p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Science and Technology, Republic of Indonesia, No. 355/DST/D.D1/HM.01.01/2026

Tropical Animal Science Journal, November 2026, 49(6):562-572
DOI: https://doi.org/10.5398/tasj.2026.49.6.562
Available online at https://journal.ipb.ac.id/index.php/tasj

Dietary Supplementation of Encapsulated *Dendrophthoe pentandra* Leaf Extract in Broilers under High Stocking Density

E. Widiastuti*, A. Puspitaningrum, I. Agusetyaningsih, T. Yudiarti, D. A. P. Sari, & S. Sugiharto
Physiology and Biochemistry Laboratory, Department of Animal Husbandry, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro
Jl. Prof. Jacub Rais, Tembalang, Semarang, Jawa Tengah 50275, Indonesia
*Corresponding author: endwidia@yahoo.co.id
(Received 10-02-2026; Revised 06-07-2026; Accepted 07-07-2026)

ABSTRACT

High stocking density can compromise broiler welfare and physiological homeostasis, but the magnitude of the challenge depends on housing and environmental conditions. This study evaluated whether encapsulated *Dendrophthoe pentandra* leaf extract modified production performance, hematological and serum biochemical variables, relative immune organ weights, and antioxidant status in broilers reared at high stocking density. A total of 300 Cobb CP 707 broiler chicks were assigned to four treatments with five pen replicates: T0, 9 birds/m²; T1, 17 birds/m²; T2, 17 birds/m² plus 0.5 g/kg encapsulated *D. pentandra* leaf extract; and T3, 17 birds/m² plus 1.0 g/kg extract. Variables measured included daily feed intake, daily weight gain, feed conversion ratio, blood profile, serum biochemistry, lymphoid organ weights, and antioxidant activity. Results showed that the treatment significantly improved daily feed intake and increased the relative weight of the bursa of Fabricius ($p<0.05$). Platelet distribution width (PDW) and thrombocyte counts were significantly lower in extract-supplemented groups compared to controls. However, the treatment did not significantly affect daily weight gain, feed conversion ratio, most blood biochemical profiles, albumin, SGOT, SGPT, SOD, or MDA activity ($p>0.05$). The T2 treatment (0.5 g extract) yielded optimal improvements without adverse effects on other measured variables. These findings suggest that encapsulated *D. pentandra* leaf extract can serve as a natural supplement to enhance feed intake and immune organ development, supporting broiler health and performance under high-density rearing conditions in tropical environments. This approach offers a promising strategy to mitigate the negative impacts of high stocking density in poultry production.

Keywords: Dendrophthoe pentandra; broiler density; immune responses; encapsulated extract; phytochemical compound

INTRODUCTION

Broiler chicken (*Gallus gallus domesticus*) is one of the sources of animal protein that is readily available and affordable compared with other types of meat. Consumption of broiler chicken meat has increased rapidly each year. It is a challenge for broiler chicken farmers to increase their production capacity. One of the efforts to increase the productivity of broiler chickens is to increase their density (Hu *et al.*, 2019). High stocking density may induce stress conditions that adversely affect growth performance, physiological status, and the balance of intestinal microflora (Li *et al.*, 2019). In addition, increased stress intensity was associated with decreased immunity, resulting in increased susceptibility to disease in broilers (Li *et al.*, 2019).

Stress arising from the high number of chickens in the cage area increases the ratio of heterophiles to lymphocytes (Astaneh *et al.*, 2018), decreases the number of lymphoid cells (Yanai *et al.*, 2018), and decreases the activity of antioxidant enzymes (Hosseini-Vashan *et al.*, 2015). According to Sugiharto *et al.* (2019), stress-induced physiological changes can increase the formation of free radicals or reactive oxygen species (ROS), thereby causing oxidative stress.

Oxidative stress refers to a condition in which the production of ROS exceeds the body's antioxidant defense capacity. An imbalance of free radicals may induce lipid peroxidation in cell membranes, particularly in unsaturated fatty acids. Lipid peroxidation is a complex process involving the reaction between polyunsaturated fatty acids in membrane phospholipids and ROS, resulting in the formation of hydroperoxides and ultimately producing malondialdehyde (MDA) (Tu *et al.*, 2024). The high MDA content in the bodies of broiler chickens indicates the presence of cellular damage and excessive oxidation (Iheagwam *et al.*, 2024).

To overcome these negative effects, synthetic antioxidant supplementation is often applied in the broiler industry (Awang *et al.*, 2023). However, prolonged use of synthetic antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole, may pose potential toxic effects and health risks to consumers (Zhou & Zhou, 2019; Taghvaei & Jafari, 2015). Therefore, natural antioxidants are considered safer and are recommended as an alternative to reduce dependence on synthetic antioxidants in broiler chicken rearing, including at high densities. Typical recommended stocking densities for broilers vary by region and climate but generally range from 10 to 15 birds/m² in tropical climates to optimize welfare and production (Iffiong *et al.*, 2021). The density of 17 birds/m² used in this study represents a high-density condition to simulate stress challenges. Broilers are usually raised from day-old chicks through three main growth phases: starter (0-14 days), grower (15-28 days), and finisher (29-42 days) (Tikate *et al*., 2020). In this study, the high stocking density treatment was applied starting at 8 days of age, corresponding to the starter phase, to evaluate the early physiological responses of broilers to density-induced stress during the rearing period (Chauhan *et al*., 2022). The application of 17 birds/m² is relevant for the entire rearing period and reflects intensive production systems in tropical climates where space constraints are common (Iffiong *et al*., 2021). This density choice is justified to investigate the physiological and performance impacts under stress-inducing conditions while considering animal welfare and production efficiency.

D. pentandra leaves are generally abundant and easily sourced in tropical regions where broiler production is intensive, particularly in Southeast Asia (Awang *et al.,* 2023). Their widespread presence on host trees and adaptability to local environments make them a sustainable resource (Alabbosh, 2024). Harvesting leaves for antioxidant supplementation can be managed without significant depletion of natural populations, especially if collection focuses on pruning rather than complete defoliation (Andreichenko *et al*., 2023). This sustainable availability supports their suitability as a long-term, eco-friendly alternative to synthetic antioxidants in broiler diets, aligning with growing demands for natural feed additives that reduce environmental impact.

Encapsulated *D. pentandra* leaf extract as a source of antioxidants contains various bioactive compounds such as flavonoids, amino acids, carbohydrates, tannins, alkaloids, and saponins, which play a role in inhibiting oxidative damage caused by free radicals (Lazuardi *et al.,* 2022). The antioxidant activity of *D. pentandra* leaves is mainly influenced by the content of flavonoids and phenolic compounds, including quercetin, which can neutralize hydroxyl radicals, superoxide anions, and fat peroxide radicals and function as antibacterial and immunomodulatory agents. In addition, secondary metabolites, such as tannins, saponins, alkaloids, and sterols (e.g., β-sitosterol and stigmasterol acetate), contribute to the suppression of oxidative stress and the strengthening of the immune response (Kong *et al.,* 2023). Therefore, encapsulation of *D. pentandra* leaf extract is expected to provide a synergistic or complementary effect in minimizing the impact of stress on broiler chickens by improving physiological balance and body resistance.

