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Effect of *Morinda citrifolia* L., citric acid, and *Lactobacillus acidophilus* on broiler growth performance, carcass traits, and litter quality

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ABSTRACT

This study evaluated the effect of a feed additive combination, *Morinda citrifolia* leaf extract, citric acid, and *Lactobacillus acidophilus* (MCL) on broiler chicken performance, carcass traits, and litter quality. MCL contains bioactive compounds such as flavonoids, saponins, citric acid, and probiotic. A total of 200 broiler chickens (8 days old with average body weight 213.88 ± 8.9 g) were randomly assigned to four treatments with five replications of bird each: T0 (control), T1 (1% MCL), T2 (2% MCL), and T3 (3% MCL). Results showed that T2 significantly improved ($P<0.05$) body weight gain (BWG) and income over feed cost (IOFC). Water consumption and carcass weight were also higher ($P<0.05$) in T2. Final body weight was significantly higher ($P<0.05$) in all MCL treatments compared to T0. Feed conversion ratio improved ($P<0.05$) in T2 and T3. MCL supplementation enhanced carcass traits and litter quality. Cooking loss was lower ($P<0.05$) in T2, while drip loss and litter pH were reduced ($P<0.05$) in all MCL treatments. White striping was higher ($P<0.05$) in T1, and litter temperature was lower ($P<0.05$) in T2 and T3. These finding suggest that MCL is a promising natural alternative to synthetic growth promotors, improving productivity and probitability in broiler production.

Keywords: Broiler Chickens, Carcass, Citric acid, Lactobacillus acidophillus, Leaf extract, Morinda citrifolia, Performance.

INTRODUCTION

Broiler chickens (*Gallus gallus domesticus*) are poultry breeds that have been specifically developed for meat production. Through intensive artificial selection, broilers have been genetically enhanced to exhibit rapid growth, high feed conversion efficiency, and favorable carcass characteristics, particularly in breast muscle (Wilcox *et al*., 2023). These genetic advancements have made broiler chickens a key commodity in the modern poultry industry (Abdel-Moneim *et al*., 2024). Broilers are highly sensitive to thermal fluctuations, with a thermoneutral zone between 20°C and 24°C during the age of 3–6 wk. Effective ventilation is essential to remove ammonia, carbon dioxide, and moisture from fecal matter and respiration (Yahav, 2009). Nonetheless, not all farmers have access to closed-house facilities because of economic constraints. Many small-scale farmers rely on open-house systems that lack environmental and biosecurity controls, which often results in reduced performance, increased mortality, and inconsistent product quality (Lara and Rostagno, 2013). In addition, cost-related limitations have led some farmers to use low-cost commercial feed that may not meet the nutritional needs of fast-growing broilers. This practice can lead to growth-related disorders, metabolic diseases, and economic losses, particularly when market prices are low. Heat stress is of major concern in tropical climates. Temperatures exceeding 30°C induce panting behavior, which leads to respiratory alkalosis owing to excessive CO_2 loss. This disrupts the acid-base balance, reduces feed intake, impairs digestive enzyme activity, and ultimately hampers growth (Lara and Rostagno, 2013).

Improving nutrient absorption and feed efficiency are critical for enhancing broiler growth performance. One strategy involves the use of natural feed additives, particularly phytobiotics, in combination with probiotics (e.g., *Lactobacillus acidophilus*) and organic acids such as citric acid. *Morinda citrifolia* (Noni) leaf extract contains bioactive compounds, including flavonoids, alkaloids, saponins, and tannins, with known antibacterial properties. As a phytobiotic feed additive, *Morinda citrifolia* can enhanced immune responeses in animal, reducing susceptibility to infection, help in scavenging free radicals, improve overall health and productivity, promoting nutrients absorption and digestion, and enhancing feed efficiency and animal growth rates (Biswas *et al*., 2024). In vitro studies have demonstrated the ability of Noni leaf extracts to inhibit the growth of *L. acidophilus* in a dose-dependent manner. *L. acidophilus* is a beneficial probiotic that produces antimicrobial compounds such as lactic acid, hydrogen peroxide, and bacteriocins. These compounds inhibit the growth of pathogenic bacteria, such as *Escherichia coli*, with an average inhibition zone of 6.91 mm. Citric acid, an organic acid widely used in the food and pharmaceutical industries, enhances the solubility of bioactive compounds and stabilizes probiotic formulations. Citric acid supplementation in broiler diets has also been reported to improve calcium retention and bone mass, thereby contributing to better skeletal development.

Although no studies to date have directly investigated the combined use of *Morinda citrifolia* leaf extract, citric acid and *L. acidophilus*, several potential synergistic effects can be inferred from their individual properties, including antimicrobial synergy between *Morinda citrifolia* leaf extract and *L. acidophilus* against pathogenic bacteria (Zhang *et al*., 2016), enhanced bioavailability of phytochemical compounds facilitated by citric acid (Shaikh *et al*., 2022), and stabilization and viability support of *L. acidophilus* in feed products through citric acid supplementation (Osman *et al*., 2019). Based on these findings, the combination of *Morinda citrifolia* leaf extract, citric acid, and *L. acidophilus*, hereafter referred to as MCL, has potential as a functional feed additive for broiler chickens. This study aimed to evaluate the effectiveness of MCL supplementation in improving broiler growth performance, carcass traits, and litter quality of broiler chicken.

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 are 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 (Wan 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* (10^{11} cfu/ml) that have been growth in MRSA (de Man Rogosa Sharpe Agar) medium before. Feed additive mixture was cooled in a sealed bottle and stored at 4ºC until used.

