancy and lactation stages, MPL and MPP groups recorded significantly increase in ewes body weight. The conception and lambing rates were tended to differ between groups, but the number of lambs born alive was significantly higher in MPP groups [19 lambs for group 2 (G2) and 18 lambs for group 3 (G3)] followed by MPL groups (16 and 18 lambs for group 4 (G4) and group 5 (G5), respectively), while the control group recorded 18 lambs. The mortality rate from birth to weaning decreased ($P<0.05$) in treated groups with 5%, 5%, 0% and 5% for G2, G3, G4 and G5, respectively, while the mortality rate increased ($P<0.05$) by 11% in control group. The milk yield tended to increase in MPP then MPL groups. The birth and weaning weights as well as average daily gain increased ($P<0.05$) in MPL and MPP groups. Thyroid hormones T_3 and T_4 concentrations increased ($P<0.05$) with enzymes mixtures supplementations. In conclusion, under the semi-arid conditions, supplementation of exogenous enzyme preparations of MPL and MPP to sheep rations, may improve weaning weight and daily gain of lambs as much as live body weight and milk production of ewes.

Key words: Biological additives, Productive performance, Reproduction, Milk, Barki sheep

INTRODUCTION

The economical and efficient production of sheep for meat, milk and others products depended on proper feeding, husbandry practices, and health care. All of these are influenced by dietary intake. The nutritional requirements for maintenance, reproduction, growth, finishing, and other production are complex because sheep are maintained under a wide variety of environmental conditions. However, attempts should be made to ensure that each production unit or individual sheep has adequate nutrient intake to be healthy and productive.

Worldwide demand for animal based products is increasing at a booming rate thus, emphasizing the necessity of applying strategies to improve animal productivity (Sujani and Seresinhe, 2015). The major constraints in today's livestock sector are high feed cost and low quality of available feed resources, especially in tropical developing countries (Fahmy et al., 2010 and Helal et al., 2018a). In arid and semi-arid regions small ruminants have a great importance (Helal et al., 2018b). They are often raised on grazing forage with little or no supplementation (El Shaer, 2004 and Aziz, 2009). Nutritional status is a major factor influencing an animal's ability to maintain health and reproduction (Grazul-Bilska et al., 2007). Over the years, animal nutritionists have developed various physical, chemical and biological methods to overcome the problems associated with livestock feed stuffs. As a biological treatment, method of utilizing exogenous enzymes has attracted attention of researches and it has become a widely discussed theme among animal nutritionists (McAllister et al., 2003). Abo Bakr (2012) suggested that enzymes can improve nutritive value of low quality roughage and bridging the feed gap for small ruminant nutrition.

The mixture of probiotic, Mixture Probiotic Liquid (MPL) and Mixture Probiotic Powder (MPP), which prepared from anaerobic bacterium, has been improved rumen fermentation, nitrogen balance, nutrient digestibility and milk yield of cows fed rations containing Egyptian by-product feeds (Gado et al., 2007), as well as gaining live weight and feed conversion of wheat straw in sheep and goats (Gado and Salem, 2008). The probiotic mixture containing enzymes such

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as cellulases, xylanases, α-amylase and proteases from an anaerobic bacterium, showed a positive effect on ruminant performance and nutrient utilization of low quality roughage *in vivo* (Gado, 1997) and *in vitro* (Gado et al., 2007).

Proposed modes of action of direct-fed enzymes include solubilization of dietary fiber before ingestion, provision of readily fermentable substrate for ruminal microorganisms and/or enhancement of microbial enzyme activity in the rumen (McAllister et al., 2001). Gado et al. (2009) observed that the addition of enzymes increased intake of Dry Matter (DM). Organic Matter (OM) was positively influenced by supplementation. Digestibility of all nutrients was higher in the total tract of supplemented cows with 40g of MPP/h/ day, although the magnitude of the improvement varied among nutrients, with the highest improvement was in Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) than other nutrients.

Sheep in North Western coast of Egypt may play a significant role in improving the animal production sector in the country (Abdalla et al., 2015). Because the farmers are able to provide its requirements and the simplicity of selling sheep especially in Religious festivals. One of the major Egyptian sheep breed is Barki (Ibrahim et al., 2018), which is highly adapted to local conditions (Nassar et al., 2017). It is the smallest breed, with white wool and a brown neck. Purebred Barki is the choice of Bedouins in the desert. All are fat-tailed sheep. What distinguishes fat-tailed sheep from other sheep is their long tails, filled with fat and having a function similar to the camel's hump. These sheep are raised mainly for lamb production, followed by wool and milk (Helal et al., 2018b).

The objective of this study was to evaluate the impacts of two forms and levels of probiotic mixture (multi-enzyme preparations, bacteria, …etc.) as a biological feed additives supplemented to sheep rations and its effect on the reproductive and productive performance as well as milk production and composition of Barki ewes under the environmental conditions of North Western coast of Egypt.

MATERIALS AND METHODS

Experimental region

The present study was carried out at the Animal Production Unit in the Sustainable Development Center for Matrouh Resources, Matrouh governorate, belongs to Desert Research Center in the North Western Coast of Egypt.

Ethical approval

This experiment was performed according to all ethics and animal rights (Desert Research Center). As much as this work had considering all rules and regulations in conformity with the European union directive for the protection of experimental animals (2010/63/EU).

Experimental animals, rations and probiotic mixture

One hundred Barki ewes, 2-3 years old and 38.9 ± 1.02 kg average body weight were used. Animals were kept in individual pens roofed with wood, and were clinically healthy and free from internal and external parasites. The MPL is a liquid multi-enzyme preparation produced from *Ruminococcus flavefaciens* at the concentration of 28×10^4 CFU with one gram of corn flour per each gram of the additive, while the MPL contains 11×10^3 CFU of the same bacteria with one ml of water per each ml of the additive. The exogenous enzyme of MPL is a biotechnical solution product made from natural sources to elevate the level of cellulose enzyme from anaerobic bacteria and contained some specific enzymes such as cellulose (8.2 µ/g), hemicellulose (6.2 µ/mg), amylase (64.4 µ/g) and protease (12.3 µ/g). While, MPP is a biotechnical powder product contained similar enzymes of MPL plus bacteria and *Saccharomyces cerevisiae* yeast.

Biological additives of multi-probiotic (i.e., MPL or MPP) were mixed well with concentrated rations before feeding. Rations were adjusted biweekly according to weight of animals to cover their requirements during their different physiological status according to Kearl (1982). Animals were fed three weeks as a transitional period, on the tested rations before the start of the experiment. Fresh water was available to all groups daily. Samples from roughage and concentrate mixture were taken monthly to determine their chemical composition according to AOAC (1990) (Table 1). Mating season started in May month and lasted for 34 days (equal to two estrous cycles). Five fertile rams were allowed to rotate among different ewe groups to avoid ram/group confounding effect. Rams were fed the control concentrate ration and removed from the ewe groups at early morning before offering rations. Once lambing took place, the born lambs were ear tagged and weighed to record their birth weight and then biweekly till weaning that took place at three months old then weaning weight was recorded and adjusted for 90 days.

Table 1. Chemical composition of concentrate feed mixture and bean straw

Items	DM	Chemical composition on DM basis (g /kg DM)							
		OM	CP	NDF	ADF	EE	NFE	TDN*	DCP**
Concentrate feed mixture	914.3	876.7	123.5	430.1	250.3	23.4	599.1	692.11	79.53
Bean straw	907.1	842.7	64.7	598.8	428.9	15.1	428.0	522.47	24.91

CFM: concentrate feed mixture; BS: bean straw; DM: dry matter; OM: organic matter; CP: crude protein; NDF: Neutral detergent fiber; ADF: Acid neutral detergent fiber; EE: ether extract; NFE: nitrogen free extract; *calculated total digestible nutrient according to Adams et al., (1964); ** calculated digestible crude protein according to Shaltout et al., (2012).

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Experimental design

Animals were randomly selected and divided into five equal groups (20 individuals each). The first group severed as control group and were fed on Concentrate Feed Mixture (CFM) without any additives, the second and third groups were fed CFM supplemented with 6 and 10 g/h/d from MPP, respectively. While the fourth and fifth groups were fed CFM supplemented with 6 and 10 ml/h/d MPL, receptively. All groups were offered same roughage as Bean (*Phaseolus vulgaris*) straw and CFM according to physiological status.

Blood sampling and hormonal assay

Blood samples were collected from all the ewes through vein puncture (using clinical needle) twice (on day 130 and 140 of pregnancy) at eight am before the morning feeding. Blood samples were centrifuged at 3000 rpm for 20 minutes for the separation of serum and kept at -20ºC until further analysis. Thyroid hormones (T3 and T4) were measured by ELISA method using immunospec kits (immunospec corporation, 7018 Owensmouth Ave, suite 103 Canoga park, CA 91303, USA).

Milk samples and analysis

Milk samples (50 ml) were taken biweekly from individual ewes within the respective groups during 12 weeks lactation period, in plastic bottle and kept under -20ºC for further analysis. Milk yield were recorded biweekly from lambing up to 12 weeks lactation period, representing early (up to the fourth week), mid (up to the eighth week) and late lactation periods (up to the 12th week), using lamb-suckling technique (Mousa and Shetawi, 1994) plus hand milking. Lambs were separated from their mothers at 8.00 p.m. on the day before measuring milk production. In the following day, lambs were weighed at 8.00 a.m., and left to suckle their dams till satisfaction, then reweighed and kept away from their mothers. While, the residual milk in the udder of each ewe was hand milked and weighed. Chemical composition of milk in terms of fat, protein, lactose, total solids and solids not fat was determined using milk scan (MilkoScan FT6000 and Bentley, Belguim).

Statistical analysis

A general linear method procedure (SAS, 2004) was used for the statistical analysis of this experiment using the following two models:

Model one for lamb weights, milk composition and thyroid hormones:

$Y_{ij} = \mu + T_i + e_{ij}$

Where,

Y_{ij} = Any observation of jth animal within ith treatment

μ = Overall mean

T_i = Effect of ith treatment (i = 1-5, 1 = CFM without additives, 2 = CFM plus 6 g/h/d MPP, 3 = CFM plus 10 g/h/d MPP, 4 = CFM plus 6 ml/h/d MPL, 5= CFM plus 10 ml/h/d MPP)

e_{ijk} = Experimental error

Model two for feed intake and milk yield:

$Y_{ijk} = \mu + T_i + S_j + (T*S)_{ij} + e_{ijk}$

Where,

Y_{ij} = Any observation of kth animal within jth treatment within ith stage

μ = Overall mean

T_i = Effect of ith treatment (i = 1-5, 1 = CFM without additives, 2 = CFM plus 6 g/h/d MPP, 3 = CFM plus 10 g/h/d MPP, 4 = CFM plus 6 ml/h/d MPL, 5= CFM plus 10 ml/h/d MPP)

S_i = Effect of ith stage (j = 1-3, 1 = Early, 2 = Mid, 3 = Late)

$(T*S)_{ij}$ = The interaction between treatment and stage

e_{ijk} = Experimental error

All statements of significance are based a probability of less than 0.05. Significant differences among means were detected using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Feed intake and live body weight changes of Barki ewes

Feed intake of ewes during pregnancy and lactation periods did not differ ($P<0.05$) among MPL and MPP ewe groups. The MPL and MPP supplementation increased ($P<0.05$) body weights in all stages. Ewes of MPL groups recorded the best values of body weight during all stages followed by MPP groups compared to control group. The highest value from all stages in body weight changes was observed in G3 (with 10 g/h/d of MPP) with values 7.9 kg (Tables 2 and 3).

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Feed intake is a factor which should be closely linked to the sheep nutrient requirements. However, variations in production levels resulting from the physiological trend of the lactation curve are not always associated with corresponding intake changes. The present study demonstrates how dry matter and crude protein intake numerically changed during pregnancy and lactation periods. During late pregnancy, the level of these parameters is much higher than during early pregnancy and then during lactation this level falls again. The present results also showed that both MPP and MPL treatments possessed a significant effect on productivity, reproductive performance and metabolic profiles of Barki ewes. The results obtained concerning the utilization of dry matter and other nutrients in groups with MPP and MPL were in agreement with those reported by Saleem et al. (2017) who obtained that dry matter intake nutrients digestibility of lambs were positively influenced by adding different levels of probiotics supplementation. Also, Gomaa et al. (2016) found that treated rice straw by bacterial enzymes (MPL) led to improve total digestible nutrient, digested crude protein and nitrogen balance of sheep. Gado et al. (2007) showed that the MPP enzyme product improved *in vitro* dry matter digestibility of wheat straw in the first 24h of incubation. The MPL and MPP forms were rich in xylanolytic, cellulose, α-amylase and protease activity, has positive effects on digestion. In the present study, increasing total feed intake may be due to the increase in body weight during different physiological stages. Kholif et al. (2017) suggested that ration supplemented by 4 gram fibrolytic enzyme per head per day showed insignificant raised for live body weight. Generally, total feed intake in all groups covered nutrient requirements of ewes according to physiological status and under desert conditions.

Ewes' body weight varied throughout the year and it changes with stage of production. In this regard, physiological stages (pregnancy, lactation …) greatly influence the body weight of ewes. In general, the ewes during the period of early lactation have lower body weight than the other periods and maximum weight was observed in the period of late pregnancy. Body weight changes from initial weight in early and late lactation period showed an increase in MPL and MPP groups compared to the control group. These results indicated that rations supplemented with biological additives (i.e., MPL and MPP) have positive effects on body weight changes during different physiological stages. Sabbah et al. (2009) and Helal et al. (2018a) suggested that higher Crude Protein (CP) digestibility and dry matter intake led to increase in body weights in Shami goats fed diets supplemented with MPL and MPP probiotic. Gado et al. (2009) reported that an exogenous enzyme of MPP has been shown to have marked positive effects on increasing the total microbial population in the rumen and increased microbial protein synthesis in sheep. However, they found that supplemental enzymes had no effects on nutrient intakes or growth performance of lambs in term of growth rate, final body weight and total gain. The present study clarifies that ewes fed on poor quality roughage and improved rumen ecosystem by feed additives for instance exogenous enzymes gives the best results with the promise animals.

Table 2. Effect of probiotic treatments on feed intake of Barki ewes during pregnancy and lactation periods

Items	G1	G2	G3	G4	G5	SEM	P value
Dry matter intake during early pregnancy							
Average BW (kg)	37.0	36.7	36.4	38.8	38.3	0.586	0.625
Roughage (g/h/d)	638	633	628	670	661	10.11	0.423
CFM (g/h/d)	425	422	418	446	441	6.741	0.233
Total (g/h/d)	1063	1055	1046	1116	1102	16.85	0.133
Total CPI (g/h/d)	93.8	93.1	92.3	98.5	97.3	1.487	0.333
R:C ratio	60:40	60:40	60:40	60:40	60:40	-	-
Dry matter intake during late pregnancy							
Average BW (kg)	40.9	40.8	41.6	42.6	42.4	0.616	0.859
Roughage (g/h/d)	606	604	616	630	628	9.124	0.234
CFM (g/h/d)	909	906	923	945	941	13.68	0.416
Total (g/h/d)	1515	1510	1539	1575	1569	22.81	0.128
Total CPI (g/h/d)	151.6	151.0	153.9	157.6	156.9	0.228	0.614
R:C ratio	60:40	60:40	60:40	60:40	60:40	-	-
Dry matter intake during early lactation							
Average BW (kg)	35.3	35.6	36.0	38.8	38.0	0.532	0.258
Roughage (g/h/d)	885	892	903	959	951	13.31	0.124
CFM (g/h/d)	590	595	602	640	634	8.874	0.331
Total (g/h/d)	1475	1487	1505	1599	1585	22.18	0.422
Total CPI (g/h/d)	130.1	131.2	132.8	141.1	139.9	1.957	0.341
R:C ratio	40:60	40:60	40:60	40:60	40:60	-	-
Dry matter intake during late lactation							
Average BW (kg)	36.1	36.9	37.1	39.1	39.2	0.539	0.242
Roughage (g/h/d)	800	818	823	869	871	11.98	0.151
CFM (g/h/d)	534	545	549	579	580	7.986	0.534
Total (g/h/d)	1334	1364	1371	1448	1451	19.96	0.452
Total CPI (g/h/d)	117.7	120.3	121.0	127.8	128.0	1.762	0.641
R:C ratio	40:60	40:60	40:60	40:60	40:60	-	-

G1: the control concentrate ration; G2: concentrate ration with 6 g mixture probiotic powder (MPP) /h/d; G3: concentrate ration with 10 g MPP /h/d; G4: concentrate ration with 6 ml mixture probiotic liquid (MPL) /h/d; G5: concentrate ration with 10 ml MPL /h/d; BW: body weight; g/h/d: gram per head per day; CFM: concentrate feed mixture; BS: bean straw; CPI: crude protein intake; R:C ratio: roughage to concentrate ratio.

