



Administration of Encapsulated of *Lactobacillus Plantarum* and *Peperomia Pellucida* Plant Extract on Performance, Protein Digestibility and Immunity of Broiler Chickens

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Abstract | This study aims to determine the effects of administration of encapsulated *Lactobacillus plantarum* and *Peperomia pellucida* plant extract (ELPPP) on performance, protein digestibility and immunity of broiler chickens. A total of 200 Ross strain broilers were arranged in a completely randomized design with 5 treatment groups and 4 replications. The treatments include: T0 (control), T1 (0.1% ELPPP), T2 (0.2% ELPPP), T3 (0.3% ELPPP), T4 (0.4% ELPPP). ELPPP was applied from day 8 to 35. The parameters observed included broiler performance, bacterial populations in the small intestine, small intestine pH, protein digestibility, malondialdehyde (MDA), superoxide dismutase (SOD), H/L ratio and relative weight of immune organs. The results showed that ELPPP increased ($p < 0.05$) performance, small intestine bacterial balance, protein digestibility, SOD level, relative weights of immune organs, but decreased small intestinal pH, MDA levels and H/L ratio. However, there was no significant effect ($p > 0.05$) on ration feed consumption. In conclusion, the administration of ESCLP improved the performance, small intestine bacterial balance, protein digestibility, and immune system of broiler chickens.

Keywords | Antioxidant, Broiler growth, Intestinal microbial, Probiotic, Phytobiotic

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INTRODUCTION

Broiler chicken is a strain of chicken that has superior genetic and productivity, especially in meat production. However, rearing in a tropical environment has its own challenges, because broilers are sensitive to high ambient temperatures, so the potential for exposure to stress is very high (Kpomasse *et al.*, 2021). Stress can have a negative im-

pact on the physiology, metabolism, immunity and performance of broiler chickens (Kikusato and Toyomizu, 2023). Nutritional manipulation such as feed additive supplementation is one strategy to overcome the adverse effects of stress (Abdel-Moneim *et al.*, 2021). Since the ban on antibiotic growth promoter (AGP), the use of natural feed additives such as a combination of probiotics and phytobiotics can be an alternative to AGP (Sapusha *et al.*, 2021).

The use of probiotics from *Lactobacillus plantarum* is known to provide beneficial effects for broiler chickens. Several studies have shown positive effects of *Lactobacillus plantarum* feeding on immune status, gut microecological balance and performance in broiler chickens (Wang *et al.*, 2023). Additionally, several studies have shown that the combined administration of probiotics and phytobiotics has a better impact on chickens compared to separate administration (Yuanita *et al.*, 2019; Sapsuha *et al.*, 2021). Ferdous *et al.* (2019) also reported that probiotics combined with phytobiotics were able to reduce intestinal pH which has an impact on reducing coliforms and increasing the performance of broiler chickens. *Peperomia pellucida* plant extract can be used as a phytobiotic. *Peperomia pellucida* plant extract contains phenolics (627.45 mg/g), flavonoids (868.78 mg/g) and tannins (13.06 mg/g) (Fakayode *et al.*, 2021). Phenolic compounds act as a source of antioxidants and antimicrobials (Srivastava *et al.*, 2023). Several studies have shown that phenolic compounds and flavonoids contained in phytobiotics can increase the activity of antioxidant enzymes in broiler chickens (Kikusato, 2021) so as to minimize the negative effects of stress. In addition, polyphenols contained in phytobiotics can support the growth of probiotic bacteria (Prakasita *et al.*, 2019). Therefore, the combination of *Lactobacillus plantarum* and *peperomia pellucida* plant extract is expected to synergize with each other.

Table 1: Composition and nutrient contents of basal feed.

Ingredients	Compositions	
	Starter (8-21)	Finisher (22-35)
Corn	50.41	55.41
Rice bran	14.74	14.74
Soybean meal	24.00	19.00
Meat bone meal	10.00	10.00
CaCO3	0.30	0.30
Premixes	0.25	0.25
Lysine	0.1	0.1
Methionine	0.20	0.20
Total	100.00	100.00
Nutrient content		
Metabolizable energy (kcal/kg)	3,062	3,073
Crude protein (%)	21.63	19.68
Crude fiber (%)	4.37	4.45
Ether extract (%)	3.39	4.33
Calcium (%)	1.44	1.42
Phosphorus (%)	0.75	0.72

¹Result of Analysis of Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang;²Metabolizable energy was calculated based on formula (Bolton 1967) as follows: 40.81 [0.87 (Crude Protein + 2.25 crude fat + nitrogen-free extract) + 2,5].