Experimental Design

This study was conducted in the Faculty of Animal and Agricultural Sciences, Universitas

Diponegoro, Indonesia. The experimental animals used were 200 broiler chickens aged 8 d, with an average body weight of 213.875 ± 8.9 grams. The experimental design employed was a completely randomized design (CRD) with four treatments and five replication, resulting in 20 experimental units. Each unit consisted of ten broiler chickens. The treatments involved the administration of a feed additive mixture containing *Morinda citrifolia* leaf extract, citric acid, and *Lactobacillus acidophilus* (MCL) through drinking water as follows: T0: drinking water only without MCL; T1: drinking water supplemented with 1% MCL; T2: drinking water supplemented with 2% MCL; T3: drinking water supplemented with 3% MCL. The nutritional composition of the feeds used in this study is listed in Table 1. The observed parameters included feed intake (FI), body weight gain (BWG), water consumption (WC), final body weight, feed conversion ratio (FCR), income over feed cost (IOFC), carcass weight, water holding capacity (WHC), cooking loss (CL), drip loss (DL), white stripping (WS), litter pH and litter temperature.

Sample Collection

Body weight, feed intake, and water consumption were measured on a weekly basis with digital scale. The feed conversion ratio (FCR) was determined by dividing the feed intake by the body weight gain. Income over feed cost (IOFC) was calculated based on total revenue minus total feed cost at the time of the experiment. Cascass weight was measured with digital scale and weighted after bleeding, feathering, and separated from head, legs, and internal organs. The water holding capacity (WHC) was assessed following the methodology outlined by Garcia *et al*. (2010). Drip loss (DL) was measured by initially weighing the breast and thigh fillets. Cooking loss (CL) was determined by calculating the weight difference between the raw and cooked meat samples. White striping (WS) was measured with measuring tape in the pectoralis major (breast muscle) that characterized by white atriations running parallel to the muscle fibers. The pH of the litter was measured using a digital pH meter. The temperatur of the litter was measured using a digital thermometer with a probe and reach into the litter layer (2-5 cm deep).

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.

Table 1. Nutritional Composition of the Feed

Items	Composition
Crude fiber (%)	9.35
Crude protein (%)	34.9
ME[1] (kcal/kg)	2.52
Ether extract (%)	0.19
Ash (%)	1.00
Calsium (%)	1.34
Phospor (%)	1.51
Urea (%)	0.27
Total aflatoxin (µg/Kg)	0.07
Amino acid :	
Lysine (%)	3.022
Methionine (%)	20.40
Methionine + Cystine (%)	2.40
Threonine (%)	3.09
Triptophan (%)	8.96

[1]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}

RESULTS AND DISCUSSION

Performance of Broiler Feed with MCL

As shown in Table 2, the addition of *Morinda citrifolia* leaf extract, citric acid, and *Lactobacillus acidophilus* (MCL) to drinking water had a significant effect (P<0.05) on broiler body weight gain. Treatment T2 showed a significantly higher (P<0.05) weight gain than T0 and T1, although the difference was not significant (P>0.05) from T3. The highest body weight gain was recorded in T2 (2,363.66 ± 11.75 g), while the lowest was observed in T0 (2093.64 ± 18.16 g). The improvement in body weight gain in the MCL group can be attributed to bioactive compounds in the leaf extract, such as flavonoids, tannins, saponins, and alkaloids, which exhibit antioxidant and antimicrobial properties. This can be proven by the increasing drinking water consumption, which is the highest water consumption at the 2% level of MCL. These compounds can inhibit the growth of pathogenic bacteria, such as *Escherichia coli* and Salmonella, resulting in a healthier intestinal environment, enhanced nutrient absorption, and reduced energy expenditure on immune responses (Palupi *et al*., 2013). Citric acid, an organic acid, reduces the gastrointestinal pH, creating an environment that inhibits the growth of pH-sensitive pathogenic microbes. Additionally, it enhances the solubility and bioavailability of essential minerals, such as Ca^{2+}, Mg^{2+}, and Zn^{2+}, which are critical for bone and tissue development (Chowdhury *et al*., 2009). Deng *et al*. (2025) stated, *Lactobacillus acidophilus* (a probiotic) supports the maintenance and restoration of beneficial gut microbiota, including *Lactobacillus* and *Bifidobacterium*, and increases the production of lactic acid and short-chain fatty acid (SCFA)-producing bacteria. These effects include improving intestinal morphology by increasing villus height and reducing crypt depth; enhancing digestive and absorptive efficiency; and suppressing pathogenic bacterial populations through competitive exclusion and bacteriostatic compound production. Thus, the combination of MCL at 2% inclusion (T2) can enhance body weight gain by improving gut health, minimizing subclinical infection-related energy losses, increasing feed conversion efficiency, and supporting immune function, thereby enabling broilers to tolerate environmental stress better. T3 treatments was not significantly different with the other its can be caused by the negative effect of the highest level of MCL suplementation. *Morinda citrifolia* have a bitter taste that can influenced the feed intake and affect body weight gain.

