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Effects of encapsulated cardamom distillation waste extract and *Lactobacillus plantarum* on intestinal profile, protein digestibility, and growth of broiler

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ABSTRACT

This research assessed the impact of incorporating encapsulated cardamom distillation waste extract and *Lactobacillus plantarum* (ECDWE-Lp) on small intestine profile, ileal protein digestibility, and broiler chicken growth performance. The study used 200 unsexed day-old Ross 308 broiler chickens in a fully randomized design with four treatments and five repetitions, each comprising 10 birds. Treatments were T0: basal diet (BD), T1: BD+0.3% ECDWE-Lp, T2: BD+0.6% ECDWE-Lp, and T3: BD+0.9% ECDWE-Lp. Parameters assessed included broiler performance, small intestine profile consisting of lactic acid bacteria (LAB) and coliform counts, intestinal pH, intestinal histomorphology (villus height (VH), crypt depth (CD), VH/CD ratio), relative intestinal segment length and weight, and ileal crude protein digestibility. Data analysis used variance analysis and Duncan's Multiple Range Test at 5% significance. Results showed ECDWE-Lp significantly affected ($P<0.05$) daily weight gain, feed intake, feed conversion ratio, LAB and coliform totals (jejunum and ileum), intestinal pH, VH and CD (jejunum and ileum), jejunal VH/CD ratio, intestinal segment measurements, and ileal crude protein digestibility. The treatment did not significantly impact ($P>0.05$) duodenal LAB and coliform counts, duodenal VH and CD, or VH/CD ratio in duodenum and ileum. Adding 0.6% ECDWE-Lp improved small intestinal profile, ileal crude protein digestibility, and broiler performance.

Introduction

Development of the broiler chicken industry in Indonesia has become one of the main sources of animal protein for the community. The Indonesian Ministry of Agriculture noted that in 2024, national broiler meat production reached 3.84 million tons, with a demand reaching 3.72 million tons. This indicates that broiler meat has become a popular source of animal protein in the community. The high demand for broiler meat has encourages poultry farmers to maximize productivity through the feed provided. Feed accounts for up to 70% of total production costs, making it a challenge for farmers to improve feed efficiency (Makkar, 2018). Efforts to improve feed efficiency include formulating feed according to nutrient requirements, and using natural feed additives such as combining encapsulated phytobiotics form cardamom distillation waste extract with probiotic *Lactobacillus plantarum* to enhance broiler production performance.

Phytobiotics are active plant compounds that act as antibacterial and antioxidant agents, thereby improving digestive tract conditions, enhancing health, and promoting nutrient absorption (Urban *et al*., 2024). Cardamom (*Amomum compactum* Soland Ex Maton) is a spice plant belonging to the Zingiberaceae family, with production in Indonesia reaching 127.926 tons by 2024 (BPS, 2024). Cardamom fruits are typically extracted for their essential oil as a herbal product, leaving behind waste that is not widely utilized. Cardamom fruit waste has the potential to be utilized as a natural feed additive because of the presence of various active compounds that are believed to remain in it. Cardamom fruit contains flavonoids such as quercetin, kaempferol, and rutin, as well as essential oil compounds such as 1,8-cineole, α-terpinyl acetate, sabinene, 4-terpinen-4-ol, and myrcene (Masoumi-Ardakani *et al*., 2016).

Probiotics are live microorganisms, including bacteria, molds, and yeasts, which play a beneficial role in maintaining digestive tract health and improving nutrient digestibility. *Lactobacillus plantarum* is a probiotic that suppresses the growth of pathogenic bacteria in the small intestine. This bacterium has several physiological, biochemical and genetic properties, and is also capable of synthesizing the family of antibacterial compounds such as peptides, extracellular polysaccharides, secondary metabolites and organic acids (Sebouai *et al*., 2024). *Lactobacillus plantarum* falls under lactic acid bacteria (LAB) which lower the pH of the intestines, inhibit the growth of pathogens and trigger digestive enzymes (Afro *et al*., 2023).

