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Effects of Nettle (*Urtica dioica*) Supplementation on Productive Performance, Biochemical Parameters, and Gut Microbiota in Broiler Chickens

Edwaldo Villanueva Pedraza [1]*, Pompeyo Ferro[1], Jeiner Alexander Villanueva Guerrero[1], Johnny Cueva Valdivia[2], Anthonny Smith Guevara Flores[1], Jose Alberto Carlos Ramos[1], Euclides Ticona Chayña[1], and Papa Pio Ascona Garcia[1]

[1]*Department of Animal Science and Biotechnology, Faculty of Natural and Applied Sciences, National Intercultural University Fabiola Salazar Leguía of Bagua, Bagua, 01721, Amazonas, Peru*
[2]*Faculty of Technology and Innovation, National University of Barranca, Legal Address: Av. Toribio Luzuriaga No. 376, Barranca, 15169, Lima, Peru*

*Corresponding author's Email: evillanueva@unibagua.edu.pe

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ABSTRACT

The global poultry industry is challenged to meet rising demands for sustainable production, prompting interest in plant-based feed additives like *Urtica dioica* due to their nutritional and functional properties. The objective of this study was to evaluate the effects of dietary inclusion of *Urtica dioica* on growth performance, serum biochemical indicators, and intestinal microbiota composition in broiler chickens. The 42-day feeding trial involved 120 male Cobb 500 broiler chickens with an average initial body weight of 41.7 ± 1.2 g. Broiler chickens were randomly allocated to four experimental groups, each consisting of six replicates with ten chickens. The treatment groups received basal diets supplemented with 1% (T1), 2% (T2), or 3% (T3) *Urtica dioica*, while the control group (T0) was fed the basal diet without additives. Productive performance parameters were recorded weekly, and on day 42, blood profiles and intestinal microbiota composition were evaluated. Results showed that 1% *Urtica dioica* significantly improved live weight and feed conversion ratio (FCR) compared to the control group, with no additional benefits observed at higher inclusion levels. The biochemical assessment showed that broiler chickens supplemented with 1% *Urtica dioica* exhibited significant reductions in total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triacylglycerol levels compared to the control group. Microbial analysis demonstrated a significant increase in *Lactobacillus* spp. populations and a decrease in *coliform* bacteria in the 1% supplementation group, suggesting improved gut health. These findings indicated that 1% *Urtica dioica* supplementation enhances growth, lipid metabolism, and intestinal health in broiler chickens.

Keywords: *Urtica dioica*, Medicinal Plant, Chicken, Productive performance, Gut microbiota

INTRODUCTION

Poultry meat production provides an economical and accessible source of animal protein, essential for meeting the increasing demand driven by global population growth (Bist et al., 2024; Mnisi et al., 2024). However, the intensified nature of modern broiler production poses challenges related to animal health, welfare, and sustainability (Teixeira et al., 2023). In this context, interest has grown in the use of natural feed additives capable of improving productive performance while enhancing the physiological resilience of chickens (Mitrović et al., 2020). *Urtica dioica* is a perennial herbaceous plant native to temperate regions of Europe, Asia, and North America, and is also found in the Peruvian Amazon, where it has long been used in traditional medicine (Bussmann and Sharon, 2006; Grauso et al., 2020; Rengifo et al., 2020). The antioxidant and anti-inflammatory properties of *Urtica dioica*, derived from its high content of phenolic compounds, flavonoids, vitamins, and essential minerals, support its potential use as a functional additive in poultry diets (Zenão et al., 2017; Kiani et al., 2020).

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The nutritional composition of *Urtica dioica* leaves includes a high content of crude protein (33.8%), fiber (9.1%), essential amino acids, vitamins A, C, and K, and trace elements (Devkota et al., 2022; Taheri et al., 2022). These characteristics have led to growing interest in its application as a functional feed additive in poultry production. Several studies have demonstrated that dietary supplementation with *U. dioica* enhances productive performance in broiler chickens, including increased body weight gain and improved FCR (Behboodi et al., 2021; Chehri et al., 2022). Furthermore, several studies have reported positive effects on serum lipid profiles, including reductions in cholesterol and triglyceride levels (Maina et al., 2023; Teixeira et al., 2023), as well as favorable modulation of intestinal microbiota, characterized by increased populations of *Lactobacillus* spp. and reduced coliform bacteria, suggesting a prebiotic effect with implications for nutrient absorption and intestinal mucosal integrity (Abed and Ali, 2022). However, additional studies are required to clarify the underlying mechanisms, define optimal inclusion levels, and evaluate the effectiveness of *Urtica dioica* under varying production conditions. Therefore, the objective of this study was to evaluate the effects of dietary inclusion of *Urtica dioica* on growth performance, serum biochemical indicators, and intestinal microbiota composition in broiler chickens.

