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Effects of dietary *Lactococcus lactis* and *Bacillus licheniformis* on growth performance and gut health of broilers

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ABSTRACT

In this study, the effects of dietary supplementation with *Lactococcus lactis* and *Bacillus licheniformis* on broiler performance, metabolizable energy, intestinal morphology, and ileal microflora were evaluated. A total of 300 unsexed Cobb strain broiler chickens were divided into three treatments. Treatment T1 served as the control with a basal diet plus 0.1% $CaCO_3$ placebo, T2 received a basal diet supplemented with 0.1% *Lactococcus lactis* and *Bacillus licheniformis* at 10^5 CFU g^{-1}, and T3 received the same probiotic combination at 10^6 CFU g^{-1}.

Probiotic supplementation improved growth performance, as indicated by increased body weight gain, improved feed efficiency (FCR), and higher metabolizable energy. Regarding intestinal microflora, T2 reduced *Escherichia coli* populations, whereas T3 increased lactic acid bacteria; however, the increase in lactic acid bacteria in T3 was not accompanied by a reduction in *Escherichia coli* ($P<0.05$). Treatment T3 produced the lowest FCR value of 1.49 compared to the control (1.60) and the highest metabolizable energy of 3372 kcal kg^{-1}, indicating improved nutrient utilization efficiency.

Histological evaluation showed a tendency to increase intestinal villus height, with the highest value observed in treatment T2 (1175.39 μm), suggesting improved intestinal absorptive capacity. It was concluded that *Lactococcus lactis* and *Bacillus licheniformis* supplementation has the potential to improve performance, intestinal health, and metabolizable energy in broiler chickens, although the effects on microbial populations depend on the dosage.

Keywords: *Bacillus licheniformis, Lactococcus lactis, Broiler chicken, Metabolizable energy. Probiotic.*

INTRODUCTION

Poultry feed containing antibiotic growth promoters (AGPs) may cause toxic residues to accumulate in animal-derived products, posing potential risks to human health, including toxic impacts and the evolution of antimicrobial resistance. The presence of antimicrobial residues in poultry tissues has also raised concerns regarding adverse health effects, such as allergic reactions and digestive disorders (Alagawany *et al.,* 2022). In Indonesia, the use of AGPs in feed has been officially prohibited since January 2018 under the Minister of Agriculture Regulation No. 22/2017 (Prasetyo *et al.,* 2020).

To overcome this problem, as an alternative to antibiotic growth promoters (AGPs) feed additives might be utilized, one of which is probiotics such as *Bacillus licheniformis* and *Lactococcus lactis*. Probiotics provide various benefits for growth performance and health, including improved body weight gain, feed conversion ratio,

resistance to bacterial infections, and regulation of gut microbiota. Although the effectiveness of probiotics may vary, their application has been widely reported to enhance poultry production through multiple mechanisms (Hossain *et al.,* 2024). Supplementation with *Lactococcus lactis* (Adel *et al.,* 2016) and *Bacillus licheniformis* (Zhou *et al.,* 2016) has been demonstrated to enhance the absorption and digestion of nutrients. In addition, *Bacillus licheniformis* increases the availability of proteolytic enzymes in the digestive tract, thereby enhancing protein utilization through the hydrolysis of complex proteins into simpler compounds such as amino acids (Ravindran and Blair, 2012). Meanwhile, *Lactococcus spp.*, particularly *Lactococcus lactis*, create an adverse environment for pathogenic bacteria by producing organic acids such as lactic and acetic acids, which reduce intestinal pH to approximately 4.0–6.5 (Lena *et al.,* 2022). In poultry diets, *Bacillus subtilis* (Soliman *et al.,* 2021; Bai *et al.,* 2016), *Bacillus coagulans*, and *Lactococcus lactis* (Zainuddin *et al.,* 2020) enhance growth performance, improve intestinal morphology, and promote overall gut health. Lactic acid bacteria contribute to pathogen inhibition primarily through the production of organic acids and bacteriocins, which lower intestinal pH and suppress the growth of harmful bacteria (Bai *et al.,* 2016; Ahmed *et al.,* 2014). Additionally, probiotic supplementation has been shown to enhance meat protein content by improving nutrient absorption, particularly protein, in the intestinal tract (Bai *et al.,* 2016). Thus, the current investigation was carried out to evaluate the effect of dietary supplementation with *Lactococcus lactis* and *Bacillus licheniformis* on growth performance, metabolizable energy, intestinal morphology, ileal microflora population of broiler.

