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Encapsulated Cardamom Waste Extract (*Amomum compactum*) Supplementation Improves Health and Performance of Broiler Chickens

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ABSTRACT

Encapsulated cardamom waste extract (ECWE) contains flavonoids with antibacterial and antioxidant properties that may enhance broiler health and performance. This study evaluated the effects of ECWE as a phytobiotic on broiler intestinal microbiota, pH, H/L ratio, lymphoid organs, and performance. A total of 200 unsexed Ross broilers (8 days old) with an average body weight of 215.08±5.1 g/bird were assigned to five dietary treatments: T0 (control), T1 (0.02% ECWE), T2 (0.04% ECWE), T3 (0.06% ECWE), and T4 (0.08% ECWE). Results showed that 0.08% ECWE significantly increased lactic acid bacteria (LAB) populations, lymphoid organ, and broiler performance. The increase in LAB given 0.08% ECWE is more effective in helping to reduce intestinal pH and to minimize the growth of pathogenic bacteria that can interfere with chicken digestion. In addition, the antioxidant properties in ECWE had a positive effect on the development of lymphoid organs, especially the thymus. It is concluded that ECWE supplementation at 0.08% was the most effective in improving digestive tract health and optimizing broiler performance.

Keywords: cardamom waste; encapsulation; flavonoids; intestinal health; lymphoid organs

INTRODUCTION

Broiler chickens are widely cultivated to meet the community's demand for animal protein. In 2023, the demand and production of chicken meat increased by 9.7% and 9.1%, respectively (BPS, 2023). Despite their rapid growth and feed efficiency, broilers are prone to stress and disease, particularly from heat stress, which adversely affects digestion and intestinal health. Since the ban on antibiotic growth promoters (AGP), various feed additives such as probiotics, prebiotics, phytobiotics, and enzymes have been used, though their effectiveness remains inconsistent.

Indonesia's rich herbal resources, which include cardamom (*Amomum compactum*), offer potential as feed additives. Cardamom contains about 8% essential oil and bioactive compounds such as flavonoids, tannins, and terpenes (Abdullah *et al*., 2021). Flavonoids possess antibacterial, antioxidant, and anti-inflammatory properties that support gut and immune health (Al-Kayri *et al*., 2022). They promote lactic acid bacteria (LAB), lower intestinal pH, and inhibit pathogen growth (Abdullah *et al*., 2022). Ouerghi *et al*. (2021) reported that cardamom exhibits antibacterial activity capable of inhibiting pathogenic bacteria. However, flavonoids are heat-sensitive and prone to oxidation, leading to degradation during processing (Antony & Farid, 2022). Encapsulation technology addresses this issue by protecting active compounds from heat, oxidation, and moisture. It also preserves organoleptic properties and enhances feed palatability and the bioavailability of the active ingredients in livestock (Sugiharto & Ayasan, 2023).

The use of waste as a feed additive supports zero-waste agriculture, where utilizing waste as a source of additives helps reduce pollution. Liu *et al*. (2024) reported that antioxidants in a Chinese herbal mixture can decrease the feed conversion ratio. Yuanita *et al*. (2022) found that red dragon fruit peel flour contains bioactive compounds that can improve the growth performance and digestive health of broiler chickens. One potential by-product that remains underutilized is cardamom waste from essential oil distillation. This waste still contains active compounds with notable antioxidant activity, but is generally not used in the feed industry. Its utilization aligns with the principles of zero-waste agriculture. Cardamom waste generally retains a fragrance due to its essential oil content, which has potential as a feed additive for broiler chickens (Saeed *et al*., 2023). The total antioxidant content of carda mom waste is 8.85 ppm (IC50) based on the DPPH test (Table 3). Hafeez *et al*. (2016) compared the addition of essential oil PFA in flour form (150 mg/kg) and encapsulated form (100 mg/kg) on performance and protein digestibility and found that encapsulation at lower levels produced similar results to the flour form. Although many studies have examined cardamom or its essential oil in powder form as a feed additive, few

have specifically evaluated the effects of encapsulated cardamom waste extract on performance, gut microflora, and lymphoid organ development in broiler chickens. Furthermore, there is no comprehensive study on the effects of varying extract levels on these outcomes. The novelty of this study lies in using encapsulated cardamom waste extract at addition levels of 0.02%, 0.04%, 0.06%, and 0.08%. This study aims to examine the effects of these levels on growth performance, intestinal bacterial populations, and lymphoid organ development in broiler chickens.