In plant extracts, the encapsulation process protects bioactive compounds from oxidation and prevents the influence of external factors such as humidity, temperature, and microbial contamination during storage (Mishra, 2016). To date, research on the potential interaction or synergy between various bioactive compounds in *D. pentandra* leaf extract is still very limited, particularly regarding its ability to mitigate oxidative stress in broiler chickens reared under high stocking density conditions. Therefore, it is hypothesized that supplementation with encapsulated *D. pentandra* leaf extract may alleviate oxidative stress induced by high stocking density (17 birds/m²), thereby improving antioxidant status, immune response, and production performance in broiler chickens. This study aimed to evaluate the effects of encapsulated *D. pentandra* extract on the production performance, blood biochemical parameters, immune organ weights, and antioxidant status of broilers under high stocking density.

MATERIALS AND METHODS

Production of Encapsulated *Dendrophthoe pentandra* Leaf Extract

D. pentandra leaves were collected from mango host trees located in Semarang, Central Java, Indonesia, specifically within the campus area of Universitas Diponegoro under natural field conditions. Leaves were dried at room temperature without exposure to direct sunlight. The dried leaves were ground using a grinder mixer until they became a fine powder and were extracted with 70% ethanol at room temperature for 72 h at a ratio of 1:5 (b/v). The resulting extracts were filtered using filter paper to obtain the filtrate. The filtrate was evaporated using a vacuum rotary evaporator to obtain a paste-shaped extract of *D. pentandra*. After the evaporation process, the extract was encapsulated with maltodextrin as a coating agent using the freeze-drying method (Jamdar *et al.,* 2021). The coating material consisted of maltodextrin dissolved in distilled water at a ratio of 1:3. The maltodextrin solution was then mixed with the *D. pentandra* leaf extract filtrate at a ratio of 5:1 and subsequently dried using the freeze-drying method to obtain encapsulated *D. pentandra* leaf extract powder. Antioxidant activity of the leaf material and encapsulated extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, following the spectrophotometric principle described by Yismairai *et al.* (2019). Briefly, the sample solution was mixed with DPPH reagent, incubated in the dark, and absorbance was measured against a reagent blank. Radical-scavenging activity was calculated as inhibition (%) = [(A control – A sample)/A control] × 100. The same sample concentration, reaction time, solvent, wavelength, and analytical batch were used for both materials. The reported values represent percentage inhibition under these assay conditions. The antioxidant activity of *D. pentandra* leaves and encapsulated *D. pentandra* leaf extract is shown in Table 1.

Ethical Approval

The research procedures were approved by the Animal Research Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (62-03/A-08/KEP-FPP).

Animals and Experimental Diets

A total of 300 unsexed Cobb CP 707 broiler chicks with an average body weight of 46.7 ± 2.2 g were randomly assigned to four treatments with five replicates following standard experimental designs in poultry studies: T0 (density of 9 birds/m²), T1 (density of 17 birds/m²), T2 (density of 17 birds/m² + 0.5 g encapsulated *D. pentandra* leaf extract), and T3 (density of 17 birds/m² + 1 g encapsulated *D. pentandra* leaf extract). The stocking density treatments (9 and 17 birds/m²) were selected based on Agusetyaningsih *et al.* (2025), who reported broiler studies using comparable low- and high-density conditions (9-10 vs 14-16 birds/m²) to induce production stress and evaluate physiological responses. Similarly, the inclusion levels of encapsulated *D. pentandra* leaf extract (0.5 and 1.0 g/kg feed) were based on prior studies reporting effective phytogenic supplementation ranges of 0.5-1.5 g/kg feed in broilers, particularly under high stocking density conditions (Agusetyaningsih *et al.*, 2025). Broiler chicks were reared in an open-house system using litter (rice husks). Chickens were fed commercial feed from 0 to 7 days of age. At 8-35 days of age, chickens were fed a pre-formulated feed (Table 2) with different doses of encapsulated *D. pentandra* leaf extract.

The sample size was determined based on previous studies to ensure adequate statistical power (Sugiharto *et al.*, 2019; Li *et al.*, 2019). Broilers were reared in an open-sided house with natural ventilation using rice husk litter (8-10 cm depth), which is commonly applied in tropical poultry production systems (Soliman & Hassan, 2020). The ambient temperature ranged from 28 to 32 °C with relative humidity between 60%-75%, and a continuous lighting program (23 h light:1 h dark) was applied (Osti *et al.*, 2017). Temperature and humidity were recorded twice daily.

All chickens were vaccinated with an active ND vaccine (Medivac ND Clone 45®, PT. Medion Farma Jaya) administered at the age of 22 days through drinking water. Body weight and feed consumption were recorded weekly from the age of 8 to 35 days. At 35 days of age, one chicken per replicate was randomly selected for blood collection via the brachial vein. The collected blood was transferred into vacutainer tubes containing an anticoagulant (EDTA) for complete blood count analysis, or into tubes without anticoagulant for serum separation. Serum was obtained by centrifuging the blood samples at 3,000 rpm for 15 min and subsequently stored at freezing temperature until further analysis. At the end of the 35-day experimental

Table 1. Antioxidant activity of *Dendrophthoe pentandra* leaves and encapsulated *D. pentandra* leaf extract

Items	Antioxidant activity (Inhibition) (%)
D. pentantra leaves	39.96
Encapsulated *D. pentandra* leaf extract	76.18

Note: *Analyzed at the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang (2025).

Table 2. Ingredients and nutritional composition of broiler diets provided on days 08-35

Type of feed / item	Broiler growth phase	
	Starter (day 8-21)	Finisher (day 22-35)
Yellow maize (%)	50.27	58.67
Soybean meal (%)	42.97	34.67
Palm oil (%)	2.9	2.9
DL-methionine (%)	0.2	0.2
Bentonite (%)	0.75	0.75
Limestone (%)	1	1
Monocalcium phosphate (%)	1.1	1
Premix (%)[2]	0.34	0.34
Choline chloride (%)	0.07	0.07
Salt (%)	0.4	0.4
Analyzed nutritional compositions:		
Metabolizable energy (kcal/kg)[1]	3249.69	3204.43
Crude protein (%)	25.11	21.87
Crude fiber (%)	3.86	3.54
Crude fat (%)	7.58	7.69
Moisture (%)	12.87	13.89
Ash (%)	4.09	4.80

Note: [1] Metabolizable energy was calculated according to Bolton's (1967) formula: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}. [2] Premix contained (per kg of diet) of Vitamin A 7750 IU, Vitamin D3 1550 IU, Vitamin E 1.88 mg, Vitamin B1 1.25 mg, Vitamin B2 3.13 mg, Vitamin B6 1.88 mg, Vitamin B12 0.01 mg, Vitamin C 25 mg, folic acid 1.50 mg, Ca-D-pantothenate 7.5 mg, niacin (Niacin) 1.88 mg, biotin 0.13 mg, Co 0.20 mg, Cu 4.35 mg, Fe 54 mg, I 0.45 mg, Mn 130 mg, Zn 86.5 mg, Se 0.25 mg, L-lysine 80 mg, choline chloride 500 mg, DL-methionine 900 mg, $CaCO_3$ 641.5 mg, dicalcium phosphate 1500 mg.

period, the sampled chickens were slaughtered, and their lymphoid organs, including the bursa of Fabricius, spleen, and thymus, were collected.

Data Collection and Laboratory Analysis

Performance of broilers. Broiler performance was evaluated based on daily body weight gain, daily feed intake, final body weight, and feed conversion ratio (FCR). Daily body weight gain was calculated following Agusetyaningsih *et al.* (2022) by recording the body weight of birds on day 35 to obtain the final weight, subtracting the initial weight measured at the end of day 7, and dividing the difference by the experimental duration (28 days). Daily feed intake was determined by dividing the total feed consumed during the trial by the length of the experimental period. The feed conversion ratio (FCR) was calculated as the ratio between feed intake and body weight gain during the treatment period. In this study, the final body weight and FCR are presented as mean ± standard deviation.

