J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
50(2): 111-121, June 2025
DOI: 10.14710/jitaa.50.2. 111-121

Improving broiler growth and immunity with encapsulated *Cosmos caudatus* and *Andrographis paniculata* extracts at high stocking density

I. Agusetyaningsih*, L. Krismiyanto, T. Yudiarti, E. Widiastuti, D.A.P. Sari, S.D. Pawesti, and S. Sugiharto
Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java, Indonesia
**Corresponding author email: kania.ikania@gmail.com*

Received January 22, 2025; Accepted May 15, 2025

ABSTRACT

The community's growing demand for chicken meat as an animal protein source has presented farmers with the challenge to alter the care of broiler chickens by using high cage density. The aim of this study is to investigate the effect of diet supplementation with encapsulated extract of kenikir (*Cosmos caudatus*) and bitter (*Andrographis paniculata*) extracts on broiler diet to growth performance, immunity status, and carcass proportion. A total of 370 day-old Cobb broiler chicks, weighing 45.25 ± 0.89 g, were divided into five groups: T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal diet + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed). At the end of the study, the blood sample and internal organ were collected to further analysis. The final weights of groups T2, T3, and T4 were better than T0 and T1, while T1 has the lowest weight among the other groups. Chicks in T0, T2, T3 and T4 had lower PDW levels compared to T1. Chicks in T4 have a higher jejunum villi height compared to T0, T1, T2 and T3 groups. The treatments did not affect the small intestine and giblets relative organ wights (P>0.05). The group of T0, T2, T3, and T4 have a fewer duodenum lesion compared to T1. The group of T2, T3, and T4 have a better structure in jejunum cells compared to T0 and T1. Lesion score of *Bursa Fabricius* and spleen were greater in T3 and T4 compared to T0, T1 and T2 groups. And T1 have the lowest ND antibody compared to other groups. In conclusion, giving encapsulation of kenikir (*Cosmos caudatus*) and bitter (*Andrographis paniculata*) extracts on broiler diet can improve production performance and immune status of broiler chickens.

Keywords: Andrographis paniculata, Broiler, Cosmos caudatus, Encapsulation, High stocking density

INTRODUCTION

Chickens raised for meat are known as broiler chickens, and they are distinguished by their quick growth, high feed efficiency, and superior meat quality. The challenge faced by broiler chicken breeders is to boost their production capacity in order to meet market demand, given the annual rise in the consumption of broiler chicken meat (Kamboh and Zhu, 2013). Raising the density of the broiler chickens' cages is one method of boosting output (Abdel-Azeem, 2005). Cage density is the quantity of chickens housed per unit area (m^2). A study conducted by

Agusetyaningsih *et al.* (2022) found that hens housed at a density of 16 birds per square meter may be stressed. This can have a detrimental effect on chickens, even though breeders frequently do it. Specifically, it can lead to the emergence of oxidative stress, which directly affects the health and performance of broiler chickens.

Oxidative stress, a condition where the formation of reactive oxygen species (ROS) overwhelms the bird's antioxidant defence mechanism, is one of the main effects of excessive stocking density. In the end, this imbalance can weaken the immune system, lower feed intake, limit growth, and compromise the quality of meat by causing lipid peroxidation, protein degradation, and DNA damage (Sahin *et al.*, 2017). The hypothalamus-pituitary-adrenal (HPA) axis is activated by prolonged exposure to stressors such as crowding, which raises corticosterone levels. This hormone reaction further weakens the birds' resistance to illness and environmental stressors by suppressing immunological function, raising metabolic demands, and changing energy allocation (Jahja *et al.*, 2022).

To mitigate the effects of oxidative stress in broilers, dietary antioxidants are often employed. Although synthetic antioxidants are commonly utilized, natural antioxidants made from medicinal plants are gaining popularity because they provide bioactive molecules with fewer negative impacts on the environment and human health. *Andrographis paniculata* (sambiloto) and *Cosmos caudatus* (kenikir) are two examples of such plants that are commonly found in Indonesia. Sambiloto contains antibacterial and antioxidant properties. Andrographolide reduces oxidative stress and supports immune cell function by scavenging free radicals and modifying inflammatory signaling pathways like NF-κB and MAPK (Ge *et al*. 2023). These systems are especially helpful when there is a high stocking density since broilers are more vulnerable to oxidative damage and immunological suppression brought on by stress.

Ascorbic acid and quercetin, two phenolic components that are strong antioxidants and immunomodulator, are abundant in kenikir and sambiloto leaves (Jiang *et al.* 2025; Jahja *et al.* 2022). Agusetyaningsih *et al.* (2022) has demonstrated that encapsulating kenikir extract acts as an immunomodulator by enhancing the structure of lymphoid organ cells, improving blood profile, and causing broiler chickens to gain weight under stressful circumstances.