On the other hand, secondary metabolite compounds contained in plant extracts (Sugihato and Ayasan, 2022) and probiotic viability (Dumitru *et al.*, 2021) are highly susceptible to storage, light, pH, temperature, heat and oxygen. This causes the stability and availability of these bioactive compounds to be low, thus reducing their effectiveness against their host (Agriopoulou *et al.*, 2023). Encapsulation is effective in protecting the active compounds contained in probiotics and plant extracts so that the release of active compounds can be controlled according to the targeted organ (Klojdova *et al.*, 2023). In addition, encapsulation also helps to mask the unpleasant taste and smell of the compound so that it does not affect the palatability of the chicken (Agustyaningsih *et al.*, 2022). To our knowledge, there is no literature regarding the encapsulated mixture of *Lactobacillus plantarum* and *Peperomia pellucida* plant extract. Therefore, this research aims to determine the effect of administration encapsulated *Lactobacillus plantarum* and *Peperomia pellucida* plant extract on the performance, protein digestibility and immunity of broiler.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND DIETS

This research was supervised and was approved by the Animal Research Ethics Committee, Faculty of Animal and Agricultural Sciences, Diponegoro University under approval number 60-07/A-14/KEP-FPP. A total of 200 Ross broilers chicks with an average body weight of 45,16 ± 0,10 g from a commercial hatchery were used in this study. The chicks were raised in a broiler house with a litter floor (rice husk) and manual feeders and drinkers. The chickens were raised for 35 days and given commercial diet and basal diet. The basal diet is formulated according to the recommended requirement standards by NRC (1994) in Table 1. Commercial diet (pre-starter) was given at the age of 1-7 days and basal diet (starter and finisher) and ELPPP was given at the age of 8-35 days. ELPPP are given as percentages, based on feed consumption. Treatment dosage selection based on research Yuanita *et al.* (2019).

EXPERIMENTAL DESIGN AND TREATMENTS

A completely randomized design was used in this study. On day 8, broiler chicks (weight 175.60 ± 3.16 g) were randomly allocated into 5 treatments and 4 replicates (each experimental unit contained 10 chicks). The treatments were as follows:

- T0: basal feed without encapsulated *Lactobacillus plantarum* and *Peperomia pellucida* plant extract (ELPPP)
- T1: basal diet + 0.1% ELPPP
- T2: basal diet + 0.2% ELPPP
- T3: basal diet + 0.3% ELPPP
- T4 : basal diet + 0.4% ELPPP

PREPARATION OF ENCAPSULATED OF *LACTOBACILLUS PLANTARUM* AND *PEPEROMIA PELLUCIDA* PLANT EXTRACT

Peperomia pellucida plants were obtained around Diponegoro University. The collected *Peperomia pellucida* was weighed, washed and dried. The drying process uses an oven with a temperature of 50 °C and is ground until powder is obtained. *Peperomia pellucida* plant extract was made based on the method described by (Gouda *et al.*, 2021). The extract was dissolved in distilled water in a ratio of 3:1 (ml:ml). Preparation of *Lactobacillus plantarum* solution (10¹⁰ cfu/ml) starts with dissolving skim milk as much as 10% of the distilled water solution. After that, the skim milk solution and *Lactobacillus plantarum* were mixed with *Lactobacillus plantarum* (2:1, ml:ml). *Peperomia pellucida* extract solution was mixed with *Lactobacillus plantarum* (1:1, ml:ml). Then encapsulation was carried out.

The encapsulation of the mixture of *Lactobacillus plantarum* and *Peperomia pellucida* plant extract refers to the method of Agustyaningsih *et al.* (2022). The coating material used for encapsulation was maltodextrin. Maltodextrin was dissolved in distilled water in a ratio of 1:3 (ml:ml). The dissolved maltodextrin was mixed with a mixture of *Lactobacillus plantarum* and *peperomia pellucida* extract in a ratio of 1:5 (ml:ml) until homogeneous and freeze drying was carried out to obtain an encapsulated mixture of *Lactobacillus plantarum* and *Peperomia pellucida* plant extract. The encapsulated mixture of *Lactobacillus plantarum* and *Peperomia pellucida* plant extract was stored in the refrigerator until use.

