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**Research article**

# Dietary effects of multi-strain probiotics as an alternative to antibiotics on growth performance, carcass characteristics, blood profiling and meat quality of broilers

**Dipankar Sardar[1,2], Sadia Afsana[2], Adnan Habib[3] and Md. Taslim Hossain[4,*]**

*[1]Poultry Production Research Division, Bangladesh Livestock Research Institute, Dhaka-1341, Bangladesh*
*[2]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh*
*[3]Animal Health Research Division, Bangladesh Livestock Research Institute, Dhaka-1341, Bangladesh*
*[4]Department of Animal Nutrition, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh*

## Abstract

The growing issue of antibiotic resistance, particularly driven by the overuse of antibiotics in poultry farming in developing countries, presents serious public health threats. This situation highlights the urgent need for alternatives that benefit both poultry and human health. Probiotics, as live microorganisms administered in proper amounts, are a promising substitute, enhancing host health without harmful residues. This study evaluated the effects of lyophilized probiotic-based starter feed versus antibiotics on the growth performance, carcass traits, serum metabolites, and meat quality of broiler chicks. As experimental feed additives, ciprofloxacin, often used in the poultry industry of developing nations, and lyophilized multi-strain probiotics ($2.8\times10^9$ CFU/g) isolated from chicken intestines, included *Bacillus tropicus* strain MCCC 1A01406, *Bacillus tequilensis* strain 10b, *Staphylococcus gallinarum* strain VIII, *Lactobacillus salivarius* strain HO 66, and *Staphylococcus hominis* strain DM 122 were utilized. A total of 300 Cobb-500 broiler chicks were divided into four groups, each with three replicates of 25 chicks, in a completely randomized design: (1) control (basal diet), (2) basal diet + 500 mg ciprofloxacin/kg feed (T1), (3) basal diet + 250 mg probiotics/kg feed (T2), and (4) basal diet + 500 mg probiotics/kg feed (T3). Growth performance was measured manually, serum biochemicals via avian diagnostic kits, and meat composition through Kjeldahl (protein) and Soxhlet (lipid) methods. After three weeks, probiotic-fed chicks showed significant increases ($P<0.05$) in body weight, feed intake, and feed conversion ratio compared to control and antibiotic groups. Serum analysis revealed increased ($P<0.05$) protein, calcium, and RBC levels and reduced cholesterol and uric acid. Meat from probiotic-fed groups had better ($P<0.05$) antioxidant properties, higher protein, and fiber, and lower fat, ash, and nitrogen-free extract levels, suggesting probiotics as a sustainable antibiotic alternative for poultry farming and public health improvement.

**Keywords**: Antibiotics, Growth performance, Meat quality, Poultry, Probiotics, Serum metabolites.

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[*] **Corresponding author**: Md. Taslim Hossain, Department of Animal Nutrition, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh. Email: taslim@kau.edu.bd.

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# INTRODUCTION

Food security and socio-economic development are closely connected, as the availability, accessibility, and affordability of nutritious food are crucial for the well-being of nations (Olubamiwa et al., 2002). Animal protein, especially from poultry products such as meat and eggs, plays a vital role in meeting global dietary needs (Mancinelli et al., 2022). As a result, there is a growing demand for higher-quality and safer protein sources that are free from infectious agents. However, the poultry industry has repeatedly encountered challenges from various diseases, particularly during periods of rapid growth (Ndukui et al., 2021). Enteric illnesses caused by viruses, bacteria, fungi, and protozoa significantly burden the poultry industry by reducing production, increasing mortality rates, contaminating poultry products for human consumption, and raising the costs of preventive measures (Patterson and Burkholder, 2003). This has led to the extensive use of antibiotics in the poultry industry for medicinal, preventive, and growth promotion purposes, especially in the early stages of life to prevent illnesses (Khan and Naz, 2013). However, widespread antibiotic use has resulted in antibiotic resistance and residues in animal products. The use of drugs that are also used to treat human diseases is particularly concerning (Dipankar and Taslim, 2022). Animals can harbor microorganisms that develop resistance to these treatments, and humans consuming such meat may acquire resistant germs and small amounts of the drugs, which can reduce their resistance to infections and beneficial microflora (Khaziahmetov et al., 2018). The global trend in poultry production now emphasizes safe production technologies, primarily by eliminating antibiotics in the production of farm animals and poultry (Mohammadi et al., 2023).

In modern industrial chicken production, newly hatched chicks have minimal contact with their mothers, resulting in a slower development of normal intestinal microbiota (Li et al., 2022). During this early period, chicks are highly susceptible to pathogen colonization due to the absence of a stable gut microbiota (Schwarzer et al., 2018). Since the European Union banned antibiotic growth promoters (AGPs) in 2006, enteric infections have become a major concern in the poultry industry (Bengtsson and Wierup, 2006). This has led to issues with production efficiency, bacterial proliferation in the small intestines, nutrient loss, and related food contamination (Huyghebaert et al., 2011). As a result, researchers have been investigating alternatives to growth-promoting antimicrobials. These alternatives aim to prevent harmful bacteria from multiplying and to enhance beneficial gut microflora to improve health, immunity, and performance (Adil and Magray, 2012). Various feed supplements have been used to replace AGPs in chickens, with varying degrees of success. Probiotics, in particular, have shown promise due to their positive effects on broiler performance and safety compared to antibiotics (Ayalew et al., 2022). Probiotics play a crucial role in achieving optimal performance criteria, though their commercial use in the poultry industry is still relatively new.