In plant extracts, the encapsulation process serves to prevent the bioactive compounds from oxidizing and being impacted by outside variables like humidity, temperature, and fungal contamination while being stored. There has never been no research done on the possible interaction (synergy) between the different types of bioactive components found in kenikir and sambiloto extracts to lessen the effects of oxidative stress brought on by high cage density. It is anticipated that encapsulating the extracts of *Andrographis paniculata* and *Cosmos caudatus*, either separately or together, will lessen the effects of stress. The purpose of this study is to ascertain how encapsulated extracts of *Andrographis paniculata* and *Cosmos caudatus* affect the immunological status, antioxidant levels, and physiological states of broiler chickens raised at high densities.

MATERIALS AND METHODS

Animals and Experimental Diets

A total of 370-day-old Cobb broiler chicks' weight of 45.25 ± 0.89 g, were purchased from a local hatchery, were employed in this present study. The chickens were divided into 5 groups, T0: control, basal diet + density of 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density of 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density of 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density of 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg of feed + *Andrographis paniculata* at 0.5 g/kg feed). The treatment starts on day 8^{th}, and the chickens were fed unrestrictedly and given *ad libitum* drinking water. The dose of 1 g/kg feed for both *Cosmos caudatus* and *Andrographis paniculata* (or a combination of 0.5 g/kg each) used in this study was selected based on a review of prior studies that demonstrated biological activity and safety in poultry at similar inclusion levels. Previous work by Agusetyaningsih *et al.* (2022**)** showed that *Cosmos caudatus* at 1 g/kg feed improved immune parameters in broilers under stress.

The first step in the production of encapsulated *Cosmos caudatus* dan *Andrographis paniculata* leaf extract was the leaf's extraction. The

leaves of the plant were acquired from Semarang's traditional markets in Central Java, Indonesia. After being weighed and spilled, the leaves were cleaned and drained. The leaves were dried indoors, shielded from the sun until the water content dropped to less than 10%. After that, the leaves were ground into a fine powder to create simplicia. Using maceration techniques, the leaves were extracted at a 1:6 ratio of simplicia to solvent (ethanol 70%) (Karimy *et al.,* 2013). The encapsulation process involved the use of freeze-dried leaf extract from *Andrographis paniculata* and *Cosmos caudatus*. Maltodextrin served as the coating material for the encapsulation. Maltodextrin was dissolved in distilled water at a ratio of 1:3 to the distilled water. The filtrate of *Cosmos caudatus* and *Andrographis paniculata* were combined with the dissolved maltodextrin at a 1:5 ratio. The encapsulated *Cosmos caudatus* and *Andrographis paniculata* leaf extract powder was obtained by freeze-drying the filtrate after it had fully dissolved in maltodextrin.

Data Collection and Laboratory Analysis
Productivity Parameters

Data on final weight and average daily gain were all measured. The chicks were weighed at the end of the research to find the final weight. And the data of average daily gain was calculated the final weight divided to the rearing total day (35 days).

The Blood Profile

A brachial vein blood sample was taken from one randomly selected chicken per experimental unit at the conclusion of the experiment. Three millilitres (ml) of blood were put in a non -EDTA tube to form serum, and one millilitres (ml) of blood was put in an EDTA tube for routine blood testing. Haematology analyzer Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia, was used to automatically determine the routine

Table 1. Feed formulation on starter and finisher period

Items	Compositions (%)	Items (%)	Composition (%)
Starter period		Finisher period	
Yellow maize	57.9	Yellow maize	61.0
Palm oil	2.55	Palm oil	2.95
Soybean meal	34.8	Soybean meal	32.0
DL-methionine	0.19	DL-methionine	0.19
Bentonite	1.00	Bentonite	0.75
Limestone	1.34	Limestone	1.00
Monocalcium phosphate	1.51	Monocalcium phosphate	1.30
Premix**	0.27	Premix**	0.34
Chlorine chloride	0.07	Chlorine chloride	0.07
Salt	0.40	Salt	0.40
Chemical compositions:		Chemical compositions:	
ME (kcal/kg)*	3539.32	ME (kcal/kg)*	3576.42
Crude protein	22.06	Crude protein	18.22
Crude fibre	1.69	Crude fibre	0.89
Crude fat	2.77	Crude fat	4.59
Water	11.01	Water	10.53
Ash	4.96	Ash	6.99

* The Bolton formula was used to calculate the amount of metabolizable energy. Bolton formula: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

**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 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, CaCO3 641.5 mg, dicalcium phosphate 1500 mg

blood profile testing of chicks. For the purpose of separating serum, the anticoagulant-free blood was centrifuged for 10 minutes at 3,000 rpm. The antibody titres against ND were measured using the hemagglutination inhibition (HI) assay. For the ND antibody titres test, U-based microtiter plates were loaded with four HA units of LaSota antigen. The test samples were serially diluted twice and mixed with an equal volume of ND antigen. To find out if the hemagglutination was totally inhibited, the number of dilutions was counted following the addition of chicken red blood cells (CRBC).