ANIMALS REARING MANAGEMENT

During rearing, all experimental units were in a healthy condition and received the same treatment of both temperature and humidity. Temperature, humidity and lighting program were set up as it recommended in Ross management manual. Chickens were firstly weighed to determine initial body weight, and subsequent weighing was performed once a week. Feed and drinking water were provided *ad libitum* during the rearing period. The Chickens were vaccinated newcastle disease (ND) and infectious bronchitis (IB) at after hatch with 0,1 ml spray technique and Gumboro/Infectious Bursal Disease (IBD) vaccine (Medivac Gumboro B vaccine) is given at 12 days of age as one dose through drinking water.

SAMPLING AND PARAMETER MEASUREMENT

PERFORMANCE OF BROILER: Body weight was obtained by weighing the chickens at 35 days of age. Body weight measurements are carried out once a week. Body weight gain was obtained by subtracting the final weight from the initial weight of the treatment. Feed intake was recorded daily by subtracting the total feed given from the remaining feed. Feed conversion ratio was calculated by dividing total feed intake by body weight gain.

MICROBIAL POPULATION: Bacterial populations in the small intestine (lactic acid bacteria and coliforms) were measured based on the method of (Sugiharto *et al.*, 2017). Samples of small intestine digesta from each replicate were taken and put into a sterile cup. Then the digesta samples were analyzed for total bacteria (LAB and Coliform) using the total plate count method. MacConkey agar medium (Merck KGaA) was used to grow coliform bacteria and incubated anaerobically for 24 hours at 38°C. The colonies of coliform bacteria counted were red and colorless. The number of lactic acid bacteria was counted on MRS agar (Merck KGaA) after aerobic incubation at 38°C for 48 hours.

PH OF SMALL INTESTINE: The pH of the small intestine of each experimental unit was measured by taking a sample of chicken small intestine digesta at 35 days of age and measured with a digital pH meter.

PROTEIN DIGESTIBILITY: Protein digestibility test was conducted for 4 days at the age of 32-35 days and measured by a combination of total collection method and indicator (Fe₂O₃) based on the method of (Afro *et al.*, 2023). During data collection, chickens were placed in battery cages with 20 excreta collection units filled with one chicken. The Fe₂O₃ indicator is 0.5% of the ration, given alternately on the first and third days. On the first day, chickens were given the treatment ration that had been added with the indicator for 24 hours. Excreta collection began when the feces first came out red until the second day changed color. This was done until the fourth day. Every day, feces were collected and sprayed with 0.2 N HCL every 2 hours (Krismiyanto *et al.*, 2022). The collected excreta were weighed, dried and analyzed for crude protein content using the Kjeldahl method. Calculation of protein digestibility based on the formula according to McDonald *et al.* (1997).

SUPEROKSIDA DISMUTASE (SOD) DAN MALONDIALDEHYDE (MDA): Blood serum from broiler chickens aged 35 days in each replication was taken for superoxide dismutase (SOD) and malondialdehyde (MDA) tests based on the method of (Agusetyaningsih *et al.*, 2022). SOD activity was tested according to the ability of the sample to suppress the auto-oxidation of pyrogallol. The mixture used consisted of samples, 50 mM Tris-HCl (pH 8.2) and 1 mM pentaacetic acid diethylenetriamine. Pyrogallol (final concentration 0.2 mM) was added to determine the reaction and the absorption value was calculated kinetically. The concentration of SOD was stated in units of U/ml. MDA activity was measured using the thiobarbituric acid (TBA) reactive substance test. Samples were vortexed, 8.1% sodium dodecyl was added, and left at room temperature for 10 minutes. Control samples were done in the same way. After incubation, the samples were added 20% acetic acid and 0.6% TBA. Then the tubes were placed in a water bath at 90-95°C for 1 hour. After that, butanol:pyridine (15:1) was applied to

the supernatant. Then the mixture will be vortexed and centrifuged. MDA concentration is expressed as nmol/ml.

