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Comparative analysis between multi-strain probiotics and antibiotic as starter feed supplement of poultry on growth performance, serum metabolites and meat quality

Md Taslim Hossain [a,*], Dipankar Sardar [b], Sadia Afsana [b], Meheta Datta [b], Md. Ahsan Habib [c]

[a] Department of Animal Nutrition, Faculty of Veterinary, Animal and Biomedical Science, Khulna Agricultural University, Khulna 9200, Bangladesh
[b] Department of Pathology and Laboratory Medicine, Doctor's Lab and Imaging, Khulna 9100, Bangladesh
[c] Department of Animal Science and Nutrition, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

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ABSTRACT

The unobstructed use of antibiotics in poultry production has emerged as a major driving force of antibiotic resistance and public health hazard, particularly in developing countries. This study aimed to determine the functional roles of lyophilized native probiotic based starter feed on performance, selective serum metabolites and meat quality of poultry. A total of 90 day-old birds (30 broilers, 30 layers and 30 ducks) were used as experimental birds which were divided into three treatment groups for each kind of bird. Isolated native probiotic strains from chicken intestine were used to prepare lyophilized probiotic samples. Growth performances were measured manually, serum biochemicals analysis were carried out using diagnostic kits, and meat quality was determined through Kjeldahl method and Soxhlet method. When compared to groups receiving antibiotics, the introduction of lyophilized probiotics in starter feed significantly ($P<0.05$) increased body weight gain, feed intake, and feed conversion ratio. The birds' serum calcium and protein levels likewise exhibited a similar pattern. Comparing the groups receiving antibiotics, the protein content of the meat revealed significant ($P<0.05$) variations. Significant ($P<0.05$) reduced level of serum total cholesterol, triglycerides and fat content in meat was observed when compared to antibiotic-fed group. It is possible to conclude that lyophilized probiotics have a significant positive impact on growth performance, serum metabolites and meat quality. The findings of the study could open up new avenues for the application and adoption of native probiotic-based poultry feeds as an alternative to antibiotic-based poultry feeds among stakeholders.

1. . Introduction

Since the beginning of the 21 st century, the poultry sector has experienced remarkable growth, becoming a highly lucrative venture to fulfill the protein demands of Bangladesh's extensive populace. The majority of Bangladesh's population lives in rural areas, where 84 % of people is engaged in agriculture and livestock-related activities (Mathur et al., 2018). The poultry industry holds a vital position within the agricultural framework, enhancing food security, granting consumers access to premium protein sources, and generating direct and indirect employment possibilities, involving ancillary services, for around 6 million individuals (Hamid et al., 2016). In recent times, Bangladesh has emerged as the swiftest expanding economy within the Asia Pacific region. In 2018, the nation achieved a notable GDP growth rate of 6.03 %, and it is anticipated that this growth momentum will persist, with an average GDP growth rate of approximately 7.5 % expected in the coming years (Bangladesh Economic Review, 2023).

The poultry industry faces a constant challenge to balance the need for increased production efficiency with the growing awareness and concerns regarding antimicrobial resistance and food safety. Antibiotics have historically played a crucial role in promoting growth and preventing diseases in poultry (Mak et al., 2022). However, the emergence of antibiotic-resistant strains and the potential transmission of resistant traits to human pathogens have raised significant public health concerns (Ding et al., 2023).

In response to these challenges, the use of probiotics as an alternative to antibiotics in poultry feed has gained momentum. Probiotics, particularly those composed of multiple strains of beneficial

* Corresponding author.
E-mail address: taslim@kau.edu.bd (M.T. Hossain).

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microorganisms, offer a holistic approach to enhancing poultry performance. Multi-strain probiotics are thought to exert positive effects on the gut microbiota, promoting a balanced microbial community that contributes to improved nutrient absorption and immune system modulation. These effects may translate into enhanced growth performance and overall health without the associated risks of antibiotic resistance (Williams, 2010).

In Bangladesh, where poultry farming is a cornerstone of agriculture, the focus lies on key breeds such as layers, broilers, and ducks. Layers, specifically raised for egg production, have become a lucrative venture, yet the prevalent use of antibiotics in their feed raises significant concerns. This has prompted a notable shift towards exploring the potential of probiotics as a more sustainable and health-conscious alternative (Yaqoob et al., 2022). Research indicates that probiotics show promising results in layers, contributing to enhanced eggshell quality, improved meat quality, bone structure, and overall growth performance (Kamruzzaman et al., 2021).

