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Effect of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* on immune system, protein digestibility and performance of broiler chicken

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ABSTRACT

This study was conducted to evaluate the effects of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* in diet on the immune system, protein digestibility, and performance of broiler chickens. There were 200 8-day-old unsexed broilers (Ross 308) with an average initial weight of 217.22±5.58 g were included to the encapsulated *Amomum compactum* waste extract-*Lactobacillus plantarum* named EAWE-Lp and assigned to 4 treatments with 5 replicates (10 chicks each). The treatments were T0 basal diet (BD), T1 BD+ EAWE-Lp 0.3%, T2 BD+EAWE-Lp 0.6%, and T3 BD+EAWE-Lp 0.9%. Data were analyzed using analysis of variance (ANOVA) to assess the impact of the treatment, followed by Duncan's multiple range test (DMRT) at 5% significance level. The result indicated that the dietary encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* influenced ($P<0.05$) several criteria such as the total count of lactic acid bacteria (LAB), total Coliform, pH of small intestine, the relative weights of the bursa fabricius, spleen, and thymus, the heterophil-to-lymphocyte ratio, protein digestibility, daily feed intake (DFI), daily body weight gain (DBWG), and feed conversion ratio (FCR). The study concludes that addition of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* level of 0.6% (T2) in the diet can improve the immune system, protein digestibility, and performance in broiler chickens.

Introduction

Broiler chickens are an essential source of animal protein at cheaper cost (Khatun *et al*., 2023). Broiler production has grown tremendously and culminate in this production. In contrast, when broiler flocks grow larger, many other problems are also encountered, such as poor body health condition, rapid disease dissemination, poor growth performance, and high mortality (Salim *et al*., 2018). This can be addressed by using different natural feed additives as substitutions with intentions to enhance overall body health and broiler performance. Feed additives added to the diet of broiler chickens can include phytobiotics and probiotics. One type of phytobiotic which can be used is waste of *Amomum compactum* and probiotics, including *Lactobacillus plantarum*.

Amomum compactum, one of the plants of the family Zingiberaceae or ginger (Nurcholis *et al*., 2021). *Amomum compactum* is known to possess significant bioactive properties, including antioxidant (Juliana *et al*., 2022), antibacterial (Hartady *et al*., 2020), antibiofilm activities (Cui *et al*., 2020), and anti-inflammatory (Arpitha *et al*., 2019). The waste generated from the distillation of *Amomum compactum* remains unutilized by the community, leading to the accumulation of waste pollution that continues to increase each year. The use of *Amomum compactum* waste could maximize utility of discarded resources, and the waste could be used as a natural feed additive for poultry, including broiler chickens. *Amomum compactum* waste possesses certain bioactive compounds which are maintained in its waste. According to the research of Sukandar *et al*. (2015), the phytochemical test result of *Amomum compactum* were phenolic, groups of terpenoids and tannin. It is more appropriate if the use of phytobiotic from *Amomum compactum* waste is combined with a probiotic. *Lactobacillus plantarum* is one of lactic acid bacteria (LAB) that belongs to genus of *Lactobacillus* which is considered as GRAS (Generally Recognized As Safe) for its non-pathogenicity potential and can inhibit pathogenic bacteria growth as probiotics (Fitrianingsih, 2023). Among *Lactobacillus* spp, *Lactobacillus plantarum* exhibits superior resistance to temperature fluctuations and acidic pH conditions (Winarti *et al*., 2019).

Bioactive compounds contained in *Amomum compactum* waste are very susceptible to damage caused by environmental conditions such as temperature, light, and other conditions so that encapsulation technology is needed. Encapsulation is a process of converting the aspects of a product from liquid form to solid particles by envelopment of these material with one or more additional materials in order to avoid interference with the composition of the material (Silalahi and Hanafi, 2015). The encapsulation can alter environmental situation as an oxidation and hydrolysis damage, causing shelf-life extension and better process stability (Kistriyani *et al*., 2020). Maltodextrin is encapsulant material. Maltodextrin is a result of oligosaccharide derivatives, which serve as an energy source for the growth of beneficial bacteria, its constituent is complex carbohydrates (Sumanti *et al*., 2016).

