Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 1, 1-6

Effectiveness of feed additive mixture on performance, gut health and antioxidant status of broiler chicken

Luthfi D. Mahfudz, Binti Ma'rifah*, Teysar Adi Sarjana, Arif Maulana

Department of Animal Science, Faculty of Animal and Agricultural Sciences Universitas Diponegoro, Semarang 50275, Central Java, Indonesia.

ARTICLE INFO

Recieved: 30 September 2025

Accepted: 09 December 2025

*Correspondence:

Corresponding author: Binti Ma'rifah
E-mail address: marifahbinti2908@gmail.com

Keywords:

Morinda citrifolia leaf extract, Citric acid, *Lactobacillus acidophilus*, Body weight gain, Antioxidant status.

ABSTRACT

This study aimed to evaluate the effect of a feed additive mixture of noni (*Morinda citrifolia*) leaf extract, citric acid, and *Lactobacillus acidophilus* (NCL) on superoxide dismutase (SOD) levels, malondialdehyde (MDA) levels, duodenal villi height, duodenal crypth depth, and body weight gain. A total of 200 broiler chickens aged 8 days, with an average body weight of 213.875 ± 8.9 g. The experimental design employed a completely randomized design (CRD) with four treatments, five replications, and 10 broilers per replicate (T0: drinking water only; T1: drinking water + 1% feed additive; T2: drinking water + 2% feed additive; T3: drinking water + 3% feed additive). Administration of NCL through drinking water significantly (P<0.05) increased SOD levels by 1% and 2%. The administration of feed additives via drinking water significantly reduced (P<0.05) MDA levels in T2. Treatments T1, T2, and T3 showed significantly higher (P<0.05) villus heights than the control group (T0), while no significant differences (P>0.05) were observed among T1, T2, and T3. The greatest crypt depth was recorded in T2, whereas the smallest crypt depth was noted in T0. The feed additive mixture (NCL) led to a significant increase (P<0.05) in body weight gain of broiler chickens. In conclusion, combined effects of phytobiotics, acidifiers, and probiotics in NCL can be an effective additive feed approach to improve antioxidant status, gut morphology, and growth performance in broiler chickens.

Introduction

Broiler chicken (*Gallus gallus domesticus*) is a type of poultry bred specifically for meat production. They are known for their fast growth and efficient feed conversion, enabling them to reach a market weight of 1.5 to 2.5 kg within 30 to 32 days. However, this rapid growth is accompanied by certain physiological challenges such as a less developed immune system, higher vulnerability to diseases, and the need for intensive management. Broilers also require feed rich in protein and energy to sustain their rapid growth. Despite their efficiency, the flavor of broiler meat is often considered inferior to that of native chickens. To mitigate these issues, feed additives are frequently incorporated into the diet or drinking water of broiler chickens. These non-nutritive additives are added to animal feed to improve livestock health, performance, and feed quality. They can be derived from plant extracts, chemical compounds, or beneficial microorganisms such as lactic acid bacteria (Shil and Chichger, 2021).

Among the various plant-based feed additives, noni (*Morinda citrifolia*) leaf extract has attracted interest due to its phytobiotic properties, which act as antibacterial agents, promote growth, and modulate the immune system (Sibi *et al*., 2012). In contrast, citric acid functions as an acidifier that reduces the gastrointestinal pH, thereby fostering microbial equilibrium and enhancing protein digestibility. The probiotic bacterium *Lactobacillus acidophilus* supports gut health by improving nutrient absorption efficiency and maintaining the balance of the intestinal microbiota (Shil and Chichger, 2021). In this study, a synergistic blend of noni leaf extract, citric acid, and *Lactobacillus acidophilus* was used as a combined feed additive. The reasoning for this mixture is based on the potential complementary benefits that each component offers to enhance the intestinal health and boost the overall performance of broiler chickens. Recent research has indicated that polyphenols found in plant extracts, such as noni, can alter the proteomic profile of *Lactobacillus acidophilus*, affecting proteins associated with metabolism and stress response pathways (Celebioglu *et al*., 2018; Felipe, 2024). These changes improve the adaptability and functionality of bacteria in the gastrointestinal tract. However, the effects of polyphenols depend on their dosage and vary according to their specific types. While polyphenols generally improve probiotic efficacy, certain polyphenols may act as pro-oxidants under particular conditions, potentially hindering bacterial growth (Piekarska-Radzik & Klewicka, 2021). This dual nature underscores the complexity of polyphenol interactions with the gut microbiota and emphasizes the need for a balanced formulation to develop feed additives.