A problem with bioactive substances is that they can degrade when exposed to high temperatures, rendering them less effective. Another possibility is that the more acidic nature of the gastrointestinal tract also causes the bio-actives to be unstable before they reach the organ, which could reduce the potency of these molecules. Utilization of encapsulation technology is a solution to reduce the decrease of the bioactivity stability. Encapsulation is technique of enrobing the active pharmaceutical ingredient with protective surface. The encapsulation procedure not only safeguards the chemical but also facilitates the regulated release of bioactive compounds, particularly inside the small intestine (Laureanti *et al*., 2023). Maltodextrin was used as the encapsulating agent in this work because to its low viscosity at elevated concentrations, excellent stability, high solubility, and comparatively inexpensive cost (Widjastuti *et al*., 2023).

Amylase in the small intestine breaks down the encapsulating material, which lets the bioactive substances in the extract out and have positive benefits on gut health. Along with *Lactobacillus plantarum*, the active chemicals make short chain fatty acids (SCFA), which may lower the pH level in the digestive system.

This acidic condition suppresses the proliferation of harmful bacteria while promoting the growth of lactic acid bacteria (LAB). The role of each section of the small intestine (duodenum, jejunum, and ileum) may lead to an increase in villus height, which is positively associated with a rise in intestinal length and weight, thus improving nutrient absorption and enhancing broiler performance.

To the best of our knowledge, utilization of combination encapsulated cardamom distillation waste extract and *Lactobacillus plantarum* study in broilers have not been reported. This research sought to scientifically assess the impact of incorporating encapsulated cardamom distillation

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waste extract and *Lactobacillus plantarum* into diets on the small intestine profile, protein digestibility, and growth performance of broilers.

Materials and methods

Materials and experimental design

The study took place between October and November 2024 at the Poultry Cage, a teaching farm affiliated with the Faculty of Animal and Agricultural Sciences at Universitas Diponegoro in Semarang. The study utilized 200 Ross 308 strain broilers that were not separated by sex. The treatments were initiated at 8 days of age with an average body weight of 217.22±5.58 g. Encapsulated cardamom distillation waste extract and *Lactobacillus plantarum* (ECDWE-Lp) was used as feed additives. In the initial phase, commercial feed (B-11S) was supplied, and the base diets were composed of yellow maize, rice bran, soybean meal, fish meal, limestone, premix, L-lysine, and DL-methionine, as detailed in Table 1. The research employed a completely randomized design featuring 4 treatments and 5 replicates, culminating in 20 experimental units, each containing 10 birds. The treatments were as follows: T0, which served as the control with the basal diet; T1, which included the basal diet plus 0.3% ECDWE-Lp; T2, which consisted of the basal diet plus 0.6% ECDWE-Lp; and T3, which involved the basal diet plus 0.9% ECDWE-Lp.

Table 1. Composition and nutrient content of basal diet.

Feed Stuff	Composition (%)	
	Starter (8 – 21 days old)	*Finisher* (22 – 35 days old)
Yellow Maize	51.35	52.41
Rice Bran	13.8	17.74
Soybean Meal	24	19
Fish Meal	10	10
Limestone	0.3	0.3
Premix	0.25	0.25
L-Lysine	0.1	0.1
DL-Methionine	0.2	0.2
Total	100	100
Nutrient Content		
Metabolic Energy (kcal/kg)[1)]	2.986.78	3.035.25
Crude Protein (%)[2)]	21.77	19.82
Crude Fat (%)[2)]	4.2	4.78
Crude Fiber (%)[2)]	4.52	4.66
Ca (%)[2)]	1.09	1.12
P (%)[2)]	0.75	0.74
Lysine (%)[3)]	1.19	1.07
Methionine (%)[3)]	0.48	0.41

1) Based on the Bolton Formula = 40.81 [0.87(CP+2.25xCF+BETN)+2.5] (Bolton, 1967).
2) Result of analysis of Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang (2024).
3) Amino acids are calculated based on the trial and error method.