Over the years, numerous definitions of probiotics have emerged. R. Fuller initially defined probiotics as live microbial feed supplements that improve the host's intestinal microbial balance (Fuller, 1989). A collaborative team from the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) describes probiotics as "live microorganisms which, when consumed in sufficient quantities, provide a health advantage to the host" (Sánchez et al., 2017). The Food and Drug Administration (FDA) classifies probiotics as Generally Regarded as Safe (GRAS) substances, as they do not leave residues in animal production or promote antibiotic resistance when consumed (Arora et al., 2019). Probiotic species such as *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have been shown to positively influence broiler performance (Dipankar and Taslim, 2022). The presence of probiotics in the digestive tract offers numerous benefits, including enhanced broiler performance and nutrient absorption, modulation of intestinal microflora and pathogen inhibition, histological changes in the intestines, immunomodulation, improved haemato-biochemical parameters, better sensory characteristics of dressed broiler meat, and improved microbiological meat quality, ultimately reducing health risks to humans (Mehr et al., 2007; Vuong et al., 2016). Furthermore, broiler starter feeds rich in probiotics increase meat protein content, high-density lipoprotein (HDL) levels, and meat yield, while reducing fat content, low-density lipoprotein (LDL), uric acid levels, and broiler chick mortality rates (Hossain et al., 2024). The method of administering probiotics to broiler chickens varies by manufacturer but is commonly done via injection into eggs, incorporation into litter, or oral administration (Krysiak et al., 2021). Traditional water-based oral applications may reduce probiotic viability due to contact with water disinfectants like chlorine or interactions with other water compounds, and they often suffer from lower stability and shelf-life issues (Meunier et al., 2016). Using dry cells produced through lyophilization is indeed a highly efficient method for incorporating probiotics into feed. The freeze-drying process preserves the viability and stability of the probiotic bacteria, ensuring that they remain effective and beneficial when added to animal feed. This method also simplifies storage and handling, as lyophilized probiotics are less sensitive to temperature and moisture compared to their liquid counterparts (Broeckx et al., 2016). Therefore, the present study investigates the effect of dietary supplementation of lyophilized probiotics on the growth performance, carcass traits, selective blood parameters, and meat quality of broiler chicks.

# MATERIALS AND METHODS

The study was conducted at Ababil Agro Farm, Chowtali, Dhamrai, Dhaka under Poultry Research Center, Bangladesh Livestock Research Institute, Bangladesh. This study protocol was reviewed and approved by Khulna Agricultural University Animal Experimentation Ethics Committee, Khulna-9208, Bangladesh (AEEC/KAU/2024-1003).

## Selection criteria of experimental birds

A total of 300 Cobb-500 strain day-old chicks, consisting of both male and female, were purchased from Nourish Poultry and Hatchery Limited, Khulna, Bangladesh. These chicks had an average body weight of 42±3.18 g upon purchase. Before acquisition, strict selection criteria were enforced to guarantee consistency in size and to ensure that there were no visible defects. These criteria involved thorough assessments of physical characteristics such as the condition of feathers, leg structure, and overall alertness. Chicks with symptoms of disease, malformations, or aberrant development were eliminated from the study.

## Experimental bird management

The study facility was carefully cleaned and sanitized prior to the trial's start. A total of 300 day-old broiler chicks were purchased from a commercial hatchery which were then quickly transported to the experimental unit within a single hour. To avoid dehydration prior to feed access, broiler chicks were kept in transportation boxes with controlled temperature (90 ± 1°F) and humidity (65 to 70%). Experimental birds were divided into four treatment groups with 3 replications in each group (25 birds per replication), following a thoroughly randomized design. Each group’s chicks were kept in a partitioned pen of about 4 square feet. For unfettered access to feed and water, each pen was prepared with a feeder and a waterer. Acclimatization of the chicks was maintained with a large chick guard under a canopy-type hover which area was about 16 square feet. The floor was covered with 3cm of fresh and dried rice husk that had been covered with shredded paper. After the first two weeks, the upper portion of the litter that had been combined with faeces was taken out and replaced with fresh litter. Every alternate day, the litter was turned over to promote fast drying and the removal of toxic gases. During 21 days of experimental period, environmental factors (lighting, temperature, humidity, ventilation) were maintained at standard levels recommended by Nourish Poultry and Hatchery Limited for broiler chickens. The temperature was kept at 95°F for the first week of life for the chicks before progressively dropping at the rate of 5°F to 85°F for the remaining three weeks. Chicks were exposed to a continuous lighting period of 23 hours and a dark period of 1 hour every 24 hours from day 2 until the end of the study.

## Immunization

All birds underwent proper immunization protocols, receiving vaccinations against Infectious Bronchitis and Newcastle Disease (BIL) on the 1st day and Infectious Bursal Disease (IBD) on the 12th day. Booster doses were administered on the 17th day for IBD to enhance immunity and protection.