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Table 3. Effect of probiotic treatments on live body weight changes of Barki ewes during pregnancy and lactation periods

Items	G1	G2	G3	G4	G5	SEM	P value
IBW	34.9	33.4	33.1	35.0	35.0	1.31	0.882
Early pregnancy weight	37.0	36.7	36.4	38.8	38.3	1.33	0.633
BW changed from IBW	2.03^{a}	2.96^{b}	2.68^{ab}	3.85^{c}	3.36^{bc}	0.208	<0.001
Late pregnancy weight	40.9	40.8	41.6	42.6	42.4	1.41	0.857
BW changes from IBW	6.00^{a}	7.07^{ab}	7.88^{b}	7.60^{ab}	7.44^{ab}	0.462	0.037
Early lactation weight	35.3	35.6	36.0	38.3	38.0	1.22	0.258
BW changes from IBW	0.37^{a}	1.88^{ab}	2.32^{b}	3.33^{b}	3.00^{b}	0.441	<0.001
Late lactation weight	36.1	36.9	37.1	39.1	39.2	1.24	0.242
BW changes from IBW	1.12^{a}	3.12^{b}	3.35^{b}	4.17^{b}	4.25^{b}	0.437	<0.001

G1: the control concentrate ration; G2: concentrate ration with 6 g mixture probiotic powder (MPP) /h/d; G3: concentrate ration with 10 g MPP /h/d; G4: concentrate ration with 6 ml mixture probiotic liquid (MPL) /h/d; G5: concentrate ration with 10 ml MPL /h/d; BW: body weight (kg); IBW: initial body weight; Different superscripts (a, b, c) within each row indicate significance (P<0.05).

Reproductive performance of Barki ewes

There were no significant differences (P<0.05) between the studied ewe groups in the measured reproductive parameters. Only in case of a few reproductive parameters such mortality, abortion rate (% of pregnancies), % of conception rate and % of lambing rate. Addition of studied enzymes decreased (P<0.05) mortality rate from birth to weaning (5.3 – 5.6%) compared to control group, where it was much higher (11.8%). The G4 group with 6 ml/h/d MPL showed the lowest values of conception rate, number of ewes lambed, lambing rate and number of born alive lambs. In this group, no lamb mortality was founded compared with other experimental groups (Table 4).

Table 4. Effect of probiotic treatments on reproductive performance of Barki ewes in the North Western Coast of Egypt

Items / Reproductive performance	G1	G2	G3	G4	G5	P value
Number of ewes joined	20	20	20	20	20	
Number of ewes conceived	18	19	19	17	19	
Conception rate (%)	90	95	95	85	95	0.732
Number of ewes aborted	0	1	0	1	0	
Abortion rate (% of pregnant)	0	5.3	0	5.9	0	0.430
Number of still births	1	0	1	1	1	0.763
Number of ewes lambed	17	18	18	15	18	
Lambing rate (%)	85	90	90	75	90	0.630
Number of lamb born alive	17	19	18	16	18	
Number of lamb weaned	15	18	17	16	17	
Lamb mortality (%)	11.8	5.3	5.6	0.0	5.6	0.593

G1: the control concentrate ration; G2: concentrate ration with 6 g mixture probiotic powder (MPP) /h/d; G3: concentrate ration with 10 g MPP /h/d; G4: concentrate ration with 6 ml mixture probiotic liquid (MPL) /h/d; G5: concentrate ration with 10 ml MPL /h/d.

In sheep production, reproductive performance is a feature of great importance especially when the meat production from small animals is the main objective. In general, the more demanding the meat production system, the more needed the production of large numbers of the offspring per breeding female. In terms of reproductive wastage, lamb losses represent a serious problem because all investments made for ewes to conceive and maintain pregnancy are wasted. In this study, 100 ewes were joined in mating season, eight ewes from 100 were barren (two in G1, one in G2, one in G3, three in G4 and one in G5). However, supplementations of MPL and MPP enzymes have positive effects on the health condition of ewes and their lambs (Table 4). The same results obtained by Abdalla et al. (2012) who found that reproductive parameters were increased in Ossimi ewes fed diet supplemented with MPP (15 g/h/d) or fed diet supplemented with melatonin (3 mg/h/d) MPP plus (15 g/h/d) compared to the control group. In a study of Abo-Bakr (2012), goats fed diet with MPL (one L/ton wheat straw) showed a high percentage of conception and kidding rats, while goats fed diet with MPP (20 g/h/d) showed the lowest percentage of conception rate compared to the control group. The MPP and the control group recorded the same percentage of kidding rate (80%). They also found that the control group recorded the highest mortality rate from birth to weaning (28.6%), while MPL and MPP treated groups recorded 15.8 and 16.7%, respectively, which support the present results.

However, conception is the point in time when the sperm fertilizes the ova and the conception rate is usually considered to be the number of ewes that lambed compared to the number of ewes joined. Using this broad definition of conception rate, more factors can influence the results, because both fertility and embryo loss can be included in the outcome. Conception rates in the present study were the highest among ewes that had addition of biological probiotics, except one group (G4 – 6 ml/h/d of MPL) compared to the other treatments. Abortions in ewes are the result of many factors that stress the pregnant animal. Intrauterine infections are the most common cause. It appears normal for about

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1.5 to 2.0% (up to 5%) of the ewes in a flock to abort. Many farmers accept abortion rates of between 5-10%. In the present experiment, this parameter ranged between 0.0 and 5.9. Percentage of lambing is still the main important factor in determining profit and even a small improvement will increase the income. Low lambing rates represent a major obstacle to the sheep industry. Regardless of G4, the highest lambing rate was in the case of ewe fed with MPL and MPP. The number of new born lambs per ewe joined is certainly an economically important trait in a commercial sheep initiative. Lamb mortality occurs in any flock, because there are so many reasons affect survival rate of lambs, it is difficult to determine the causes that could affect lamb survival in the flock. Many factors influence and potentially cause problems in these areas - nutrition of the ewe during gestation, sanitation, ventilation, size of litter and birth weight. In the treatment group of the present study, the lamb mortality was around 5%. It was much less than that of the control group.

Birth and weaning weights of Barki lambs

Birth, weaning weights and average daily gain were increased (P<0.05) in all treated groups. G4 and G5 groups recorded the best (P<0.05) birth weights with values being 3.19 and 3.33 kg for G4 and G5, respectively, weaning weight (22.0 and 22.0 kg) and average daily gain was 209 and 207 g, respectively. The weaning weight was on the same level, regardless of the amount of additive of MPP and MPL. The same result was observed by Saleem and Zanouny (2016) as they noted that addition of different level (0.5 or 1 gram/head/day) of probiotic led to insignificant increase of daily gain for weaned lambs. The obtained results showed that the larger addition of biological enzymes slightly decreased (P<0.05) average daily gain (Table 5). The lamb weight of at birth has a great impact on the ability of the lamb to survive. Nutrition of the ewe at the end of gestation and the number of lambs per ewe are the two main factors affecting the weight of the lambs. In addition, the present results showed that higher value of lamb birth weight, weaning weight and average daily gain were recorded in MPL and MPP groups in compared to the control group. Improvement of average daily gain and weaning weight in treated groups are probably due to increased ruminal fermentation activities and nutrient digestion (Gado et al., 2011), improve synthesis of microbial protein that resulted in increased post-ruminal amino acids supply (El-Katch et al., 2016) or may be due to the highest milk production for their mothers. Thakur et al. (2010) investigated the effect of supplementing exogenous enzymes on the growth performance in buffalo calves. They reported that high dose of enzymes enhanced the average body weight and average daily gain. In Previous study carried out by Titi and Lubbadeh (2004), the influence of fibrolytic enzyme treatment on birth and weaning weights of Awassi lambs and Shami kids was investigated. They found that there were no significant differences in average birth weight between control and enzyme treated groups for both lambs and kids. However, weaning weight of lambs from ewes fed enzyme supplementation diets were higher (P<0.05) than those of the control group, while no differences were observed between treated and control groups in weaning weights of kids. Probiotic is usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and increased flow of microbial protein from the rumen which may be beneficial for lambs live weight (Salem et al., 2004). On the other hand, Bueno et al. (2013) evaluated the effect of high doses of exogenous enzymes on lamb performance, and doses were 0, 5, 10g of enzymes per one kg oat straw. They reported that increasing enzyme doses resulted in decreasing enzyme treatment intake without changing the weight gain of lambs.

Table 5. Effect of probiotic treatments on productive performance of Barki lambs in the North Western Coast of Egypt

Traits	G1	G2	G3	G4	G5	SEM	P value
Birth weight (Kg)	2.88^{a}	3.04^{ab}	3.17^{ab}	3.19^{ab}	3.33^{b}	0.094	0.012
Weaning weight, (Kg)	20.0^{a}	21.5^{b}	21.5^{b}	22.0^{b}	22.0^{b}	0.39	0.002
Average daily gain, (Kg)	0.190^{a}	0.205^{b}	0.203^{ab}	0.209^{b}	0.207^{b}	0.0041	0.008

G1: the control concentrate ration; G2: concentrate ration with 6 g mixture probiotic powder (MPP) /h/d; G3: concentrate ration with 10 g MPP /h/d; G4: concentrate ration with 6 ml mixture probiotic liquid (MPL) /h/d; G5: concentrate ration with 10 ml MPL /h/d; Different superscripts (a, b, c) within each row indicate significance (P<0.05).

Thyroid hormones

Concerning thyroid activity, G3 group (10g/h/d MPP) recorded the highest (P<0.05) value of T_3 (1.72 ug/dl), while G5 group (10ml/h/d MPL) recorded the highest (P<0.05) value of T_4 (7.34 ug/dl). Moreover, all treated groups had higher (P<0.05) values of two thyroid hormones than the control group (Table 6). Thyroid hormones, which are mainly function in the regulation of tissue growth and metabolism, are affected by many factors including nutrition (Baruah et al., 1993). Many studies reported a marked seasonal variation in thyroid activity and in blood thyroid hormone concentration (Chavhan and Dhamani, 2013; Dardente et al., 2014 and Qasim et al., 2018). These hormonal differences in free and grazing animals are particularly important that major physiological functions (feed intake, reproduction and hair growth) are outstandingly seasonal. This is the situation with the traditional breeding of small ruminants. In fact, such differences in hormone concentrations allow animals to adjust their metabolic balance with different environmental

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conditions, differences in nutrient requirements and availability, and to homeoretic changes during different physiological stages (Ganong, 1995). Data of the present study showed that thyroid hormones (T_3 and T_4) were tended to increase in groups fed rations supplemented with MPL and MPP. Groups 3 and 5 recorded the highest values of T_3 and T_4 hormones, while the control group recorded the lowest values of thyroid hormones. Animals fed rations supplemented with the level of 6 g or ml from MPL and MPP showed nearly concentrations of T_3 and T_4 hormones that revealed by control group. The increase in thyroid hormones in ewes fed ration supplemented with probiotic mixture as compared to the control group indicated that these probiotics may be due to the increased protein level, improve dry matter and other nutrient digestibility (Gado et al., 2009). Mohamed and Abou-Zeina (2008) who found that T_3 and T_4 concentration were higher in goats fed diets supplemented with high doses of exogenous enzyme compared to the low level and the control group. They suggested that the enzymes directly or indirectly promoted an enhanced activity of deiodinase in liver and kidney tissues, promoting the transformation of T_4 into the biologically active hormone (T_3).

Table 6. Effect of probiotic treatments on thyroid hormones concentrations of Barki ewes in the North Western Coast of Egypt

Thyroid hormone	G1	G2	G3	G4	G5	SEM	P value
T_3 (ng/ml)	1.57	1.58	1.72	1.60	1.63	0.054	0.327
T_4 (µg/ml)	7.01	7.05	7.29	7.20	7.34	0.263	0.873

G1: the control concentrate ration; G2: concentrate ration with 6 g mixture probiotic powder (MPP) /h/d; G3: concentrate ration with 10 g MPP /h/d; G4: concentrate ration with 6 ml mixture probiotic liquid (MPL) /h/d; G5: concentrate ration with 10 ml MPL /h/d.

Milk yield and composition

The present treatments tended to increase (P<0.05) the total milk yield of all treated ewe groups. The best results in this case were observed in MPP (G2 and G3). Milk yield increased (P<0.05) with the supplementation of probiotic during the early, mid and late of lactation period. These results agreed with Refat et al. (2018) who reported that pretreating dairy cows' rations with fibrolytic enzyme led to increase milk production. However, the value of milk composition was the highest in G4 group (6 ml/h/d MPL). Other treatment groups were lower in comparison with the control group (Table 7). Supplementation of probiotic preparation (i.e., MPP or MPP) did not affect (P<0.05) the total milk yield but it improved (P<0.05) the milk yield during the early, mid and late of the lactation stages. The improvement (P <0.05) was in MPP supplemented groups for G2 and G3, respectively, followed by MPL supplemented groups for G4 and G5, respectively, while milk production in the control group recorded the lowest value. The present study showed that treated groups (P<0.05) increased in milk production in early and mid-lactation stages as compared to the control group. Mid lactation recorded high milk production followed by the early stage, while late stages recorded the lowest values in all groups. These results are in agreement with those reported by Titi and Lubbadeh (2004) who found that milk production was increased (P<0.05) by 10-12% affected by the enzyme supplementation in Awassi ewes and Shami goats. They suggested that the improved milk production in ewes and goats could be attributed to the increase total tract dry matter digestibility. Moreover, the improvement of milk production by exogenous enzyme supplementation for dairy cows fed alfalfa hay or total mixed ration was a direct result of increased feed digestibility rather than to increased feed intake and energy available for milk production (Yang et al., 2000).