Digestive Tract's pH and Gut Microbiota

The duodenum, ileum, and cecum segments of the intestine were measured using a digital pH meter (ST300 Portable pH-Meter, OHAUS). The duodenum, ileum, and cecum mucosa were all touched with the pH-meter's pen tip until the intestinal pH values showed up on the screen. At 38°C for 24 hours, total coliform was counted on MacConkey agar (Merck KGaA, Darmstadt, Germany). Following anaerobic incubation for 48 hours at 38°C, the lactic acid bacteria colony was counted using Merck KGaA's de Man, Rogosa, and Sharpe agar (MRS).

Lymphoid Organ Weight and Histology Parameters

The weights of the lymphoid organs (spleen, thymus, and bursa of Fabricius) were divided by the live body weight and multiplied by 100% to determine their relative weight. The histologic examination was performed on the spleen and small intestine. After cutting histologic organs 5 µm transversely, haematoxylin-eosin (HE) staining was applied. Every intestinal segment's villus height and crypt depth were measured using an optical microscope equipped with a digital camera (Leica Mycrosystems GmBH, Wetzlar, Germany). The villus-crypt ratio (VH/CD) was calculated by dividing the villus height by the crypt depth.

Based on the extent of tissue damage, the lesions in the lymphoid organs and small intestine were classified. The following criteria were used to score the distribution of injuries: necrosis, inflammatory infiltration, loss of cilia, hypertrophy or hyperplasia of epithelial cells, and focal or multifocal injuries. A severity score of 0 (no lesion, 0% cell damage), 1 (mild, 5-25% of cell damage), 2 (moderate, 26-50% of cell damage), and 3 (severe, >50% of cell damage) was assigned by earlier research (Alabi *et al.,* 2020; Agusetyaningsih *et al.,* 2022). The average of three observation sites per small intestine sample was computed to determine the villi height and crypt depth.

Statistical Analysis

In this study, the data were analyzed using the SPSS software version 22.0. With a 5% significance level, one-way ANOVA was used to statistically analyze the collected data. It was decided to assess the differences between the treatment groups using the Duncan's multiple range test (DMRT). The Kruskal-Wallis's analysis method was used to analyze the histopathological lesion scores on the small intestine and lymphoid organ non-parametrically.

RESULTS

Performance of Broilers

Data on final body weight and daily weight gain are listed in Table 2. The table shows that administration of kenikir and sambiloto encapsulated extract affects final body weight and daily weight gain ($P < 0.05$). The final weights of groups T2, T3 and T4 were better than T0 and T1. while T1 has the lowest weight among the other groups.

Complete Blood Counts

The data of complete blood counts listed in Table 3. Chicks in T0, T2, T3 and T4 have a lower PDW level compared to T1. The treatment of kenikir and sambiloto encapsulated extract did not affect the other blood parameters (Hb, erythrocytes, Haematocrit, MCV, MCH, MCHC, RDW, PMV, leucocytes, heterophils, thrombocytes and H/L).

Small Intestine Histomorphometry and Ileum-Caecum pH

Small intestine histomorphometry data stated in Table 4, and the ileum-caecum pH listed in Table 5. Chicks in T4 have a higher jejunum villi height compared to T0, T1, T2 and T3 groups. Also, they have a ratio between jejunum villi and crypts higher in T3 compared to other groups.

The treatments did not affect the villi and crypt dept of duodenum and ileum, and also on ileum-caecum pH (P>0.05).

Small Intestine and Giblets Relative Organ Weight

The data of small intestine and giblets weight are listed in Table 6. The treatments did not affects the small intestine and giblets relative organ weight (P>0.05).

Small Intestine Lesion Score

The data of small intestine lesion scores are shown in Table 7. The groups of T0, T2, T3 and T4 have a fewer duodenum lesion compared to T1. The group of T2, T3, and T4 have a better structure in jejunum cells compared to T0 and T1.

