








Effects of Yogurt Supplementation on Feed Efficiency, Growth Performance, and Ileal Nutrient Digestibility in Broiler Chicken

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ABSTRACT

The use of probiotics, particularly fermented yogurt, in poultry diets has gained substantial interest due to their capacity to enhance growth performance, feed conversion efficiency, and nutrient absorption in broiler chickens. This study evaluated the effects of yogurt supplementation on broiler performance and nutrient utilization. Two hundred one-day-old Ross-308 male broiler chicks were randomly assigned to five dietary treatments using a completely randomized design. Each treatment group included five replicates with eight chicks per replicate. The dietary treatments consisted of a control diet (without yogurt), locally prepared yogurt (5 mL/L in drinking water), yogurt fermented with *Lactobacillus acidophilus* (LA, 5 mL/L), yogurt fermented with *Streptococcus thermophilus* (ST, 5 mL/L), and yogurt co-fermented with *L. acidophilus* and *S. thermophilus* (LA+ST, 5 mL/L). The performance and ileal digestibility of nutrients were measured. Results indicated that the average daily feed intake (ADFI) significantly decreased in the LA+ST group at 0-14 days, with an 11.7% reduction compared to the control. Broilers receiving yogurt demonstrated a higher average daily gain (ADG) at 0-14 days, with the LA+ST group showing an 8% improvement over the control. At 0-28 days, the LA+ST group maintained the highest ADG, 6.8% higher than the control. The feed conversion ratio (FCR) significantly improved with yogurt supplementation at 0-14 days. Compared to the control, FCR improved by 3.6%, 7.9%, 5.7%, and 15.7% in the Local, LA, ST, and LA+ST groups, respectively. Additionally, yogurt fermented with specific lactic acid bacteria (LAB) significantly enhanced the ileal digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), and total ash (TA). These findings highlight the efficacy of yogurt fermented with *L. acidophilus* and *S. thermophilus* as a dietary supplement to enhance growth performance and nutrient utilization in broiler chickens.

Keywords: Broiler, Daily gain, Feed conversion ratio, Feed intake, Yogurt

INTRODUCTION

Chickens are unable to efficiently digest lactose present in yogurt due to the absence of lactase, the enzyme required for its digestion. However, when administered in limited quantities, yogurt may provide beneficial probiotics that support gut health, although excessive intake may result in gastrointestinal disturbances. The intricate interplay between diet, the gut microbiome, and host health is fundamental, as gut health plays a pivotal role in efficient nutrient absorption, optimal performance, and economic

viability in broiler production systems (İpçak, 2023; Perler et al., 2023). Tight junctions between the epithelium of the intestine are critical for maintaining gut barrier function, regulating nutrient assimilation, and safeguarding against pathogenic invasion. However, their disruption due to stress or disease can trigger inflammation and metabolic dysfunction (Casula et al., 2023; Chu et al., 2023). Emerging nutritional and microbiological interventions are increasingly recognized for their potential to enhance broiler productivity by modulating gut barrier function and

overall gut health (Ducatelle et al., 2023; Szabó et al., 2023).

Yogurt is a dairy product prepared by fermenting milk with probiotic bacterial cultures, commonly known as lactic acid bacteria (LAB). During the fermentation of milk, the exponential multiplication of bacteria causes the production of lactic acid sufficient enough to drop the pH of the milk to 4.4-4.8, resulting in the development of a distinctive color, a classic tangy taste, and a tart flavor (Aleman et al., 2023). The LAB count in yogurt may range from 90 to 500 billion colony forming units (CFU) per serving. The plenty of beneficial probiotics in yogurt can improve the health and performance of broiler chickens. Additionally, it supports digestive health by inhibiting pathogenic bacteria and maintaining a balance of beneficial bacteria. Furthermore, *Lactobacillus acidophilus* has been shown to reduce cholesterol absorption in the intestines thereby lowering blood cholesterol levels (Momin et al., 2023; Song et al., 2023). Above all, LAB can improve the digestibility of dietary protein and minerals such as Cu, Mn, Fe, Ca, and P in broiler chickens (Jin et al., 2000; Khayoon et al., 2024; Rodjan et al., 2018).

Previous studies have highlighted that the recommended levels of dietary yogurt improved ADG, ADFI, and FCR in broiler chickens (Mahmmod et al., 2014; Ghasemi-Sadabadi et al., 2019; Hossain and Momu, 2022). Yogurt further influenced carcass traits, meat yield, meat quality, intestinal length, and abdominal fat deposition in broiler chickens (Hossain and Momu, 2022). It is indicated that yogurt, being a probiotic-rich feed, may enhance gut health, improve nutrient absorption, and strengthen the immune response in broiler chickens. Understanding these effects can lead to optimized feed formulations, resulting in significant improvements in growth rates, feed conversion ratios, and overall health in broiler chickens (Mirsalami and Mirsalami, 2024). Therefore, this study aimed to investigate the effects of dietary yogurt supplementation on the performance and nutrient utilization of broiler chickens.

MATERIALS AND METHODS

Ethical approval

The experimental procedures involving animals were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals, with approval obtained from the relevant Bangladeshi regulatory authorities overseeing animal welfare (Memo No. CVASU/Dir (R&E) EC/2021/244) (6).

Study design

A total of 200 day-old Ross-308 male broiler chickens, with an average weight of 46.64 g, were randomly assigned to a completely randomized design into five dietary treatment groups designated as a diet without yogurt supplementation (control), a diet containing locally prepared (Local) yogurt (5 mL/L of drinking water), a diet containing *Lactobacillus acidophilus* (LA) fermented yogurt (5 mL/L of drinking water), a diet containing *Streptococcus thermophilus* (ST) fermented yogurt (5 mL/L of drinking water), and a diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* (LA+ST) fermented yogurt (5 mL/L of drinking water). Each treatment had five replications, with eight broiler chickens per replicate. The experiment was conducted between May and June 2024 at the experimental poultry station of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram-4225, Bangladesh. The broiler chickens used in the study were sourced from Nourish Agro Limited, Chattogram, Bangladesh.