MATERIALS AND METHODS

Animal Management

All experimental procedures were approved by the Animal Ethics Committee of IPB University. The study used 300 unsexed day-old Cobb broiler chicks randomly divided into three treatments, and 5 replicates, 20 birds per replicate, and were reared for 35 days old. The chickens were housed in floor pens with wood shavings litter (1m x 2m dimensions).

Barn temperature was automatically regulated between 22–30°C using mechanical ventilation, fans, and evaporative cooling pads controlled by temperature sensors. Lighting was provided by LED lamps with a photoperiod of 23 h light and 1 h dark, and light intensity was maintained at 5–20 lux with uniform distribution. The broiler house was sanitized prior to chick placement. Upon arrival, day-old chicks were provided with sugar water and vaccinated against Newcastle Disease and Infectious Bursal Disease. Twenty birds per litter-floor enclosures were used to raise the birds. Diets were formulated based on the growth phases (starter: 1–14 days; grower: 15–35 days), and feed and drinking water were provided ad libitum. Probiotic supplementation was applied according to the assigned treatments for each replicate.

Diet and Treatments

The Probiotic strains *Lactococcus lactis* D1813 and *Bacillus licheniformis* D3270 were provided by Kyushu Medical Japan. The treatments were control group, (T1) fed a basal diet supplemented with 0.1% placebo ($CaCO_3$); T2 fed the basal diet supplemented with 0.1% of *Bacillus licheniformis* and *Lactococcus lactis* (10^5 CFU g^{-1}); T3 fed the basal diet supplemented with 0.1% of *Bacillus licheniformis* and *Lactococcus lactis* (10^6 CFU g^{-1}). The experimental broiler diet's nutritional makeup and composition are shown in Table 1.

Measurement of Variables

1. Intake of Feed (g/bird). Weekly feed intake was determined by deducting the residual feed from the offered feed.
2. Body weight (g/bird). Body weight was determined by weighing all chickens in each replicate and expressed in grams per bird (g/bird).
3. Body Weight Gain (g/bird). The weekly body weight growth, which was measured in grams per bird (g/bird), was determined by comparing body weight at the beginning and end of each observation week.
4. Feed Conversion Ratio (FCR). The total feed consumption (g) divided by the total weight increase (g) during the rearing period yields the cumulative feed conversion ratio.

Table 1. Composition and Nutrients Content of the Experimental Diets (as-fed basis)

Ingredients (%)	Starter (1–14 d)	Grower (15–35 d)
Yellow corn	63.00	66.50
Soybean meal	25.50	25.50
MBM	9.00	5.00
CPO	1.00	1.00
$CaCO_3$	0.20	0.70
NaCl	0.20	0.20
Premix	0.50	0.5
DL-Methionine	0.25	0.2
L-Lysine	0.25	0.3
Probiotic	0.10	0.10
Total	100	100
Nutrient Content (%)		
Dry matter[1]	87.21	89.96
Ash[1]	6.51	6.16
Metabolizable energy (kcal/kg)[2]	3257	3277
Crude protein[1]	23.83	20.86
Crude fiber[1]	1.27	2.11
Crude fat[1]	2.70	6.91
Nitrogen-free extract (NFE)[1]	52.90	53.92
Calcium[1]	1.51	1.59
Available phosphorus[2]	0.63	0.43
Sodium chloride (NaCl)[1]	0.22	0.19
Lysine[2]	1.27	2.11
Methionine[2]	0.54	0.48
Methionine + Cystine[2]	0.82	0.73

[1]Analytical results from the Laboratory of Feed Science and Technology, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University (2023). [2]Based on calculation [3]Meat and Bone Meal; Crude Palm Oil

