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RESEARCH

Hematological, Histological, and NMR Analysis of Meat Quality and Growth Performance of Broilers Fed with Probiotics *Bacillus licheniformis*-Supplemented Poultry Feed

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Abstract

The present study evaluated the effect of probiotics (*Bacillus licheniformis*) on the growth pattern, hematological parameters, metabolic profile, and meat quality of broiler birds. Total of 75 Ross 308 broiler chicks were divided into three groups (25 broilers in each) and fed with poultry feed supplemented with probiotics (15 g/ton of feed for starter diet and 35 g/ton for grower diet), antibiotic (amoxicillin 900 mg/ton of feed) and basal poultry feed, respectively, for 5 weeks. The broilers of control group exhibited higher feed conversion rate (FCR), i.e., 1.932 ± 0.10 followed by the FCR of antibiotic group (1.84 ± 0.10) and probiotic (1.714 ± 0.10) group broilers. It indicated that the chicks fed with probiotic rich feed gained more weight while ingesting comparatively less quantity of feed. The difference in weight of body organs (heart, liver, spleen, and gallbladder) among all groups showed no significant change ($p > 0.05$). However, significant difference of intestinal length was observed; control group with longest intestine (213.5 ± 15.22 cm) and probiotic group with shortest intestine of length 194 ± 5.51 cm. Moreover, probiotics group showed an improved temperature (30.75 ± 1.75 °C) and pH (6.05 ± 0.05) in meat after 20 min of slaughter. The breast muscles of probiotic group chickens exhibited greater cooking loss, yellowness (b^*) (13.15 ± 2.23) and lightness (L^*) (53.9 ± 1.6) with reduced redness (a^*) (12.305 ± 1.35) and shear force (17.265 ± 0.82) compared to the control and antibiotic-treated group. The histological examination of probiotics group chickens pectoral muscles indicated significant ($p < 0.05$) increase in fascicle diameter (654.08 ± 5.55 µm) and fiber density (29.26 ± 0.65 n/µm) of meat compared to the antibiotics and control group. Probiotic-treated chickens showed improved intestinal health while the blood glucose level of the probiotics group chicken was reported to be higher. Metabolic analysis of leg and breast meat of chickens fed with probiotics supplemented feed with 600 MHz 1H-NMR spectra revealed 23 identified metabolites, of which seven metabolites (carnitine, phenylalanine, histidine, O-acetylcarnitine, omega 3 fatty acid, uridine, lactate) were only reported in probiotics group. The findings suggest that *B. licheniformis* enhances growth, carcass yield, organ weights, meat quality, and overall health of broiler birds. Consequently, *Bacillus licheniformis* can be utilized as a substitute growth promoter instead of antibiotics.

Keywords Probiotics · *Bacillus licheniformis* · NMR analysis · Broiler · Meat quality

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Introduction

Broiler chickens constitute a valuable source of high-quality protein for human nutrition. In poultry farms, these broilers are reared specifically for meat production because of its tender meat with low fats and shorter cooking period [1]. The poultry industry is the second-largest sector of livestock, making a significant contribution of 1.3% to Pakistan's GDP [2]. Pakistan ranks 11th in the world for poultry production, and poultry products accounts for almost 40 to 45% of the total meat requirement in Pakistan

[3]. Despite being a profitable venture, commercial poultry farming also faces challenges, with infectious and non-infectious diseases being a major concern. Traditionally, antibiotics have been extensively utilized as antimicrobial feed additives, in conventional broiler production, to enhance animal health and growth performance [4].

Like all other industries, poultry industry also requires maximum production with minimum input, and the largest expenditure (70%) of the poultry industry is poultry feed. A constant rise in the cost of poultry feed and its components can pose serious effect on the profit margin of poultry industry, therefore, a balanced poultry fees is an important requisite for economic poultry production. Several hormones and antibiotics have been used as synthetic feed additives in poultry feed. In 1951, FDA approved the utilization of certain antibiotics in poultry feed to prevent several bacterial and viral infections [5]. However, development of antibiotic resistance in chicken gut microbiota and accumulation of antibiotics residues in meat and eggs causing serious health issues for consumers. The use of low doses of antibiotic in poultry feed increases gut health of the chicken and prevents it from subclinical infections by reducing the pathogen load [6, 7]. Besides this, antibiotics also thickens the gut, improving nutrients absorption. However, excessive use of antibiotics has potential to disturb the normal microflora of chicken gut, impregnate antibiotic residues in muscles and also develop new drug resistant strains by plasmid-mediated transfer [8]. On consuming the meat and eggs from such broilers, exposes the human population with drug resistant strains posing a serious threat to health. Keeping in view of all these circumstances, antibiotic supplementation in poultry feed was banned in 2005 by European Union Commission. Several other countries are also considering the ban of antibiotics in poultry feed, and hence, there is a huge scope of discovering alternatives to antibiotics [9, 10].