The gut microbiota is essential for the digestion of feed and absorption of nutrients in rapidly growing poultry such as broiler chickens. These microorganisms aid in breaking down fiber and complex carbohydrates that poultry digestive enzymes cannot process (Rowland *et al*. 2018). Furthermore, gut microbes can transform certain feed components into vital vitamins that are needed by the body (Foster *et al*., 2013). These microbes have specific enzymes that decompose complex food molecules and release important nutrients and metabolites that are crucial for the host. Short-chain fatty acids (SCFAs) are a significant by-product of bacterial fermentation in the intestine and are vital for health and metabolism (Turnbaugh *et al*., 2006). Complementary effects among the active compounds from *Morinda citrifolia*, citric acid, and *Lactobacillus acidophilus* can be understood through their synergistic, additive, or antagonistic interactions. Research has shown that certain phenolic compounds display synergistic effects with organic acids like citric acid. This synergy is often due to enhanced stability of the phenolic compounds in acidic environments, which could potentially enhance their efficacy (Vicol & Duca, 2023). This suggests that *Morinda citrifolia*, known for its rich phenolic content, might exhibit enhanced antioxidant activity in the presence of citric acid. *Lactobacillus acidophilus* is a well-known probiotic with health benefits, including antimicrobial activity. When combined with plant-derived compounds, such as those found in *Morinda citrifolia*, the effects might be complementary, resulting in improved antimicrobial efficacy. This could be due to the probiotics' ability to enhance the bioavailability and stability of phytochemicals (Zhu *et al*., 2025). Studies on combinations of natural compounds show that mixtures can prevent the formation of harmful biofilms and reduce antimicrobial resistance, likely due to their ability to target multiple microbial pathways simultaneously (Tan *et al*., 2019; Zhu *et al*., 2025). This indicates that a combination of *Morinda citrifolia* and *Lactobacillus acidophilus*, possibly stabilized or enhanced by citric acid, could act more effectively against pathogenic microbes. Synergistic effects often arise from the interaction of different compounds that have complementary mechanisms of action. In the case of natural compounds, their diverse functional groups may interact with multiple targets within microbial cells, thereby enhancing each other's efficacy while reducing required dosages and side effects (Zhu *et al*., 2025).

Additionally, the gut microbiota plays a crucial role in affecting various performance indicators in broilers, such as feed conversion efficiency, growth rate, apparent metabolizable energy, residual feed intake, and time needed to achieve the target market weight. These elements directly affect the health and productivity of chicken. Understanding the specific interactions between polyphenols, citric acid, and gut microbiota is essential for creating a synergistic feed additive that combines noni leaf extract, citric acid, and *Lactobacillus acidophilus* (NCL). This formulation was designed to boost intestinal health, enhance nutrient absorption, and optimize the overall performance of broiler chickens.

Materials and methods

Ehical approval

The Committee of Animal Ethics of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, approved the present experiment (Approval Number: 61-04/A-09/KEP-FPP).

Feed additive preparation

Mature *Morinda citrifolia* leaf were collected from around Universitas Diponegoro Semarang Central Java Indonesia. The leaf was cleaned and oven-dried at 30ºC. Once dry, they are ground and sieved. 10 g of the *Morinda citrifolia* leaf powder is dissolved in 1 L of water, then heated in a water bath at 100ºC for 30 minutes (Osman *et al*., 2019). Then, it is filtered to obtain the *Morinda citrifolia* leaf extract solution. For citric acid preparation, weigh 1.2 g of citric acid and dissolve it in the *Morinda citrifolia* leaf extract, and mix with *Lactobacillus* acidhopilus (1011 cfu/ml) that have been growth in MRSA (de Man Rogosa Sharpe Agar) medium before.

Experimental design

This study was conducted in July 2025 at the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. The used experimental animals were 200 broiler chickens aged 8 days, with an average body weight of 213.875±8.9 grams. A mini closed-house (MCH) system was used, partitioned into 20 pens, each measuring 80×60 cm. The experimental design employed was a completely randomized design (CRD) with four treatments and five replications, resulting in 20 experimental units. Each unit consisted of ten broiler chickens. Treatments involved the administration of a feed additive mixture containing noni leaf extract, citric acid, and *Lactobacillus acidophilus* (referred to as NCL) through drinking water as follows: T0 (Drinking water only); T1 (Drinking water + 1% feed additive NCL); T2 (Drinking water + 2% feed additive NCL); T3 (Drinking water + 3% feed additive NCL). The nutritional composition of the feeds used in this study is listed in Table 1.

Sample collection

The observed parameters included superoxide dismutase (SOD) and malondialdehyde (MDA) levels, villus height, crypt depth of the duodenum, and body weight gain of broiler chickens. SOD and MDA activities were assessed following to the procedures outlined by Agusetyaningsih *et al*. (2022). Superoxide dismutase (SOD) activity was determined based on the sample's ability to inhibit pyrogallol auto-oxidation and results were presented in U/mL. Malondialdehyde activity were measured using a thiobarbituric acid (TBA) reactive substances assay and reported in nmol/mL. The villus height and crypt depth in each segment were determined using an optical microscope with a digital camera (Leica Microsystems Gmbh, Wetzlar, Germany) (Dokou *et al*., 2023). Body weight were recorded weekly.

Table 1. Nutritional composition of the feed used.

