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The effect of encapsulated Japanese Papaya leaf extract supplementation in diets on protein digestibility, immunity, and performance of broiler chickens

Muhammad N. Nabhan[1*], Vitus D. Yunianto[2], Mulyono Mulyono[2], Lilik Krismiyanto[2]

[1]Master of Animal Science, Faculty of Animal and Agricultural Sciences Universitas Diponegoro, Semarang 50275, Central Java, Indonesia.
[2]Department of Animal Science, Faculty of Animal and Agricultural Sciences Universitas Diponegoro, Semarang 50275, Central Java, Indonesia.

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*Correspondence:

Corresponding author: Muhammad Naufal Nabhan
E-mail address: nabhannaufal81@gmail.com

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ABSTRACT

The research propose to evaluate the impact of addition encapsulated Japanese papaya leaf extract (EEDPJ) to diets on protein digestibility, health status, and broiler performance. This study used 200 unsexed day-old Ross 308 broiler chickens with fully randomized design, four treatments, five repetitions, and each comprising 10 birds. The treatments were as follows: T0, basal diet (control); T1, basal diet + 0.09% Japanese papaya leaf extract (EDPJ); T2, basal diet + 0.03% EEDPJ; T3, basal diet + 0.06% EEDPJ and T4, basal diet + 0.09% EEDPJ. Measurement the parameters using total lactic acid bacteria (LAB), total coliforms, small intestine pH, protein digestibility, lymphoid organ weight, heterophil/lymphocyte ratio (H/L), feed intake, daily weight gain, and feed conversion ratio (FCR). Data analysis used variance analysis and Duncan's Multiple Range Test at 5% significance. Supplementation with EEDPJ gives effect to led a significant improvement ($P<0.05$) in daily weight gain, feed conversion ratio (FCR), lactic acid bacteria population, coliform count, intestinal pH, relative weights of the bursa of Fabricius and spleen, heterophil/lymphocyte (H/L) ratio, and protein digestibility. In contrast, not significantly influenced ($P>0.05$) on daily feed intake and thymus weight. In inclusion, the addition of 0.06% EEDPJ in the diet enhanced protein digestibility, strengthened immune response, and improved the broiler chickens growth performance.

Introduction

Broiler chickens are the product of intensive breeding programs that started in the early 20th century. The broiler chicken industry in Indonesia has an important role in meeting national protein needs. Antibiotic growth promoters (AGPs) was used by farmer to enhance production of broiler chicken, aiming to fullfill the demand for animal-based prorein in Indonesia. However, due to concerns over their negative impacts, the Indonesian government prohibited the use of AGPs under Regulation No. 14/PERMENTAN/PK.350/5/2017. According to Ronquillo and Hernandez (2017), antibiotics improve the risk of antibiotic-resistant bacteria, that has a bad impact for human health and the environment.

Prohibition of AGPs has driven farmers to explore natural alternatives as feed additives. One potential option is the Japanese papaya leaf (*Cnidoscolus aconitifolius*), which contains various phytochemicals, including flavonoids, saponins, and alkaloids, known for their bioactive activities (Wongnhor *et al*., 2023). Previous studies informed that the levels of flavonoids, saponins, and alkaloids in Japanese papaya leaves were 23.72%, 12.49%, and 17.45%, respectively (Obichi *et al*., 2015). Similarly, Chikezie *et al*. (2016) documented concentrations of flavonoids, saponins, and alkaloids, each 1200 mg/100 g, 3900 mg/100 g, and 490 mg/100 g.

Phytobiotics are highly bioavailable but easily to degradation under the acidic conditions of the digestive tract (Velasco & Williams, 2011; Sugiharto, 2021). To maintain their stability and effectiveness, encapsulation is applied. This technique coats the active substances with carrier materials, ensuring their controlled and targeted release (Vinceković *et al*., 2017). Recent study explains that maltodextrin was used as the encapsulating agent. Maltodextrin, a starch hydrolysate, is easily soluble, resistant to oxidation, and does not produce pigments (Xiao *et al*., 2022). It is hydrolyzed by amylase, which enables the release and absorption of phytobiotics in the chicken intestine. According to Aderibigbe *et al*. (2020), most starch is digested by amylase in the duodenum and jejunum, that serves as an energy source and promotes the growth of LAB by producing glucose (Yu *et al*., 2021). Once absorbed, bioactive compounds enhance nutrient utilization, improve digestion, and support immune function by increasing the relative weights of lymphoid organs such as the bursa of Fabricius, thymus, and spleen in broilers (Kamboh *et al*., 2016).