Broiler production holds a prominent and promising position within the poultry industry due to its capacity for rapid returns, which significantly contribute to economic advancement and serve as a vital source of animal protein for human consumption. Scientific evidence has confirmed that incorporating probiotics into the diets of broiler chickens leads to enhancements in their feed consumption, growth performance, and characteristics of the carcass (Ahmed et al., 2019; Bai et al., 2013; Shabani et al., 2012). The utilization of probiotics is leading to increasing recognition for their impact on the immune responses of broilers (Mahdavi et al., 2005).

Duck farming, another economically significant aspect of Bangladesh's poultry industry, has also seen a shift towards probiotic inclusion in feed. Studies reveal that 0.2 g per kilogram of diet from a blend of *Lactobacillus acidophilus* and *Lactobacillus casei* lead to notable changes in the blood's biochemical profile, improvements in egg composition, and the establishment of a stable gut microbiota (Khattab et al., 2021; Sun et al., 2022). These outcomes contribute to enhanced growth performance and a reduction in the incidence of diseases, reinforcing the potential of probiotics in promoting the health and productivity of ducks (Li et al., 2011).

However, a prominent area of unmet research need in Bangladeshi poultry production is the lack of comprehensive studies examining the distinct impacts of probiotic and antibiotic supplementation on several parameters. While a great deal of research has been done on the benefits of specific feed additives, not as much has been done on multi-strain probiotics. The rationale behind this comparative analysis stems from the need to address the dual objectives of promoting growth and ensuring the production of high-quality meat, all while adhering to contemporary concerns related to antibiotic usage in animal husbandry. By investigating the effects of both multi-strain probiotics and antibiotics on growth parameters, serum metabolites, and meat quality, this study aims to provide valuable insights into the efficacy of these supplements, aiding in the formulation of evidence-based strategies for sustainable and responsible poultry production.

2. Materials and methods

This study protocol was reviewed and approved by the Department of Animal Science and Nutrition, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh (HSTU/VAS/ASN/EA/008).

2.1. Selection criteria of the experimental birds

A total of 90 day-old birds of mixed sex (male and female) including 30 boiler chicks, 30-layer chicks, and 30 ducklings were purchased from a reliable agent in Khulna, Bangladesh. Boiler chicks, layer chicks, and ducklings had average initial body weights of 42±2.6 gm, 40±2.1 gm, and 43±2.8 gm, respectively. Strict selection criteria were applied before purchasing to ensure consistent size and the absence of any noticeable defects. Any chicks showing signs of disease, deformity, or abnormal development were not included in the study.

2.2. Experimental design

The experiment was carried out using a randomized control trial. In the experiment, birds were randomly assigned to treatment and control groups. A total of 90 birds were divided evenly and randomly into the treatment group 1 (T1), treatment group (T2), and treatment group (T3). For every species of bird, three unique experimental units, or replications, with ten birds per replication, were designed under the same treatment. Among three treatment groups, one of which included an antibiotic and two probiotic treatment groups. All of the birds were given a corn-soybean meal base diet (Table 1) and water, while feed and drink were provided *ad libitum* at all times during the broiler trial. The diets were formulated with some modifications, but it keeps the metabolizable energy and crude protein consistent with three earlier studies' recommendations (Kumari et al., 2011; Miah et al., 2010; Nath et al., 2023). The dietary treatments were:

(1) Basal diet + 500 mg of Oxytetracycline antibiotic per kg of feed (T1),
(2) Basal diet + 250 mg of lyophilized probiotics per kg of feed (T2),
(3) Basal diet + 500 mg of lyophilized probiotics per kg of feed (T3).

2.3. Experimental probiotics and antibiotics

Probiotics: In this study, probiotics were prepared in the Cell Culture Laboratory, Doctor's Lab and Imaging, Khulna, Bangladesh. It was a multi-strain preparation in lyophilized form (2.8×10^9 CFU/gm) that consists of *Bacillus tequilensis* strain 10b, *Bacillus tropicus* strain MCCC 1A01406, *Lactobacillus salivarius* strain HO 66 and *Staphylococcus gallinarum* strain VIII1 and *Staphylococcus hominis* strain DM 122 (Dipankar Sardar, 2022).

Antibiotic: The experimental antibiotic utilized in this trial was oxytetracycline, which is sold under the trade name Renamycin Vet. Approximately 20 % of this antibiotic is made up of oxytetracycline hydrochloride USP.

Table 1
Composition of basal diet (as dry basis) used in the study.

