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Effects of Substitution of Corn with Dried Orange Pulp on Health and Growth Performance of Broiler Chickens

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ABSTRACT

The rising cost of cereal-based ingredients and restrictions on the use of antibiotic growth promoters in poultry have increased demand for sustainable alternatives. Agro-industrial by-products, particularly dried orange pulp (DOP), offer an economical, nutrient-rich solution with bioactive compounds that may improve broiler health and growth. The present study aimed to investigate the effects of incorporating different levels of dried orange pulp (*Citrus sinensis*) on growth performance and blood serum biochemical parameters in broiler chickens. A total of 200 one-day-old Arbor Acres chickens (45 ± 1.7 g) were randomly divided into four dietary groups. Each group consisted of 50 chickens, with five replicates of 10 chickens, and was reared for 49 days. The dietary groups included a control diet without DOP and three experimental diets in which DOP was partially replaced by corn at inclusion levels of 5% (50 g/kg of dry matter [DM]), 10% (100 g/kg of DM), and 15% (150 g/kg of DM). Growth performance was monitored throughout the entire rearing period. Blood samples were collected at 37 and 49 days of age to assess glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Broiler chickens fed DOP-substituted diets generally exhibited lower serum lipid levels, especially triglycerides and total cholesterol, compared to the control group at both sampling times. However, there was a slight increase in triglycerides in the 5% DOP group at 49 days compared to the control group. Glucose concentrations generally increased with higher levels of DOP inclusion, especially at 15%. Regarding growth performance, chickens fed the 5% DOP diet had the highest live weight, whereas those fed the 10% DOP diet demonstrated an improvement in feed conversion ratio compared to the control group. The dietary incorporation of DOP enhanced growth performance and positively influenced serum biochemical profiles in broiler chickens, with the 5% DOP level demonstrating the most significant improvements in performance and productivity parameters, while 10% DOP exerted the most beneficial effects on blood biochemical parameters.

Keywords: Cholesterol, HDL, LDL, Lipid fraction, Triglyceride

INTRODUCTION

In 2022, global poultry meat production reached 141 million tons, representing a 1.9% increase from the previous year. In 2021, chicken meat demonstrated the highest absolute and relative growth since 2000 (+107%, or 63 million tons), and remained the most produced type of meat (Ortiz-Sanchez et al., 2024). In recent years, there has been a steady rise in the costs of livestock production, mainly due to increasing prices of feedstuffs such as soybean products and cereal grains (Lawrence et al., 2008). Implementing a resilient feeding strategy enhances production efficiency, reduces costs, and ensures the delivery of high-quality products to consumers (Ebrahimi et al., 2013).

Simultaneously, the use of synthetic growth promoters and antibiotics has been investigated due to their association with antimicrobial resistance, risks to human health, and consumer demand for residue-free, organic poultry products (Nhung et al., 2017). In response, natural feed additives such as probiotics, prebiotics, essential oils, organic acids, and plant-derived by-products rich in polyphenols and flavonoids have emerged as promising alternatives to antibiotics (Imran, 2022; Saeed et al., 2024). Among these, phytobiotics, plant-derived compounds with antioxidant, antimicrobial, and anti-inflammatory properties, have shown potential in improving gut health, enhancing immunity, and regulating lipid metabolism, including the mitigation of hypercholesterolemia in quail (Tufan et al., 2023). Large amounts of orange processing waste (OPW) are produced worldwide, totaling 24 million tons (Bouaita et al., 2022). To mitigate environmental damage and promote a circular economy, new methods are being explored to valorize OPW within the citrus value chain. Dried orange byproducts (*Citrus sinensis*) could be a potential source of valuable nutrients, such as carbohydrates, lipids and fatty acids, vitamins, minerals, phenolic compounds and flavonoids, and organic acids, serving as a natural source of antioxidants for poultry feed (Ebrahimi et al., 2013; 2014; 2015). Orange peels contain high concentrations of phenols, flavonoids (Wang et al., 2015), and vitamins, especially vitamin C (Manthey et al., 2004).

