OPEN ACCESS Journal of Animal Health and Production

Research Article

Effects of Multi-Strain Probiotics on Growth Performance, Meat Composition, and Hematobiochemical Attributes of Broiler Chickens

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Abstract | The objective of this study was to assess the effects of adding multi-strain probiotics on the growth performance, meat quality, and hematobiochemical parameters of broiler chickens. A hundred eighty (180) one-day-old chicks were obtained for the study and randomly assigned to three groups; each group was divided into six replications of ten birds each. The study was conducted for six weeks, during which the birds were kept under uniform management practices. Three different diets were formulated for the study: control (CONT) (birds were fed the basal diet), multi-strain probiotics (PROBO) (basal diet supplemented with 100g/100kg multi-strain probiotics), and antibiotics (ABT) (basal diet supplemented with 10g/100kg lincomycin antibiotics). During the starter, finisher, and overall periods of the experiment, the PROBO group consistently exhibited significantly higher live body weight (LBW), average daily gain (ADG), and improved feed conversion ratio (FCR) compared to the other groups ($P < 0.05$). However, no significant difference was noted in average daily feed intake (ADFI) among all groups ($P > 0.05$). A significant decline was seen in cooking loss and drip loss ($P < 0.01$) in the PROBO compared to the CONT. Whereas, the water holding capacity (WHC) of meat significantly increased in the PROBO ($P < 0.05$). The pH, ash, and moisture content did not significantly differ among the groups ($P > 0.05$). Conversely, PROBO and ABT displayed markedly higher crude protein content in meat compared to CONT ($P < 0.01$). Fat content of meat exhibited an inverse relationship with CONT, the highest fat levels were found in CONT ($P < 0.05$). No significant difference ($P > 0.05$) was found in white blood cell (WBC) and hemoglobin count among all treatments. Whereas packed cell volume (PCV) and total red blood cell (RBC) count were significantly higher ($P < 0.05$) in the PROBO group as compared with CONT and ABT groups. However, there is no significant difference ($P > 0.05$) found in albumin, glucose, creatinine, low-density lipoprotein (LDL), and total leukocyte count (TLC) among all the dietary treatment groups. A highly significant difference ($P < 0.05$) was found in total protein, globulin, and high-density lipoprotein (HDL) in PROBO groups compared to CONT and ABT groups. Overall, the inclusion of multi-strain probiotics in broiler diets may serve as a promising alternative to antibiotics, enhancing growth performance and meat quality without adverse effects on health parameters.

Keywords | Multistrain probiotics, Growth performance, Meat quality, Hematobiochemical attributes, Broilers

Received | June 22, 2025; **Accepted** | August 04, 2025; **Published** | October 06, 2025
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Citation | Mangi B, Khan S, Zakria M, Bilawal M, Koondhar IH, Leghari IH, Pirzado SA (2025). Effects of multi-strain probiotics on growth performance, meat composition, and hematobiochemical attributes of broiler chickens. J. Anim. Health Prod. 13(4): 884-892.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.4.884.892
ISSN (Online) | 2308-2801

INTRODUCTION

Various feed additives like vitamins, minerals, enzymes, and probiotics are included in the broiler diets to improve growth performance, feed efficiency, and nutrient utilization (Pirzado *et al*., 2022; Soomro *et al*., 2024; Zakria *et al*., 2025). In addition to enhancing productivity, such additives help in making animal farming sustainable by reducing the excretion of nutrients, minimizing the environmental impact, and benefiting the feed at large (Arsène *et al*., 2021; Ayoob *et al*., 2023). Antibiotic is routinely used in the poultry industry to stimulate growth, to improve feed efficiency, and to reduce disease conditions to promote meat production (Chattopadhyay, 2014; Diarra and Malouin, 2014; Rajput *et al*., 2024). Nonetheless, the misuse of antibiotics causes serious problems in the world, not limited to gut dysbiosis, multidrug-resistant bacteria, and the presence of antibiotic residues in poultry products such as meat and eggs (Popova, 2017; Purba *et al*., 2020). These challenges have driven the search for natural, effective alternatives to enhance the health and performance of birds without sacrificing food safety.