Complete blood counts. To assess the hematological parameters, one bird was randomly selected from each replicate, and blood samples were collected via the brachial vein. Blood was drawn using a 3 mL syringe fitted with a 25G needle and then transferred into EDTA tubes (0.5 mL). The samples were subsequently subjected to hematological analysis in the laboratory. The parameters measured included Red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Complete blood counts were determined using the dilution flask method as described by Almuthaybiri *et al*. (2025). Hemoglobin levels were measured using the Sahli method. Erythrocyte and leukocyte counts were determined based on the dilution method, and a Bürker chamber was used for cell counting. Hematocrit values were determined using the microhaematocrit technique, while differential leukocyte counts were assessed using a light microscope.

Serum protein profiles of broilers. During the fifth week of the experiment, one bird was randomly selected from each replicate pen for blood sampling. Approximately 2 mL of blood was collected from the brachial vein, resulting in five independent serum samples per treatment. Blood sampling was performed using a 3 mL syringe fitted with a 25 G needle. The collected blood was transferred into plain tubes and allowed to stand for approximately 15 minutes to facilitate serum separation. The serum was then separated from the remaining cellular components by aspirating it with a pipette and subsequently used for biochemical analysis. The analyses followed the procedures described by Sugiharto *et al.* (2016) with minor modifications. The albumin levels were determined by the bromocresol green (BCG) method. SGOT and SGPT levels were determined using the International Federation of Clinical Chemistry (IFCC) method.

Relative immune organ weights of broilers. The relative weights of immune organs (bursa of Fabricius, spleen, and thymus) were calculated following the method described by Choi *et al.* (2021) by dividing the organ weight by live body weight and multiplying the result by 100%.

Antioxidant activity. Malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity were determined as described by Agusetyaningsih *et al.* (2022). SOD activity was evaluated based on the ability of the sample to inhibit pyrogallol auto-oxidation. The reaction mixture consisted of 50 mM Tris-HCl buffer (pH 8.2), 1 mM diethylenetriaminepentaacetic acid, and the sample. The reaction was initiated by adding pyrogallol to achieve a final concentration of 0.2 mM, followed by kinetic measurement of absorbance. SOD levels were expressed in units (U/ml). Malondialdehyde (MDA) levels were determined using a thiobarbituric acid (TBA) reactive substances assay. Each sample was vortexed, mixed with 8.1% sodium dodecyl sulfate, and incubated at room temperature for 10 min. The control samples were processed similarly. Subsequently, 20% acetic acid and 0.6% TBA were added, and the mixture was incubated in a water bath at 90-95 °C for 1 h. After incubation, a butanol/pyridine solution (15:1) was added to the supernatant, vortexed, and centrifuged. MDA concentrations were reported in nmol/mL.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) based on a completely randomized design (CRD). The statistical model used was:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

where Y_{ij} is the observed value, μ is the overall mean, τ_i is the treatment effect, and ϵ_{ij} is the experimental error. Prior to analysis, data normality was tested using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. Differences among treatments were further analyzed using Duncan's multiple range test when significant effects were observed ($p<0.05$). All statistical analyses were performed using SPSS version 25.0.

RESULTS

Growth Performance of Broilers

Dietary supplementation of encapsulated *D. pentandra* leaf extract significantly affected daily feed intake (DFI) ($p<0.05$), whereas no significant effects were observed on daily weight gain (DWG), final body weight, or feed conversion ratio (FCR) (Table 3). Broilers in the T2 group exhibited the highest feed intake, which increased by 6.1% compared to T0 (107.07 vs 100.89 g/day) and by 1.7% compared to T1 (107.07 vs 105.29 g/day). In contrast, no significant differences were observed in DWG (p = 0.737), final body weight (p = 0.647), or FCR (p = 0.504).

Complete Blood Counts of Broilers

Most hematological variables, including erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, leukocytes, heterophils, lymphocytes, and H/L ratio, were not significantly affected by treatments (p>0.05) (Table 4). However, significant differences were observed in thrombocyte count and platelet distribution width (PDW). Thrombocyte count decreased significantly in T3 by 42.8% compared to T0 (15.80 vs 27.60 ×10³/μL; p = 0.003). Similarly, PDW values were reduced by 41.3% in T2 compared to T0 (8.00 vs 13.64; p = 0.010).

Serum Protein Profiles of Broilers

Serum protein profile data are presented in Table 5. No significant differences were observed in albumin (p = 0.401), SGOT (p = 0.495), or SGPT (p = 0.185) levels among treatments. Although SGPT levels in T3 were numerically higher (5.98 U/L) compared to T0 (2.70 U/L), this increase (+121.5%) was not statistically significant.

Relative Immune Organ Weights of the Broilers

The relative weight of the bursa of Fabricius was significantly affected by treatment (p = 0.015), while spleen and thymus weights remained unchanged (p = 0.711 and P = 0.438, respectively) (Table 6). Broilers in T2 exhibited the highest bursa weight, which increased by 50.0% compared to T0 (0.06 vs 0.04%).

Antioxidative Status of Broilers

No significant differences were observed in superoxide dismutase (SOD) activity (p = 0.205) or malondialdehyde (MDA) levels (p = 0.883) among treatments (Figure 1). However, SOD activity showed an increasing trend in T3, with a 29.3% higher value compared to T0 (5.433 vs 4.203 U/mL). In contrast, MDA levels remained relatively stable across treatments, with only a slight increase of 7.4% in T3 compared to T0 (1.721 vs 1.602 nmol/mL). While encapsulated *D. pentandra* did not significantly alter antioxidant status, the numerical increase in SOD activity in T3 warrants

Table 3. Performance of broilers (days 8-35) with supplemented encapsulated *Dendrophthoe pentandra* leaf extract in high stocking density

Variables	Treatments				SEM	p value
	T0	T1	T2	T3		
DWG (g)	61.94	57.22	63.73	62.35	0.720	<0.737
DFI (g)	100.89^{d}	105.29^{b}	107.07^{a}	104.77^{c}	0.084	<0.000
Final BW (g)	1958.98	1943.78	2011.26	1961.87	19.510	<0.647
FCR	1.63	1.72	1.68	1.68	0.088	<0.504

Note: Different superscripts (a, b, c, d) within the same row indicate significant differences (p<0.05). T0: broiler chickens were reared at a density of 9 birds/m² without additive supplementation; T1: broilers were reared at a density of 17 birds/m² without additive supplementation; T2: broilers were reared at a density of 17 birds/m² and supplemented with 0.5 g/kg encapsulated *D. pentandra* leaf extract; T3: broilers were reared at a density of 17 birds/m² and supplemented with 1.0 g/kg encapsulated *D. pentandra* leaf extract. SEM: standard error of the mean; DWG: daily weight gain; DFI: daily feed intake; Final BW: final body weight; FCR: feed conversion ratio.