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Finally, dried citrus pulp (DCP) contains high levels of fermentable carbohydrates and sugars, making it a valuable energy source in poultry nutrition (Bampidis et al., 2006) with antibacterial, antioxidant properties, and immune-stimulating activities (Ebrahimi et al., 2015; Pourhossein et al., 2015). Therefore, the present study aimed to evaluate the effects of replacing dried orange pulp (DOP) with maize in broiler diets on growth performance, blood biochemical parameters, and production efficiency over a 49-day rearing period.

MATERIALS AND METHODS

Ethical approval

All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of experimental animals established by the National Committee for Ethics in Animal Experimentation (Algeria). The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee of the Higher School of Agronomy, Mostaganem, Algeria (ESAM-PA-2023-011).

Dried orange pulp preparation

Fresh Citrus sinensis pulp residues were collected from a local juice processing facility and spread onto thin layers (2-3 cm) of clean, food-grade plastic sheets. The pulp was sun-dried under greenhouse conditions with proper ventilation for 2-4 days, depending on ambient humidity and temperature, until moisture content dropped below 10%. Moisture content was determined by oven-drying 5 g of the sample at 105°C for 24 hours to stabilize the weight, and expressed as percentage loss in weight relative to the initial sample mass (AOAC, 2005). Manual turning was performed twice daily to ensure uniform drying and avoid microbial contamination. The dried material was then ground using a hammer mill, sieved through a 1 mm mesh, and stored in airtight containers at room temperature (Fernández-López et al., 2009).

Animals and experimental design

A total of 200 one-day-old unsexed Arbor Acres broiler chickens, weighing 45 ± 1.7 g, were used for the present study. During the first 15 days, all chickens were fed a standard starter diet containing 3035 kcal/kg of metabolizable energy (ME) and 22% crude protein formulated according to the nutritional requirements established by the Algerian West Poultry Group (ORAVIO) for chickens. From day 16 onward, chickens were randomly assigned to four dietary treatments, each comprising five replicates with ten chickens, including a control group without DOP and three experimental diets in which DOP was replaced by corn at 5%, 10%, or 15%. The experimental diets were formulated to be isonitrogenous and isocaloric and were balanced according to the nutrient recommendations of the nutrient requirements of poultry (NRC, 1994). Diet formulation was performed using linear programming (least-cost formulation) with feed formulation software (Win Feed/Feed soft or equivalent), entering the analyzed nutrient values of local raw materials. Dried orange pulp replaced corn at 5, 10, and 15% (w/w) on a grower-finisher basis (15-49 days). Diets were supplemented with DL-methionine and L-lysine HCl to ensure they met or exceeded NRC targets for amino-acid requirements in grower-finisher broiler chickens (lysine ≈ 0.9 -1.1% total diet; methionine ± cystine ≈ 0.45-0.55% total diet). The ME values were calculated from ingredient tables and confirmed by proximate analysis according to the nutritional requirements established by the Algerian West Poultry Group (ORAVIO) for chickens. All diets included a commercial vitamin-mineral premix containing vitamins A, D3, E, K, and B-complex, and trace minerals, provided per kg of diet to meet the micronutrient requirements listed in Table 1.

Table 1. Ingredients and nutrient composition of different diets in broiler chickens' feed

Ingredients (%) / Diets (16 to 49 days of age)	Control	DOP 5%	DOP 10%	DOP 15%
Corn grains	67.00	62.00	57.00	52.00
Soybean meal	27.00	27.00	27.00	27.00
Wheat bran	4.00	4.00	4.00	4.00
Dicalcium phosphate	1.00	1.00	1.00	1.00
Vit-Min Premix *	1.00	1.00	1.00	1.00
Dried orange pulp	0	5.00	10.00	15.00
Chemical analysis				
Metabolizable energy	3121.49	3140.41	3162.50	3187.45
Proteins content	21.77 ± 0.12	20.60 ± 0.78	20.43 ± 0.51	20.22 ± 0.89
Ash	3.07 ± 2.28	3.20 ± 0.98	3.51 ± 2.73	3.82 ± 1.99
Fat content	4,36 ± 0,41	3,65 ± 0,48	3,21 ± 0,13	3,16 ± 0,8
Crude cellulose	5.09 ± 0.68	6.43 ± 0.54	6.59 ± 0.34	6.68 ± 0.79