Probiotics have recently received attention as a potential sustainable alternative. They support the intestinal microbial balance, maximize nutrient utilization, stimulate the immune system, and have a positive effect on the quality of meat (Wang *et al*., 2018; Bughio *et al*., 2021; Ebeid *et al*., 2021). Numerous microbial strains, including *Bacillus subtilis, Streptococcus faecium, Lactobacillus casei,* and *Saccharomyces cerevisiae,* have been found to enhance growth performance, immunity, and reduce enteric pathogens of broilers (Reis *et al*., 2017; Tabashsum *et al*., 2020; Gopal and Dhanasekaran, 2021).

While single-strain probiotics have been widely studied, multi-strain formulations are increasingly attracting attention due to their potential synergistic effects targeting different aspects of gut health and metabolism simultaneously (Hussein and Selim, 2018; Zheng et al., 2019). Studies have suggested that combining different strains may provide broader and more consistent benefits than individual strains alone. However, limited research is available on the comparative advantages of multi-strain probiotics over antibiotic use in broiler chickens, particularly with growth performance, meat quality, and hematobiochemical health parameters. Therefore, this study aimed to evaluate the effects of a multi-strain probiotic blend (*Lactobacillus casei, Bacillus subtilis, Streptococcus faecium,* and *Saccharomyces cerevisiae*) on the growth performance, meat quality, and hematobiochemical attributes of broiler chickens.

MATERIALS AND METHODS

Housing management

To initiate the research, one hundred eighty (180) one-day-old healthy broiler chicks were acquired from a nearby hatchery in Kotri, Hyderabad, and then brought to the poultry experimental station, Department of Poultry Husbandry, Sindh Agriculture University, Tandojam. Before the chicks' arrival, the house was thoroughly cleaned, washed, and disinfected to prevent any potential bacterial or viral contamination. Wood shavings were placed to a depth of 2 inches to serve as bedding material. The brooders were set up and activated before the birds' arrival to ensure the experimental house was at the necessary temperature. The house's temperature was kept at 32°C initially, later it was reduced regularly by 2°C weekly till it became 22°C. The relative humidity remained between 55 and 65%. Throughout the experiment, Food and water were always available for the broiler chickens. There was availability of light 24 hours in the first three days, and then lighting was reduced by one hour each week. The birds' health was monitored daily. All the birds were vaccinated according to the schedule recommended by the Pakistan Poultry Association (PPA).

Diet-based treatments

The broiler birds were randomly assigned to three dietary treatment groups, each group comprised of six replicates, having 10 birds per replicate. The experiment lasted 6 weeks and was divided into two phases: the starter phase (days 1-21) and the finisher phase (days 22-42). Three different diets were formulated for the study: CONT (birds were fed the basal diet), PROBO (basal diet supplemented with 100g/100kg Probiotics), and ABT (basal diet supplemented with 10g/100kg Lincomycin antibiotics). The formulation of basal diets is presented in Table 1. The probiotics and antibiotics, both in powder form, were premixed with a small quantity of the basal diet to ensure uniform distribution and then thoroughly incorporated into the complete feed before daily feeding. The probiotics and antibiotics were purchased from Hanvet, Vietnam. The multi-strain probiotics composition is presented in Table 2.

Growth performance

On the 21st and 42nd days of the experiment, measurements of live body weight (LBW) and feed intake were taken for each replicate. The average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) (Depar *et al*., 2021) were computed using the following formulas:

$$\text{Feed Intake} = (\text{Total feed offered} - \text{Total feed refused}) / \text{Total number of broilers}$$

$$\text{FCR} = \text{Feed intake} / \text{Weight gain}$$

Table 1: Feed formulation and nutrient composition of basal diets.

Ingredient percentage	Starter phase	Finisher phase
Maize	60	72.5
Soyabean meal	36	24.43
Vegetable oil		0.5
L-Lysine sulphate	0.46	0.4
DL-methionine	0.35	0.24
L-threonine	0.18	0.07
MCP	1.06	0.15
Lime stone	1.15	0.97
Salt	0.25	0.25
Soda	0.25	0.25
NSP	0.01	0.01
Phystase	0.01	0.01
Toxin binder	0.05	0.05
Vitamin premix	0.05	0.05
Mineral premix	0.05	0.05
L- valine	0.07	0.01
L-Isoleucine		
Total	100	100
Calculated nutritive composition (%)		
ME(kcal/kg)	2905	3104.30
CP	21.50	19.45
Ca	0.96	0.72
Ph	0.58	0.36
Na	0.22	0.20
Cl	0.20	0.20
D. Lysine	1.26	0.98
D.M+C	0.94	0.74
D. threonine	0.86	0.62
D. valine	0.96	0.74
D. isoleucine	0.85	0.67

Table 2: Multi-strain probiotics composition.