Table 4. Complete blood counts of broilers with supplemented encapsulated *Dendrophthoe pentandra* leaf extract in high stocking density

Variables	Treatments				SEM	p value
	T0	T1	T2	T3		
Erythrocytes (10⁶/μL)	2.07	1.87	1.90	1.86	0.049	<0.424
Hemoglobin (g/dL)	8.40	7.66	7.76	8.28	0.178	<0.387
Haematocrit (%)	34.78	31.26	31.66	31.44	0.826	<0.408
MCV (fL)	1.46	1.38	1.28	1.36	0.893	<0.823
MCH (pg)	40.48	40.76	40.82	44.36	0.611	<0.119
MCHC (g/dL)	23.60	23.90	24.02	25.74	0.356	<0.181
RDW-SD (fL)	47.96	46.46	45.72	46.84	0.631	<0.658
RDW-CV (%)	9.84	9.58	9.44	9.56	0.105	<0.602
MPV (fL)	10.18	10.06	10.08	9.74	0.116	<0.579
PDW (fL)	13.64^{a}	8.72^{b}	8.00^{b}	9.76^{b}	0.551	<0.010
Leukocytes (10³/μL)	60.04	69.50	71.88	72.88	2.594	<0.316
Heterophils (10³/μL)	2.62	3.40	2.60	3.92	0.258	<0.240
Lymphocytes (10³/μL)	57.40	66.10	69.28	68.96	2.439	<0.311
Thrombocytes (10³/μL)	27.60^{a}	24.40^{a}	22.00^{a}	15.80^{b}	0.924	<0.003
Ratio H/L	0.04	0.05	0.03	0.05	0.106	<0.772

Note: Different superscripts (a, b) within the same row indicate significant differences (p<0.05). T0: broiler chickens were reared at a density of 9 birds/m² without additive supplementation; T1: broilers were reared at a density of 17 birds/m² without additive supplementation; T2: broilers were reared at a density of 17 birds/m² and supplemented with 0.5 g/kg encapsulated *D. pentandra* leaf extract; T3: broilers were reared at a density of 17 birds/m² and supplemented with 1.0 g/kg encapsulated *D. pentandra* leaf extract. SEM: standard error of the mean; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red blood cell distribution width-standard discrepancy; RDW-CV: red blood cell distribution width-coefficient of variation; MPV: mean platelet volume; PDW: platelet distribution width; Ratio H/L: ratio heterophils/ lymphocytes.

Table 5. Serum protein profiles of broilers with supplemented encapsulated *Dendrophthoe pentandra* leaf extract in high stocking density

Variables	Treatments				SEM	p value
	T0	T1	T2	T3		
Albumin (g/dL)	1.46	1.38	1.28	1.36	0.036	<0.401
SGOT (U/L)	273.72	275.10	290.80	320.46	11.900	<0.495
SGPT (U/L)	2.7	3.3	3.0	5.98	0.232	<0.185

Note: T0: broiler chickens were reared at a density of 9 birds/m² without additive supplementation; T1: broilers were reared at a density of 17 birds/m² without additive supplementation; T2: broilers were reared at a density of 17 birds/m² and supplemented with 0.5 g/kg encapsulated *D. pentandra* leaf extract; T3: broilers were reared at a density of 17 birds/m² and supplemented with 1.0 g/kg encapsulated *D. pentandra* leaf extract. SEM: standard error of the mean.

Table 6. Relative immune organ weights of the broilers with supplemented encapsulated *Dendrophthoe pentandra* leaf extract in high stocking density

Variables (% live body weight)	Treatments				SEM	p value
	T0	T1	T2	T3		
Bursa of Fabricius	0.04[c]	0.05[bc]	0.06[a]	0.06[ab]	0.055	<0.015
Spleen	0.11	0.10	0.09	0.09	0.174	<0.711
Thymus	0.17	0.14	0.21	0.18	0.374	<0.438

Note: Different superscripts (a, bc, c) within the same row indicate significant differences (p<0.05). T0: broiler chickens were reared at a density of 9 birds/m² without additive supplementation; T1: broilers were reared at a density of 17 birds/m² without additive supplementation; T2: broilers were reared at a density of 17 birds/m² and supplemented with 0.5 g/kg encapsulated *D. pentandra* leaf extract; T3: broilers were reared at a density of 17 birds/m² and supplemented with 1.0 g/kg encapsulated *D. pentandra* leaf extract. SEM: standard error of the mean.

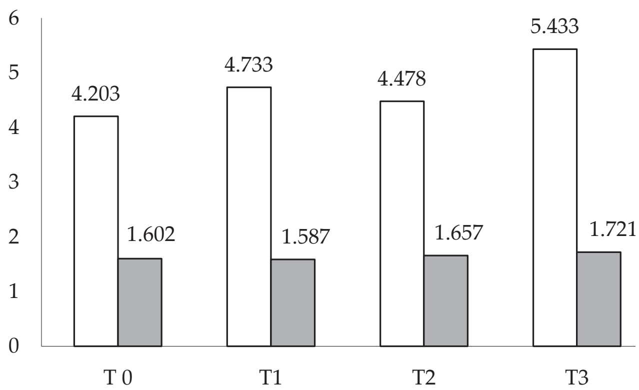

Figure 1. Serum superoxide dismutase (SOD) and malondialdehyde (MDA) with supplemented encapsulated *Dendrophthoe pentandra* leaf extract in high stocking density. T0: broiler chickens were reared at a density of 9 birds/m² without additive supplementation; T1: broilers were reared at a density of 17 birds/m² without additive supplementation; T2: broilers were reared at a density of 17 birds/m² and supplemented with 0.5 g/kg encapsulated *D. pentandra* leaf extract; T3: broilers were reared at a density of 17 birds/m² and supplemented with 1.0 g/kg encapsulated *D. pentandra* leaf extract; SEM: standard error of the mean; SOD (□): superoxide dismutase, U/mL; MDA (■): malondialdehyde, mmol/mL.

further investigation to determine if a biologically relevant effect exists under different experimental conditions.

DISCUSSION

Growth Performance of Broilers

Data on daily weight gain (DWG), final body weight, and feed conversion ratio (FCR) observed in this study were within the normal range for broiler chickens, indicating that the applied high stocking density (17 birds/m²) did not markedly impair growth performance under the current experimental conditions. This suggests that the physiological stress induced was not severe enough to adversely affect growth-related parameters, which represents a limitation in fully evaluating the extract's impact under strong stress. This finding agrees with previous reports by Chauhan *et al.* (2022) indicating that broilers can maintain productive performance under moderate stocking densities when environmental management and nutritional conditions are adequate. Consistent with this interpretation, the high-density control group (T1) showed no significant deterioration in DWG, final body weight, FCR, SGOT, or SGPT compared with the low-density control group (T0), further indicating that the stocking-density treatment did not impose a sufficiently severe physiological challenge under the present experimental conditions.

The higher daily feed intake (DFI) observed in the T2 group indicates that supplementation with encapsulated *D. pentandra* leaf extract may help maintain feed consumption under relatively challenging conditions. This response may be attributed to the biological activities of flavonoids, tannins, saponins, and phenolic compounds present in the extract (Saavedra-Molina *et al.*, 2024). These phytochemicals have been reported to improve gastrointestinal health by enhancing intestinal integrity, modulating gut microbiota composition, reducing oxidative damage to intestinal epithelial cells, and stimulating digestive enzyme secretion (Kwon *et al.*, 2023). Improved intestinal functionality may subsequently increase feed palatability and nutrient utilization efficiency, leading to higher feed consumption (Michalak *et al.*, 2025).