## Experimental feed additives

### Probiotic

Isolation, biochemical and molecular characterization (Dipankar and Taslim, 2022), and lyophilization of the probiotic were conducted at Animal Cell Culture Laboratory, Khulna University, Bangladesh. The isolation of probiotic strains from the ceca, crop, and small intestine of chickens from five different government and non-government poultry farms was the first step in the production of lyophilized probiotics. The 16S rRNA gene was sequenced to identify the isolated native probiotic bacteria. The bacterial solution was combined with the protective medium in a 2:1 ratio before lyophilization. The protective medium was made up of an equal proportion of sugar solution and antioxidant solution, with the following composition: 11.29 g/L sucrose, 9 g/L sodium chloride, and 5 g/L ascorbic acid. 60ml bacterial solution and 30ml protective medium were mixed in a 300ml lyophilization tube and pre-frozen at -20°C in a deep freezer in such a way that there was enough space in the tube. The cooling temperature was set to -35°C after the lyophilizer was started. When the vacuum pressure dropped to 20 mmtor or less, the vacuum valves were closed and the vacuum was turned off. The procedure takes between 30 and 36 hours to complete. The vacuum valves were closed and vacuum was switched off when the vacuum pressure reached 20 mmtor or less. The flasks were then disconnected, and samples were taken out. It was a multi-strain preparation in lyophilized form ($2.8\times10^9$ CFU/g) that consists of *Bacillus tropicus* strain MCCC 1A01406, *Bacillus tequilensis* strain 10b, *Staphylococcus gallinarum* strain VIII, *Lactobacillus salivarius* strain HO 66, and *Staphylococcus hominis* strain DM 122.

### Antibiotic

Ciprofloxacin, a member of Floroquinolone, which is marketed under the trade name CiproCare (AdvaCare Pharma). Each bolus contains Ciprofloxacin Hydrochloride USP equivalent to Ciprofloxacin 1 g. Ciprofloxacin has been extensively used in commercial poultry farms in Bangladesh and other developing countries (Tasmim et al., 2023). The desire to replace this antibiotic is the rationale behind its selection for our experimental trial.

## Experimental design

Each group was subjected to a feeding treatment. Among them, three were treatment groups consisting of two probiotic treatment groups and one antibiotic treatment group and the remaining one was the control group with no probiotic or antibiotic. The dietary treatments were:

1. Control: Basal diet
2. Treatment 1: Basal diet + 500mg of Ciprofloxacin antibiotic/kg feed
3. Treatment 2: Basal diet + 250mg of lyophilized probiotics/ kg feed
4. Treatment 3: Basal diet + 500mg of lyophilized probiotics/ kg feed

Diets were formulated to provide the recommended requirements (Table 1) for broilers by Nourish Poultry and Hatchery Limited, Bangladesh (without added antibiotics, or growth promoters). The probiotics were thoroughly mixed with the feed by hand, ensuring the correct ratio was maintained, and then supplied to the chickens. The mixing process was carefully carried out to guarantee even distribution of the probiotics throughout the feed. Throughout the trial, feed and water were provided *ad libitum* to the chicks.

**Table 1** Ingredients and nutritional composition of broiler chicks' diet provided on days 1-21 days.

| Ingredient[1*], % | Composition |
|---|---|
| Maize | 58.65 |
| Rice Polish | 3 |
| Vegetable Oil | 1.8 |
| Molasses | 0.50 |
| Soybean Meal | 28.55 |
| Fishmeal | 5.60 |
| Meat and Bone Meal | 0.30 |
| Limestone | 1.00 |
| Dicalcium phosphate | 0.10 |
| Vitamin and Mineral Premix[2] | 0.25 |
| Common Salt | 0.25 |
| Nutrient analysis* (Calculated) | |
| Metabolizable energy, kcal | 3002.91 |
| Crude protein, % | 22.02 |
| Crude fiber, % | 3.72 |
| Calcium, % | 1.10 |
| Phosphorus, % | 0.79 |
| Methionine, % | 0.37 |
| Lysine, % | 1.36 |

[1]Ingredient nutrient compositions were analyzed before formulating the diet; [2]Supplied per kg of feed - Vitamin A: 12500 IU; vitamin D3: 1250 IU; vitamin E: 18 IU; vitamin K3: 3.7 mg; thiamine: 1.8 mg; riboflavin: 6.6 mg; calcium pantothenate: 10 mg; niacin: 37.5 mg; pyridoxine: 32.5 mg; vitamin B12: 2.5 mg; Mn: 50 mg; Zn: 37.5 mg; Fe: 25 mg; Cu: 7.5 mg; [*]Ingredients and nutritional contents were calculated on a dry matter basis.

## Growth performance assessment

To assess body weight gain (BWG), each chicken was weighed at the beginning and end of each week, and the weekly weight gain was calculated by subtracting the initial weight from the final weight. Live weight was measured using a digital weighing scale, and this process was conducted weekly for each treatment group. For feed intake (FI), the daily feed intake of each chicken was measured and recorded to determine weekly feed intake. To calculate the feed conversion ratio (FCR), the total weight gain for each week was divided by the total amount of feed consumed during that week.

## Carcass characteristics assessment

At the end of the experimental trial, 10 birds from each replicate group, selected for their average weight, were deprived of food for 12 hours while still having access to water. After that, the birds were transported to a slaughter facility, where they were immediately bled by partially slicing the neck and cutting the carotid arteries with a manual neck cutter. To guarantee complete bleeding, the birds were hanged upside down. After that, the feathers were removed by hand pinning while the birds were submerged in hot water (51-55°C) for 2 minutes. For the purpose of calculating the carcass yield characteristics, the head, shank, viscera, giblet (heart, liver, and gizzard), and abdominal fat were removed. Dressed broilers were divided into different parts such as breast, thigh, drumstick, wing etc. Finally, each piece that had been cut up was then weighed and recorded as grams of slaughter weight.