MATERIALS AND METHODS

This study was conducted at the Faculty of Animal and Agricultural Science, Universitas Diponegoro, Semarang, using 200 unsexed Ross strain broiler chickens aged 8 days, with an average body weight of 215.08 ± 5.1 g/bird. The study followed ethics and animal welfare guidelines with an approval number 61-01/A-01/KEP-FPP/2025. The chickens were housed in 20 pens, each containing 10 birds. From 1–7 days of age, they were given commercial rations, and from 8–35 days, they were fed basal rations supplemented with encapsulated cardamom waste extract (ECWE). Maintenance was conducted in an open-house cage, with temperature and humidity regulated by adjusting curtain height and pen lighting. At 12 days of age, the chickens received the ND II vaccine via eye drops, with two drops per bird. Drinking water was provided *ad libitum*. The composition and nutrient content of the feed are presented in Tables 1 and 2.

A completely randomized design was used with five treatments and four replications, each replication consisting of 10 chickens. The treatments were as follows:

T0: basal rations (control)
T1: basal ration + ECWE 0.02%
T2: basal ration + ECWE 0.04%
T3: basal ration + ECWE 0.06%
T4: basal ration + ECWE 0.08%

Chemical Composition of Cardamom Waste

Antioxidant activity was analyzed using the DPPH IC_{50} method (Kozmelj *et al*., 2024). A 0.01 mM DPPH solution and cardamom waste extract were prepared, mixed, and incubated for approximately 30 minutes at room temperature. The absorbance of the mixture was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The antioxidant activity results are shown in Table 3.

Preparation of Cardamom Extract Encapsulation

Cardamom waste was extracted following the method of Gouda *et al.* (2021). The waste was dried in an oven at 50 °C and then ground into flour. The flour was dissolved in 96% ethanol (25 g/250 mL) and stirred until homogeneous. The solution was then sonicated at 37 °C with a wavelength of 50 Hz for 30 minutes, followed by incubation for 3 days. The filtrate was filtered and evaporated to remove ethanol. The drying process followed the method of Pham *et al.* (2015), in which the evaporated material was dried in an incubator at 40 °C to produce a thick extract.

Encapsulation followed the method of Agusetyaningsih *et al*. (2022). Maltodextrin was used as the coating material, dissolved in a ratio of 1:3. The dissolved maltodextrin was mixed with cardamom waste extract in a 5:1 ratio and dried using the freeze-drying method. Freeze-drying produced dry lumps of

Table 1. Composition and nutrient content of starter phase rations

Feed ingredients	Composition (%)				
	T0	T1	T2	T3	T4
Yellow corn	50.91	50.91	50.91	50.91	50.91
Rice bran	14.24	14.24	14.24	14.24	14.24
Soybean meal	24.00	24.00	24.00	24.00	24.00
Meat bone meal (MBM)	10.00	10.00	10.00	10.00	10.00
Limestone	0.30	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25	0.25
L-lysine	0.10	0.10	0.10	0.10	0.10
DL Methionine	0.20	0.20	0.20	0.20	0.20
Encapsulated cardamom waste extract (ECWE)	0	0.02	0.04	0.06	0.08
Total	100.00	100.02	100.04	100.06	100.08
Nutrient content (**)					
ME (Kcal/kg)*	3,064.35	3,063.74	3,062.51	3,062.51	3,061.90
CP (%)*	21.62	21.61	21.60	21.60	21.60
CF (%)*	4.34	4.33	4.33	4.33	4.33
CFib (%)*	4.34	4.34	4.34	4.34	4.34
Ca (%)*	1.43	1.43	1.43	1.43	1.43
P total (%)*	0.75	0.75	0.75	0.75	0.75