Phytobiotic from *Amomum compactum* waste which contain flavonoid compounds has antioxidant and antibacterial activities which can increase the immunity response of the immune system through the activation of lymphocytes and macrophage, and prevent the small intestinal mucosa from being colonized by pathogenic bacteria. *Lactobacillus plantarum* in lactic acid bacteria is one of probiotics, which can regulate the balance of microbial flora in small intestine, control the growth of pathogens, improve the digestibility of nutrition, stimulate the systemic and mucosal immunity. According to study of Yin *et al*. (2023), reported that *Lactobacillus plantarum* enhances humoral immune response, improves barrier function of small intestine and feed conversion ratio in chicken. The combination of *Amomum compactum* waste and *Lactobacillus plantarum* may be used as a potential solution with a synergetic effect of broiler performance improvement through positively modify the immune status, enhanced nutrient digestibility (especially protein digestibility), and support growth. Dibamehr *et al*. (2023) found that increased relative weight of lymphoid organs like bursa of fabricius and spleen due

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to combination of *Lactobacillus plantarum* along with phytobiotics which also enhanced the immune responses. Furthermore, the use of this blend could not only improve immune system and protein digestibility, but also body weight gain and FCR. This research assessed the effects of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* on immune system, protein digestibility, and performance of broiler chickens.

Materials and methods

Experimental animals and diet

There were 200 8-day-old unsexed broilers (Ross 308) with an average body weight of 217.22±5.58 g. The tools and materials used for extraction and encapsulation were *Amomum compactum* waste, *Lactobacillus plantarum*, maltodextrin, skim milk, sterile distilled water, 96% ethanol, aluminium foil, oven, grinder, measuring cylinder, beaker glass sonicator, vacuum rotary evaporator, magnetic stirrer, freeze dryer, and fine filter paper. Tools and materials used in rearing broiler chickens include encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum*, Fe_2O_3, cages, feeders, hanging drinkers, lamps, thermohygrometers, digital scales, sprayers, and sanitation equipment.

Broiler chickens were maintained starting from DOC until 7 days and fed with commercial starter feed B-11S. Feed and drinking water are available ad libitum. At the age of 8 to 21 days, they were given a starter basal diet and at the age of 22 to 35 days, they were given a finisher basal diet with encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum*. The composition of the feed stuff and nutrient content of the broiler basal diet are listed in Table 1.

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
Nutrients Content		
Metabolic Energy (kcal/kg)[2]	2.986.78	3.035.25
Crude Protein (%)[1]	21.77	19.82
Crude Fat (%)[1]	4.2	4.78
Crude Fiber (%)[1]	4.52	4.66
Kalsium (%)[1]	1.09	1.12
Posphor (%)[1]	0.75	0.74
Lysine (%)[3]	0.19	1.07
Methionine (%)[3]	0.48	0.41

[1]Results of Analysis of Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia (2025).
[2]Based on the Bolton Formula = 40.81 [0.87(CP+2.25×CF+BETN)+k] (1967).
Description: CP= Crude Protein; CF= Crude Fat; BETN= Nitrogen-Free Extract; k=Correction factor
[3]Amino acids are calculated based on the Trial and Error Method.

Preparation of extract and encapsulation

Amomum compactum waste extract was obtained by an extraction process based on the method of Gouda *et al*. (2021), which has been modified. Extraction began with *Amomum compactum* waste was dried in an oven at 50ºC with the aim of reducing the water content in *Amomum compactum* waste and facilitating the pulverization process. The dried *Amomum compactum* waste was pulverized into flour. *Amomum compactum* waste flour was dissolved in 96% ethanol at a ratio of 1:10 (b/v) and stirred to ensure homogeneity. The solution was sonicated at 37ºC and room temperature at a wavelength of 50 Hz for 60 min. The solution was allowed to stand for 24 h (maceration) and, filtered using filter paper. The obtained filtrate was subjected to evaporation to eliminate 96% ethanol, yielding a concentrated extract.