Table 7. Effect of probiotic treatments on ewes milk yield and milk composition (%) during lactation period (12 weeks)

Traits	G1	G2	G3	G4	G5	SEM	P-
Milk yield (ml/h/d)	510	584	599	543	556	34.12	0.575
Early (ml/h/d)	502^{a}	604^{b}	639^{b}	550^{ab}	577^{b}	28.16	0.001
Mid (ml/h/d)	537^{a}	646^{b}	634^{b}	582^{b}	594^{b}	25.02	0.001
Late (ml/h/d)	492^{a}	504^{b}	524^{b}	499^{b}	499^{b}	22.04	0.001
Milk composition							
Fat (%)	3.98^{a}	3.40^{ab}	3.52^{ab}	3.96^{a}	3.25^{a}	0.212	0.055
Protein (%)	3.77	3.68	3.75	3.90	3.84	0.132	0.798
Lactose (%)	5.02	5.03	5.06	5.09	5.06	0.046	0.823
Total solids (%)	13.53	12.85	13.08	13.78	12.97	0.279	0.105
Ash (%)	0.76^{b}	0.74^{b}	0.75^{b}	0.83^{a}	0.82^{a}	0.011	<0.001

G1: the control concentrate ration; G2: concentrate ration with 6 g mixture probiotic powder (MPP) /h/d; G3: concentrate ration with 10 g MPP /h/d; G4: concentrate ration with 6 ml mixture probiotic liquid (MPL) /h/d; G5: concentrate ration with 10 ml MPL /h/d; ml/h/d: millilitre per head per day; Different superscripts (a, b, c) in a row indicate significance (P<0.05).

Moreover, Morsy et al. (2016) reported that milk production was increased in Egyptian buffaloes fed diets supplemented with Veta-Zyme plus or Tomoko enzyme product. Gado et al. (2009) found that milk production was higher in enzyme supplemented cows compared to control cows (12.75 vs. 15.70 kg/h/d) for control and supplemented group. In Shami goats, Abo Bakr (2012) found that milk yield was higher in goats fed wheat straw treated with MPL solution (1.07 L/h/d) followed by goats fed wheat straw treated by 3% molasses plus 3% urea and added 20 g/h/d of MPP . Also, he reported that there is insignificant increase in milk production between two groups fed rations

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supplemented with two forms of enzymes (liquid versus powder) compared with control group. Also in this study explained that the increase in milk production might be due to higher nutrient digestibility and more effects on manipulation of rumen environment which lead to more feed efficiency and more productivity. In contrast, Dean et al. (2007) revealed that enzyme supplementation did not improve *in vivo* digestibility of lactating cows, therefore milk production was not significantly affected. Flores et al. (2008) found that no effects on lactation when fibrolytic enzyme product was added to the concentrate of dairy ewes. Milk protein was higher in treated groups but differences were not significant. While milk fat tended to increase in control and G4 groups compared to other groups. While, milk lactose and total solids were not affected by treatments. The higher milk components of probiotic treated ewes than the control group were most likely due to more availability of nutrients as a result of improved digestibility following enzyme treatment (Yang et al., 2000). In contrary, Rode et al. (1999) found a drop in milk fat and protein of dairy cows fed fibrolytic enzymes treated diet. Sabbah et al. (2009) reported that milk protein was increased in MPL and MPP treated diets compared to the untreated group, while other compounds (fat, lactose, total solids and solid not fat) were not affected. The higher milk protein content by probiotic supplementation may be due to stimulation of rumen microbes that cause altering in microbial protein synthesis and increase protein yield in the milk (Dawson, 1993).

Feed efficiency and economical evaluation of ewes during lactation period

Feed efficiency, feed conversion and economical evaluation are presented in table 8. The best feed conversion in term of Kg DM intake/one litter milk was in G3 and G2 (Rations supplemented with MPP). This may be due to its higher milk production. These results were agreed with those obtained by Abo Bakr (2012) who found that goats fed biologically treated straw showed better feed efficiency than control group. Moreover, Kewan et al. (2019) suggested that treated *Moringa oleifera* tree stalks by different type of probiotic (fungi or yeast) had better economical feed efficiency compared to the control group. Also, G2 was better for Kg DMI / Kg weaned live body weight followed by G3while the worst recorded by G1 (control group). This may be due to variation in milk yield which higher for G2 and G3 (Table 7). In the same trend, the low cost to produce one litter milk recorded by G2. While, the high cost of production (gain or milk) recorded by G4 and G5 because the high price of MPL and milk production did not cover this cost. On behave of the cost to produce one kg gain, the lowest cost was in G1 because did not supplemented with any additives while, the highest cost recorded by G5 supplemented with high dosage of MPL and expensive price of it. Differences between the price of feed additives plus and the price of live body weight (Kg) as return from selling weaning kids showed G2 was higher income followed by G3 and G4 while lower income recorded by G5 and G1 respectively. The differences in additives dosages, price of additives, daily and total gain led to this results. Generally, feed efficiency for ewes fed biologically additives (especially MPP) was better than those fed control rations and other groups. The same result was obtained by Abd El-Ghani and Metawli (2003) suggested biologically treated roughage improved feed efficiency (DM intake/milk).

Table 8. Effect of probiotic treatments on feed efficiency and economical evaluation of ewes during lactation periods

Items	G1	G2	G3	G4	G5
Feed efficiency					
Kg DMI/litter milk	2.75	2.44	2.40	2.80	2.73
Suckling milk / kg gain	2.69	2.85	2.95	2.60	2.69
Kg DMI / Kg weaned live body weight	16.16	15.11	15.24	15.65	15.56
Feed cost LE/h/d					
Roughage cost	1.69	1.71	1.73	1.83	1.82
CFM cost	2.25	2.28	2.30	2.44	2.43
MPP cost	0	0.45	0.75	0	0
MPL cost	0	0	0	0.9	1.5
Total cost	3.94	4.44	4.78	5.17	5.75
Feed cost / litter milk	7.71	7.59	7.98	9.50	10.33
Feed cost / kg gain	20.70	21.66	23.54	24.72	27.78
Cost of total gain	354.38	399.82	431.43	464.94	518.61
Price of total gain	1198.4	1292.2	1283.1	1316.7	1306.9
Return from selling	844.02	892.38	851.67	851.76	788.29

G1: the control concentrate ration; G2: concentrate ration with 6 g mixture probiotic powder (MPP) /h/d; G3: concentrate ration with 10 g MPP /h/d; G4: concentrate ration with 6 ml mixture probiotic liquid (MPL) /h/d; G5: concentrate ration with 10 ml MPL /h/d; CFM: concentrate feed mixture; BS: bean straw; DMI: dry matter intake; LE: Egyptian pound; Price of ton CFM = 4000 LE; price of ton Bean straw = 2000 LE; Price of litter MPL = 150 LE; price of Kg MPP = 75 LE; price of kg live body weight = 70 LE.

CONCLUSION

The novel of this study was the evaluation of the impact of two forms of probiotic preparations, as sources of exogenous enzymes, on some productive and physiological reproductive parameters in ewes during different physiological stages of pregnancy and lactation. However, the results provided quantitative information about the impact of MPL and MPP

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enzymes on utilization of feed intake, live body weight, reproductive performance, daily gain, milk yield and composition, feed efficiency and thyroid hormones in Barki ewes. The best treatment was the MPL 6 ml/h/d, which improved the productive and reproductive parameters. While, the best feed efficiency and economical evaluation recorded by G2 (MPP). Generally, all groups fed biologically treated rations showed positive effect in all parameters compared with control group. Based on these results, it was concluded that the probiotic mixture preparations (MPL and MPP), sourced from anaerobic bacterium and added to sheep rations, increased utilization of feed intake and body weight changes during pregnancy and lactation stages. In addition, probiotic mixture improved the weaning weight, daily gain of lambs, live body weight changes of ewes and milk production. The present study indicated that both MPP and MPL treatments possessed a significant effect on productivity, reproductive performance and metabolic profile of Barki ewes.

DECLARATIONS

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Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

Author's contribution

Dr. Ahmed Sobhy El-Hawy designed the experiment, article writing and revision. Dr. Moharram Fouad El-Bassiony designed the experiment, statistical analysis, tabulation of experimental data, manuscript writing, commenting and approval. Dr. Salah Abo Bakr helped in field study, collected data, tabulation of experimental data and article writing. Dr. Hamdi Abdel-Aziz Gawish facilitates the field study, manuscript writing and revision. Dr. Mohamed Tarek Badawy facilitates the field study. While, Dr. Hany Mohamed Gado, provides the probiotic mixtures, designed the experiment, manuscript writing. All authors have read and approved the final manuscript.

REFERENCES

Abd El-Ghani AA and Metawli AA (2003). Effect of *leucaena leucocephala* silage treated with different levels of Lactic acid bacteria on performance of Ossimi ewes. Egyptian Journal of Nutrition and feeds, 6 (Special Issue): 833-842.

Abdalla EB, Al-Momani AQ, Khalil FA, Gado HM and Al-Barakeh FS (2012). Improving reproductive performance of Ossimi ewes using hormonal and enzymatic treatments. Dairy Science, 95(2): 71.

Abdalla EB, El-Hawy AS, Gawish HA and Madany EM (2015). Reproductive and productive efficiency of Barki ewes fed on Nigella Sativa as a source of ration protein. Egyptian Journal of Nutrition and feeds, 18(2): 213-221.

Abo-Bakr S (2012). Nutritional studies on goats fed treated straw under North Sinai conditions. Ph. D. Thesis, Faculty of Agriculture, Cairo University, Egypt, pp. 49-93.

Adams RS, Moore JH, Kesler EM and Stevens GZ (1964). New relationships for estimating TDN content of forages from chemical composition. Journal of Dairy Science, 47: 1461. Agriculture (27): 436-441.

AOAC (1990). Official Methods of Analysis. Association of official analytical chemists international, 16th Edition, Vol. 1, Agricultural, chemicals, contaminants Drugs Washington DC, USA.

Aziz HAA (2009). Effect of feeding olive tree pruning by-products in Sinai on sheep performance, Ph. D. Thesis, Faculty of Agriculture, Ain Shams University, Egypt, pp. 51-89.

Baruah KK, Baruah A, Baruah RN and Zaman J (1993). Relation of serum thyroxin and triiodothyronin to body weight in kid. Indian Veterinary Journal, 1: 118-1121.

Beauchemin KA, Rode LM, Macaw M, Morgan DP and Kampent R (2000). Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. Journal of Dairy Science, 83: 543. DOI: https://doi.org/10.3168/jds.S0022-0302(00)74914-9.

Bueno AL, Martinez GM, Garcia PH, Garcia JM and Perez FP (2013). Evaluation of high doses of exogenous fibrolytic enzymes in lambs fed oat straw based ration. Animal Nutrition and Feed Technology, 13: 355-362. https://www.researchgate.net/publication/290225972.

Chavhan P and Dhamani A (2013). Seasonal Variation in Thyroid Gland of the Female Bat, Taphozous Kachhensis (Dobson) During Pregnancy. International Journal of Molecular Zoology, 13(2): 4-9. DOI: http://dx.doi.org/10.5376/ijmz.2013.03.0002

Dardente H, Hazlerigg DG and Ebling FJP (2014). Thyroid Hormone and Seasonal Rhythmicity. Frontiers in Endocrinology, 5(19): 1-11. DOI: https://doi.org/10.3389/fendo.2014.00019

Dawson KA (1993). Biotechnology in the feed industry. Proceedings of All tech's, 9th Annual Symposium, 269.

Dean DB, Adesogan AT, Staples CR, Littell RC and Kim SC (2007). Effect of method of adding a fibrolytic enzyme to different components of a dairy caw diet on feed intake, digestibility, milk production, ruminanl, fermentation and blood metabolites. Animal Nutrition and Feed Technology, 13: 337-353.

Duncan DB (1955). Multiple range and multiple f tests. Biometrics, 11: 1-42.

To cite this paper: El-Hawy AS, El-Bassiony MF, Abo Bakr S, Gawish HA, Badawy MT and Gado HM (2019). Productive and Reproductive Performance and Metabolic Profile of Barki Ewes Supplemented with Two Forms of Probiotics as Feed Additives. *World Vet. J.* 9(2): 135-145. www.wvj.science-line.com

El Shaer HM (2004). Potentiality of halophytes as animal fodder under arid conditions of Egypt. Rangeland and Pasture Rehabilitation in Mediterranean Areas. *In*: Cahier Options Méditerranéennes, 62: 369–374. Available at: http://om.ciheam.org/article.php?IDPDF=4600190

El-Katch MI, Soltan MA and Essi MS (2016). Effect of Pediococcus spp. Supplementation on growth performance, nutrient digestibility and some blood serum biochemical changes of fattening lambs. Alexandria Journal of Veterinary Science, 49(1): 44-54. DOI: http://dx.doi.org/10.5455/ajvs.210911

Fahmy AA, Youssef kM and El Shaer HM (2010). Intake and nutritive value of some salt-tolerant fodder grasses for sheep under saline conditions of South Sinai, Egypt. Small Ruminant Research, (91): 110-115. DOI:https://doi.org/10.1016/j.smallrumres.2009.11.023

Flores C, Caja G, Casals R, Albanell E and Such X (2008). Performance of dairy ewes fed diets with a fibrolytic enzyme product included in the concentrate during the suckling period. Animal, 2:962-968. DOI: 10.1017/S1751731108001961.

Gado H (1997). Effect of enzymatic treatments for poor quality roughages on fiber digestibility and nitrogen metabolism in Baladi goats. Egyptian Journal of Nutrition and Feeds, 1(Special Issue): 49-56.

Gado HM and Salem AZM (2008). Influence of exogenous enzymes from anaerobic source on growth performance, digestibility, ruminal fermentation and blood metabolites in lambs fed of orange pulp silage in total mixed ration. In: 59th Annual Meeting of the European Association for Animal Production, Vilnius, Lithuania, August 24-27, p. 228.

Gado HM, Metwally HM, El-Basiony AA, Soliman HS and Abd El-Galil ER (2007). Enzymatic treatments of sugarcane bagasse by different sources of cellulase enzymes. Egyptian Journal of Nutrition and Feeds, 10 (2): 607-620. https://www.researchgate.net/publication/320586091

Gado HM, Salem AZM, Odongo NE and Borhami BE (2011). Effect of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. Animal Feed Science and Technology, 165: 131-136. DOI: 10.1016/j.anifeedsci.2011.02.016.

Gado HM, Salem AZM, Robinson PH and Hassan M (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Animal Feed Science and Technology, 154: 36-46. DOI: org/10.1016/j.anifeedsci.2009.07.006.

Ganong WF (1999). Thyroid gland. In: review of medical physiology. 7th edition, Appleton and Lange California p.o.box954, beirot, Lebanon, pp. 290-305.

Gomaa AAAI, Mohamed MY, Saba FE, Ibrahim EMM, El Badawy AA and El-Giziry AA (2016). Growth performance of Ossimi lambs as affected by treated rice straw by enzymes ZAD effective microorganisms. Egyptian Journal of Nutrition and Feeds, 19(2): 243-254.

Grazul-Bilska AT, Caton JS, Borowczyk E, Arndt W, Bilski JJ, Weigl RM, Kirsch JD, Redmer DA, Reynolds LP and Vonnahme KA (2007). Effects of overnutrition and undernutrition on serum metabolic hormones and estradiol-17β concentration in sheep. Proceedings, Western Section, American Society of American Science, 58: 299-303.

Helal HG, Abo Bakr S, Eid EY and El Shaer HM (2018a). Productive performance of Barki ewes fed halophytes added with Propionibacteria freudenreichii under saline conditions, Research Journal of Animal and Veterinary Sciences, 10(2): 18-27. DOI: 10.22587/rjavs.2018.10.2.3.

Helal HG, Eid EY, Nassar MS, Badawy HS and El Shaer HM (2018b). Comparative nutritional studies of ewes and does fed salt tolerant plants under desert condition. Journal of Nature and Science, 16: 62-72. DOI:10.7537/marsnsj160618.10

Ibrahim NH, El-Hawy AS, El-Bassiony MF, Younis FE and Abo Bakr S (2018). Effect of Feeding Salt Tolerant Plants Silage on Productive Performance and Biochemical Changes of Barki Ewes and their Lambs during the First Month Post-Partum. Journal of Animal and Poultry Production, Mansoura University, 9(8): 337-344.