Probiotics are the microbial cultures that facilitates the normal microflora of gut, when ingested [11]. Probiotics serve as a nutritional supplement in poultry feed to enhance broiler growth rates. They promote meat quality by modulating the intestinal microflora and preventing the occurrence of infections. Probiotics provide different enzymes such as cellulose, phytase, and xylanase in the gut of chicken which transforms less degradable compounds to degradable. Consequently, the population of normal gut microbiota changes leading to the change in immunity of chicken. When broiler feed is supplemented with probiotics, its nutritional price increases due to the production of epoxy saccharides and antioxidants along with the regular nutrient value. Probiotics enhance the activity of normal gut flora, improves the function of digestive enzymes, improves humoral and cell-mediated immunity, and neutralises a variety of enterotoxins [12]. Research indicates that specific probiotics include *Bacillus* spp., *Bifidobacterium* spp., *Lactobacillus* spp., *Streptococcus* spp., *Lactococcus* spp., and *Candida* spp. [13, 14].

The proposed mechanisms of probiotic action in chickens involve maintaining a beneficial microbial population through competitive exclusion and microbial antagonism, enhancing the feed intake and digestion, and altering the metabolism of bacteria. However, there is a lack of comprehensive data on the direct-fed microbial effects on the histomorphology of broiler chickens' small intestines, as noted by Awad et al. (2009) [15]. Nowadays, probiotics have developed into one of potential substitutes for antibiotics, and their application in livestock and poultry diets has become a research hotspot. In this study, the effect of *Bacillus licheniformis* as a probiotic supplementation in broiler feed was evaluated on the basis of broiler's growth performance (FCR, body weight, feed intake, weight increase, and mortality rate), hematology, meat quality (on the basis of NMR analysis), and inner organs weight compared to the antibiotic and control group in 35-day long experimental trial.

Materials and Methods

Poultry Feed Preparation

Probiotics, *Bacillus licheniformis* KT443923 pure culture was obtained from Dr. Ikram-ul-Haq Institute of Industrial Biotechnology at GC University Lahore, while the poultry feed was obtained from Hi-Tech Feeds (Pvt) Ltd., Pakistan. Table 1 provides the information for preparation of the probiotic poultry feed (PPF) by supplementing the feed with probiotics and categorized as starter diet (with 15 g/ton probiotic culture) and grower diet (with 35 g/ton probiotic culture), while maintaining the quantity of probiotic at 5.6×10^9 cells/g [16]. On the other hand, antibiotic poultry feed (APF) was prepared by adding 900 mg/ton of amoxicillin in poultry feed. The selected chickens were fed with starter diet for the first 2 weeks and then with grower feed until fifth week, the completion of trial.

Experimental Trial

For experimental trial, 75 healthy Ross 308 broiler chickens (1 day old) were selected and purchased from Hi-Tech hatchery. The chickens were divided equally in three treatment groups, i.e., control group (fed with poultry feed without probiotics and antibiotics), antibiotics group (fed with poultry feed supplemented with amoxicillin) and probiotic group (fed with poultry feed supplemented with probiotic). The chicks in different groups were fed for 35 days. Each group was further subdivided into three sub-groups each with 5 birds. In probiotic group, chickens were fed on PPF with

Table 1 Composition and nutritional value of control feed probiotic poultry feed and antibiotic poultry feed

Feed type		Composition	Nutritional value
Control Feed	Starter diet	Standard	MC = 10–12%, CP = 21–22%, CF = 3–5%, CFib = 3–6%, AC = 5–8%, NFE = 50–60%, energy content = 2800–32000 kcal/kg Minerals = calcium (0.9–1%), phosphorus (0.45–0.55%), sodium (0.15–0.2%), potassium (0.8–0.9), and magnesium (0.05–0.1%) Amino acid = lysine (1.2–1.4%), methionine (0.4–0.5%), threonine (0.8–0.9%)
	Grower diet	Standard	MC = 10–12%, CP = 18–20%, CF = 4–6%, CFib = 3–5%, AC = 5–8%, NFE = 55–60%, energy content = 2900–33000 kcal/kg Minerals = calcium (0.8–0.9%), phosphorus (0.4–0.5%), sodium (0.15–0.18%), potassium (0.75–0.85%), and magnesium (0.04–0.08%) Amino acid = lysine (1.1–1.3%), methionine (0.35–0.45%), threonine (0.75–0.85%)
Probiotic poultry feed (PPF)	Starter diet	Standard feed supplemented with *Bacillus licheniformis* KT443923 15 g/ton of feed	MC = 10–12%, CP = 21–22%, CF = 3–5%, CFib = 3–6%, AC = 5–8%, NFE = 50–60%, energy content = 2800–32000 kcal/kg Minerals = calcium (0.9–1%), phosphorus (0.45–0.55%), sodium (0.15–0.2%), potassium (0.8–0.9), and magnesium (0.05–0.1%) Amino acid = lysine (1.2–1.4%), methionine (0.4–0.5%), threonine (0.8–0.9%)
	Grower diet	Standard feed supplemented with *Bacillus licheniformis* KT443923 35 g/ton of feed	MC = 10–12%, CP = 18–20%, CF = 4–6%, CFib = 3–5%, AC = 5–8%, NFE = 55–60%, energy content = 2900–33000 kcal/kg Minerals = calcium (0.8–0.9%), phosphorus (0.4–0.5%), sodium (0.15–0.18%), potassium (0.75–0.85%), and magnesium (0.04–0.08%) Amino acid = lysine (1.1–1.3%), methionine (0.35–0.45%), threonine (0.75–0.85%)
Antibiotic poultry feed (APF)	Starter diet	Standard feed supplemented with amoxicillin 900 mg/ton of feed	MC = 10–12%, CP = 21–22%, CF = 3–5%, CFib = 3–6%, AC = 5–8%, NFE = 50–60%, energy content = 2800–32000 kcal/kg Minerals = calcium (0.9–1%), phosphorus (0.45–0.55%), sodium (0.15–0.2%), potassium (0.8–0.9), and magnesium (0.05–0.1%) Amino acid = lysine (1.2–1.4%), methionine (0.4–0.5%), threonine (0.8–0.9%)
	Grower diet	Standard feed supplemented with amoxicillin 900 mg/ton of feed	MC = 10–12%, CP = 18–20%, CF = 4–6%, CFib = 3–5%, AC = 5–8%, NFE = 55–60%, Energy content = 2900–33000 kcal/kg Minerals = calcium (0.8–0.9%), phosphorus (0.4–0.5%), sodium (0.15–0.18%), potassium (0.75–0.85%), and magnesium (0.04–0.08%) Amino acid = lysine (1.1–1.3%), methionine (0.35–0.45%), threonine (0.75–0.85%)