MATERIALS AND METHODS

Ethical approval

The experimental protocol was approved by the Institutional Research Ethics Committee of the National University Pedro Ruiz Gallo (UNPRG), located in Lambayeque, Peru. The study was conducted in accordance with the Peruvian Animal Protection and Welfare Law (Law No. 30407) and followed the guidelines established by the World Organization for Animal Health (OIE) to ensure the welfare of animals used for scientific purposes.

Harvesting and preparation of *Urtica dioica* meal

Fresh leaves and stems of *Urtica dioica* L. were collected during their flowering season from the Tsuntsuntsa Native Community, Bagua, Amazonas, Peru (709 m.a.s.l.; Latitude 5°23'8.1" S, Longitude 78°28'4"W). Only healthy plant material was selected, ensuring the exclusion of damaged or contaminated parts. The collected plant material was first washed with distilled water to remove contaminants, dust, and foreign particles. The preliminary drying process was conducted at room temperature (approximately 25°C) for 24 hours under controlled ventilation to prevent microbial growth. Afterward, the material was placed in a Memmert® UN75 forced-air oven (Memmert GmbH, Germany) and dried at 40°C for 24 hours to achieve consistent moisture reduction while maintaining the integrity of bioactive constituents. Once dried, the plant material was subjected to a two-step milling process to produce a uniform fine powder. Initially, it was coarsely ground using a Fritsch® Universal Cutting Mill PULVERISETTE 19 (Fritsch GmbH, Germany) to reduce the particle size. The resulting material was then finely milled and passed through a 1 mm mesh sieve to ensure consistency in particle size distribution. The resulting *Urtica dioica* meal was immediately stored in airtight containers under dry, dark conditions to preserve its nutritional and bioactive properties until further incorporation into the experimental diets. This preparation process followed the procedure described by Devkota et al. (2022).

Animal handling and experimental design

A total of 120 male Cobb 500 broiler chickens, each one-day-old and averaging 41.7 ± 1.2 g in body weight, were used in the experimental trial. Broiler chickens were randomly assigned to four dietary groups, each comprising six replicates with ten chickens per replicate. The treatment groups received basal diets supplemented with 1% (T1), 2% (T2), and 3% (*Urtica dioica*), while the control group (T0) was fed the basal diet without additives. The chickens were placed in 24 separate pens (0.30 m² each) within a facility equipped with controlled temperature and adequate ventilation. The ambient temperature was maintained at 32-34°C during the first week and was gradually decreased by approximately 2-3°C per week to reach 24°C by the end of the experimental period. Throughout the 42-day experimental period, all broiler chickens had *ad libitum* access to feed and water and were maintained under a lighting regimen of 23 hours of light and 1 hour of darkness per day.

Experimental diets

Feed for this study was prepared from conventional components, including corn, soybean meal, wheat by-products, and soybean oil. None of the treatments contained antibiotics, growth-stimulants, or anticoccidial additives. Broiler chickens were fed using an age-specific nutritional plan divided into three distinct phases: starter (days 1-14), grower (days 15-28), and finisher (days 29-42). The diets corresponding to each production phase were formulated to meet the nutritional demands of broiler

chickens, providing approximately 22% crude protein (CP) at the start and decreasing to 18% toward the end, with energy levels ranging between 3,200 and 3,100 kcal/kg. The basal diet was chemically analyzed following standardized procedures outlined by AOAC (1990). Throughout the 42-day growth period, broiler chickens had *ad libitum* access to feed and fresh water. A detailed composition of ingredients and nutritional values is presented in Table 1.