Ingredients	Broiler (%)	Layer (%)	Duck (%)
Maize (Crush)	58.65	56.30	41.00
Rice polish	3.00	13.20	10.00
Vegetable oil	1.80	0.60	
Molasses	0.50	1.00	
Soybean meal	28.55	21.90	12.00
Fish meal	5.60	4.00	10.00
Meat and bone meal	0.30	1.70	5.50
Limestone	1.00	0.65	
Di-calcium phosphate	0.10		
Vitamin and mineral premix	0.25	0.30	1.50
Common salt	0.25	0.30	0.25
Methionine		0.05	
Till oil cake			10.00
Broken rice	5.00		
Wheat (Crush)	5.00		
Calculated nutrients content			
Metabolizable energy (Kcal/kg)	3002.91	2950.00	2707.00
Crude protein (%)	22.02	20.00	19.19
Calcium (%)	1.10	1.00	1.27
Phosphorus (%)	0.79	0.50	0.81
Methionine (%)	0.37	0.50	0.35
Lysine (%)	1.36	1.00	0.97

2.4. Management of birds

All of the birds were raised for 21 days using best practices in an experimental poultry farm at Khulna Agricultural University. All poultry birds were raised in a climate-controlled environment. An automatic thermo-hygrometer was used to measure the room's temperature and humidity levels. The floor was covered in 3 centimeters of fresh and dried rice husk used as litter material. Over the rice husk, old newspaper was also used as litter. The upper layer of the litter, which had been mixed with feces, was removed and replaced with new litter. After two weeks, all of the old litter was replaced with fresh litter. Every alternate day, the litter was disturbed to promote fast drying and the removal of toxic gases. The environment was maintained at 95 ^{o}F for the first week of life before progressively decreasing by 5 °F each week until the experiment's completion. The birds were subjected to 23 hours of nonstop lights. The dark period arrangement was designed to keep the chickens accustomed to darkness in the event of an electrical outage. Environmental parameters (lighting, temperature, humidity, and ventilation) were maintained during the experiment's 21-day run. The experimental period was conducted with appropriate sanitary precautions. On days 1, 3, and 10 respectively, the boiler and layer chicks received eye drops vaccinations against Marek's disease, Newcastle Disease (ND), and Infectious Bursal Disease (IBD) in accordance with the manufacturer's recommendations. Booster doses for Newcastle Disease (ND) and Infectious Bursal Disease (IBD) were given on days 17 and 20, respectively, of the experiment. Ducklings don't need to be immunized for 30 days. Therefore, during the experiment's 21 days, we don't provide any vaccines.

2.5. Sample collection and analysis

Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) for birds were recorded separately. As soon as the birds arrived at the experimental farm from the hatchery, they were all individually weighed and documented. Daily feed records were kept, and weekly assessments of body weight and feed intake (FI) were made. The birds were weighed each week before being fed using an automated balance. Throughout the trial period, mortality was tracked through daily visual observation. Body weight gain (BWG), FI, and feed conversion ratio (FCR) were calculated using the following formula:

$$\mathrm{BWG} = \mathrm{Final\ body\ weight\ (gm)} - -\mathrm{Initial\ body\ weight\ (gm)}$$

$$\mathrm{FI} = [\mathrm{Feed\ supplied\ in\ a\ week\ (gm)} - \mathrm{feed\ weigh\ back\ in\ a\ week\ (gm)}]/\mathrm{No.\ of\ bird}$$

$$\mathrm{FCR} = \mathrm{FI(gm)}/\mathrm{BWG(gm)}$$

Three weeks into the trial, 5 birds from each replication were arbitrarily chosen and put to death. Birds were allowed enough access to water but were starved for 10 hours prior to slaughter. They were promptly bled by using a manual neck cutter to partially slice the neck and cut the carotid arteries. Each bird had its feathers removed by being scalded after bleeding for five minutes. For calculating the carcass yield characteristics, the head, viscera, shank, giblet, and belly fat were cut on. Dressed birds were divided into many pieces, including wings, breast, and drumsticks.

After dressing properly, 50 gm sample of drumstick muscle was overnight frozen at -20 °C with sufficient leveling. Each chosen bird's (Broiler and Duck) drumstick was harvested for its meat, which was then tested for lipid and protein levels. Kjeldahl and Soxhlet methods were used to analyze the protein and fat that make up the fundamental chemical makeup of the bird's drumstick muscles. The crude protein content of a muscles is estimated by applying a conversion factor to the total nitrogen content obtained using the Kjeldahl technique (Varelis, 2016). The sample is digested at about 420 °C using concentrated sulfuric acid, potassium sulphate to raise the boiling point, and a catalyst like copper to speed up the digestion process to measure the nitrogen content. As a result, the sample's nitrogen is changed into nonvolatile ammonium sulphate. Ammonium sulphate is heated with sodium hydroxide to produce volatile ammonia gas after the digest has cooled and been diluted. After being steam-distilled, the ammonia is trapped by creating ammonium borate in an excess of boric acid solution. The remaining boric acid is then titrated using a standard acid and an appropriate end-point indicator to determine the sample's overall nitrogen content (Evers & Hughes, 2002; Goulding et al., 2019). The process of Soxhlet extraction is highly beneficial for preparative work where the analyte needs to be isolated from specific interfering compounds or concentrated from the matrix as a whole. During the process of a traditional Soxhlet, condensed new solvent from a distillation flask is progressively added to the sample, which is held in a thimble holder. Upon reaching an overflow level, the liquid is extracted using a syphon, which then returns the entire contents of the thimble-holder to the distillation flask, containing the extracted analytes in the bulk liquid. Until total extraction is achieved, this process is repeated (Luque de Castro & García Ayuso, 2000).