The addition of MCL to drinking water did not have a significant effect (P>0.05) on broiler feed intake. The presence of flavonoids in herbal ingredients gives a bitter taste, which tends to

Table 2. Broiler Performance Feed with MCL

Variables	T0	T1	T2	T3	P-value
BWG (g/bird)	2093.64±18.16^{b}	2188.66±11,27^{b}	2363.66±11,75^{a}	2276.92±84.34^{ab}	0.025
FI (g/bird)	3495.0±12.93	3581.18±15,79	3677.22±15,72	3617.35±58.02	0.179
WC (ml)	9958.04±640.17^{b}	10195.89±131,05^{b}	11012.67±427,12^{a}	10291.13±465.94^{b}	0.016
Final BW (g)	2374.40±83.03^{b}	2580.00±21,13^{a}	2710.00±94,14^{a}	2678.00±91.41^{a}	0.014
FCR	1.67±0.09^{a}	1.63±0.05^{ab}	1.55±0.02^{b}	1.58±0.04^{b}	0.028
IOFC (IDR/bird)	8,334±1.757^{b}	8,508±1.181^{b}	10,510±0.765^{a}	9,088±0.23^{ab}	0.031

^{a,b}Values in the same row with different letters reflect significant differences (P < 0.05).

T0: drinking water only without MCL, T1: drinking water supplemented with 1% MCL, T2: drinking water supplemented with 2% MCL, T3: drinking water supplemented with 3% MCL, BWG: body weight gain, FI: feed intake, WC: water consumption, BW: body weight, FCR: feed conversion ratio, IOFC: income over feed cost, IDR: Indonesian Rupiah (Indonesian currency).

Table 3. Carcass Traits of Broiler Chicken Feed with MCL

Parameter	Treatment				P Value
	T0	T1	T2	T3	
Carcass Weight (g)	1740.60±127.01[b]	1890.00±120.80[a]	1999.00±93.09[a]	1920.00±83.36[a]	0.012
WHC	23.00	25.00	26.80	27.00	0.508
CL	19.26[a]	19.40[a]	18.12[b]	19.07[ab]	0.047
DL	4.49[a]	3.44[b]	3.41[b]	3.34[b]	0.042
WS	6.28[b]	6.63[a]	6.43[ab]	6.22[b]	0.023

[a,b]Values in the same row with different letters reflect significant differences ($P < 0.05$)

T0: drinking water only without MCL, T1: drinking water supplemented with 1% MCL, T2: drinking water supplemented with 2% MCL, T3: drinking water supplemented with 3% MCL, WHC: water holding capacity, CL: cooking loss, DL: drip loss, WS: white striping.

reduce feed intake. However, owing to the presence of citric acid, this effect is diminished, resulting in similar feed consumption. This combination may reduce the bitter taste of broiler feed. Chowdhury *et al*. (2009) reported that citric acid lowers gastrointestinal pH, which can enhance mineral availability and inhibit pathogenic bacterial growth; however, in excessive amounts, it may also irritate the intestinal mucosa and reduce appetite. Another factor that influences feed intake uniformity is the homeostatic mechanism in chickens, which regulates feed consumption based on nutritional needs. McDonald *et al*. (2011) stated that when energy and protein contents are balanced, there is no significant factor driving an increase or decrease in feed intake. Insignificant feed intake but allowed with increasing in body weight indicates that the treatment resulted in better feed efficiency, this is also indicated the decreasing feed conversion ratio value which means it is increasingly effisient.

The addition of MCL to drinking water had a significant effect ($P<0.05$) on broiler water consumption. Water consumption increased in the 2% MCL supplementation group compared to the other groups. *Morinda citrifolia* leaf extracts have been shown to exhibit antibacterial effects against various bacteria, which can help maintain a healthier gut environment in chickens, potentially reducing pathogen burdens that can negatively affect water consumption (Almeida *et al*., 2019; Zhang *et al*., 2016). Citric acid serves as a pH modulator and preservative, potentially preserving the water quality and reducing the growth of undesirable microbes. It acts as a natural acidulant, enhancing the acidity of water, which can inhibit the growth of pathogenic bacteria, thereby potentially promoting better hydration levels in broilers by ensuring that the water remains safe and palatable. *Lactobacillus acidophilus* is a probiotic that supports gut health by enhancing feed efficiency, improving the immune response, and maintaining a healthy gut microbial balance (De Cesare *et al*., 2020; Liu *et al*., 2025). Supplementation of water with *Lactobacillus acidophilus* can promote beneficial microbial populations in the intestinal tract of broilers, thereby possibly improving water intake indirectly by enhancing digestive health and metabolic functions. The introduction of such probiotics has been shown to improve the overall health status and performance outcomes of poultry by optimizing nutrient absorption and utilization (De Cesare *et al*., 2020). The combination of these three feed additives creates a synergy that maximizes feed intake, intestinal health, and metabolic needs that encourage higher drinking water (Sunder *et al*., 2015).

The addition of MCL to drinking water significantly ($P<0.05$) increased the live body weight of broiler chickens. This improvement is attributed to the synergistic action of the bioactive compounds in MCL, which counteract free radicals and promote a healthy gastrointestinal environment. According to Sunder *et al*. (2015), bioactive compounds in *Morinda citrifolia* can enhance nutrient metabolism, thereby contributing to muscle growth and body weight gain. Furthermore, Sunder *et al*. (2015) reported that *Lactobacillus acidophilus* and *Morinda citrifolia*, when used as feed additives, improved immune responses and intestinal health, which are key factors for optimal nutrient absorption and muscle development. The combination of probiotics (*Lactobacillus acidophilus*), acidifiers (citric acid), and phytobiotics (*Morinda citrifolia* leaf extract) may exert a synergistic effect on enhancing gut health, metabolic efficiency, and the balance of the intestinal microflora (Huyghebaert *et al*., 2011). This synergy supports more efficient feed-to-biomass conversion, even when energy and protein intake and digestibility are not significantly different (Prakash *et al*., 2020).

