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Effects of *Bacillus amyloliquefaciens* and Organic Acids on Growth Performance, Carcass Traits, Blood Parameters, and Cecal Microbiota in Cockerels

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ABSTRACT

The increasing restrictions on antibiotic growth promoters have drawn attention to natural feed additives that enhance gut health and contribute to sustainable poultry production. The present study aimed to investigate the individual and combined effects of organic acids and *Bacillus amyloliquefaciens (B. amyloliquefaciens)* on growth performance, organ development, carcass characteristics, blood biochemical and hematological parameters, and cecal microbiota in cockerels. A total of 200 cockerels, weighing 68.5 ± 4.44 grams, were randomly assigned to four treatment groups. Four treatments consisted of a control group (A0B0), organic acids alone (A1B0), *B. amyloliquefaciens* alone (A0B1), and their combination (A1B1), with five replicates of ten cockerels each over 42 days. Two factors were employed, with organic acids supplemented in the drinking water at two levels (0% [A0] and 0.08% [A1]) and *B. amyloliquefaciens* incorporated into the diet at two levels (0% [B0] and 0.01% [B1]) at a concentration of 1×10^9 CFU/g feed. Organic acids enhanced body weight gain during the starter phase (599.6 versus 546.1 g), whereas *B. amyloliquefaciens* reduced cumulative feed intake over the entire period (3044.9 versus 3330.9 g). Group A1B1 did not enhance growth beyond that of the individual additives, indicating no synergistic effect. Organic acids decreased liver and spleen weights but increased proventriculus weight, while *B. amyloliquefaciens* increased spleen weight and decreased gizzard and proventriculus weights. A significant interaction was detected for spleen weight. Most blood biochemical and hematological parameters were unaffected by the treatment. However, organic acids reduced serum total protein and globulin, while hepatic enzyme activities remained unchanged, indicating no adverse effects on liver function. Cecal microbiota profiling demonstrated that organic acids were more effective than the combined treatment in reducing *Escherichia coli* and coliform populations. In contrast, Group A1B1 resulted in the highest *Bacillus* count. Both additives modulated cecal microbial populations compared to the control group. Organic acids improved early growth and reduced pathogenic bacteria, whereas *B. amyloliquefaciens* reduced feed intake and increased *Bacillus* abundance. The combined supplementation of organic acids and *B. amyloliquefaciens* did not provide synergistic benefits, indicating a parameter-dependent response. Neither additive adversely affected hematological indices or liver function.

Keywords: Antibiotic, *Bacillus amyloliquefaciens*, Cecal microbiota, Cockerel, Growth performance, Organic acid

INTRODUCTION

Poultry production is recognized as the most significant supplier among different food supply sectors (FAO, 2023). The increasing global consumption of poultry products is driven by population growth and ongoing urbanization (Mottet and Tempio, 2017). Maintaining high-quality, safe nutrients is crucial for the poultry industry to meet consumer expectations and regulatory standards. In the poultry industry, the use of dietary antibiotics has historically been widespread (Abreu et al., 2023). For many decades, antibiotics were routinely used as a key management practice, considered essential for maintaining production efficiency and protecting birds from infectious diseases (Bao et al., 2022). Nevertheless, the long-term

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misuse of antimicrobial agents has contributed to the emergence of bacterial resistance, a major global challenge in livestock production (Haulisah et al., 2021). The worldwide prohibitions on antibiotic growth promoters (AGPs) in poultry production, notably in organic systems, necessitated the development of innovative alternatives (Salim et al., 2018). The suggested non-antibiotic options should aim to boost innate immunity and influence the cecal microbiome to support the bird's health and performance (Paneru et al., 2024). As a result, multiple studies have examined non-antibiotic alternatives such as organic acids (Khan et al., 2022; Abd El-Ghany, 2024) and *Bacillus*-based probiotics (Bao et al., 2022; Bahaddad et al., 2023). Different categories of feed additives have been suggested as alternatives to AGPs, such as organic acids, enzymes, probiotics, prebiotics, herbal extracts, essential oils, and immunostimulants. The suggested feed additives are used to mitigate production losses following the ban on antibiotics (Khan et al., 2022; Abd El-Ghany, 2024). Organic acids and probiotics have generated substantial interest due to their potential not only to enhance poultry growth but also to maintain a well-balanced gut microbiota and stimulate immune activity (Ebeid et al., 2021). Among the alternatives to AGPs, organic acids, particularly propionic, formic, citric, and acetic acids, have emerged as promising candidates (Khan et al., 2022). Organic acids, classified as short-chain fatty acids (SCFAs), are characterized by their volatility and broad-spectrum antimicrobial activity against enteric pathogens (Abd El-Ghany, 2024). Organic acids enhance gut health through multiple mechanisms, including reducing intestinal pH, improving nutrient digestibility and absorption, and suppressing pathogenic bacterial proliferation (Adil et al., 2020). Consequently, SCFAs, including propionic, acetic, butyric, lactic, and fumaric acids, have been demonstrated to enhance poultry health and performance (Khan et al., 2022). The positive effects of SCFAs include modulating the gut microbiota, enhancing carcass traits (Hamid et al., 2018), inhibiting pathogenic microorganisms (Melaku et al., 2021), enhancing nutrient digestibility (Ghazalah et al., 2011), and improving intestinal morphology (Deng et al., 2023).

Probiotics included in poultry diets are beneficial microorganisms that colonize the gut of homeothermic animals (Khan, 2013). The strains of *Bacillus*, *Bifidobacterium*, and different yeasts are the most commonly used probiotics for broiler chickens and laying hens (Khan, 2013). *Bacillus* species produce different digestive enzymes, including cellulase, α-amylase, and proteases, which optimize nutrient utilization and enhance nutrient digestibility and absorption (Lee et al., 2008). *Bacillus* species have distinct advantages in feed applications due to their ability to form resilient endospores and inhibit gut-pathogenic bacteria (Bahaddad et al., 2023). Spore formation and antimicrobial activity significantly encourage the viability and utility of *Bacillus* species in commercial feed production (Elisashvili et al., 2019). *Bacillus amyloliquefaciens (B. amyloliquefaciens)*, in particular, produces antimicrobial substances such as subtilin and barnase, which invariably reduce the abundance of pathogenic microorganisms in the gut (Lisboa et al., 2006). As a microbial feed additive, *B. amyloliquefaciens* has been recognized as a suitable replacement for AGPs in poultry production (Lei et al., 2015), mainly due to its ability to produce extracellular enzymes that promote feed utilization (Lee et al., 2008). Although different studies have investigated the effects of organic acids (Dibner and Buttin, 2002; Adil et al., 2011; Khan et al., 2022) and *B. amyloliquefaciens* (Ahmed et al., 2014; Lei et al., 2015; Bao et al., 2022) individually, few studies have examined their combined effect during the growth phase of cockerels, a period essential for enhancing growth efficiency and immune system development. The cecal microbiota is a major factor determining the metabolic fate of nutrients, immune status, and the health status of chickens (Stanley, 2014). However, the effects of concurrent use of *B. amyloliquefaciens* and organic acids on physiological indicators, including blood biochemical parameters and microbial diversity, remain largely unexplored in cockerels. Previous studies have indicated that each additive offers notable benefits individually (Khan et al., 2022; Abd El-Ghany, 2024). For instance, supplementation with *B. amyloliquefaciens* has been indicated to improve growth performance in broiler chickens (Ahmed et al., 2014), although similar studies in native cockerels remain limited. Additionally, organic acids have been indicated to effectively control enteric pathogens, including *Clostridium perfringens*, *Escherichia coli* (*E. coli*), and *Salmonella spp*. (Islam et al., 2024).