Five birds were selected at random on day 21 from each replication to have their blood drawn. Samples of blood were drawn from the brachial vein. Using a sterile syringe, approximately 3cc of blood was extracted from each bird and stored upright in the refrigerator. Serum was isolated from blood samples after centrifugation at 3000×g for 10 min, and it was then stored at -20°C for subsequent study. Using a Cholesterol Liquicolor kit, the enzymatic colorimetric approach was used to measure the cholesterol level (GmbH, Wiesbaden, Germany). Suitable commercial diagnostic kits for avian species were used to measure the amounts of albumin, globulin, and total protein (Bio-Systems, S.A. Barcelona, Spain and GmbH, Wiesbaden, Germany). Using a Calcium (CPC) Liquicolor kit (Stanbio Laboratory, L.P, Boerne, TX, USA), the manufacturer's recommendations were followed to assess the calcium level using the enzymatic colorimetric method.

Table 2
Growth performances of birds in different dietary groups.

Performance Analysis[1]	Broiler	Layer	Duck	Experimental Groups
Body weight gain (gm)	581.91 ±7.18^{a}	182.16 ±0.51^{a}	205.87 ±7.14^{c}	T1
	592.73 ±7.13^{a}	185.91 ±0.58^{a}	235.80 ±10.10^{b}	T2
	617.67 ±6.79^{b}	194.08 ±3.55^{a}	272.47 ±12.84^{a}	T3
Feed intake (gm)	629.71 ±10.67^{a}	284.28 ±0.48^{b}	384 ±12.08^{b}	T1
	636.80 ±10.45^{a}	256.54 ±0.49^{a}	386 ±11.22^{b}	T2
	652.84 ±8.45^{b}	261.54 ±0.73^{a}	394 ±13.26^{a}	T3
Feed conversion ratio	1.08^{a}	1.56^{a}	1.87^{c}	T1
	1.07^{a}	1.38^{a}	1.64^{b}	T2
	1.05^{b}	1.34^{b}	1.44^{a}	T3

^{abc} Within the same column of each parameter, means with different superscripts are significantly different ($p < 0.05$) by Tukey's test.
[1] Data represent mean(n=30) ±standard error mean (SEM).

2.6. Statistical analysis

Data were analyzed using SPSS statistical software version 26.0 for Windows (IBM, USA). The differences among groups were analyzed by one-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Result

3.1. Growth performance

Table 2 displays the effects of antibiotic supplementation and increasing lyophilized probiotic levels in the feed on growth performance. The probiotic supplemented group (T3) outperformed both the antibiotic-fed group (T1) and the probiotic-fed group with a lower dose (T2) in terms of body weight gain (BWG). However, T1 and T2 did not differ statistically significantly, indicating that a larger dose may be advantageous to broiler production in terms of body weight gain. There was no significant difference between the experimental groups in the case of layer chicks when BWG was taken into consideration in the current study. Both lower and higher dosages of probiotic supplemented feed had a significant ($P<0.05$) impact when compared to the antibiotic-fed group, with the higher dose producing the best outcomes in ducklings.

When it came to feed intake, broiler chickens followed the same pattern as BWG. In layer chicks, the antibiotic-fed group performed significantly better than the probiotic-fed group. In this case, ducklings behaved similarly to broiler chickens. Both broiler and layer chicks had comparable feed conversion ratio (FCR) values, with higher doses of probiotic demonstrating a significant ($P<0.05$) difference from antibiotic fed group (T1). Probiotics functioned significantly ($P<0.05$) better in the case of ducklings than antibiotics, and higher probiotic dose also outperformed.