The addition of MCL to drinking water had a significant effect (P<0.05) on the feed conversion ratio (FCR) of broiler chickens. The improvement in FCR was influenced by the presence of saponins in the non-leaf extract, which enhanced intestinal cell wall permeability, leading to increased nutrient absorption, contributing to greater body weight gain, and improved feed efficiency (Youssef *et al*., 2020). This resultd also similar with the research from Ma'rifah *et al*. (2025) that mention leaves extract can enhanced feed efficiency with the reducing of feed conversion ratio value. Another contributing factor to improved FCR is the inclusion of the probiotic *Lactobacillus acidophilus*, which is highly effective in fermenting low molecular weight carbohydrates in the diet into lactic acid and short-chain fatty acids (SCFAs). These compounds inhibit the growth of harmful microorganisms in the gastrointestinal tract of broilers (Sari *et al*., 2013). Additionally, probiotics indirectly enhance digestive enzyme activity, thereby improving nutrient breakdown and absorption (Ravindran and Abdollahi, 2021). The improvement in feed conversion is also attributed to the strong correlation between feed intake and body weight gain, where lower feed consumption accompanied by higher weight gain reflects more efficient feed conversion (Benyi *et al*., 2015).

The addition of MCL in drinking water had a significant effect (P<0.05) on the income over feed cost (IOFC) of broiler chickens. Treatment T2 significantly (P<0.05) increased IOFC compared to T0 and T1, whereas T3 was not significantly different from T2. The improvement in IOFC can be attributed to the inclusion of *Lactobacillus acidophilus*, which produces short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. These compounds enhance the efficiency of metabolizable energy utilization and stimulate the production of B-complex vitamins, which are essential for energy metabolism and growth. The combination of citric acid and bioactive compounds also enhances the solubility of polyphenols, thereby optimizing their antioxidant and antibacterial activities. Polyphenolic compounds in noni leaves can cause protein denaturation, which damages the permeability of bacterial cell walls, leading to the death of pathogenic bacteria and promotion of the proliferation of beneficial bacteria, such as lactic acid bacteria, in the digestive tract (Castaldo *et al*., 2021). Lactic acid bacteria can increase digestive enzyme secretion, which maximizes nutrient absorption. This ultimately contributes to improved body weight gain and feed efficiency, both of which significantly influence the IOFC. In this study, the average IOFC values in broilers receiving the MCL combination ranged from IDR 8,334.00 to IDR 10,510.00 with final body weights between 2307.34 and 2577.36 g per bird. An increase in IOFC is closely linked to more efficient nutrient absorption, which allows for greater body weight gain with the same amount of feed consumed. This is supported by Su *et al*. (2016), who stated that good feed conversion is characterized by low feed intake and high body weight gain. Shah *et al*. (2019) also emphasized that IOFC in broilers is primarily influenced by two factors, they are average final body weight and total cost of feed during the rearing period. Additionally, feed prices and market prices of broilers at the time of sale also play important roles in determining IOFC (Al-Sultan, 2003).

Carcass Traits Feed with MCL

The addition of MCL to drinking water significantly increased (P < 0.05) the carcass weight of broilers . The average carcass weights in the T1, T2, and T3 treatments were significantly higher (P<0.05) than those in the control group (T0). However, there were no significant differences (P>0.05) between T1, T2, and T3, indicating that 1% MCL supplementation per 1000 mL of drinking water was sufficient to enhance carcass weight in broilers. This improvement is likely due to the synergistic effects of the MCL feed additive in enhancing nutrient absorption, particularly protein absorption, by stimulating digestive enzyme activity, improving gut health, and reducing pathogenic bacterial populations. These findings are supported by those of Mojer *et al*. (2024), who reported that the combination of probiotics and acidifiers promotes optimal digestion. Similarly, Lipinski *et al*. (2019) stated that phytobiotics, acidifiers, and probiotics as feed additives could prevent pathogenic bacterial colonization and enhance the utilization of dietary protein and energy for muscle development. The study found that the average broiler carcass weight ranged from 1,740.60 to 1,999.00 g per bird. The inclusion of MCL in drinking water contributed to improved gastrointestinal health and activation of digestive enzymes, thereby increasing nutrient absorption. Flavonoid bioactives in MCL are believed to enhance carcass weight by improving gut health, which in turn facilitates optimal digestion. This aligns with Mishra and Jha (2019), who stated that flavonoids can improve poultry productivity by optimizing gastrointestinal function, thus maximizing nutrient digestion and absorption, especially of proteins. *Lactobacillus acidophilus* plays a role in maintaining the intestinal microflora balance. In agreement with this, Kaushik *et al*. (2009) reported that probiotics from the Lactobacillus genus help restore the microbial balance and suppress coliform bacterial populations. Shokryazdan *et al*. (2017) further explained that supplementation with Lactobacillus probiotics enhanced nutrient absorption and enzymatic activity in the digestive tract of chickens, leading to improved production performance and increased carcass weight.