Then the evaporation extract was continued with the encapsulation process using a modified method carried out by Agusetyaningsih *et al*. (2022). To initiate the encapsulation are preparing a mixture of skim milk and sterile distilled water in a 1:2 (b/v) ratio. This blend was mixed with *Lactobacillus plantarum* in a ratio of 2:1 (v/v). A blend of *Amomum compactum* waste extract and *Lactobacillus plantarum* solution were mixed as a volume ratio of 1:1 (v/v). Maltodextrin was solubilized in sterile distilled water as a dressing solution at a concentration of 1:3 (w/v). Finally, *Amomum compactum* waste extract and *Lactobacillus plantarum* solution were mixed with the maltodextrin solution in a 1:5 (v/v) ratio and was freeze dried at -50 ºC until dried.

Research design

The experiment was established mainly as a completely randomized design with 4 treatment groups and 5 replicates (10 chicks in each replicate). The experimental treatments were:
T0= Basal Diet/BD (Control).
T1= BD + EAWE-Lp 0.3%.
T2= BD + EAWE-Lp 0.6%.
T3= BD + EAWE-Lp 0.9%.

Parameters and measurement procedures

The pH of the small intestine was measured by homogenizing the entire contents of the small intestine and measuring them with a digital pH meter. The enumeration of total LAB and total Coliforms was done by culturing the samples on de Man Rogosa Sharpe (MRS) agar and Eosin Methylene Blue Agar (EMBA) media. The growth of bacteria was performed by Total Plate Count (TPC) method according to Fardiaz (1993).

Data on the relative weight of lymphoid organs were collected from 35-day-old chickens, and then each lymphoid organ (bursa fabricius, spleen, and thymus) was collected. Each organ was weighed using an analytical balance and the relative weight was calculated.

Blood samples were taken from the brachial vein of 35-day-old chickens to measure the heterophil-lymphocyte ratio before they were processed for carcassing. Blood±3 ml, collected and placed in a test tube containing an anticoagulant in the form of Ethylene Diamine Tetraacetic Acid (EDTA) to avoid blood clots. Blood samples were analyzed by placing the blood sample in the adapter on the hematology analyzer, closing the sample container, and pressing the "Run" button. The analysis results appear on the monitor screen automatically, and the H/L ratio can be calculated.

Protein digestibility of broiler chicken was assessed using the total collection method with an Fe_2O_3 indicator for four days. Broilers were kept in battery cages and fed basal diets with the addition of treatments mixed with Fe_2O_3 indicator (as a marker for the start of total excreta collection) at a 0.5% of daily feed intake. Excreta were collected on days 2 and 4, and excreta were weighed to determine wet and dry weights. Excreta were analyzed for nitrogen content to calculate digestibility. The method for determining protein digestibility followed the formula outlined by Tillman *et al*. (2005).

Daily feed intake (DFI) data were collected by recording the daily feed minus the remaining daily feed for 28 days.Daily body weight gain

(DBWG) data were measured by weighing the initial body weight (8 days old) and the final body weight (35 days old) of broiler chickens, and was calculated.The feed conversion ratio (FCR) is determined by dividing the amount of feed consumed daily by the daily increase in body weight over the course of the study.

Statistical analysis

In this study, the data were analyzed using analysis of variance (ANOVA) to assess the impact of the treatment, followed by Duncan's multiple range test (DMRT) at a 5% level of significance (Steel *et al.*, 1997).

Results

Total bacteria and pH of small intestine

Total bacteria and pH of the small intestine are also provided in Table 2. Analysis of variance revealed significant (P<0.05) effects of the addition of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* to the diet on total LAB, total Coliform, and small intestine pH. The total LAB in T2 and T3 were significantly higher (P<0.05) than those at T0 and T1. The total Coliform in T1, T2, and T3 were significantly lower (P<0.05) than those at T0. The pH of the small intestine in T2 and T3 was significantly lower (P<0.05) than that in T0 and T1.

Table 2. Total bacteria and pH in the small intestine.