Nutrient	Composition
Crude fiber (%)	9.5*
Crude Protein (%)	19.95*
ME (kcal/kg)	3497.16***
Crude Lipid (%)	7.74*
Crude Fiber (%)	7.55*
Ash (%)	6.50*
Calcium (Ca) (%)	0.80-1.10**
Phospor (P)	Min. 0.50**
Total (%) Urea	ND
Total aflatoksin (µg/Kg)	Maks. 40**
Amino acids:	
Lisin (%)	Min. 1.30**
Metionin (%)	Min. 0.50**
Metionin + Sistin (%)	Min 0.90**
Treonin (%)	Min. 0.80**
Triptofan (%)	Min. 0.20**

*: Proximate Analysis of Feed Nutrition Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University; **: Feed Label Starfeed B 168 SP PT. Sinar Indochem; ***ME : Metabolizable energy was calculated according to Bolton's (1967) formula: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

Data analysis

Collected data were analyzed using analysis of variance (ANOVA) with an F-test to determine the effect of the treatments. If a significant treatment effect was observed, Duncan's multiple range test was conducted to assess differences among treatments.

Results

Blood superoxide dismutase (SOD) activity

The results of the administration of a feed additive mixture consisting of noni leaf extract, citric acid, and *Lactobacillus acidophilus* are presented in Table 2. Superoxide dismutase (SOD) activity in broiler chickens supplemented with NCL ranged from 4.132 to 9.344 units/L. As shown in Table 2, results of the analysis of variance indicated that administration of NCL through drinking water had a significant effect ($P < 0.05$) on increasing SOD activity at the 1% and 2% inclusion levels. However, increasing the dosage to 3% resulted in SOD activity similar to those of the control group (T0).

Malondialdehyde (MDA) levels

Malondialdehyde (MDA) level in broiler chickens resulting from the addition of NCL ranged from 9.215 to 13.080 nmol/mL. As shown in Table 2, the analysis of variance indicated that the administration of feed additives via drinking water significantly ($P<0.05$) influenced MDA level in broiler chickens.

Duodenal villus height

The addition of NCL to the drinking water had a significant effect ($P < 0.05$) on duodenal villus height in broiler chickens (Table 2). Treatments T1, T2, and T3 showed significantly higher villus heights than the control group (T0), while no significant differences were observed among T1, T2, and T3. The highest villus height was observed in T2, whereas the lowest

Table 2. SOD, MDA, duodenal villus height and crypt depth of broiler chickens.

Variables	Treatments				P (Value)
	T0	T1	T2	T3	
SOD (Units/L)	4.36±0.58[b]	8.87±3.05[a]	9.34±4.00[a]	4.13±0.36[b]	0.003*
MDA (ηmol/mL)	10.51±3.13[ab]	10.91±0.70[a]	9.21±1.77[b]	13.08±0.81[a]	0.019*
Duodenal Villi Height (μm)	1.05 ±28.30[b]	1.23±98.47[ab]	1.50 ±,25.26[a]	1.20 ± 26.70[ab]	0.048*
Duodenal Crypt Depth (μm)	153.46±39.38[b]	215.50±23.34[b]	256.37±63.30[a]	207.90±53.05[ab]	0.009*
Body Weight Gain (g)	2093.64±184.16[b]	2188.66±113.27[b]	2363.66±109.75[a]	2276.92±84.34[ab]	0.03

[a,b]Different superscript letters within the same row indicate significant differences (P < 0.05); SOD: superoxide dismutase, MDA: malondialdehyde, T0: drinking water only, T1: drinking water + 1% feed additive NCL, T2: drinking water + 2% feed additive NCL, T3: drinking water + 3% feed additive NCL.

was recorded in T0.

Duodenal crypt depth

Based on the data presented in Table 2, the addition of NCL through drinking water to low-cost feed had a significant effect (P < 0.05) on increasing the crypt depth of the duodenum in broiler chickens. The highest crypt depth was observed in T2 (low-cost feed + 2% feed additive), whereas the lowest was found in T0 (low-cost feed only).

Body weight gain

As shown in Table 2, the addition of NCL to drinking water significantly influenced (P<0.05) body weight gain in broiler chickens.

Discussion

The observed increase in blood SOD activity was attributed to the presence of flavonoid compounds in the leaf extract, which act as antioxidants by inhibiting the formation of free radicals. Noni leaf extract is a phytochemical rich in bioactive compounds such as polyphenols and flavonoids. Polyphenols are closely associated with antioxidant enzyme activity, enhancing their ability to neutralize superoxide radicals. Flavonoids, a subclass of polyphenols, also contribute to antioxidant activity. The synergistic combination of NCL components is believed to enhance metabolic efficiency, maintain the intestinal microbial balance, and improve gastrointestinal function. This condition may support a more efficient conversion of feed into body mass, even though there may be no significant differences in feed intake, energy digestibility, or protein digestibility among the treatments. The increase in SOD activity observed in broiler chickens given the NCL feed additive combination suggests a decrease in oxidative stress. Increased SOD activity helps to preserve the integrity of intestinal epithelial cells, which in turn aids in effective nutrient digestion and absorption. This situation aligns with better intestinal health, as demonstrated by the observed increases in villus height and crypt depth. Optimal crypt depth is associated with improved enterocyte turnover and enhanced growth performance in broilers. As noted by Tang *et al.* (2018), SOD is crucial in the antioxidant defense system of the visceral organs.