The addition of MCL to drinking water had no significant effect (P>0.05) on the water-holding capacity (WHC) of broiler chicken meat (Table 2). According to Mir *et al*. (2017b), water holding capacity has a direct bearing on the color and tenderness of meat, which is the most important functional property of raw meat. An increase in the water content of muscles enhances tenderness, juiceness, firmness, and appearance, improving the quality and economic value of meat. WHC is the ability of meat to retain water during processing and cooking, and is influenced by pH, protein structure, and cell membrane integrity, all of which are influenced by nutritional status. Because consumption is not significantly different, nutritional intake is the same, so WHC is the same. This insignificant effect can be directly caused by feed consumption. In all treatments, the addition of MCL had no significant effect on feed consumption. Energy supply from feed consumption results in the accumulation of actinomyosin complexes, which causes loss of space between myofibrillar proteins and the consequent enhancement in water holding capacity (Mir *et al*., 2017a). The accumulation of actinomyosin complexes and the resulting effects on the water-holding capacity of broiler meat can be significantly influenced by feed consumption and the energy supply provided. This energy supply, typically derived from the nutrients available in the feed, affects the biochemical and physiological processes within the muscle fibers of broilers, contributing to meat quality attributes, such as WHC. First, the energy derived from the feed is critical for metabolic pathways that control muscle development and maintenance. Glycolytic energy production in muscles influences the rigor mortis process post-slaughter, which is essential for structural integrity and water retention in the muscle tissues (Ponsuksili *et al*., 2008).

The cooking loss values observed at T0 and T1 did not demonstrate a statistically significant difference, indicating that the addition of the 1% combination was insufficiently effective. Treatment T3, with 3% feed additive, resulted in a reduction in cooking loss; however, this reduction was not significantly different from the values recorded for T0, T1, and T2. In the case of T2, the antioxidant properties of *Morinda citrifolia* leaf extract and citric acid contributed to the preservation of muscle protein structure, thereby inhibiting the escape of water during cooking. Additionally, *Lactobacillus acidophilus* enhances gut health and chicken metabolism, leading to improved muscle quality and higher water-holding capacity. A feed additive concentration of 2% appeared to represent the optimal level at which the synergistic effects of the three components were most effectively realized. Previous research has demonstrated that citric acid enhances the oxidative stability of muscle protein isolates by lowering the pH levels and eliminating existing peroxides, thereby reducing lipid oxidation (Vareltzis *et al*., 2008). Maintenance of stability is essential for preserving the structural integrity of muscle proteins. This suggests that citric acid can aid in preserving muscle protein integrity and potentially minimizing water loss during cooking. This antioxidant activity can help to protect the structure of muscle proteins by preventing oxidative damage. The extraction of bioactive compounds from *Morinda citrifolia* leaf using citric acid as a variable demonstrated that the concentration of citric acid significantly influences the antioxidant activity of the extracts (Somat *et al*., 2025). This indicates that noni extract, which is rich in antioxidants, can assist in preserving muscle proteins. Supplementation with *L. acidophilus* enhances meat quality in broiler chickens by reducing cooking loss, which is a critical indicator of muscle quality. Additionally, *L. acidophilus* can decrease lipid oxidation in meat, thereby contributing to improved meat stability and quality (Yuanita *et al*., 2019; Dev *et al*., 2020). The ability of probiotics to modulate lipid metabolism and enhance antioxidant capacity further supports these findings.

As shown in Table 3, MCL decreased drip loss in all treatments compared to the control. Supplementation of drinking water with *Morinda citrifolia* leaf extract significantly affected drip loss ($P < 0.05$). Drip loss is a common indicator of water holding capacity (WHC). This research is in line with Ma'rifah *et al*. (2023), which states that the higher cooking loss, the lower water holding capacity. The presence of flavonoids in *Morinda citrifolia*, which act as antioxidants, plays an important role in suppressing lipid and protein oxidation in muscle tissues, thereby improving membrane stability. Supplementation with natural antioxidants such as *Morinda citrifolia* leaf extract has been shown to reduce drip loss in meat. Antioxidants scavenge reactive oxygen species (ROS) generated during metabolism and post-mortem processes. ROS promotes lipid and protein oxidation in muscle cell membranes, leading to membrane damage and reducing the ability of myofibrillar proteins to retain water. This condition accelerates fluid loss during storage, thereby increasing the drip loss. With antioxidant protection, lipid and protein oxidation are suppressed, membrane structures remain stable, and the water-holding capacity of meat is improved. Consequently, exudate loss is minimized, resulting in reduced drip loss (Estevez, 2015; Falowo *et al*., 2014; Irmawati *et al*., 2020).

The combination of feed additives in MCL decreased white stripping in T3 with 3% supplementation of MCL compared with 1% (T1). White stripping is a condition in poultry where white fat deposits replace muscle in stripes along the muscle fibers. It is considered a type of myopathy, primarily involving the pectoral muscle (Petracci *et al*., 2014). White stripping is correlated to hypoxia and oxidative stress that a role in emergence. White stripping also influenced higher calcium levels in the tissue between cells and the muscle fiber chaching type (Prisco *et al*., 2021). The high growth rate of broiler chickens leads to oxygen deprivation, which leads to degeneration, eventually being replaced by fat or white connective tissue (Petracci *et al*. 2014). These effects are not harmful, but they affect meat quality by affecting its texture and appearance.

Table 4. Litter Quality of Broiler Chicken Feed with MCL

Parameter	Treatment				P Value
	T0	T1	T2	T3	
Litter temperature (°C)	37.12[a]	36.68[a]	34.98[b]	34.90[b]	0.012
Litter pH	8.60[a]	8.34[b]	8.20[b]	8.21[b]	0.001

[a,b]Values in the same row with different letters reflect significant differences ($P < 0.05$).

Larger white striping is usually positively correlated with other meat quality defects, such as wooden breast score, which affects consumer preference (Petracci *et al*., 2014).