Parameters	T0	T1	T2	T3	*p*-value
LAB (log cfu/g)[1]	8.75 ± 0.31^{b}	8.89 ± 0.11^{b}	9.41 ± 0.12^{a}	9.47 ± 0.21^{a}	<0.000
Coliform (log cfu/g)	3.08 ± 0.19^{a}	2.80 ± 0.05^{b}	2.69 ± 0.08^{b}	2.72 ± 0.06^{b}	<0.000
pH of Small Intestine	6.56 ± 0.07^{a}	6.40 ± 0.10^{b}	6.29 ± 0.07^{bc}	6.23 ± 0.07^{c}	<0.000

^{a,b,c}Means marked with superscript letter in the same row are significantly different (P<0.05).
T0: Basal diet as control, T1: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.3%); T2: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.6%); T3: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.9%). 1 LAB : Lactic Acid Bacteria.

Relative weight of lymphoid organs and heterophil-lymphocyte ratio

The relative weight of lymphoid organs and heterophil-to-lymphocyte ratio are presented in Table 3. The relative weight of lymphoid organs and the H/L ratios was significantly influenced by the inclusion of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* in diet as in analysis of variance (P<0.05). The relative weight of bursa fabricius in T1, T2, and T3 was significantly (P<0.05) increased compared with T0. The relative weight of the spleen in T1, T2, and T3 was significantly (P<0.05) greater than that in T0. The relative weights of the thymus in T1, T2, and T3 were significantly higher (P<0.05) than those in T0. The heterophil-to-lymphocyte ratio at T3 was significantly lower (P<0.05) than that at T0, T1, and T2.

Table 3. Relative weight of lymphoid organs and Heterophil-Lymphocyte ratio.

Parameters	T0	T1	T2	T3	*p*-value
Bursa Fabricius (%)	0.12 ± 0.03^{b}	0.16 ± 0.04^{a}	0.19 ± 0.01^{a}	0.18 ± 0.03^{a}	<0.005
Spleen (%)	0.15 ± 0.03^{b}	0.21 ± 0.01^{a}	0.21 ± 0.02^{a}	0.22 ± 0.06^{a}	<0.016
Thymus (%)	0.29 ± 0.04^{b}	0.39 ± 0.05^{a}	0.42 ± 0.10^{a}	0.47 ± 0.04^{a}	<0.005
H/L Ratio[1]	0.90 ± 0.03^{a}	0.77 ± 0.02^{b}	0.71 ± 0.01^{c}	0.61 ± 0.01^{d}	<0.000

^{a,b,c,d}Means marked with superscript letter in the same row are significantly different (P<0.05).
T0: Basal diet as control, T1: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.3%); T2: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.6%); T3: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.9%).1 H/L: Heterophil/Lymphocyte.

Protein digestibility and performance of broiler chickens

Protein digestibility and performance of broiler are shown in Table 4. The addition of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* in the diet significantly affected (P<0.05) on protein digestibility, daily feed intake, daily body weight gain, and feed conversion ratio in broiler chickens. Protein digestibility were the lowest in T0 as compared to the T1 or T2, but the highest in T3. The highest daily feed intake was observed in T3 which was significantly different from T0, but showed no significant difference from T1 and T2. Feed conversion ratios in T2 and T3 were significantly (P<0.05) lower compared in T0 and T1.

Table 4. Protein digestibility and performance of broiler chickens.

Parameters	T0	T1	T2	T3	*p*-value
Protein Digestibility (%)	71.92 ± 4.04^{c}	79.84 ± 4.56^{b}	83.41 ± 2.16^{ab}	85.42 ± 0.59^{a}	<0.000
DFI (g)[1]	94.71 ± 4.16^{b}	99.27 ± 3.13^{a}	99.61 ± 1.84^{a}	100.04 ± 2.80^{a}	<0.051
DBWG (g)[2]	57.09 ± 1.57^{c}	59.54 ± 1.11^{b}	62.29 ± 0.66^{a}	62.44 ± 0.39^{a}	<0.000
FCR[3]	1.66 ± 0.04^{a}	1.67 ± 0.05^{a}	1.60 ± 0.03^{b}	1.60 ± 0.04^{b}	<0.027