*Vitamin-mineral premix: Provided (in mg kg of diet), Vitamin E: 6, Vitamin K3: 0.80, Vitamin B1: 1, Vitamin B2: 3, Pantothenate of Ca: 6, Vitamin B6: 1.5, Vitamin B12: 0.006, Folic acid: 0.2, Nicotinic acid: 12, Copper: 5, Cobalt: 0.65, Manganese: 65, Zinc: 65, Selenium: 0.25, Iron: 50, Iode: 0.8, Magnesium: 100, C: Control diet, CP: Calcium + Phosphore, DOP 5,10, and 15%: Dried orange pulp at 5, 10, and 15%.

Housing

The feeding trial was conducted in a thoroughly fumigated, washed, and disinfected poultry facility with a surface area of 20 m², following the Arbor Acres management guidelines (2018). Broiler chickens were randomly distributed into pens measuring 5 m² (10 birds/m²), equipped with wood-shaving litter, a feeder, and a drinker. Environmental conditions were maintained according to standard broiler rearing protocols. The brooding temperature was initially set at 34°C during the first week and gradually reduced to 24°C by the fourth week, while relative humidity was maintained between 60-70%. The lighting schedule consisted of 23 hours of light and 1 hour of darkness during the first three days, and was subsequently adjusted to 22 hours of light and 2 hours of darkness from Day 21 until Day 49. Feed and water were supplied *ad libitum* throughout the experimental period. Ventilation was regulated using a thermostat-controlled cross-ventilation system.

Vaccination

All chickens were vaccinated following a standardized protocol. At hatch, chickens received vaccination against Marek's disease (HVT strain, 2000 PFU, Cevac® Rispens, Ceva Santé Animale, France), Newcastle (LaSota strain, Zoetis, USA), and infectious bronchitis (Massachusetts strain, Bronhivit®, Spain) on Days 7 and 21 via drinking water. Vaccination against infectious bursal disease (Intermediate strain, Cevac® IBD L, Ceva Santé Animale, France) was administered on Days 14 and 28. The vaccination program was identical for all experimental groups (Wegner et al., 2024).

Zootechnical parameters

Growth performance parameters, including body weight and feed conversion ratio (FCR), were measured and recorded weekly. Feed intake was determined by recording the quantity of feed offered and the residues remaining at the end of the rearing period. Body weight was measured individually at the beginning of the experiment and at the end of the rearing period. The FCR was calculated as the ratio of feed intake to body weight gain for each replicate group over the entire 49-day rearing period. Mortality, if it occurred, was recorded daily and used to adjust feed intake and performance data.

Sampling

Blood samples were collected on days 37 and 49 by randomly selecting two chickens from each pen, yielding a total of 10 chickens per treatment group. Approximately 3 mL of blood per chicken was drawn from the brachial vein using sterile syringes and collected into plain vacutainer tubes without anticoagulant. Sampling and serum preparation followed the protocol of Readh et al. (2023). Samples were centrifuged at 8000 × g for 8 minutes to obtain serum, which was subsequently stored at -20°C until further analysis. Biochemical analyses included measurements of glucose, triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol using commercial diagnostic kits (BIOLABO SAS, France) and quantified with a SPECORD® 210 spectrophotometer (Analytik Jena GmbH, Germany). All analyses were performed at the Laboratory of Applied Biotechnology, Mostaganem, Algeria.

Statistical analysis

All data were analyzed using IBM SPSS Statistics version 26.0. A one-way analysis of variance (ANOVA) was structured to detect significant differences in body weight, FCR, and serum biochemical parameters with 80% statistical power and a significance level (α) of 0.05. Data normality was verified using the Shapiro-Wilk test, and homogeneity of variances was confirmed before ANOVA. When significant differences were found ($p < 0.05$), Tukey's HSD post hoc test was used for pairwise comparisons, with Bonferroni adjustments where appropriate. Results were expressed as Mean ± Standard Deviation (SD).