Flavonoids, particularly quercetin derivatives identified in *D. pentandra*, are known to exert anti-inflammatory and antioxidant effects within the gastrointestinal tract, thereby preserving villus structure and absorptive capacity (Parasuraman *et al.*, 2016). Likewise, saponins may suppress pathogenic microorganisms while promoting beneficial microbial populations, contributing to improved digestive health and nutrient assimilation (Zhang *et al.*, 2023). Similar increases in feed intake following phytogenic supplementation with encapsulated *Cosmos caudatus* have been reported by Agusetyaningsih *et al.* (2022) in broilers receiving polyphenol-rich plant extracts under environmental stress conditions.

Despite the increase in feed intake, no significant improvements were observed in DWG or FCR. This finding suggests that the increased feed consumption was not sufficient to produce measurable improvements in growth performance (Kavoi *et al.*, 2016). One possible explanation is that the physiological challenge imposed by the experimental stocking density was relatively mild, limiting the opportunity for phytogenic supplementation to demonstrate growth-promoting effects (Li *et al.*, 2024). Previous studies by Li *et al.* (2019) have shown that the beneficial effects of phytogenic additives become more evident when broilers are exposed to severe oxidative, thermal, or management-related stressors. Therefore, future studies should evaluate the efficacy of encapsulated *D. pentandra* leaf extract under more pronounced stress conditions.

Complete Blood Counts of Broilers

The hematological parameters, including erythrocytes, hemoglobin, hematocrit, and erythrocyte indices (MCV, MCH, MCHC), remained within normal physiological ranges and did not differ significantly among treatments. This indicates that neither high stocking density nor supplementation with encapsulated *D. pentandra* leaf extract adversely affected oxygen transport capacity or erythropoiesis. These findings are consistent with previous studies reporting that broilers can maintain normal hematological profiles under moderate stocking density when environmental and nutritional conditions are adequately managed (Rashid *et al.*, 2016; Tella, 2025).

The current results are consistent with those reported by Rashid *et al.* (2016), who observed stable hematological profiles in broilers receiving antioxidant-rich phytogenic additives. Similarly, Tella (2025) reported that plant-derived bioactive compounds did not significantly alter erythrocyte-related parameters when birds were maintained under relatively stable environmental conditions.

Leukocyte counts and heterophil-to-lymphocyte (H/L) ratios also remained unaffected by treatments. Leukocyte values observed in this study were within the normal physiological range reported for healthy broilers, suggesting that birds maintained adequate immune surveillance and physiological homeostasis throughout the experimental period (Lebedev *et al.*, 2024). The H/L ratio is considered one of the most reliable indicators of chronic stress in poultry because elevated corticosterone concentrations typically increase heterophil numbers while reducing circulating lymphocytes (Cotter, 2022). Generally, H/L ratios exceeding 0.5 are associated with significant physiological stress, whereas lower values indicate normal adaptation to environmental conditions (B, 2018). The low H/L ratios observed in the present study (0.03-0.05) therefore suggest that the birds did not experience substantial chronic stress despite being reared at a higher stocking density (Uysal *et al.*, 2025).

In contrast, thrombocyte counts and PDW were significantly lower in T1, T2, and T3 compared to T0, with only minor numerical differences among the high-density groups. Thrombocytes play important roles in hemostasis and innate immune responses in avian species (Ferdous & Scott, 2023). The reduction in PDW may indicate a more homogeneous platelet population and reduced platelet activation status (Shubha Sangeetha & A Manjunatha, 2019). This effect may be associated with flavonoids present in *D. pentandra* leaves, which have been reported to possess antiplatelet and antioxidant properties through inhibition of thromboxane synthesis, suppression of oxidative stress-induced platelet activation, and modulation of intracellular calcium signaling pathways (Santoso *et al.*, 2017; Zverev, 2017).

Previous studies by Santoso *et al.* (2017) have demonstrated that flavonoid-rich phytogenic supplements can influence platelet activity without inducing pathological changes in hematological status. Because thrombocyte counts remained within physiological limits, the observed reductions likely represent modulation of platelet function rather than suppression of thrombopoiesis (Vallance *et al.*, 2019). Nevertheless, the biological significance of these findings requires further investigation, particularly regarding the potential anti-inflammatory and immunomodulatory properties of *D. pentandra* bioactive compounds.

Serum Protein Profiles of Broilers

Data on serum concentrations, including albumin, SGOT, and SGPT, did not differ significantly among treatments and remained within normal physiological ranges, indicating that liver function and protein metabolism were not adversely affected by high stocking density or dietary supplementation. Similar findings have been reported in broilers, where stable serum biochemical parameters reflect normal hepatic function and metabolic homeostasis under controlled rearing conditions (Punyatong *et al*., 2025)

These findings suggest that encapsulated *D. pentandra* leaf extract is physiologically safe and does not cause hepatotoxicity. The stability of liver enzyme activity may also reflect the protective role of antioxidant compounds in maintaining hepatocyte integrity, as flavonoids and polyphenols are known to mitigate oxidative damage and support liver function in poultry (Siyanova & Zalyubovskaya, 2025)

However, the absence of significant variation among treatments may also indicate that the experimental conditions did not impose sufficient metabolic or hepatic stress to challenge liver function in broilers. Under mild or non-stress conditions, hepatic biomarkers often remain stable, thereby limiting the ability to detect functional improvements from dietary interventions (Mukul Ray *et al*., 2020). As a result, the capacity of the extract to demonstrate hepatoprotective effects under stress conditions may not have been fully expressed. This highlights the need for future studies under more challenging physiological conditions to evaluate better the functional benefits of the extract on liver health.

Relative Immune Organ Weights of the Broilers

Data on the relative weight of the bursa of Fabricius were significantly higher in the T2 group, indicating a positive effect of encapsulated *D. pentandra* leaf extract on the development of this primary lymphoid organ. The bursa of Fabricius is a primary lymphoid organ unique to avian species and serves as the principal site for B-lymphocyte proliferation, differentiation, and maturation (Szőcs *et al*., 2024). Enlargement of this organ is generally associated with enhanced humoral immune competence and improved antibody production capacity (Szőcs *et al*., 2024).

The observed increase may be attributed to the immunomodulatory activities of flavonoids, quercetin derivatives, tannins, and other phenolic compounds present in *D. pentandra*. These bioactive compounds have been reported to stimulate lymphocyte proliferation, regulate cytokine secretion, enhance macrophage activity, and protect immune cells from oxidative damage (Abubakar *et al*., 2019). Ang *et al.* (2014), demonstrated that *D. pentandra* extract significantly stimulated splenocyte and thymocyte proliferation, supporting its immunostimulatory potential.

Furthermore, flavonoids may promote B-cell differentiation through modulation of intracellular signaling pathways involved in immune cell activation and cytokine production (Liu & Jiao, 2025). Enhanced antioxidant protection may also preserve the viability and functionality of developing lymphocytes within the bursa, thereby contributing to increased organ development (Ke *et al*., 2023). Similar improvements in lymphoid organ development and humoral immunity have been reported by Ke *et al*. (2023) in broilers receiving phytogenic supplements rich in polyphenols and flavonoids.

Interestingly, the positive response was most evident in the T2 (0.5 g/kg), whereas the higher supplementation level T3 (1.0 g/kg) did not produce additional improvements. This finding suggests that moderate supplementation may provide optimal immunomodulatory stimulation, whereas excessive concentrations may not confer further benefits due to saturation of physiological response mechanisms. Similar dose-dependent responses have been reported for various phytogenic feed additives used in poultry nutrition.