Multi-strain probiotics	
Component	**Amount per 1 kg**
Bacillus subtilis	1.0×10^{11} CFU
Streptococcus faecium	1.0×10^{11} CFU
Lactobacillus casei	1.0×10^{11} CFU
Saccharomyces cerevisiae:	5.0×10^{11} CFU
Antibiotics	
Component	Amount per kg
Lincomycin	10g/100kg

Physical properties of meat

PH value: To assess post-slaughter pH changes, meat samples were homogenized with deionized water (10g:90ml) and their pH measured within 60 minutes using a calibrated portable meter (Hanna Italia, Mauritius). Measurements were obtained with an electrode connected to the instrument.

Drip loss: After slaughter, a standardized procedure was used to assess drip loss (Torres-Filho *et al*., 2017). Fresh meat samples weighing 50 grams were placed in polyethylene bags, sealed securely, and refrigerated for 24 hours at 4°C. Following refrigeration, the samples were unpacked, dried gently, and reweighed. Drip loss was then calculated as a percentage using the following formula:

$$\text{Drip loss (\%)} = [(\text{Initial weight} - \text{Final weight}) / \text{Initial weight}] \times 100$$

Cooking loss measurement

Meat samples weighing 20 grams were individually sealed in plastic bags and cooked in a water bath at 80°C for an hour. The internal temperature of the meat was monitored, and cooking stopped once it reached 72°C. After cooling at room temperature, the cooked samples were weighed again. The percentage of lost weight, relative to the initial weight (Pang *et al*., 2020), was calculated as cooking loss.

Water holding capacity assessment

The water holding capacity (WHC) of breast meat was assessed using a modified centrifugation method aimed at evaluating its ability to retain moisture. In this procedure, ten grams of finely crushed meat were combined with 12 milliliters of 0.6M sodium chloride (NaCl) solution in a centrifuge tube. Subsequently, the mixture underwent centrifugation at 10,000 revolutions per minute (rpm) for 15 minutes at 4°C, effectively separating the liquid (supernatant) from the meat solids. The WHC was then calculated by measuring the volume of the collected supernatant, with the difference between the initial volume of the NaCl solution (12 ml) and the measured supernatant volume representing the water holding capacity. A higher WHC value suggests enhanced water retention in the meat, indicating the potential for juicier and more tender cooked products (Kudryashov and Kudryashova, 2023).

Chemical properties of meat

The breast and leg muscle samples were analyzed for their nutritional composition using established methods from the Association of Official Analytical Chemists (AOAC, 2005). Each sample underwent triplicate analysis for accuracy. Moisture content was determined by drying a 2-gram sample at 105°C in a hot air oven for one hour. Crude protein content was determined using the Kjeldahl method, involving digestion with sulfuric acid and calculating CP as N% multiplied by 6.25. Fat content was estimated using a Soxhlet apparatus with N-hexane as the solvent. Finally, the ash content was determined by incinerating the samples at 550°C in a muffle furnace (Young *et al*., 2012).

Hematology and blood biochemistry

Sample collection

At the end of the research experiment, 3 birds from each replicate were slaughtered for blood collection. 5ml of blood collected from the wing vein, in vacutainers with Na-EDTA as an anticoagulant for the hematological analysis using the automated hematological analyzer, followed by the Bakari *et al*. (2019).

Hematology

The hemoglobin was determined by Sahli's acid hematin method, Packed cell volume (PCV) by using the micro hematocrit method, whereas total leukocyte count (TLC) and the total erythrocyte count were estimated using the hemocytometer by the protocol of Hussein and Selim (2018).