3.2. Carcass characteristics

In Table 3, it is depicted how various dietary groups affect the qualities of the carcass yield. Probiotic-fed groups fared significantly better than antibiotic-fed groups when it came to dressing yield. Numerically, T3 was found to have the highest dressed weight fed with higher dose of probiotic. In the case of ducks, T3 differed significantly from T1 and T2, while there was no significant difference between T1 and T2. Both breast and drumstick yield in broilers revealed comparable results, with probiotic-fed groups showing a significant ($P<0.05$) difference when compared to antibiotic-fed groups.

Regarding ducks, T3 had a significant ($P<0.05$) impact when compared to T1 and T2, but there was no discernible difference when T2 was considered. Drumstick yield also followed a similar pattern. Between the probiotic-fed and antibiotic-treated groups, the breast yield did not significantly change. In either the broiler or duck experimental groups, there was no observable change in wing yield.

Table 3
Carcass parameters of birds in different dietary groups.

Carcass Yield, gm[2]	Broiler	Layer	Duck	Experimental Groups
Dressing Yield	446±9.28^{a}	142 ±0.44^{a}	232.23 ±0.90^{b}	T1
	581±7.25^{c}	141 ±0.57^{a}	236.03 ±0.97^{b}	T2
	602±8.07^{b}	142 ±0.29^{a}	241.51 ±0.98^{a}	T3
Breast	82.52 ±2.67^{b}	23± 0.57^{a}	1.476 ±0.26^{a}	T1
	122.93 ±3.69^{a}	26±0.88^{a}	1.36±0.20^{b}	T2
	129.74 ±2.27^{a}	28±0.88^{a}	1.49±0.19^{a}	T3
Drumsticks	83.63 ±2.19^{a}	23±0.28^{a}	36.83 ±0.72^{b}	T1
	117.75 ±2.16^{b}	22±0.57^{a}	38.93 ±0.63^{b}	T2
	123.25 ±1.12^{b}	23.5 ±0.44^{a}	42.06 ±0.52^{a}	T3
Wings	27.88 ±1.06^{a}	9.5 ±0.14^{a}	3.57±0.29^{a}	T1
	31.06 ±1.17^{a}	9.1 ±0.03^{a}	3.70±0.36^{a}	T2
	31.14 ±1.13^{a}	9.7 ±0.17^{a}	5.40±0.30^{a}	T3

^{abc} Within the same column of each parameter, means with different superscripts are significantly different ($p<0.05$) by Tukey's test.
[2] Data represent mean(n=30) ±standard error mean (SEM).

3.3. Serum metabolites

The blood parameters are listed in Table 4. In the probiotic supplemented treatments, blood total cholesterol was lower ($P<0.05$) than in the antibiotic-treated group, and the contents of the different cholesterol

Table 4
Blood parameters of birds receiving different dietary groups.

Indicators	Broiler	Layer	Duck	Experimental Groups
Cholesterol, mg/dl[1]				
Total	128.71 ±2.334^{b}	76.06 ±1.416^{a}	165.17 ±1.397^{b}	T1
	102.34 ±3.667^{a}	59.17 ±0.726^{b}	147.27 ±1.334^{c}	T2
	90.25 ±1.231^{c}	56.76 ±0.846^{b}	134.05 ±1.534^{a}	T3
High-density lipoprotein	72.40 ±0.056^{a}	26.23 ±0.788^{a}	69.6267 ±1.088^{a}	T1
	75.61 ±0.023^{b}	37.11 ±0.482^{b}	62.13 ±1.095^{a}	T2
	82. 88 ±0.038^{b}	39.28 ±0.360^{b}	44.12 ±1.108^{b}	T3
Low-density lipoprotein	61.61 ±0.056^{a}	22.05 ±0.534^{b}	55.217 ±1.146^{a}	T1
	34.63 ±0.027^{b}	17.14 ±0.708^{a}	44.50 ±0.910^{b}	T2
	31. 29 ±0.019^{b}	15.59 ±0.858^{a}	34.81 ±0.692^{c}	T3
Triglycerides, mg/dl	113.5 ±7.678^{a}	98.93 ±0.635^{b}	193.87 ±1.509^{b}	T1
	92.33 ±3.033^{c}	78.21 ±0.616^{a}	175.15 ±1.52^{a}	T2
	80.06 ±3.084^{b}	75.35 ±0.900^{a}	153.49 ±1.346^{c}	T3
Calcium, gm/dl	10.38 ±0.20^{a}	11.21 ±0.069^{a}	10.383 ±0.39^{a}	T1
	11.55 ±0.12^{b}	11.80 ±0.023^{a}	11.53 ±0.44^{a}	T2
	11.79 ±0.07^{b}	11. 98 ±0.021^{b}	12.33 ±0.41^{b}	T3
Protein, gm/dl[1]				
Total	1.15 ±0.022^{b}	1.52 ±0.081^{a}	2.24 ±0.257^{a}	T1
	0.73 ±0.050^{a}	2.97 ±0.036^{b}	2.07 ±0.188^{b}	T2
	0.68 ±0.057^{a}	3.91 ±0.030^{c}	2.31 ±0.149^{a}	T3
Albumin	0.47 ±0.038^{a}	0.52 ±0.014^{b}	0.77 ±0.067^{a}	T1
	0.21 ±0.024^{b}	1.19 ±0.011^{c}	0.70 ±0.062^{a}	T2
	0.10 ±0.020^{c}	1.78 ±0.017^{a}	0.81 ±0.049^{b}	T3
Globulin	0.56 ±0.019^{b}	1.00 ±0.057^{b}	1.48 ±0.266^{a}	T1
	0.52 ±0.015^{b}	1.78 ±0.031^{c}	1.36 ±0.201^{b}	T2
	0.58 ±0.020^{a}	2.13 ±0.088^{a}	1.49 ±0.199^{a}	T3
Albumin/Globulin (A/G) Ratio	0.84:1	0.52:1	0.52:1	T1
	0.40:1	0.67:1	0.52:1	T2
	0.17:1	0.83:1	0.54:1	T3