In the T1 and T2 treatment groups, SOD activity was notably higher than in T0 and T3. Nevertheless, no significant differences were observed between T1 and T2 or between T0 and T3. Interestingly, increasing the NCL dosage to 3% (T3) did not further boost SOD activity; instead, they were comparable to those of the control group (T0). This indicates that high doses of feed additives may reduce SOD activity. One possible reason is that higher concentrations of reactive oxygen species (ROS) can oxidize and deactivate crucial proteins, including antioxidant enzymes, such as SOD, resulting in diminished enzymatic activity (Phaniendra *et al.*, 2014). Additionally, administration of high levels of citric acid as an acidifier can hinder the function of digestive enzymes and decrease broiler productivity. According to Khan *et al.* (2013), excessive supplementation with citric acid can adversely affect growth, mainly because of a decrease in water and feed consumption. The polyphenols found in noni leaf extracts aid probiotic activity by encouraging the production of anti-inflammatory substances and adjusting immune responses in the gut. Bahmani *et al.* (2015) showed that using probiotics, prebiotics, or synbiotics can significantly elevate blood SOD levels. This finding is further corroborated by Lee *et al.* (2012), who discovered that plant extracts high in flavonoids and other bioactive compounds boosted SOD activity in broiler blood. Flavonoids function as antioxidants that help to reduce oxidative stress and lower the risk of metabolic syndrome.

The probiotic *Lactobacillus acidophilus* contributes to gut microbiota balance and stimulates the production of endogenous antioxidant enzymes. The combination of polyphenols and *L. acidophilus* is believed to enhance the immune function (Lenhardt and Mozes, 2013). Furthermore, citric acid acts as an antioxidant acidifier, capable of neutralizing free radicals. Reduced levels of free radicals allow animals to maintain or increase their SOD production. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide (H_2O_2), as described by Yasmeen and Hasnain (2015).

The increase in SOD activity observed in T1 and T2 coincided with a reduction in malondialdehyde (MDA) levels, suggesting that adding 1% and 2% NCL effectively boosted antioxidant capacity. The higher SOD activity in these groups implied a reduced oxidative stress, likely due to the ability of antioxidants to neutralize reactive oxygen species (ROS). As noted by Yuniastuti (2016), antioxidants function by scavenging free radicals, such as ROS. The enhancement of SOD activity in T1 and T2 was also linked to increased carcass weight and notable body weight gain. These results align with those of Hikmat *et al.* (2020), who observed that vitamin C and palm oil supplementation increased SOD activity and carcass yield. Broilers that can effectively manage stress are more likely to attain their growth potential.

The notable decrease in MDA level (P<0.05) was linked to the use of the combined feed additive, NCL. Noni leaf extract is rich in bioactive substances such as flavonoids, saponins, and alkaloids, which serve as both immunostimulants and antioxidants. These antioxidants play a role in halting lipid peroxidation by neutralizing free radicals and preventing further oxidation processes. Lower levels of MDA suggest better health in chickens, as they are associated with a reduction in reactive oxygen species (ROS) and polyunsaturated fatty acids (PUFAs), which in turn decrease lipid peroxidation and MDA production. This shift allows energy that would have been used to fight stress or illness to be redirected towards growth, thereby enhancing performance. Additionally, reduced oxidative stress improves feed efficiency, leading to greater live body weight in broiler chickens. This is consistent with the findings of Razak *et al.* (2016), who observed a positive association between body weight and nutrient absorption. Enhanced feed absorption is facilitated by increased villus height and crypt depth, which, as noted by Lisnahan *et al.* (2019), indicate the absorptive capacity of the small intestine

Incorporating citric acid as an acidifier in the formulation of noni leaf extract helps diminish pathogenic bacteria, which in turn reduces the generation of harmful free radicals. This reduction in free radical activity helps limit oxidative damage to lipids. According to Iheagwam *et al.* (2012), ketapang leaves have antioxidant properties that neutralize free radicals. Furthermore, the use of *L. acidophilus* as a probiotic has been found to lower blood cholesterol levels and decrease MDA level. Utami *et al.* (2017) found that taking 1.5 mL/day of L. casei strain Shirota for 21 days led to a 60.15% reduction in serum MDA level. Interestingly, the T2 treatment group, which was given 2% feed additive in their drinking water, showed a significant (P< 0.05) reduction in MDA level, along with an increase in superoxide dismutase (SOD) levels. This indicates that a 2% feed additive combination is effective in lowering MDA level in broiler chickens. In contrast, there was no significant difference between treatments T1 and T3, with the T3 group showing an increase in MDA level. The elevated MDA level in T3, which received 3% feed additive, might be attributed to an excessive amount of noni leaf extract, causing toxic effects and increased ROS production. High ROS can overwhelm the antioxidant defense system, leading to oxidative stress and the subsequent buildup of MDA (Chen *et al.*, 2019).