Preparation of encapsulated cardamom distillation waste extract and Lactobacillus plantarum

The *Lactobacillus plantarum* used in the investigation was obtained from commercial sources. For encapsulation of the extract that is derived from cardamom distillation waste an extraction method performed by Gouda *et al*. (2021). Dehydration Cardamom (*Amomum compactum* Soland Ex Maton) distillation waste was dried completely at 50°C. It was then pulverized into fine powder. We stirred this powdered garbage in 96% ethanol (1:10 w/v) until it was all one. Then the solution was sonicated for 60 min at 37°C and 50 Hz and room temperature. After being sonicated, the mixture was allowed to stand for 24 h and filtered. Instead of ethanol, the filtrate was evaporated, and it yielded a concentrated extract.

The subsequent step was encapsulation performed using the methods described by Agusetyaningsih *et al*. (2022). The first step involved preparing a coating composition by mixing the maltodextrin with distilled water (1:3 (w/v)). Cardamom distillation waste extract to *Lactobacillus plantarum* ratio was 1:1 (v/v). At the beginning, *Lactobacillus plantarum* were reconstituted in skim milk and distilled water (1:2 w/v). It was then mixed with the extract in a proportion of 1:2 (v/v). The second concentration was mixed at 1:5 (v/v) with the coating solution and was freeze-dried with a drying temperature of -50°C until a dry encapsulated product was formed.

Maintenance and management

Rearing was done from day-old chicks (DOC) to 7 days using 4 units of cage (50 chicks/cage unit) and 8-35 days using 20 units of cages (10 birds/unit). The lighting provided during DOC until 4 days old was 23-24 hours, 5-7 days old 18 hours, 8-22 days old for 19 hours and 23-35 days old for 23 hours. Light intensity was gradually reduced from day 7 from 20 lux to 10 lux. The temperature in the cage at the age of 1-7 days was 30-32oC and at the age 8-35 days was 23-29oC with a humidity of 50-70%. The diet used from 1 to 7 days of age was the commercial diet B-11S. A basal starter diet was introduced on day 8-21 and a basal finisher diet was provided on day 22-35, both in combination with the addition of ECDWE-Lp.

Sampling and analyses

The parameters measured included broiler performance, lactic acid bacteria (LAB) total, coliform total, intestinal pH, histomorphology of intestinal segments (villus height (VH), crypt depth (CD), and VH/CD ratio), relative length and weight of intestinal segments, and ileal crude protein digestibility.

Broiler performance was assessed by examining daily feed intake (DFI), daily weight gain (DWG), and the feed conversion ratio (FCR). To calculate daily weight gain, the initial body weight at the beginning of the treatment (day 8) was subtracted from the final body weight at the conclusion of the treatment (day 35), and the difference was divided by 28 days. The FCR was obtained by dividing the daily feed intake by the daily weight gain.

The Total Plate Count (TPC) method was employed to measure the total counts of LAB and coliforms. The populations of LAB and coliforms were calculated using the formula provided by Fardiaz (1993):

Total LAB or coliform (cfu/g)= Number of colonies x 1 /Dilution Factor

The pH levels in the small intestine sections, namely the duodenum, jejunum, and ileum, were assessed using a digital pH meter (Eco Test pH 1) with a precision of 0.01. This measurement involved inserting the pH meter into the digesta until a consistent pH reading was achieved.

Histomorphological data from the small intestine segments by taking 2 cm samples from each segment and putting them in a pot jar with a 10% formalin buffer solution. We employed hematoxylin and eosin (HE) staining to get the tissue ready. The light microscope at 100× magnification revealed villi and crypts. The observations were recorded using the AmScope digital microscope camera and software. The Miconos Image Raster 3 software was used to find out how deep the crypts and how high the villi were (Samanya and Yamauchi, 2001). We measured the height of the villi from the tip to the base and the depth of the crypt from the base of the villi to the base of the crypt. We used the formula from Nguyen *et al*. (2021) to get the VH/CD ratio:

VH/CD ratio= Villus height (µm)/Crypt depth (µm)