The absence of significant changes in spleen and thymus weights suggests that systemic immune activation and T-cell development remained stable (Mehrzad *et al*., 2024). This observation may indicate that the birds were not exposed to severe immunological challenges during the experimental period (Mehrzad *et al*., 2024). Therefore, future studies employing pathogen challenge models or vaccination response assessments are warranted better to characterize the immunomodulatory properties of *D. pentandra* leaf extract.

Antioxidative Status of Broilers

Serum SOD activity and MDA levels are widely used indicators of antioxidant defense and oxidative stress in broilers, reflecting the balance between reactive oxygen species production and antioxidant capacity (Nawaz & Zhang, 2021). Data on SOD activity did not differ significantly among treatments, although a numerical increase was observed in supplemented groups. Similarly, MDA levels remained stable across all treatments, indicating that lipid peroxidation and oxidative damage were not markedly elevated.

These results suggest that broilers maintained oxidative balance under the given experimental conditions and that the endogenous antioxidant system was sufficient to prevent excessive oxidative stress. Similar findings have been reported in broilers under moderate environmental stress, where antioxidant biomarkers remain stable due to effective physiological adaptation (Cimpean *et al*., 2025). The absence of significant differences in SOD and MDA levels suggests that under the present experimental conditions, the broilers' endogenous antioxidant system was sufficient, or the oxidative challenge was not severe enough to elicit a detectable antioxidative effect from the supplement. This limits the ability to clearly demonstrate the extract's antioxidative potential in this specific context.

This condition represents an important limitation in evaluating the efficacy of antioxidant supplementation. When oxidative stress is not strongly induced, the measurable benefits of exogenous antioxidants may be minimal or undetectable, as also discussed in studies on antioxidant supplementation under low-stress conditions (Carmo De Carvalho E Martins *et al*., 2022; Ngestiningsih *et al*., 2019). Therefore, the absence of significant differences in SOD and MDA should not be interpreted as a lack of biological activity, but rather as a consequence of limited oxidative challenge within the experimental system.

In addition, the relatively short experimental duration and controlled environmental conditions may have further contributed to the stability of oxidative parameters. Previous studies have shown that stronger stressors, such as heat stress or higher stocking densities, are required to significantly elevate oxidative stress markers in broilers (Sharma *et al*., 2020). Future studies employing stronger stress models, such as higher stocking densities, heat stress, or longer rearing periods, are needed to assess better the antioxidative potential of encapsulated *D. pentandra* leaf extract.

CONCLUSION

Supplementation with 0.5 g/kg encapsulated *D. pentandra* leaf extract improved daily feed intake and increased the relative weight of the bursa of Fabricius in broilers reared under high stocking density without adversely affecting growth performance, hematological and serum biochemical parameters, or antioxidant status. These findings indicate that encapsulated *D. pentandra* leaf extract has potential as a safe natural feed additive to support immune development and contribute to sustainable broiler production under intensive rearing conditions.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENT

The authors acknowledge Universitas Diponegoro for funding this research, contract number 222-459/UN7.D2/PP/IV/2025. Gratitude was also expressed to the laboratory assistants, laboratories involved, and students who actively participated in this research.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

We declare that generative AI and AI-assisted technologies were used for language refinement and reference organization. All content has been critically reviewed and edited to ensure it reflects the authors' understanding, analysis, and academic integrity.

REFERENCES

Abubakar, S., Al-Mansoub, M. A., Murugaiyah, V., & Chan, K. (2019). The phytochemical and anti-inflammatory studies of Dillenia suffruticosa leaves. Phytotherapy Research, 33(3), 660-675. https://doi.org/10.1002/ptr.6255

Agusetyaningsih, I., Krismiyanto, L., Yudiarti, T., Widiastuti, E., Sari, D. A. P., Pawesti, S. D., & Sugiharto, S. (2025). Improving broiler growth and immunity with encapsulated *Cosmos caudatus* and *Andrographis paniculata* extracts at high stocking density. Journal of the Indonesian Tropical Animal Agriculture, 50(2), 111-121. https://doi.org/10.14710/jitaa.50.2.111-121

Agusetyaningsih, I., Widiastuti, E., Wahyuni, H. I., Yudiarti, T., Murwani, R., Sartono, T. A., & Sugiharto, S. (2022). Effect of encapsulated *Cosmos caudatus* leaf extract on the physiological conditions, immune competency, and antioxidative status of broilers at high stocking density. Annals of Animal Science, 22(2), 653-662. https://doi.org/10.2478/aoas-2021-0043

Alabbosh, K. F. (2024). Leveraging the potential of environmental microorganisms: an extensive examination of their capacity in tackling global and local environmental and ecological challenges. Polish Journal of Environmental Studies, 34(1), 1-11. https://doi.org/10.15244/pjoes/185701

Almuthaybiri, A. R., Bawazir, A. A. B., Almutairi, F. A. M., Almutairi, A. A., Faqeehi, M. H., & Al-Harbi, T. F. M. (2025). Complete blood count (CBC) interpretation and analysis: a comprehensive review for medical laboratory professionals. Journal of Posthumanism, 5(1), 1683–1699. https://doi.org/10.63332/joph.v5i1.3144

Andreichenko, S., Didenko, I., Amber, A., & Vasylenko, O. (2023). Ecological and economic efficiency of harvesting medicinal plant raw materials. Journal of Vasyl Stefanyk Precarpathian National University. Biology, 10, 113-119. https://doi.org/10.15330/jpnubio.10.113-119

Ang, H. Y., Subramani, T., Yeap, S. K., Omar, A. R., Ho, W. Y., Abdullah, M. P., & Alitheen, N. B. (2014). Immunomodulatory effects of *Potentilla indica* and *Dendrophthoe pentandra* on mice splenocytes and thymocytes. Experimental and Therapeutic Medicine, 7(6), 1733-1737. https://doi.org/10.3892/etm.2014.1657

Astaneh, I. Y., Chamani, M., Mousavi, S. N., Sadeghi, A. A., & Afshar, M. A. (2018). Effect of stocking density on performance and immunity in Ross 308 broiler chickens. Kafkas Universitesi Veteriner Fakultesi Dergisi, 24(4), 483-489. https://doi.org/10.9775/kvfd.2017.18869

Awang, M. A., Nik Mat Daud, N. N. N., Mohd Ismail N. I., Abdullah, F. I. & Benjamin, M. A. Z. (2023). A review of *Dendrophthoe pentandra* (Mistletoe): Phytomorphology, extraction techniques, phytochemicals, and biological activities. Processes, 11(8), 2348. https://doi.org/10.3390/pr11082348

Belousov, A. (2018). Hematocrit isn't targeted indicators for hemotransfusion. Medical Journal of Clinical Trials & Case Studies, 2(2), 000148. https://doi.org/10.23880/MJCCS-16000148

Bolton, W. (1967). Poultry nutrition (3rd rev. ed.). Her Majesty's Stationery Office.