Kearl LC (1982). Nutrient requirements of ruminants in developing countries. Int. Feedstuffs Inst., Utah, USA.

Kewan KZ, Salem FA, Salem AZM, Abdou AR, El-Sayed HM, Eisa SS, Zaki EA and Odongo NE (2019). Nutritive utilization of *Moringa oleifera* tree stalks treated with fungi and yeast to replace clover hay in growing lambs. Agroforestry systems, 93: 161–173. DOI: http://dx.doi.org/10.1007/s10457-017-0158-6

Kholif AE, Abdoa MM, Aneleb UY, El-Sayed MM and Morsy TA (2017). *Saccharomyces cerevisiae* does not work synergistically with exogenous enzymes to enhance feed utilization, ruminal fermentation and lactational performance of Nubian goats. Livestock Science, 206: 17-23. DOI: https://doi.org/10.1016/j.livsci.2017.10.002

Krause M, Beauchemin KA, Rode LM, Farr BI and Norgaard P (1998). Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. Journal of Animal Science, 67: 2912-2920. DOI: 10.2527/1998.76112912x.

McAllister TA, Hristov AN, Beauchemin KA, Rode LM and Cheng KJ (2003). Enzymes in ruminant diets. Agriculture and Agri-Food Canada (AAFC), Department of Animal Science, University of British Columbia, Lethbridge, Canada. DOI:10.3923/ajas.2015.85.99.

McAllister TA, Hristov AN, Beauchemin KA, Rode LM and Cheng KJ (2001). Enzymes in ruminant diets. In: Bedford, M., Partridge, G. (Eds.), Enzymes in Farm Animal Nutrition. CABI Publishing, Oxon, UK, pp. 273–298.

Mohamed MI and Abou-Zeina AA (2008). Effect of dietary supplementation with biologically treated suger beet pulp on performance and organs function in goat kids. American-Eurasian Journal of Agricultural and Environmental Sciences, 4(4): 410-416.

Morsy TA, Kholif AE, Kholif SM, Kholif AM, Sun E and Salem AZM (2016). Effect of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. Annals of Animal Science, 16 (1): 209-222. DOI: https://doi.org/10.1515/aoas-2015-0039.

Mousa MT and Shetaewi MM (1994). Crossing local Ossimi sheep with imported Chios to improve milk production and pre-weaning lamb gains. Assiut Veterinary Medical Journal, 30: 76-86.

To cite this paper: El-Hawy AS, El-Bassiony MF, Abo Bakr S, Gawish HA, Badawy MT and Gado HM (2019). Productive and Reproductive Performance and Metabolic Profile of Barki Ewes Supplemented with Two Forms of Probiotics as Feed Additives. *World Vet. J.* 9(2): 135-145. www.wvj.science-line.com

Nassar MS, El Shereef Afaf and Abo Bakr S (2017). Influence of feeding garlic plant either as powder or oil on reproductive performance of ewes. GSC Biological and Pharmaceutical Sciences, 1(3): 59-61. DOI:https://doi.org/10.30574/gscbps.2017.1.3.0064

Qasim SA, Abeer AS, Alia AE and Hadeel ARH (2018). The Effect of Seasonal Variation on Thyroid Hormones in Iraqi Patients. International Journal of Pharmaceutical Quality Assurance, 9(4): 405-409. Available at: http://impactfactor.org/PDF/IJPQA/9/IJPQA,Vol9,Issue4,Article9.pdf

Refat B, Christensen DA, McKinnon JJ, Yang W, Beattie AD, McAllister TA, Eun JS, Abdel-Rahman GA and Yu P (2018). Effect of fibrolytic enzymes on lactational performance, feeding behavior, and digestibility in high-producing dairy cows fed a barley silage-based diet. Journal of Dairy Science,101(9): 7971-7979. https://doi.org/10.3168/jds.2017-14203

Rode LM, Yang WZ and Beauchemin KA (1999). Fibrlytic enzyme supplements for dairy cows in early lactation. Journal of Dairy Science, 82: 2121. DOI: 10.3168/jds.S0022-0302(99)75455-X.

Sabbah MA, El-Shaer HM, Youssef KM, Ali MA and Abo Bakr S (2009). Impact of feeding biologically treated wheat straw on the production performance of goats in North Sinai. World Journal of Agricultural Science, 5(5): 535-543.

Saleem AM and Zanouny AI (2016). Effect of two levels of probiotic supplementation on performance, digestibility and some blood constituents in weaned lambs. Egyptian Journal of Nutrition and Feeds, 19(2): 265-275.

Saleem AM, Zanouny AI and Singer AM (2017). Growth performance, nutrients digestibility, and blood metabolites of lambs fed diets supplemented with probiotics during pre- and post-weaning period. Asian- Australasian Journal of Animal Science, 30(4): 523–530. DOI:https://doi.org/10.5713/ajas.16.0691

Salem AZM, Alsersy H, Camacho LM, El-Adawy MM, Elghandour MM, Kholif AE, Rivero N, Alonso MU and Zaragoza A (2015). Feed intake, nutrient digestibility, nitrogen utilization and ruminal fermentation activities in sheep fed Atriplex halimus ensiled with three developed enzymes cocktails. Czech Journal of Animal Science, 60(4):185-194. DOI: 10.17221/8133-CJAS.

Salem AZM, El-Adawy MM, Salem MZM and Hassan AA (2004). Effect of probiotics fed as additives on the activity of isolated and characterized lactic acid intestinal bacteria to inhibit Escherichia coli-10 in sheep. Egyptian Journal of Nutrition and Feeds, 7: 167-184.

SAS (2004). SAS User's Guide: Statistics. Release. 9.1. Ed., SAS Inst. Inc., Cary, N.C., USA.

Shaltout KH, EL-Komi THM and Ebrahem M (2012). Seasonal variation in the phytomass, chemical composition and nutritional value of Azolla filiculoides Lam. Along the water courses in the Nile Delta, Egypt. Feddes Repertorium, 123(1): 37-49. DOI: 10.1002/fedr.201200001.

Sujani S and Seresinhe RT (2015). Exogenous enzymes in ruminant nutrition: A review. Asian Journal of Animal Science, 9(3):85-99. DOI: 10.3923/ajas.2015.85.99.

Thakur SS, Verma MP, Ali B, Shelke SK and Tomar SK (2010). Effect of exogenous fibrolytic enzyme supplementation on growth and nutrient utilization in Murrah buffalo calves. Indian Journal of Animal Science, 80: 1217-1219.

Titi HH and Lubbadeh WF (2004). Effect of feeding cellulose enzyme on productivity responses of pregnant and lactating ewes and goats. Small Ruminant Research, 52:137-143. DOI:org/10.1016/S0921-4488(03)00254-2.

Yang WZ, Beauchemin KA and Rode LM (2000). A comparison of methods of adding fibrolytic enzymes to lactating cow diets. Journal of Dairy Science, 83: 2512-2516. DOI: 10.3168/jds.S0022-0302(00)75143-5.

To cite this paper: El-Hawy AS, El-Bassiony MF, Abo Bakr S, Gawish HA, Badawy MT and Gado HM (2019). Productive and Reproductive Performance and Metabolic Profile of Barki Ewes Supplemented with Two Forms of Probiotics as Feed Additives. *World Vet. J.* 9(2): 135-145. www.wvj.science-line.com

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Potential Ameliorative Effect of Bee Honey on Experimentally Induced Melamine Formaldehyde Toxicity in Male Rats

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ABSTRACT

Melamine is considered as one of urea derivatives. Recently it is added to feed stuffs for industrial purposes (falsely elevate its protein contents), however addition of melamine resulted in marked oxidative stress and toxic effect on different body organs, especially the nephrotoxicity and urolithiasis. Therefore, this work is designed to explore the beneficial effect of bee's honey to alleviate the harmful effect induced by melamine toxicity and to show the histological changes on male albino rats. In this work seven animal groups (five rats for each), group 1; negative control, while groups 2, 4, 6 received melamine-formaldehyde orally at dose 0.9, 90, 9000 ppm, respectively while groups 3, 5, 7 received the same melamine dose beside bee's honey (dose of 2.5 gm/kg body weight (B. w) for 45 days. Results declared that melamine treated rats showed marked oxidative, biochemical, hematological changes as well as pathological alterations in vital assets especially liver and urinary system. As distension of the urinary bladder, crystals deposition and stone formation were detected with variable degrees in all groups treated only with melamine. Microscopically, various pathological changes in kidneys, liver, lung, heart and intestine were also demonstrated. The severity of these changes varied from mild to severe changes depending upon the dose of melamine. Interestingly, rats treated with melamine plus the bee's honey showed mild changes in comparison to the only melamine treated rats. These findings assured that, marked antioxidant and ameliorative effect of bee's honey successfully reduced the noxious effect of melamine on different body organs.

Key words: Melamine, Vital assets toxicity, Bee's honey, White albino rats

INTRODUCTION

Recently milk was supplemented with melamine for false elevation of its protein contents, melamine is liquefied in water without forming precipitate as well as its solubility increases at elevated temperature, these facts make it is possible to add excessive amount of melamine that can be dissolved in warm water, the addition of melamine to milk in dose of 1g per 1L falsely elevates the protein content by 0.4% (Hau et al., 2009). Melamine accepted human oral dose is 0.2 mg/kg body weight/day (Zhang et al., 2009). In March 2007, several cases of acute renal dysfunction were reported in cats and dogs ingested food contaminated with melamine, it became a discussion topic when, melamine contamination of pet's food had led to hundreds of deaths (Li et al., 2010). More over by September 2008, melamine was illegally contaminated in infant formulas which caused thousands of deaths in china children (Zhang et al., 2009). Melamine contaminated tainted products consumption had led to children several cases of renal complications (Chan et al., 2008; Filigenzi et al., 2008). Contamination of milk-derived products was detected in China (Bhalla et al., 2009). Animal's study showed that rats treated with melamine showed listlessness, anorexia, loss in condition, decreased body weight, reduced litter size, and average fetal body weight (Stine et al., 2014), toxic influence on hippocampus and causes impairments of synaptic plasticity (An et al., 2011). Stones formation were the most prominent findings in majority of subacute and chronic melamine exposure (Hau et al., 2009). According to gender, melamine doses and amount of water intake the incidence varied from 5 to 100% (Ogasawara et al., 1995). The stones composition is either a mixture of uric acid and melamine or melamine in protein matrix, phosphate and uric acid (Hau et al., 2009). It is a complicated process from crystals to stone formation for all types of stone. Crystals could be excreted with urine flow and would not form stone unless it remains in the kidney tubules (Zhang et al., 2009). In general, renal tubules injury, dysfunction can favor nucleation, aggregation and retention of crystal (Khan, 2006). Several efforts have been done for reducing melamine produced oxidative stress. Honey is formed of a compound mix of proteins, carbohydrates, enzymes (glucose oxidase, catalase, phosphatases and invertase), organic and amino acids (acetic acid, gluconic acid, etc.), vitamins, lipids (pyridoxine, niacin, ascorbic acid, catalase, tocopherols, and phenolic compounds), phenolic acids, volatile chemicals, flavonoids, minerals and carotenoid-like materials that possess antioxidants properties (Blasa et al., 2006). Honey is a normal antioxidant that eliminating and

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scavenging free radicals (Johnston et al., 2005). Honey possess a protecting role against the sodium nitrite feed additives induced renal malfunction, besides its antioxidant and hepatoprotective effects (Erejuwa et al., 2012). Honey increases the epididymal weight and augments spermatogenesis in male Wistar rats (Abdul-Ghani et al., 2008). Accordingly, the purpose of the current study was to examine the protective effect of the bee's honey on the melamine induced toxicity as well as to illustrate the histopathological changes on vital body assets in male albino rats.

MATERIALS AND METHODS

Experimental animals

35 male albino rats weighing 180-200g were used throughout the experiments. The rats obtained from animal house of national research center, Giza, Egypt. The animals were kept in the animal house in the faculty of veterinary medicine, Benha university at 21-22◦C in a12/12 h light/dark cycle and were allowed free access water and standard food pellets throughout the experimental period. Rats accommodated to the laboratory conditions for two weeks before starting the experiment.

Ethical approval

All the experiment animals were carried out under the approved protocols by the institutional animal house of faculty of veterinary medicine, Benha, University, Egypt.

Experimental design

Rats were randomly located into seven groups (five rats each). Group 1 (G1) was kept as a negative control given distilled water only. Group 2 (G2), group 4 (G4) and group 6 (G6) were administered melamine formaldehyde (Sigma-Aldrich, Germany) orally at doses of 0.9, 90 and 9000 ppm, respectively for 45 days. While, group 3 (G3), group 5 (G5) and group 7 (G7) were treated for the same period, with the same melamine doses (0, 9, 90 and 9000 ppm) plus bee's honey (2.5gm/kg BW), bought from agriculture faculty, Benha University, Egypt. Melamine and bee's honey doses were dissolved in distilled water and given orally using stomach tube in daily basis.

Blood samples collection and analyses

Hematological parameter evaluation.

At day 45, two blood samples were collected from retro-orbital venous plexus one sample was collected in EDTA containing tubes for the hematological parameters while the other blood sample was collected without anticoagulant, left in room temperature for 20 minutes then centrifugated for 20 minutes at speed of 3500 (RPM) for serum separation, serum then kept at -20^{o}C and stored to be used in biochemical analysis. Hematological parameters (Table 2), Red Blood Cells (RBC) counts, Packed Cell Volume (PCV), Hemoglobin (Hb), and White Blood Cells (WBC) counts were done according to Feldman and Jain (2000) using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy).

Biochemical analysis.

Separated serum was used for determination of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in serum was done according to Reitman and Frankel (1957). Alkaline phosphatase was measured according to Kochmar and Mossa (1976). Creatinine was calculated according to Henry (1974). Urea was measured according to Patton and Crouch (1977) and uric acid was calorimetrically determined according to Morris and Macleod (1922). Sodium (Na), potassium (k) and chlorine (Cl) were determined using Lustgarten et al. (1974) method. Calcium (Ca^{++}) was determined following Leary et al. (1992) method, while phosphorus determination was done according to Munoz and Balon (1983).

Redox state evaluation.

Determination of Glutathione (GSH) in liver tissue homogenate was done using the method ascribed by Beutler (1963). Malondialdehyde was determined calorimetrically according to the method described by Ohkawa et al. (1979) and Catalase was measured using Hadwan (2016).

Histopathological examination.

Small tissue specimens from liver, kidneys, heart, intestine and lungs of rats in different groups were taken at 45 days and immediately fixed in 10% neutral buffered formalin. Following to proper fixation, samples were dehydrated using ethyl alcohol, then cleared in xylol, embedded and casted in paraffin. According to Suvarna et al. (2018), preparation of thin paraffin sections, staining with Hematoxylin and Eosin stain was done.

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Statistical analysis

SPSS (version 20, USA) was used for the statistical analysis. Data were evaluated using one-way ANOVA, followed by Duncan's test. Results expressed as means ± S.E. Accepted significant values were P<0.05.

RESULTS

Clinical signs

No clinical signs appeared, except in the last week that two animals from G6 have died, but before death these animals showed some sort of convulsions and diarrhea (Figure 1a).

Postmortem examination

Postmortem changes (G6) were represented in congested intestine, distended urinary bladder with urine (Figure 1b), with accumulation of white crystals in the two ureters (Figure 1c).

Redox state

Concerning changes in the hepatic tissue reduced GSH and malondialdehyde level, there were significant increase (P<0.05) in all melamine treated groups (G4 and G6) compared to the control negative group (G1), however, these elevations were lesser in G2. Meanwhile, a significant decrease (P<0.05) of reduced GSH and GSH in bee's honey treated groups (G3, G5 and G7) in comparison to only melamine treated groups (Table 1). While, concentration of tissue catalase was significantly (P<0.05) increased in the melamine treated groups than negative control group however there was no significant (P<0.05) variation among the only melamine treated groups (G2, G4 and G6) and melamine plus bee's honey treated groups (G3, G5 and G7).