## Blood parameters analysis

At the end of the experiment on day 21, blood samples from 10 randomly selected birds from each group were collected by vena subcutanea ulnaris puncture before slaughtering in sterile tubes without anticoagulant. Serum was extracted and kept at -20°C until analysis after centrifuging (3,000 × *g*, 10 min, room temperature), and clotting at room temperature for 1 hour. Cholesterol level was tested using the enzymatic colorimetric method by means of a Cholesterol Liquicolor kit (GmbH, Wiesbaden, Germany). Albumin, Globulin, and total protein levels were determined by appropriate commercial diagnostic kits for avian species (BioSystems, S.A. Barcelona, Spain, and GmbH, Wiesbaden, Germany). Calcium level was also tested using the enzymatic colorimetric method by means of a Calcium (CPC) Liquicolor kit (Stanbio Laboratory, L.P, Boerne, TX, USA) according to the manufacturer's instructions. Urea nitrogen was measured following the method described by Chawla et al. (2013); random blood sugar (RBS) was measured through Contour Plus One Blood Glucose Monitoring System (Square Glucometer, Bangladesh).

## Meat composition analysis

Approximately 100 g of meat from the thigh muscle of each slaughtered bird was collected for proximate analysis. The crude fat content of the meat was determined using the Soxhlet method (López-Bascón, 2019) and the protein content was measured using the Kjeldahl method (Owusu-Apenten, 2002). Crude fibre, ash and nitrogen free extract (NFE) was calculated following the method described by (Mishra et al., 2023). The press technique was used to determine the water holding capacity (WHC) in triplicates described by Tsai and Ockerma (1981). The proportion of bound water, or WHC, was computed as 100% less than the percentage of free water. Antioxidants (Total Phenolic Compound, Total Flavonoids) were quantified through spectrophotometric method, which measures the amount of light absorbed by a substance at specific wavelengths. The concentration of a solute in a solution by analyzing how much light is absorbed at a particular wavelength is directly related to the concentration according to the Beer-Lambert law.

## Statistical analysis

Data were analyzed using the General Linear Model (GLM) of SPSS statistical software version 26.0 for Windows (IBM, USA). To find differences between treatment means, Tukey pairwise comparisons were used, with a significance level set at $P<0.05$. The statistical model used was as follows: $Y_{ij} = \mu + \alpha_i + e_{ij}$; where $Y_{ij}$: Growth performance, carcass quality, blood parameters, and meat biochemistry; μ is the overall mean averaged over all treatments; $\alpha_i$ is the treatment effect; $e_{ij}$ is a random error associated with treatment and replicated within the treatment.

# RESULTS

## Growth performance

Table 2 illustrates the effects of antibiotics and varying concentrations of lyophilized probiotics added to the feed on growth performance. In case of body weight gain (BWG), each treatment group fed with antibiotics and probiotics outperformed the control group. However, when compared to control, there was no significant difference between T1 and T2. T3, on the other hand, had the best response (P<0.05) among the dietary treatments. Feed intake (FI) followed the same trend as BWG. In the case of feed conversion ratio (FCR), the antibiotic-fed group did not exhibit significant improvement when compared to the control group during the starter phase. T2 showed results comparable to those observed with T1. T3 had a lower FCR (P<0.05) than the other dietary treatments and control.

**Table 2** Growth performance analysis of broilers (21 days) of different dietary treatments

| Growth performance | Dietary Treatment$^{1}$ | | | | SEM | P |
|---|---|---|---|---|---|---|
| | Control | Treatment 1 (T1) | Treatment 2 (T2) | Treatment 3 (T3) | | |
| BWG, g | 593.70$^{b}$ | 681.91$^{a}$ | 692.73$^{a}$ | 707.67$^{c}$ | 21.65 | 0.03 |
| FI, g | 640.54$^{b}$ | 729.71$^{a}$ | 736.80$^{a}$ | 748.84$^{c}$ | 14.79 | 0.04 |
| FCR | 1.08$^{a}$ | 1.07$^{a}$ | 1.06$^{a}$ | 1.05$^{b}$ | 0.02 | 0.02 |

BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio; $^{1}$Data represents mean where n=3; $^{abc}$Within the same row, means with different superscripts are significantly different (P<0.05) by Tukey's test; SEM: Standard error mean.

## Carcass characteristics

Table 3 outlines the carcass characteristics of broiler chicks under different dietary treatments. For dressing weight, all treatment groups outperformed the control group, with both probiotic-fed groups showing significantly (P<0.05) higher weights than the antibiotic-fed group (T1). Notably, the higher dose probiotic group (T3) achieved significantly (P<0.05) better results than the lower dose probiotic group (T2). Regarding breast weight, T1 did not significantly differ from the control, while both T2 and T3 showed significantly (P<0.05) higher results, with the highest value observed in T3. For drumstick weight, all treatment groups surpassed the control group, with no significant difference between T1 and T2, whereas T3 showed a significantly (P<0.05) higher result. Head weight followed a similar pattern to drumstick weight, and neck weight mirrored the trend of dressing weight. Although abdominal fat, kidney, lung, liver, heart, and gizzard weights were numerically higher in the treatment groups—with probiotic groups outperforming the antibiotic group and T3 showing the highest values—no significant variations were observed among the treatment groups.