Litter Quality

The addition of MCL to drinking water had a significant effect ($P<0.05$) on the temperature and pH of the litter as shown in Table 4. Temperature and pH are two factors that significantly influence bacterial growth. Feed additives can have a significant effect on the temperature and pH of broiler litter. Based on available research, the integration of feed additives influences litter quality by modulating its chemical properties. Specifically, when clinoptilolite was incorporated into chicken diets and bedding, it effectively reduced the organic content of the litter and managed the ammonia concentration, which was directly linked to litter pH and environmental temperature control (Karamanlis *et al*., 2008). The pH of poultry litter is crucial because it influences microbial activity and the emission of gases such as ammonia. The application of feed additives as part of management strategies can help regulate the pH, thereby reducing ammonia losses and potential emissions. These additives decrease the pH below neutral, which has been shown to diminish ammonia volatilization, leading to more stable litter conditions. Regarding temperature, studies indicate that water content and management practices influence litter conditions, suggesting that feed additives indirectly affect temperature by altering moisture and microbial processes (Sharma *et al.*, 2017). The presence of additives leads to improved thermal regulation within litter by controlling nitrogen transformations, which are exothermic reactions (Moore *et al*., 2011). We presumed that the inclusion of MCL was able to increase relative protein efficiency and litter quality, since higher body weight and better feed conversion resulted in relatively insignificant digested protein. This means that a smaller amount of bypass protein is released through the excreta. The optimum litter fermentation process which lead to higher temperature and pH commonly caused by higher nitrogen materials and water content (Swiatkiewicz *et al*., 2017). This condition resulted a comfort environment to the broiler. Lower litter temperature can reduced microbial activity in litter, improved digestion and nutrient utilization. Improved litter quality also reduces amonia levels that can enhancing respiratory health and overall welfare (Almeida *et al*., 2019).

CONCLUSION

Supplementation of broiler drinking water with a combination of *Morinda citrifolia* leaf extract, citric acid, and *Lactobacillus acidophilus* (MCL) can enhanced broiler performance, particularly at the 2% inclusion level. Improvements in body weight gain, feed conversion ratio, and income over feed cost (IOFC) highlight the economic and biological benefits of MCL supplementation. 1-2% MCL is optimal for practical application. MCL presents a promising natural alternative to synthetic growth promotors, contributing to sustainable and profitable poultry production. Further research is needed to determine the impact of MCL supplementation under different environmental conditions and housing systems.

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CONFLICT OF INTEREST

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

REFERENCES

Abdel-Moneim, A.M.E., B. Yang, A.M. Shehata, M.A. Elsayed, A.M. Kamal, T.A. Ebeid, S.A. Siddiqui, N.M. Mesalam, M.S. Abougabal, X. Teng and A. Biswas. 2024. Impact of light wavelength on growth and welfare of broiler chickens – overview and future perspective. Ann. Anim. Sci. 24:731–

748. https://doi.org/10.2478/aoas-2023-0090

Al-Sultan, S.I. 2003. The effect of *Curcuma longa* (tumeric) on overall performance of broiler chickens. Int. J. Poult. Sci. 2:351–353. https://doi.org/10.3923/ijps.2003.351.353

Almeida, E.S., D. De Oliveira and D. Hotza. 2019. Properties and applications of *Morinda citrifolia* (Noni): a review. Compr. Rev. Food Sci. Food Saf. 18:883–909. https://doi.org/10.1111/1541-4337.12456

Benyi, K., K.T. Mahlako, T.S. Tshilate and A.J. Netshipale. 2015. Effects of genotype and sex on the growth performance and carcass characteristics of broiler chickens. Trop. Anim. Health Prod. 47:1225–1231. https://doi.org/10.1007/s11250-015-0850-3

Biswas, S., J.M. Ahn and I.H.Kim. 2024. Assessing the potential of phytogenic feed additives: a comprehensive review on their effectiveness as a potent dietary enhancement for nonruminant in swine and poultry. J. Anim. Phy. Anim. Nut. 108:711-723. https://doi.org/10.1111/jpn.13922

Castaldo, L., Y. Rodriguez-Carrasco, A. Ritieni, A. Narváez, L. Izzo and S. De Pascale. 2021. Chemical composition, in vitro bioaccessibility and antioxidant activity of polyphenolic compounds from nutraceutical fennel waste extract. Molecules 26:1968. https://doi.org/10.3390/molecules26071968

Chowdhury, S.S., M.D. Al-Amin, M. Jamila, S. Haque, T. Ahmed and M.E.H. Mazumder. 2009. Effect of citric acid and acetic acid on performance and gut health of broilers. J. Appl. Poult. Res. 18:96–102.

De Cesare, A., G. Castellani, G. Manfreda, A. Astolfi, A. Giardini, C. Sala and V. Indio. 2020. Effect of *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation in drinking water on chicken crop and caeca microbiome. PLoS ONE 1:e0228338. https://doi.org/10.1371/journal.pone.0228338

Deng, J., Y. Hu, P. Zhu, Y. Yu, Q. Chen, H. Wu, Z. Zha, H. Wang and Y. Ma. 2025. Probiotic delivery for editing of the gut microbiota to mitigate colitis and maintain hepatic homeostasis via gut-liver axis. ACS Nano 19:10500–10514. https://doi.org/10.1021/acsnano.5c00325

Dev, K., N.A. Mir, A. Biswas and A. Mandal. 2020. Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. Anim. Nutr. 6:389–397. https://doi.org/10.1016/j.aninu.2020.07.001

Estevez, M. 2015. Oxidative damage to poultry: from farm to fork. Poult. Sci. 94:1368–1378. https://doi.org/10.3382/ps/pev094

Falowo, A.B., P.O. Fayemi and V. Muchenje. 2014. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: a review. Food Res. Int. 64:171–181. https://doi.org/10.1016/j.foodres.2014.06.022

Garcia, R., J. Graciano, R. Farias, C. Komiyama, A. Gabriel, I. Almeida Paz, A. Schwingel, L.D. Freitas, and F. Caldara. 2010. Incidence and physical properties of PSE chicken meat in a commercial processing plant. Revista Brasileira de Ciencia Avicola. 12:233-237.