Prior to the study, the shed underwent a thorough cleaning process using tap water and caustic soda. The floor, ceiling, corners, rearing cages, and brooding boxes were disinfected with a 1% (v/v) phenyl solution. After sanitization and disinfection, the building was left empty for 24 hours to ensure adequate drying. The shed entrances were securely sealed, and the shed was fumigated overnight using a gaseous fumigant comprising formalin and potassium permanganate (KMnO₄). Feeders and drinkers were cleaned and washed daily using a 0.3% (v/v) solution of Timsen® (San Vet, Brazil).

Each chick was examined for abnormalities and to confirm uniformity in size before starting the study. The shed was constructed with brick cement walls and included grooved metal wiring at the lower section. Each chick was allocated 0.17 square feet (sq. ft) of floor space in the brooding pen and 1 sq. ft in the cage. For the first three days, the broiler chickens were kept under light for 23 hours, with the lighting duration reduced by one hour each subsequent day until it reached 8 hours. The broiler chickens were raised for a period of 28 days for growth and performance trial and an additional three days for the digestibility trial. The temperature was maintained at 95°F during the first week, 90°F in the second week, 85°F in the third week, and 80°F in the fourth week and onward. The broiler chickens were vaccinated against Newcastle disease (RaniVax Plus Vet Initial, Incepta vaccine Ltd, Bangladesh) on days 5 and 17 and against Infectious Bursal Disease (GumboMed Vet, Incepta vaccine Ltd, Bangladesh) on days 12 and 22 via the ocular route.

Experimental diet and feeding trial

Three types of yogurts were prepared, and one type was purchased from the local market, all of which were tested for beneficial microorganisms before being supplemented with drinking water. The concentration of beneficial bacteria in the yogurt was 1.90×10^7 CFU for LA, 1.96×10^7 CFU for ST, and 1.98×10^7 CFU for the LA+ST-supplemented group. The broiler chickens were provided with mash-type feed (Formulated according to the breeder manual for Ross-308; Table 1). During the growth and performance trial (For the first 28 days), the broiler chickens had continuous access to feed and water.

Table 1. Starter (1-14 d) and finisher (15-28 d) diet for the experimental broiler chicken based on the broiler breeder manual (Ross-308)

Ingredients (kg)	Starter	Finisher
Maize	52.96	56.51
Rice polish	2.01	2.01
DDGS ¹	0.51	0.51
Fish oil	0.01	0.01
Soybean oil	1.81	3.01
Soybean meal	37.11	33.11
Protein concentrate	2.81	2.01
Limestone	1.11	1.21
Dicalcium phosphate	0.81	0.81
L-Lysine	0.165	0.135
DL-Methionine	0.21	0.19
Vitamin premix ²	0.25	0.25
Feedzyme ³	0.025	0.025
Common salt	0.21	0.21
Total	100.0	100.0
Calculated value		
Metabolizable energy (kcal/kg)	3001.1	3103.6
Crude protein (%)	23.06	21.13
Calcium (%)	0.96	0.95
Phosphorus (%)	0.70	0.68
Phosphorus _(avail) (%)	0.43	0.41
Lysine (%)	1.50	1.34
Methionine (%)	0.56	0.52
Cystine-methionine	0.88	0.82
Tryptophan (%)	0.32	0.30
Crude fiber (%)	3.44	3.25
Ether extract (%)	4.52	5.61

¹DDGS (Distiller's dried grain soluble): Dry matter (DM), 88.11%; Crude protein (CP), 30.69%; Ether extract (EE), 10.89%; Crude fiber (CF), 5.94%; Nitrogen free extracts (NFE), 44.56%; Total ash (TA), 7.92%; Calcium (Ca), 0.25%; P, 0.75%; Apparent gross energy (AGE), 3169.21 kcal/kg DM. ²Per 2500 g contained: Beta-carotene (vitamin A) at 12,000,000 IU, cholecalciferol (vitamin D3) at 2,400,000 IU, alpha-tocopherol (vitamin E) at 23 g, menadione (vitamin K3) at 2 g, thiamine (vitamin B1) at 2.5 g, riboflavin (vitamin B2) at 5 g, pyridoxine (vitamin B6) at 4 g, vitamin B3 at 40 g, Ca-D-pantothenate at 12.5 g, vitamin B12 at 12 mg, folic acid at 800 mg, vitamin B7 at 100 mg, cobalt at 400 mg, copper at 10 g, iron at 60 g, iodine at 400 mg, manganese at 60 g, zinc at 50 g, selenium at 150 mg, DL-methionine at 100 g, L-lysine at 60 g, and calcium (Ca) at 679.6 g. ³Per 100 g contained: cellulase at 20,000 IU, xylanase at 200,000 IU, protease at 20 IU, amylase at 40,000 IU, phytase at 20 IU, pectinase at 1,400 IU, invertase at 400 IU, hemi-cellulase at 500 IU, lipase at 20 IU, and α -galactosidase at 100 IU.

Ileal digestibility of nutrients

An additional 3 days (Days 28-30) beyond the actual growth and performance trial (Days 1-28) of the broiler chicken were considered for the digestibility trial. The earlier study thoroughly outlined the methodology (Hossain et al., 2023a). To summarize, titanium oxide (TiO₂) was administered to the diet of broiler chickens at a dosage of 5 g/kg for three days (Days 28-30) as a marker to evaluate digestibility (Short et al., 1996). On day 30 of rearing, three broiler chickens per group were ethically euthanized by severing the jugular veins and carotid arteries after a fasting period of three hours. To prevent contamination from the large intestine, ileal digesta was meticulously collected from the Meckel's diverticulum to the ileal-cecal-colon junction and preserved at -20°C (Hossain et al., 2023a). The collected digest was then freeze-dried and ground into a fine powder using a 0.25 mm mesh. Subsequently, the proximate composition of both the feed and ileal contents was analyzed (AOAC, 2019). The concentration of TiO₂ in both the experimental diet and ileal samples (3 replicates per treatment) was measured after the trial to calculate the digestibility of the nutrients using a UV-VIS spectrophotometer (UV 2600, Shimadzu, Japan). Following established procedures (Maynard, 2018), apparent ileal nutrient digestibility (AID) was calculated using specific formulae.