5. Mortality (%). To calculate the mortality rate, the number of dead chickens was multiplied by 100, and the result was divided by how many chickens there were overall when the trial started.
6. Metabolizable Energy. At 35 days of age, metabolizable energy and nitrogen retention were determined following the method of Farrell (1978). Fifteen male broilers were housed individually in metabolic cages and adapted for three days, while three fasted broilers were used to determine endogenous energy losses. One male chicken was selected from each replicate (five chickens per treatment) randomly chosen at 35 days of age based on secondary sexual characteristics such as comb size and body posture for further analysis. Prior to excreta collection, all birds were fasted for 24 h with free access to water. Excreta were collected in trays sprayed with 0.01% H_2SO_4 every 2 h to prevent nitrogen volatilization, then weighed, frozen, thawed, homogenized, dried at 60°C for 48 h, and reweighed. The AOAC (2005) was used to assess the dry matter, ether extract, and crude protein contents. Using an adiabatic bomb calorimeter, the gross energy of the feed and excreta was calculated. (Parr 1261®, USA). In accordance with Farrell (1978), Lopez and Leeson (2007) revised the formulas for true metabolizable energy (TME), apparent metabolizable energy (AME), and their nitrogen-corrected values (AMEn and TMEn). Using the following formulae, the values were determined:

TME (kcal/kg) = (GEi − (GEe − GEendogenous)) / Feed intake (kg) whilst AME (kcal/kg) = (GEi − GEe) / Feed intake (kg)

AMEn = AME − 8.22 × Nr (kcal/kg)

TMEn (kcal/kg) = TME − 8.22 × Nr

Where endogenous indicates the endogenous energy expelled by starved birds (kcal), GEi is the gross energy intake (kcal), GEe is the gross energy of excreta (kcal), and Nr is the nitrogen retained per gram of feed intake. The formula for calculating nitrogen retention (RN) was RN (%) = [(Nintake − Nexcreta) / Nintake] × 100.

7. Intestinal Morphology. A total of 15 male chickens were selected, with one male chicken from each replicate (five chickens per treatment) randomly chosen at 35 days of age based on secondary sexual characteristics such as comb size and body posture for further analysis and their digestive tracts were removed after exsanguination and evisceration. Ileal samples (2 cm) were collected and preserved for 24–48 hours in 10% buffered formalin. The tissues underwent a sequence of graded alcohol dehydration, xylol clearing, paraffin embedding, and sectioning using a microtome, and stained with hematoxylin and eosin. Villus surface area was measured using a light microscope with 4× objective magnification. Two villi were measured per field of view, and villus surface area was established using the procedure outlined by Iji *et al.* (2001).
8. Ileal Microflora Population. On day 35, A total of 15 male chickens were selected, with one male chicken from each replicate (five chickens per treatment) randomly chosen at 35 days of age based on secondary sexual characteristics such as comb size and body posture for further analysis. The abdominal cavity was opened, the intestines were removed, and digesta from the small intestine were collected (Ahmed *et al.,* 2014). 9 mL of buffered peptone water (1 g/L peptone, 8 g/L NaCl, 0.5 g/L L-cysteine hydrochloride) was mixed with about 1 g of intestinal digesta (M614®, HiMedia), then serially diluted up to 10−5. Dilutions (10−1–10−5) were plated in triplicate on selective media. Lactic acid bacteria were isolated on MRS agar (M1164®, HiMedia) *E. coli* on EMB agar (M022S®, HiMedia) in anaerobic conditions for 48 hours and in aerobic conditions for 24 at 37°C. After counting, colony-forming units (CFU) were represented as log10 CFU/mL (Ahmed *et al.,* 2014).

Statistical Analysis

The effects of the treatment were evaluated. If significant differences ($p<0.05$) were discovered using analysis of variance (ANOVA) as a post hoc analysis, Duncan's Multiple Range Test was used to compare the mean values.

RESULTS AND DISCUSSION

Broiler Performance

The performance of broilers at 35 days of age is presented in Table 2. Dietary probiotic supplementation affected broiler performance, but the response differed between doses. The T3 group (10^6 CFU g^{-1}) improved feed efficiency, as indicated by the lowest FCR of 1.49 with a BWG of 1822.12 g/bird, which was comparable to the control group (1854.43 g/bird) despite lower feed intake. Feed intake was highest in the control group, followed by T3, while T2 showed the lowest intake. Overall, dietary probiotic supplementation, particularly at 10^6 CFU g^{-1}, improved feed efficiency and performance of broilers at 35 days of age.

The improved BWG and FCR observed in T3 indicate enhanced feed efficiency and nutrient utilization due to probiotic supplementation. This finding is consistent with previous studies, where *Bacillus licheniformis* supplementation reduced FCR from 1.66 to 1.54 and increased final body weight from 1745 g to 1820 g at 35 days of age (Trela *et al.* 2020). Similarly, probiotic supplementation, particularly *Bacillus*-based strains, has been reported to improve growth performance by reducing FCR and increasing BWG in broiler chickens (Bai *et al.,* 2016; Arif *et al.,* 2021).