HETEROPHIL-LIMFOSIT RATIO: Measurement of the heterophil/lymphocyte ratio (H/L) was carried out using the leukocyte differential observation method based on the method described by [Sunu et al. \(2021\)](#). Measurement of leukocyte differential was done by making a blood smear preparate. Blood samples were taken on day 35 of each replicate and put in EDTA tubes. Blood smears were made on object glass, then fixed with methanol, stained with giemsa, washed with water and allowed to dry at room temperature. The finished smear preparation was observed with a microscope and the percentage of heterophils and lymphocytes obtained was calculated. Then the percentage of heterophils and lymphocytes was multiplied by the number of leukocytes to obtain the number of heterophils and lymphocytes. Determination of the H/L ratio was obtained by dividing the number of heterophils by the number of lymphocytes.

Table 2: Performance of 35-day-old broilers.

Variabel	Treatment					SEM	p- value
	T0	T1	T2	T3	T4		
Body weight (g)	1651 ^d	1717 ^c	1884 ^b	1908 ^{ab}	1952 ^a	27.57	0.000
Weight gain (g)	1477 ^d	1543 ^c	1711 ^b	1732 ^{ab}	1773 ^a	27.28	0.000
Feed intake (g)	2567	2445	2644	2595	2608	26.03	0.131
Feed conversion	1.73 ^a	1.58 ^b	1.54 ^b	1.49 ^b	1.47 ^b	0.026	0.01

a. b. c. d means in the same row with superscripts are significantly different ($p < 0,05$).

RELATIVE WEIGHT OF IMMUNE ORGANS: Measurement of the relative weight of immune organs of each replicate was carried out on day 35. Chickens were slaughtered and removed immune organs (Bursa fabricius, thymus and spleen), then weighed and expressed as a percentage of live weight according to [Fajrih et al. \(2014\)](#).

$$\text{Immune organ weight (\%)} = \frac{\text{Immune organ (g)}}{\text{Live weight (g)}} \times 100\%$$

STATISTICAL ANALYSIS

The data has met the assumptions of normality and homogeneity tests. The data were analyzed by analysis of variance (ANOVA) using SPSS software and if there was a significant effect then continued with the Duncan test.

RESULTS AND DISCUSSION

PERFORMANCE OF BROILERS

The results of statistical analysis showed that the provision of encapsulated *Lactobacillus plantarum* and *Peperomia pellucida* plant extract (ELPPP) in this study (Table 2) had

a positive effect on increasing ($p < 0.05$) body weight gain by 1.03 (T1), 1.14 (T2), 1.15 (T3) and 1.18 (T4) times that of the control and broiler body weight gain by 1.09 (T1), 1.12 (T2), 1.16 (T3) and 1.20 (T4) times that of the control. It also reduced ($p < 0.05$) the feed conversion ratio by 1.09 (T1), 1.12 (T2), 1.16 (T3) and 1.17 (T4) times that of the control. However, feed intake was not affected ($p > 0.05$) the treatment.

This research shows that administration ELPPP can increase body weight and body weight gain. Several studies have shown that giving a mixture of probiotics and phyto-biotics to broiler chickens can increase broiler body weight ([Ferdous, 2019](#); [Sapsuha et al., 2021](#)). In this case, it is possible that the metabolite compounds contained in the mixture of *Lactobacillus plantarum* and *Peperomia pellucida* plant extract that are encapsulated are optimally absorbed so that it can be utilized properly. [Natsir et al. \(2019\)](#) reported that encapsulation treatment was more effective in improving intestinal performance, ecology and morphology compared to non-encapsulation treatment. There was no significant ($p > 0.05$) difference in feed intake in this study. This may occur because encapsulation can mask the taste and smell of ELPPP so that it does not affect palatability and feed intake. [Sugiharto and Ayasan. \(2022\)](#) reported that undesirable taste and smell can be masked by encapsulation. However, this providing evidence that the synergistic effect of ELPPP can improve feed efficiency, as indicated by a lower feed conversion ratio compared to the control. This means that the nutrients in the feed are optimally absorbed. [Sapsuha et al. \(2021\)](#) reported that probiotics and phyto-biotics have pharmacological effects as antibacterials that can improve intestinal health so that the absorption of nutrients is optimal and has an impact on improving broiler performance.

Table 3: Microbial population, pH of small intestine and protein digestibility.