The duodenum, jejunum, and ileum were separated from each other so that we could measure the length and weight of the small intestine. We used a measuring tape to figure out how long each piece was. Weigh-

ing the cleansed segments on an analytical scale that could measure to within 0.001 g came after cleaning away the digesta from each part. We utilized Chen *et al.* (2021) formula to get the relative length and Assis *et al.* (2021) formula to obtain the relative weight, as shown below:

Relative length= Intestinal length (cm)/Live weight (kg)

Relative weight= Intestinal weight (g) /Live weight (g) x100%

We employed the ileal approach to see how efficiently crude protein could be digested, using lignin as a marker. We utilized Huang *et al.* (2006) approach to find out how digestible ileal crude protein (ICPD) is:

ICPD = (Protein diet/Lignin diet) - (Protein digesta/Lignin digesta) /(Protein diet/Lignin diet) x 100%

Statistical analysis

The data from the study was tested for normality and homogeneity test first until they meet the ANOVA assumptions. The data underwent analysis through analysis of variance (ANOVA) with a significance level set at 5%. When a significant effect was identified, Duncan's Multiple Range Test was employed at the same significance level to assess differences among the treatments (Steel and Torrie, 1960).

Results

Growth performance of broiler

Table 2 displays the Daily Feed Intake (DFI), Daily Weight Gain (DWG), and Feed Conversion Ratio (FCR). The variance analysis indicated that the treatment significantly influenced ($P<0.05$) the daily feed intake, daily weight gain, and feed conversion ratio. Incorporating encapsulated cardamom distillation waste extract and *Lactobacillus plantarum* in T1, T2, and T3 led to a notably higher DFI (days 7–35) compared to T0. This finding aligns with the DWG results, where T2 and T3 exhibited significantly

Table 2. Broiler performance.

Variables	Treatment				SEM	p-value
	T0	T1	T2	T3		
DWG (g)						
Week 1 (7-14)	15.13^{c}	18.89^{b}	21.41^{a}	22.45^{a}	0.75	0
Week 2 (14-21)	70.91^{b}	72.15^{b}	75.24^{ab}	80.15^{a}	1.11	0.01
Week 3 (21-28)	68.43	67.58	72.11	71.72	1.38	0.59
Week 4 (28-35)	74.15	79.56	80.39	75.46	1.14	0.14
DWG total (28 days)	57.09^{c}	59.55^{b}	62.29^{a}	62.44^{a}	0.55	0
DFI (g)						
Week 1 (7-14)	34.40^{c}	40.18^{b}	42.13^{ab}	43.86^{a}	0.96	0
Week 2 (14-21)	73.06^{b}	81.06^{a}	85.52^{a}	86.77^{a}	1.53	0.00
Week 3 (21-28)	123.91	128.11	126.63	127.4	0.97	0.47
Week 4 (28-35)	147.48	147.73	144.17	142.13	1.31	0.39
DFI total (28 days)	94.71^{b}	99.27^{a}	99.61^{a}	100.04^{a}	0.8	0.05
FCR						
Week 1 (7-14)	2.32^{a}	2.13^{ab}	1.98^{b}	1.96^{b}	0.46	0.01
Week 2 (14-21)	1.03^{b}	1.12^{ab}	1.14^{a}	1.08^{ab}	0.17	0.13
Week 3 (21-28)	1.82	1.9	1.78	1.78	0.32	0.55
Week 4 (28-35)	2	1.86	1.8	1.89	0.35	0.25
FCR total (28 days)	1.66^{a}	1.67^{a}	1.60^{b}	1.60^{b}	0.01	0.02

abc Different superscripts in the same row indicate significant differences ($P<0.05$). T0: (basal diet as control); T1: (basal diet + 0.3% ECDWE-Lp); T2: (basal diet + 0.6% ECDWE-Lp); T3 (basal diet + 0.9% ECDWE-Lp). DWG: Daily Weight Gain. DFI: Daily Feed Intake. FCR: Feed Conversion Ratio. SEM: standard error of the mean.