Table 1. Nutritional composition of experimental diets for broiler chickens at different growth phases (Starter, grower, and finisher)

Ingredient	Starter (1-14 days) (%)	Grower (15-28 days) (%)	Finisher (29-42 days) (%)
Yellow corn	55.00	58.00	61.00
Soybean meal (44% CP)	36.00	30.00	26.00
Wheat by-product	2.00	5.00	6.00
Soybean oil	3.50	3.50	3.50
Dicalcium phosphate	1.80	1.60	1.50
Calcium carbonate	1.00	1.00	0.90
Salt	0.40	0.35	0.30
DL-Methionine	0.30	0.28	0.25
L-Lysine	1.20	1.10	1.00
Choline chloride (60%)	0.10	0.10	0.10
Vitamin-mineral premix[1]	0.50	0.50	0.50
Mycotoxin binder	0.10	0.10	0.10
Calculated Composition (%)			
Metabolizable energy (kcal/kg)[2]	3.2	3.15	3.1
Crude protein (%)	22.00	20.00	18.00
Crude fiber (%)	3.50	3.80	4.00
Calcium (%)	1.00	0.90	0.85
Available phosphorus (%)	0.45	0.42	0.40
Sodium (%)	0.18	0.17	0.16
Methionine + Cystine (%)	0.85	0.80	0.75
Lysine (%)	1.30	1.10	1.00

Vitamin and Mineral Premix per kg of diet: Vitamins: Retinol, 10,000,000 IU; Cholecalciferol, 3,000,000 IU; Tocopherol, 15,000 IU; Menadione, 2.5 g; Riboflavin, 6 g; Calcium pantothenate, 6 g; Niacin, 20 g; Pyridoxine, 4 g; Cyanocobalamin, 0.012 g; Biotin, 0.15 g; Folic acid, 0.5 g; Thiamine, 2 g. Minerals: Copper (Cu), 6 g; Zinc (Zn), 60 g; Manganese (Mn), 60 g; Iron (Fe), 40 g; Iodine (I), 1 g; Selenium (Se), 0.3 g; Cobalt (Co), 0.15 g. [2]The metabolizable energy (kcal/kg) was estimated using the equation by Carpenter and Clegg (1956).

Production parameters

Production performance was evaluated by measuring body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) during the 42-day experimental period. Measurements were taken in three feeding phases: starter (1-14 days), grower (15-28 days), and finisher (29-42 days), as well as for the overall period (1-42 days).

Body weight gain

BWG was calculated as the difference between the final and initial body weights, recorded on days 0, 21, and 42. All measurements were performed in the morning before feed distribution.

$$\text{BWG (g)} = \text{Final live weight (g)} - \text{Initial live weight (g)} \quad \text{(Formula 1)}$$

Feed intake

FI was determined daily by subtracting the weight of the residual feed from the amount of feed offered. Residual feed was weighed each morning before new feed was provided. Values were expressed per chicken per day.

$$\text{FI (g/chicken/day)} = \frac{\text{Feed offered (g)} - \text{Residual feed (g)}}{\text{Number of chickens}} \quad \text{(Formula 2)}$$

Feed conversion ratio

FCR was calculated as the ratio of total FI to total body weight gain for each replicate.

$$\text{FCR} = \frac{\text{Total FI (g)}}{\text{Total BWG (g)}} \quad \text{(Formula 3)}$$

Blood serum parameters

At day 42, a total of 48 broiler chickens (three per replicate and twelve per treatment) were randomly chosen for blood sampling. Around 6 mL of blood was collected from each chicken during slaughter by exsanguination and placed into sterile centrifuge tubes. The samples were then centrifuged at 3000 rpm for 15 minutes to separate the serum, which was promptly stored at -20 °C for subsequent analysis. Biochemical parameters, including total protein, albumin, globulin, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were assessed using commercial diagnostic kits from Wiener Lab® (Rosario, Argentina) and BIOLABO® (Maizy, France), following the manufacturers' instructions. Total protein was measured using the colorimetric method described by Henry et al. (1974), while albumin was determined following the procedure of Doumas et al. (1971). Globulin concentration was obtained by subtracting albumin from total protein.

The activities of AST and ALT were interpreted based on the protocol outlined by the Center (2007).