Note: (*) AOAC proximate analysis results; Ca and P analysis conducted at the Nutrition and Feed Science Laboratory. ME = Metabolizable energy, CP = crude protein, CF = crude fat, CFib = crude fiber, Ca = calcium, P = phosphorus. Premix composition: Ca 32.5%, P 1%, Fe 6 g, Mn 4 g, I 0.075 g, Cu 0.3 g, Zn 3.75 g, Vit B_{12} 0.5 mg, Vit D_3 50,000 IU. (**) Equalized to 100%

Table 2. Composition and nutrient content of rations in the finisher phase

Feed ingredients	Composition (%)				
	T0	T1	T2	T3	T4
Yellow corn	55.91	55.91	55.91	55.91	55.91
Rice bran	14.24	14.24	14.24	14.24	14.24
Soybean meal	19.00	19.00	19.00	19.00	19.00
Meat bone meal (MBM)	10.00	10.00	10.00	10.00	10.00
Limestone	0.30	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25	0.25
L-lysine	0.10	0.10	0.10	0.10	0.10
DL Methionine	0.20	0.20	0.20	0.20	0.20
Encapsulated cardamom waste extract (ECWE)	0	0.02	0.04	0.06	0.08
Total	100.00	100.02	100.04	100.06	100.08
Nutrient content (**)					
ME (Kcal/kg)*	3,075.19	3,075.19	3,075.19	3,075.19	3,075.19
CP (%)*	19.67	19.67	19.66	19.66	19.65
CF (%)*	4.41	4.41	4.41	4.41	4.40
CFib (%)*	4.28	4.28	4.28	4.28	4.28
Ca (%)*	1.42	1.42	1.42	1.42	1.42
P total (%)*	0.72	0.72	0.72	0.72	0.72

Note: (*) AOAC proximate analysis results; Ca and P analysis conducted at the Nutrition and Feed Science Laboratory. ME = Metabolizable energy, CP = crude protein, CF = crude fat, CFib = crude fiber, Ca = calcium, P = phosphorus. Premix composition: Ca 32.5%, P 1%, Fe 6 g, Mn 4 g, I 0.075 g, Cu 0.3 g, Zn 3.75 g, Vit B_{12} 0.5 mg, Vit D_3 50,000 IU. (**) Equalized to 100%

Table 3. Chemical composition of cardamom waste

Chemical composition	Contents
Water (%)**	25.43
Polyphenol (%)**	8.84
Tannin (%)**	1.94
Antioxidant activity (ppm)*	8.85
Saponin (ppm)**	49.31
Flavonoids (ppm)**	104.175

Note: *Testing using the IC_{50} method, **the DPPH method, and **AOAC Proximate at CV Chem-Mix Pratama.

encapsulated material, which were then ground into particles approximately 20 μm.

Sampling

Daily feed consumption and weight gain data were recorded from day 8 to day 35 of maintenance. Feed consumption was measured each morning by weighing the remaining feed (g) in each pen, using the following formula:

Feed intake (FI)=feed offered (g) - leftover feed (g) / number of birds

Body weight gain was calculated by weighing the chickens weekly in the morning, using the formula:

Average daily gain (ADG)=Final weight (g) - Initial weight (g) /Time (days)

On day 31, total excreta collection was conducted to measure crude protein digestibility. One chicken per replication, with body weight close to the group average (20 samples in total), was selected and placed in a battery cage coded according to the treatment. Excreta collection lasted 4 days, beginning with 24 hours of fasting, during which chickens had ad libitum access to water (Ruvini *et al.*, 2021). The feed was supplemented with Fe_2O_3 as a digestibility marker. During collection, excreta were sprayed with 0.2 N HCl to bind nitrogen (Kikusato & Namai, 2025), then cleaned of feathers and other debris. The excreta were weighed, sun-dried under a net to prevent contamination, and reweighed before being ground. A 5 g sample of dry excreta was placed into a coded plastic container for laboratory analysis. Protein digestibility was calculated using the formula:

Protein digestibility (%) = (Protein consumption (g) - Protein in excreta(%)) /Protein consumption (g) × 100