Blood biochemistry

A total of 10 ml of blood was collected in a vacutainer without using anticoagulants, and the serum was centrifuged at 3000 rpm for 15 minutes at 4 °C and stored at -20 °C for the estimation of the biochemical indices. The cholesterol was estimated using the enzymatic photometric test. The triglyceride was analyzed by using a Colorimetric enzymatic test. Glucose was estimated by the method CHOD-PAP. The serum contents of MDA, SOD, and catalase (CAT) activity were determined by using commercial kits (Cell Biolabs Inc., San Diego, CA, USA).

Statistical analysis

The data was analyzed using one-way ANOVA in SPSS Statistics software version 19.0. Before performing ANOVA, its prerequisites, such as normality and homogeneity of variances, were tested and confirmed. The Shapiro-Wilk test was used for a normality check, while Levene's test confirmed homogeneity of variances. Post hoc analyses using Tukey's HSD test were conducted for significant differences among groups. Results are presented as means ± SE, and significance was declared at $P < 0.05$.

RESULTS

During the initial phase (starter), no significant variation in LBW and ADFI was noted among all groups (Table 3). The probiotics-fed group (PROBO) exhibited a markedly higher ADG with statistical significance (P <0.01) and a commendable improvement in FCR with a highly significant difference (P <0.001) compared to both the control (CONT) and antibiotics-fed (ABT) groups. Similarly, during the 22-42 days phase and the overall 1-42 days period, the PROBO group consistently exhibited significantly higher LBW, ADG, and improved FCR compared to the other groups. However, no statistical differences were noted in ADFI among all groups.

Table 3: Effect of dietary treatments on growth performance in broilers (gm).

Parameters	CONT	ABT	PROBO	SE	P. value
Feeding phases (Days)					
1-21 days					
LBW	811.25^{a}	836.50^{a}	887.25^{a}	14.41	0.075
ADG	38.41^{ab}	37.63^{b}	42.02^{a}	0.773	0.026
ADFI	60.90^{a}	58.69^{a}	62.18^{a}	1.19	0.526
FCR	1.37^{a}	1.31^{b}	1.24^{c}	0.016	0.0001
22-42					
LBW	1579.93^{b}	1589.45^{b}	1848.98^{a}	49.17	0.019
ADG	75.01^{b}	73.48^{b}	87.82^{a}	2.44	0.012
ADFI	137.03^{a}	130.30^{a}	144.67^{a}	2.97	0.141
FCR	1.82a	1.72^{b}	1.64^{c}	0.02	0.0001
1-42 days					
LBW	2509.31^{b}	2524.42^{b}	2894.85^{a}	73.86	0.034
ADG	59.63^{b}	59.00^{b}	68.81^{a}	1.80	0.025
ADFI	98.96^{a}	94.50^{a}	103.43^{a}	2.06	0.224
FCR	1.65^{a}	1.57^{b}	1.50^{c}	0.20	0.0001

Superscripts with different letters in rows varied significantly (P<0.05). LBW= Live Body Weight, ADG= Average Daily Gain, ADFI=Average Daily Feed Intake, and FCR= Feed Conversion Ration, Control= basal diet, PROBO= Probiotics, ABT= Antibiotics

Table 4: Effect of dietary treatments on physical properties of meat in broilers.

Parameters	CONT	ABT	PROBO	SE	P. value
Physical parameters (%)					
Cooking loss	26.52^{a}	22.49^{b}	21.95^{b}	0.62	0.0001
Drip loss	3.79^{a}	2.59^{b}	2.44^{b}	0.18	0.0001
WHC	70.53^{b}	75.05^{a}	75.25^{a}	0.66	0.0001
PH	5.68^{a}	5.61^{a}	5.59^{a}	0.02	0.149

Superscripts with different letters in rows varied significant (P<0.05) Control= basal diet, PROBO= Probiotics, ABT= Antibiotics. SE= Standard Error, WHC= Water Holding Capacity.

A statistically significant improvement was seen in cooking loss (P <0.05) and drip loss (P <0.01) in the probiotic-treated group (PROBO) compared to the control group (Table 4). Similarly, drip loss percentages were 3.79% (CONT), 2.59% (ABT), and notably reduced to 2.44% in the PROBO group. The water holding capacity (WHC) increased in the PROBO group compared to both the antibiotics-treated (ABT) and control (CONT) groups (P <0.05). The WHC percentages were 70.53% (CONT), 75.05% (ABT), and notably higher at 75.25% in the PROBO group. pH values did not exhibit a significant difference among the groups (*P* >0.05).