Carmo de Carvalho e Martins, M. d., da Silva Santos Oliveira, A. S., da Silva, L. A. A., Primo, M. G. S., & de Carvalho Lira, V. B. (2022). Biological indicators of oxidative stress [malondialdehyde, catalase, glutathione peroxidase, and superoxide dismutase] and their application in nutrition. In Patel, V. B., Preedy, V. R. (eds), Biomarkers in nutrition. biomarkers in disease: methods, discoveries and applications (pp. 833-856). Springer, Cham. https://doi.org/10.1007/978-3-031-07389-2_49

Chauhan, P., Singh, K. D., Pramanik, P. S., Kumar, S., Srivastav, A. K., Tripathi, K. K., & Nandan, D. (2022). Impacts of stocking density on broiler chicken performance stress reaction and mortality in broiler chicken. Indian Journal of Veterinary Sciences & Biotechnology, 18(4), 36-39. https://doi.org/10.48165/ijvsbt.18.4.08

Choi, W. J., Kim, J. H., Han, G. P., Kwon, C. H., & Kil, D. Y. (2021). Effects of dietary hatchery by-products on growth performance, relative organ weight, plasma measurements, immune organ index, meat quality, and tibia characteristics of broiler chickens. Animal Bioscience, 34(7), 1181. https://doi.org/10.5713/ab.20.0755

Cimpean, A., Brozan, M. M., & Pojar, T. N. (2025). The impact of oxidative stress on growth performance and metabolic health in broiler chickens. Cluj Veterinary Journal, 30(1), 19-31. https://doi.org/10.52331/cvj..v30i1.52

Cotter, P. F. (2015). An examination of the utility of heterophil-lymphocyte ratios in assessing stress of caged hens. Poultry Science, 94(3), 512-517. https://doi.org/10.3382/ps/peu009

Ferdous, F., & Scott, T. (2023). The immunological capacity of thrombocytes. International Journal of Molecular Sciences, 24(16), 12950. https://doi.org/10.3390/ijms241612950

Hosseini-Vashan S. J., Golian, A., & Yaghobfar, A.. (2015). Growth, immune, antioxidant, and bone responses of heat stress-exposed broilers fed diets supplemented with *tomato pomace*. International Journal of Biometeorology, 60, 1183-1192. https://doi.org/10.1007/s00484-015-1112-9

Hu, R., He, Y., Arowolo, M. A., Wu, S., & He, J. (2019). Polyphenols as potential attenuators of heat stress in poultry production. Antioxidants, 8(3), 67. https://doi.org/10.3390/antiox8030067

Iffiong, S. A., Fulani, I. J., & Olomu, J. M. (2021). The effects of stocking density on the performance of broiler chickens. Nigerian Journal of Animal Production, 6, 21-25. https://doi.org/10.51791/njap.v6i.2652

Iheagwam, F. N., & Chinedu, S. N. (2024). High eggplant (*Solanum aethiopicum L.*) fruit intake for short duration enhances the antioxidant activity and biochemical parameters in rats. Journal of Phytology, 16, 121-126. https://doi.org/10.25081/jp.2024.v16.8962

Jamdar, F., Ali Mortazavi, S., Asl, M. R. S., & Sharifi, A. (2021). Physicochemical properties and enzymatic activity of wheat germ extract microencapsulated with spray and freeze drying. Food Science & Nutrition, 9(2), 1192-1201. https://doi.org/10.1002/fsn3.2104

Kavoi, B., Gakuya, D., Mbugua, P., & Kiama, S. (2016). Effects of dietary *Moringa oleifera* leaf meal supplementation on chicken intestinal structure and growth performance. Journal of Morphological Sciences, 33(04), 186-192. https://doi.org/10.4322/jms.095915

Ke, Y., Khaliq, H., Jiafu, L., Waqas, M. Y., Javid, M. A., Basit, M. A., Bhatti, S. A., Saleem, M. U., Farooq, A. A., & Murtaza, S. (2023). Distribution and developmental changes of IL-21 immunopositive cells in the bursa of Fabricius of Jinhu silky chicken. Journal of Experimental Zoology Part A Ecological and Integrative Physiology, 341(1), 41-47. https://doi.org/10.1002/jez.2759

Kong, D., Wang, L., Niu, Y., Cheng, L., Sang, B., Wang, D., & Jia, R. (2023). *Dendrophthoe falcata* (Lf) Ettingsh. and *Dendrophthoe pentandra* (L.) Miq.: A review of traditional medical uses, phytochemistry, pharmacology, toxicity, and applications. Frontiers in Pharmacology, 14, 1096379. https://doi.org/10.3389/fphar.2023.1096379

Kwon, C., Ediriweera, M. K., & Kim Cho, S. (2023). Interplay between phytochemicals and the colonic microbiota. Nutrients, 15(8), 1989. https://doi.org/10.3390/nu15081989

Lazuardi, M., Suharjono, S., Chien, C. H., He, J. L., Lee, C. W., Peng, C. K., & Maslachah, L. (2022). Toxicity test of flavonoid compounds from the leaves of *Dendrophthoe pentandra* (L.) Miq. using in vitro culture cell models. Veterinary World, 15(12), 2896. https://doi.org/10.14202/vetworld.2022.2896-2902

Lebedev, S. V., Kazakova, T. V., & Marshinskaya, O. V. (2024). Immune status of broiler chickens during different times of physiological development. Вестник Российской Сельскохозяйственной Науки, 3, 92-96. https://doi.org/10.31857/S2500208224030205

Li, W., F. Wei, B. Xu, Q. Sun, W. Deng, H. Ma, J. Bai, & S. Li. (2019). Effect of stocking density and alpha-lipoic acid on the growth performance, physiological and oxidative stress and immune response of broilers. Asian-Australasian Journal of Animal Sciences, 32(12), 1914-1922. https://doi.org/10.5713/ajas.18.0939

Li, Z., Gao, Q., Dong, S., Dong, K., Xu, Y., Mei, Y., & Hou, Z. (2024). Effects of chronic stress from high stocking density in mariculture: evaluations of growth performance and lipid metabolism of rainbow trout (*Oncorhychus mykiss*). Biology, 13(4), 263. https://doi.org/10.3390/biology13040263

Liu, Y., & Jiao, A. (2025). Flavonoids as immunoregulators: molecular mechanisms in regulating immune cells and their therapeutic applications in inflammatory diseases. Frontiers in Immunology, 16, 1703672. https://doi.org/10.3389/fimmu.2025.1703672

Mehrzad, J., Shojaei, S., Keivan, F., Forouzanpour, D., Sepahvand, H., Kordi, A., & Hooshmand, P. (2024). Avian innate and adaptive immune components: a comprehensive review. Journal of Poultry Sciences and Avian Diseases, 2(3), 73-96. https://doi.org/10.61838/kman.jpsad.2.3.7

Michalak, K., Janowski, K., Piotrowicz, E., & Góras, S. (2025). Impact of dietary phytochemicals on gut microbiota and intestinal barrier integrity - nutritional interventions and their role in chronic diseases prevention. Quality in Sport, 39, 59218. https://doi.org/10.12775/QS.2025.39.59218

Mishra, M. (2016). Materials of natural origin for encapsulation, In Mishra M. (Ed.), Handbook of Encapsulation and Controlled Release (pp 493). CRC Press Taylor & Francis Group.