**Table 3** Carcass characteristics of broilers (21 days) of different dietary treatments

| Carcass yield, g | Dietary treatments$^{1}$ | | | | SEM | P |
|---|---|---|---|---|---|---|
| | Control | Treatment 1 (T1) | Treatment 2 (T2) | Treatment 3 (T3) | | |
| Dressing weight | 493.49$^{a}$ | 507.34$^{b}$ | 520.84$^{c}$ | 537.52$^{d}$ | 12.40 | 0.03 |
| Breast weight | 82.52$^{b}$ | 84.79$^{b}$ | 122.93$^{a}$ | 129.74$^{c}$ | 9.40 | 0.02 |
| Drumsticks weight | 83.63$^{a}$ | 116.91$^{b}$ | 117.75$^{b}$ | 123.25$^{c}$ | 9.02 | 0.02 |
| Wings weight | 27.88 | 32.12 | 31.06 | 31.14 | 0.92 | 0.19 |
| Abdominal fat weight | 5.67 | 5. 32 | 5.13 | 5.03 | 0.19 | 0.23 |
| Head weight | 2.11$^{c}$ | 2.23$^{a}$ | 2.32$^{a}$ | 2.38$^{b}$ | 0.05 | 0.04 |
| Neck weight | 4.12$^{d}$ | 4.20$^{a}$ | 4.35$^{b}$ | 4.42$^{c}$ | 0.06 | 0.01 |
| Kidney weight | 0.98$^{c}$ | 1.29$^{a}$ | 1.37$^{b}$ | 1.44$^{b}$ | 0.10 | 0.01 |
| Lung weight | 1.15 | 1.25 | 1.27 | 1.31 | 0.03 | 0.55 |
| Liver weight | 3.38 | 3.52 | 3.58 | 3.65 | 0.05 | 0.42 |
| Heart weight | 1.16 | 1.27 | 1.27 | 1.31 | 0.03 | 0.63 |
| Gizzard weight | 5.16 | 5.32 | 5.85 | 5.93 | 0.19 | 0.27 |

$^{1}$Data represents mean where n=10; $^{abcd}$Within the same row, means with different superscripts are significantly different (P<0.05) by Tukey's test; SEM: Standard error mean.

## Serum biochemistry

Table 4 lists the blood parameters of the broiler chicks. Compared to the control, total blood cholesterol was significantly lower (P<0.05) in the probiotic-supplemented groups. Additionally, there was a shift in the cholesterol fractions: HDL levels increased, while LDL and VLDL levels decreased. The antibiotic-fed group exhibited a similar pattern, but overall cholesterol levels were higher compared to the probiotic groups.

In compared to all other treatments and controls, the T1 group had significantly ($P<0.05$) higher mean plasma triglycerides. When compared to the birds who did not receive probiotics, the birds who got probiotics had significantly ($P<0.05$) lower mean total triglycerides while T3 group had the lowest ($P<0.05$) plasma triglyceride level. When it came to serum protein, the probiotic-fed group with the lower dose exhibited a significant ($P<0.05$) difference from the control group, while the higher dose of probiotics showed no significant change. However, the antibiotic-fed group (T1) outperformed the other groups by a substantial ($P<0.05$) margin. Serum albumin showed the similar pattern, with the greatest score ($P<0.05$) coming from probiotic therapy at a lower dose. T1 and T3 had clearly higher blood globulin levels, but there was no statistically significant difference between the groups. When it comes to the A/G ratio, T3 had the lowest and T1 had the greatest when compared to the control group. Antibiotics lower serum calcium levels when compared to the control, whereas those who were given probiotics had significantly ($P<0.05$) higher levels. However, T3 with a higher dose of probiotics had the highest ($P<0.05$) benefit. Regarding random blood sugar (RBS), the antibiotic-fed group exhibited a significantly ($P <0.05$) lower level compared to the control group. In contrast, both probiotic-fed groups showed significantly ($P <0.05$) higher levels, with T3 having the highest score, which was significantly ($P <0.05$) different from T2. For uric acid levels, T1 and T2 did not outperform the control group. However, T3 showed a significantly ($P <0.05$) reduced level of uric acid compared to both the control and antibiotic-fed groups.

**Table 4** Biochemical analysis of blood serum from 21-day-old broilers of different dietary treatments

| Parameters | Dietary Treatment[1] | | | | SEM | P |
|---|---|---|---|---|---|---|
| | Control | Treatment 1 (T1) | Treatment 2 (T2) | Treatment 3 (T3) | | |
| Total cholesterol, g/dL | 132.86$^{b}$ | 156.71$^{c}$ | 129.34$^{a}$ | 131.25$^{d}$ | 6.43 | 0.01 |
| HDL, g/dL | 69.73$^{a}$ | 72.40$^{a}$ | 75.61$^{b}$ | 82. 88$^{b}$ | 1.69 | 0.01 |
| LDL, g/dL | 40.79$^{a}$ | 61.61$^{b}$ | 34.63$^{a}$ | 31. 29$^{a}$ | 8.16 | 0.02 |
| VLDL, g/dL | 22.34$^{a}$ | 22.70$^{a}$ | 19.10$^{b}$ | 17.08$^{a}$ | 1.34 | 0.02 |
| Triglycerides, g/dL | 111.7$^{a}$ | 113.5$^{b}$ | 92.33$^{c}$ | 80.06$^{d}$ | 8.03 | 0.01 |
| Total protein, g/dL | 2.63$^{a}$ | 3.03$^{b}$ | 2.73$^{c}$ | 2.68$^{a}$ | 0.08 | 0.01 |
| Albumin, g/dL | 1.11$^{bc}$ | 1.47$^{a}$ | 1.21$^{bd}$ | 1.10$^{bc}$ | 0.08 | 0.03 |
| Globulin, g/dL | 1.52 | 1.56 | 1.52 | 1.58 | 0.01 | 0.56 |
| A/G ratio | 0.73:1 | 0.94:1 | 0.79:1 | 0.69:1 | | |
| Calcium, mg/dL | 11.44$^{a}$ | 10.38$^{b}$ | 11.55$^{a}$ | 11.79$^{c}$ | 0.31 | 0.01 |
| RBS mg/dl | 181.48$^{c}$ | 168.64$^{d}$ | 196.76$^{b}$ | 216.18$^{a}$ | 10.23 | 0.02 |
| Uric Acid mg/dl | 4.21$^{b}$ | 4.27$^{d}$ | 4.25$^{a}$ | 4.18$^{c}$ | 0.02 | 0.03 |

HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; A/G: Albumin/Globulin; RBS: Random blood sugar; [1]Data represents mean where n=10; $^{abcd}$ Within the same row, means with different superscripts are significantly different ($P<0.05$) by Tukey's test; SEM:  Standard error mean.

## Meat quality

Table 5 presents the proximate analyses of broiler meat from each experimental group. For total protein content, chicks given probiotics throughout their rearing period had significantly more ($P<0.05$) protein in their meat compared to other groups. T3 had the highest protein level, with a significant difference ($P<0.05$) from the antibiotic-fed group (T1). T2 had slightly higher protein levels than T1, but the difference was not statistically significant. The crude fat content in meat was lower in the probiotic-fed groups than in other groups, reaching statistical significance (P<0.05), though there was no discernible difference between T2 and T3. Regarding crude fibre, there was no significant difference among the treatment groups. For ash content, each treatment group showed a significant (P<0.05) difference compared to the control. T1 had significantly (P<0.05) higher ash content, while the probiotic-fed groups had significantly (P<0.05) lower ash content compared to the control, with T3 having the lowest value. The nitrogen-free extract (carbohydrate content) followed the same trend as the ash content. Under chemical analysis, each dietary treatment showed significant (P<0.05) variance compared to control considering drip loss. However, both probiotic fed groups significantly (P<0.05) outperformed than antibiotic fed group where T3 had significantly (P<0.05) lowest value. This pattern is also oppositely true for water holding capacity where T3 experienced the highest score. In case of cooking loss, both antibiotic and probiotic feed groups showed significantly (P<0.05) lower score than control where only T3 with higher dose of probiotics had significantly (P<0.05) lowest result than antibiotic fed group. When it comes to antioxidant content, no group outperformed than control except T3 which had significantly (P<0.05) higher result than control as well as antibiotic fed group considering total phenolic content. In case of total flavonoid compound, each dietary treatment had significantly (P<0.05) higher value than control. However, T2 did not exhibit significant (P<0.05) result comparing T1, in opposite, T3 had significantly (P<0.05) highest result.

**Table 5** Selective chemical composition of femoral muscles (skinless) from 21-day-old broilers of different dietary treatments

| Proximate analyses (%) | Dietary Treatment[1] | | | | SEM | P |
|---|---|---|---|---|---|---|
| | Control | Treatment 1 (T1) | Treatment 2 (T2) | Treatment 3 (T3) | | |
| Crude protein | 15.68$^{b}$ | 17.13$^{a}$ | 18.70$^{a}$ | 20.35$^{b}$ | 1.01 | 0.01 |
| Crude fat | 3.40b$^{c}$ | 5.87$^{b}$ | 4.98$^{a}$ | 4.56$^{a}$ | 0.51 | 0.01 |
| Crude fiber | 0.76 | 1.16 | 1.13 | 1.21 | 0.10 | 0.42 |
| Ash | 1.20$^{b}$ | 1.31$^{a}$ | 1.17$^{c}$ | 1.15$^{d}$ | 0.03 | 0.04 |
| NFE | 5.81$^{a}$ | 5.56$^{c}$ | 5.45$^{b}$ | 5.25$^{d}$ | 0.11 | 0.03 |
| Chemical analysis (%) | | | | | | |
| Drip loss | 8.43$^{a}$ | 7.38$^{c}$ | 6.52$^{b}$ | 6.27$^{b}$ | 0.48 | 0.04 |
| Cooking loss | 41.56$^{a}$ | 37.25$^{c}$ | 36.60$^{c}$ | 35.89$^{b}$ | 1.27 | 0.02 |
| WHC | 70.33$^{c}$ | 80.39$^{a}$ | 86.40$^{b}$ | 89.73$^{d}$ | 4.25 | 0.01 |
| Antioxidant | | | | | | |
| Total Phenolic (mg GAE/100g sample) | 77.28$^{b}$ | 64.24$^{d}$ | 76.44$^{c}$ | 119.28$^{a}$ | 8.42 | 0.01 |
| Total Flavonoids (mg QE/100g sample) | 77.39$^{c}$ | 82.24$^{b}$ | 81.60$^{b}$ | 86.59$^{a}$ | 1.88 | 0.01 |

NFE: Nitrogen free extract; WHC: Water holding capacity; [1]Data represents mean where n=10; $^{abcd}$Within the same row, means with different superscripts are significantly different (P<0.05) by Tukey's test; SEM: Standard error mean.