The tallest villi were found in T2, whereas T0 had the shortest villi. These results align with the findings of Khasanah *et al.* (2024), who reported that bioactive compounds such as flavonoids and saponins in noni leaf extracts have anti-inflammatory effects that promote the growth of intestinal epithelial cells. This growth leads to an increase in both the number and height of villi, which is essential for enhancing the surface area available for nutrient absorption in the small intestine. Similarly, Satimah *et al.* (2019) indicated that a higher count of epithelial cells resulted in a larger surface area and more pronounced villi. Citric acid, serving as an acidifier, was another factor contributing to the increased villus height in T2. It helps to maintain an optimal intestinal pH that encourages the growth of beneficial lactic acid bacteria. Jamilah *et al.* (2014) highlighted that such favorable gastrointestinal conditions are crucial for villus development, as they allow lactic acid bacteria to thrive and positively impact gut morphology. *Lactobacillus acidophilus*, which is included in feed additives, functions as a probiotic by boosting the population of lactic acid bacteria. These bacteria generate short-chain fatty acids (SCFAs), notably butyrate, acetate, and propionate, which are vital to intestinal health. Butyrate is a key energy source for villus development. According to Collins and Gibson (1999), SCFAs are used by the host as energy for villus growth and help lower the intestinal pH. A more acidic environment suppresses the growth of harmful bacteria, thereby reducing their number in the digestive tract.

According to Prihambodo *et al.* (2020), flavonoids enhance the intestinal structure by increasing villus height and crypt depth. Additionally, *Lactobacillus acidophilus*, a member of the *Lactobacillus* genus, is a crucial component of the normal gut microbiota, helping inhibit harmful bacteria and maintain microbial equilibrium (Sasmita *et al.*, 2021). In this study, the average villus height was 1050–1501 μm. This finding aligns with Sunu *et al.* (2021), who found that adding *Lactobacillus acidophilus* to broilers resulted in villus heights from 1160.54 to 1552.55 μm, with significant statistical results. According to Ma'rifah *et al.* (2025), the increase in villus height was associated with a improvement in body weight gain, further supporting the beneficial physiological effects of the feed additives used.

The presence of antioxidant compounds in the feed additive is linked to an increase in crypt depth. These antioxidants mainly originate from the noni leaf extract, which contributes to enhanced mucus secretion by boosting the number of goblet cells. As noted by Hamid *et al.* (2014), a greater number of goblet cells is associated with deeper intestinal crypts, suggesting increased mucus production that aids in epithelial regeneration. Furthermore, these antioxidant compounds protect the intestinal cells against oxidative damage. Ekawasti *et al.* (2019) observed that antioxidants might disrupt the protective mechanisms of parasites against oxidative stress, thereby decreasing their viability. The inclusion of *Lactobacillus acidophilus* in the feed additive serves as a probiotic that fosters gut microbial health and fortifies the mucosal barrier, which, in turn, improves the efficiency of nutrient absorption. According to Budiansyah (2010), probiotics operate solely within the digestive system and attach to the intestinal cells without entering the bloodstream. Devi *et al.* (2020) also noted that the presence of probiotics is associated with an increase in crypt depth, which corresponds to greater mucosal thickness.

Moreover, the inclusion of citric acid in the feed additive creates an environment that supports the growth and renewal of epithelial cells. Increased acidity in the intestinal environment can help reduce the inflammatory responses triggered by infections or irritation. Such inflammation can harm epithelial cells and lead to a decrease in the crypt depth. Thus, the acidifying effect of citric acid aids in the maintenance of intestinal integrity by reducing this damage. In this study, the average crypt depth was found to be between 153.46 and 256.32 μm, which is significantly greater than the 110–122 μm range reported by Nourmohammadi and Afzali (2013). According to Kusuma *et al.* (2022), deeper crypts signify active cell regeneration, with a constant production of new cells to replace those that are damaged, thereby enhancing the digestive capacity and nutrient absorption of the intestine. These results indicate that the feed additive mixture improved intestinal morphology, particularly by increasing crypt depth, which is crucial for digestive efficiency and nutrient absorption in broiler chickens.

As shown in Table 2, incorporating the feed additive NCL into the drinking water had a significant effect (P<0.05) on the weight gain of broiler chickens. This improvement in weight gain is linked to the presence of *L. acidophilus*, which helps maintain a balanced gut microbiota by suppressing the growth of harmful bacteria (Fuller, 1989). Furthermore, probiotics, such as *L. acidophilus*, can enhance the secretion of digestive enzymes, such as protease and amylase, leading to better digestion and nutrient absorption. The inclusion of *L. acidophilus* in the diet had a beneficial effect on weight gain. Noni leaf extract is thought to affect weight gain owing to its bioactive components, such as flavonoids, saponins, and alkaloids. These components function as immunostimulants, boost metabolism, and reduce oxidative stress. By enhancing overall health, these bioactive substances allow energy that would typically be used to fight stress or illness to be redirected towards growth.