Ileal and cecal bacterial counts

On day 42 of the trial, ileal and cecal digesta were aseptically collected from 48 broiler chickens (three chickens per replicate, 12 per treatment) immediately post-slaughter to prevent microbial alterations. Approximately one g of fresh intestinal content was homogenized in 9 mL of sterile buffered peptone water (Condalab®, Madrid, Spain) using a vortex mixer for one minute. Serial dilutions were prepared, and 0.1 mL aliquots were plated onto selective culture media. All media were prepared one day prior and poured into sterile Petri dishes under aseptic conditions. Collection tubes were autoclaved at 121°C for 10 minutes and sealed with aluminum foil until use. After homogenization and shaking for 30 minutes to facilitate microbial suspension, 1 mL of each sample was further diluted in 9 mL of phosphate-buffered saline (PBS; Oxoid™, Basingstoke, UK). *Lactobacillus* spp. were cultured on de Man, Rogosa, and Sharpe (MRS) agar (Oxoid™, UK) under anaerobic conditions at 37°C for 72 hours. Total aerobic bacteria and *coliform*s were cultured on MacConkey agar (Oxoid™, UK) under aerobic conditions at 37°C for 48 hours. Colony counts were performed using an automatic colony counter (Scan® 500, Interscience, France), and results were expressed as $\log_{10}$ colony-forming units (CFU) per gram of intestinal content.

Statistical analysis

All collected data were first subjected to a normality check using the Shapiro–Wilk test to ensure appropriate distribution for parametric analysis. Subsequently, one-way analysis of variance (ANOVA) was conducted using the General Linear Model (GLM) procedure in SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). When significant treatment effects were detected ($P < 0.05$), Tukey's post hoc test was applied to identify differences among group means. Results are reported as mean values ± standard error (SE), and statistical significance was declared at $P < 0.05$.

RESULTS

Growth and feed efficiency in broiler chickens

There was no significant difference in feed consumption across the treatment groups throughout the experimental period, with values ranging between 3460 and 3475 g/chicken ($p > 0.05$). However, significant differences were observed in live weight at days 21 and 42, with the highest values recorded in chickens supplemented with 1% *Urtica dioica*, showing an improvement of 8.96% and 7.40% compared to the control group, respectively ($p < 0.05$). At Day 21, broilers in the 1% group showed the best growth performance (730 g), followed by the 3% group (720 g). A similar trend was noted at Day 42, where the 1% group achieved the highest live weight (2395 g), significantly higher than the control (2230 g), the 3% group (2250 g), and slightly higher than the 2% group (2360 g; $p < 0.05$). The FCR also exhibited significant differences ($p < 0.05$), with the control group showing the least efficient feed utilization. Significant differences ($p < 0.05$) were observed in the overall FCR, with the most efficient value recorded in the 2% group (1.78), followed by the control (1.79), 3% (1.84), and 1% (1.86; Table 2).

Biochemical parameters in broiler chickens

It is clear from Table 3 that the treatments with *Urtica dioica* inclusion resulted in a significant decrease ($p < 0.05$) in total cholesterol, HDL, and LDL levels, with the 1% inclusion group showing the most notable reductions. The 1% group had significantly reduced total cholesterol (125 mg/dL) compared to the control group, and although triacylglycerol levels were numerically lower (45 mg/dL), this difference was not statistically significant ($p > 0.05$). However, there were no significant differences in glucose, total protein, albumin, globulin, or liver function parameters (AST and ALT) among the treatment groups when compared to the control group ($p > 0.05$; Table 3).

Duodenal and cecal microbial populations in broilers

Feeding broiler chickens with diets containing *Urtica dioica* at different inclusion levels significantly affected the microbial population in both the duodenum and cecum ($p < 0.05$). The count of *Lactobacillus* spp. in the duodenum increased significantly ($p < 0.05$) in the 1% *Urtica dioica* group (4.00 CFU/g) compared to the control group (2.10 CFU/g). Similarly, a significant decrease in *coliform* bacteria was observed with increasing levels of *Urtica dioica*, with the 3% group showing the lowest count (6.90 CFU/g) compared to the control (7.90 CFU/g; $p < 0.05$). In the cecum, the count of *Lactobacillus* spp. also increased significantly ($p < 0.05$) with 1% inclusion, reaching 3.60 CFU/g compared to 2.20 CFU/g in the control group. The *coliform* bacteria count in the cecum significantly decreased in the 1% group (6.10 CFU/g) compared to the control group (9.10 CFU/g; $p < 0.05$; Table 4).

Table 2. Effects of *Urtica dioica* inclusion levels on feed intake, live weight, and feed conversion in broiler chickens aged 1 to 42 days.