Immunology Parameters

Immunological parameter data are listed on Table 8. According to this table, treatment of encapsulated kenikir and sambiloto extract did not alter the relative immune organ weight (P>0.05), but give some impact on lymphoid organ lesions and ND antibody titre. Based on Table 8, lesion scores of Bursa Fabricius and spleen were greater in T3 and T4 compared to T0, T1 and T2 groups. And T1 have the lowest ND antibody compared to other groups.

Histopathologic Scoring of the Lymphoid Tissue and Small Intestines

Table 9 shows the data of carcass proportions in broiler. The treatment did not make a significant effect in carcass proportion on broiler chickens (P>0.05).

DISCUSSION

The results of this study demonstrated that dietary administration of encapsulated Cosmos caudatus and Andrographis paniculata extract had a significant effect on broiler chicken growth performance. Data on the group treated with encapsulated *Cosmos caudatus* and *Andrographis paniculata* extract had a better final weight than the other groups, likewise with daily body weight gain. In general, the broiler's growth, development, and overall performance are greatly influenced by the nutritional makeup of the feed they are fed (Table 1). *Cosmos caudatus* and *Andrographis paniculata* have an antioxidant activity that can involves the neutralization of reactive oxygen species (ROS) and oxidative stress within the chicks's body. hen the body's antioxidant defenses are unable to completely remove reactive oxygen species (ROS), such as free radicals, an imbalance arises that leads to oxidative stress. Antioxidants promote healthy growth and development by lowering oxidative damage to tissues. This is especially crucial when broiler growth is at its fastest. This is consistent with research by Jahja *et al.* (2022) that shows supplementing broiler chickens with bittersweet (*Andrographis paniculata*) can increase body weight and daily body weight gain. Additionally, Agusetyaningsih *et al.* (2022) reported that supplementing broiler chickens with kenikir (*Cosmos caudatus*) extract at a dose of 0.5–0.1 g/kg feed may raise the birds' daily body weight gain (ADG). This suggests that the phenolic components in sambiloto and kenikir contribute to the broiler chicken bodies anti-oxidative processes, reducing the amount of oxidative stress brought on by overcrowding in the cage.

Table 3 displays the blood profile data for broiler chickens. Empirical evidence indicates that the administration of encapsulated bitter and kenikir extracts has no discernible impact on blood parameters or hematological parameters. On the other hand, the data indicate that raising

Table 2. Performance of broilers (days 0-35)

Items	T0	T1	T2	T3	T4	SEM	P value
Final weight (g)	1558.00^{b}	1310.80^{c}	1605.40^{ab}	1677.80^{ab}	1711.80^{a}	34.34	<0.001
ADG (g)	44.51^{b}	37.43^{c}	45.86^{ab}	47.57^{ab}	48.90^{a}	0.98	<0.001

^{a,b,c}The means in the same row indicated by superscript letters differ significantly (P<0.05). T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean, DWG: daily weight gain, DFI: daily feed intake, FE: feed efficiency

Table 3. Complete blood counts and blood biochemical of broilers

Items	T0	T1	T2	T3	T4	SEM	P value
Haemoglobin (g/dL)	7.66	7.36	7.86	7.44	7.48	0.10	0.583
Erythrocytes (10^6/µL)	1.85	1.84	1.82	1.85	1.82	0.02	0.996
Haematocrit (%)	30.88	30.88	30.86	31.10	30.24	0.46	0.986
MCV	167.58	168.06	169.72	168.42	165.98	0.71	0.609
MCH	41.44	39.96	43.00	40.06	40.04	0.47	0.247
MCHC	24.32	23.34	24.84	23.26	24.26	0.29	0.404
RDW-SD	46.48	48.34	48.34	48.70	46.08	0.50	0.342
RDW-CV	9.56	9.94	9.82	9.98	9.58	0.09	0.446
MPV	7.08	7.72	7.14	6.94	6.58	0.18	0.380
PDW	5.04[b]	6.80[a]	4.04[b]	4.32[b]	4.96[b]	0.29	0.012
Leukocytes (10^3/µL)	70.12	68.76	70.52	60.76	64.52	1.76	0.355
Heterophils (10^3/µL)	2.20	2.12	2.16	1.30	1.56	0.15	0.198
Lymphocytes (10^3/µL)	67.92	66.64	68.36	59.46	62.96	1.65	0.399
Thrombocytes (10^3/µL)	34.80	32.20	40.60	37.80	44.60	2.14	0.416
H/L	3.22	3.19	3.12	2.17	2.32	0.19	0.262

[a, b,c]The means in the same row indicated by superscript letters differ significantly (P<0.05). T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW-SD: red cell distribution width-standard deviation, RDW-CV: red cell distribution-coefficient variation, MPV: mean platelet volume, PDW: platelet distribution width, H/L: heterophils/lymphocytes ratio, ACR: albumin to creatinine ratio.