This study has novelty in the application of encapsulated Japanese papaya leaf extract in broiler diets, which has not been previously reported. Unlike earlier studies that focused on direct supplementation of herbal extracts, this research introduced an encapsulation technique to improve bioavailability and stability, thereby enhancing protein digestibility, immune response, and broilers performance.

Materials and methods

Experimental design

In November 2024 this study began in the Poultry House, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang. The experimental animals were 200 Ross strain broiler chickens, with 247.0±10.0 g body weight at 8 days of age. Encapsulated Japanese papaya leaf extract (EEDPJ) was used as the feed additive treatment. The dietary ingredients included yellow corn, pollard, soybean meal, meat bone meal, $CaCO_3$, premix, lysine, and methionine (Table 1). The materials used for encapsulation included Japanese papaya leaves, 96% ethanol, fine filter paper, oven, grinder, water bath, refrigerator, and aluminum foil. The maintenance equipment consisted of the main house, experimental units, battery cages, digital scales (accuracy 1 g), thermohygrometer, brooder, 60- and 40-watt lamps, feed and water containers, pH meter, pot jars, ethylenediaminetetraaceticacid (EDTA) tubes, and cool boxes. The encapsulation equipment included 2-liter beaker glass, stirring rods, 500 ml measuring cylinders, glass funnels, sonicator, evaporator, and freeze dryer.

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The study was arranged in a completely randomized design with five treatments and four replications, totaling 20 experimental units. Each unit be composed of 10 chickens. The treatments were as follows: T0, basal diet (control); T1, basal diet + 0.09% non-encapsulated Japanese papaya leaf extract (EDPJ); T2, basal diet + 0.03% EEDPJ; T3, basal diet + 0.06% EEDPJ; T4, basal diet + 0.09% EEDPJ.

Table 1. Feed ingredient composition and nutrient content of the diet.

Feed Ingredient	Composition (%)	
	Starter (8-21 days)	Finisher (22-35 days)
Corn Yellow	50.26	53.01
Rice Bran	14.89	17.14
Soybean Meal	24	19
Meat Bone Meal	10	10
$CaCO_3$	0.3	0.3
Premix	0.25	0.25
Lysine	0.1	0.1
Methionine	0.2	0.2
Total	100	100
Nutrient Content		
Metabolizable Energy (kcal/kg)[1)]	2.989.53	3.078.82
Crude Protein (%)[2)]	21.63	19.74
Crude Fat (%)[2)]	4.19	4.62
Crude Fiber (%)[2)]	4.35	4.58
Calcium (%)[3)]	1.11	1.18
Phosphorus (%)[3)]	0.75	0.71

[1)]Based on the Bolton Formula = 40.81 [0.87(CP+2.25xCF+NFE)k] (Bolton, 1967).
[2)]Result of analysis of Laboratory of Animal Nutrition Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang (2024).

Extraction and encapsulation procedure

The encapsulation process was taken on based to the method by Gouda *et al*. (2021). First, Japanese papaya leaves were oven-dried at 50°C and grinded into a powder. The powder was dissolved in 96% ethanol at a ratio of 1:10 (b/v) and stirred until it was homogeneous. Then be sonicated at 37°C for 60 minutes with 50 Hz of frequency at room temperature. The cells were left for 24 h (maceration). The filtrate was evaporated to remove the ethanol.

Encapsulation followed a modified method by Agusetyaningsih *et al*. (2022), using a coating solution made from maltodextrin and distilled water at a 1:3 (b/v) ratio. The evaporated extract was mixed with the coating solution at a 1:5 (v/v) ratio and then dried using a freeze dryer at −50°C to obtain a powdered form.

Broiler management

Chickens were housed in litter-floor pens with 20 experimental units (10 birds per unit). From days 1 to 7, the commercial feed was mixed with the experimental diet to allow for adaptation. From days 8 to 35, the chickens were fed the with experimental diet supplemented with encapsulated Japanese papaya leaf extract according to the treatment groups. Feed was provided based on daily intake and then ad libitum on drinking water.

Data collection

The following parameters were measured:

The blood collected from the Vena brachialis in 3 ml volumes and placed in EDTA tubes to analyze H/L ratio. The H/L analysis was accomplished as described by Gil *et al*. (2023).

Lymphoid organ weights: The relative weights of lymphoid organs calculated with formula described by Hidayat *et al*. (2020).