Parameter	Control	Percentage of dietary inclusion of *Urtica dioica* (%)			SEM	P-value
		1%	2%	3%		
Feed consumption g/chicken						
1-21 days	$0.60^{a} \pm 0.03$	$0.61^{a} \pm 0.03$	$0.62^{a} \pm 0.03$	$0.60^{a} \pm 0.03$	0.03	0.311
22-42 days	$1,120^{a} \pm 12.0$	$1,115^{a} \pm 12.0$	$1,122^{a} \pm 12.0$	$1,118^{a} \pm 12.0$	12.0	0.352
1-42 days	$3,460^{a} \pm 25.0$	$3,450^{a} \pm 25.0$	$3,475^{a} \pm 25.0$	$3,468^{a} \pm 25.0$	25.0	0.622
Live weight (g)						
Initial	$43.00^{a} \pm 1.40$	$43.30^{a} \pm 1.40$	$43.50^{a} \pm 1.40$	$42.90^{a} \pm 1.40$	1.40	0.939
Day 21	$670.00^{c} \pm 15.0$	$730.00^{a} \pm 15.0$	$698.00^{b} \pm 15.0$	$720.00^{a} \pm 15.0$	15.0	< 0.01
Day 42	$2,230.00^{c} \pm 20.0$	$2,395.00^{a} \pm 20.0$	$2,360.00^{b} \pm 20.0$	$2,250.00^{c} \pm 20.0$	20.0	< 0.01
Feed conversion ratio						
0-21 days	$1.23^{c} \pm 0.01$	$1.27^{a} \pm 0.01$	$1.26^{b} \pm 0.01$	$1.26^{b} \pm 0.01$	0.01	< 0.01
22-42 days	$1.88^{c} \pm 0.04$	$2.05^{a} \pm 0.04$	$1.92^{b} \pm 0.04$	$1.94^{b} \pm 0.04$	0.04	< 0.01
1-42 days	$1.79^{c} \pm 0.03$	$1.86^{a} \pm 0.03$	$1.78^{b} \pm 0.03$	$1.84^{b} \pm 0.03$	0.03	< 0.01

SEM: Standard error of mean. ^{a,b,c} Values in a row with different superscripts are significantly different ($p < 0.05$).

Table 3. Effects of *Urtica dioica* inclusion levels on serum biochemical parameters and liver function in broiler chickens at 42 days of age

Parameter	Control	Dietary inclusion of *Urtica dioica* (%)			SEM	P-value
		1%	2%	3%		
Total cholesterol (mg/dL)	$150.00^{a} \pm 1.31$	$125.00^{c} \pm 1.31$	$145.00^{b} \pm 1.31$	$143.00^{b} \pm 1.31$	1.31	< 0.01
HDL (mg/dL)	$97.00^{a} \pm 0.07$	$90.00^{c} \pm 0.07$	$94.00^{b} \pm 0.07$	$93.50^{b} \pm 0.07$	0.07	< 0.01
LDL (mg/dL)	$54.00^{a} \pm 0.07$	$48.00^{c} \pm 0.07$	$52.00^{b} \pm 0.07$	$51.80^{b} \pm 0.07$	0.07	< 0.01
Triacylglycerols (mg/dL)	$60.00^{a} \pm 1.28$	$45.00^{a} \pm 1.28$	$58.00^{a} \pm 1.28$	$56.50^{a} \pm 1.28$	1.28	0.424
Glucose (mg/dL)	$184.32^{a} \pm 3.55$	$191.44^{a} \pm 3.55$	$185.02^{a} \pm 3.55$	$186.62^{a} \pm 3.55$	3.55	0.504
Total protein (g/dL)	$2.80^{a} \pm 0.17$	$3.06^{a} \pm 0.17$	$2.95^{a} \pm 0.17$	$2.99^{a} \pm 0.17$	0.17	0.720
Albumin (g/dL)	$1.30^{a} \pm 0.14$	$1.39^{a} \pm 0.14$	$1.42^{a} \pm 0.14$	$1.56^{a} \pm 0.14$	0.14	0.638
Globulin (g/dL)	$0.04^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.05^{a} \pm 0.01$	$0.05^{a} \pm 0.01$	0.01	0.537
Liver function						
AST (UI/L)	$308.70^{a} \pm 20.54$	$353.38^{a} \pm 20.54$	$360.38^{a} \pm 20.54$	$355.94^{a} \pm 20.54$	20.54	0.285
ALT (UI/L)	$2.90^{a} \pm 0.37$	$3.20^{a} \pm 0.37$	$3.10^{a} \pm 0.37$	$2.98^{a} \pm 0.37$	0.37	0.940