Table 4. Small intestine histomorphometry

Items	T0	T1	T2	T3	T4	SEM	P value
Duodenum							
VH	998.27	965.85	974.22	1173.8.	1231.03	50.07	0.30
CD	182.88	156.21	161.34	183.21	153.72	8.01	0.66
VH/CD	5.64	6.34	6.45	6.64	8.47	0.43	0.34
Jejunum							
VH	1173.10[b]	945.61[b]	1165.02[b]	1176.12[b]	1419.60[a]	42.62	<0.01
CD	216.70	196.86	150.74	199.48	173.46	9.45	0.20
VH/CD	5.70[bc]	5.06[c]	7.92[ab]	6.33[bc]	8.60[a]	0.44	0.04
Ileum							
VH	678.20	634.06	690.70	618.41	676.15	21.88	0.82
CD	120.73	128.42	160.63	128.31	126.87	10.19	0.78
VH/CD	5.68	5.46	4.81	5.86	5.38	0.34	0.19

[a,b]The means in the same row indicated by superscript letters differ significantly (P<0.05). T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean. VH: villi height, CD: crypt depth, VH/CD: villi height and crypt depth ratio

broiler chickens at high densities may result in a reduction in the chickens' platelet distribution width (PDW) levels (T1). The platelet distribution width (PDW) measures how differently the platelets that are circulating in peripheral blood vessels are sized. Low PDW values signify small -sized platelets. The process of blood clotting depends heavily on platelets. The body's 5-hydroxytryptamine serotonin system may be impacted by oxidative stress, potentially influencing PDW levels (Audhya *et al.,* 2012). This demonstrates how high cage density-related oxidative stress impacts broiler chickens' ability to form platelets.

The data of small intestine lesion scores are shown in Table 7. The groups of T0, T2, T3 and T4 have a fewer duodenum lesion compared to T1. The group of T2, T3, and T4 have a better structure in jejunum cells compared to T0 and T1. This indicates that phenolic compound in *Cosmos caudatus* and *Andrographis paniculata* exhibits antioxidant properties, which may protect cells from oxidative stress. Oxidative stress can negatively impact cell viability (Wasti and Mishra, 2020). By reducing oxidative stress, antioxidants properties may create a better condition for increasing cell integrity. *Cosmos caudatus* and *Andrographis paniculata* have the antimicrobial compound that aid in preventing the development of harmful bacteria in the gastrointestinal tract. It can be related on the villi data that shown in Table 7. Chicks in T4 have a higher jejunum villi height compared to T0, T1, T2 and T3 groups. Also have a ratio between jejunum villi and crypts higher in T3 compared to other groups. The treatments did not affects the villi and crypts dept of duodenum and ileum, and also on ileum-caecum pH ($P>0.05$). Increased villus height, particularly in the jejunum, indicates a larger surface area for nutrient absorption. The jejunum is the primary site for the absorption of carbohydrates, amino acids, vitamins, and minerals in broiler chickens. A higher villus height suggests enhanced absorptive efficiency, leading to improved nutrient uptake from the feed. Meanwhile, a higher VH/CD ratio is a reliable indicator of intestinal health, where deeper crypts typically suggest increased cell turnover due to inflammation or stress (Uni *et al.* 2003). Bioactive compound in *Cosmos caudatus* and *Andrographis paniculata* may regulate key proteins involved in the cell cycle, such as cyclins and cyclin-dependent kinases (CDKs) contributing on cell integrity and proliferation (Wang *et al.,* 2023). These improvements in intestinal morphology correspond well with the higher daily weight gain and final body weight observed in groups supplemented with *Cosmos caudatus* and *Andrographis paniculata*, likely due to improved feed conversion and nutrient utilization efficiency.

The small intestine lesion scores are listed in Table 7. It shows that supplementation with encapsulated *Cosmos caudatus* and *Andrographis paniculata* singly or in combination have a significant effect on duodenum and jejunum cell integrity. Polyphenols are a diverse group of naturally occurring compounds found in plants, and they are known for their antioxidant properties. Reactive oxygen species (ROS) and free radicals can be neutralized by polyphenols, which are potent antioxidants. Polyphenols aid in preventing oxidative damage to cellular constituents such as lipids, proteins, and DNA by scavenging these dangerous molecules. This antioxidant action helps to preserve the integrity of cell membranes and the general health of cells. In addition, Numerous polyphenols have anti-inflammatory qualities. Persistent inflammation can weaken cell integrity and cause cellular damage. Polyphenols have the potential to mitigate inflammation and its deleterious impact on cells by regulating inflammatory pathways. Through their interactions with cell signaling pathways, polyphenols can affect cellular responses and gene expression. It has been demonstrated that certain polyphenols regulate pathways related to cell cycle control, apoptosis (programmed cell death), and cell survival. This modulation may help to preserve the integrity and appropriate operation of cells.