Despite promising findings in individual applications, potential synergistic interactions among these additives, particularly regarding metabolic and microbiological responses and pharmacodynamic interactions within the gastrointestinal tract of native cockerels, remain largely unexamined. It remained uncertain whether their concurrent supplementation could enhance beneficial microbiota, antagonistically suppress pathogens, or lead to unexpected interactions in organ development and metabolic functions. Therefore, the present study aimed to investigate the individual and combined effects of organic acids and *B. amyloliquefaciens* on growth performance, organ development, blood parameters, and cecal microbiota in Saudi native cockerels.

MATERIALS AND METHODS

Ethical approval

The study protocol was reviewed and approved by the Internal Scientific Committee of the National Organic Agriculture Center, Ministry of Environment, Water and Agriculture, Saudi Arabia (Approval No. NOAC/2023/014, dated 15 March 2023). The experimental chickens were obtained from the Organic Poultry Unit of the National Organic Agriculture Center. All experimental procedures were conducted in accordance with local regulations (Royal Decree No. M / 59, dated 14/09/1431 H) and internationally accepted guidelines for the care and use of animals in research.

Cockerels and management

The current experiment was conducted at the National Organic Agriculture Center of the Ministry of Environment, Water, and Agriculture in Saudi Arabia. One-day-old Saudi native cockerels were obtained from the Organic Poultry Unit of the National Organic Agriculture Center. The cockerels hatched from eggs laid by an in-house breeding flock of Saudi native chickens raised at the center. Eggs were incubated at 37.5˚C and 55-60% humidity in the hatchery center. After hatching, male chickens (cockerels) were individually selected, with an initial body weight of 68.5 ± 4.44 g, and randomly assigned to experimental treatments. All cockerels were housed in 20 floor wire pens (100 × 100 × 100 cm) within an environmentally controlled house. The floor of each pen was covered with a 5-cm layer of wood shavings as litter. Each pen had a feeder and a drinker to provide *ad libitum* feed and water throughout the experimental period. The temperature was maintained at 35˚C for the first week of age, then gradually reduced to 24-27˚C at 3˚C per week, with relative humidity ranging from 50 to 70%. Continuous lighting was kept throughout the experiment. The same lighting program was applied to all treatment groups to ensure uniform management conditions and to avoid potential confounding effects of lighting on the evaluation of the dietary treatments.

Experimental design and treatments

A total of 200 cockerels were randomly allocated to four treatments in a completely randomized design, employing a 2 × 2 factorial experiment with five replications of ten cockerels each. The two factors included the use of organic acids and *B. amyloliquefaciens*. The organic acid was used at two levels in the drinking water; 0% (drinking water without organic acids; A0) and 0.08% (A1). The two levels of *B. amyloliquefaciens* were 0% (basal diet without supplementation; B0) and 0.01% in the diet, at a concentration of 1 × 10^9 CFU/g of feed (B1). Consequently, four treatments included a control group without supplementation of either additives (A0B0), a group with only organic acids at 0.08% (A1B0), a group supplemented with *B. amyloliquefaciens* at 0.01% (1 × 10^9 CFU/g feed; A0B1), and a combined group receiving both organic acids at 0.08% and *B. amyloliquefaciens* at 0.01% (1 × 10^9 CFU/g of feed; A1B1). For organic acid supplementation, a commercial organic acid blend (Agrocid Super, CID LINES N. V., Belgium) containing formic acid, propionic acid, lactic acid, citric acid, and sorbic acid was added to the drinking water at a final concentration of 0.08%, which was within the safe range and below toxicity-associated levels (Adil et al., 2011). Based on the manufacturer's specifications, the addition of 0.08% organic acid blend (Agrocid Super) reduced the pH of drinking water from approximately 7.2 (plain water) to approximately 4.0-4.5. A fresh organic acid solution was prepared daily and freely accessible to chickens during the 42-day experimental period. For probiotic supplementation, *B. amyloliquefaciens* CECT 5940 (Evonik España y Portugal S. A. U., Spain) was incorporated into the feed at 0.01% (w/w), yielding a final concentration of 1 × 10^9 CFU/g of feed. The probiotic was mixed thoroughly with the basal diet. Treated feed was prepared weekly and stored in sealed bags at room temperature until further use. The stability of *B. amyloliquefaciens* CECT 5940 in feed during the 7-day storage period was verified by laboratory testing using the spread plate method on nutrient agar after heat treatment at 80˚C for 10 minutes. Feed samples were collected on days 0 and 7, and viable *Bacillus* counts were determined using standard serial dilution and plating methods. Colony counts were calculated from plates containing 30-300 colonies. The current results confirmed that the probiotic remained stable throughout storage, with viability exceeding 95% of the initial count on day 7. Basal starter (0-21 days) and finisher (22-42 days) diets were formulated to meet or exceed the nutrient requirements recommended by the National Research Council (NRC, 1994) for poultry. All experimental diets were designed to be isoenergetic with equal metabolizable energy (ME) and iso-nitrogenous, containing the same crude protein

(CP) to ensure that any observed effects resulted from the additives themselves rather than from differences in energy or protein levels. The composition of the experimental diets is detailed in Table 1.