On day 35, chickens from each pen were randomly selected for hematology sampling. Blood was taken from the wing vein and collected in an ethylenediaminetetraacetic acid (EDTA) tube. The remaining blood was collected in a tube without an anticoagulant and allowed to clot for 2 hours at room temperature. After centrifugation at 2000 rpm for 15 minutes, serum was obtained and stored at -20°C until biochemical analysis. The chickens from which blood was taken were then weighed and slaughtered. The carcasses were separated into feathers, skin, head, feet, and internal organs before being weighed. Lymphoid organs, including the thymus, bursa of Fabricius, and spleen, were weighed using an analytical balance, and relative organ weight was calculated as follows:

Relative organ weight (%) = (Lymphoid organ weight (g))/live weight (g) × 100

Digesta from the ileum and cecum were collected aseptically into coded tubes for microbiological analysis, and pH was measured using an electric pH meter. The number of microbes in the intestinal digesta was determined following the method of Yudiarti *et al.* (2024). Total bacteria were counted on potato dextrose

agar (PDA) after aerobic incubation at 38 °C for 24 hours. Coliform bacteria were counted on MacConkey agar after aerobic incubation at 38 °C for 24 hours. Lactic acid bacteria were counted on de Man Rogosa Sharpe (MRS) agar after anaerobic incubation at 38 °C for 48 hours.

The number of leukocytes was determined using the Bürker chamber method (Ciborowska *et al.*, 2024). Differential leukocyte counts were obtained by examining smear preparations under a light microscope with an immersion lens. The coverslip was used to prepare blood smears. The heterophil-to-lymphocyte (H/L) ratio was calculated by dividing the number of heterophils by the number of lymphocytes (Madej *et al.*, 2024).

Statistical Analysis

Data were analyzed using a completely randomized design (ANOVA) with SPSS Version 29. Significant differences among treatments were further tested using Duncan's multiple range test.

RESULTS

Data on the effects of ECWE on broiler performance are presented in Table 4. ECWE at 0.08% significantly increased feed intake, protein digestibility, and average daily gain, while reducing feed conversion ratio (FCR) compared with ECWE at 0.02%, 0.04%, and 0.06% ($p<0.05$).

The effects of ECWE on bacterial populations and intestinal pH are shown in Table 5. Supplementation significantly increased LAB counts and reduced coliform counts and intestinal pH ($p<0.05$). As presented in Table 6, ECWE also significantly increased the relative weights of lymphoid organs, including the bursa of Fabricius, spleen, and thymus ($p<0.05$). However, supplementation had no significant effect on the H/L ratio ($p>0.05$).

DISCUSSION

Feed consumption increased with higher ECWE levels. This effect may be attributed to bioactive compounds in cardamom extract, such as flavonoids and essential oils, which improve the performance of digestive organs in broiler chickens and stimulate the release of ghrelin, a hormone that increases appetite. The administration level also influences flavonoid content; higher supplementation results in higher flavonoid levels. Arista *et al.* (2023) reported that essential oils contain sylvestrene, α-pinene, and β-pinene, which can improve palatability, stimulate appetite, and enhance enzyme activity. According to Kikusato (2021), phytobiotics can improve the feed status and feed consumption.

Table 4. Performance of broiler chickens in the finisher phase

Variables	Treatments					SEM	p-value
	T0	T1	T2	T3	T4		
Consumption of rations (g/bird/day)	93.53 ^{b}	97.21 ^{b}	101.19 ^{a}	104.98 ^{a}	102.78 ^{a}	1.064	<0.05
Protein digestibility (%)	75.66 ^{d}	80.29 ^{c}	81.48 ^{bc}	83.77 ^{ab}	85.53 ^{a}	0.876	<0.05
ADG (g/bird/day)	53.27 ^{d}	55.59 ^{c}	57.89 ^{b}	61.27 ^{a}	62.25 ^{a}	0.808	<0.05
FCR	1.76 ^{b}	1.75 ^{b}	1.75 ^{b}	1.72 ^{b}	1.65 ^{a}	0.012	<0.05
Final Weight (g/bird/day)	1,700.63^{d}	1,769.05^{c}	1,837.10 ^{b}	1,930.68^{a}	1,964.15^{a}	23.46	<0.01

Note: Means in the same row with different superscripts differ significantly ($p<0.05$) or ($p<0.05$). ADG= average daily gain; FCR= feed conversion ratio; SEM= Standard error of the mean. T0= control, T1= basal ration + ECWE 0.02%, T2= basal ration + ECWE 0.04%, T3= basal ration + ECWE 0.06%, T4= basal ration + ECWE 0.08%.