Variable	Treatment					SEM	p-value
	T0	T1	T2	T3	T4		
LAB (log cfu/g)	8.73 ^c	9.18 ^b	9.30 ^{ab}	9.40 ^{ab}	9.49 ^a	0.37	0.000
Coliforms (log cfu/g)	2.81 ^a	2.57 ^b	2.51 ^{bc}	2.45 ^{bc}	2.39 ^c	0.68	0.000
pH	6.51 ^a	6.35 ^b	6.22 ^c	6.21 ^c	6.10 ^c	0.36	0.000
Protein digestibility	78.94 ^c	82.92 ^b	83.10 ^{ab}	84.10 ^{ab}	86.00 ^a	0.62	0.000

a.b.c means in the same row with superscripts are significantly different ($p < 0.05$).

MICROBIAL POPULATION AND PROTEIN DIGESTIBILITY

Data on microbial population, small intestine pH, and protein digestibility in treated broiler chickens are presented in Table 3. ELPPP feeding increased ($p < 0.05$) LAB population by 1.05 (T1), 1.07 (T2), 1.08 (T3) and 1.09 (T4)

times that of the control and protein digestibility by 1.05 (T1), 1.05 (T2), 1.07 (T3) and 1.09 (T4) times that of the control. It also reduced ($p < 0.05$) coliform population by 1.09 (T1), 1.12 (T2), 1.15 (T3) and 1.18 (T4) times that of the control and small intestinal pH by 1.03 (T1), 1.05 (T2), 1.05 (T3) and 1.07 (T4) times that of the control.

The results of this study showed that chickens given ELPPP had a high ($p < 0.05$) population of lactic acid bacteria (LAB) and there was a decrease ($p < 0.05$) in the population of coliform compared to controls. This condition may be due to the antibacterial activity contained in ELPPP. Thus, beneficial bacteria are able to compete for nutrients in the small intestine through competitive exclusion mechanisms with pathogenic bacteria. Through this mechanism, beneficial bacteria can help maintain the balance of the intestinal microbiota. In addition, the decrease ($p < 0.05$) in pH in this study played a role in improving the balance of bacteria in the small intestine. This study is in line with Sapsuha *et al.*, (2023) who showed a decrease in pH in the small intestine when given a mixture of nutmeg extract and *Lactobacillus plantarum*. The decrease in pH in the small intestine seems to be caused by organic acids or short chain fatty acids (SCFA) produced by *Lactobacillus plantarum*, although SCFA levels were not measured. Vimon *et al.* (2023) that lactic acid and SCFA caused a condition of the intestine to become acidic, which affected the decrease in the intestinal pH. In addition, acidic pH conditions in the digestive tract are needed as a precursor to increase the activity of digestive enzymes (Pearlin *et al.*, 2020). This was proven to increase ($p < 0.05$) protein digestibility in this study. The increased LAB condition allows protein to be absorbed optimally. Mangisah *et al.* (2020) reported that increasing LAB has a positive impact on intestinal health and the growth of intestinal villi, which indirectly improves nutrient digestibility and absorption. LAB also produce proteolytic enzymes that are useful in digesting protein (Kieliszek *et al.*, 2021).

IMMUNE RESPONSE

The Administration of ELPPP (Table 4) to the diet increased ($p < 0.05$) the relative weight of bursa by 1 (T1), 1.15 (T2), 1.17 (T3) and 1.20 (T4) times that of the control and thymus by 1.41 (T1), 1.64 (T2), 1.72 (T3) and 1.76 (T4) times that of the control. But had no effect ($p > 0.05$) on the relative weight of spleen (Table 4).

The results of this study showed a significant ($p < 0.05$) increase in the relative weight of the bursa and thymus compared to the control. These results are consistent with the study by Mangisah *et al.* (2021) that the administration of phytobiotics and probiotics increased the relative weight of the bursa fabricius. It has a positive effect on improved immunity. In some cases, the ability and function of the immune system in chickens can be seen from the relative weight of immune organs (Sugiharto *et al.*, 2017). The me-

tabolite compounds contained in ELPPP may contribute to increased number of immune cells and improved immune response thus increasing the relative weight of the fabricius and thymus bursa. In addition, increased balance of intestinal microflora is able to stimulate improved immune response (Liu *et al.*, 2022) (Table 4) thereby reducing the work of immune organs. Interestingly, the relative weight of the spleen in this study was not significantly different ($p > 0.05$). It seems that the bursa and thymus have been able to overcome the negative effects of oxidative stress so that antigens entering the blood are minimal and the work of the spleen becomes lighter. Fajrih *et al.* (2014) reported that the high amount of antigen entering the spleen causes the spleen to work harder, resulting in an increase in the size of the spleen.