Huyghebaert, G., R. Ducatelle and F. Van Immerseel. 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187:182–188. https://doi.org/10.1016/j.tvjl.2010.03.003Get rights and content

Irmawati, A., E. Darmawan and T. Wahyono. 2020. The effect of noni (Morinda citrifolia) leaf extract supplementation on meat quality of broiler chicken. IOP Conf. Ser.: Earth Environ. Sci. 492:012085. https://doi.org/10.1088/1755-1315/492/1/012085

Karamanlis, X., C. Batzios, D. Papaioannou, A. Kamarianos, P. Fortomaris, G. Arsenos and I. Dosis. 2008. The effect of a natural zeolite (clinoptilolite) on the performance of broiler chickens and the quality of their litter. Asian-Aust. J. Anim. Sci. 21:1642–1650. https://doi.org/10.5713/ajas.2008.70652

Kaushik, J.K., A.K. Mohanty, A. Kumar, S. Grover, V.K. Batish and R.K. Duary. 2009. Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum*. PLoS ONE 4:e8099. https://doi.org/10.1371/journal.pone.0008099

Lara, L.J.C. and M.H. Rostagno. 2013. Impact of heat stress on poultry production. Animals 3:356–369.

Lipinski, K., Z. Antoszkiewicz, S. Kotlarczyk,

M. Mazur-Kusnirek, J. Kaliniewicz and Z. Makowski. 2019. The effect of herbal feed additive on the growth performance, carcass characteristics and meat quality of broiler chickens fed low-energy diets. Arch. Anim. Breed. 62:33–40. https://doi.org/10.5194/aab-62-33-2019

Liu, J., H. Gu, R. Jia, S. Li, Z. Chen, A. Zheng, W. Chang and G. Liu. 2025. Effects of *Lactobacillus acidophilus* on production performance and immunity of broiler chickens and their mechanism. Front. Vet. Sci. 12:1554502. https://doi.org/10.3389/fvets.2025.1554502

Ma'rifah, B., I. Agusetyaningsih, T.A. Sarjana, S. Kismiati and S. Sugiharto. 2023. Effect of *Moringa oleifera* leaves extract, whey protein, and their combination on growth, carcass and meat quality of broiler chicken. Trop. Anim. Sci. J. 46:313-320. https://doi.org/10.5398/tasj.2023.46.3.313.

Ma'rifah, B., E. Suprijatna, D. Sunarti, L.D. Mahfudz, S. Kismiati, T.A. Sarjana, R. Muryani, H.D. Shihah, N.M. Wahyuni, and S. Sugiharto. 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. https://doi.org/10.1016/j.vas.2025.100504.

Mishra, B. and R. Jha. 2019. Oxidative stress in the poultry gut: potential challenges and interventions. Front. Vet. Sci. 6. https://doi.org/10.3389/fvets.2019.00060

Moore, P.A., I.H. Choi, D. Pote, R. Burns, D. Miles and K. Berg. 2011. Ammonia emission factors from broiler litter in barns, in storage, and after land application. J. Environ. Qual. 40:1395–1404. https://doi.org/10.2134/jeq2009.0383

Mojer, A.M., K.S. Al-Niaeem and K.Y. Al-Dubakel. 2024. Effect of Bacflora®F symbiotic mixture on growth and the health of common carp, Cyrinus carpio. Int. J. Aquat. Biol. 12:2300. https://doi.org/10.22034/ijab.v12i6.2300

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan. 2011. Animal Nutrition. Pearson Education.

Mir, N.A., V. Shukla, A. Rafiq, V. Singh and F. Kumar. 2017a. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 54:2997–3009. https://doi.org/10.1007/s13197-017-2789-z

Mir, N.A., A.K. Biswas, C. Deo, A.K. Verma, S.A. Sheikh, A.B. Mandal, D. Sharma, P.K. Tyagi and P.K. Tyagi. 2017b. Impact of feeding chromium supplemented flaxseed based diet on fatty acid profile, oxidative stability and other functional properties of broiler chicken meat. J. Food Sci. Technol. 54:3899–3907. https://doi.org/10.1007/s13197-017-2846-7

Osman, W.N.W., N.A.C.A. Tantowi, S.F. Lau and S. Mohamed. 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:e12755. https://doi.org/10.1111/jfbc.1275

Palupi, E., F. Anwar, I. Tanziha, M.A. Gunawan and A. Khomsan. 2013. Indigenous soybean-alternatives from Gunungkidul, Yogyakarta. IOP Conf. Ser.: Mater. Sci. Eng. 546:022014. https://doi.org/10.1088/1757-899X/546/2/0220

Petracci, M., S. Mudalal, E. Babini and C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:3138. https://doi.org/10.4081/ijas.2014.3138

Prakash, A., V.K. Saxena and M.K. Singh. 2020. Genetic analysis of residual feed intake, feed conversion ratio and related growth parameters in broiler chicken: a review. World's Poult. Sci. J. 76:304–317. https://doi.org/10.1080/00439339.2020.173597