$$\text{AID (\%)} = 100 - \left(\left[\frac{\text{percentage of feed indicator}}{\text{percentage of ileal indicator}} \right] \times \left[\frac{\text{percentage of ileal nutrient}}{\text{percentage of feed nutrient}} \right] \times 100 \right) \text{ (Formula 1)}$$

$$\text{Apparent DM digestibility (\%)} = 100 - \left(\left[\frac{\text{percentage of feed indicator}}{\text{percentage of ileal indicator}} \right] \times \left[\frac{\text{percentage of ileal DM}}{\text{percentage of feed DM}} \right] \times 100 \right) \text{ (Formula 2)}$$

$$\text{Apparent OM digestibility (\%)} = 100 - \left(\left[\frac{\text{percentage of feed indicator}}{\text{percentage of ileal indicator}} \right] \times \left[\frac{\text{percentage of ileal OM}}{\text{percentage of feed OM}} \right] \times 100 \right) \text{ (Formula 3)}$$

$$\text{Apparent CP digestibility (\%)} = 100 - \left(\left[\frac{\text{percentage of feed indicator}}{\text{percentage of ileal indicator}} \right] \times \left[\frac{\text{percentage of ileal CP}}{\text{percentage of feed CP}} \right] \times 100 \right) \text{ (Formula 4)}$$

$$\text{Apparent CF digestibility (\%)} = 100 - \left(\left[\frac{\text{percentage of feed indicator}}{\text{percentage of ileal indicator}} \right] \times \left[\frac{\text{percentage of ileal CF}}{\text{percentage of feed CF}} \right] \times 100 \right) \text{ (Formula 5)}$$

$$\text{Apparent EE digestibility (\%)} = 100 - \left(\left[\frac{\text{percentage of feed indicator}}{\text{percentage of ileal indicator}} \right] \times \left[\frac{\text{percentage of ileal EE}}{\text{percentage of feed EE}} \right] \times 100 \right) \text{ (Formula 6)}$$

$$\text{Apparent TA digestibility (\%)} = 100 - \left(\left[\frac{\text{percentage of feed indicator}}{\text{percentage of ileal indicator}} \right] \times \left[\frac{\text{percentage of ileal TA}}{\text{percentage of feed TA}} \right] \times 100 \right) \text{ (Formula 7)}$$

Performance parameter

Mortality among the chickens was monitored and recorded daily. Dead broiler chickens were excluded from the study, for accurate calculation of feed intake per broiler chicken, the cumulative feed intake of the dead broiler chickens was subtracted from the total feed intake of a particular replicate. Similarly, to calculate weight gain, the weight of dead chickens on the last weighing day was deducted from a replicate's total weight. On days 14 and 28, ADG and ADFI were calculated to determine FCR. The ADG was determined by deducting the initial body weight of the broiler chickens from their final weight. Feed intake was calculated by subtracting the remaining feed from the initial amount provided. The FCR was then obtained by dividing the total feed intake by weight gain.

Statistical analysis

The detailed statistical procedures have been described in previous studies (Hossain and Akter, 2022; Hossain et al., 2023a; 2023b). Performance and digestibility data from each pen were averaged before further analysis, with each pen treated as an independent experimental unit. Outliers and multicollinearity in the data were assessed using variance inflation factors and interquartile range tests. The normal distribution of the response variable was evaluated using a normal probability plot, and the Shapiro-Wilk test was employed to assess the equality of variances. The analysis of the data was conducted using a generalized linear model. To assess the suitability of the dataset for principal component analysis, the Kaiser-Meyer-Olkin test for sampling adequacy and Bartlett's test of sphericity were performed using the SAS 2022 platform. Orthogonal 'varimax' rotation (Kaiser off) was performed, leading to the identification of two primary components based on the top 'eigen' values in the 'scree plot. Duncan's New Multiple Range Test (DMRT) was utilized to compare means when significant effects were detected ($p < 0.05$). Statistical analyses were performed using SAS JMP Pro 16.2 2022 (SAS Institute, Cary, North Carolina, USA) and Stata 14.1 SE 2015 (Stata Corp LP, College Station, Texas, USA). The study employed the following additive model.

Y_{ijk}	$\mu + \alpha_i + \beta_j + \gamma_k + \dots + \epsilon_{ijkn}$
Y_{ijk}	The effect observed for the ' n^{th} ' repetition of the combination involving the ' i^{th} ' level of factor ' α ', the ' j^{th} ' level of factor ' β ', and the ' k^{th} ' level of factor ' γ ';
μ	The intercept of the regression model;
α_i	The effect of the ' i^{th} ' level of factor ' α ' on the observed value in

	Y_{ijk} ;
β_j	The effect of the ' j^{th} ' level of factor ' β ' on the observed value in Y_{ijk} ;
γ_k	The effect of the ' k^{th} ' level of the factor ' γ ' on the value observed in Y_{ijk} ;
ϵ_{ijk}	The random sampling error due to the ' i^{th} ' level of the factor ' α ', the ' j^{th} ' level of the factor ' β ', and the ' k^{th} ' level of the factor ' γ ' distributed as $\epsilon_i \sim \text{NID}(0, \sigma^2)$.

RESULTS

Average daily feed intake

The ADFI of the broiler chickens differed significantly ($p < 0.001$) among treatment groups during days 0-14 (Table 2). The lowest ADFI was recorded in the LA + ST- supplemented group which was 11.7% lower than the control group during this period. However, at days 15-28 and 0-28, no substantial differences in ADFI were observed between the control and treated groups ($p > 0.05$). At days 15-28, ADFI increased by 7.3%, 6%, 3.1%, and 5.4% in local, LA, ST, and LA + ST treated groups, respectively, compared to the control. Similarly, at days 0-28, ADFI was 5.2% higher in the local group, 3.8% in the LA group, and 2.2% in the ST-supplemented group compared to the control. An increased ADFI was associated with a concomitant increase in ADG at the expense of FCR (Figures 1-3). A strong positive correlation was observed between FW, ADG (Days 15-28), and ADG (0-28) while a strong negative correlation was noted between ADG (Days 15-28 and 0-28) and FCR (Days 15-28 and 0-28) (Figure 4). The FW, ADG (Days 0-28), and ADFI (Days 0-28) were identified as the principal eigenvectors determining major variations in broiler performance (Figures 5 and 6).