Table 3. Bacterial population and pH of intestinal segments.

Variables	Treatment				SEM	p-value
	T0	T1	T2	T3		
LAB (log cfu/ml)						
Duodenum	9.4	9.31	9.58	9.59	0.64	0.32
Jejunum	8.70^{b}	9.14^{ab}	9.65^{a}	9.80^{a}	0.16	0.05
Ileum	8.15^{b}	8.21^{ab}	9.01^{a}	9.01^{a}	0.15	0.04
Coliform (log cfu/ml)						
Duodenum	2.64	2.6	2.61	2.59	0.01	0.40
Jejunum	3.25^{a}	2.86^{b}	2.76^{b}	2.76^{b}	0.06	0.00
Ileum	3.33^{a}	2.93^{b}	2.70^{b}	2.81^{b}	0.08	0.01
Intestinal pH						
Duodenum	5.93^{a}	5.66^{b}	5.65^{b}	5.48^{c}	0.04	0
Jejunum	6.81^{a}	6.66^{ab}	6.52^{b}	6.52^{b}	0.04	0.00
Ileum	6.94^{a}	6.87^{ab}	6.72^{bc}	6.69^{c}	0.03	0.02

abc Different superscripts in the same row indicate significant differences ($P<0.05$). T0: (basal diet as control); T1: (basal diet + 0.3% ECDWE-Lp); T2: (basal diet + 0.6% ECDWE-Lp); T3 (basal diet + 0.9% ECDWE-Lp). LAB: Lactic acid bacteria. SEM: standard error of the mean.

greater (P<0.05) DWG than T0 and T1. The FCR values for T2 and T3 were significantly lower (P<0.05) than those for T0 and T1.

Bacteria populations and pH of intestinal segment

Table 3 displays the data on lactic acid bacteria (LAB), pH, and coliforms for each section of the small intestine, namely the duodenum, jejunum, and ileum. The analysis of variance results indicated that the treatment significantly influenced (P<0.05) the total LAB and coliforms in the jejunum and ileum, as well as the pH levels in the duodenum, jejunum, and ileum. However, the treatment did not significantly affect (P>0.05) the total LAB and coliforms in the duodenum. Total LAB and coliforms in the duodenum showed similar results across treatments; however, T3 had asignificantly lower pH response (P<0.05) than T0, T1, and T2. Treatments T2 and T3 in the jejunum significantly increased total LAB (P<0.05) compared to T0, and reduced pH and total coliforms compared to T0. The ileum segment showed that total LAB, pH, and coliforms treatments T2 and T3 produced the same response as the jejunum, with significantly (P<0.05) increased total LAB, reduced pH, and coliforms compared to T0.

Histomorphology of intestinal segments

Table 4 displays the measurements of villus height, crypt depth, and the villus height to crypt depth ratio (VH/CD ratio) for each section of the small intestine, including the duodenum, jejunum, and ileum. The analysis of variance demonstrated that treatment significantly influenced (P<0.05) villus height and crypt depth in the jejunum and ileum, as well as the VH/CD ratio in the jejunum. Conversely, no significant impact (P>0.05) was found on VH and CD in the duodenum, nor on the VH/CD ratio in duodenum and ileum. In the jejunum, the villus height, crypt depth, and VH/CD ratio in T2 and T3 were notably higher compared to T0. The VH/CD ratio of the ileum was also not affected (P>0.05), although combine T2 and T3 they improved VH and CD compared to T0.

Relative length and weight of intestinal segment

Table 5 displays the comparative lengths and weights of each section of the small intestine. The analysis of variance results revealed that the treatment significantly influenced (P<0.05) the relative length and weight of the duodenum, jejunum, and ileum. In the duodenum, treatments T2 and T3 led to a notably greater (P<0.05) relative length and weight compared to T0. For the jejunum, segments indicated that T1, T2, and T3 significantly (P<0.05) increased in relative length and weight when compared to T0. Similarly, in the ileum, T2 and T3 demonstrated a higher relative length and weight response (P<0.05) than T0.