Hematological parameter evaluation

Regarding RBCs counts and its related parameters HB and PCV % among examined groups, there was a significant decrease (P<0.05) in only melamine fed groups (G2, G4 and G6) compared to control group. Conversely, the melamine and bee's honey fed groups (G3, G5 and G7) showed non-significant decrease (P<0.05) in RBCs counts and other related parameters compared to control group. Generally, there was an inverse correlation between melamine dose and RBCs count, Hb and PCV% (Table 2). In respect to WBC a significant reduction (P<0.05) has been observed in groups G4 and G6 compared to the control one. Though the bee's honey treated groups (G3, G5 and G7) showed significant increase (P<0.05; Table 2) in the WBCs than the only melamine treated groups (G2, G4 and G6).

Table 1. Enzymatic changes among different melamine doses and honey feed groups in male rats

Groups	Reduced glutathione Mg/gm tissue (Mean ± SE)	MDH nmol/gm tissue (Mean ± SE)	Catalase U/gm tissue (Mean ± SE)
Group 1	13.04±0.61^{d}	1394.42±46.63^{g}	201.63±13.97^{d}
Group 2	17.68±0.36^{c}	2411.45±80.08^{e}	375.58±12.96^{c}
Group 3	15.99±0.16^{c}	1989.00±77.96^{f}	376.73±13.10^{c}
Group 4	25.11±0.64^{b}	3389.95±163.70^{c}	433.17±2.90^{b}
Group 5	21.28±0.75^{bc}	2906.58±27.43^{d}	418.44±4.88^{b}
Group 6	61.20±8.53^{a}	4975.82±264.88^{a}	489.22±8.58^{a}
Group 7	31.43±2.27^{b}	4307.87±61.05^{b}	465.32±2.98^{a}

Mean label different superscripts ($^{abcd\cdots}$) letters at the same column are significantly different at P<0.05. MDH: malondialdehyde. Group 1 is negative control, groups 2, 4, and 6 received melamine at dose of 0.9, 90 and 9000 ppm respectively while groups 3, 5, 7 received melamine in dose of 0.9, 90 and 9000 ppm beside 2.5gm/kg B.W

Table 2. Hematological changes among different melamine doses and honey treated male rat's groups

Groups	RBCS ($\times10^{6}$/µL) (Mean ± SE)	TLC ($\times10^{3}$µL) (Mean ± SE)	Hb (gm/dl) (Mean ± SE)	PCV% (Mean ± SE)
Group 1	6.72±0.20^{a}	9.91±0.17^{a}	13.95±0.26^{a}	41.28±0.33^{a}
Group 2	6.30±0.11^{b}	6.84±0.12^{c}	9.98±0.30^{c}	38.47±0.64^{bc}
Group 3	6.71±0.04^{a}	8.11±0.48^{b}	11.74±0.30^{b}	41.00±0.24^{a}
Group 4	6.32±0.06^{b}	5.88±0.17^{d}	9.32±0.32^{c}	38.60±0.33^{b}
Group 5	6.72±0.05^{a}	7.76±0.17^{bc}	9.74±0.25^{c}	41.02±0.27^{a}
Group 6	5.91±0.07^{c}	2.63±0.58^{e}	5.56±0.90^{e}	36.07±0.46^{d}
Group 7	6.11±0.08^{bc}	5.13±0.30^{d}	7.44±0.71^{d}	37.30±0.51^{c}

Means label different superscripts ($^{abcd\cdots}$) letters at the same column are significantly different at P<0.05. RBCS: erythrocyte counts, TLC: Total leucocyte counts, Hb: hemoglobin, PCV: packed cell volume, Group1 is negative control, groups 2, 4, and 6 received melamine at dose of 0.9, 90 and 9000 ppm respectively while groups 3, 5, 7 received melamine in dose of 0.9, 90 and 9000 ppm beside 2.5gm/kg B.W

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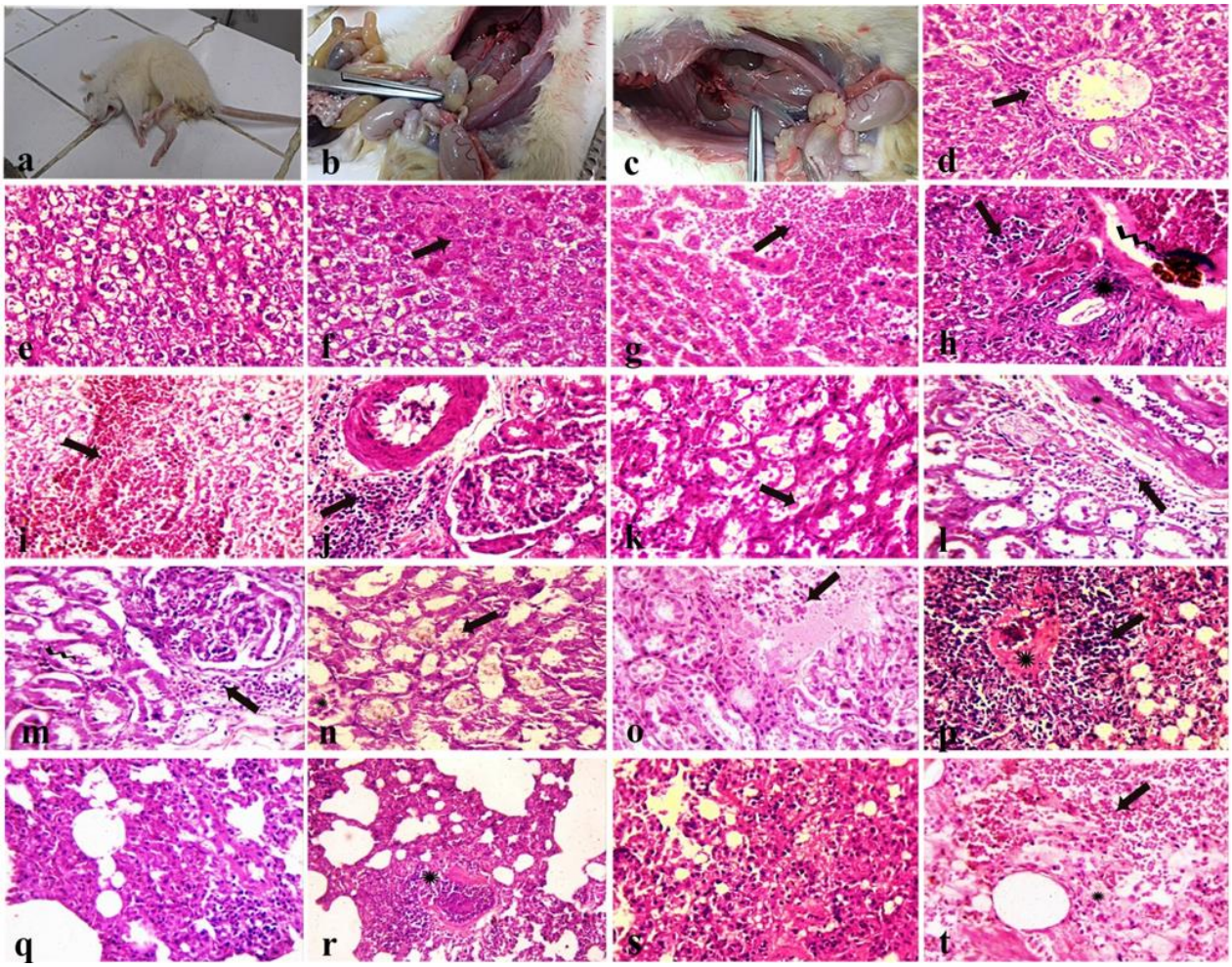

Figure 1. Postmortem changes and changes among different organs of male rats treated with different melamine concentration and bee's honey**.** Convulsion (**a**), urinary bladder distension with urine and crystals (**b**), distension in ureter with crystals (**c**) stained sections with H & E. liver obtained from group 2 (**d** and **e**), group 4 (**f** and **g**) and group 6 (**h** and **i**), **d**: showing congestion of portal blood vessels with mild mononuclear leukocytic cellular infiltration (arrow, x 400), **e**: Diffuse hydropic degeneration of hepatocytes in the hepatic parenchyma (x 200), **f**: Focal area of coagulative necrosis in the hepatic parenchyma (arrow, x 200), **g**: Area of lytic necrosis in the hepatic parenchyma that characterized by disappearance of hepatocytes and replaced by erythrocytes and eosinophilic necrotic debris (arrow, x 200), **h**: Moderate proliferation of the biliary epithelium (asterisk) with mild mononuclear leukocytic cellular infiltration (arrow, x200), with accumulation of melamine substance in the portal blood vessels (zigzag arrow, x 200), **i**: Extensive lytic necrosis scattered in the hepatic parenchyma (arrow) with marked hydropic degeneration in the remnant hepatocytes (asterisk, x 200). Renal changes stained sections with H&E for group 2 (**j** and **k**) group 4 (**l** and **m**) and group 6 (**n** and **o**) taken from animals treated with different concentrations of melamine, perivascular edema admixed with mononuclear leukocytic infiltration (arrow, x200) with vacuolar degeneration of mesangial cells of glomeruli (**j**) (x 200), **k**: Necrosis of the lining epithelium of renal tubules with the presence of hyaline casts in the lumen of some renal tubules (arrow, x 200), **m**: Degenerative changes in the wall of renal blood vessels (asterisk) with perivascular edema admixed with inflammatory cells (arrow) with marked necrosis of the epithelial cell lining of renal tubules (**l**). Necrosis of the endothelial cell lining of the glomerular tuft with peri-glomerular leukocytic infiltration (arrow) with necrotic eosinophilic debris in the lumen of some renal tubules (zigzag arrow, x 200), **n**: Entire necrosis of the glomerular tuft (asterisk) and the lining epithelium of renal tubules with accumulation of melamine substance in the lumen of some renal tubules (arrow, x 200), **o**: Focal areas of lytic necrosis that characterized by disappearance of renal tissues and replaced by proteinaceous eosinophilic substance with necrotic debris (arrow, x 200). Pulmonary changes stained sections of lung with H&E in group 2 (**p** and **q**), group 4 (**r** and **s**) and group 6 (**t**). **p**: Peri-vascular edema admixed with mononuclear leukocytic cellular infiltration (arrow, x200), with hyalinization of the wall of pulmonary blood vessels (asterisk, x200), **q**: Diffuse alveolar consolidation (arrow) filed with eosinophilic exudate with inflammatory cells and alternated with emphysema (x 200), **r**: Rupture of the wall of pulmonary blood vessels (asterisk, x200) with consolidated alveoli (x 200), **s**: Diffuse alveolar consolidation of most pulmonary alveoli (x 200), **t**: Diffuse hemorrhage in pulmonary tissue (arrow, x200), with distension of pulmonary alveoli with edematous eosinophilic substances (asterisk, x200).

Biochemical analysis

Kidney function.

Parameters assessing the kidney function Blood Urea Nitrogen (BUN), creatinine, uric acid] showed a significant ($P<0.05$) serum elevation (Table 3) in all melamine treated groups (G2, G3, G4, G5, G6 and G7) than control group (G1). Although the bee's honey treated groups (G3, G5 and G7) showed significant reduction ($P<0.05$) in the serum level of these parameters than the only melamine treated groups with the same doses, respectively (G2, G4 and G6).

Liver function.

It was observed that the liver function assessing factors (AST, ALT and total protein) showed marked changes. About serum level of both AST and ALT, it showed significant increases ($P<0.05$) in the melamine treated groups (G4,

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G6 and G7), while the melamine plus bee's honey treated groups (G3, G5 and G7) showed a significant reduction (P<0.05) than the melamine treated groups (G2, G4 and G6). Concerning serum total protein, the melamine treated groups showed a significant reduction (P<0.05) than control one, however the melamine plus bee's honey treated groups showed a significant increase (P<0.05) than only melamine treated groups.

Electrolyte state evaluation.

A significant decrease (P<0.05) in serum electrolytes levels (Table 4) was indicated in all treated groups compared to the control group, the melamine treated groups (G2, G4 and G6) caused a significant decrease (P< 0.05) in the electrolytes level than the melamine plus bee's honey groups (G3, G5 and G7).

Histopathological changes.

Various degrees of pathological alterations were demonstrated in different investigated organs mainly in rats that have high concentrations of melamine (G4 and G6). Animals received melamine at concentration of 0.9 ppm (G2) revealed marked congestion of central vein, blood sinusoids and portal blood vessels with mild perivascular mononuclear leukocytic cellular infiltration (Figure 1d), mild hyperplastic proliferation of biliary epithelium with formation of newly formed bile ductless as well as periductal mononuclear inflammatory cells were seen in most treated animals. Enlarged hepatocytes, with diffuse hydropic degeneration was observed (Figure 1e). Small focal areas of coagulative necrosis of hepatocytes, characterized by retention of hepatic cell outline with hypereosinophilic cytoplasm of hepatocytes and karyorrhectic nuclear changes. Equally, marked congestion of hepatic blood vessels with biliary epithelium hyperplasia was seen in G4. Additionally, extensive hydropic degeneration associated with pyknosis of the nuclei of individual hepatocytes as well as multiple focal areas of coagulative necrosis was demonstrated in G4 (Figure 1f). Scattered area of lytic necrosis in the hepatic parenchyma that characterized by disappearance of hepatocytes and replaced by erythrocytes and eosinophilic necrotic debris (Figure 1g) was also detected. Meanwhile, moderate proliferation of the biliary epithelium with mild mononuclear leukocytic cellular infiltration with accumulation of melamine substance in the portal blood vessels (Figure 1h) was detected in the liver tissue obtained from G6. Furthermore, scattered hemorrhagic areas with multiple area of lytic necrosis (Figure 1i) that characterized by disappearance of hepatocytes and replaced by erythrocytes with marked hydropic degeneration in the remnant hepatocytes was seen in the hepatic parenchyma of treated rats of G6. The microscopical examination of kidneys revealed perivascular mononuclear cellular infiltration with degeneration in the wall of the renal blood vessels (Figures 1j and 1L) that characterized by hyperplasia of its endothelial cell lining with vacuolation of the sarcoplasm of its muscular layer was detected in all treated groups with different concentrations of melamine (G2, G4 and G6) with variable degree. Moreover, vacuolar degeneration of the endothelial cell lining of the glomerular tuft was demonstrated in rats of G2 (Figure 1j). Additionally, the lining epithelium of proximal and distal convoluted tubules were swollen and exhibited vacuolar and hydropic degeneration. Moreover, the renal tubules in the renal cortex showed coagulative necrosis of their lining epithelium evidenced by hyper-eosinophilic cytoplasm and pyknosis and karyorrhexis of nuclei with homogenous eosinophilic casts were also seen in the lumen of some convoluted tubules (Figure 1k). Moreover, severe degree of degeneration of the lining epithelium of proximal and distal convoluted tubules with periglomerular and perivascular leukocytic cellular infiltration was noticed in G4 (Figures 1L and 1m). Meanwhile, severe shrinkage and necrosis of glomerular tuft and the lining epithelium of renal tubules with the presence of hyaline and cellular casts in their lumen in association with accumulation of melamine like substance in the lumen of some renal tubules (Figure 1n). Focal areas of lytic necrosis that characterized by disappearance of renal tissues and replaced by proteinaceous eosinophilic substance with necrotic debris (Figure 1o).