RESULTS

The effects of different levels of DOP on growth performance, body weight, and FCR in broiler chickens during the whole rearing period are presented in Table 2. At day 49 of rearing, dietary inclusion with DOP significantly affected body weight ($p < 0.05$). Broiler chickens fed the DOP 5% diet exhibited the most significant improvement, with an increased average body weight of 14.4% compared to the control group. The DOP 10% and 15% diets increased final body weight by 6.0% and 2.9%, respectively, compared to the control group. These findings showed that moderate levels of DOP, especially at 5%, had a strong positive impact on growth performance. Higher levels of DOP (10-15%) improved growth performance, but to a smaller degree. The dose-dependent trend indicated that DOP can effectively replace corn in broiler diets, with the best results at lower substitution levels.

Feed conversion ratio was not significantly affected by dietary inclusion of DOP ($p > 0.5$). The control group recorded the highest FCR, whereas the 5%, 10%, and 15% of DOP demonstrated numerical improvements at approximately 6.1%, 9.7%, and 4.0%, respectively. Although these differences were not statistically significant, the trend suggested a potential improvement in feed efficiency with moderate levels of DOP substitution, particularly at DOP 10%.

Table 2. Effect of dried orange pulp on the performance of broiler chickens aged 49 days (Mean ± SD)

Item \ Treatments	Control	DOP 5%	DOP 10%	DOP 15%	SE	p value
Whole rearing period 1st–49st day						
Weight (g/chick/duration)	$2994^{a} \pm 163$	$3424^{c} \pm 126$	$3174^{bc} \pm 117$	$3081^{b} \pm 102$	28.91	0.01
Feed conversion ratio	$2.47^{a} \pm 0.13$	$2.32^{a} \pm 0.11$	$2.23^{a} \pm 0.17$	$2.37^{a} \pm 0.14$	0.25	0.19

DOP: Dried orange pulp, SE: Standard deviation, each least squares mean represents 12 replicated pens. Means within each row of dietary treatments with no common superscript differ significantly at $p < 0.05$. $^{a–c}$ Means in each row with no common superscript letter differ significantly at ($p < 0.05$).

Table 3 presents the results regarding the impact of DOP on different blood parameters, including glucose, cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in broiler chickens, measured at two distinct time points, 37 and 49-days post-hatch. The inclusion of DOP in broiler diets significantly influenced serum glucose and lipid levels, with responses depending on diet and age. At day 37, glucose concentrations were significantly increased in the DOP 10% (+26%) and DOP 15% (+39%) compared to the control group ($p < 0.05$). However, glucose concentrations in DOP 5% did not differ from those in the control group. Triglyceride and total cholesterol concentrations were significantly reduced in the DOP 10% by 37% and 48%, respectively ($p < 0.05$).

On day 49, glucose levels steadily increased with higher DOP inclusion, reaching a peak in DOP 15% (68%) compared to the control group ($p < 0.05$). For lipid levels, total cholesterol and LDL were significantly decreased in all DOP-fed groups ($p < 0.05$), while HDL concentrations were significantly elevated, particularly in the DOP 10% (5%) compared to the control group ($p < 0.05$). Chickens fed the DOP 5% diet exhibited a moderate decrease in cholesterol of approximately 32% compared to the control group, whereas the DOP 10% and 15% diets markedly reduced cholesterol levels by 48% and 35%, respectively. These results suggested that increasing dietary inclusion of DOP exerted a hypocholesterolemic effect, particularly at 10% substitution, which produced the most pronounced reduction.

Triglyceride levels tended to decrease in DOP-fed groups; however, this effect was less consistent and was not statistically significant ($p < 0.05$).

Table 3. The impact of dried orange pulp diets on serum glucose and lipid fractions at 37 and 49 days of rearing