Average daily gain

The yogurt-supplemented groups exhibited a statistically significant improvement in ADG compared to the control group during days 0 -14 ($p = 0.031$; Table 2). Higher ADG was recorded in all the yogurt supplemented groups compared to the control. The ADG increased by 3.7%, 6.7%, 5.9%, and 4.3% in the Local, LA, ST, and LA+ST-supplemented groups, respectively, compared to the control ($p = 0.031$). Similarly, on days 15-28, the lowest ADG was recorded in the control group, while the highest ADG was recorded in the LA+ST-supplemented group, which was 8% higher than the control ($p = 0.484$). Accordingly, at days 0-28, the highest ADG was recorded

in the LA+ST-supplemented group, which was 6.8% higher than the control ($p = 0.374$).

Feed conversion ratio

The feed conversion ratio varied significantly among treatment groups at days 0-14 ($p < 0.001$) and 0-28 ($p = 0.023$). However, no significant differences were observed during days 15-28. During days 0-14, FCR improved by 3.6%, 7.9%, 5.7%, and 15.7% in local, LA, ST, and LA+ST yogurt-supplemented groups, respectively, compared to the control. Over the entire period (Days 0-28), the best FCR was observed in the LA+ST supplemented group, which was 6.6% better ($p = 0.023$) than the control group (Table 2).

Ileal digestibility

Overall, yogurt supplementation in the broiler diet significantly ($p < 0.001$) improved the ileal digestibility of DM, OM, CP, CF, EE, and TA except in the group supplemented with local yogurt, where digestibility of DM, CF, EE, and TA rather decreased than in the control group (Table 3). The highest digestibility of DM, OM, CP, and EE was recorded in the LA+ST supplemented group, with improvements of 23.82%, 29.51%, 20.27%, and 23.76%, respectively, compared to the control group ($p < 0.001$).

Table 2. Performance parameter of the broiler chicken provided with different types of yogurt supplements

Parameter	Dietary treatments ¹					SEM	p-value
	Control	Local	LA	ST	LA+ST		
IW ² (g/b)	46.7	46.8	46.7	46.4	46.6	0.42	0.970
FW ³ (g/b)	1806.6	1834.0	1872.7	1853.6	1927.3	41.5	0.374
ADFI ⁴ (0-14 d, g/bird/d)	52.2 ^a	52.3 ^a	51.7 ^a	52.2 ^a	46.1 ^b	0.80	<0.001
ADG ⁵ (0-14 d, g/bird/d)	37.4 ^b	38.8 ^{ab}	39.9 ^a	39.6 ^a	39.0 ^a	0.53	0.031
FCR ⁶ (0-14 d)	1.40 ^a	1.35 ^b	1.29 ^c	1.32 ^b	1.18 ^d	0.02	<0.001
ADFI (15-28 d, g/bird/d)	118.4	127.0	125.4	122.1	124.8	3.16	0.373
WG (15-28 d, g/bird/d)	88.3	88.9	90.5	89.5	95.4	2.92	0.484
FCR (15-28 d)	1.34	1.43	1.39	1.36	1.31	0.03	0.133
ADFI (0-28 d, g/bird/d)	85.3	89.7	88.5	87.2	85.4	1.74	0.346
WG (0-28 d, g/bird/d)	62.9	63.8	65.2	64.5	67.2	1.48	0.374
FCR (0-28 d)	1.36 ^b	1.41 ^a	1.36 ^b	1.35 ^b	1.27 ^c	0.02	0.023

¹Control: A diet without yogurt; Local: A diet containing locally prepared yogurt at 5 mL/L drinking water; LA: A diet containing *Lactobacillus acidophilus* fermented yogurt at 5 mL/L drinking water; ST: A diet containing *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; LA+ST: A diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; ²IW: Initial weight; ³FW: Final weight; ⁴ADFI: Average daily feed intake; ⁵ADG: Average daily gain; ⁶FCR: Feed conversion ratio; ^{abcd} Means bearing different superscripts in the same row differ significantly at $p < 0.05$.

Table 3. Nutrient digestibility in broiler chickens provided diets supplemented with various types of yogurts

Parameter (%)	Dietary treatments ¹					SEM	p-value
	Control	Local	LA	ST	LA+ST		
Dry matter	65.9 ^d	64.0 ^e	75.3 ^c	78.0 ^b	81.6 ^a	1.83	<0.001
Organic matter	61.0 ^e	64.9 ^d	71.1 ^c	75.7 ^b	79.0 ^a	1.77	<0.001
Crude protein	67.1 ^e	69.6 ^d	72.4 ^c	73.1 ^b	80.7 ^a	1.22	<0.001
Crude fiber	61.1 ^d	58.9 ^e	77.3 ^b	78.7 ^a	75.4 ^c	2.27	<0.001
Ether extract	60.6 ^d	57.8 ^e	69.6 ^c	71.5 ^b	75.0 ^a	1.76	<0.001
Total ash	76.0 ^d	73.7 ^e	77.8 ^b	81.2 ^a	77.5 ^c	0.66	<0.001

¹Control: diet without yogurt; Local: A diet containing locally prepared yogurt at 5 mL/L drinking water; LA: A diet containing *Lactobacillus acidophilus* fermented yogurt at 5 mL/L drinking water; ST: A diet containing *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; LA+ST: A diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; ^{abcde} Means bearing different superscripts in the same row differ significantly at $p < 0.05$.