Table 1. Ingredients and chemical composition of the experimental diets

Ingredients (%)	Starter diet with *Bacillus amyloliquefaciens*	Starter diet without *Bacillus amyloliquefaciens*	Finisher diet with *Bacillus amyloliquefaciens*	Finisher diet without *Bacillus amyloliquefaciens*
Corn	56.9	57.65	57.6	57.20
Soybean meal (40% CP)	32.55	32.5	32.8	32.80
Corn germ cake	0.59	0.59	0.00	0.00
Wheat bran	2.40	2.26	1.49	1.90
Salt	0.20	0.20	0.20	0.20
Vegetable oil	3.90	3.50	4.10	4.10
Dicalcium phosphate	1.75	1.75	1.80	1.80
Limestone	1.20	1.20	1.30	1.30
DL-Methionine	0.10	0.10	0.20	0.20
Lysine	0.20	0.05	0.30	0.30
Vitamin-mineral premix[1]	0.20	0.20	0.20	0.20
Bacillus amyloliquefaciens	0.01	0.00	0.01	0.00
Calculated analysis				
ME (kcal/kg)	3198	3196	3201	3202
Crude Protein	20.06	20.09	20.01	20.03
Calcium	0.98	0.98	1.01	1.01
Available phosphorus	0.36	0.36	0.35	0.35
Lysine	1.45	1.45	1.43	1.43
Methionine	0.54	0.54	0.53	0.53

[1]Vitamin-mineral premix provided the following per kilogram of diet. Vitamin A (retinyle acetate): 10,000 IU, Vitamin D_3 (cholecalciferol): 2,500 IU, Vitamin E (α-tocopheryl acetate): 60 mg, Vitamin K_3 (menadione sodium bisulfite complex): 15 mg, Vitamin B_1 (thiamine hydrochloride): 2 mg, Vitamin B_2 (riboflavin): 8 mg, Vitamin B_6 (pyridoxine hydrochloride): 4 mg, Vitamin B_{12} (cyanocobalamin): 0.04 mg, Pantothenic acid: 15 mg, Nicotinic acid: 40 mg, Folic acid: 1.5 mg, Biotin: 0.2 mg, Choline chloride: 200 mg, Iron: 50 mg, Manganese: 50 mg, Copper: 10 mg, Zinc: 50 mg, Calcium: 352 mg, Iodine: 1.46 mg, Cobalt: 0.5 mg, Selenium: 0.2 mg. ME: Metabolizable energy (Kcal/Kg), CP: Crude protein, DL-Methionine: A racemic mixture of the D- and L- isomers of methionine (synthetic methionine). Experimental diets were formulated according to NRC (1994).

Growth performance

Feed consumption and body weight gain were recorded weekly for each replicate. The collected data were used to calculate the feed conversion ratio (FCR). Growth performance parameters were evaluated across three periods, including starter (0-21 days), finisher (22-42 days), and the entire experimental period (0-42 days).

Slaughter performance

At the end of the trial, two cockerels from each pen were selected based on their proximity in weight to the average weight of the cockerels within their respective pen (experimental unit). The selected cockerels were subsequently slaughtered to determine the dressing percentage, as well as the relative weights of the proventriculus, gizzard, liver, pancreas, and spleen, along with the measurements of the intestine and ceca lengths. After slaughter, the abdominal cavity was opened, and the internal organs were carefully excised. The proventriculus and gizzard were opened, and their contents were completely removed by gentle scraping and rinsing with saline solution. All organs, including the liver, spleen, proventriculus, gizzard, and pancreas, were gently dried with filter paper to remove surface blood and moisture before weighing. The relative weights of the organs were calculated as a percentage of live body weight. The lengths of the intestine and ceca were measured following gentle expression of their contents. The cockerel's carcass sections, such as breast, thigh, and drumstick, were measured relative to their live body weights.

Blood sample collection

On day 42, two cockerels were selected randomly from each pen (10 cockerels per treatment) to collect the blood samples. The cockerels were fasted for 4 to 6 hours prior to blood collection, with feed removed, and water, free of organic acids, was available *ad libitum*. Two blood samples were collected via wing veins from each selected cockerel before slaughter to avoid post-mortem changes. The first sample was collected into a tube containing anticoagulant (EDTA) for hematological parameters using an automated hematology analyzer (HumaCount, 30TS, Germany). The hematological parameters analyzed were red blood cells (RBCs), packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBCs), differential leukocyte counts, and mean corpuscular hemoglobin concentration (MCHC). The second blood sample was placed in a plain test tube, centrifuged (DLAB, DM0412, China) at 6,000 × g for 10 minutes to separate the serum, and stored at -20˚C until further biochemical analysis. Blood biochemical parameters were measured spectrophotometrically using a HumaLyzer 4000 (HUMAN, Germany) according to the manufacturer's instructions. Commercial diagnostic kits (HUMAN, Germany) were used to determine glucose, total protein, albumin, cholesterol, calcium, phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), and high-density lipoprotein (HDL). The globulin concentration was calculated as the difference between the total protein and albumin (Busher, 1990). Low-density lipoprotein (LDL) and Very-low-density lipoprotein (VLDL) concentrations were calculated using the Friedewald formula (Friedewald et al., 1972).

Cecal microbial count

Cecal samples (1 g each) were collected aseptically from two cockerels per replicate (10 samples per treatment) for microbial analysis. All dissecting tools were autoclaved at 121˚C for 15 minutes and flamed with 70% ethanol between samples. Cecal contents were collected directly into pre-sterilized tubes using sterile forceps and scalpels. All procedures were performed on a sterile surface near a Bunsen burner flame. Plating was conducted in a laminar flow cabinet. Gut microbial analysis involved quantifying total aerobic bacteria, *E. coli*, and *Bacillus* spp., using established methods (McDonald et al., 1983; van Horn et al., 1996; Hossain et al., 2015). Serial decimal dilutions of the homogenized digesta were prepared with sterile buffered peptone water (Liofilchem, Italy; Cat. No. 611014) for microbial counting. Selective agar media were employed for each target organism. Nutrient agar (Mast Group, Germany; Cat. No. DM179D) for total aerobic bacteria, chromatic coliform agar (Liofilchem, Italy; Cat. No. 610610) for coliforms, chromatic *E. coli* O157 agar (Liofilchem, Italy; Cat. No. 610614) for *E. coli*, and polymyxin egg yolk mannitol bromothymol blue agar (PEMBA; Neogen, USA; Cat. No. LAB193) for *Bacillus cereus* were used. All plates were aerobically incubated at 37˚C. Bacterial counts were reported as log10 colony-forming units (CFU) per gram of digesta based on colony morphology and distinctive growth characteristics.

Statistical analysis

All data were analyzed using SPSS, version 21. Two-way analysis of variance (ANOVA) was used to assess the effects of two independent variables, namely organic acids and *B. amyloliquefaciens*, on performance, blood parameters, carcass characteristics, and cecal microbiota of cockerels. The model analyzed the primary effects of each factor along with their interactions. Differences between mean values of the treatments were determined using Duncan's multiple-range test (Statistical significance was defined at $p \leq 0.05$). The results were expressed as the means and standard error of the mean (SEM).