Ileal Crude Protein Digestibility

Table 6 displays the data on ileal protein digestibility. The variance analysis indicated that the treatments substantially affected (P<0.05) the ileal crude protein digestibility in broilers. Adding T3 did not have a significant (P>0.05) impact on T2, however it did considerably (P<0.05) enhance protein digestibility when compared to T0 and T1.

Table 4. Histomorphology of intestinal segments.

Variables	Treatment				SEM	p-value
	T0	T1	T2	T3		
Villus height (µm)						
Duodenum	1341.02	1370.43	1385.26	1385.96	6.83	0.05
Jejunum	937.49^{c}	1354.48^{b}	1563.86^{a}	1577.49^{a}	60.49	0
Ileum	432.06^{b}	484.20^{b}	458.81^{b}	629.89^{a}	19.34	0
Crypt depth (µm)						
Duodenum	254.67	251.01	254.93	252.44	6.46	0.10
Jejunum	217.33^{c}	259.00^{b}	277.31^{b}	324.31^{a}	10.56	0
Ileum	153.68^{c}	158.22^{bc}	165.73^{b}	196.56^{a}	4.18	0
VH/CD ratio						
Duodenum	5.41	5.48	5.48	5.53	0.13	0.99
Jejunum	4.32^{b}	5.26^{a}	5.68^{a}	4.97^{ab}	0.17	0.02
Ileum	2.81	3.07	2.77	3.21	0.07	0.06

^{abc}Different superscripts in the same row indicate significant differences (P<0.05). T0: (basal diet as control); T1: (basal diet + 0.3% ECDWE-Lp); T2: (basal diet + 0.6% ECDWE-Lp); T3 (basal diet + 0.9% ECDWE-Lp). VH: villus hight. CD: crypt depth. SEM: standard error of the mean.

Table 5. Relative length and weight of intestinal segments.

Variables	Treatment				SEM	p-value
	T0	T1	T2	T3		
Relative length (cm/kg)						
Duodenum	13.50^{b}	14.58^{ab}	15.09^{a}	15.10^{a}	0.23	0.04
Jejunum	36.23^{c}	40.49^{b}	41.22^{b}	42.63^{a}	0.58	0
Ileum	36.44^{b}	38.41^{b}	42.97^{a}	43.14^{a}	0.74	0
Relative weight (%)						
Duodenum	0.46^{b}	0.53^{a}	0.53^{a}	0.56^{a}	0.01	0.02
Jejunum	0.98^{c}	1.24^{b}	1.31^{ab}	1.33^{a}	0.03	0
Ileum	0.98^{b}	1.04^{b}	1.24^{a}	1.24^{a}	0.03	0

abc Different superscripts in the same row indicate significant differences (P<0.05). T0: (basal diet as control); T1: (basal diet + 0.3% ECDWE-Lp); T2: (basal diet + 0.6% ECDWE-Lp); T3 (basal diet + 0.9% ECDWE-Lp). SEM: standard error of the mean.

Table 6. Ileal crude protein digestibility.

Variables	Treatment				SEM	p-value
	T0	T1	T2	T3		
ICPD (%)	65.11[c]	69.76[b]	71.80[ab]	73.21[a]	0.79	0

[abc] Different superscripts in the same row indicate significant differences (P<0.05). T0: (basal diet as control); T1: (basal diet + 0.3% ECDWE-Lp); T2: (basal diet + 0.6% ECDWE-Lp); T3 (basal diet + 0.9% ECDWE-Lp). ICPD: ileal crude protein digestibility. SEM: standard error of the mean.