Additionally, marked cystic dilatation of some renal tubules. Periglomerular, perivascular and intertubular leukocytic cellular infiltration was noticed in G6. On the other hand, various pathological changes with different severity were displayed in the lungs of rats fed diet supplemented with different concentrations of melamine (G2, G4 and G6). Congestion of pulmonary blood vessels, perivascular mononuclear inflammatory cells mainly lymphocytes (Figure 1p) was observed in the lungs of groups G2, G4 and G6. Meanwhile, small focal areas of alveolar consolidation that filled with inflammatory exudate composed of fibrin threads admixed with macrophages and lymphocytes (Figure 1q) in association with focal areas of emphysema were demonstrated in rats of G2. On the other side, rupture of the wall of pulmonary blood vessels (Figure 1r) as well as scattered areas of consolidated alveoli with focal areas of atelectasis with compensatory emphysema (Figure 1s) was noticed in lungs of rats of G4. Meanwhile, diffuse hemorrhagic areas in the pulmonary tissue (Figure 1t) with extensive consolidation of pulmonary alveoli was seen in lungs of G6 (Figure 2a). The most common pathological changes in the heart of G2, G4 and G6 were intermuscular hemorrhages (Figure 2b) myocardial degeneration characterized by presence of clear vacuoles in the sarcoplasm of myocardium in combination with hyaline degeneration of myocardium that characterized by highly eosinophilic sarcoplasm with loss of muscular striation (Figures 2c and 2d). Additionally, extensive degeneration in the wall of myocardial blood vessels was also detected with severe intermuscular hemorrhage (Figure 2e). In the heart of group 6. The intestinal microscopical examination of G2 showed mild desquamation of the lining epithelial cells of the intestinal villi. Accidentally, small necrotic areas represented by more eosinophilic cytoplasm with pyknotic nuclei.

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Table 3. Biochemical changes among different melamine doses and honey treated male rat's groups

Factors (Mean ± SE) / Groups	BUN (mg/dl)	Creatinine mg/dl	Uric acid mg/dl	AST (U/l)	ALT (U/l)	Total protein (g/dI)
Group 1	22.06±1.08^{h}	0.41±0.20^{d}	0.60±0.02^{d}	119.81±2.82^{d}	75.87±3.11^{d}	3.86±0.13^{a}
Group 2	63.81±0.85^{e}	0.73±0.017^{c}	0.79±0.03^{d}	314.87±22.69^{b}	85.03±2.33^{c}	2.66±0.08^{bc}
Group 3	61.12±0.2^{g}	0.55±0.04^{d}	0.69±0.01^{d}	248.34±19.41^{c}	78.27±1.29^{d}	2.90±0.12^{b}
Group 4	71.70±0.25^{c}	0.83±0.01^{c}	1.13±0.04^{c}	395.43±22.53^{a}	91.25±1.94^{bc}	2.32±.25^{cd}
Group 5	68.85±0.69^{b}	0.72±0.02^{c}	1.22±.19b^{c}	267.28±15.25^{bc}	86.50±1.83^{c}	2.34±0.13^{cd}
Group 6	78.80±1.01^{a}	1.42±0.10^{a}	1.92±0.13^{a}	390.36±20.66^{a}	103.39±2.18^{a}	1.96±0.08^{d}
Group 7	75.00±0.51^{b}	1.07±0.07^{b}	1.43±0.03^{b}	306.76±24.66^{bc}	94.98±2.19^{b}	2.32±0.14^{cd}

Means label different superscripts (^{abcd}) letters at the same column are significantly different at P<0.05. BUN: blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase group1 is negative control, groups 2, 4, and 6 received melamine at dose of 0.9, 90 and 9000 ppm respectively while groups 3, 5, 7 received melamine in dose of 0.9, 90 and 9000 ppm beside 2.5gm/kg B.W.

Table 4. Electrolytes changes among different melamine doses and honey treated male rat's groups

Groups	Ca mg/dl (Mean ± SE)	Phos mg/dl (Mean ± SE)	Na mgEq/I (Mean ± SE)	K mgEq/I (Mean ± SE)	Cl mgEq/I (Mean ± SE)
Group 1	9.36±0.31^{a}	9.70±0.21^{a}	147.00±1.00^{a}	6.82±0.10^{a}	107.60±0.87^{a}
Group 2	3.36±0.08^{c}	6.20±0.27^{d}	135.20±1.53^{b}	5.86±0.11^{c}	98.20± 1.36^{bc}
Group 3	6.51±0.61^{b}	8.14±0.12^{b}	136.40±1.03^{b}	6.74±0.47^{ab}	101.00±1.52^{b}
Group 4	1.49±0.09^{e}	6.76±0.21^{c}	121.20±2.80^{c}	4.68±0.43^{de}	93.60±1.08^{c}
Group 5	3.14±0.05^{cd}	5.06±0.12^{e}	131.80±1.07^{b}	5.94±0.27^{bc}	85.40± 3.59^{d}
Group 6	1.44±0.04^{e}	3.84±0.11^{g}	89.20±2.15^{e}	4.20±0.26^{e}	84.40± 1.29^{d}
Group 7	2.47±0.10^{d}	4.52±0.17^{f}	107.40±2.94^{d}	5.38±0.12^{cd}	65.80± 1.98^{e}

Means label different superscripts ($^{abcd\cdots}$) letters at the same column are significantly different at P< 0.05. Ca: calcium, phos: phosphorus, Na: sodium, K: potassium, CL: chlorine Group1 is negative control, groups 2, 4, and 6 received melamine at dose of 0.9, 90 and 9000 ppm respectively while groups 3, 5, 7 received melamine in dose of 0.9, 90 and 9000 ppm beside 2.5gm/kg B.W.

Moreover, blood capillaries presented mild congestion in submucosa and lamina propria. On the other side, fused intestinal villi with necrosis of their lining epithelial cells were noticed in rats obtained from G4 and G6. Additionally, desquamation of the necrosed villar epithelium with mononuclear cells infiltration into the lamina propria (Figures 2f and 2g) with mild hemorrhage was seen in other cases. Edema and necrosis of the muscularis mucosae was demonstrated in some rats of G6. In addition to some lymphocytic infiltration in sub-mucosa, necrosis of the epithelial cells lining the intestinal glands with sever infiltration of mononuclear inflammatory cells and increase in the goblet cells of the intestinal villi was observed as well as infiltration of inflammatory cells in between the intestinal glands. Vacuolation of the glandular epithelium with its engorgement with lymphocytes was also seen among group 6. Remarkably, the microscopical examination of different organs obtained from rats treated with different concentrations of melamine (0.9, 90 and 9000 ppm) and honey treated animals (G3, G5 and G7) for 45 days revealed marked ameliorative effect of honey with variable degrees to the histopathological alterations induced by melamine in different investigated organs especially in rats of G3. Accordingly, improvement in the hepatocellular architecture with more regular and less altered hepatocytes except dilatation of central vein (Figure 2h) when compared to melamine treated rats only was demonstrated. In contrast, the hepatic tissues obtained from rats of G5 revealed mild reduction in the severity of pathological changes induced by melamine as congested central veins and mild sinusoidal dilatation with a mild degree of degenerative changes in hepatocytes with mild perivascular edema (Figure 2i) However, hydropic degeneration of hepatocytes with small area of coagulative necrosis was detected in the hepatic parenchyma of only two animals. Meanwhile, treatment of melamine-intoxicated rats with honey (G7) resulted in a mild improvement in the lesions induced by melamine, but it still having some pathological alterations, as the liver showed numerous swollen hepatocytes with vacuolar degeneration in combination with focal areas of hemorrhage in hepatic parenchyma. Focal areas of coagulative necrosis were detected in two examined cases. Slight hyperplasia of the biliary epithelium and activation of Von Kupffer cell (Figure 2j) in comparable to those of the control group. Kidneys of G3 showed nearly normal histological structure in most rats except mild degeneration of the lining epithelium of renal tubules with vacuolation of the endothelial cell lining of glomerular tuft (Figure 2k). Meanwhile, the renal tissue obtained from G5 revealed tubular changes, some tubules showed disintegration of the glomerular tuft with lining epithelium degenerative changes. Moreover, cellular and hyaline casts (Figure 2L) was also noticed in the lumen of some renal tubules. Furthermore, few leukocytic infiltration mainly macrophage and lymphocytes as well as necrosis of the lining epithelium with cellular and hyaline cast was observed. Degenerative changes in the endothelial cell lining of the glomerular tuft (Figure 2m) was seen in the renal tissues of G7. Hyperplasia of the glomerular tuft with reduction in Bowman's space was also observed in few animals among this group. Microscopically, the most common pathological changes detected in the pulmonary tissues obtained from groups G3, G5 and G7 were emphysema in which pulmonary alveoli were enlarged with few consolidated alveoli (Figures 2n

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and 2o). Interestingly, cardiac tissues appeared normal in most examined cases of G3 (Figure 2p). Mild degenerative changes in the sarcoplasm of cardiac muscle were common pathological changes (Figure 2q) seen in the heart of G5. On the other side, congestion of myocardial blood vessels with degeneration in the wall of myocardial blood vessels in combination with mild hyaline degeneration of the cardiac muscles (Figure 2r) was observed in some rats mainly from G7. Intestine showed mild degeneration of the lining epithelium of intestinal villi with hyperplasia of goblet cells was noticed in intestine from G3. Meanwhile, less necrotic changes with few inflammatory cells in the lumen of the villi and in lamina propria of intestine of rats from G5 and G7 (Figure 2s).

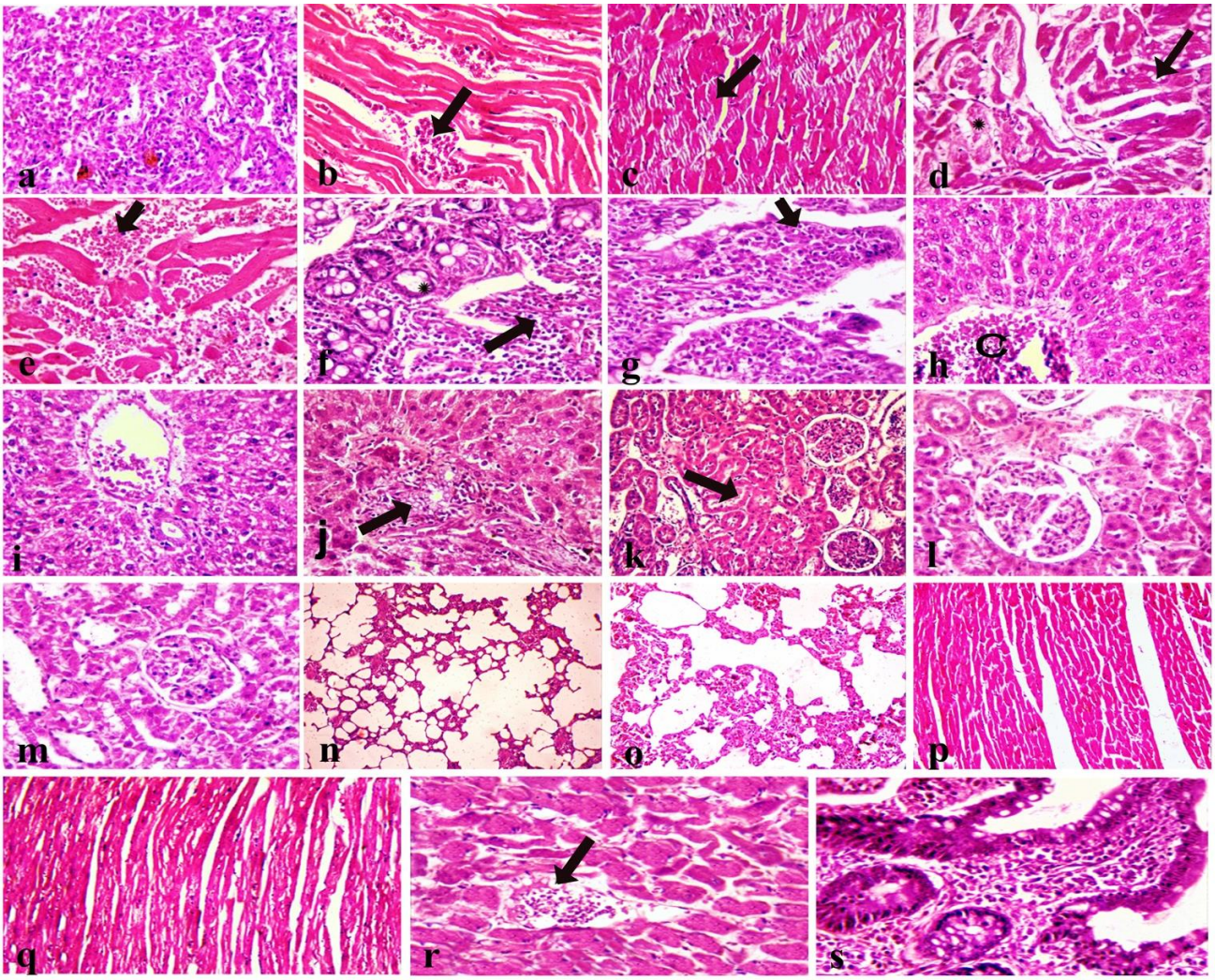

Figure 2. Histological changes among different organs of male rats treated with different melamine concentration and bee's honey**,** Pulmonary changes among melamine treated groups, H & E stained section of lung obtained from group 6 (**a**) showing Extensive diffuse consolidation of pulmonary alveoli (x 200). Cardiac changes among melamine treated groups (**b**, **c**, **d** and **e**). H&E stained section of heart, group 2 (**b** and **c**), group 4 (**d**), group 6 (**e**), **b**: showing Intermuscular hemorrhage (arrow, x200), **c**: Hyaline degeneration of some cardiac muscles (arrow, x 200), **d**: Clear vacuolation of the sarcoplasm of cardiac muscles (arrow) with myomalacia of some muscle fiber (asterisk, x 200), **e**: Marked intermuscular hemorrhage admixed with few leukocytic infiltration (arrow) with hyaline degeneration of the cardiac muscles (x 200), Intestinal changes of melamine treated groups (**f** and **g**) (H&E stained section), **f**: Mononuclear leukocytic cellular infiltrations in the lamina propria of intestinal villi (arrow) with vacuolation and necrosis of the lining epithelium of sub-mucosal gland (asterisk, x 400), **g:** Entire necrosis of the lining epithelium of intestinal villi (arrow) with leukocytic cellular infiltrations in the lamina propria (x 400). Liver and kidney histological changes, H&E stained section obtained from liver, group3 (**h**), group5 (**i**), group7 (**j**), **h**: showing normal histological structure of hepatic tissue except dilatation of central vein (C, x 200), **i**: Congestion of portal blood vessels with mild perivascular edema with mild vacuolar degeneration of hepatocytes (x 200), **j**: Mild proliferation of the lining epithelium of bile duct (arrow) with mild vacuolar degeneration of hepatocytes (x 200). While kidney's, group$_3$ (**k**), group$_5$ (**l**), group$_7$ (**m**), **k:** Clear vacuolation of the endothelial cell lining of glomeruli with the presence of eosinophilic hyaline casts in the lumen of some renal tubules (arrow, x 100), **l**: Disintegration of the glomerular tuft with the presence of cellular casts in the lumen of some renal tubules (arrow, x 200), **m**: Degenerative changes in the endothelial cell lining of the glomerular tuft with necrosis of the lining epithelium of some renal tubules (x 200). Histological changes, H&E stained section of lung (**n** and **o**), **n:** obtained from group3 showing emphysema in the pulmonary tissue (x 200), **o:** obtained from group7 showing compensatory emphysema with few consolidated alveoli (x 200), **p**: obtained from group3 showing normal histological structure of cardiac muscle (x 200), **q:** obtained from group5 showing mild degenerative changes in the sarcoplasm of cardiac muscle (x 200), **r:** obtained from group7 showing hyaline degeneration in some cardiac muscle fiber with vacuolar degeneration in the wall of myocardial blood vessels (x 200), **s**: obtained from group7 showing mild degenerative changes in the lining epithelium of the intestinal villi with few mononuclear leukocytic cellular infiltration in the lamina propria (x 400).