^{abc} Within the same column of each parameter, means with different superscripts are significantly different ($p<0.05$) by Tukey's test.
[1]Data represents mean(n=15) ±standard error mean (SEM).

fractions changed: HDL content rose while LDL level fell. However, broiler chicks fed a larger dose of probiotics had the best results. The total cholesterol in layer chicks did not differ statistically significantly between the probiotic-treated groups, but the probiotic-fed group did differ significantly from the antibiotic-treated group. In this case, HDL was significantly ($P<0.05$) higher, and LDL was significantly ($P<0.05$) lower with no significance variance between the probiotic fed groups. In duckling, total cholesterol followed the same pattern as broiler had. When it turns into triglycerides of broiler, the birds who got probiotics had significantly ($P<0.05$) lower mean total triglycerides than the birds who received antibiotic. The T3 group had the lowest plasma triglyceride level. Ducklings showed the same trend as broiler chicks. While there was no significant difference between the probiotic fed groups (T2, T3) in the case of layer, the probiotic treated group displayed a significant ($P<0.05$) difference in comparison to the antibiotic fed group.

When compared to the antibiotic-fed group in broiler chicks, Serum calcium levels were significantly different ($P<0.05$) in the probiotic-fed group, but not significantly ($P>0.05$) from the probiotic-fed groups (T2, T3). Probiotic treated groups with higher dose demonstrated a significant ($P<0.05$) difference when compared to antibiotic treated groups in both layer chicks and ducklings.

When compared to probiotic-treated groups in broiler chicks, the antibiotic-treated group had a noticeably superior outcome in case of serum protein. T3 had a significantly lower level of albumin and higher amount of globulin, which resulted in a lower A/G ratio. In comparison to the antibiotic-fed group in layer chicks, the probiotic-treated groups (T2, T3) had considerably greater ($P<0.05$) levels of total blood protein, albumin, and globulin, even though T3 had a significant ($P<0.05$) difference from T2. In the case of ducklings, there was no discernible difference between the groups that received antibiotics and probiotics. Similar patterns were visible in serum globulin levels, while albumin levels in the probiotic-fed group were considerably greater than in the antibiotic-treated group.

3.5. Meat quality

Table 5 is a list of the selected chemical makeup of meat. The broiler chickens which received the probiotic during their entire raising period had much higher protein in their flesh overall. With a significant difference ($P<0.05$) between it and the antibiotic-fed group, T3 had the greatest protein level. Although T2 had marginally higher protein levels than T1, the difference was not statistically significant. Additionally, there were significant differences ($P<0.05$) in the treatment groups for ducks, where the probiotic fed group receiving the maximum dose (T3), exhibiting the highest score.

The probiotic-fed animals had lower crude fat content in broiler meat than the other groups, and this tendency was statistically significant ($P<0.05$). T2 and T3, however, did not appear to differ from one another. In addition, T3 had the highest score across all treatment groups in the case of duck, which indicated significant ($P<0.05$) differences.

Table 5
Selective chemical composition of leg meat (5 gm) of birds receiving different dietary groups.

Indicator	Broiler	Duck	Experimental Groups
Protein, gm[1]	1.68±0.029[b]	0.97±0.11[a]	T1
	1.75±0.023[b]	1.17±0.10[b]	T2
	2.08±0.041[a]	1.25±0.04[c]	T3
Fat, gm[1]	0.51±0.028[a]	1.79±0.067[b]	T1
	0.25±0.021[b]	1.24±0.083[a]	T2
	0.20±0.017[c]	1.15±0.036[c]	T3

[abc]Within the same column of each parameter, means with different superscripts are significantly different ($p<0.05$) by Tukey's test.
[1]Data represent mean(n=15) ± standard error mean (SEM).