The addition of citric acid increased the solubility of polyphenols, which in turn enhanced their bioavailability. This improvement maximizes the antioxidant and antibacterial properties of the polyphenols. Polyphenols found in noni leaves, such as anthraquinones, can cause protein denaturation in harmful bacterial cells, resulting in weakened cell wall permeability and promoting the growth of beneficial lactic acid bacteria in the gut (Bintang *et al.*, 2007). Lactic acid bacteria are essential for boosting the immune response, inhibiting the growth of harmful microorganisms, releasing digestive enzymes, and increasing the production of fatty acids (Coates and Fuller, 1977; Fuller, 1989; Rolfe, 2000). The decrease in harmful microorganisms and the optimal release of enzymes improve the ability of the digestive system to absorb nutrients, thereby aiding in weight gain.

The combination of polyphenols and *L. acidophilus* is thought to modify the gut microbiota and promote the proliferation of lactic acid bacteria, while suppressing harmful bacteria. An increase in lactic acid bacteria can lead to thickening of the duodenal mucosa, which increases the surface area for nutrient absorption. This in turn enhances nutrient deposition for meat production, ultimately resulting in greater body weight gain. Devi *et al.* (2020) support this by noting that the thickening of the small intestine's mucosa is associated with increased crypt depth due to probiotics. Additionally, Yalcinkaya *et al.* (2008) indicated that probiotics boost resistance to infections by pathogenic bacteria and fortify the mucosal immune system, thereby lowering pathogen levels and improving broiler health.

The combination of probiotics (*Lactobacillus acidophilus*), acidifiers (citric acid), and phytobiotics (noni leaf extract) improves the health of the digestive system, enhances metabolic efficiency, and maintains a balanced gut microflora. This collaborative effect allows for a more effective conversion of feed into biomass, despite no significant differences in energy and protein intake and digestibility. The most significant body weight gain was recorded in treatment T2 (drinking water + 2% feed additive), which demonstrated the most efficient nutrient conversion into body mass. Feed additive levels below or above 2% did not result in the highest body weight gain.

Broiler chickens experienced a notable increase in overall body weight, which was reflected in the weight gain in various body parts. This observation is consistent with the effects of feed additive combination on carcass weight, with the most pronounced effect observed in T2 (drinking water + 2% feed additive). The increase in body weight was proportionately similar to that in carcass weight (Herlina and Ibrahim, 2019). As shown in Table 2, the average body weight gain of the broiler chickens in this study ranged from 2,192.41 to 2,361.66 g/bird. These findings are consistent with those reported by Kurniasih *et al.* (2019), who observed that the combination of Dayak onion extract and *L. acidophilus* led to a body weight gain of 1,377.25 and 1,519.16 g/bird.

Conclusion

Administering a 2% blend of NCL feed additives, including *Morinda citrifolia* (noni) leaf extract, citric acid, and *Lactobacillus acidophilus*, through drinking water to broiler chickens on a commercial diet markedly enhance intestinal health and overall performance, through increase in superoxide dismutase (SOD) levels, decrease in malondialdehyde (MDA) levels, increased villus height and crypt depth in the duodenum, and improved body weight gain. The combined effects of phytobiotics, acidifiers, and probiotics in NCL can be an effective feed additive strategy to boost the antioxidant status, gut morphology, and growth performance in broiler chickens.

Acknowledgments

The authors acknowledge the financial support and sponsorship for this research by the Faculty of Animal and Agricultural Sciences Grant (grant number: 21/UN7.F5/PP/I/2025). And we would like to thank to Poultry Production Laboratory, Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Indonesia.

Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Agusetyaningsih, I., Widiastuti, E., Wahyuni., H.I., Yudiarti, T., Murwani, R., Sartono, T.A., Sugiharto, S., 2022. Effect of encapsulated Cosmos caudatus leaf extract on the physiological conditions, immune competency, and antioxidative status of broilers at high stocking density. Ann. Anim. Sci. 22, 653–662.

Bahmani, F., Ebrahimi, M.T., Kolahdooz, F., Mazouchi, M., Hadegh, H., Jamal, A.S., Mazroii, N., Asemi, S., Asemi, Z., 2015. The consumption of synbiotic bread containing *Lactobacillus* sporogenes and inulin affects nitric oxide and malondialdehyde in patients with type 2 diabetes mellitus: randomized, double-blind, placebo-controlled trial. J. Am. Coll. Nutr. 0, 1–8.

Bintang, I.A.K., Sinurat, A.P., Purwadaria, T., 2007. Penambahan ampas mengkudu sebagai senyawa bioaktif terhadap performans ayam broiler. JITV 12, 1–5.

Budiansyah, A., 2010. Performan ayam broiler yang diberi ransum yang mengandung bungkil kelapa yang difermentasi ragi tape sebagai pengganti sebagian ransum komersial. J. Ilm. Ilmu-Ilmu Peternak. 260–268.

Celebioglu, A., Yildiz, Z.I., Uyar, T., 2018. Fabrication of electrospun eugenol/cyclodextrin inclusion complex nanofibrous webs for enhanced antioxidant property, water solubility, and high temperature stability. J. Agric. Food Chem. 66, 457–466.