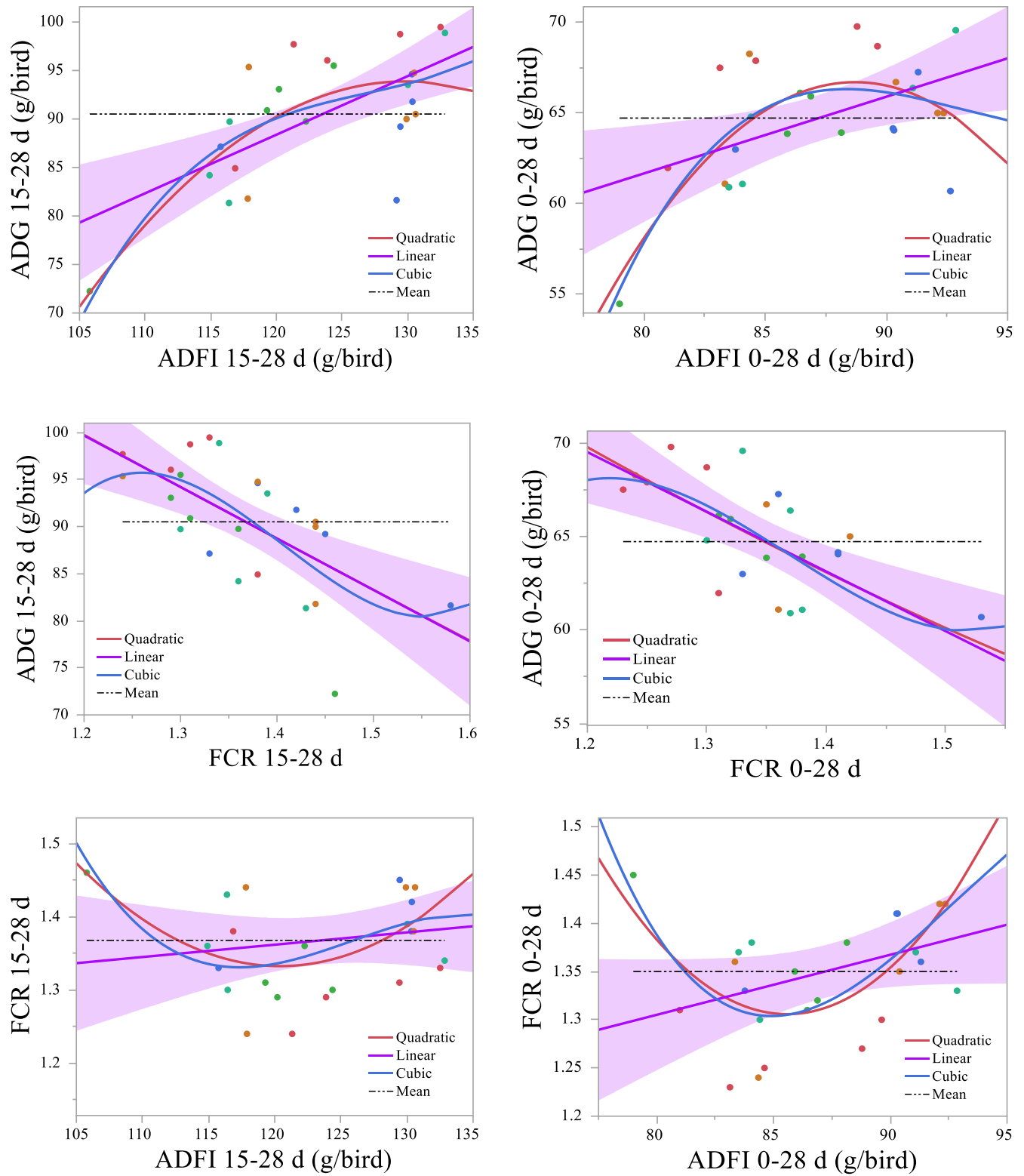


Figure 1. Bivariate distributions showing linear effects of diets supplemented with different types of yogurts on average daily feed intake, average daily gain, and feed conversion ratio in broiler chicken.

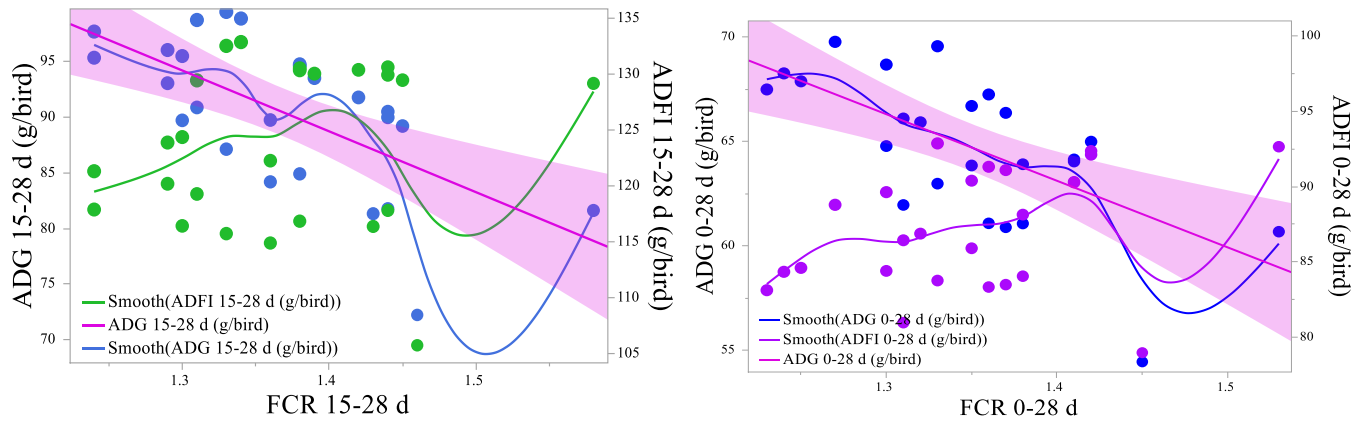


Figure 2. Locally weighted scatterplot smoothing showing effects of diets supplemented with different types of yogurts on feed conversion ratio, average daily feed intake, and average daily gain of the broiler chicken.