4. Discussion

Probiotic supplementation improved BWG, FI, and FCR in broiler chicks when compared to the antibiotic treatment, according to some studies, which is consistent with our findings (Li et al., 2011; Samanya et al., 2002). However, both authors pointed out that the benefits were greatest at the highest supplementation. The results of the current study are consistent with a certain investigation which found that dietary probiotics increased final body weights in a significant manner (Sobczak & Kozłowski, 2015). Additionally, other studies that found no significant effects of dietary probiotic addition on feed intake quantity in case of layer have confirmed findings (Anjum et al., 2005). In accordance with our findings, some research hypothesized that probiotic supplementation had a significant impact on the feed conversion ratio (FCR) impacted by dietary probiotics (Sobczak & Kozłowski, 2015). These results also align with those of other research that observed higher body weight, feed intake, and feed conversion in ducklings treated with probiotics as opposed to antibiotic-treated group (Neijat et al., 2019). Based on some studies, probiotics can enhance the fermentation of non-digestible components of the feed, leading to the production of short-chain fatty acids which serve as an energy source for the host and contribute to improved performance (Patterson & Burkholder, 2003). Probiotics may also stimulate the production of digestive enzymes in the gastrointestinal tract. Increased enzyme activity can improve the breakdown of complex nutrients in the feed, making them more readily available for absorption (Awad et al., 2009). Additionally, probiotics may enhance the absorption of nutrients, such as vitamins and minerals, by promoting the expression of nutrient transporters in the intestinal lining which can contribute to better overall nutrient utilization, improved growth performance, and organ weights (Adil & Magray, 2012).

Probiotic treatment significantly improves carcass status in terms of carcass yield, according to certain research. After that, some research found that giving probiotics raised the percentage of breast tissue and carcass weight (Fathi et al., 2017). Our recent study found a notable rise in carcass, breast, and drumstick yields, continuing this trend. While some studies found that adding probiotics to broiler diets increased wings yield and decreased abdominal fat weight, contrary to the present study's findings, others discovered that adding probiotics to broiler diets increased abdominal fat weight (Kalavathy et al., 2003). These outcomes matched those of a study conducted on ducklings, which discovered that the carcass and breast meat ratios of the ducks given probiotic treatment were higher than those of the birds given antibiotics (Balevi et al., 2001). When probiotics were given to layer chicks in place of antibiotics, there was a greater increase in carcass output. However, as compared to the antibiotic-treated group, probiotics had no significant influence on carcass output in case of layer. The addition of probiotics to the diet boosted weight gain and improved feed intake and feed conversion. As opposed to that, the effect of probiotic supplementation on carcass features has not been independently verified by other researchers, who attribute this effect to the unique environmental circumstances of the experiment (Panda et al., 2008).

The results of current study confirm with the report which reported a reduced cholesterol in broilers diets containing probiotics in starter phase by mechanisms such as bile salt hydrolase activity, short-chain fatty acid production, anti-inflammatory effects, and modulation of gut microbiota (Pambuka et al., 2014). Some researchers also found that probiotic administration boosted blood HDL levels while decreasing serum LDL levels (Sun & Kim, 2021). Layers treated with probiotics have been shown to lower blood cholesterol levels because to their incorporation (Bidura et al., 2019). In another study, a significant reduction in total cholesterol with increasing HDL and decreasing LDL content was evident in accordance with the recent study. The findings were comparable to some studies shown that the probiotics diets resulted in a significant increase in concentration HDL and decrease in LDL level resulted in a considerable drop in serum cholesterol content (M. Ahmed