The previous mentioned data have a correlation with lymphoid organ lesion score that shown on Table 8. According to this table, treatment of encapsulated *Cosmos caudatus* and *Andrographis paniculata* extract did not alter the relative immune organ weight ($P>0.05$), but give some impact on lymphoid organ lesion and ND antibody titre. Based on Table 8, lesion score of Bursa Fabricius and spleen were greater in T3 and T4 compared to T0, T1 and T2 groups. Pre-

Table 5. Ileum and caecum pH

Items	T0	T1	T2	T3	T4	SEM	P value
Ileum	6.21	6.44	6.54	6.92	6.42	0.12	0.49
Caecum	7.59	7.48	7.33	7.44	7.65	0.05	0.43

T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean

Table 6. Small intestine and giblets relative organ weight

Items	T0	T1	T2	T3	T4	SEM	P value
Duodenum	0.56	0.54	0.60	0.46	0.46	0.02	0.39
Jejunum	1.21	1.10	1.07	0.85	0.96	0.05	0.27
Ileum	1.15	0.88	0.98	0.82	1.02	0.07	0.66
Proventriculus	0.50	0.50	0.54	0.52	0.51	0.01	0.94
Gizzard	1.72	1.69	1.66	1.72	1.59	0.04	0.89
Pancreas	0.26	0.29	0.26	0.24	0.25	0.01	0.68

T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean

Table 7. Small intestine lesion score

Items	T0	T1	T2	T3	T4	SEM	P value
Duodenum	1.80^{a}	3.20^{b}	1.40^{b}	1.80^{b}	1.00^{b}	0.18	<0.01
Jejunum	2.60^{a}	3.20^{a}	1.40^{b}	1.00^{b}	1.40^{b}	0.21	<0.01
Ileum	1.60	2.60	1.40	1.80	1.20	0.16	0.06

^{a,b} Means marked with superscript letters in the same row are significantly different (P<0.05). T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean

Table 8. Immunological parameters

Items	T0	T1	T2	T3	T4	SEM	P value
Relative immune organ weight							
Bursa of Fabricius	0.15	0.15	0.15	0.13	0.11	0.01	0.85
Spleen	0.09	0.12	0.13	0.11	0.07	0.01	0.60
Thymus	0.17	0.15	0.14	0.18	0.17	0.01	0.95
Limfoid organ lesion score							
Bursa of Fabricius	2.40^{a}	2.40^{a}	1.80^{ab}	1.00^{b}	1.00^{b}	0.21	0.05
Spleen	2.80^{a}	2.80^{a}	2.00^{ab}	1.40^{b}	1.00^{b}	0.24	0.04
Thymus	1.80	1.80	1.80	1.20	1.00	0.14	0.61
Antobody titre							
Newcastle disease (ND)	3.60^{a}	1.60^{b}	3.20^{a}	3.80^{a}	3.80^{a}	0.26	0.02

^{a,b} Means marked with superscript letters in the same row are significantly different (P<0.05). SEM : standard error mean, ND: Newcastle Disease. T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean

vious study stated that polyphenol in plants have the potential to impact cell-matrix interactions, thereby aiding in the preservation of tissue integrity. These substances have the ability to modify the expression of proteins that are essential for tissue structure and function, such as those involved in cell adhesion, migration, and extracellular matrix formation.

According to the ND antibody titer, bioactive compound in *Cosmos caudatus* and *Andrographis paniculata* (polyphenol) may influence various signaling pathways involved in immune responses. This includes pathways related to cytokine production, which can impact the differentiation and activation of immune cells, including those involved in antibody production (Widowati *et al.,* 2022). Polyphenols might influence the presentation of antigens to immune cells, facilitating a more robust immune response. This can contribute to a heightened antibody production, especially in response to specific pathogens or vaccines. Otherwise, polyphenols, with their anti-inflammatory properties, may help create an environment conducive to optimal immune function. By reducing inflammation in immune organ (Bursa of Fabricius and Spleen), polyphenols may indirectly support antibody production. This condition indicates that broiler chickens vaccinated against ND can have higher levels of ND antibody titer when they receive the treatment (Fayed *et al.,* 2023). Antigens are typically proteins present on the surface of ND viruses' vaccine. Bioactive compound in *Cosmos caudatus* and *Andrographis paniculata* has been documented that it can trigger the release of specific cytokines, which can stimulate B cells and encourage their proliferation and activation. Upon activation, B cells differentiate into two primary cell types: memory B cells, which can retain the specific antigen for more rapid and powerful responses upon antigen exposure, and plasma cells, which are specialized for producing antibodies (Horns *et al.,* 2020; Klein, 2021; Li *et al.,* 2021).