Table 5 shows that Moisture content exhibited no difference among the control (CONT), antibiotic treatment (ABT), and probiotic treatment (PROBO) groups, with no statistically significant differences noted (*P* >0.05). Conversely, the crude protein (CP) increased in PROBO supplemented groups, then CONT (*P* <0.01), while no difference was found with the ABT group (*P* >0.05). Fat content exhibited an inverse relationship with CONT, the highest fat levels were found in CONT, ABT showed intermediate values, and PROBO revealed the lowest fat content. The observed differences in fat content were statistically significant (*P* <0.05). Similarly, ash content followed a similar trend, the PROBO exhibiting the highest ash content, followed by ABT and CONT. PROBO showed statistically significant differences with other groups (*P* < 0.05).

Table 5: Effect of dietary treatments on chemical properties of meat in broilers (%).

Parameters (%)	CONT	ABT	PROBO	SE	P. value
Moisture	73.28^{a}	73.44^{a}	74.66^{a}	0.44	0.502
CP	20.99^{b}	22.68^{a}	23.02^{a}	1.01	0.0001
Fat	3.15^{a}	2.86^{ab}	2.44^{b}	0.29	0.004
Ash	1.24^{b}	1.25^{b}	1.50^{a}	0.04	0.015

Superscripts with different letters in rows varied significant (P<0.05) Control = basal diet, PROBO=Probiotics, ABT= Antibiotics. SE = Standard Error, WHC = Water Holding Capacity, CP = rude protein.

Table 6: Effect of dietary treatments on hematology of broiler chicken.

Parameters	CONT	ABT	PROB	SE	P. value
WBCS ($\times10^3/\mu L$)	8000.0^{a}	7780.0^{a}	9100.00^{a}	259.28	0.067
RBCs ($10^6/\mu L$)	5.53^{b}	5.40^{b}	7.14^{a}	0.266	0.001
Hemoglobin g/dL	12.87^{a}	12.10^{a}	13.85^{a}	0.334	0.088
PCV%	27.99^{b}	28.50^{ab}	29.23^{a}	0.200	0.019

Superscripts with different letters in rows varied significant (P<0.05). WBCS=White blood cells, RBCs=Red blood cells, PCV=Packed cell volume, and Control = basal diet, PROB=Probiotics, ABT= Antibiotics.

Table 6 describes the effect of treatments on the hematological parameters. No significant difference was found in WBCS and hemoglobin count among all treatments. Whereas the total RBC count was significantly higher (*P* <0.05) in the PROBO group as compared with the control and ABT group. The PCV was significantly higher (*P* <0.05) in the PROBO group as compared to the control and ABT groups.

Table 7 describes the effect of dietary treatments on biochemical blood indices. No significant difference (*P* >0.05) was found in albumin, glucose, creatinine, LDL, and TC among all the dietary treatment groups. While highly significant differences (*P* <0.05) were found in total protein, globulin, and HDL in PROBO treatment group with comparison to the control and ABT treatment groups.

Table 7: Effect of dietary treatments on blood biochemistry in broilers.

Parameters	CONT	ABT	PROB	SE	P. value
TP (g/dL)	5.50^{b}	5.87^{b}	8.27^{a}	0.431	0.002
ALB (g/dL)	2.33^{a}	2.32^{a}	2.56^{a}	0.134	0.757
GLB (g/dL)	3.16^{b}	3.55^{b}	5.71^{a}	0.402	0.004
Glucose (mg/dL)	66.50^{a}	65.75^{a}	70.00^{a}	1.357	0.434
Creatinine (mg/dL)	1.38^{a}	1.45^{a}	1.47^{a}	0.272	0.897
LDL (mg/dL)	27.75^{a}	27.00^{a}	23.25^{a}	0.954	0.105
HDL (mg/dL)	55.25^{b}	53.00^{b}	75.00^{a}	3.417	0.002
TC (mg/dL)	80.00^{a}	80.00^{a}	75.25^{a}	2.734	0.630

Superscripts with different letters in rows varied significantly (*P* <0.05). TP= total protein, ALB= Albumin, GLB=Globulins, LDL = low density lipid, HDL= high density lipoprotein, TC= total cholesterol and Control = basal diet, PROB=Probiotics, ABT= Antibiotics.