^{a,ab,b,c}Means marked with superscript letter in the same row are significantly different (P<0.05).
T0: Basal diet as control, T1: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.3%); T2: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.6%); T3: Basal diet + (Encapsulated *Amomum compactum* Waste Extract-*Lactobacillus plantarum* 0.9%). 1 DFI: Daily Feed Intake; 2 DBWG: Daily Body Weight Gain;3 FCR: Feed Conversion Ratio.

Discussion

The addition of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* in the diet significantly improved the microflora of the small intestine of broiler. Increased levels of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* influenced an increase in total lactic acid bacteria, a decrease in total Coliform bacteria, and a decrease in the pH of the small intestine. Table 2, shows that the total lactic acid bacteria was highest in the T3 treatment compared to that in control treatment (T0). Bacteriocins derived from herbal ingredients can have an antagonistic effect on the growth of pathogenic bacteria, such as Coliform. Bacteriocins are antibacterial compounds secreted by lactic acid bacteria (LAB), but are not harmful to LAB because bacteriocin-producing bacteria have specific immunity (Martinez *et al.*, 2013). Lactic acid bacteria (LAB) have the functionality to control the growth of pathogenic bacteria because LAB can grow at acidic pH. High lactic acid produced by LAB will cause the potential of hydrogen (pH) of the broiler digestive tract to become acidic, then Coliform bacteria or other pathogens cannot grow (McNaught and MacFie, 2001). The BAL-Coliform ratio is classified into three categories, namely >1 (high), 1 (balanced), and <1 (low). The BAL-Coliform ratio in each treatment was as follows T0 (28.40×10^{6}), T1 (31.75×10^{6}), T2 (34.98×10^{6}), and T3 (32.81×10^{6}). These results indicating that the dominance of beneficial lactic acid bacteria over Coliforms promotes a favorable gut microbial balance and reflects optimal fermentation activity in broiler chickens. Increased total lactic acid bacteria can suppress the growth of Coliform bacteria attached to the villus wall of the small intestine through its ability to produce short-chain acids. Acidic pH conditions in the small intestine of broilers cause the total Coliform bacteria to decrease, so that villi can grow and develop through an increase in the number of villi and an increase in the width and height of the villi. Morphological improvements in villus height and width contribute to an increased mucosal surface area, enhancing the capacity for nutrient absorption in the intestine (Fitrianingsih, 2023). Setiawan *et al.* (2018) stated that flavonoids can increase the height of duodenal villi, which has an impact on the optimal nutrient absorption process, thus increasing the slaughter weight of livestock.