SAMP	Diets	GLU (mg/dl)	TRIG (mg/dl)	CHOL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
37d (S1)	Control	127.07^{a}	121.08^{c}	137.16^{c}	74.67^{ab}	36.56^{a}
	DOP 5%	120.84^{a}	92.94^{b}	117.44^{b}	74.67^{ab}	32.89^{a}
	DOP 10%	160.47^{b}	67.33^{a}	71.82^{a}	78.00^{b}	32.56^{a}
	DOP 15%	177.39^{c}	76.61^{ab}	105.07^{b}	68.44^{a}	32.40^{a}
49d (S2)	Control	175^{a}	137.15^{c}	212.23^{c}	98.67^{a}	51.00^{b}
	DOP 5%	163^{a}	143.70^{c}	140.71^{b}	98.00^{a}	47.24^{ab}
	DOP 10%	209^{b}	71.82^{a}	101.98^{a}	102.44^{a}	45.71^{ab}
	DOP 15%	252.50^{c}	112.73^{b}	137.21^{b}	89.73^{a}	40.71^{a}
S1	**P value**	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p > 0.05$
S2		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p > 0.05$	$p < 0.01$
Age		$P < 0.01$	$P < 0.01$	$P < 0.01$	$p < 0.01$	$p < 0.01$
Diet*Age		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p > 0.05$	$p < 0.01$

Glu: Glucose; Trig: Triglycerides; Chol: Cholesterol; HDL: High density lipoprotein, and LDL: Low density lipoprotein. For each group, n = 10. SAMP: Samples; S1: First sample; S2: Second sample. Diet × Age: Represents the interaction effect between the type of diet and the age of the chickens. $^{a–c}$ Means in each row with no common superscript letter differ significantly at ($p < 0.05$). The results are expressed as the mean.

The global analysis confirmed that diet, age, and their interaction significantly affected glucose and lipid metabolism ($p < 0.05$). The current results demonstrated that DOP supplementation induced a beneficial hypolipidemic effect, reducing total cholesterol, triglycerides, and LDL while enhancing HDL, with the most consistent improvements observed at the DOP 10% dietary group.

The serum glucose and lipid profiles revealed that dietary DOP had a significant and age-dependent impact (Table 3). Importantly, DOP incorporation had a dual effect; it regularly decreased lipid levels, notably triglycerides and total cholesterol, while also causing a dose-dependent rise in serum glucose. This glycemic effect was significantly more pronounced in the older cohort (S2). Statistical analysis confirmed significant interactions between diet and age for glucose, triglycerides, and cholesterol ($p < 0.01$), underscoring that the host's age is a crucial factor in the metabolic response to DOP.

DISCUSSION

The present findings are consistent with those reported by Parobali et al. (2024), who demonstrated that supplementing broiler diets with *Citrus sinensis* seed at inclusion levels of 0.25%, 0.5%, and 0.75% considerably improved body weight gain and FCR at 49 days of rearing period compared to the control group. Similarly, the current results align with those of Abu Al-Makarem et al. (2024), who reported that supplementing a basal corn-soybean meal diet with 1% and 2% DOP enhanced FCR in broiler chickens.

The current results indicated age-related increases in serum glucose levels, reflecting metabolic development. Similar trends were reported by Tůmová et al. (2019).

Ahmad et al. (2024) suggested that the positive effect of orange peel inclusion on broiler weight gain might be related to the citric acid content, which helps modulate gut microbial balance by inhibiting pathogenic bacteria. This antimicrobial activity can improve digestive efficiency, thereby enhancing nutrient absorption and overall growth performance. The low pH of these acids inhibits pathogenic gastrointestinal microbes, thereby reducing the toxicity of bacterial products, improving protein digestibility and energy utilization, and ultimately enhancing weight gain. Additionally, Jiang et al. (2020) demonstrated that naringin content in DOP improved performance parameters and reduced FCR in the naringin-treated group.

Similarly, Nazok et al. (2010) reported that the inclusion of citrus by-products enhanced growth performance. Mehmet and Simsek (2016) observed elevated glucose levels in broilers fed *Citrus sinensis* essential oil, likely due to impaired glucose uptake. This response may be attributed to the action of citrus flavonoids such as hesperidin, naringin, and narirutin, which are known to modulate carbohydrate metabolism and glucose transport (Chen et al., 2017). Flavonoids can influence glucose homeostasis by affecting the expression or activity of key enzymes involved in glycolysis and gluconeogenesis, such as hexokinase, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase. Moreover, flavonoids may alter insulin signaling pathways and the translocation of glucose transporter proteins in cell membranes, thereby reducing cellular glucose uptake and contributing to increasing the blood glucose levels.