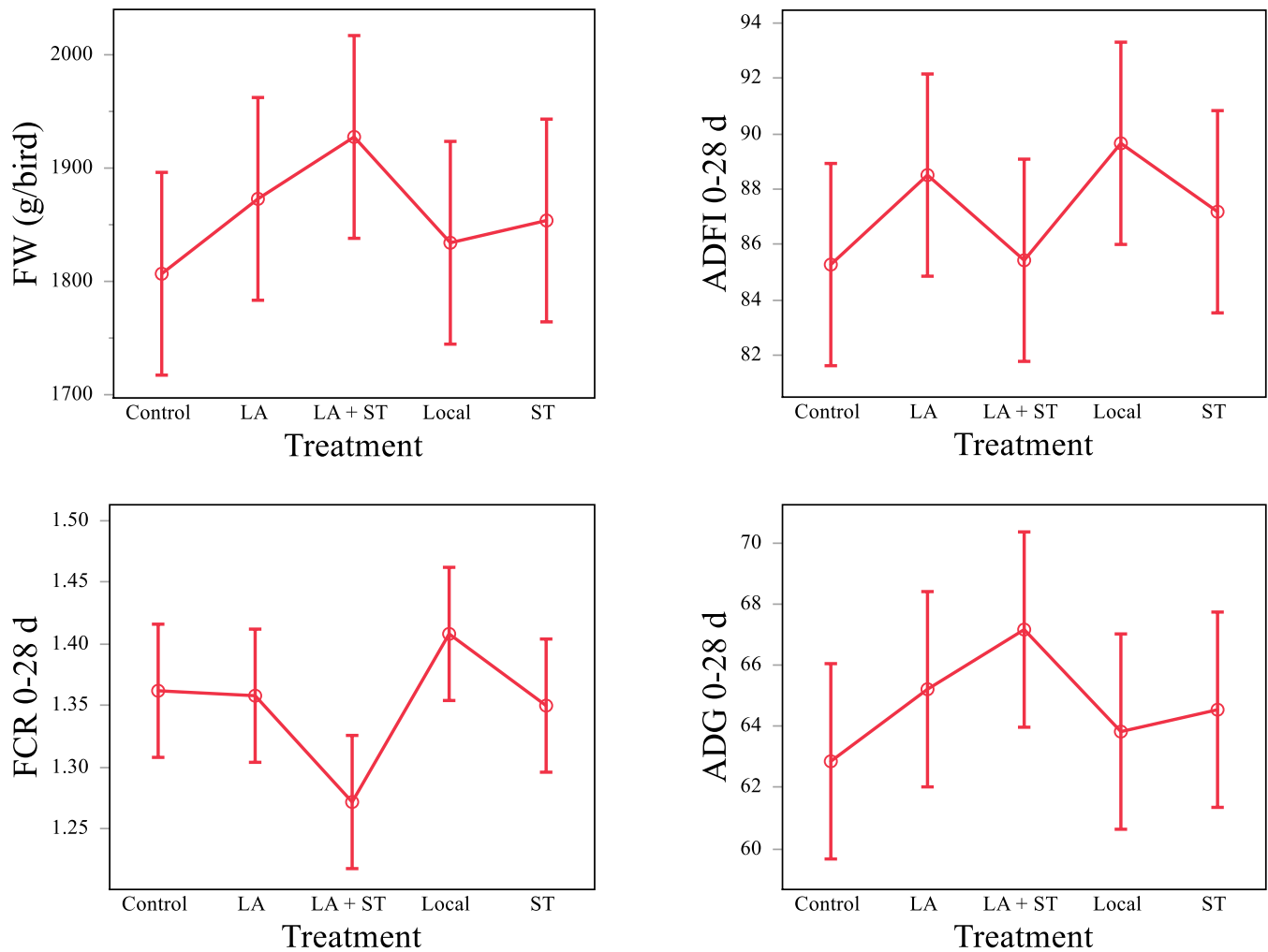


Figure 3. Least square means showing linear effects of different types of yogurts on final weight, average daily feed intake, average daily gain, and feed conversion ratio of the broiler chicken. Control: A diet without yogurt; Local: A diet containing locally prepared yogurt at 5 mL/L drinking water; LA: A diet containing *Lactobacillus acidophilus* fermented yogurt at 5 mL/L drinking water; ST: A diet containing *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water; LA+ST: A diet containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* fermented yogurt at 5 mL/L drinking water.

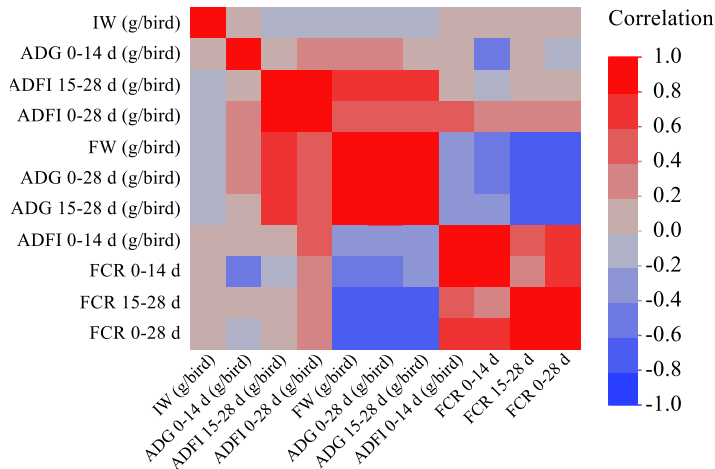
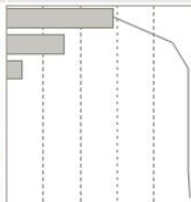


Figure 4. Heatmap showing clustered multiple correlation coefficient matrix of the performance parameter of the broiler chicken fed different yogurt supplemented diets