The relative weight of the lymphoid organs consists of the bursa fabricius, spleen, and thymus. The results of this research showed the highest values for the T3 and T2 treatments. It can be suggested that the diet nutrients and active ingredients can help keep the performance of the bursa fabricius, spleen and thymus. Flavonoids with antioxidant power are also produced in stress conditions when antioxidant enzyme activity is exhausted or in the case of abnormal events (Agati *et al*., 2012). It was previously found that dietary supplementation with genistein, a flavonoid compound, at doses of 20 to 80 mg/kg feed affected significantly the immunological reactions and performance in broilers (Rasouli and Jahanian, 2015). It can be inferred from the above that, under normal condition, the supplementation with flavonoid compounds and LAB enables the body of the chickens to keep healthy of the organism without imposing excessive burden on immune organs, thereby maintaining the relative weights of lymphoid organs at a relatively stable and normal level. The organosomatic index of the bursa fabricius was 0.12–0.19 %, which was in the large range of normal size. Satrian *et al*. (2022), bursa fabricius relative weight varied from 0.12–0.29%. The bursa of fabricius can experience follicular depletion and atrophy, which is accompanied by a decrease in the lymphocyte population if it frequently forms antibodies, which ultimately reduces the levels of antibodies produced by the animal (Rokhmana *et al*., 2013). The spleen of broiler chickens is a secondary lymphoid organ responsible for the production of lymphocyte cells and storing antigens that go into the blood circulation (Ain *et al*., 2020). The encapsulation of *Amomum compactum* waste and *Lactobacillus plantarum* contains active compound, mainly flavonoids, which could increase the immune cell counts of lymphocytes, thereby promoting spleen development and growth. Yang *et al*. (2021) also proposed that the increased splenic macrophage phagocytotic activity and peritoneal leukocyte phagocytotic clearance efficiency observed were perhaps owed to the supplement of flavonoids, which could aid in reducing the pathogenic bacteria-induced damage to the visceral organs. The organosomatic index of the spleen weight was 0.15–0.22%, which still fell within the normal limit. The relative weight of the normal spleen of broiler ranges from 0.10–0.23% (Putnam, 1991). The relatively small spleen weight indicates that the spleen does not contain too many antigens, and thus the performance of the spleen is not excessive (Wulansari *et al*., 2016). However, if there is an enlarged spleen in the body, the chicken is infected with bacteria (Akbar *et al*., 2022). The thymus regulates the immune response of chickens to infected cells. The thymus will experience a change in shape to become small due to stress and death due to illness (Sulistiyanto *et al*., 2019). As shown in Table 3, the relative weight of the thymus in the T3 treatment group was higher than that in the control treatment (T0). The organosomatic index of the thymus organ in the results ranged from 0.29 – 0.47% (Table 2) and can be categorized in the normal range, because the thymus does not metabolize many active substances. Thymus size varies greatly depending on several factors, including sex, body weight, species, feed, physiological body, hormonal stress, immunosuppressive drugs, environment, and disease (Fadhilah *et al*., 2022). The relative weight of lymphoid organs, especially the spleen, is closely related to the heterophil-to-lymphocyte ratio (H/L), because the function of the spleen is to take antigens from the blood. According to Jamilah *et al*. (2013), the spleen functions to absorb antigens in the blood that interact to lymphocytes. If the size of the spleen is enlarged, it indicates that it holds more antigens, which results in reduced free lymphocytes in the blood and increases the H/L ratio. An enlarged thymus indicates a more active and healthier immune system, resulting in increased lymphocyte production. Conversely, a reduced thymus size signifies that the broiler is experiencing stress, which leads to a decrease in lymphocyte count and a consequent increase in the heterophil to lymphocyte (H/L) ratio. Larger thymus and spleen sizes in broiler chickens indicate lower physiological stress and optimal immune function, thus correlating negatively with the H/L ratio. The H/L ratio results showed that in T3 treatment was the lowest compared with the other treatments. In this research, the percentage of H/L ratios ranged between 0.61–0.90. Thus, the H/L ratio in broilers was lower when the birds were relatively comfortable. The broiler chicken stress resistance are indicated by the values of H/L ratio, among them 0.2 (lower), 0.5 (normal), and 0.8 (higher) (Emadi and Kermanshahi, 2007). Stress conditions in broiler may decrease lymphocyte and increase H/L ratio (Abdullah *et al*., 2018). In general, the encapsulation of *Amomum compactum* waste extract and *Lactobacillus plantarum* did not compromise the comfort of the broilers, seen from the H/L ratio value.

The addition of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* in the diet can enhance the protein digestibility of broilers. In Table 4. present the highest protein digestibility of T3 treatment in comparison with T0 treatment. The combination of herbal ingredients and probiotics provides a synergistic mechanism that involves improving gut health and nutrient absorption. Giving *Amomum compactum* waste extract contains flavonoids that can affect intestinal acidity, so that the intestinal pH is low. Low intestinal pH conditions create a balance of microorganisms in the intestine, such as LAB, which increases and Coliform which decrease. Based on the research of Ramaiyulis *et al*. (2023), stated that flavonoids act as antioxidants by increasing protein digestion activity and reducing protein digestion in the small intestine of broiler chickens. Flavonoids act as antibacterial agents that can increase the efficiency of feed digestibility, resulting in improved absorption of feed nutrients (Lestariningsih *et al*., 2015). An acidic gut environment is caused by the production of lactic acid by *Lactobacillus plantarum*, which activates digestive enzymes and accelerates protein hydrolysis into amino acids that are easily absorbed (Sampath *et al*., 2021). Thus, broilers have an adequate supply of nutrients for growth, health, and productivity. The combination of flavonoids and *Lactobacillus plantarum* can significantly improve the ration protein efficiency and digestibility in broiler chickens.