RESULTS

Growth performance

The effects of water supplemented with organic acids and the dietary incorporation of *B. amyloliquefaciens* on the growth performance of the cockerels during the starter (0-21 days), finisher (22-42 days), and entire experimental periods are presented in Table 2. Organic acid supplementation did not significantly affect feed consumption during the starter period (0-21 days), finisher period (22-42 days), or the entire experimental period (0-42 days; $p > 0.05$). In contrast, the dietary inclusion of *B. amyloliquefaciens* significantly reduced the cumulative feed intake (3044.9 g) in comparison to B0 (3330.9 g) throughout the entire experimental period (0-42 days; $p < 0.05$). However, no significant effects were observed during the starter or finisher phases when considered separately ($p > 0.05$). Supplementing with organic acids significantly increased body weight gain in Group A1, reaching 599.6 g compared to 546.1 g in Group A0 during the starter period (0-21 days; $p < 0.05$). No significant effects on weight gain were observed during the finisher period or the entire experimental period ($p > 0.05$). *Bacillus amyloliquefaciens* supplementation did not significantly affect weight gain during any period (starter, finisher, or entire period; $p > 0.05$). Organic acid supplementation significantly impaired FCR throughout the entire 0-42 day experimental period, with A1 at 3.2 compared to A0 at 2.9 ($p < 0.05$); however, no significant effects were observed during the starter or finisher periods ($p > 0.05$). *Bacillus amyloliquefaciens* supplementation did not significantly affect FCR during any period ($p > 0.05$). No significant interaction effects between organic acids and *B. amyloliquefaciens* were observed for any growth parameters, including feed consumption, weight gain, or FCR, during any period ($p > 0.05$).

Table 2. Effects of organic acids and *Bacillus amyloliquefaciens* on cockerels' growth performance during starter, finisher, and entire experimental periods

Treatments	Weight gain (g)			Feed consumption (g)			Feed conversion ratio (g:g)		
	Starter	Finisher	Entire period	Starter	Finisher	Entire period	Starter	Finisher	Entire period
Control (A0B0)	565.4	409.8	1043.6	1777.4	1445.4	3223	3.00	3.52	2.86
Bacillus amyloliquefaciens (A0B1)	526.8	397	986.6	1660	1375	2998.4	3.16	3.46	2.96
Organic acids (A1B0)	595.2	406.2	1014.4	1824.4	1428.6	3438.8	3.36	3.52	3.38
Combination (A1B1)	604.0	390.6	1001.6	1699	1394.4	3091.4	2.82	3.58	3.10
SEM	24.8	7.35	27.59	143.1	58.52	133.2	0.18	0.127	0.162
Organic acids effects									
A0	546.1[b]	403.4	1016.6	1718.7	1410.2	3110.7	3.1	3.5	2.9[b]
A1	599.6[a]	398.4	1019	1761.7	1411.5	3265.1	3.1	3.6	3.2[a]
SEM	17.5	5.2	19.5	101.2	41.38	94.19	0.13	0.09	0.12
p-value	0.046	0.506	0.759	0.768	0.983	0.263	0.956	0.644	0.048
***Bacillus amyloliquefaciens* Effects**									
B0	580.3	408	1029	1800.9	1437	3330.9[a]	3.2	3.5	3.1
B1	565.4	393.8	995.6	1679.9	1384.7	3044.9[b]	3.0	3.5	3.0
SEM	17.5	5.2	19.5	101.2	41.38	94.2	0.13	0.09	0.12
p-value	0.556	0.071	0.244	0.409	0.385	0.047	0.307	0.981	0.587
Organic acids × *Bacillus amyloliquefaciens* (p-value)	0.353	0.851	0.466	0.978	0.761	0.657	0.07	0.644	0.259

A0 and A1: Levels of organic acids at 0 and 0.08%, respectively, B0 and B1: Levels of *Bacillus amyloliquefaciens* as probiotics at 0 and 0.01%, respectively, with a concentration of 1×10^9 CFU/g. SEM: Standard error of the mean, starter: From day 0 to 21, finisher: From day 22 to 42, entire period: From day 0 to 42. [a,b] Mean different superscript letters in the same column were significantly different at $p \leq 0.05$.

Slaughter performance

The percentages of internal organs in relation to body weight, as well as the lengths of the intestine and ceca of the cockerels affected by organic acids and *B. amyloliquefaciens*, were presented in Table 3. The present results indicated that organic acids significantly decreased the relative weight of the liver and spleen ($p < 0.05$), compared with Group A0. Conversely, the proventriculus weight was significantly increased in cockerels receiving organic acids ($p < 0.05$). No statistically significant differences were observed in the weights of the gizzard and pancreas, nor the lengths of the intestines and ceca between treated and non-treated groups ($p > 0.05$). Similarly, adding *B. amyloliquefaciens* to the cockerels' diet did not significantly affect the weight of the pancreas or the lengths of the intestine and ceca compared to Group B0 ($p > 0.05$). Furthermore, the liver weight was not influenced by the inclusion of *B. amyloliquefaciens* in the diet. In contrast, cockerels receiving diets with *B. amyloliquefaciens* revealed a significant increase in spleen relative weight ($p < 0.05$), while the relative weights of the gizzard and proventriculus were significantly decreased compared to cockerels receiving diets without *B. amyloliquefaciens* ($p < 0.05$). No significant effects of *B. amyloliquefaciens* supplementation were observed on pancreas weight or the lengths of the intestine and ceca ($p > 0.05$). Liver weight remained unaffected. A significant interaction between organic acids and *B. amyloliquefaciens* was detected for spleen weight ($p < 0.05$). Group A0B1 significantly increased spleen weight (0.396%) compared to Group A0B0 (0.184%; $p < 0.05$). However, Group A1B1 (0.192%) demonstrated no statistically significant difference compared to Group A1B0 (0.176%; $p > 0.05$). No significant effects were observed in any carcass parameter, including carcass weight, dressing percentage, breast, thigh, and drumstick, for either organic acids (A1 and A0) or *B. amyloliquefaciens* groups (B1 and B0; $p > 0.05$; Table 4).