Eigenvectors					
	Prin1	Prin2	Prin3	Prin4	Prin5
ADG 0-28 d (g/bird)	0.45798	0.07878	0.03576	0.35536	0.20695
FW (g/bird)	0.45796	0.07856	0.03535	0.35860	0.27601
ADG 15-28 d (g/bird)	0.45295	0.07299	0.16641	-0.48720	0.38306
ADFI 15-28 d (g/bird)	0.27262	0.48565	-0.20899	-0.57597	-0.27868
ADFI 0-28 d (g/bird)	0.17068	0.57588	0.03440	0.40841	-0.41651
FCR 0-14 d	-0.24075	0.29046	0.84750	-0.04929	-0.07736
FCR 15-28 d	-0.31448	0.38921	-0.45442	0.08089	0.11138
FCR 0-28 d	-0.33918	0.42302	-0.01267	0.01596	0.68171

Eigenvalues							
Number	Eigenvalue	Percent	20	40	60	80	Cum Percent
1	4.6622	58.277					58.277
2	2.5815	32.269					90.546
3	0.7126	8.907					99.452
4	0.0399	0.499					99.951
5	0.0030	0.037					99.988
6	0.0008	0.010					99.999
7	0.0001	0.001					100.000
8	0.0000	0.000					100.000

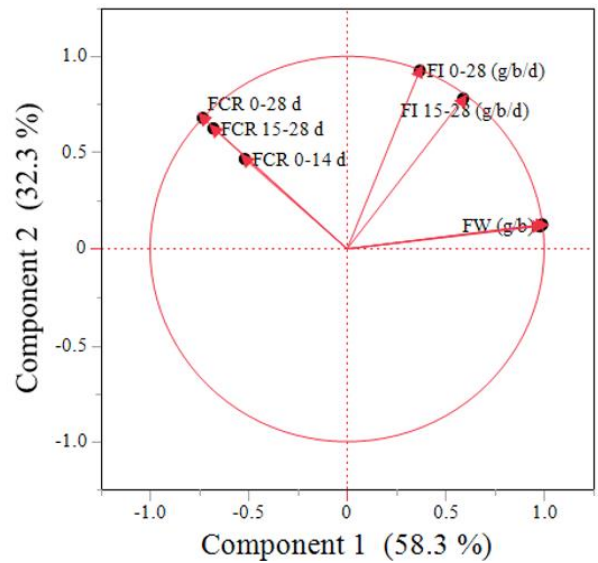


Figure 5. Principal component analysis with eigenvectors and values showing effects of different types of yogurts on the performance of the broiler chicken. Plotted on “x” as component 1 (58.3%) and “y” as component 2 (32.3%)

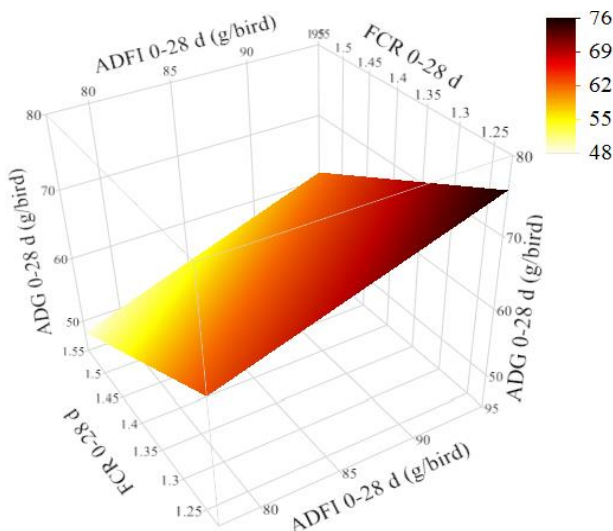


Figure 6. Response surface model with the central composite design showing desirable zone of average daily gain (ADG) optimized by linear combinations of the different values of average daily feed intake (ADFI) and feed conversion ratio (FCR) in broiler chickens provided with diets supplemented with different types of yogurts.

DISCUSSION

Average daily feed intake

In the present study, the ADFI ranged from 46.1 to 52.3 g/bird/d at 0-14 days. Among the study groups, broiler chickens supplemented with LA+ST yogurt showed the lowest ADFI, whereas those provided with local yogurt showed the highest ADFI. Although significant differences were noticed between the yogurt and control groups at 0-14 days, no marked difference was found at 15-28 or over the entire 28-day period. These findings align with a previous study, which reported that supplementing broiler diets with yogurt at a concentration of 5 mL/L in drinking water did not significantly affect overall ADFI (Sultan et al., 2006). Likewise, Hossain and Momu (2022) reported that yogurt supplementation had a negligible effect on feed consumption in chickens. In contrast, earlier studies reported significantly higher feed intake in the broiler chickens supplemented with dried yogurt powder (1 kg/100 kg diet) compared to the probiotic-supplemented and control groups, which was consistent with the present study (Mamun et al., 2021; Hossain and Momu, 2022). Accordingly, another study found that dietary yogurt supplementation did not affect ADFI in broiler chickens during the first 10 days but had a substantial impact in the later stages (Ghasemi-Sadabadi et al., 2019). It implies that the influence of yogurt on feed intake may diminish over time, with noticeable differences in the early stages that do not persist. Hence, it may be concluded that the overall effects of yogurt on ADFI in broiler chickens are complex and vary depending on the study duration, the form and quantity of yogurt used, and the specific conditions under which the broilers were raised (Ghasemi-Sadabadi et al., 2019).

Average daily gain

In the current study, the ADG differed significantly among the dietary groups. The LA-supplemented yogurt group showed the highest gain at 0-14 days, with no notable difference in overall weight gain at 15-28 days. According to a former study, yogurt supplementation at 5 mL/L of drinking water significantly improved weight gain during both the starter and finisher phases in comparison to the control group (Sultan et al., 2006). The highest body weight gain at later stages in the LA+ST supplemented group was also aligned with an earlier study which reported better weight gain in broiler chicken supplemented with duo-strain probiotic (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Adriani et al., 2015). The highest ADG in the LA supplemented group at

the initial stage obtained in the present study was also consistent with some other studies. For example, one study reported that supplementation with *Lactobacillus acidophilus* in broiler chickens infected with *Clostridium perfringens* improved ADG (Li et al., 2018). The observed improvement in performance may be ascribed to enhanced nutrient digestibility and absorption, maintenance of a stable intestinal microbiota, improved intestinal health, a stronger immune response, and reduced stress levels (Revolledo et al., 2006; Prado-Rebolledo et al., 2017; Li et al., 2018).