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DISCUSSION

Falsely, melamine could be used to increase the feedstuffs nitrogen concentration. Mammalian bodies can't metabolize melamine (Jutzi et al., 1982; Hammond et al., 1986), so once it is ingested it results in insoluble crystals that obstruct and damage renal tubules and leads to renal failure (Brown et al., 2007; Puschner et al., 2007). World Health Organization (WHO) stated that the human tolerable daily melamine dose is 0.2 mg per kilogram of body weight whereas, the Food and Drug Administration (FDA) recommended dose is 2.5 part per million in food and one part per million for infant formula (Ingelfinger, 2008). Melamine contaminated milk products results in infants and children urinary system calculi (Ingelfinger, 2008; Lam et al., 2008; Xin and Stone, 2008). Above recommended dose, toxicity and renal calculus formation increase with melamine dose increase (Lam et al., 2008). Attempts have been made to select ameliorative agents to reduce obvious melamine toxicity to the exposed individuals. Bee's honey is known to have a wide antioxidant and protective properties range (Al-Hindi et al., 2011). The histopathological changes in the most of examined vital assets (liver, kidney, heart, intestine and lungs) was melamine concentrations dependent, as the severity of the alterations was increased by increasing the concentration of melamine. These results controversy with those of Shehu et al. (2015) who showed no histopathological changes in melamine treated male albino rats at 28 days, this may be because of the variation in exposure period of melamine toxicity. However, the bee's honey treated animals revealed some pathological changes than the only melamine treated animals and this finding matched with the result of Wilson et al. (2011) who ascribed the protective rule of bee's honey on liver of adult Wister rats. In present study, convulsions and nervous manifestation have been recorded in some animals before death, these finding agreed with Zhang et al. (2009), they attributed similar findings to the toxic effect of melamine on brain tissue and azotemia. Besides An et al. (2012) showed that the melamine could pass the blood brain barrier and affected potassium and sodium channel in hippocampal neurons causes synaptic plasticity impairments. Regarding the postmortem (PM) changes, urinary bladder and ureters showed distention with white crystals that might be ascribe to melamine crystals deposition on the urinary system. This observation was consistent with Xin and Stone (2008). Available suggestion about susceptibility of hepatocytes to damage during oxidative stress and the proven antioxidants beneficial effect was reported by Dias et al. (2005) and Gumieniczek (2005). In the current study significant increases in the oxidative stress marker Glutathione (GSH), Malondialdehyde (MDH) and catalase) in all melamine treated animals was obtained compared to control animals, this result disagree with the finding of Kılıcoglu et al. (2008) and Lv et al. (2013), who showed marked reduction in the oxidative stress markers after melamine administration for 13 weeks in mice, this controversy may be due to the longer period that is enough to deplete cellular antioxidant reserve. Bee's honey treated rats caused marked reduction in antioxidant markers (GSH, MDH) compared to corresponding groups that received melamine only, this finding matched with report of Yao et al. (2011), who proved that the bee's honey effectively restored the antioxidant (GSH, MDH) reserve in male rat's hepatocytes and Al-Waili (2003) who proved the bee`s honey had valuable antioxidant effect on sheep. Although the antioxidant effect of bee's honey on catalase level in the hepatocytes was moderate this finding was according to with report of Petrus et al. (2011) who indicated moderate effect of bee's honey on catalase enzyme concentration in hepatocytes. It is truly reported that the oxidative stress and inflammation are correlated (Peake et al., 2007). Bee's honey possessed both antioxidant and anti-inflammatory, so this fact makes the ameliorative effect of bee's honey to be more pronounced (Kassim et al., 2010; Omotayo et al., 2010; Erejuwa et al., 2011). Blood is considered as sensitive and good index of any physiological changes, so any toxic stress would show great hematological parameters changes. This study showed a significant decrease in RBCs, HB%, and PCV% in the melamine treated groups (for 45 days) than control group, this may be due to the cytotoxic effect of melamine on different body organs, this observation is in concordant with Zhang et al. (2009). Reduction in these parameters may be up to produced enteritis that caused thickening and shortening in the intestinal villi with fibrous tissue formation in mucosa and submucosa (Bhor and Sivakami, 2003; Weiss and Wardrop, 2011), these changes resulted in nutrients poor absorption. Strakova et al. (2014) reported that the reduction in RBCs counts mainly is due to the negative impact of melamine toxicity on the RBCs life span through increasing their membrane fragility via decreasing Na+/K- ATPase activity. The melamine induced nephrotoxicity may have negative effect in the erythropoietin hormone production as stated by Hau et al. (2009); Doubek et al. (2010). Many authors (Zheng et al., 2013) referred the reduction on RBCs, HB% and PCV% to suppressor effect of melamine toxicity on bone marrow multiplication. The bee's' honey treated animal groups obtained significant increases in the RBCs, HB% and PCV%. Yao et al. (2011) stated this increase is due to the marked antioxidant and protective rule of bee's' honey that restore the activity of catalase and glutathione peroxidase. Some researchers (Yao et al., 2004; Michalkiewicz et al., 2008) attributed the hematological parameters increases to the antioxidant's properties of honey as well as its high contents of moisture, sugars such as fructose, glucose, enzymes like glutathione reductase and catalase, in addition to essential elements such as iron, copper, zinc, and calcium, vitamins such as vitamin A, C, and E, and some flavonoids and phenolic acids. This study indicated significant decrease the total leukocyte counts this finding is concordant with Strakova et al. (2014), this might be due to the undesirable effect of melamine on the cell membrane integrity. Some authors (El Rabey et al., 2013) correlated the reduction in the leukocyte counts to the hepatoxicity and

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decreased the liver activities. Meanwhile the bee's honey treated groups revealed significant increase in the leukocyte count which might be due to the antioxidant effect of the bee's honey, this finding was agreed with Al-Waili (2003) who reported similar finding in human. Concerning the biochemical evaluation, the result obtained in this study showed marked elevation in the BUN, creatinine, and uric acid in melamine treated rats' group, this finding concordant with the finding of Jingbin et al. (2010). Dogs and cats presented alike results (Cianciolo et al., 2008; Dobson et al., 2008). This elevation in the urea, uric acid and creatinine may be attributed to renal ischemia and focal lesion produced due to melamine toxicity, this hypothesis agrees with Ogasawara et al. (1995), who has found similar results on rats. Bee's honey fed groups indicated significant reduction in the serum level of urea, uric acid and creatinine. These findings matched with those proved by Erejuwa et al. (2012) who stated that bee's honey has marked antioxidant and protective effect on the kidney. Regarding to liver assessing serum parameters (AST, ALT, and total protein), significant increase in the serum level of AST and ALT have been recorded in this study, this may be due to the oxidative stress produced through the melamine toxic effect of the liver cells that resulting in increased the lysis of hepatocyte and release of its enzymes (AST and ALT). This finding agrees with Khan et al. (2005), in this respect Hayes et al. (1999) stated that increased serum level of AST and ALT indicate pronounced liver cell damages. However, this finding is disagreed with Puschner and Reimschuessel (2011) who showed decrease in the AST and ALT in dogs and cats exposed to melamine toxicity. The bee's honey treated animals (at 2.5gm\kg\body weight) showed marked reduction in the liver enzyme (AST and ALT) this could be due to the antioxidant effect of bee's honey on the hepatic cells, this result concurred with Erejuwa et al. (2012). It is true that most of plasma protein synthesis occurs in liver, so total plasma protein can be use as indicator about the liver cell function (Yang and Chen, 2003). The study presented marked reduction in total serum protein in the melamine treated rats, which may be caused by hepatotoxicity, this result matched with those ascribed with Chen et al. (2009) who stated that the melamine toxicity results in nephritis, and may lead to increase the filtration and loss of albumen in urine. Whenever the bee's honey treated animals showed marked increase in serum total protein, this result may be due to the bee's honey ameliorative effect on liver cells, and concordant with findings of El-Khayat and Ahmed (2000).

The current study showed a significant reduction in electrolytes (Ca, ph, Na, K, and Cl). Serum, these findings concordant with the declaration of Abdel-Gayoum and Ahmida (2017) who presented noticeable decrease in serum electrolytes, and ascribed this finding to the nephrotoxicity that hindered the tubular reabsorption of these electrolytes and increased their renal excretions. Bee's honey treated groups showed marked elevation in the serum level of these electrolytes (Ca, ph, Na, K, and Cl), which may be due to the proven antioxidant effect that caused decrease in melamine nephrotoxic effect. These findings are in line with report of Wilson et al. (2011) who stated that, the protective effect of bee's' honey could be attributed to their biologically active compounds such as vitamins, flavonoids, and antioxidants that synergistically work to clean the oxidative stress produced free radicals.

CONCLUSION

The current study, disclosed the protective effect of bee's' honey against melamine induced toxicity. This fact was concluded from its ameliorative effect in biochemical, hematological and tissue histopathological alterations induced by different concentrations of melamine. Thus, bee's' honey might be used to protect

DECLARATIONS

Author's contribution

Ahlam F. Hamouda and Samar S have designed and performed experimental work. Aziza Amin has performed the histopathological changes. Mohamed A Mahmoud has performed the serum analysis, statistical analysis, writing and publication.

Consent to publish

The authors allow the publisher the only and sole license of the patent in the contribution. So, the publisher shall have the special right throughout the world to publish and sell the contribution in all languages and all other forms of electronic publication.

Competing interests

All authors have no conflict of interest.

REFERENCES

Abdul-Ghani A-S, Dabdoub N, Muhammad R, Abdul-Ghani R and Qazzaz M (2008). Effect of palestinian honey on spermatogenesis in rats. Journal of medicinal food, 11 (4): 799-802. Doi: 10.1089/jmf.2008.0085

Abdel-Gayoum AA and Ahmida MHS (2017). Changes in the serum, liver, and renal cortical lipids and electrolytes in rabbits with cisplatin-induced nephrotoxicity. Turkish journal of medical sciences, 47 (3): 1019-1027. Doi: 10.3906/sag-1602-136

To cite this paper: Hamouda AF, Amin AAE, Ibrahim SS, and Mahmoud MA (2019). Potential Ameliorative Effect of Bee Honey on Experimentally Induced Melamine Formaldehyde Toxicity in Male Rats. *World Vet. J.* 9(2): 146-157. www.wvj.science-line.com

Al-Hindi RR, Bin-Masalam MS and El-Shahawi MS (2011). Antioxidant and antibacterial characteristics of phenolic extracts of locally produced honey in saudi arabia. International journal of food sciences and nutrition, 62 (5): 513-517. Doi: 10.3109/09637486.2010.550276

Al-Waili NS (2003a). Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. Journal of medicinal food, 6 (2): 135-140. Doi:10.1089/109662003322233549

Al-Waili NS (2003b). Intravenous and intrapulmonary administration of honey solution to healthy sheep: Effects on blood sugar, renal and liver function tests, bone marrow function, lipid profile, and carbon tetrachloride-induced liver injury. Journal of medicinal food, 6 (3): 231-247. Doi:10.1089/10966200360716652

An L, Li Z, Yang Z and Zhang T (2011). Cognitive deficits induced by melamine in rats. Toxicology letters, 206 (3): 276-280.Doi: 10.1016/j.toxlet.2011.08.009

An L, Li Z, Yang Z and Zhang T (2012). Melamine induced cognitive impairment associated with oxidative damage in rat's hippocampus. Pharmacology Biochemistry and Behavior, 102 (2): 196-202. Doi: 10.1016/j.pbb.2012.04.009

Beutler E (1963). Improved method for determination of blood glutathione. Journal of Laboratory and Clinical Medicine, 61 (5): 882-888.

Bhalla V, Grimm PC, Chertow GM and Pao AC (2009). Melamine nephrotoxicity: An emerging epidemic in an era of globalization. Kidney international, 75 (8): 774-779. Doi: 10.1038/ki.2009.16

Bhor VM and Sivakami S (2003). Regional variations in intestinal brush border membrane fluidity and function during diabetes and the role of oxidative stress and non-enzymatic glycation. Molecular and Cellular Biochemistry, 252 (1-2): 125-132. Doi: 10.1023/a:1025599126840

Blasa M, Candiracci M, Accorsi A, Piacentini MP, Albertini MC and Piatti E (2006). Raw millefiori honey is packed full of antioxidants. Food Chemistry, 97 (2): 217-222. Doi: 10.1016/j.foodchem.2005.03.039

Brown CA, Jeong K-S, Poppenga RH, Puschner B, Miller DM, Ellis AE, Kang K-I, Sum S, Cistola AM and Brown SA (2007). Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. Journal of Veterinary Diagnostic Investigation, 19 (5): 525-531.Doi: 10.1177/104063870701900510

Chan EYY, Griffiths SM and Chan CW (2008). Public-health risks of melamine in milk products. The Lancet, 372 (9648): 1444-1445. Doi: 10.1016/S0140-6736(08)61604-9

Chen K-C, Liao C-W, Cheng F-P, Chou C-C, Chang S-C, Wu J-H, Zen J-M, Chen Y-T and Liao J-W (2009). Evaluation of subchronic toxicity of pet food contaminated with melamine and cyanuric acid in rats. Toxicologic pathology, 37 (7): 959-968. Doi: 10.1177/0192623309347910

Cianciolo RE, Bischoff K, Ebel JG, Van Winkle TJ, Goldstein RE and Serfilippi LM (2008). Clinicopathologic, histologic, and toxicologic findings in 70 cats inadvertently exposed to pet food contaminated with melamine and cyanuric acid. Journal of the American Veterinary Medical Association, 233 (5): 729-737. Doi: 10.2460/javma.233.5.729

Dias AS, Porawski M, Alonso M, Marroni N, Collado PS and Gonzalez-Gallego J (2005). Quercetin decreases oxidative stress, nf-κb activation, and inos overexpression in liver of streptozotocin-induced diabetic rats. The Journal of nutrition, 135 (10): 2299-2304. https://doi.org/10.1093/jn/135.10.2299

Dobson RLM, Motlagh S, Quijano M, Cambron RT, Baker TR, Pullen AM, Regg BT, Bigalow-Kern AS, Vennard T, Fix A, et al. (2008). Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. Toxicological Sciences, 106 (1): 251-262. https://doi.org/10.1093/toxsci/kfn160

Doubek J, Slosarkova S, Rehakova K, Bouda J, Scheer P, Piperisova I, Tomenendalova J and Matalova E (2010). The interpretation of basic biochemical and haematological indices in animals. Noviko, Czech Republic (in Czech), p. 102.