Serum biochemical parameters and liver enzymes

The effects of organic acids and *B. amyloliquefaciens* on serum biochemical parameters, liver enzymes, and calcium and phosphorus of the cockerels are shown in Tables 5 and 6. Most serum biochemical parameters, including glucose, cholesterol, triglycerides, HDL, LDL, VLDL, albumin, calcium, and phosphorus, were not significantly affected by any treatment ($p > 0.05$). However, Group A1 exhibited significantly lower total protein and globulin levels (3.47 g/dL and 2.23 mg/dL, respectively) in comparison to those observed in Group A0 (3.88 g/dL and 2.61 mg/dL, respectively; $p < 0.05$). There were no significant differences between Group B1 and B0 in terms of reduced total protein and globulin levels (p > 0.05). Supplementation with organic acids and *B. amyloliquefaciens* had no significant effect on serum AST or ALT activities (p > 0.05).

Hematological parameters

The results of the hematological parameters influenced by organic acids and/or *B. amyloliquefaciens* are summarized in Table 7. Supplementation with organic acids and/or *B. amyloliquefaciens* had no significant effect on any hematological parameter, including RBC, PCV, Hb, WBC, and MCHC (p > 0.05). Similarly, supplementation with organic acids or *B. amyloliquefaciens* did not significantly affect the differential leukocyte profile, including heterophils, lymphocytes, monocytes, or the heterophil-to-lymphocyte ratio (p > 0.05). Additionally, no significant interactions were observed for any hematological parameters (p > 0.05).

Cecal microbiota

The present results indicated that both organic acids and *B. amyloliquefaciens* significantly affected cecal microbiota populations (Table 8). Group A1 had lower coliform, *E. coli*, and total aerobic bacterial counts than Group A0. Conversely, Group B1 exhibited an increase in *Bacillus* spp. while concurrently decreasing the populations of coliform and *E. coli* compared to Group B0. Significant interactions between the two factors were detected for all microbial variables (p < 0.001). Group A1B1 had the highest *Bacillus* spp. count (5.11 log_{10} CFU/g), whereas the lowest coliform (5.91 log_{10} CFU/g) and *E. coli* (4.71 log_{10} CFU/g) counts were observed in Group A1B0. In contrast, *B. amyloliquefaciens* increased total aerobic bacterial counts, potentially mitigating the suppressive effect of organic acids and resulting in higher counts in the combined treatment group than in the cockerels receiving organic acids alone.

Table 3. Effects of organic acids and *Bacillus amyloliquefaciens* on the relative weights of internal organs and the lengths of the intestine and ceca of the cockerels at 42 days of age

Treatments	Liver (%)	Spleen (%)	Proventriculus (%)	Gizzard (%)	Pancreas (%)	Intestinal length (cm)	Cecal length (cm)
Control (A0B0)	1.654	0.184	0.412	1.528	0.188	119.0	18.6
Bacillus amyloliquefaciens (A0B1)	1.482	0.396	0.164	1.472	0.194	116.4	14.6
Organic acids (A1B0)	1.404	0.176	0.448	1.708	0.19	119.6	14.0
Combination (A1B1)	1.352	0.192	0.36	1.254	0.152	114.7	14.9
SEM	0.081	0.026	0.05	0.111	0.015	6.358	1.529
Organic acids effects							
A0	1.568[a]	0.290[a]	0.288[b]	1.5	0.191	117.7	16.6
A1	1.378[b]	0.184[b]	0.404[a]	1.48	0.171	117.2	14.5
SEM	0.057	0.019	0.035	0.078	0.011	4.5	1.08
p-value	0.031	0.001	0.034	0.866	0.211	0.932	0.179
***Bacillus amyloliquefaciens* effects**							
B0	1.529	0.180[b]	0.430[a]	1.62[a]	0.189	119.3	16.3
B1	1.417	0.294[a]	0.262[b]	1.36[b]	0.173	115.6	14.8
SEM	0.057	0.019	0.035	0.078	0.011	4.5	1.08
p-value	0.183	0.001	0.004	0.035	0.313	0.564	0.326
Organic acids × *Bacillus amyloliquefaciens* (p-value)	0.467	0.002	0.129	0.091	0.171	0.859	0.129

A0 and A1: Levels of organic acids at 0 and 0.08%, respectively, B0 and B1: Levels of *Bacillus amyloliquefaciens* as probiotics at 0 and 0.01%, respectively, with a concentration of 1×10^9 CFU/g. SEM: Standard error of the mean. [a,b] Mean different superscript letters in the same column were significantly different at $p \leq 0.05$.

Table 4. Effects of organic acids and *Bacillus amyloliquefaciens* on carcass weight, dressing percentage, and cut-up part yields of the cockerels at 42 days of age

Treatments	Carcass weight (g)	Dressing (%)	Breast (%)	Thigh (%)	Drumstick (%)
Control (A0B0)	700.6	68.13	13.78	5.59	5.26
Bacillus amyloliquefaciens (A0B1)	673.6	65.74	11.79	5.84	4.90
Organic acids (A1B0)	657.4	64.46	12.3	5.83	5.38
Combination (A1B1)	697.2	64.76	11.594	5.25	4.80
SEM	38.742	2.617	0.878	0.369	0.292
Organic acids effects					
A0	687.1	66.93	12.78	5.71	5.08
A1	677.3	64.61	11.95	5.54	5.09
SEM	27.4	1.85	0.621	0.261	0.206
p-value	0.804	0.389	0.355	0.646	0.973
***Bacillus amyloliquefaciens* effects**					
B0	679	66.3	13.04	5.71	5.32
B1	685.4	65.25	11.69	5.54	4.85
SEM	27.4	1.85	0.621	0.261	0.206
p-value	0.871	0.694	0.145	0.653	0.128
Organic acids × *Bacillus amyloliquefaciens* (p-value)	0.401	0.614	0.477	0.273	0.701

A0 and A1: Levels of organic acids at 0 and 0.08%, respectively, B0 and B1: Levels of *Bacillus amyloliquefaciens* as probiotics at 0 and 0.01%, respectively, with a concentration of 1× 109 CFU/g. SEM: Standard error of the mean.