Protein digestibility was considered using the total collection method combined with Fe_2O_3 as the marker. The collection was conducted from days 32 to 35. During collection, excreta were sprayed with 0.2 N HCl every hour. The fresh weight was recorded, the samples were dried and 50 g of dried excreta was homogenized, labeled, and sent for laboratory analysis. Digestibility was deliberated using the following formula (Li *et al*., 2017).

Daily weight gain (DWG) was measured using the following formula from Wijayanto (2022).

Intestinal pH measured at slaughter. Samples from the small intestine were collected and measured using a digital pH meter (Ecotest pH 1).

The total plate count (TPC) was used to count Total LAB and Coliform according to Fardiaz (1993).

Statistical Analysis

All data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level. If significant effects were found, Duncan's Multiple Range Test was applied to compare treatment means (Steel and Torrie, 1960).

Results

Broiler performance

The impact of EEDPJ supplementation on key performance indicators daily feed intake (DFI), daily weight gain (DWG), and feed conversion ratio (FCR) is presented in Table 2. The results showed that EEDPJ supplementation had improved (P <0.05) on DWG and FCR. The best results were observed in treatments T3 (0.06% EEDPJ) and T4 (0.09% EEDPJ). However, there was no significant difference (P>0.05) in DFI among the treatment groups.

Table 2. Daily feed intake (DFI), daily weight gain (DWG), and feed conversion ratio (FCR) of day 28.

Variables	Treatment					SEM	p-value
	T0	T1	T2	T3	T4		
DWG (g/bird/day)	60.87^{b}	62.36^{b}	61.00^{b}	66.44^{a}	67.00^{a}	0.74	0.00
DFI (g/bird/day)	112.59	109.7	111	111.98	111.29	0.43	0.56
FCR	1.85^{a}	1.76^{b}	1.82^{a}	1.68^{c}	1.66^{c}	0.02	<0.001

[abc]Different superscripts in the same row indicate significant differences (P<0.05). T0: basal diet (control); T1: basal diet + 0.09% non-encapsulated Japanese papaya leaf extract; T2: basal diet + 0.03% EEDPJ; T3: basal diet + 0.06% EEDPJ; T4: basal diet + 0.09% EEDPJ. SEM: standard error of the mean.

Total bacteria and small intestine pH

Data on total LAB, coliforms, and small intestine pH are presented in Table 3. The findings indicated that EEDPJ supplementation had improved (P<0.05) the total LAB count, coliform count, and intestinal pH of broiler chickens. Treatments T1, T2, T3, and T4 had higher LAB counts than the control treatment (T0). A significantly lower pH was showed in treatments T2, T3, and T4 than in T0. Additionally, coliform counts were significantly reduced in all treatments compared with T0.

Relative weight of lymphoid organs, H/L ratio, and protein digestibility

Data on the relative weights of lymphoid and the H/L are shown in Table 4. Analysis of variance revealed that EEDPJ supplementation had improved (P<0.05) the relative weights of the bursa of Fabricius and spleen, H/L ratio, and protein digestibility. There was no significant effect (P>0.05) on the relative weight of the thymus. Bursa of Fabricius relative height was higher at T3 and T4 than at T0 and T1. The spleen weight was significantly higher at T2, T3, and T4 than at T0. The H/L ratio was significantly lower in all treatment groups (T1-T4) that a T0.

Table 3. Total bacteria and small intestine pH.

Parameter	T0	T1	T2	T3	T4	SEM	p-value
LAB (Log cfu/g)	6.37^{b}	7.16^{a}	7.42^{a}	7.62^{a}	7.55^{a}	0.12	<0.001
Coliform (Log cfu/g)	3.39^{a}	2.85^{b}	2.84^{b}	2.64^{bc}	2.59^{c}	0.71	<0.001
pH	6.51^{a}	6.44^{ab}	6.40^{b}	6.29^{c}	6.22^{c}	0.27	0.00

^{abc}Different superscripts in the same row indicate significant differences (P<0.05). T0: basal diet (control); T1: basal diet + 0.09% non-encapsulated Japanese papaya leaf extract; T2: basal diet + 0.03% EEDPJ; T3: basal diet + 0.06% EEDPJ; T4: basal diet + 0.09% EEDPJ. SEM: standard error of the mean.

Table 4. Relative lymphoid organ weight, heterophil/lymphocyte ratio, and protein digestibility.