# DISCUSSION

Measuring parameters like body weight gain, average daily feed intake, and feed conversion ratio are important for evaluating the growth rate of an animal (FAO, 2023). These measurements were used to assess the weight gain of the birds, their feed conversion efficiency, and their daily feed consumption. In agreement with our results, some study found that probiotic supplementation improved BWG, FI, and FCR in broiler chicks when compared to the control and antibiotic treatment, but the researchers stated that at the maximum dosage of supplementation, the effects were more apparent (Anjum et al., 2005; Mehr et al., 2007). In contrast, according to Argañaraz Martínez et al. (2016) and Fathi et al.

(2017), probiotic supplementation has no influence on broiler growth performance. According to Mountzouris et al. (2010) findings make it impossible to draw any firm conclusions about how increasing probiotic administration level affects growth performance. They also suggested that each of the probiotics tested may have an ideal concentration that varies depending on the strain. However, it has been shown that most probiotics' efficiency in birds is dosage dependent (Mountzouris et al., 2010; Shim et al., 2012), which could explain why the T2 treatment did not improve performance attributes when compared to the T3. On the other hand, most of the above-mentioned studies used chicks kept in cages or did not define the rearing strategy, whereas the current study was done on the floor. The raising system may have an impact on the observed yields. Furthermore, because to changes in hygienic conditions, the benefits of broiler feed supplementation with probiotics may vary depending on the raising system (Pirgozliev et al., 2014). As a result, for a fuller interpretation of experimental findings from probiotic supplementation effects studies, rearing conditions should be included.

When it comes to carcass yield, there are some contradictory outcomes. Probiotic supplementation improves carcass status significantly, according Mehr et al. (2007) and Olnood et al. (2015). On the other hand, Awad et al. (2009) showed no differences in the percentage of carcass between a control and a probiotic supplemented treatment in their experiments. Following the same trend, our current study revealed significant increase in carcass, breast and drumstick yields. When compared to controls, probiotic supplemented diets have been found by some researchers to have no effects on abdominal fat, heart, liver, lung, kidney and wings yield in broilers, which is consistent with the study's findings (Shabani et al., 2012; Rehman et al., 2020). However, other researchers have discovered that probiotic supplemented diets increase abdominal fat weight in broilers (Anjum et al., 2005; Awad et al., 2009). These findings suggest that the efficiency of probiotics may be influenced by factors such as chicken type and age, the strain of probiotic organisms used, duration of supplementation as well as the environmental and management conditions (housing conditions, stress levels, biosecurity measures).

The results of the current study align with those reported by El-Baky (2013), which showed a decrease in cholesterol levels in broilers given probiotics during the starter phase. Mansoub (2010) and Panda et al. (2006) found that probiotic administration boosted blood HDL levels while decreasing serum LDL levels. However, some findings disagree with the study which reported that probiotic supplementation had no effect on blood HDL and LDL concentrations in broiler chicks (Ashayerizadeh et al., 2011). Although the mechanisms are unknown, it is thought that certain probiotic strains may incorporate cholesterol into their cells, favorably altering bird lipoprotein metabolism by breaking down the bile salts or inhibiting hydroxyme thylglutaryl-CoA, a rate-limiting enzyme in cholesterogenesis, and thus lowering the body's cholesterol pool (Kalavathy et al., 2003; Yazhini et al., 2018). The breakdown of food components and the absorption of fatty acids produce triglycerides in the intestinal mucosa and liver. Serum triglyceride levels in poultry, however, are affected by sex, diet, and hormonal factor (Rezende et al., 2017). Some research found significant drop in serum triglycerides level in broiler chickens on probiotic supplementation which is similar to the current study (Al-Saad et al., 2014; Abeer and Mosaad, 2015). When it came to serum protein, the probiotic-fed group with the lower dose exhibited a significant difference from the control group. Serum proteins are mainly produced in the liver and have a number of functions, such as regulating metabolism, carrying hormones and medications, assisting in cell coagulation, preserving blood volume via the colloidal osmotic effect, buffering blood pH, and supporting the body's defence mechanisms against foreign invaders (Melillo, 2013). It is theorized that probiotics can outcompete harmful microbes in the body, reducing the breakdown of proteins into nitrogen compounds. This, in turn, could improve the absorption and utilization of amino acids and proteins from the diet (Peng et al., 2019). Another interesting findings was higher globulin level and a lower A/G ratio which have been used as indications of immunological response, a source of antibody production, and disease resistance (Lewis, 2003). The findings of this study is aligned with a work which found that probiotic treatment enhanced plasma protein levels (Yazhini et al., 2018). The results of this investigation, however, did not correspond with some research which found that the serum total protein concentration of probiotic-supplemented birds was substantially ($P<0.05$) lower than that of control birds (Abdel-Hafeez et al., 2017; Li et al., 2014). Ciprofloxacin, commonly used in the poultry industry, is a strong chelating agent. When ciprofloxacin chelates with dietary metals, it can reduce the bioavailability of metal ion such as calcium, magnesium, or iron (Hasan et al., 2011). Calcium, on the other hand, has been shown to boost growth performance, carcass features, meat quality, activities of immunological and digestive enzymes, as well as inhibiting harmful bacteria while increasing beneficial bacteria (Xing et al., 2020). The finding of this study is closer to the finding of certain study which found significant differences in serum calcium levels between probiotic supplemented treatments and controls (Panda et al., 2006). The increase in calcium levels in blood serum caused by the addition of probiotics could be attributed to the organic acids from probiotics lowering the PH in the gastrointestinal system, hence improving mineral absorption from the gastrointestinal tract into the bloodstream (Dousa et al., 2013). Studies have shown that the use of antibiotics can lead to microbial dysbiosis in the gut, which can lead to altered production of short-chain fatty acids (SCFAs), which play a role in glucose regulation (Dipankar and Taslim, 2022). Besides antibiotics can induce stress and an immune response, potentially impacting metabolic processes and glucose levels (Fujisaka et al., 2016). In opposite, research suggests that probiotics can positively influence glucose metabolism by inhibiting harmful bacteria, reducing the chances of inflammation as well as modulating the immune system, potentially leading to reduced stress and better regulation of metabolic functions (Isolauri et al., 2002). This explains the reasons why probiotics showed significant results than control and antibiotic in our current study. This elevated blood sugar levels but in normal range comparing antibiotic can provide a buffer against stress. When broilers encounter stressors (like temperature changes, handling, or transport), having higher glucose reserves can help sustain energy levels and reduce the impact on growth and health (Humam et al., 2019). In this study, probiotic fed group showed significantly reduce level of uric acid than antibiotic treated group. Studies indicate that antibiotics increase uric acid levels in broilers by disrupting gut microbiota and protein metabolism, potentially leading to inefficient nitrogen utilization and stress on the kidneys whereas probiotics generally support lower or stable uric acid levels by enhancing gut health, improving nutrient absorption, and supporting metabolic and excretory functions (Rahman et al., 2014).