DISCUSSION

The results of the current study show that while feed intake remained relatively constant, the probiotics-fed group experienced substantial improvements in growth parameters, evaluating the efficacy of the probiotic mix in promoting enhanced growth performance in broilers. This effect could be attributed to the improved activities of digestive enzymes such as amylase and protease (Denli *et al*., 2003; Soomro *et al*., 2024). These results align with numerous studies that have confirmed the beneficial impacts of probiotics on the growth performance of broilers. Earlier results have indicated that diets enriched with multi-strain probiotics (Zhang *et al*,. 2022). Notably enhanced the growth performance of the treated broiler chicken compared to the control group. Shabani *et al*. (2012) observed the same findings, that adding three commercial probiotics to the broiler feed enhanced the average daily gain (ADG) and feed conversion ratio (FCR). Whereas the ADFI remained the same in all groups, indicating that probiotics may improve the growth and feed efficiency of broilers. Another study found that mixed probiotics improved the growth by aiding the intestinal development in broilers with subclinical necrotic enteritis (Sun *et al*., 2021).

A finding by Ignatova *et al*. (2009) assessed the impact of adding probiotics to the diet on the performance of chickens. The study involved 200 one-day-old male White Plymouth Rock mini chickens. The findings demonstrated that mixing probiotics in the feed positively affected final body weight, increasing it by 14.4%. It also led to a 7.7% increase in feed intake and improved feed utilization by 8.1%. However, the impact of dietary supplements on broiler performance is not always significant, as per various studies. For instance, the study indicated that when *Saccharomyces cerevisiae* was used as a substitute for a portion of fish meal and poultry by-products in their feed, it did not increase the weight but increased feed conversion. However, these changes were not statistically significant (Zhang *et al*., 2022). The results collectively underscore the potential of probiotics to positively influence the growth dynamics of broilers, presenting a promising avenue for improving productivity and efficiency in poultry farming.

The current study showed that PRBO exhibited improved RBCs, Hb, and PCV in broiler chickens. Our findings are aligned with those of Deraz (2018), who observed that broilers supplemented with *L. lactis* and *L. plantarum* had a significant increase in red blood cell counts and hemoglobin concentrations. Qureshi *et al*. (2020) also obtained similar results and revealed that diet supplementation with *Bacillus pumilus* significantly improved the hematological parameters such as WBC, hemoglobin, RBCs, and PCV. These improvements could be evidence of enhanced immune activity, better oxygen-carrying capacity and generally improved health status of broilers. The current study showed a marked elevation in the serum total protein (TP), globulin (GLOB), and high-density lipoprotein (HDL) levels in broilers on probiotic-supplemented diets when compared with the control and antibiotic groups. These results are consistent with Abdel-Hafeez et al. (2017), who documented increased serum protein levels in broilers receiving probiotics. The increase in total protein and globulin is likely indicative of more proficient protein metabolism, enhanced immune function and metabolism, as globulins are involved in antibody response and resistance to infections. The improvement in HDL levels shown in the PROBO group indicates possible favorable changes in lipid metabolism. Moreover, the role of HDL plays the main role in maintaining lipid balance and supporting physiological health of broilers. Probiotics might hold this lipid-regulating effect due to modification of gut microbiota, thus lowering cholesterol absorption and increasing bile acid deconjugation during absorption (Shang *et al*., 2021). Overall, these results support the potential of multi-strain probiotics in promoting physiological health and metabolic performance of broilers. The altered levels of hematobiochemical indices may indicate that birds in the PROBO group had better nutritional status, immune competence and metabolic function. These improvements can provide better growth performance, feed conversion efficiency, and economic values in the broiler production system (Ali *et al*., 2023).