Discussion

The results showed that adding ECDWE-Lp made grow more weight and feed intake, but it also made the feed conversion ratio go down. This result is due to the combined effects of the encapsulated probiotics and phytobiotics. Sapsuha *et al*. (2021) found that combining phytobiotics and probiotics helped broilers gain weight. This phenomenon may be related to the active components in the extract and the potential of the *Lactobacillus plantarum* entrapped to colonise effectively and be uptaken in the small intestine. Akinyemi *et al.* (2025) showed improved intestinal morphology in animals fed bioactive ingredients in encapsulated form compared with those fed non-encapsulated material. Cardamom distillation waste extract is rich in beneficial compounds such as polyphenols, tannins, flavonoids and saponins which kill germs and protect cells from damage. *Lactobacillus plantarum*, a LAB, as a probiotic produces some short-chain fatty acids (SCFA) and bacteriocins which acidify the digestive tract inhibiting pathogenic bacteria from growth (Vimon *et al*., 2023).

Insawake *et al*. (2024) observed that broilers fed phytobiotics from citrus extracts could have enabled the broilers to develop faster. Xiao *et al*. (2024) also found that the addition of the probiotic *Lactobacillus plantarum* resulted in a significant increase in body weight gain and feed intake in broilers. Wishna-Kadawarage *et al.* (2024) showed that mixing phytobiotics and probiotics may make broilers' gut health better. Changes in the pH of the intestines are associated to this improvement. These changes may change the makeup of gut microbiota and help the small intestine's villi grow properly. Villi are essential for expanding the small intestine's surface area for nutrient absorption. Increased villus height enhances nutrient absorption, particularly of proteins (Zhang *et al*., 2023), leading to greater nutrient deposition in the meat and ultimately increasing the body weight of broilers.

The findings indicated that incorporating ECDWE-Lp into diets could enhance the growth of microflora in the small intestine. While the treatment did not significantly impact the total counts of LAB and coliform in the duodenum, it successfully increased LAB totals and decreased coliform phyla in the jejunum and ileum. This is due to the antibacterial activity effects from ECDWE-Lp. In addition, the presence of *Lactobacillus plantarum* also increases the synergistic effect of the treatment owing to its ability to adhere and colonize the intestinal wall, compete for nutrients to grow, and produce antimicrobial substances to stimulate LAB growth and reduce colonization of coliform (Naeem and Bourassa, 2025).

Adding ECDWE-Lp to meals made the coliform phyla less common than in the control group. This is because the therapy kills bacteria and has *Lactobacillus plantarum*, which produces short-chain fatty acids (SCFA) and bacteriocins. LAB may boost SCFA synthesis, reduce pH, and turn on digestive enzymes, which makes it easier for the body to absorb nutrients (Sapsuha *et al*., 2023). This is in line with the results of a study that showed that the addition of ECDWE-Lp resulted in a lower pH value (P<0.05) than that without treatment (control). The condition of the digestive tract, which tends to be acidic, suppresses the growth of pathogenic bacteria and increases the population of LAB because pathogenic bacteria are less tolerant to acidic conditions and grow optimally under neutral pH conditions, whereas LAB grow optimally under acidic (Li *et al*., 2024). In addition, low pH in the digestive tract can also enhance mucus production, which functions as a protective surface of the digestive tract so that pathogenic bacteria cannot penetrate the small intestinal cells (Duangnumsawang *et al*., 2021).

Villi are small protrusions on the surface of the small intestine, increasing its surface area for nutrient absorption. Crypts are indentations or gaps at the base of the villi, which regeneration and proliferation of intestinal epithelial cells. Introducing ECDWE-Lp did not notably alter villus height and crypt depth in the duodenum, but it did lead to a significant increase in both villus height and crypt depth in the jejunum and ileum. The medication that inhibits dangerous bacteria from growing, maintains the intestinal mucosa healthy, and helps the villi develop to their maximum capacity is what makes the villi taller. Pathogenic bacteria penetrate small intestinal cells, perhaps causing inflammation in the intestinal mucosa and hindering villus growth (Awad *et al*., 2017). The metabolite compounds in ECDWE-Lp also protect epithelial cells from oxidative stress since they are antioxidants. Oxidative stress may lead to inflammation and cellular damage in the small intestine (Kim *et al*., 2023). A more favorable intestinal environment, devoid of oxidative stress-induced inflammation, promotes optimal epithelial cell proliferation and villus formation.