The feed supplementation with encapsulated *Cosmos caudatus* and *Andrographis paniculata* did not affect the carcass proportion on broiler chickens. Despite the fact that broiler growth performance improved after receiving the phytogenic supplements, there were no appreciable changes in carcass proportions (e.g., breast meat, thigh, wings) for a number of reasons. First, muscle-specific deposition affects carcass yield in addition to growth rate, and it may not react consistently to antioxidant administration. Although polyphenolic substances may improve development in general by lowering oxidative stress, selective muscle hypertrophy is not always the result of this action. Second, the age at slaughter and the length of supplementation might not have been long enough for the difference in muscle deposition to show. And last, instead of rerouting energy especially toward carcass yield, the bioactive chemicals might have given priority to immunological resilience and interior tissue health (Kishawy *et al.* 2023; Zhao *et al.* 2022).

CONCLUSION

In conclusion, broiler chickens raised in high-density conditions (16 birds/m2), the addition of encapsulated extracts of kenikir (*Cosmos caudatus*) and sambiloto (*Andrographis panicu-*

Table 9. Carcass proportion of broilers

Items (%)	T0	T1	T2	T3	T4	SEM	P value
Carcass							
Breast	34.12	32.83	34.80	34.59	32.90	0.49	0.60
Wings	11.18	11.63	10.96	11.37	10.91	0.16	0.68
Thight	16.74	16.61	16.82	15.98	16.70	0.20	0.75
Drumstick	15.41	16.30	15.36	15.57	16.26	0.23	0.56
Back	14.70	14.77	114.80	13.15	15.90	0.58	0.72
Abdominal fat	1.48	1.21	1.11	1.30	1.28	0.06	0.49

T0: control, basal diet + density 10 chicks/m^2, T1: basal diet + density 16 chicks/m^2, T2: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 1 g/kg feed, T3: basal feed + density 16 chicks/m^2 + *Andrographis paniculata* 1 g/kg feed, T4: basal feed + density 16 chicks/m^2 + *Cosmos caudatus* 0.5 g/kg feed + *Andrographis paniculata* 0.5 g/kg feed, SEM : standard error mean

lata) at a dose of 1 g/kg feed, or their combination at 0.5 g/kg each, markedly enhanced growth performance, intestinal health, and immune response. Increased jejunum villi height and improved lymphoid organ integrity are signs that these bioactive plant extracts aid in lowering oxidative stress and promoting improved nutrient absorption.

ACKNOWLEDGEMENT

The authors express would like to thank to Universitas Diponegoro, which funded this research (Contract Number 609-13/UN7.D2/PP/VIII/2023). Also, to the laboratory assistants and laboratory technicians, as well as the students who actively participated in conducting this research.

CONFLICT OF INTEREST

All authors declare that there has no conflict of interests.

REFERENCES

Abdel-Azeem, F.J.E.P.S.J. 2005. Green tea flowers (Camellia sinensis) as natural antioxidants feed additives in growing Japanese quail diets. Egypt. Poult. Sci. J. 25(3):569-588.

Agusetyaningsih, I., E. Widiastuti, H.I. Wahyuni, R. Murwani, T.A. Sartono and S. Sugiharto. 2022. Effect of encapsulated Cosmos caudatus leaf extract on the physiological conditions, immune competency, and antioxidative status of broilers at high stocking density. Ann. Anim. Sci. 22 (1):653-662.

Alabi, O.J., J.W. Ng'Ambi and E.F. Mbajiorgu. 2020. Aqueous extract of Moringa (Moringa oleifera) leaf (AEMOL) on the growth, sensory and histology parameters of broiler chickens. Appl. Ecol. Environ. Res. 18(5):6753-6764.

Audhya, T., J.B. Adams and J. Johansen. 2012. Correlation of serotonin levels in CSF, platelets, plasma and urine. Biochim. Biophys. Acta. 1820:496-501.

Fayed, R.H., E. Rashad, S.I. El-Samannoudy, H.M.R. Elsherif, H.A. Ella and E. Ismael. 2023. Mitigative effect of dietary Tinospora cordifolia and Andrographis paniculata on health and hepato-renal expression of Caspase-3 and TNF-α of broiler chickens fed on aflatoxin and ochratoxin contaminated diet. J. Adv. Vet. Res. 13(8):1668-1677.