Table 5. Bacterial populations and intestinal pH of finisher phase broiler chickens

Variables	Treatments					SEM	p-value
	T0	T1	T2	T3	T4		
LAB (Log cfu/g)	7.17 ^{c}	7.20 ^{c}	7.26 ^{b}	7.33 ^{a}	7.34 ^{a}	0.016	<0.05
Coliform (Log cfu/g)	2.70 ^{c}	2.64 ^{bc}	2.61 ^{b}	2.56 ^{ab}	2.54 ^{a}	0.016	<0.05
Intestinal pH	6.45 ^{b}	6.38 ^{b}	6.14 ^{ab}	6.10 ^{ab}	6.03 ^{a}	0.047	<0.05

Note: Means in the same row with different superscripts differ significantly ($p<0.05$). LAB= lactic acid bacteria; SEM= Standard error of the mean. T0= control, T1= basal ration + ECWE 0.02%, T2= basal ration + ECWE 0.04%, T3= basal ration + ECWE 0.06%, T4= basal ration + ECWE 0.08%.

Table 6. Relative weight of lymphoid organs and H/L ratio of finisher phase broiler chickens

Variables	Treatments					SEM	p-value
	T0	T1	T2	T3	T4		
Thymus weight (%)	0.42 ^{b}	0.50 ^{a}	0.52 ^{a}	0.54 ^{a}	0.55 ^{a}	0.014	<0.05
Spleen weight (%)	0.18 ^{b}	0.24 ^{a}	0.23 ^{a}	0.24 ^{a}	0.25 ^{a}	0.008	<0.05
Bursa of Fabricius weight (%)	0.12 ^{b}	0.20 ^{a}	0.21 ^{a}	0.22 ^{a}	0.23 ^{a}	0.009	<0.05
H/L ratio (%)	0.83	0.73	0.74	0.78	0.76	0.023	>0.05

Note: Means in the same row with different superscripts differ significantly ($p<0.05$). H/L= heterophil-to-lymphocyte ratio; SEM= Standard error of the mean. T0= control, T1= basal ration + ECWE 0.02%, T2= basal ration + ECWE 0.04%, T3= basal ration + ECWE 0.06%, T4= basal ration + ECWE 0.08%.

Flavonoid compounds act as antibacterials by damaging bacterial cell membranes and inhibiting peptidoglycan synthesis, preventing complete formation of the cell wall in pathogenic bacteria and leading to cell death. The reduction in pathogenic bacteria allows LAB to grow and produce lactic acid and short-chain fatty acids in the small intestine as protective agents. Bedford and Juha (2021) noted that increasing LAB populations can enhance intestinal nutrient absorption, improve digestion, and increase feed consumption.

Daily weight gain increased with higher ECWE levels, likely due to flavonoid compounds that enhance digestion, thereby increasing ADG. Consistent with these results, Dosu *et al.* (2023) noted that ginger root extract supplementation increased growth performance, while Rafeeq *et al.* (2023) indicated that herbal concoctions also improved broiler weight gain. Flavonoids improve digestion by stimulating the production of digestive enzymes. Increased enzyme production enhances digestive efficiency, allowing nutrients such as protein to be absorbed effectively, which supports optimal growth. The antibacterial properties of flavonoids help maintain a balanced intestinal microbiota and suppress nutrient competition from pathogenic bacteria, enabling more efficient nutrient absorption. Similarly, El-Saadony *et al.* (2023) reported that plants containing active compounds act as antimicrobials and stimulate the digestive system, improving nutrient absorption. These findings align with the observed increase in protein digestibility as feed consumption rises. Maintaining bacterial balance in the intestine supports the health and function of digestive organs, allowing optimal nutrient absorption. This ensures that more protein from the consumed ration is digested and utilized for muscle formation. Sugiharto (2023) reported that fruit peels contain compounds with antibacterial, antioxidant, and immunostimulant activity that can improve intestinal microflora and broiler chicken performance.