Mukul Ray, S., Banik, A., & Bhagat, G. (2020). Effects of proprietary hepatoprotective additives (Cadliv™ liq.) supplementation on the growth performance and hepatic histological architecture of commercial broiler chickens. International Journal of Poultry Science, 19(7), 338-345. https://doi.org/10.3923/ijps.2020.338.345

Nawaz, A., & Zhang, L. (2021). Oxidative stress in broiler chicken and its consequences on meat quality. International Journal of Life Science Research Archive, 1(1), 045-054. https://doi.org/10.53771/ijlsra.2021.1.1.0054

Ngestiningsih, D., Rahayu, R. A., & Batubara, L. (2019). Effect of superoxide dismutase (SOD) supplementation on plasma levels of malondialdehyde (MDA), total cholesterol and LDL cholesterol in the elderly. Journal of Biomedicine and Translational Research, 5(2), 29-33. https://doi.org/10.14710/jbtr.v5i2.4679

Osti, R., Bhattarai, D., & Zhou, D. (2017). Climatic variation: effects on stress levels, feed intake, and bodyweight of broilers. Revista Brasileira de Ciência Avícola, 19(3), 489-496. https://doi.org/10.1590/1806-9061-2017-0494

Parasuraman, S., Anand David, A., & Arulmoli, R. (2016). Overviews of biological importance of quercetin: a bioactive flavonoid. Pharmacognosy Reviews, 10(20), 84. https://doi.org/10.4103/0973-7847.194044

Punyatong, M., Thapingkae, W., Srinual, O., & Lamsaengkul, C. (2025). Effects of glutamine supplementation on performance, carcass traits, and oxidative stress markers in native crossbred chickens under heat stress conditions. Veterinary Integrative Sciences, 24(1), 1-9. https://doi.org/10.12982/VIS.2026.027

Rashid, M. H. A., Ahmad, N., Amin, M. R., & Mollah, M. L. (2016). Effects of selected vitamins and minerals on growth rate and hematological parameters in broilers. Asian Journal of Medical and Biological Research, 1(3), 487-494. https://doi.org/10.3329/ajmbr.v1i3.26466

Saavedra-Molina, A., Lemus-De La Cruz, J., Landa-Moreno, C., Murillo-Villicaña, M., García-Berumen, C., Montoya-Pérez, R., Manzo-Avalos, S., Aguilera-Méndez, A., Salgado-Garciglia, R., & Cortés-Rojo, C. (2024). Antioxidant activity of natural products from medicinal plants. Biochemistry Intechopen. 1-21. https://doi.org/10.5772/intechopen.1004272

Santoso, U., Fenita, Y., & Kususiyah, K. (2017). The effect of medicinal herb inclusion on hematologic status and blood lipid profiles in broiler chickens. International Journal of Poultry Science, 16(10), 415-423. https://doi.org/10.3923/ijps.2017.415.423

Sharma, A., Swamy, M., Tiwari, A., Dubey, A., & Verma, Y. (2020). Oxidative stress markers in broiler chickens under heat stress condition. Indian Journal of Poultry Science, 55(2), 139. https://doi.org/10.5958/0974-8180.2020.00024.0

Shubha Sangeetha, R., & A Manjunatha, Y. (2019). Platelet distribution width (PDW): A novel platelet marker in thrombocytosis: An insight. IP Journal of Diagnostic Pathology and Oncology, 4(4), 264-267. https://doi.org/10.18231/j.jdpo.2019.055

Siyanova, I., & Zalyubovskaya, E. (2025). Effect of plant-based hepatoprotectors on the functional state of the liver in chickens. Genetics and Breeding of Animals, 2, 67-76. https://doi.org/10.31043/2410-2733-2025-2-67-76

Soliman, E. S., & Hassan, R. A. (2020). Influence of housing floor on air quality, growth traits, and immunity in broiler chicken farms. Advances in Animal and Veterinary Sciences, 8(9), 997-1008. https://doi.org/10.17582/journal.aavs/2020/8.9.997.1008

Sugiharto, S., Turini, Y., Isroli, I., Widiastuti, E., Wahyuni, H. I., & Sartono, T. A. (2019). Fermented feed as a potential source of natural an tioxidants for broiler chickens - a mini review. Agriculturae Conspectus Scientificus, 84(4), 313-318. https://hrcak.srce.hr/228920

Sugiharto, S., Yudiarti, T., & Isroli, I. (2016). Performances and haematological profile of broilers fed fermented dried cassava (*Manihot esculenta* Crantz). Tropical Animal Health and Production, 48, 1337-1341. https://doi.org/10.1007/s11250-016-1098-2

Szőcs, E., Balic, A., Soós, Á., Halasy, V., & Nagy, N. (2024). Characterization and ontogeny of a novel lymphoid follicle inducer cell during development of the bursa of Fabricius. Frontiers in Immunology, 15, 1449117. https://doi.org/10.3389/fimmu.2024.1449117

Taghvaei M., & Jafari S.M. (2015). Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. Journal of Food Science and Technology. 52: 1272-1282. https://doi.org/10.1007/s13197-013-1080-1

Tella, A. (2025). Effect of strain on haematology and serum biochemical profile of broiler chickens fed aqueous extract of bitter leaf. Jordan Journal of Agricultural Sciences, 21(2), 147-157. https://doi.org/10.35516/jjas.v21i2.3326

Tikate, K., Wade, M., Ranade, A. S., Patodkar, V. R., Dhaygude, V. S., & Bhalerao, S. M. (2020). Influence of dietary multiple phase feeding on growth performance of commercial broiler chicken. Indian Journal of Animal Research, 55(1), 66-70 https://doi.org/10.18805/ijar.B-3928

Tu, J., Kang, M., Zhao, Q., Xue, C., Bi, C., & Dong, N. (2024). Oleanolic acid improves antioxidant capacity and the abundance of *Faecalibacterium prausnitzii* in the intestine of broilers. Poultry Science, 103(12), 104340. https://doi.org/10.1016/j.psj.2024.104340

Uysal, A., Özentürk, U., Laçin, E., & Laçin, B. B. (2025). The effect of different cage densities on selected stress and welfare indicators in brown and white laying hens. Acta Veterinaria Brno, 94(2), 145-154. https://doi.org/10.2754/avb202594020145

Vallance, T. M., Ravishankar, D., Albadawi, D. A. I., Osborn, H. M. I., & Vaiyapuri, S. (2019). Synthetic flavonoids as novel modulators of platelet function and thrombosis. International Journal of Molecular Sciences, 20(12), 3106. https://doi.org/10.3390/ijms20123106

Yanai, T., Abo-Samaha, M. I., El-Kazaz, S. E., & Tohamy, H. G. (2018). Effect of stocking density on productive performance, behaviour, and histopathology of the lymphoid organs in broiler chickens. European Poultry Science, 82, 1-15. https://doi.org/10.1399/eps.2018.247

Yismairai, E., Hemelda, N. M., Yasman, & Handayani, W. (2019). Antioxidant activity of extract of mistletoe, *Dendrophthoe pentandra* (L.) Miq., lived in three different host plants, collected from Kampus UI, Depok. AIP Conference Proceedings, 2168, 020100. https://doi.org/10.1063/1.5132527

Zhang, Y., Hao, R., Chen, J., Li, S., Huang, K., Cao, H., Farag, M. A., Battino, M., Daglia, M., Capanoglu, E., Zhang, F., Sun, Q., Xiao, J., Sun, Z., & Guan, X. (2023). Health benefits of saponins and its mechanisms: perspectives from absorption, metabolism, and interaction with gut. Critical Reviews in Food Science and Nutrition, 64(25), 9311-9332. https://doi.org/10.1080/10408398.2023.2212063

Zhou Y., Mao S., & Zhou M. (2019). Effect of the flavonoid baicalein as a feed additive on the growth performance, immunity, and antioxidant capacity of broiler chickens. Poultry Science, 98(7), 2790-2799. https://doi.org/10.3382/ps/pez071

Zverev, Y. (2017). Antiplatelet activity of flavonoids. Voprosy Pitaniia, 86(6), 6-20. https://doi.org/10.24411/0042-8833-2017-00001