Suryadi et al. (2019) discovered that supplementing feed with probiotics significantly increased meat protein content while lowering fat content in broiler meat which agree with our current study. This can be explained in a way that probiotic could enhance nutrient absorption and improve gut health, leading to more efficient digestion and utilization of dietary proteins which means that broilers can better convert the protein in their diet into muscle mass, thus increasing the protein content in their meat (Wang and Ji, 2018). Additionally, probiotics help balance the gut microbiota, which can enhance the metabolic processes related to protein synthesis and fat metabolism, thereby lowering the fat content in muscle (Ghasemi et al., 2016). Broiler meat from probiotic fed group had lower ash content than antibiotic fed group in our study. This can be explained in a way that probiotics can enhance the overall nutrient absorption and metabolism, leading to better utilization of minerals in the diet. This can potentially result in more balanced mineral levels in the meat, but it does not typically reduce ash content (Skrypnik and Suliburska, 2018). Thus, probiotics ensure the appropriate mineral content is maintained, contributing to optimal health and meat quality. Nitrogen free extract or carbohydrate content also followed the same trend that of ash content in this study which is comparable to the result of a recent study (Omara, 2012). Probiotics can enhance the breakdown and absorption of nutrients, including carbohydrates, by maintaining a balanced gut microbiome. This can lead to a more efficient use of dietary carbohydrates for energy, reducing the likelihood of carbohydrates being stored as glycogen in muscles (Kashmir, 2005). Drip loss refers to the amount of fluid that leaks out of meat during storage and cooking loss is the reduction in weight of meat during cooking due to the evaporation of water and the melting of fat (Mir et al., 2017). Probiotics showed reduced drip loss and cooking loss with increased water holding capacity than antibiotics in this study which collectively contribute to better meat quality, texture, and economic value. These results are aligned with some recent research which also explain why probiotics are better than antibiotics in this case. By reducing inflammation and immune activation, probiotics decrease the muscle degradation processes that can lead to higher drip and cooking losses (Popova, 2017). Besides, probiotics enhance the integrity of muscle cell membranes by promoting the synthesis of phospholipids and maintaining cellular functions which reduce the leakage of intracellular fluids (Cramer et al., 2018). Considering antioxidant content, higher phenolic compounds can help reduce oxidative stress, contributing to better health outcomes by lowering the risk of chronic infections and improving the oxidative stability of the meat, which helps it stay fresh longer and resist rancidity (Reddy et al., 2018). In addition, higher flavonoid levels suggest better anti-inflammatory, antiviral, and antioxidant properties, with improved colour stability of the meat (Manessis et al., 2020). This is particularly true for this study where probiotic showed significantly increased level but in normal range in case of both phenolic and flavonoid compound comparing control and antibiotic.

## CONCLUSIONS

The main goal of this study was to investigate using lyophilized (freeze-dried) probiotics as an alternative to harmful antibiotics in chicken feed for broiler chickens. The findings showed that probiotics in broiler chick diets can promote growth rate, feed intake, feed conversion ratio, and carcass content while also improving broiler meat quality by raising protein content and antioxidant properties with decreasing fat content. Probiotic-based broiler chick feeds reduced serum cholesterol and triglyceride levels but had little effect on decreasing LDL and increasing HDL levels. Lyophilized native probiotics in broiler chick feeds had significant effects on boosting globulin levels and decreasing A/G ratio, indicating that probiotics may have a favorable influence on immunological response and disease resistance. Overall, this research supports the use of probiotic-supplemented feed as a way to reduce uncontrolled antibiotic use while enabling more sustainable and healthier broiler chicken production practices.

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# AUTHOR CONTRIBUTIONS

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

# CONFLICT OF 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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