FCR decreased with increasing ECWE supplementation. The values in this study were lower than those reported by Mariadi *et al.* (2025), who found that the addition of herbal extract emulsions (HEE) in broiler rations produces an FCR value of around 1.55-1.60. FCR reflects the efficiency of feed utilization; according to Szőllősi *et al.* (2021), higher FCR values indicate less economical feed use.

The addition of ECWE to the ration increased feed consumption and supported digestive organ function, enhancing nutrient absorption. As a result, protein digestibility and average daily gain (ADG) improved, leading to lower FCR. Nuryati (2019) reported that other factors influencing FCR include feed form, temperature, environmental conditions, feed intake, body weight, and the adequacy of feed energy and nutrient content. The favorable FCR values in this study are reflected in the final body weights, which ranged from 1700.63 to 1964.15 g. Moreover, ECWE supplementation at 0.06% and 0.08% produced similar final body weights.

Bacteria and Intestinal pH

The addition of 0.08% ECWE (T4) to the ration significantly ($p<0.05$) improved small intestine health. In this treatment, the LAB population increased to 7.34 log cfu/g. This increase was influenced by active compounds in cardamom extract, particularly phenolic compounds. According to Shehata *et al*. (2022), flavonoids are phenolic compounds that act as natural prebiotics metabolized by beneficial microbiota such as LAB. Flavonoids provide fermentation substrates for LAB and stimulate the expression of enzymes that accelerate the metabolism of simple carbohydrates into organic acids, including lactic and acetic acids.

Soundararajan *et al*. (2023) reported that flavonoid compounds in Moringa (*Moringa oleifera*) leaf meal act as modulators that support the growth of beneficial chicken gut microbiota and facilitate colonization. Lactic acid bacteria ferment glucose into lactic acid via the glycolysis pathway, lowering pH in the intestinal lumen. This acidic environment not only benefits LAB growth but also inhibits pathogenic bacteria such as *E. coli* and *Salmonella*. In this study, intestinal pH ranged from 6.03 to 6.45, lower than the 6.4–6.7 range reported by Sunu *et al*. (2019), in which prebiotic activity from garlic reduced pH.

Cardamom essential oils, including cineole, terpineol, and α/β-pinene, possess antibacterial properties but tend to be selective against pathogenic bacteria. These compounds damage gram-negative bacterial cell membranes by disrupting permeability and causing leakage of cell contents such as ions, ATP, and nucleic acids. This selectivity gives LAB a competitive advantage in dominating gut microbiota. Acidic pH changes in the intestine further promote the growth of *Lactobacillus* spp. and *Bifidobacteria*, suppressing pathogenic bacteria. Encapsulation technology protects flavonoids and essential oils from degradation during gastric digestion. The maltodextrin coating in the encapsulated particles allows gradual release in the small intestine, where LAB colonization occurs.

H/L ratio

The H/L ratio is an indicator of stress levels in poultry; a higher ratio reflects a greater stress level. According to Jumadin *et al.* (2020), factors influencing the H/L ratio include biological activity, environmental conditions, age, and feed. The normal H/L ratio is approximately 0.3–0.5. In this study, the ratio ranged from 0.73 to 0.83. The H/L ratio can also indicate the poultry immune system, with values of around 0.2 considered low, 0.5 normal, and 0.8 high.

The inclusion of ECWE in rations did not reduce stress levels in broiler chickens. Environmental conditions may have contributed to stress in the flock. According to Wang *et al.* (2023), heterophils are part of the nonspecific immune response, functioning to limit pathogen spread before activation of the specific immune response, which involves lymphocytes.

Relative Weight of Lymphoid Organs

The effects of encapsulated cardamom waste extract (ECWE) on the relative weights of lymphoid organs are presented in Table 6. The treatment significantly ($p<0.05$) increased the relative weights of the bursa of Fabricius, spleen, and thymus. The addition of ECWE to the ration resulted in a thymus relative weight of approximately 0.52%–0.55%, higher than the 0.33% average reported by Akbar *et al*. (2022). The thymus, located in the neck, plays a key role in the maturation of T lymphocytes. Factors affecting thymus size include lymphocyte production and protein intake. In this study, protein digestibility was high (75.66%–83.77%), indicating efficient amino acid absorption for immune cell formation.