Table 4: Relative weight of immune organs.

Variable	Treatment					SEM p-value	
	T0	T1	T2	T3	T4		
Bursa of fabricius	0.078 ^b	0.078 ^b	0.090 ^{bc}	0.092 ^a	0.094 ^a	0.002	0.019
Spleen	0.118	0.129	0.131	0.132	0.133	0.003	0.680
Thymus	0.265 ^b	0.375 ^a	0.436 ^a	0.457 ^a	0.467 ^a	0.207	0.001

a,b,c means in the same row with superscripts are significantly different ($p < 0.05$).

There was a significant difference ($p < 0.05$) between broilers fed ELPPP compared to the control. Data on superoxide dismutase (SOD), malondialdehyde (MDA) and H/L ratio of broiler chickens are presented in Table 5. SOD levels increased ($p < 0.05$) by 2.72 (T1), 3.09 (T2), 3.18 (T3) and 3.15 (T4) times that of the control. It also a decrease ($p < 0.05$) in MDA levels by 1.41 (T1), 1.58 (T2), 1.70 (T3) and 1.83 (T4) times that of the control and a decrease ($p < 0.05$) in H/L ratio by 1.46 (T1), 1.52 (T2), 1.54 (T3) and 1.85 (T4) times that of the control.

Table 5: Stress indicators.

Variable	Treatment					SEM p-value	
	T0	T1	T2	T3	T4		
SOD (U/ml)	5.61 ^c	15.30 ^b	17.34 ^{bc}	17.85 ^{bc}	19.89 ^a	1.22	0.000
MDA (nmol/ml)	4.25 ^a	3.01 ^b	2.68 ^{bc}	2.49 ^{bc}	2.31 ^c	1.79	0.000
H/L Ratio	1.11 ^a	0.76 ^b	0.73 ^{bc}	0.72 ^{bc}	0.60 ^c	0.428	0.000

a,b,c means in the same row with superscripts are significantly ($p < 0.05$).

This study shows that the administration of ELPPP in feed is able to increase SOD levels and decrease MDA levels in broiler chickens. These findings are consistent with those reported by Dong *et al.* (2019) that there was a decrease in MDA levels and an increase in antioxidant enzymes in chickens treated with probiotics and phytobiotics. The antioxidant content, especially flavonoids contained in ELPPP, may provide protection against the negative effects of oxidative stress during

maintenance. SOD plays a role in providing protection from free radicals. This causes the MDA levels of broiler chickens to be lower in the administration of ELPPP.

The increased in SOD levels in this study, seems to have a positive effect on immune enhancement, which is marked a low the H/L ratio ($p < 0.05$). Low H/L ratio values in ELPPP feeding indicate that chickens experience mild stress. Changes in the H/L ratio were associated with the level of stress experienced by the chickens (Sugiharto *et al.*, 2017). in this case, the ELPPP-treated feed helped to reduce oxidative stress. Several studies have shown that the administration of phytobiotics to chickens experiencing oxidative stress can reduce H/L ratio levels (Isroli *et al.*, 2017). Changes in SOD, MDA (Saracila *et al.*, 2021) and H/L ratio (Mohammed *et al.* 2021) are a response to oxidative stress caused by excessive production of reactive oxygen species (ROS) in the body.

CONCLUSIONS AND RECOMMENDATIONS

The administration of encapsulated *Lactobacillus plantarum* and *Peperomia pellucida* plant extract extracts (ELPPP) improves broiler chicken performance, small intestine bacterial balance, protein digestibility, and immune system of broiler chickens. The optimal dosage for T4 treatment (basal diet + 0.4% ELPPP).

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NOVELTY STATEMENT

The novelty of this research is the use of encapsulated *Lactobacillus plantarum* and *Peperomia pellucida* plant extract in broiler chicken

AUTHOR'S CONTRIBUTIONS

Muhammad Luthfi Hidayat: Compiled the research concept, methodology, collected and processed research data and compiled the article.

Istna Mangisah: Supervised this research.

Lilik Krismiyo: Conducted data curation, methodology and supervised this research.

Vitus Dwi Yunianto: Conceptualized and supervised this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests in the publication of this article.

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