Parameter	T0	T1	T2	T3	T4	SEM	p-value
Bursa of Fabrisius (%)	0.31^{c}	0.36^{c}	0.36^{bc}	0.40^{ab}	0.41^{a}	0.01	0.00
Spleen (%)	0.16^{c}	0.22^{abc}	0.18^{ab}	0.27^{ab}	0.28^{a}	0.15	0.02
Thymus (%)	0.56	0.58	0.58	0.58	0.59	0.01	0.96
H/L Ratio (%)	0.92^{a}	0.74^{b}	0.78^{b}	0.78^{b}	0.77^{b}	0.01	<0.001
Protein Digestibility (%)	73.21^{c}	76.39^{b}	78.75^{a}	78.86^{a}	78.99^{a}	0.57	<0.001

^{abc}Different superscripts in the same row indicate significant differences (P<0.05). T0: basal diet (control); T1: basal diet + 0.09% non-encapsulated Japanese papaya leaf extract (EDPJ); T2: basal diet + 0.03% encapsulated extract (EEDPJ); T3: basal diet + 0.06% EEDPJ; T4: basal diet + 0.09% EEDPJ. SEM: standard error of mean.

Discussion

Statistical analysis indicated that EEDPJ supplementation did not significantly imrpoved daily feed intake of broiler chickens. Feed consumption was relatively uniform across all treatments, indicating that the inclusion of EEDPJ did not interfere with fundamental energy regulation or basal energy needs.

The significant improvement in DWG observed in T3 and T4 was likely the cumulative result of the multiple positive effects of EEDPJ. Improvements in digestive tract health, indicated by increased LAB and reduced coliform counts, created a more favorable gut environment for nutrient digestion and absorption, while reducing nutrient competition from pathogenic microbes (Choct, 2009). The increased protein digestibility indicated that more essential amino acids were available for muscle protein synthesis and tissue growth. Amino acids are essential for tissue development and growth in broilers, and their increased availability directly contributes to improved weight gain (Alagawany *et al*., 2021). Protein and amino acid intake has a function in maintaining immunocompetence, protecting against disease, and enhancing performance (Beski *et al*., 2015).

Improved immune status, evidenced by increased bursa of Fabricius and spleen relative weights and a reduced H/L ratio as a stress indicator, suggested that EEDPJ-treated broilers were in better physiological conditions. Healthy and non-stressed chickens can allocate more energy and nutrients to growth rather than fight infections or manage stress (Lara and Rostagno, 2013). Previous studies on phytogenic feed additives have also reported improvements in DWG due to their bioactive compounds (Brenes & Roura, 2010; Diaz-Sanchez *et al*., 2015). The encapsulation of EEDPJ likely enhances the bioavailability and efficacy of the active compounds, leading to improved growth responses.

The significant improvement in FCR following EEDPJ supplementation indicates enhanced digestive tract function in broilers. This feed efficiency improvement resulted from the higher DWG in T3 and T4 without a corresponding increase in feed intake. The ability to convert the same amount of feed into greater body weight gain reflects better nutrient utilization efficiency (Prakash *et al*., 2020), which is supported by the observed increase in protein digestibility. FCR average in this study was low, indicating excellent efficiency. Sugiharto *et al*. (2011) reported an average FCR of 1.87 when turmeric extract was added to broiler diets.

The gain of LAB at EDPJ and EEDPJ treatment is a positive indication of improvement gut health in broilers. Lactic acid bacteria (BAL) keeping the balance of intestinal microbiota. (Abdel-Moneim *et al*., 2020; Xiao *et al*., 2024). LAB generating lactic acid and short-chain fatty acids (SCFAs), via fermentation of indigestible carbohydrates (Gibson *et al*., 2017). SCFAs, especially butyrate, help to maintain intestinal mucosal sincerity and barrier function (Peng *et al*., 2009). Additionally, LAB generating antimicrobial substances, like a hydrogen peroxide and bacteriocins, that specifically forbid pathogen growth (Lagha *et al*., 2017). They also drive competitive exclusion, where LAB collide with pathogens for nutrients and sticking sites in the gut, thus minimalize pathogen colonization (Schneitz, 2005). Li *et al*. (2022) prove that LAB exhibit antimicrobial activity and can tolerate low-pH environments.

Japanese papaya leaf (*Cnidoscolus aconitifolius*) have many bioactive compounds such as flavonoids, saponins, and alkaloids (Chikezie *et al*., 2016), which possibly contribute to increased LAB counts. Flavonoids, such as quercetin and kaempferol, own prebiotic effects that provoke the growth of beneficial bacteria, with providing fermentation substrates or modifying gut conditions to favor LAB (Dueñas *et al*., 2015). At definited concentrations, saponins have also been shown to reduce E. coli and coliform populations in the gut (Bera *et al*., 2019). Encapsulation, as used in T2, T3, and T4, possibly enhances the efficacy of these bioactive compounds by keeping them from degradation and assure targeted release in the intestine.