Drip and cooking losses are valuable measures of the water-holding capacity (WHC) of meat. The WHC has an important contribution in determining the meat's appearance before it is cooked, the characteristics of the meat during cooking, and its juiciness. Probiotic supplementation has been shown to enhance water-holding capacity (WHC) by improving muscle integrity and antioxidant status (Ebeid *et al*., 2019). Similarly, multi-strain probiotics improved WHC and reduced drip loss, likely through modulation of gut microbiota and nutrient absorption (Hussein and Selim, 2018). Zhou *et al*. (2010) also observed a decrease in the drip loss in the breast muscle of chicken birds that were given *Bacillus coagulans*. Ali (2010) has shown that feeding probiotics to chickens can lead to lower drip loss in their breast meat compared to control birds, especially within the first 8 days. This suggests that certain probiotics can potentially enhance the water-holding capacity of meat, thereby improving its quality. These findings collectively underscore the positive impact of probiotics on crucial meat quality parameters, specifically mitigating cooking and drip losses while enhancing water holding capacity, thus contributing to the overall improvement of broiler meat. However, there was no significant variation in pH across all treatment groups. In chickens that are in good health, metabolic activities like glycolysis continue to generate glycogen, thereby preventing any significant alteration in the pH value (Suryadi *et al*., 2019). The mean pH value in broilers remained consistent across both the control group and those treated with probiotic supplements. This uniformity in pH value in broiler meat is believed to be a result of the chickens in this study maintaining a healthy state, which persisted even after the introduction of probiotics.

Broilers supplemented with probiotics showed the highest levels of crude protein (CP) and ash, along with lowered fat content, compared to the other dietary groups. Widiyaningsih (2011) indicates that probiotics, especially those with lactic acid bacteria, generate digestive enzymes that decompose intricate nutrient molecules by severing chemical bonds. This enzymatic action diminishes nutrient particle size, increasing their bioavailability and absorption rates, which ultimately fosters better protein and ash utilization in the host. Several studies support the observation that probiotic supplementation can modulate fat deposition in broiler meat. The reduced fat content in PROBO group augurs well with the findings of Sardar *et al*. (2025), who observed that dietary multi-strain probiotics fed to broilers decreased their abdominal fat as well as intramuscular fat deposition-probably by bettering lipid metabolism. This is in line with the discoveries of Zhou *et al*. (2022), who found that probiotics supplementation reduced meat fat percentage and improved profiles of fatty acids toward a healthier quality of meat. There are, however, contrary reports such as from Ahmed *et al*. (2021), which indicated no significant effect of probiotics on meat fat content-maybe the impact is strain-specific and depends on dosage as well as diet composition.

Multi-strain probiotics can be considered a resultant antibiotic replacement due to a better feed conversion ratio and reduced mortality that will eventually improve the economic return of the farmer. Probiotics supplementation has been known to improve gut health as well as immune response, hence reducing therapeutic costs. The improvement in physiological and hematological status means less stress and better welfare of broilers, which is good news for a more enlightened population that cares about animal welfare.

CONCLUSION

Multi-strain probiotics elicited enhancements of growth performance, hematobiochemical indexes, and quality of meat in broiler chickens hence validating their use as substitutes for antibiotic growth promoters. The integration of probiotics into the feeding regimen enhances production efficiency and healthiness of birds; thus, sustaining the sustainability pillar in poultry farming systems. Probiotic-based feeding strategies should be taken up by poultry farmers to create a reduction in antibiotic usage among poultry tenantry that is on the rise due to higher demands for more secure and more residue-free poultry products.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Department of Poultry Husbandry, Sindh Agriculture University, Tandojam, for extending the basic requisite arrangements and assistance towards the undertaking of this study.

NOVELTY STATEMENT

This research analyzes the impact of a multi-strain probiotic blend on growth performance, meat quality, and blood parameters in broiler chickens. It enhances understanding of probiotics' health- and productivity-optimizing effects, emphasizing their promise as natural substitutes for antibiotic growth promoters in poultry production.

AUTHOR'S CONTRIBUTION

SAP: Designed and supervised the study.
BM: Performed experimental trial, data collection and initial draft the manuscript.
S: conducted laboratory analyses.
MZ: Performed statistical analysis.
MB: Helped in sample collection.
IA: Reviewed literature and wrote discussion section.
IHL: Reviewed and edited the manuscript and language clarity.

Ethics approval

All the methods and experimental protocols were approved by the Animal Care and Ethics Committee (AN-FAHVS-56-2024) of Sindh Agriculture University, Tandojam.

Data availability

The data of the current experiment is available on reasonable request from the corresponding author.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI-assisted technologies were used in the writing, editing, data analysis, and preparation of manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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