El-Khayat Z and Ahmed HH (2000). Antitumer efficacy of edible portulaca oleracea and bee's honey in mice inoculated with ehrlich ascttes tumer cells. Journal of Union of Arab Biologists, 13: 583-605. Doi:10.1007/springerreference_69205

El Rabey HA, Al-Seeni MN and Al-Solamy SM (2013). Bee's' honey protects the liver of male rats against melamine toxicity. BioMed research international, 2013: 1-8. Doi: 10.1155/2013/786051

Erejuwa OO, Sulaiman SA and Ab Wahab MS (2012). Honey: A novel antioxidant. Molecules, 17 (4): 4400-4423. Doi: 10.3390/molecules17044400

Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KNS, Salleh SM and Gurtu S (2011). Effect of glibenclamide alone versus glibenclamide and honey on oxidative stress in pancreas of streptozotocin-induced diabetic rats. International Journal of Applied Research in Natural Products, 4 (2): 1-10. Doi: 10.3390/ijms11052056

Filigenzi MS, Puschner B, Aston LS and Poppenga RH (2008). Diagnostic determination of melamine and related compounds in kidney tissue by liquid chromatography/tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 56 (17): 7593-7599. Doi: 10.1021/jf801008s

Grindem CB (2011). Schalm's veterinary hematology, 6th edition. Editors: Douglas j. Weiss, k. Jane wardrop. Veterinary Clinical Pathology, 40 (2): 270-270. Doi:10.1111/j.1939-165x.2011.00324.x

Gumieniczek A (2005). Oxidative stress in kidney and liver of alloxan-induced diabetic rabbits: Effect of repaglinide. Acta diabetologica, 42 (2): 75-81. Doi: 0.1007/s00592-005-0182-2

Hadwan MH (2016). New method for assessment of serum catalase activity. Indian Journal of Science and Technology, 9 (4). Doi: 10.17485/ijst/2016/v9i4/80499

Hammond BG, Barbee SJ, Inoue T, Ishida N, Levinskas GJ, Stevens MW, Wheeler AG and Cascieri T (1986). A review of toxicology studies on cyanurate and its chlorinated derivatives. Environmental Health Perspectives, 69: 287. Doi: 10.1289/ehp.8669287

To cite this paper: Hamouda AF, Amin AAE, Ibrahim SS, and Mahmoud MA (2019). Potential Ameliorative Effect of Bee Honey on Experimentally Induced Melamine Formaldehyde Toxicity in Male Rats. *World Vet. J.* 9(2): 146-157. www.wvj.science-line.com

Hau AK-c, Kwan TH and Li PK-t (2009). Melamine toxicity and the kidney. Journal of the American Society of Nephrology, 20 (2): 245-250. Doi: 10.1681/asn.2008101065

Hayes JM, Strauss H and Kaufman AJ (1999). The abundance of 13c in marine organic matter and isotopic fractionation in the global biogeochemical cycle of carbon during the past 800 ma. Chemical Geology, 161 (1-3): 103-125. Doi: 10.1016/s0009-2541(99)00083-2

Henry RJ (1974). Determination of serum creatinine. Clinical Chemistry: Principles and Techniques. second edition. Harper and Row Publishers, New York, pp. 104-106

Ingelfinger JR (2008). Melamine and the global implications of food contamination. New England Journal of Medicine, 359 (26): 2745-2748. Doi: 10.1056/nejmp0808410

Jingbin W, Ndong M, Kai H, Matsuno K and Kayama F (2010). Placental transfer of melamine and its effects on rat dams and fetuses. Food and Chemical Toxicology, 48 (7): 1791-1795. Doi: 10.1016/j.fct.2010.03.043

Johnston JE, Sepe HA, Miano CL, Brannan RG and Alderton AL (2005). Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat science, 70 (4): 627-631. Doi: 10.1016/j.meatsci.2005.02.011

Jutzi K, Cook AM and Hütter R (1982). The degradative pathway of the s-triazine melamine. The steps to ring cleavage. Biochemical Journal, 208 (3): 679-684. Doi: 10.1042/bj2080679

Kassim M, Achoui M, Mansor M and Yusoff KM (2010). The inhibitory effects of gelam honey and its extracts on nitric oxide and prostaglandin e2 in inflammatory tissues. Fitoterapia, 81 (8): 1196-1201. Doi: 10.1016/j.fitote.2010.07.024

Khan SM, Sobti RC and Kataria L (2005). Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract. Clinica Chimica Acta, 358 (1-2): 131-138. Doi: 10.1016/j.cccn.2005.02.015

Khan SR (2006). Renal tubular damage/dysfunction: Key to the formation of kidney stones. Urological research, 34 (2): 86-91. Doi: 10.1007/s00240-005-0016-2

Kılıcoglu B, Gencay C, Kısmet K, Kılıcoglu SS, Erguder I, Erel S, Sunay AE, Erdemli E, Durak I and Akkus MA (2008). The ultrastructural research of liver in experimental obstructive jaundice and effect of honey. The American Journal of Surgery, 195 (2): 249-256. Doi: 10.1016/j.amjsurg.2007.04.011

Kochmar J and Mossa D (1976). Foundamental of clinical chemistry. first edition. Philadelphia: W.B. Saunders Company, p. 606.

Lam HS, Ng PC, Chu WCW, Wong W, Chan DFY, Ho SS, Wong KT, Ahuja AT and Li CK (2008). Renal screening in children after exposure to low dose melamine in hong kong: Cross sectional study. British Medical Journal, 337: 2991. Doi: 10.1136/bmj.a2991

Leary NO, Pembroke A and Duggan PF (1992). Single stable reagent (arsenazo iii) for optically robust measurement of calcium in serum and plasma. Clinical Chemistry, 38 (6): 904-908. Doi: 10.1177/000456329202900113

Li L, Li B, Cheng D and Mao L (2010). Visual detection of melamine in raw milk using gold nanoparticles as colorimetric probe. Food Chemistry, 122 (3): 895-900.

Lustgarten JA, Wenk RE, Byrd C and Hall B (1974). Evaluation of an automated selective-ion electrolyte analyzer for measuring na+, k+, and cl-in serum Clinical Chemistry, 20 (9): 1217-1221. Doi: 10.1093/ajcp/59.4.542

Lv Y, Liu P, Xiang C and Yang H (2013). Oxidative stress and hypoxia observed in the kidneys of mice after a 13-week oral administration of melamine and cyanuric acid combination. Research in Veterinary Science, 95 (3): 1100-1106. Doi: 10.1016/j.rvsc.2013.10.001

Michalkiewicz A, Biesaga M and Pyrzynska K (2008). Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey. Journal of chromatography A, 1187 (1-2): 18-24. Doi: 10.1016/j.chroma.2008.02.001

Morris JL and Macleod AG (1922). Colorimetric determination of uric acid: Estimation of 0.03 to 0.5 mg. Quantities by a new method Journal of Biological Chemistry, 50: 55. Doi: 10.1021/ac60058a044

Munoz MA, Balon M and Fernandez C (1983). Direct determination of inorganic phosphorus in serum with a single reagent. Clinical Chemistry, 29 (2): 372-374. Doi: 10.1039/an9790401124

Ogasawara H, Imaida K, Ishiwata H, Toyoda K, Kawanishi T, Uneyama C, Hayashi S, Takahashi M and Hayashi Y (1995). Urinary bladder carcinogenesis induced by melamine in f344 male rats: Correlation between carcinogenicity and urolith formation. Carcinogenesis, 16 (11): 2773-2777. Doi: 10.1093/carcin/16.11.2773

Ohkawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95 (2): 351-358. Doi: 10.1016/0003-2697(79)90738-3

Omotayo EO, Gurtu S, Sulaiman SA, Wahab MSA, Sirajudeen KNS and Salleh MSM (2010). Hypoglycemic and antioxidant effects of honey supplementation in streptozotocin-induced diabetic rats. International Journal for Vitamin and Nutrition Research, 80 (1): 74. Doi: 10.1024/0300-9831/a000008

Patton GJ and Crouch SR (1977). Determination of urea (urease modified berthelot reaction). Analytical Chemistry, 49: 464-469. Doi: 10.1021/ac50011a034

Peake JM, Suzuki K and Coombes JS (2007). The influence of antioxidant supplementation on markers of inflammation and the relationship to oxidative stress after exercise. The Journal of Nutritional Biochemistry, 18 (6): 357-371. Doi: 10.1016/j.jnutbio.2006.10.005

Petrus K, Schwartz H and Sontag G (2011). Analysis of flavonoids in honey by hplc coupled with coulometric electrode array detection and electrospray ionization mass spectrometry. Analytical and bioanalytical chemistry, 400 (8): 2555-2563. Doi: 10.1007/s00216-010-4614-7

Puschner B, Poppenga RH, Lowenstine LJ, Filigenzi MS and Pesavento PA (2007). Assessment of melamine and cyanuric acid toxicity in cats. Journal of Veterinary Diagnostic Investigation, 19 (6): 616-624. Doi: 10.1177/104063870701900602

To cite this paper: Hamouda AF, Amin AAE, Ibrahim SS, and Mahmoud MA (2019). Potential Ameliorative Effect of Bee Honey on Experimentally Induced Melamine Formaldehyde Toxicity in Male Rats. *World Vet. J.* 9(2): 146-157. www.wvj.science-line.com

Puschner B and Reimschuessel R (2011). Toxicosis caused by melamine and cyanuric acid in dogs and cats: Uncovering the mystery and subsequent global implications. Clinics in laboratory medicine, 31 (1): 181-199. Doi: 10.1016/j.cll.2010.10.003

Reitman S and Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28 (1): 56-63. Doi: 10.1093/ajcp/28.1.56

Shehu S, Wasagu RSU, Lawal M and Albert AK (2015). Effect of melamine adulteration on body weight, liver and kidney histology and liver function indices of albino rats. Asian Journal of Biochemistry, 10 (3): 125-131. Doi: 10.3923/ajb.2015.125.131

Stine CB, Reimschuessel R, Keltner Z, Nochetto CB, Black T, Olejnik N, Scott M, Bandele O, Nemser SM, Tkachenko A, et al. (2014). Reproductive toxicity in rats with crystal nephropathy following high doses of oral melamine or cyanuric acid. Food and Chemical Toxicology, 68: 142-153. Doi: 10.1016/j.fct.2014.02.029

Strakova E, Karaskova K, Zapletal D and Suchy P (2014). Effect of melamine and cyanurid acid contaminated diets on blood indicators in broiler chickens. Czech Journal of Animal Science, 59: 564-570. Doi: 10.17221/7796-cjas

Van Kampen EJ and Zijlstra WG (1961). Standardization of hemoglobinometry ii. The hemiglobincyanide method. Clinica Chimica Acta, 6 (4): 538-544. Doi: 10.1016/0009-8981(61)90145-0

Wilson JI, George BO and Umukoro GE (2011). Effects of honey on the histology of liver in adult wistar rats. Biology and Medicine, 3 (1): 1-5. Doi: 10.15406/mojbm.2017.02.00046

Xin H and Stone R (2008). Chinese probe unmasks high-tech adulteration with melamine. Science, 322 (5906): 1310-1311. Doi: 10.1126/science.322.5906.1310

Yang J-L and Chen H-C (2003). Effects of gallium on common carp (cyprinus carpio): Acute test, serum biochemistry, and erythrocyte morphology. Chemosphere, 53 (8): 877-882. Doi: 10.1016/s0045-6535(03)00657-x

Yao L, Jiang Y, D'Arcy B, Singanusong R, Datta N, Caffin N and Raymont K (2004). Quantitative high-performance liquid chromatography analyses of flavonoids in australian eucalyptus honeys. Journal of Agricultural and Food Chemistry, 52 (2): 210-214. Doi: 10.1021/jf034990u

Yao LK, Razak SLA, Ismail N, Fai NC, Asgar MHAM, Sharif NM, Aan GJ and Jubri Z (2011). Malaysian gelam honey reduces oxidative damage and modulates antioxidant enzyme activities in young and middle aged rats. Journal of Medicinal Plants Research, 5 (23): 5618-5625. http://www.academicjournals.org/JMPR

Zhang L, Wu L-L, Wang Y-P, Liu A-M, Zou C-C and Zhao Z-Y (2009). Melamine-contaminated milk products induced urinary tract calculi in children. World Journal of Pediatrics, 5 (1): 31-35. Doi: 10.1007/s12519-009-0005-6

Zheng X, Zhao A, Xie G, Chi Y, Zhao L, Li H, Wang C, Bao Y, Jia W, Luther M, et al. (2013). Melamine-induced renal toxicity is mediated by the gut microbiota. Science translational medicine, 5 (172): 172ra22. Doi: 10.1126/scitranslmed.3005114

Zhou Y, Li C-Y, Li Y-S, Ren H-L, Lu S-Y, Tian X-L, Hao Y-M, Zhang Y-Y, Shen Q-F and Liu Z-S (2012). Monoclonal antibody based inhibition elisa as a new tool for the analysis of melamine in milk and pet food samples. Food Chemistry, 135 (4): 2681-2686. Doi: 10.1016/j.foodchem.2012.07.053

To cite this paper: Hamouda AF, Amin AAE, Ibrahim SS, and Mahmoud MA (2019). Potential Ameliorative Effect of Bee Honey on Experimentally Induced Melamine Formaldehyde Toxicity in Male Rats. *World Vet. J.* 9(2): 146-157. www.wvj.science-line.com

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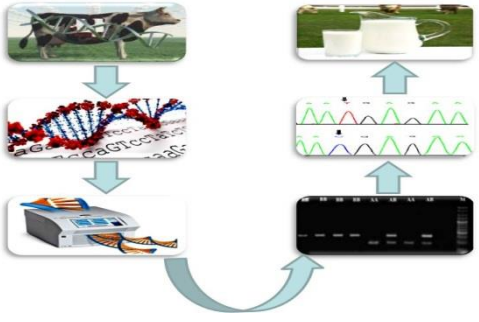

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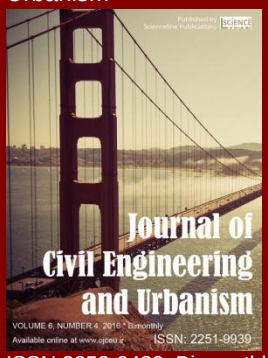

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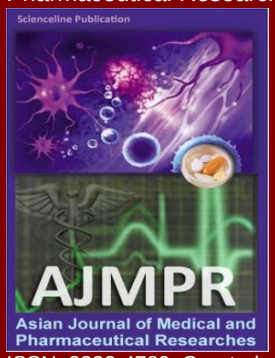

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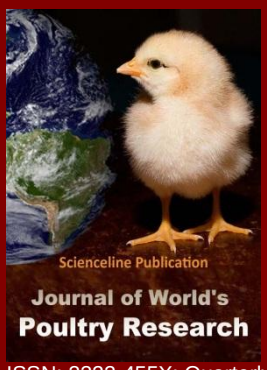

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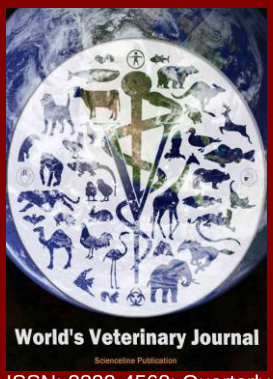

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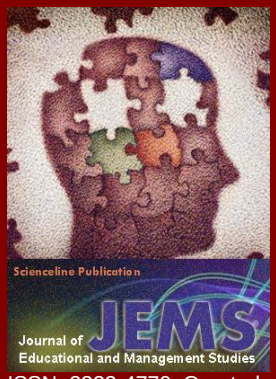

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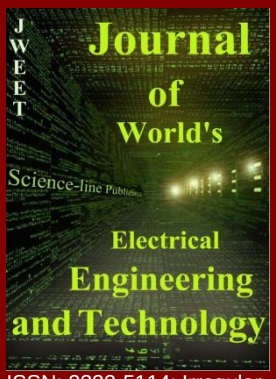

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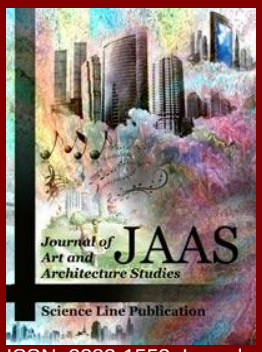

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