et al., 2018). Similar to the current investigation, some studies reported a substantial decrease in serum triglycerides in broiler chickens given probiotic supplements (Wang & Zhou, 2007). According to some investigations, utilizing probiotics considerably decreased the blood triglyceride level of layer chicks as compared to the antibiotic-treated group. This finding is consistent with the significantly decreased serum triglyceride level (Anna et al., 2005). Additionally, the results were consistent with a certain study which also demonstrated that probiotic diets caused a considerable reduction in the content of serum triglycerides in ducks (Jukna et al., 2005). Although the mechanisms are unknown, it is believed that some bacterial probiotic strains can incorporate cholesterol into their cells, alter the lipoprotein metabolism of birds favorably by hydrolyzing bile salts or blocking hydroxyme thylglutaryl-CoA, the rate-limiting enzyme in cholesterogenesis, and subsequently lower the body pool of cholesterol (Kalavathy et al., 2003). The results of this investigation are more comparable to the investigation which discovered substantial changes in the serum calcium levels of broilers between treatments with probiotic supplements and those treated with antibiotics (Awad et al., 2009). In addition, recent experiments found that layers fed probiotics had significantly higher serum calcium levels than the antibiotic-fed group as antibiotics act as strong chelating agent. Because of this ability to chelate metals like calcium, the amount of calcium in blood serum is reduced (Hlavka et al., 2000; Panda et al., 2006). As like our research finding, some researchers reported that, supplementing with probiotics had a substantial impact in raising serum calcium levels (Sen et al., 2012). The increase in calcium levels in blood serum brought on by the addition of probiotics may be due to the organic acids from probiotics reducing the PH in the gastrointestinal system, which enhances the absorption of such mineral from the gastrointestinal tract into the blood stream (Dousa et al., 2013). The results of this investigation are corroborated by a research showing that probiotic treatment enhanced plasma protein levels of broilers (Silva et al., 2020). Probiotics are thought to compete with pathogenic bacteria, reducing protein degradation to nitrogen. As a result, amino acid and protein consumption is improved (Mansoub, 2010). This study is also similar to another study that found that total protein, albumin, and globulin showed significant results depending on the time of collection in case of layer (Rahman et al., 2014). The outcomes are consistent with a study that probiotics treated ducks exhibited significant variance in serum albumin and globulin level as well as in A/G ration indicating that probiotics may have a favorable influence on immunological response and disease resistance. (Panda et al., 2006).

Research has demonstrated that supplementing feed with probiotics considerably raised the protein content of meat while reduced the fat content of broiler meat, which concur with the findings of the current inquiry (Chen et al., 2014). Current research has also confirmed that the probiotics' effects were evident in the treated ducks' higher levels of protein and decreased levels of fat in their meat (Kokoszyński et al., 2021).

5. Conclusion

All things considered, this study highlights the potential benefits of adding multi-strain probiotics as an alternative of antibiotics to the diets of poultry, such as broiler, layer, and duck, as these probiotics have not gotten as much attention as they should. This study showed that feeding lyophilized multi-strain probiotic supplements to birds can increase their feed intake, growth rate, and feed conversion ratio. while simultaneously raising the protein content and lowering the fat content of the broiler and duck carcasses to improve the quality of the meat. Probiotic-based feeds reduce serum cholesterol and triglyceride levels as well as have significant effects on boosting globulin levels and decreasing A/G ratio, indicating that probiotics may have positive impact on immunological response and disease resistance. These findings suggest that using this multi-strain probiotics could be a practical way to improve the health and performance of birds without using antibiotics, which would assist the production of poultry in a sustainable manner. By reducing reliance on antibiotics, these findings may contribute to mitigating antibiotic resistance issues, benefiting both animal and human health. Besides, the adoption of this probiotic-based feed could create new market opportunities for producers and suppliers of probiotic products. It may also meet consumer demands for poultry products produced with fewer antibiotics.

Limitation

The current investigation was a small laboratory scale research. That is why, this study was conducted on a limited starting size, using 30 birds per group to observe the effects of lyophilized native probiotics *in vivo* which could be a shortcoming.

Ethical statement

This study protocol was reviewed and approved by the Department of Animal Science and Nutrition, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh (HSTU/VAS/ASN/EA/008).

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To whom it may concern

This is to certified that the research work entitled as "Comparative analysis between multi-strain probiotics and antibiotic as starter feed supplement of poultry on growth performance, serum metabolites and meat quality" was conducted under the supervision of Dr. Md. Taslim Hossain, Department of Animal Nutrition, Khulna Agricultural University, Khulna and Dr. Md. Ahsan Habib, Department of Animal Science and Nutrition, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh in accordance to the research ethics and guidelines followed by Department of Animal Science and Nutrition of this university. The experimental design of this study was not objectionable or subversive to animal ethics, therefore this research work has been approved at the meeting held in the department on 27th November, 2022 at 10 AM bearing the resolution NO. 008.

CRediT authorship contribution statement

Md Taslim Hossain: Investigation, Supervision, Validation, Visualization. **Dipankar Sardar:** Conceptualization, Methodology, Investigation, Data curation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sadia Afsana:** Investigation, Formal analysis, Writing – original draft. **Meheta Datta:** Investigation, Formal analysis, Writing – original draft. **Md. Ahsan Habib:** Investigation, Supervision, Validation, Visualization.

Declaration of competing interest

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

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