Ge, S., W. Lian, Y. Bai, L. Wang, F. Zhao, H. Li, D. Wang and Q. Pang. 2023. TMT-based quantitative proteomics reveals the targets of andrographolide on LPS-induced liver injury. BMC Vet. Res. 19:199.

Horns, F., C.L. Dekker and S.R. Quake. 2020. Memory B cell activation, broad anti-influenza antibodies, and bystander activation revealed by single-cell transcriptomics. Cell Rep. 30(3):905-913.

Jahja, E.J., R. Yuliana, W.T. Simanjuntak, N. Fitriya, A. Rahmawati and E. Yulinah. 2022. Potency of Origanum vulgare and Andrographis paniculata extracts on growth performance in poultry. Vet. Anim. Sci. 19:100274.

Jiang, Z., G. Lhamo, M. Ma, X. Ye, J. Chen, Y. He, J. Xu and L. Huang. 2025. Quercetin as a therapeutic agent for acute pancreatitis: a comprehensive review of antioxidant, anti-inflammatory, and immunomodulatory mechanisms. Front. Pharmacol. 16

Kamboh, A.A. and W.Y. Zhu. 2013. Individual and combined effects of genistein and hesperidin supplementation on meat quality in meat-type broiler chickens. J. Sci. Food Agric. 93(13):3362-3367.

Karimy, M.F., Julendra, Hayati, Sofyan, Damayanti and Priyowidodo. 2013. Efektifitas ekstrak daun kenikir (Cosmos caudatus), daun mengkudu (Morinda citrifolia), dan tepung cacing tanah (Lumbricus rubellus) dalam sediaan granul larut air sebagai koksidiostat alami terhadap infeksi Eimeria tenella pada ayam broiler. JITV. 18:88-98.

Kishawy, A.T.Y., D. Ibrahim, E.M. Roushdy, A. Moustafa, F. Eldemery, E.M. Hussein, F.A.M. Hassan, S.T. Elazab, M.T. Elabbasy, R. Kanwal, W.M. Kamel, M.R. Atteya and A.W. Zaglool. 2023. Impact of resveratrol-loaded liposomal nanocarriers on heat-stressed broiler chickens: Effects

on performance, sirtuin expression, oxidative stress regulators, and muscle building factors. Front. Vet. Sci. 10:1137896.

Klein, B.G. 2021. Cunningham's Textbook of Veterinary Physiology, 6th ed. Saunders.

Li, W., J. Li, N. He, X. Dai, Z. Wang, Y. Wang and K. Pan. 2021. Molecular mechanism of enhancing the immune effect of the Newcastle disease virus vaccine in broilers fed with Bacillus cereus PAS38. Food Funct. 12(21):10903-10916.

Sahin, K., C. Orhan, M. Tuzcu, F. Akdemir and N. Sahin. 2017. The role of oxidative stress and antioxidants in the pathogenesis of poultry diseases. Poultry Sci. 96:4133-4142.

Tan, Z., B. Halter, D. Liu, E. Gilbert and M.A. Cline. 2022. Dietary flavonoids as modulators of lipid metabolism in poultry. Front. Physiol. 13.

Uni, Z., A. Smirnov and D. Sklan. 2003. Pre and post-hatch development of goblet cells in the broiler small intestine: Effect of delayed access to feed. Poult. Sci. 100 (1):234-245.

Wang, Y., Z. Zhong, R. Wang, N. Munawar, L. Zan and J. Zhu. 2023. Effects of proanthocyanidins and dialdehyde chitosan on the proliferation and differentiation of bovine myoblast for cultured meat production. Int. J. Biol. Macromol. 246:125618.

Wasti, S., N. Sah and B. Mishra. 2020. Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals. 10(8):1266.

Widowati, M., C. Hardiyanti, R. Nisak, M.M.P. Sirat and R. Ermawati. 2022. Suplementasi tepung daun sambiloto (Andrographis paniculata) melalui air minum ayam broiler terhadap titer antibodi Newcastle disease, avian influenza, dan infectious bursal disease. J. Ilm. Peternak. Terpadu. 10 (1):114-123.

Zhao, Y., Z. Li, X. Wang, F. Zhao, C. Wang, Q. Zhang, X. Chen, Z. Geng and C. Zhang. 2022. Resveratrol attenuates heat stress-induced impairment of meat quality in broilers by regulating the Nrf2 signaling pathway. Animals. 12:1889.