Triglyceride levels fell within the Arbor Acres strain's typical range, varying from 0.17 to 1.61 g/l. Moreover, serum cholesterol concentrations ranged from 0.20 to 1.50 g/L, aligning with the reference range for *Arbor Acres* broiler chickens (Arzour-Lakehal and Boudebza, 2021), except for a deviation observed in the control group during the second sampling.

In the present study, triglyceride and cholesterol levels increased with age, which was consistent with the findings of Almeida et al. (2006), who reported that elevated lipid levels in older chickens were associated with higher energy demands during the finishing phase. As reported by Silva et al. (2007), the elevation of cholesterol and triglyceride levels during the rearing period is also linked to reduced mobility and increased synthesis of these lipids within the body.

Incorporating DOP into chicken diets effectively modulated serum lipid metabolism by reducing cholesterol, triglyceride, and LDL levels, while promoting higher glucose concentrations, particularly at higher inclusion levels (10–15% DOP). Similar findings were reported when orange peel or pulp was incorporated into chicken diets by Alefzadeh et al. (2016), Akpe et al. (2019), Abd El Latif et al. (2023), and Hussein et al. (2023). Moreover, the lipid analysis in the present study was consistent with that reported by Younis et al. (2025), who observed that dietary supplementation with dried pomegranate reduced serum cholesterol and triglyceride levels. The current findings corroborated the results reported by Abbasi et al. (2015) and Alefzadeh et al. (2016), who demonstrated that dietary DOP and orange pulp reduced blood cholesterol and triglycerides. This effect might be attributed to the hypocholesterolemic properties of citrus fruits. The flavonoids have been shown to inhibit cholesterol synthesis in the liver by inhibiting the hepatic enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (Gilani et al., 2018).

Moreover, supplementation with DOP and citrus waste in broiler diets decreased blood cholesterol, triglyceride, and LDL levels (Abu Al-Makarem et al., 2024). In 37 days, broiler chickens fed DOP 10% had the highest HDL levels compared to the control group, indicating an improved lipid profile. Additionally, the supplementation of polyphenols from green tea at 50 and 100 mg/kg of body weight for 20 days has been shown to reduce serum lipid levels in chickens (*Gallus gallus domesticus*) by inhibiting fatty acid synthesis and promoting lipolysis (Huang et al., 2013). Although

Huang et al. (2013) used green tea polyphenols, DOP also has a high content of polyphenolic compounds, which are recognized for their similar lipid-lowering and antioxidant properties.

CONCLUSION

The inclusion of DOP in broiler diets demonstrated positive effects on both growth performance and serum biochemical parameters. The DOP 5% exhibited the highest live weight, showing an improvement of approximately 15% compared to the control group, while the DOP 10% recorded the FCR. In terms of biochemical responses, increasing DOP inclusion level reduced serum cholesterol and triglyceride concentrations, particularly in DOP 10% and 15%, and increased HDL levels, indicating a healthier lipid profile. These findings confirmed that DOP, rich in polyphenols and natural antioxidants, can effectively replace part of the corn in broiler diets without compromising performance or metabolic health. Further studies are recommended to investigate the long-term effects of DOP on meat quality, immune response, and gut microbiota composition, and to evaluate its optimal inclusion level across different rearing and environmental conditions.

DECLARATIONS

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Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Authors' contributions

Ahmed Readh Chaib Eddour contributed to conceptualization, experimental design, data collection, statistical analysis, data interpretation, manuscript drafting, figure and table preparation, and final manuscript revision. Miloud Litim assisted in the experimental setup and contributed to data interpretation and manuscript review. Kaddour Bouderoua supervised the experimental procedures and ethical compliance, verification of analytical accuracy, and critical revision of the manuscript. All authors read and approved the final edition of the manuscript.

Competing interests

The authors declared no competing interests in the present study.

Ethical considerations

All ethical considerations related to this study, including but not limited to plagiarism, consent for publication, research misconduct, data fabrication or falsification, redundant publication, and simultaneous submission, have been thoroughly reviewed and addressed by all authors. The research was conducted in accordance with ethical standards and institutional guidelines. The authors confirm that no AI tools were used to generate, manipulate, or fabricate any data in this study. All data were obtained through standard experimental procedures and analyzed by the research team

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