The inclusion of *L. acidophilus* in poultry feed prevents the growth of harmful bacteria and regulates intestinal flora via competitive exclusion (Forte et al., 2018). However, despite many similarities, these results contradict a previous study that found no effect of probiotic supplementation (*Lactobacillus spp.*) on ADG during phage feeding (Hosamani et al., 2006). The differences in weight gain could be attributed to factors such as sex, weather conditions, infectious diseases, and other variables (Aftahi et al., 2006). This study further revealed the highest body weight gain in the LA+ST-supplemented group at 15-28 days, indicating a synergistic effect of the multi-strain probiotic on broiler performance. The findings suggest that yogurt supplementation, particularly with *Lactobacillus acidophilus* and multi-strain probiotics (LA+ST), enhances early-stage ADG in broiler chickens. While initial improvements in weight gain are consistent with previous studies, long-term effects may vary due to environmental and health factors.

Feed conversion ratio

In the current study, notable differences in FCR were observed among the yogurt-supplemented groups compared to the control at 0-14 days. Among the yogurt supplemented groups, the LA + ST group showed the best FCR. Overall, FCR differed significantly, with the LA+ST group showing the highest feed efficiency. However, from days 15-28, no significant difference was found between the yogurt-supplemented group and the control group ($p = 0.133$). These results observed a better FCR in the LA + ST supplemented group compared to the control. Consistent with these findings, another study reported better FCR in the duo-strain probiotic supplementation in broiler chickens (Mirsalami and Mirsalami, 2024). Several previous studies also reported substantial impacts of yogurt supplementation on the FCR in broiler chicken (Sultan et al., 2006; Mansoub and Nezhady, 2011; Ghasemi-Sadabadi et al., 2019).

In contrast to the current study, a previous study reported that there was no statistically significant effect of yogurt supplementation on FCR in the broiler chicken (Mahmmod et al., 2014). Accordingly, no combined effect of *Streptococcus thermophilus* and *Lactobacillus sp.* on FCR was found, possibly due to insufficient bacterial count, as well as genotype and growth stage variations among experimental broiler chickens (Nafees and Pagthinathan, 2018). The present study indicated that yogurt supplementation, particularly with the LA+ST multi-strain probiotic, enhances FCR in broiler chickens, especially during the initial growth phase. However, discrepancies in findings across the studies may be due to variations in bacterial count, genetic factors, and growth stages (Mirsalami and Mirsalami, 2024).

Nutrient digestibility

Yogurt supplementation significantly improved the ileal digestibility of DM, OM, CP, CF, EE, and TA in the LA, ST, and LA + ST groups. Consistent with these results, a previous study reported enhanced ileal protein digestibility following dried yogurt powder supplementation (Abbas et al., 2020). The improved protein digestion within the gastrointestinal tract was likely due to increased lactic acid concentration, which enhances protease enzyme activity (Abbas et al., 2020). The reduction in pH induced by lactic acid bacteria boosts the enzyme activity, thereby facilitating feed breakdown, inhibiting the growth of pathogenic microorganisms, and promoting the proliferation of beneficial bacteria (Flint and Garner, 2009; Recoules et al., 2017). Another study demonstrated that a multi-strain probiotic containing lactic acid bacteria significantly enhanced the ileal digestibility of DM, CP, and gross energy compared to the control group (Kim et al., 2012). Similarly, another study reported that supplementation with lactic acid bacteria, specifically *Lactobacillus bulgaricus*, enhanced the digestion of nitrogen and fat, though it had no significant impact on fiber digestion. These observations suggested that lactic acid bacteria can improve the ability of broiler chickens to digest certain nutrients (Apata, 2008). On the contrary, it was indicated that the total tract apparent digestibility of DM, OM, CP, EE, and ash in broiler chickens remained unaffected despite the inclusion of yogurt acid whey powder at concentrations of 2.5%, 5%, and 10% of the diet (Paraskeuas et al., 2023). Similarly, it was reported that supplementation of dry whey powder and whey concentrate did not affect the digestibility of DM and CP, however, they significantly influenced the digestibility of

minerals, particularly calcium, and phosphorus (Pineda-Quiroga et al., 2018).

CONCLUSION

Supplementation of yogurt in broiler diets improved their growth performance, feed intake, and nutrient utilization. Fermented yogurt containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* (LA+ST) distinctively enhance feed efficiency, ileal nutrient digestibility, and growth performance of broiler chickens over 28 days. It may be concluded that probiotic-enriched yogurt was a viable dietary supplement to promote efficient growth and enhance nutrient utilization in broiler chickens. Future research should explore gut barrier functionality, intestinal histo-morphometry, bacterial translocation, and humoral immune responses in chickens fed yogurt supplemented diets.

DECLARATIONS

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Authors' contributions

Md. Emran Hossain conceptualized the study, managed the project, curated the data, conducted the generalized linear model, principal component analysis, and response surface modeling, interpreted the results, and finalized the initial draft. Minara Begum Munni, Umme Salma Amin, and Mahabub Alam prepared the yogurt culture, carried out the feeding trial, immunization, and ileal nutrient digestibility, and contributed to drafting the initial manuscript. Shilpi Islam, Nasima Akter, and Md. Ahasanul Hoque provided critical insights and oversaw the entire study. All authors reviewed and approved the final version of the manuscript.

Competing interests

The authors declare that there are no conflicts of interest.

Ethical considerations

The authors affirm that they have adhered to ethical research practices, avoiding plagiarism, misconduct, data fabrication or falsification, and duplicate submission or publication, and have provided their consent for this article's publication.

Availability of data and materials

The datasets used and/or analyzed data during the current study are available from the corresponding author upon reasonable request.

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