Moniei *et al*. (2024) reported that the addition of turmeric flour to feed positively affected intestinal microflora, thereby improving thymus gland function. Cardamom's main bioactive compounds—flavonoids, terpenoids, and essential oils—possess immunomodulatory activity. According to Gharechopogh *et al*. (2021), antioxidants in medical plants and prebiotics can enhance thymus function in antibody production. However, flavonoids are heat- and oxidation-labile, making them susceptible to degradation before reaching the intestine. Encapsulation technology protects these compounds during digestion in the proventriculus and gizzard, allowing controlled release in the small intestine for optimal absorption.

Flavonoids can donate hydrogen atoms from hydroxyl groups (-OH) to neutralize free radicals, preventing lipid peroxidation of immune cell membranes in lymphoid organs. This antioxidant protection reduces lymphocyte apoptosis caused by oxidative stress, leading to an increased immune cell population.

The average relative weight of the spleen in this study ranged from 0.18 to 0.25%. Ampode and Federico *et al*. (2022) reported that adding 5% oregano powder (OP) significantly increased lymphoid organ weight by about 3%. The relative spleen weight observed here may be attributed to the activity of cardamom bioactive compounds and the protective effect of encapsulation, which maintains their availability in the digestive tract, thereby supporting lymphoid tissue function and development. Encapsulation preserves the integrity of the flavonoid chemical structure until it reaches the small intestine and ensures gradual release, allowing the concentration of absorbed active substances to remain stable and continuously stimulate the immune system.

The spleen is a lymphoid organ responsive to antigen stimulation and plays a role in lymphocyte formation for antibody production. According to Akbar *et al*. (2022), flavonoids in cardamom neutralize free radicals (ROS) by donating hydrogen atoms from hydroxyl groups, preventing lipid peroxidation in lymphocyte membranes. This process reduces lymphocyte apoptosis caused by oxidative stress, thereby increasing immune cell numbers in the lymph and contributing to greater organ mass.

Khukhodziinai *et al*. (2024) further noted that an increase in LAB in the intestine, promoted by flavonoids and essential oils, lowers intestinal pH, inhibits pathogenic bacteria, and improves amino acid absorption efficiency. Essential amino acids such as lysine and methionine are utilized in the synthesis of immunoglobulins and lymphocyte structural proteins, contributing to lymphocyte growth.

The bursa of Fabricius is a round-shaped lymphoid organ located between the cloaca and sacrum that plays a role in capturing antigens and forming antibodies (Wu *et al*. 2021). In this study, increasing ECWE supplementation raised the relative weight of the bursa of Fabricius to approximately 0.12%–0.23%. Morais *et al*. (2023) also found that feeding garlic flour significantly increased bursa weight.

Cardamom essential oils, including cineole, terpineol, and α-pinene, are selectively antibacterial, inhibiting pathogenic bacteria without affecting beneficial bacteria by disrupting the phospholipid layer of pathogenic bacterial membranes. ECWE reduces intestinal infection burden and decreases the need for nonspecific immune system activation, allowing energy and nutrients to be directed toward the development of primary immune organs, including the bursa of Fabricius. Flavonoids further enhance the expression of immunostimulatory cytokines that stimulate B-cell proliferation and differentiation, leading to increased lymphoid follicles in the bursa and, consequently, greater organ weight and size.

CONCLUSION

Supplementing broiler diets with 0.08% encapsulated cardamom waste extract significantly improved gut health and performance, indicating its potential as an effective phytobiotic feed additive.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to the material presented in this manuscript.

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

During the preparation of this work, the author(s) used generative AI and AI-Assisted technologies in order to refine the language and organizational structure of the text. After using this tool/service, all material in the manuscript has been carefully reviewed and edited, ensuring it is consistent with the author's understanding and take full responsibility for the content of the publication.

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