The significant decrease in total coliforms observed in all EDPJ and EEDPJ treated groups, can be related to the increase in LAB levels (Arreguin-Nava *et al*., 2020) and the antimicrobial effects of EEDPJ bioactive compounds, such as saponins, which interfere bacterial membranes, particularly in gram-negative bacteria (Wei *et al*., 2025). Flavonoids gives antimicrobial effects through mechanisms such as the hitch of nucleic acid synthesis or cytoplasmic membrane disruption (Cushnie & Lamb, 2005). Various plant extracts reported can reducing E. coli and other coliforms in poultry gut (Tiihonen *et al*., 2010).

EEDPJ supplementation also significantly reduced the pH of the small intestine (Table 3), especially in T2 (6.29), T3 (6.27), and T4 (6.22). This acidification is a key finding that link increased LAB counts with reduced coliform populations. LAB ferment carbohydrates into organic acids, mainly lactic acid and SCFAs, which accumulate in the intestinal lumen and lower the pH (Gibson *et al*., 2017). This acidic environment favors the growth of acidophilic LAB, while inhibiting acid-sensitive pathogens (Li *et al*., 2022).

The superior acidification effects of EEDPJ treatments (T2, T3, T4) compared to non-encapsulated EDPJ (T1) suggest the added value of encapsulation. As a controlled-delivery system, encapsulation preserve bioactive compounds from early degradation and assure gradual release at distal intestinal segments (jejunum and ileum), where they are more effective in modulating the microbiota (Alemzadeh *et al*., 2020). Targeted release gives higher bioactive concentrations to reach active sites, enhancing LAB stimulation, organic acid production, and overall pH reduction.

Supplementation with EEDPJ significantly improved the relative weights of the bursa of Fabricius and spleen in broilers, particularly at the highest dose (T4). This shows a potential immunostimulatory effect. It mean that EEDPJ can enhance the development and function of lymphoid organs, which play key roles in the immune defense system of chickens. Flavonoids and saponins as are known to exhibit immunomodulatory properties. Flavonoids can affect lymphocyte proliferation and cytokine production, while saponins act as adjuvants that boost immune responses (Rajput *et al*., 2007; Martínez *et al*., 2019).

The H/L ratio across all EEDPJ-treated groups showed that both encapsulated and non-encapsulated Japanese papaya leaf extract supplementation reduced physiological stress in broilers. This stress reduction might result from improved overall health and decreased subclinical pathogenic challenges in the digestive tract. The immunomodulatory effects of EEDPJ, proven in increased lymphoid organ weight and decreased H/L ratio, possibly contributed indirectly to the improved production performance. Chickens with lower stress levels and stronger immune systems desire less energy and nutrients for stress management and infection control (Calefi *et al*., 2017). This allows more metabolic resources to be serve for growth and development.

The absence of a significant effect on thymus weight may be attributed to natural thymic involution with age. According to Song *et al*. (2021), the peak development of thymus reaches at a very early age, and then undergoes gradual regression. Early signs of involution include reduced cortex-to-medulla ratio and increased adipose tissue deposition (Youm *et al*., 2010).

The differing responses among lymphoid organs may indicate specific target actions of the bioactive compounds in EEDPJ or differences in the sensitivity of each lymphoid organ to the type and concentration of the modulators administered.

The significantly higher protein digestibility observed in T2, T3, and T4 suggests Japanese papaya leaves bioactive compunds, effectively enhance protein digestion in broilers. These compounds help modulate the gut microbial balance, suppress pathogens, and support beneficial bacteria. A healthy microbial environment minimizes nutrient competition by pathogens and prevents mucosal damage, thus promoting optimal digestive function (Pacheco and Sperandio, 2015; Horrocks *et al*., 2023). Flavonoids, owing to their antioxidant properties, protect intestinal mucosal cells, so that preserving absorptive integrity and function (Carioca *et al*., 2022). At appropriate concentrations, saponins may increase intestinal cell membrane permeability, facilitating amino acid and small peptide absorption (Johnson *et al*., 1986). These findings indicate that EEDPJ at doses ranging from 0.03% to 0.09% enhanced protein digestion efficiency. This aligns with Viveros *et al*. (2011) research, that reported a certain plant extracts improve nutrient digestibility in broilers.

Conclusion

Supplementation with encapsulated Japanese papaya leaf extract at a level of 0.06% (T3) in the diet can improve protein digestibility, immune health, and overall performance of broiler chickens.

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Conflict of interest

The authors have no conflict of interest to declare.

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