Table 5. Effects of organic acids and *Bacillus amyloliquefaciens* on cockerels' serum biochemical parameters at 42 days of age

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control (A0B0)	244.0	99.0	60.8	34.46	52.4	12.15	3.93	1.26	2.67
Bacillus amyloliquefaciens (A0B1)	246.4	106.4	63.4	34.06	59.5	12.76	3.84	1.31	2.55
Organic acids (A1B0)	262.4	100.4	45.4	37.14	54.2	9.08	3.48	1.28	2.202
Combination (A1B1)	219.6	103.8	62.8	35.98	55.2	12.55	3.45	1.19	2.26
SEM	17.503	9.281	5.246	3.127	6.978	1.049	0.165	0.076	0.164
Organic acids effects									
A0	245	103	62.0	34.2	56.0	12.5	3.88[a]	1.29	2.61[a]
A1	241	102	54.0	36.6	54.7	10.8	3.47[b]	1.23	2.23[b]
SEM	12.4	6.6	3.7	2.2	4.9	0.74	0.12	0.05	0.12
p-value	0.81	0.949	0.147	0.473	0.86	0.138	0.022	0.501	0.035
***Bacillus amyloliquefaciens* effects**									
B0	253	100	53.0	35.8	53.3	10.6	3.7	1.27	2.44
B1	233	105	63.0	35.0	57.4	12.7	3.65	1.25	2.41
SEM	12.4	6.6	3.7	2.2	4.9	0.74	0.022	0.05	0.12
p-value	0.265	0.569	0.075	0.806	0.568	0.07	0.73	0.835	0.857
Organic acids × *Bacillus amyloliquefaciens* (p-value)	0.215	0.832	0.178	0.905	0.67	0.192	0.867	0.335	0.599

A0 and A1: Levels of organic acids at 0 and 0.08%, respectively, B0 and B1: Levels of *Bacillus amyloliquefaciens* as probiotics at 0 and 0.01%, respectively, with a concentration of 1× 10^9 CFU/g. TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, SEM: Standard error of the mean. [a,b] Mean different superscript letters in the same column were significantly different at $p \leq 0.05$.

Table 6. Effects of organic acids and *Bacillus amyloliquefaciens* on cockerels' liver enzymes and serum calcium and phosphorus at 42 days of age

Treatments	ALT (U/L)	AST (U/L)	Calcium (mg/dL)	Phosphorus (mg/dL)
Control (A0B0)	4.43	248.2	7.48	6.22
Bacillus amyloliquefaciens (A0B1)	4.77	249	8.72	5.5
Organic acids (A1B0)	5.58	271.4	7.66	5.02
Combination (A1B1)	5.25	284.8	7.56	5.1
SEM	0.46	23.65	0.446	0.432
Organic acids effects				
A0	4.6	248.6	8.1	5.86
A1	5.42	278.1	7.61	5.06
SEM	0.323	16.72	0.315	0.305
p-value	0.092	0.23	0.288	0.082
***Bacillus amyloliquefaciens* effects**				
B0	5.0	259.8	7.57	5.62
B1	5.01	266.9	8.14	5.3
SEM	0.323	16.72	0.315	0.305
p-value	0.991	0.768	0.315	0.469
Organic acids × *Bacillus amyloliquefaciens* (p-value)	0.474	0.793	0.152	0.368

A0 and A1: Levels of organic acids at 0 and 0.08%, respectively, B0 and B1: Levels of *Bacillus amyloliquefaciens* as probiotics at 0 and 0.01%, respectively, with a concentration of 1× 10^9 CFU/g. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, SEM: Standard error of the mean. All measurements were performed on serum samples collected at 42 days of age. No significant differences were observed for any parameter ($p > 0.05$).

Table 7. Effects of organic acids and *Bacillus amyloliquefaciens* on cockerels' hematological profile at 42 days of age

Treatments	RBC (10^6/µL)	PCV (%)	Hb (g/dL)	WBC (10^3/µL)	Heterophils (10^3/µL)	Lymphocytes (%)	Monocytes (%)	H/L ratio	MCHC (g/dL)
Control (A0B0)	3.01	30.72	21.23	24.60	7.87	53.46	8.22	0.56	69.40
Bacillus amyloliquefaciens (A0B1)	3.06	31.16	21.65	31.08	9.63	54.86	9.30	0.71	69.62
Organic acids (A1B0)	2.89	30.38	20.80	26.28	7.23	54.80	8.86	0.53	68.42
Combination (A1B1)	2.84	28.72	19.97	21.60	7.67	52.60	8.12	0.57	69.50
SEM	0.120	1.335	0.808	4.14	0.719	0.856	0.609	0.052	1.397
Organic acids effects									
A0	3.03	30.94	21.44	27.84	8.75	54.16	8.76	0.63	69.51
A1	2.87	29.55	20.38	23.94	7.45	53.70	8.49	0.55	68.96
SEM	0.085	0.994	0.572	2.93	0.508	0.605	0.431	0.55	0.988
p-value	0.188	0.313	0.210	0.360	0.089	0.598	0.664	0.137	0.699
***Bacillus amyloliquefaciens* effects**									
B0	2.95	30.55	21.01	25.44	7.55	54.13	8.54	0.55	68.91
B1	2.95	29.94	20.81	26.34	8.65	53.73	8.71	0.54	69.56
SEM	0.085	0.944	0.572	2.93	0.508	0.605	0.431	0.037	0.988
p-value	0.993	0.654	0.803	0.831	0.145	0.647	0.784	0.104	0.648
Organic acids × *Bacillus amyloliquefaciens* (p-value)	0.677	0.443	0.448	0.196	0.371	0.062	0.155	0.295	0.762

A0 and A1: Levels of organic acids at 0 and 0.08%, respectively, B0 and B1: Levels of *Bacillus amyloliquefaciens* as probiotics at 0 and 0.01%, respectively, with a concentration of 1× 10^9 CFU/g. RBC: Red blood cells, PCV: Packed cell volume, Hb: Hemoglobin, WBC: White blood cells, H/L: Heterophils/Lymphocyte's ratio, MCHC: mean corpuscular hemoglobin concentration, SEM: standard error of the mean.

Table 8. Effects of organic acids and *Bacillus amyloliquefaciens* on cockerels' cecal microbial count (log_{10} CFU/g) at 42 days of age

Treatments	*Bacillus* count	Coliforms	Aerobic bacteria	*Escherichia coli*
Control (A0B0)	3.65	10.14	13.045	7.49
Bacillus amyloliquefaciens (A0B1)	4.826	6.85	11.675	5.11
Organic acids (A1B0)	3.554	5.905	9.86	4.705
Combination (A1B1)	5.11	7.912	12.765	5.90
SEM	0.041	0.126	0.132	0.11
Organic acids effects				
A0	4.238^{b}	8.495^{a}	12.360^{a}	6.300^{a}
A1	4.332^{a}	6.908^{b}	11.313^{b}	5.303^{b}
SEM	0.029	0.089	0.093	0.078
p-value	0.035	0.0001	0.0001	0.0001
***Bacillus amyloliquefaciens* effects**				
B0	3.602^{b}	8.022^{a}	11.452^{b}	6.098^{a}
B1	4.968^{a}	7.381^{b}	12.220^{a}	5.505^{b}
SEM	0.029	0.089	0.093	0.078
p-value	0.0002	0.0001	0.0001	0.0002
Organic acids × *Bacillus amyloliquefaciens* (p-value)	0.0001	0.0002	0.0001	0.0002

A0 and A1: Levels of organic acids at 0 and 0.08%, respectively, B0 and B1: Levels of *Bacillus amyloliquefaciens* as probiotics at 0 and 0.01%, respectively, with a concentration of 1×10^9 CFU/g. SEM: Standard error of the mean. [a,b] Mean different superscript letters in the same column are significantly different at $p \leq 0.05$.