SEM: Standard error of mean. ^{a,b,c} Values in a row with different superscripts are significantly different ($p < 0.05$). HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

Table 4. Microbial counts in the duodenum and cecum (CFU/g) of broiler chickens at 42 days of age fed diets with different *Urtica dioica* inclusion levels

Parameter	Control	Dietary inclusion of *Urtica dioica* (%)			SEM	P-value
		1%	2%	3%		
Duodenum						
Lactobacillus spp.	$2.10^{c} \pm 0.14$	$4.00^{a} \pm 0.14$	$3.70^{b} \pm 0.14$	$3.40^{b} \pm 0.14$	0.14	0.020
Coliform bacteria	$7.90^{a} \pm 0.18$	$7.20^{b} \pm 0.18$	$7.00^{b} \pm 0.18$	$6.90^{c} \pm 0.18$	0.18	0.030
Cecum						
Lactobacillus spp. (Cecum)	$2.20^{b} \pm 0.16$	$3.60^{a} \pm 0.16$	$3.30^{ab} \pm 0.16$	$3.10^{b} \pm 0.16$	0.16	< 0.01
Coliform bacteria	$9.10^{a} \pm 0.23$	$6.10^{c} \pm 0.23$	$7.40^{b} \pm 0.23$	$7.00^{b} \pm 0.23$	0.23	0.020

* SEM: Standard error of mean. ^{a,b,c} Values in a row with different superscripts are significantly different ($p < 0.05$).

DISCUSSION

In the present study, the inclusion of 1% *Urtica dioica* in the diet was associated with improvements in BW and FCR in Cobb 500 broiler chickens from 1 to 42 days of age. These results are consistent with the findings of Teixeira et al. (2023), who reported that dietary supplementation with 1% *Urtica dioica* significantly improved growth performance and feed efficiency in Ross 308 broilers, attributing these effects to the presence of bioactive compounds with antioxidant, immunomodulatory, and digestive properties. It is worth noting that, although the 3% inclusion of *Urtica dioica* resulted in higher BW compared to the 2% group, no improvement in FCR was observed. This phenomenon may be related to the presence of antinutritional factors, such as tannins and alkaloids, which are found in higher concentrations of *Urtica dioica* and can interfere with nutrient absorption and utilization (Keshavarz et al., 2014). However, in the present study, differences in FCR between treatments indicate variations in feed utilization efficiency. While the control and 2% *Urtica dioica* groups exhibited the best FCR, the 1% and 3% inclusion groups demonstrated higher body weights. These findings align with those reported by Maina et al. (2023), who observed that supplementing Cobb 500 broiler diets with 1.5% *Urtica dioica* enhanced weight gain, while a 2% inclusion level led to improved FCR, indicating a dose-dependent effect. Similarly, Keshavarz et al. (2014) observed that *Urtica dioica* supplementation influenced lipid metabolism in broiler chickens, which may explain the observed differences in BWG and FCR among treatments. Higher dietary inclusion levels of *Urtica dioica* may introduce antinutritional factors, such as tannins and alkaloids, which could impair nutrient absorption and utilization (Gadde et al., 2017).