Optimal broiler performance can be achieved by focusing on the ration content. Broiler diets containing encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* resulted in a significant increase in broiler performance. Flavonoids from *Amomum compactum* waste were able to increase the growth of beneficial gut bacteria and inhibit the growth of harmful bacteria. The growth of *Lactobacillus plantarum* has a positive effect on intestinal permeability and increases nutrient digestibility, resulting in a better FCR and a higher average daily body weight gain in broilers (Pertiwi *et al*., 2021). The results in Table 4, show that the daily feed intake in the T3 treatment was higher than that of T0 treatment. Sugiharto and Ayasan (2023), stated that the use of encapsulation methods for herbal ingredients has a positive impact on physiological and health conditions as well as nutrient digestibility, thus increasing growth performance and feed conversion in broiler chickens. The encapsulation method can protect the active compounds in *Amomum compactum* waste to optimally reach the small intestine. Antibacterial and antioxidant activities can suppress the growth of pathogenic bacteria, thus giving *Lactobacillus plantarum* the opportunity to grow and develop in the intestinal tract of broiler. Acidic pH conditions in the intestinal tract can stimulate the activity of digestive enzymes (Fadhiila *et al*., 2022). This condition creates healthy and balanced intestinal microflora, which can reduce digestive disorders and increase appetite in broiler chickens. An increase in high daily feed intake will support faster growth, so that the daily body weight gain is higher. This is supported by Rasyaf (2012), who stated that rapid growth in chickens is supported by high feed intake. High feed intake provides an opportunity for broilers to digest and absorb nutrients in diet, so that the availability of higher nutrients will support the metabolic process and productivity of broilers. The feed conversion ratio (FCR) value in this research showed the lowest results in T3 and T2 compared to T1 and T0 (Table 4). Encapsulation of *Amomum compactum* waste extract and *Lactobacillus plantarum* at a dose of 0.6% (T2) has been able reduced the FCR value. According to Akter *et al*. (2024), supplementation of a flavonoid blend at a level of 0.6 gm/kg in broiler diets was found to enhance nutrient digestibility, increase beneficial intestinal bacteria in the small intestine, improve growth performance, and ameliorate meat quality. Zhou *et al*. (2019), reported that dietary supplementation with the flavonoid compound baicalein at a concentration of 200 mg/kg significantly increased the feed conversion ratio and enhanced the average daily weight gain in broiler chickens. The FCR value indicates the level of feed conversion use, meaning that the lower the FCR value, the higher the feed conversion value and the more economical (Razak *et al*., 2016). Moreover, an increasing FCR value indicates a decline in the nutritional quality of the diet. The low FCR value can be attributed to changes in pH of intestinal caused by the combination of compounds present in *Amomum compactum* waste and *Lactobacillus plantarum*. Low intestinal pH conditions will affect the composition of microorganisms, especially the increase in LAB, resulting in the assimilation of nutrients that have a positive impact on increasing feed conversion utilization. Hussein *et al*. (2020), reported that addition probiotics to diet chickens can be utilized as an alternative growth promoter that can improve the performance and quality of the final product. According to Listyasari and Purnama (2022), FCR can be influenced by several factors, including diet, genetics, environmental conditions, feed intake, sex, and body weight.

Conclusion

Addition of encapsulated *Amomum compactum* waste extract and *Lactobacillus plantarum* level of 0.6% (T2) in the diet can improve the immune system, protein digestibility, and performance in broiler chickens.

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Conflict of interest

The authors declare no conflict of interest with other relationships or organizations related to the materials discussed in the manuscript.

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