Growth performance

The current study revealed that supplementing drinking water with organic acids notably improved body weight gain in cockerels during the starter period (0-21 days) by 9.8% compared to the control group. Improved body weight gain observed during the starter phase in the present study was consistent with findings of Adil et al. (2011) and Dibner and Buttin (2002), who reported that organic acids enhanced early growth by improving nutrient digestibility, reducing intestinal pH, and suppressing pathogenic bacteria. Although early growth improved, supplementing with organic acids considerably increased the FCR throughout the experimental period. The impaired FCR observed during the experimental period may be attributed to microbial adaptation to organic acids over time, changes in gut microbiota dynamics, or a decline in the effectiveness of organic acids as the cockerels' age (Ricke, 2003). The present findings indicated that careful optimization of the timing and duration of organic acid administration was required in cockerel production. Throughout the growth periods, body weight gain and FCR in cockerels were not affected by the dietary inclusion of *B. amyloliquefaciens*. Despite the substantial reduction in cumulative feed intake, no improvement in FCR was observed during the present study. *Bacillus* strains, including *B. amyloliquefaciens* and *B. subtilis*, have been demonstrated to modulate the gut microbiota, suppress pathogenic bacteria, and produce beneficial enzymes and antimicrobial substances (Jeong and Kim, 2014; Wang et al., 2021). No notable interactions have been recorded between organic acids and *B. amyloliquefaciens* across all investigated growth parameters. The absence of interaction indicated that organic acids and *B. amyloliquefaciens* may exert their effects through distinct physiological pathways that did not overlap sufficiently to generate interactive responses under the conditions examined, which is consistent with previous reports in broilers showing variable responses to their combined use depending on the parameter assessed (Rodjan et al., 2018; Ebeid et al., 2021). The present findings reinforce that combining feed additives does not inherently yield interactive benefits and emphasize the need for parameter-specific evaluation.

Slaughter performance

In the present study, organic acid supplementation was associated with reduced relative weights of the spleen and liver. Reduced spleen and liver weights occurred in the absence of physiological stress indicators, suggesting that the observed changes were not related to pathological conditions. Nonetheless, caution was necessary when interpreting reductions in lymphoid organ size, since these changes alone did not definitively indicate altered immune function. The antimicrobial properties of organic acids, such as formic, propionic, lactic, citric, and sorbic acids, likely helped modulate the intestinal microbiota and decrease microbial antigen exposure, potentially affecting the development of immune organs (Dibner and Buttin, 2002; Klasing, 2007; Adil et al., 2010).

In contrast, dietary inclusion of *B. amyloliquefaciens* was associated with an increase in spleen relative weight. However, spleen enlargement was interpreted with caution, since organ size alone did not definitively indicate immune activation, especially in the absence of additional immunological data. The lack of changes in the heterophil-to-lymphocyte ratio further supported the absence of physiological stress. Therefore, the biological importance of this finding remained unclear. Zhyla et al. (2022) observed a similar increase in lymphoid organ weights after probiotic supplementation. It is interesting to note that acidification of the upper gastrointestinal tract can influence the gut environment and microbial populations (Van Immerseel et al., 2006), potentially indirectly affecting the development of digestive organs, including the proventriculus. Conversely, the absence of notable alterations in gizzard and pancreas weights, in addition to intestinal and cecal lengths, indicated that organic acids had a limited impact on the structural development of the lower gastrointestinal tract. Gunal et al. (2006) likewise reported limited morphological changes despite functional responses to organic-acid supplementation. The gut-modulatory and antimicrobial properties of organic acids support their potential use as alternatives to antibiotic growth promoters in poultry (Van Immerseel et al., 2003; Ebeid and Al-Homidan, 2022).

The absence of notable changes in pancreatic weight and intestinal and cecal length values following *B. amyloliquefaciens* supplementation suggested that this probiotic strain did not directly alter the structural development of cockerels' digestive and metabolic organs. The current results demonstrated that organic acids or *B. amyloliquefaciens* did not remarkably affect the carcass weight, dressing percentage, or yields of cut-up parts such as breast, thighs, wings, and drumsticks in cockerels. The absence of carcass responses indicates that these additives act primarily through physiological and microbial pathways rather than by altering tissue deposition. The current findings were consistent with those of Cengiz et al. (2015), Rehman et al. (2020), and Ebeid et al. (2021), who reported that such additives may enhance gut function without necessarily resulting in measurable changes in carcass composition under standard production conditions.

Blood biochemical parameters and liver enzymes

The current findings indicated that supplementation with organic acids and/or *B. amyloliquefaciens* did not remarkably affect the main blood biochemical parameters or liver enzyme activities in cockerels. The stability of glucose and lipid profiles (cholesterol, TG, HDL, LDL, VLDL) was consistent with the findings of Adil et al. (2010), who reported that organic acids did not affect blood lipid metabolites or glucose levels. The lack of notable changes in liver enzymes (AST and ALT) suggested that neither organic acids nor *B. amyloliquefaciens* affected liver function. The present findings were consistent with those of Qiu et al. (2023), who reported that the organic acidifier did not affect AST and ALT activities. However, the reduction in total protein and globulin levels following organic acid supplementation may reflect an immunomodulatory effect, potentially attributed to organic acids' role in modulating the gut microbiota and reducing antigenic stimulation (Adil et al. 2010).

Hematological parameters

Hematological parameters did not change with the addition of organic acids, *B. amyloliquefaciens*, or their combination, indicating that neither additive disrupted hematopoiesis nor systemic physiological homeostasis under the conditions of the present investigation. The absence of changes in differential leukocyte profiles and the heterophil-to-lymphocyte ratio indicated that the treatments did not elicit measurable stress or inflammatory responses. The unchanged hematological indices after probiotic supplementation were consistent with the findings of Nguyen et al. (2018), who similarly reported no changes. The current findings indicated that the organic acids and *B. amyloliquefaciens* additives primarily act at the gastrointestinal level without causing systemic hematological changes, supporting their safe use in poultry diets under similar production conditions.