In addition, Song *et al.* (2017) demonstrated that high-dose *Lactococcus lactis* supplementation increased final body weight by 3–5%, which was associated with improved villus morphology and enhanced intestinal absorptive surface area. The BWG trend observed in the present study suggests a dose–response relationship, in which the 10^6 CFU g^{-1} dose provided more optimal probiotic colonization and functional activity. Similar dose-dependent responses have also been reported for combinations of *Lactococcus lactis* and *Bacillus licheniformis* (Lena *et al.,* 2022) as well as for *Bacillus licheniformis* alone (Qin *et al.,* 2024).

Table 2. Broiler Chicken Performance During the Study (1-35 days)

Parameters	T1	T2	T3	p-value
Feed intake (g bird^{-1})	3030.40±164.21^{A}	2670.16±46.18^{B}	2784.52±112.29^{B}	0.001
BWG (g bird^{-1})	1854.43±95.05^{a}	1691.35±80.60^{b}	1822.12±98.63^{a}	0.036
BW (g bird^{-1})	1900.93±96.90^{a}	1737.70±80.27^{b}	1868.52±98.77^{a}	0.037
FCR	1.60±0.03^{a}	1.54±0.07^{ab}	1.49±0.04^{b}	0.016

AME: Apparent Metabolizable Energy; TME: True Metabolizable Energy; AMEn: Apparent Metabolizable Energy corrected for Nitrogen; TMEn: True Metabolizable Energy corrected for Nitrogen; ^{a,b,c} Differences exist between means in rows with distinct superscripts ($p < 0.05$). ; T1: 0.1% Calcium carbonate ($CaCO_3$); T2: 0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^5 CFU g^{-1}); T3: 0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^6 CFU g^{-1})

Table 3. Effects of Dietary *Bacillus licheniformis* and *Lactococcus lactis* on Metabolizable Energy

Parameter	T1	T2	T3	p-value
AME (kcal kg^{-1})	3023.60±49.89^{a}	2934.15±79.93^{a}	3385.68±100.59^{b}	0.003
TME (kcal kg^{-1})	3023.78±90.72^{a}	2934.32±113.73	3385.85±146.62^{b}	0.000
AMEn (Kkcal kg^{-1})	3015.52±52.67^{a}	2925.24±162.53^{a}	3372.15±150.44^{b}	0.026
TMEn (kcal kg^{-1})	3015.70±44.78^{a}	2925.42±108.67^{a}	3372.33±123.48^{b}	0.000
Retained N (%)	86.06±2.18	84.19±5.20	86.65±0.45	0.484

AME: Apparent Metabolizable Energy; TME: True Metabolizable Energy; AMEn: Apparent Metabolizable Energy corrected for Nitrogen; TMEn: True Metabolizable Energy corrected for Nitrogen; ^{a,b,c} Differences exist between means in rows with distinct superscripts ($p < 0.05$); T1: 0.1% Calcium carbonate ($CaCO_3$); T2: 0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^5 CFU g^{-1}); T3: 0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^6 CFU g^{-1})

The improvement in growth performance may be attributed to enhanced digestive enzyme activity and intestinal morphology at appropriate probiotic inclusion levels (Pan *et al.,* 2022; Kan *et al.,* 2021; Arif *et al.,* 2021). In the present study, supplementation at 10^6 CFU g^{-1} (T3) was more effective than 10^5 CFU g^{-1} (T2), as indicated by comparable BWG to the control despite lower feed intake, suggesting improved nutrient utilization efficiency. Probiotics have been reported to increase digestive enzyme activities, including amylase, lipase, and trypsin, thereby improving nutrient digestion and absorption (Zhang *et al.,* 2022). Specifically, *Bacillus licheniformis* can enhance α-amylase, maltase, and sucrase activities while improving intestinal morphology and microbial balance (Zhang *et al.,* 2023). Furthermore, probiotics may modulate gut microbiota by suppressing pathogenic bacteria and promoting beneficial populations, as well as influence gastrointestinal hormone secretion related to feed intake regulation (Markowiak and Śliżewska, 2018).