Chen, D., Huang, C., Chen, Z., 2019. A review for the pharmacological effect of lycopene in central nervous system disorders. Biomed. Pharmacother. 111, 791–801.

Coates, M.E., Fuller, R., 1977. The genetobiotic animal in the study of gut microbiology. In: Microbial Ecology of the Gut. Clarke, R.T.J., Bauchop, T. (Eds.), Academic Press, London, pp. 311–346.

Collins, M.D., Gibson, G.R., 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am. J. Clin. Nutr. 69, 825–1020.

Devi, R., Kaur, T., Kour, D., Rana, K.L., Yaday, A., 2020. Beneficial fungal communities from different habitats and role in plant growth promotion and soil health. Microbiol. J.

Dokou, S., Mellidou, I., Savvidou, S., Stylianaki, I., Panteli, N., Antonopoulo, E., Wang, J., Grigoriadou, K., Tzora, A., Jin, L., Skoufos, I.A., Giannenas, I., 2023. A phytobiotic extract, in an aqueous or in a cyclodextrin encapsulated form, added in diet affects meat oxidation, cellular responses and intestinal morphometry and Microbiota of broilers. Front. Anim. Sci. 4.

Ekawasti, F., Nurcahyo, W., Wardhana, A.H., Shibahara, T., Tokoro, M., Sasai, K., Matsubayashi, M., 2019. Molecular characterization oh highly pathogenic Eimeria species among beef cattle on Java Island, Indonesia. Parasit. Inter. 72, 101927.

Felipe, A. de O., 2024. Latin American perspectives. 51, 2.

Foster, J.A., McVey Neufeld, K.A., 2013. Gut-brain axis: How the microbiome influences anxiety and depression. Trends Neurosci. 36, 305–312.

Fuller, R., 1989. A review, probiotics in man and animals. Appl. Bacteriol. 66, 365–378.

Hamid, Y., Tang, L., Hussain, B., Usman, M., Guraja, H.K., Rashid, M.S., He, Z., Yang, X., 2014. Efficiency of lime, biochar, Fe containing biochar and composite amendments for Cd and Pb immobilization in a co-contaminated alluvial soil. Environ. Pollut. 257.

Herlina, B., Ibrahim, W., 2019. Penambahan tepung daun salam dalam ransum terhadap konsumsi ransum, bobot potong, bobot karkas dan organ dalam ayam kampung super. J. Sain Peternak. Indon. 14, 254–269.

Hikmat, N., Kurniawan, W., Syamsuddin, 2020. Bobot potong, persentase karkas dan lemak abdominal ayam broiler yang diberi gula aren dalam air minum. JIPHO 2, 420–426.

Iheagwam, I.W., Ezeokonkwo, R.C., Ezeh, I.O., Onunkwo, J.I., 2012. Comparative serum biochemical changes in mongrel dogs following single and mixed infections of Trypanosoma congolense and Trypanosoma brucei brucei. Vet. Parasitol. 190, 56–61.

Jamilah, N., Suthama, L.D., Mahfudz, L.D., 2014. Pengaruh penambahan jeruk nipis sebagai acidifier pada pakan stepdown terhadap kondisi usus halus ayam pedaging. JITP, 90–95.

Khan, S., Wang, N., Reid, B.J., Freddo, A., Cai, Ch., 2013. Reduced bioaccumulation of PAHs by Lactuca satuva L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar. Environ. Pollut. 175, 64–68.

Khasanah, H., Kusbianto, D.E., Purnamasari, L., Cruz, J.F.D., Widianingrum, D.C., Hwang, S.G., 2024. Modulation of chicken gut microbiota for enhanced productivity and health: A review.

Kusuma, A.A.Ng.J., Arya, M.B.D., Mahendra, N.B., Susato, D.H., 2022. Description of neutrophil-to-lymphocyte ratio, C-reactive protein, and procalcitonin levels in pregnancy with COVID-19 at Sanglah General Hospital period of April 2020–April 2021. J. South Asian Fed. Obstet. Gynaecol. 14, 4.

Lee, S.H., Yang, J., Goddard, M.E., Visscher, P.M., Wray, N.R., 2012. Estimation of pleiotropy between complex diseases using single-nucleotide polymorphism-derived genomic relationships and restricted maximum likelihood. Bioinformatics 28, 2540–2542.

Lenhardt, L., Mozes, S., 2013. Morphological and functional changes of the small intestine in growth-stunted broilers. Acta Vet. Brno 72, 353–358.

Lisnahan, C.V., Wihandoyo, W., Zuprizal, Z., Harimurti, S., 2019. Morfologi usus ayam kampung umur 20 minggu yang disuplementasi DL-methionin dan L-lisin HCL dalam pakan. J. Trop. Anim. Sci. Technol. 1, 14–21.

Ma'rifah, B., Suprijatna, E., Sunarti, D., Mahfudz, L.D., Kismiati, S., Sarjana, T.A., Muryani, R., Shihah, H.D., Wahyuni, N.M., Sugiharto, S. 2025. Effect of encapsulated Averrhoa carambola L. leaf extract on the growth performance, haemato-biochemical parameters, gut health, and antioxidant activity in broiler chickens. Vet. Anim. Sci. 30. 100504.