Cecal microbiota

The current results confirmed that supplementing organic acids, *B. amyloliquefaciens*, or their combination altered the gut microbiota composition, encouraging the growth of beneficial bacteria and reducing pathogenic microorganisms. *Bacillus amyloliquefaciens* contains a variety of bioactive substances, including antimicrobial peptides that inhibit pathogenic bacteria, promote the proliferation of beneficial gut microorganisms, and help maintain intestinal homeostasis (Sutyak et al., 2008; Luan et al., 2019). Probiotics have been shown to increase *Lactobacillus* and *Bifidobacterium* populations and decrease *Clostridium* and coliform populations in broiler chickens, including Ross 308 (Giannenas et al., 2012), Ross (Al-Fataftah and Abdelqader, 2014), and Arbor Acres (Song et al., 2014) strains. The observed increase in *Bacillus* counts following organic acid supplementation suggested a favorable modulatory effect of acidification on the establishment of spore-forming probiotic strains. The increase in *Bacillus* counts following organic acid supplementation was likely mediated through pH reduction and competitive inhibition of acid-sensitive microbial populations (Rodjan et al., 2018). In contrast, the stronger effect observed with *B. amyloliquefaciens* alone likely resulted from its natural ability to produce antimicrobial compounds and competitively outcompete pathogenic bacteria (Tang et al., 2017).

Organic acids and *B. amyloliquefaciens* demonstrated strong antimicrobial effects, notably reducing *E. coli* and coliform populations. The antimicrobial effects of both additives likely resulted from distinct yet complementary mechanisms, such as pH-dependent microbial inhibition by organic acids and the production of bacteriocins (subtilin, subtilosin) by *Bacillus* spp. (Lisboa et al., 2006; Liu et al., 2021; Rathnayake et al., 2021). However, the combined treatment was less effective than organic acids alone at inhibiting *E. coli*, indicating a parameter-dependent interaction that needs mechanistic investigation, which may reflect differences in the responses of intestinal microbial populations to organic acids and probiotics when administered individually or in combination, as similarly reported in poultry by Rodjan et al. (2018). Rapid acidification may impose transient acid stress on *B. amyloliquefaciens*, potentially affecting its growth and metabolic activity during adaptation to a low-pH environment (Chowdhury et al., 2021). Furthermore, the establishment of *B. amyloliquefaciens* may require an extended adaptation period, especially under low-pH and bile-rich intestinal conditions (Rathnayake et al., 2021). The differing impacts of the two additives on total aerobic bacteria reflected their distinct mechanisms of action. Organic acids notably decreased aerobic counts by broadly inhibiting microbes, a reduction linked to lower pH (Papatsiros et al., 2014). Whereas *B. amyloliquefaciens* increased total aerobic bacterial counts, likely through competitive exclusion and metabolic cross-feeding. Similar increases in aerobic and lactic acid bacteria following probiotic supplementation have been reported previously by Shargh et al. (2012).

The total aerobic bacterial counts in the combined treatment were more similar to those in the control than to those in the organic acids' treatment alone. The observed similarity in total aerobic counts between the combined treatment and the control may be attributed to the direct contribution of *B. amyloliquefaciens*, an aerobic spore-forming bacterium, to the total aerobic population. Additionally, *B. amyloliquefaciens* can secrete extracellular enzymes, such as proteases, amylases, and cellulases, which facilitate the release of fermentable substrates and promote the proliferation of other aerobic commensals (Lee et al., 2008). The acidifying effect of organic acids may be less pronounced in the cecum compared to the upper gastrointestinal tract, thereby facilitating the survival of acid-tolerant aerobes (Gunal et al., 2006; Van Immerseel et al., 2006). Based on the present findings, the interaction between organic acids and *B. amyloliquefaciens* was not consistently synergistic. In the current study, synergistic effects mainly appeared in the growth of beneficial *Bacillus* strains and overall aerobic bacteria. However, the reduced ability to suppress pathogens indicated some functional interference when applied together. Taken together, these findings indicate a parameter-dependent interaction between organic acids and *B. amyloliquefaciens*, with the combined treatment increasing *Bacillus* counts but providing less suppression of *E. coli* and coliforms than organic acids alone.

CONCLUSION

The present study revealed that organic acids and *B. amyloliquefaciens* had distinct effects on growth, physiological traits, and cecal microbiota in native cockerels. Organic acids improved body weight gain during the starter phase and significantly reduced *E. coli*, coliforms, and total aerobic bacteria. In contrast, *B. amyloliquefaciens* reduced feed intake without adversely affecting growth and increased *Bacillus* counts in the cecum. The combined supplementation did not consistently enhance performance or microbial responses compared to individual treatments, indicating that the interaction between the two additives was parameter-dependent. No significant effects were observed on carcass characteristics, hematological indices, or liver enzyme activities, suggesting that the treatments did not adversely affect physiological status. The present study acknowledged limitations. One notable constraint was that the cecal microbiota was evaluated using culturedependent methods, which may not comprehensively capture the microbial diversity. In addition, molecular indicators of immune function were not evaluated. Therefore, future studies should focus on dose-response relationships using advanced microbiome techniques (16S rRNA sequencing) to further clarify the effects of organic acids and probiotic supplementation on poultry nutrition.

DECLARATIONS

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Authors' contributions

All authors contributed equally to the investigation, writing, reviewing, editing, and approval of the final edition of the manuscript. Mutaz Babiker contributed to conceptualization, methodology, data curation, formal analysis, supervision, and drafting the original edition. Saleh Alhomaidan carried out the methodology. Abdulmajeed Alhumud and Fahd Alsaleem contributed to software analysis. Saleh Almithn validated the study. Abdulrahman Alhumimidi, Abdullah Alobaydaa, and Sultan Al-Eid contributed to the validation process, with Sultan supervising resources and overseeing the project. Salman Aloudah was responsible for project management and securing funding. All authors read and approved the final edition of the manuscript.

Availability of the data and materials

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to institutional and ethical restrictions.

Competing interests

The authors declared that they have no competing interests.

Ethical considerations

The study protocol was reviewed and approved by the Internal Scientific Committee of the National Organic Agriculture Center, Ministry of Environment, Water and Agriculture, Saudi Arabia (Approval No. NOAC/2023/014, dated 15 March 2023). All procedures involving birds were conducted in accordance with applicable local regulations (Royal Decree No. M/59, dated 14/09/1431 H) and internationally accepted guidelines for the care and use of animals in research. Animal welfare considerations were observed throughout the experimental period. During the preparation and revision of this manuscript, the authors used ChatGPT (OpenAI) solely as an AI-assisted tool for language refinement and grammatical editing. The tool was not used to generate, analyze, or interpret experimental data, perform statistical analyses, or independently formulate scientific conclusions. The authors confirmed that the original experimental results and data reported in this study were generated independently of AI and were verified by the authors before submission and again before acceptance and publication. The authors take full responsibility for the content, accuracy, originality, and integrity of the manuscript.

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