The biochemical blood evaluation is a key tool for the continuous monitoring of poultry health, as it allows for the early detection of physiological alterations and facilitates the timely diagnosis of various diseases (Franciosini et al., 2023). In the present study, the dietary inclusion of *Urtica dioica* significantly reduced total cholesterol and LDL levels in broilers, with the most pronounced effect observed in the 1% treatment. However, as the supplementation rate increased, cholesterol levels also increased, suggesting a dose-dependent response. Despite higher cholesterol levels in the 2% and 3% groups, other biochemical parameters remained stable across treatments, indicating that the main effect of *Urtica dioica* was on cholesterol modulation. These findings aligned with previous studies suggesting that nettle's bioactive compounds, such as phytosterols and flavonoids, contribute to lowering cholesterol by inhibiting its intestinal absorption and promoting its excretion (Righi et al., 2021). The reduction in LDL levels observed in the 1% and 3% treatments further supports the role of *Urtica dioica* in modulating lipid metabolism (Hashem and Salem, 2022). Interestingly, the present study showed significantly lower HDL concentrations in all *Urtica dioica* groups compared to the control, with the most pronounced reduction in the 1% group (90.00 mg/dL vs. 97.00 mg/dL). These findings are consistent with those of Teixeira et al. (2023), who also reported reduced HDL levels in broilers fed *Urtica urens*, whereas higher values were observed in the control group. In concordance with the findings of Safamehr et al. (2012), the inclusion of *Urtica dioica* in broiler diets did not significantly alter other biochemical parameters, including triacylglycerols, glucose, total protein, albumin, and globulin. Similarly, Adam et al. (2020), who conducted their study with Arbor Acres broiler chickens, found no significant changes in blood metabolites with nettle supplementation, further supporting its safety as a dietary additive. Additionally, the stability of AST and ALT levels across treatments in the present study suggests that *Urtica dioica* does not negatively impact liver function, confirming previous reports that its supplementation did not induce hepatic stress or toxicity in broilers (Özen and Korkmaz, 2003).

In the present study, a significant decrease in the logarithmic total number of *coliform*s was observed, along with an increase in *Lactobacillus* spp. (cfu/g) in the duodenal and cecal contents of broiler chickens, with the most notable effect seen in the 1% *Urtica dioica* supplementation. These results align with those of Abed and Ali (2022), who studied Ross 380 broiler chickens and found that *Urtica dioica* can promote intestinal microbial balance by boosting beneficial bacteria and reducing pathogens. Specifically, supplementation with *Urtica dioica* significantly enhanced the growth of beneficial bacteria such as *Lactobacillus* spp., which are vital for maintaining a healthy gut environment. This effect may be due to bioactive compounds in *Urtica dioica*, like flavonoids and phenols, which have antimicrobial properties capable of disrupting pathogenic bacterial cell membranes and interfering with their metabolism (Kupnik et al., 2021). The reduction in *coliform* bacteria supports previous studies highlighting *Urtica dioica's* ability to inhibit both Gram-positive and Gram-negative bacteria

because of its phenolic compounds with antibacterial activity (Tabari et al., 2016). However, increasing the supplementation to 2% and 3% did not produce significant differences between these levels, indicating that higher doses of *Urtica dioica* do not offer additional benefits. This aligns with findings by Kiani et al. (2020), who reported that *Lactobacillus* spp. produces bacteriocins with stronger antibacterial effects than *Urtica dioica* extracts against antibiotic-resistant bacteria. In this context, the microbial changes caused by *Urtica dioica* may depend on interactions between its bioactive compounds and the gut microbiota, underscoring the importance of identifying the optimal dosage to maximize benefits for broiler gut health without causing adverse effects.

CONCLUSION

Dietary inclusion of 1% *Urtica dioica* significantly enhanced growth performance, modulated lipid metabolism by reducing serum total cholesterol and LDL levels, and improved gut microbial balance by increasing *Lactobacillus* counts and reducing *coliform* populations in broiler chickens. No adverse effects were observed on liver enzymes or other biochemical markers, supporting its safety as a feed additive. Future studies should investigate other *Urtica* species, explore their potential as a natural growth promoter, and evaluate their effects on gut microbiota dynamics, immune responses, and carcass traits under commercial conditions.

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Availability of data and materials

Data generated or analyzed during this study are available upon reasonable request from the corresponding author.

Authors' contributions

Edwaldo Villanueva Pedraza contributed to the conceptualization of the study, drafted the original manuscript, and managed the overall project. Pompeyo Ferro participated in the conceptualization and contributed to the review and editing of the manuscript. Jeiner Alexander Villanueva Guerrero was responsible for the execution of the experiment and the collection of experimental data. Johnny Cueva Valdivia performed the formal data analysis and statistical evaluations. Anthonny Smith Guevara Flores assisted with data collection and fieldwork. José Alberto Carlos Ramos supervised the study process and validated the results. Euclides Ticona Chayña provided supervision and contributed to the critical review and editing of the manuscript. Papa Pio Ascona García supported the development of the methodology. All authors have read and approved the final version of the manuscript before publication in the present journal.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors affirm that this manuscript is original and has not been submitted for publication elsewhere. Furthermore, they assure that the data included in this manuscript is truthful and has not been manipulated.

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