Metabolizable Energy

The supplementation with *Lactococcus lactis* and *Bacillus licheniformis* significantly influenced metabolizable energy (ME) and nitrogen retention (NR) in broilers (Table 3). Among the treatments, the T3 group exhibited the highest ME and NR values compared to the other groups. These results indicate significant differences in energy utilization and nitrogen retention among treatments.

The higher ME and NR values observed in the T3 treatment indicate improved energy utilization efficiency and nitrogen balance in broilers supplemented with a higher dose of the probiotic combination. Enhanced nutrient absorption and digestive efficiency are commonly associated with probiotic supplementation. During fermentation in the digestive tract, beneficial bacteria such as *Lactococcus lactis* and *Bacillus licheniformis* produce organic acids, particularly lactic acid, which lower gut pH and inhibit pathogenic bacteria, thereby promoting nutrient absorption and overall gut health (He *et al.,* 2023).

Probiotic supplementation has been reported to enhance protease activity, thereby improving protein degradation, nitrogen retention, and net metabolizable energy by reducing nitrogen excretion and energy loss (Wang *et al.,* 2017). A

Table 4. The Effect of Dietary *Lactococcus lactis* and *Bacillus licheniformis* on Intestinal Morphology

Parameters	T1	T2	T3	p-value
Villus Height (μm)	1065.31±53.06	1175.39±50.60	1106.69±48.49	0.066
Villus surface area (μm^2)	0.30± 0.05	0.35± 0.18	0.25± 0.14	0.230
Crypt depth (μm)	262.15± 80.03	238.92±55.41	261.78±54.26	0.349

T1: 0.1% Calcium carbonate ($CaCO_3$); T2: 0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^5 CFU g^{-1}); T3:0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^6 CFU g^{-1})

Table 5. Ileal Microflora Population

Parameter	T1	T2	T3	p-value
Lactic acid bacteria (Log_{10} CFU g^{-1})	6.35± 0.28^{ab}	4.91± 0.75^{a}	7.28± 0.75^{b}	0.002
E. coli (Log_{10} CFU g^{-1})	7.61± 1.08^{b}	5.07± 0.85^{a}	8.15± 0.54^{b}	0.001

^{a,b,c} Significant differences exist between means in a column with distinct superscripts ($p < 0.05$); T1: 0.1% Calcium carbonate ($CaCO_3$); T2: 0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^5 CFU g^{-1}); T3: 0.1% *Lactococcus lactis* and *Bacillus licheniformis* (10^6 CFU g^{-1})

positive relationship between digestive health, metabolizable energy, and nitrogen retention has also been documented (Wealleans *et al.,* 2017). *Bacillus licheniformis* further contributes to nutrient utilization by hydrolyzing complex proteins into absorbable amino acids and increasing digestive enzyme activities, including trypsin and lipase, in the jejunum and ileum, which enhances protein and fat digestibility (Wang *et al.,* 2017). These mechanisms likely explain the increased metabolizable energy and nitrogen retention observed in the T3 treatment.

Intestinal Morphology

Probiotic supplementation did not significantly affect intestinal villus height in broiler chickens ($P > 0.05$; Table 4). Although numerical differences in villus height were observed among the treatment groups, these differences were not statistically significant, indicating that the dietary probiotic supplementation resulted in relatively similar villus height among treatments.

Villus height is an important indicator of intestinal morphology and is generally associated with the absorptive surface area of the intestine. However, because no significant differences were observed in the present study, the probiotic supplementation cannot be concluded to have improved intestinal villus development or nutrient absorptive capacity. Hartono et al. (2016) reported that probiotic supplementation could increase villus height and surface area and was associated with improved nutrient absorption and digestive enzyme activity. However, such effects were not statistically demonstrated in the present study.

The absence of a significant effect on villus height may indicate that the dietary supplementation of *Lactococcus lactis* and *Bacillus licheniformis* at the levels used in this study was insufficient to induce measurable changes in intestinal villus morphology. Probiotic microorganisms may influence intestinal structure through modulation of gut microbiota and their effects on intestinal epithelial development. *Lactococcus lactis* produces lactic acid and bacteriocins that may inhibit pathogenic bacteria and support intestinal mucosal integrity (Adel et al., 2016). Similarly, *Bacillus licheniformis* has been reported to enhance digestive enzyme secretion and support intestinal epithelial development (Kan et al., 2021; Cheng et al., 2021). Nevertheless, these potential mechanisms did not result in a statistically significant change in villus height under the conditions of the present study.