Prisco, F., D. De Biase, G. Piegari, I. d'Aquino, A. Lama, F. Comella, R. Mercogliano, L. Dipineto, S. Papparella and O. Paciello. 2021. Pathologic characterization of white striping myopathy in broiler chickens. Poult. Sci. 100:101150. https://doi.org/10.1016/j.psj.2021.101150

Ponsuksili, S., E. Jonas, E. Murani, C. Phatsara, T. Srikanchai, C. Walz, M. Schwerin, K. Schellander and K. Wimmers. 2008. Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. BMC Genomics 9:367. https://doi.org/10.1186/1471-2164-9-367

Ravindran, V. and M.R. Abdollahi. 2021. Nutrition and digestive physiology of the broiler chick: state of the art and outlook. Animals 11:2795. https://doi.org/10.3390/ani11102795

Sari, M.L., A. Abrar and M. Merint. 2013. Isolasi dan karakterisasi bakteri asam laktat pada usus ayam broiler. J. Agripet 13:43–48.

Sharma, N.K., M. Choct, S. Wu and R.A. Swick. 2017. Nutritional effects on odour emissions in broiler production. World's Poult. Sci. J. 73:257–280. https://doi.org/10.1017/s0043933917000046

Shah, T.M., J.G. Patel, T.P. Gohil, D.P. Blake and C.G. Joshi. 2019. Host transcriptome and microbiome interaction modulates physiology of full-sibs broilers with divergent feed conversion ratio. NPJ Biofilms Microbiomes 5:96. https://doi.org/10.1038/s41522-019-0096-3

Shaikh, I.A., F. Alsaikhan, U.M. Muddapur, D. Yaraguppi, M.M. Ghoneim, B.A. Mannasaheb, A. Al Ali, M.H. Mahnashi, F.N. Niyonzima, S. Chiniwal, S.M.S. Iqubal, S.S. More, A.A. Khan, Z.K. Bagewadi and A. Asiri. 2022. Characterization of bioactive compounds from *Acacia concinna* and Citrus limon, silver nanoparticles' production by *A. concinna* extract, and their biological properties. Molecules 27:2715. https://doi.org/10.3390/molecules27092715

Shokryazdan, P., Y.W. Ho, K. Ramasamy, M. Faseleh Jahromi, C.C. Sieo and J.B. Liang. 2017. Effects of a *Lactobacillus salivarius* mixture on performance, intestinal health and serum lipids of broiler chickens. PLoS ONE 12:e0175959. https://doi.org/10.1371/journal.pone.0175959

Somat, H.A., I.S. Saipul Anwar, W.A. Wan Mustapha and N.M. Thani. 2025. Subcritical water extraction (SWE) and ultrasonic-assisted extraction (UAE) of bioactive compounds from *Morinda citrifolia*: comparative evaluation of process efficiency. Sains Malaysiana 54:321–330.

Sunder, J., S. Tamilvanan and A. Kundu. 2015. Efficacy of feeding of *Morinda citrifolia* fruit juice and Lactobacillus acidophilus in broiler. Asian J. Anim. Vet. Adv. 10:352–359. https://doi.org/10.3923/ajava.2015.352.359

Su, J.L., B.L. Shi, S.M. Yan, P.F. Zhang, T.Y. Li, and D.S. Sun. 2016. Effects of Yucca extract on feed efficiency, immune and antioxidative function in broiler. Braz. Archives. Biol. Technol. 59. https://doi.org/10.1590/1678-4324-2016150035

Swiatkiewicz, S., D. Jozefiak and A. Arczewska-Wlosek. 2017. The nutrition of poultry as a factor affecting litter quality and foot pad dermatitis – an updated review. J. Anim. Physiol. Anim. Nutr. 101:e14–e20. https://doi.org/10.1111/jpn.12630

Vareltzis, P., H.O. Hultin and W.R. Autio. 2008. Hemoglobin-mediated lipid oxidation of protein isolates obtained from cod and haddock white muscle as affected by citric acid, calcium chloride and pH. Food Chem. 106:116–126. https://doi.org/10.1016/j.foodchem.2007.05.065

Wilcox, C.H., V. Sandilands, N. Mayasari and I.Y. Asmara. 2023. A literature review of broiler chicken welfare, husbandry, and assessment. World's Poult. Sci. J. 80:3–32.

Yahav, S. 2009. Thermal comfort for poultry. Poult. Sci. 88:1456–1464. https://doi.org/10.3382/ps.2008-00293

Youssef, I.M.I., J. Zentek and K. Männer. 2020. Effect of essential oils or saponins alone or in combination on productive performance, intestinal morphology and digestive enzymes' activity of broiler chickens. J. Anim. Physiol. Anim. Nutr. 105:99–107. https://doi.org/10.1111/jpn.13431

Yuanita, I., D. Sunarti, H.I. Wahyuni and N. Suthama. 2019. Feeding Dayak onion (*Eleutherine palmifolia*) extract and *Lactobacillus acidophilus* mixture on blood biochemicals, meat quality characteristics and growth performance in broiler chickens. Livest. Res. Rural Dev. 31. http://www.lrrd.org/lrrd31/9/iyua31139.html

Zhang, W., W. Hao, W. Wang, Z. Wang, Y. Wang, J. Zhang and W. Huang. 2016. Antibacterial constituents of Hainan *Morinda citrifolia* (Noni) leaves. J. Food Sci.81: M1192–M1196.