The inclusion of ECDWE-Lp in that facilitates the proliferation of intestinal epithelial cells at the base of crypts (Du *et al*., 2024; Iwasaki *et al*., 2019). These factors help the small intestine's crypt depth increase. The VH/CD ratio tells us how effectively nutrients can be absorbed (which depends on the height of the villus) and how well epithelial cells can grow back (which depends on the depth of the crypt). The findings indicated that the incorporation of ECDWE-Lp elevated the VH/CD ratio in the jejunum compared to the control group. This indicates that ECDWE-Lp may enhance the morphological integrity of the intestinal mucosa in a more functional capacity. According to Zhang *et al*. (2023), taller villi improve the surface area for absorbing nutrients. According to Sobolewska *et al*. (2017), deeper crypts demonstrate that epithelial cells are actively and effectively renewing. The height of the villus should likewise grow higher as the depth of the crypt goes up. A deeper crypt without a corresponding increase in villus height may indicate pathogenic conditions such as inflammation or mucosal damage (Parker *et al*., 2019). The increase in crypt depth and villus height post-treatment indicates that the regeneration of epithelial cells is synchronized with the maturation and differentiation processes at the apex of the villi. A greater balance between absorption and the regeneration of epithelial cells helps nutrients get into the body and broilers thrive.

Dosing with ECDWE-Lp extended the duodenum, jejunum and ileum in relation to each other. Treatments which increased the relative lengths of these sections of the small intestine compared to the control group represented increased growth of the intestine. This enhancement is attributed to the bioactive ingredients present in ECDWE-Lp. As antibacterial and antioxidant agents, they inhibit the growth of pathogenic bacteria, and contribute to intestinal health (Zhang *et al*., 2021). In addition, the addition of *Lactobacillus plantarum* in the treatment inhibits the proliferation of pathogenic bacteria and promotes the abundance of lactic acid bacteria (LAB) in the small intestine by generating metabolites including lactic acid and short-chain fatty acids (SCFA) and creating an acidic environment in the small intestine (Sampath *et al*., 2021). The greater number of LABs in the gut helps digestive tract in staying healthy by stimulating the growth and regeneration of villi, which makes the surface area for absorbing the nutrients bigger. When the small intestine absorbs nutrients better, it becomes longer, which helps it grow.

ECDWE-Lp administration led to an increase in the weight of the duodenum, jejunum, and ileum, indicating that the treatment improved the

ability of the small intestine to function using effective ingredients and *Lactobacillus plantarum*. Bioactive substances could either kill bacteria or shield cells from damage, which is supportive of the balance of intestinal microflora (Rodsatian *et al*., 2023). The other way, *Lactobacillus plantarum* inhibits the unwanted bacteria's growth and produces more LAB in the small intestine (Sampath *et al*., 2021). Increase in LAB population favours the length of the villi which helps to improve food absorption and stimulate digestion, particularly protein (Oyeagu *et al*., 2023). Greater protein digestibility leads to more intestinal epithelial cells. Proteins help epithelial cells grow by allowing the villi to be longer and more numerous (Sohel *et al*., 2019). As the villi grow longer and multiply, the small intestine gets heavier.

The addition of ECDWE-Lp treatment was able to increase ileal crude protein digestibility compared to the control treatment. This is due to the synergistic effect of encapsulated phytobiotics and probiotics which can improve small intestinal health thereby optimizing villi growth. Villi play a role in increasing the surface area of the small intestine so that nutrient absorption is more optimal, increasing the height of the villi will indicate an increasing surface area of the small intestine to absorb nutrients (Attia *et al*., 2020). increasing the height of the villi will increase the absorption of nutrients, especially protein, thereby increasing the digestibility value of ileal crude protein (Zhang *et al*., 2023).

Conclusion

Adding encapsulated cardamom distillation waste extract and *Lactobacillus plantarum* (ECDWE-Lp) at 0.6% (T2) to meals made the small intestinal profile better, made protein easier to digest, and enhanced the performance of broilers.

Conflict of interest

The authors state that there are no conflicts of interest related to the publication of this paper.

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