Overall, probiotic supplementation with *Lactococcus lactis* and *Bacillus licheniformis* did not significantly alter intestinal villus height in broiler chickens. Therefore, the numerical differences observed among treatments should be interpreted as variation within the experimental groups rather than evidence of an improvement

in intestinal morphology.

The increased villus height observed in the T2 and T3 treatment groups suggests a potential improvement in intestinal absorptive capacity, which is commonly associated with enhanced nutrient absorption and gut health. Probiotic supplementation has been reported to improve intestinal structure by stimulating villus growth through the enhancement of beneficial gut microbiota. Hartono *et al.* (2016) reported that increased villus height and surface area following probiotic supplementation were associated with improved nutrient absorption and enhanced digestive enzyme activity, which is consistent with the present findings.

The elevated villus height observed in the T2 group may reflect an improvement in intestinal mucosal structure resulting from probiotic-mediated modulation of gut microbiota. *Lactococcus lactis* produces lactic acid and bacteriocins that suppress pathogenic bacteria, thereby supporting mucosal integrity and promoting villus elongation (Adel *et al.,* 2016). In addition, *Bacillus licheniformis* has been shown to enhance digestive enzyme secretion and stimulate epithelial development in the intestine, contributing to increased villus height (Kan *et al.,* 2021; Cheng *et al.,* 2021).

The combination of *Lactococcus lactis* and *Bacillus licheniformis* in the T2 diet likely created a synergistic effect that promoted beneficial microbial colonization and intestinal development, resulting in improved villus morphology. Overall, these findings indicate that probiotic supplementation may improve intestinal morphology, particularly villus height, which is closely associated with enhanced nutrient absorption and gut health in broiler chickens.

Ileal Microflora Population

Table 5 shows that probiotic supplementation significantly affected the intestinal microflora of broiler chickens ($P<0.05$). The T2 treatment exhibited the lowest *Escherichia coli* population, indicating a strong inhibitory effect against this bacterium. However, this treatment also resulted in the lowest lactic acid bacteria population. In contrast, the highest lactic acid bacteria population was observed in the T3 treatment, which was accompanied by a higher *Escherichia coli* population compared to the control group. These findings indicate that probiotic supplementation produced varying effects on intestinal microbial composition depending on the treatment.

The increased population of lactic acid bacteria observed in the T3 treatment suggests that probiotic supplementation promoted beneficial microbial growth. Similar results have been reported by Pertiwi *et al.* (2008), showing that probiotics support digestive health by enhancing beneficial bacterial populations. However, in the present study, the increase in lactic acid bacteria in T3 was not accompanied by a reduction in *Escherichia coli*, indicating that the modulation of intestinal microbiota was not uniformly beneficial across all microbial groups.

The reduction of *Escherichia coli* observed in the T2 treatment suggests inhibition of pathogenic bacteria, potentially through competitive exclusion mechanisms, as reported in broiler studies (Dame-Korevaar *et al.,* 2020). Lactic acid bacteria contribute to this effect by producing organic acids that lower intestinal pH, thereby creating unfavorable conditions for pathogenic bacteria (Ferdous *et al.,* 2019). However, the simultaneous decrease in lactic acid bacteria in T2 indicates a possible imbalance or competitive interaction among microbial populations.

In addition, probiotics may influence microbial dynamics through competition for adhesion sites, interference with quorum sensing, and modulation of host immune responses (Vinayamohan *et al.,* 2024). The differing responses observed among treatments suggest that the effectiveness of probiotic supplementation depends on factors such as strain combination, dosage, and microbial interactions. Therefore, while probiotic supplementation has the potential to modulate intestinal microflora, its effects on specific bacterial populations, including *Escherichia coli*, may vary depending on the treatment conditions.

CONCLUSION

Dietary supplementation with the combination of *Bacillus licheniformis* and *Lactococcus lactis* at 10^6 CFU g^{-1} improved feed efficiency (FCR 1.49) and increased metabolizable energy, while intestinal morphology tended to improve. Probiotics modulated intestinal microflora, with T2 reducing *Escherichia coli* but also lowering

lactic acid bacteria, while T3 increased beneficial bacteria without suppressing *Escherichia coli*. These findings indicate that the probiotic combination has potential as an alternative feed additive, but the optimal dose requires further evaluation.

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CONFLICT OF INTEREST

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