Nourmohammadi, R., Afzali, N., 2013. Effect of citric acid and microbial phytase on small intestinal morphology in broiler chicken. Ital. J. Anim. Sci. 12, 44–47.

Osman, W.N.W., Tantowi, N.A.C.A., Lau, S.F., Mohamed, S., 2019. Epicatechin and scopoletin rich *Morinda citrifolia* (Noni) leaf extract supplementation mitigated osteoarthritis via anti-inflammatory, anti-oxidative, and anti-protease pathways. J. Food Biochem. 43, 12755.

Piekarska-Radzik, A., Klewicka, E., 2021. Mutual influence of polyphenols and *Lactobacillus* spp. bacteria in food: a review. European Food Research and Technology 247, 9-24.

Phaniendra, A., Dinesh, J., Latha, P., 2014. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J. Clin. Biochem. 30, 11–26.

Prihambodo, T.R., Sholikin, M.M., Qomariyah, N., Jayanegara, A., Batubara, I., Utomo, D.B., Romli, N., 2020. Effects of dietary flavonoids on performance, blood constituents, carcass composition and small intestinal morphology of broilers: a meta-analysis. J. Anim. Physiol. Anim. Nutr. 34, 434–442.

Razak, I., Nirwanto, N., Triatmanto, B., 2016. The impact of product quality and price on customer satisfaction with the mediator of customer value. J. Mark. Consum. Res. 30, 59–68.

Rolfe, R.E., 2000. The role of probiotic cultures in the control of gastrointestinal health. J. Nutr. 130, 396–402.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., Tuohy, K., 2018. Gut microbial function on metabolism of nutrients and other food components. Eur. J. Nutr. 57, 1–24.

Satimah, S., Yunianto, V.D., Wahyono, F., 2019. Bobot relatif dan panjang usus halus ayam broiler yang diberi ransum menggunakan cangkang telur mikropartikel dengan suplementasi probiotik *Lactobacillus* sp. J. Sain Peternak. Indon. 14, 396–403.

Sasmita, H.A., Sapriati, A.N., Kursia, S., 2021. Isolasi dan identifikasi bakteri asam laktat dari liur basa (limbah sayur bayam dan sawi). As-Syifaa 10, 2.

Shil, A., Chichger, H., 2021. Artificial sweeteners negatively regulate pathogenic characteristics of two model gut bacteria, E. coli and E. faecalis. Int. J. Mol. Sci. 22, 5228.

Sibi, G., Chatly, P., Adhikari, S., Rafikumar, K.R., 2012. Phytoconstituents and their influence on antimicrobial properties of *Morinda citrifolia* L. Res. J. Med. Plant. 6, 441-448.

Sunu, P., Sunarti, D., Mahfudz, L.D., Yunianto, V.D., 2021. Effect of synbiotic from Allium sativum and *Lactobacillus acidophilus* on hematological indices, antioxidative status and intestinal ecology of broiler chicken. J. Saudi Soc. Agric. Sci. 20, 103-110.

Tan, F., Luo, Z., Liu, Y., Zhou, L., She, P., Wu, Y., Chen, L., 2019. Bactericidal and anti-biofilm activity of the retinoid compound CD437 against Enterococcus faecalis. Front. Microbiol. 10, E1603.
Tang, D., Wu, J., Jiao, H., Wang, X., Zhao, J., Lin, H., 2018. The development of antioxidant system in the intestinal tract of broiler chickens. Poult. Sci. 98, 664–678.
Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., Gordon, J.I., 2006. An obesity associated gut microbiome with increased capacity for energy harvest. Nature 444, 1027-1031.
Utami, K.S., Aulanni'am, Mahdi, C., 2017. Potential of *Lactobacillus* casei Shirota strain probiotic toward total cholesterol levels and SOD activity in rat with high cholesterol diet. Molecules 12, 153–158.
Vicol, C., Duca, G., 2023. Synergistic, additive and antagonistic interactions of grapes' phenolic compounds and organic acids. Acta Chim. Slov. 70, 588–600.
Yalcinkaya, I., Gungor, T., Basalan, M., Erdem, E., 2008. Mannan oligosaccharides (MOS) from Saccharomyces cerevisiae in broilers: effects on performance and blood biochemistry. Turkish J. Vet. Anim. Sci. 32, 43-48.
Yasmeen, H., Hasnain, S., 2015. In vitro antioxidant effect of Camellia sinensis on human cell cultures. Pak. J. Pharm. Sci. 28, 1573–1581.
Yuniastuti, A., 2016. Dasar molekuler glutation dan perannya sebagai antioksidan. FMIPA Press, Semarang.
Zhu, W., Dong, Y., Wu, T., Jiang, T., Xiao, Y., Yu, X., Zhong, T., 2025. Synergies of plant-derived compounds in controlling foodborne microorganisms: antimicrobial mechanisms, determination methods and combined effects. Food Rev. Int. 41, 1-20.