*Here *MC*, moisture content; *CP*, crude protein; *CF*, crude fat; *CFib*, crude fiber; *AC*, ash content; *NFE*, nitrogen-free extract

5.6×10^6 cell/gm probiotics (*B. licheniformis* KT443923) per ton feed, the antibiotic group chickens were fed on APF with 900 mg amoxicillin antibiotic per ton feed, and the control group chickens were fed with basal diet with no antibiotic or probiotics. The poultry group chicken were fed with starter diet in the first 2 weeks and followed by the grower diet until the end of experimental trials.

A controlled shed house was used for experimental purpose. The area of the room was 500 square foot. 09 pens of equal size were constructed in the room. Area of each pen was 25 square feet (5ft x 5 ft). The experimental room was

thoroughly brushed, swiped, and properly washed by water. Afterwards bleaching powder @ 1 kg/500 square foot was spread over the floor and was kept for 24 h. The bleaching powder was cleaned by using forced tap water. The room was then disinfected by TH4 + solution. Fresh and dry rice husks were spread on the floor of the pens as a litter material [17].

The chickens were kept and fed in the shed house with maintained relative humidity ranging between 40 and 60%. The temperature of shed house was maintained at 32 °C on the first day with gradual reduction in temperature until it reached 24 °C in the second week and then maintained at 24 °C until the completion of experimental trial. The chickens were vaccinated against Newcastle disease on the 7th day. The birds were kept under daily management practices in a hygienic environment until the end of the experiment. The feed was provided on a regular basis throughout the experimental trial.

Broilers Growth Performance

The broilers performance, during the experimental trials, was determined via average body weight gain, feed intake, feed conservation ratio, and mortality rate.

Weight Gain

For weight gain measurements, the body weight of chickens was first recorded before feeding and then regularly throughout the experiment. The final body weight gain was determined by the difference of first day weight and final day weight of chickens.

$$Average\ weight\ gain = average\ final\ body\ weight - average\ initial\ body\ weight$$

Feed Consymption

Feed consumption was also regularly evaluated throughout the experimental period. For this, the difference between the total grams of feed provided to the birds and the total grams of feed left behind in each feeding period was calculated.

Feed Conversion Ratio

Feed conversion ratio (FCR) was determined based on feed intake that resulted in the weight gain of chicks. If FCR is higher it means that the chicks intake more feed to achieve weight gain while lower FCR means the chick's take less feeds to gain more body weight.

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ consumption\ (g)}{Body\ weight\ gain\ (g)}$$

Mortality Rate

The mortality of broiler birds refers to the total broilers died, in respective group, during the whole duration of the study. It was calculated as the ratio of number of birds died to the total number of birds in the trial multiplying it with 100.

$$Mortality\ charge = \frac{Number\ of\ birds\ died}{Total\ birds\ range\ in\ trial} \times 100$$

All growth factors, notably FCR, feed intake, and chickens body weight, were determined.

Slaughtering of Broilers

After the completion of trial, five chicks from each group were slaughtered, and their pectoral muscles were analyzed for serological and histological characteristics. After slaughtering, birds were allowed to bleed for 2 min and immersed in hot water (51–55 °C) for 120 s in order to loose the feathers and known as semi-scalding procedure. The feathers were removed by hand pinning manually. Then, head, shank, viscera, giblet (heart, liver, and gizzard), and abdominal fat were removed for determination of meat yield parameters. Dressed broilers were cut into different parts such as breast, thigh, drumstick, wing, and back. Finally, every cut up parts were weighed and recorded for broiler of all replicates. For serological investigations, sterile syringes were used to take blood from the wing vein of birds and was transferred in separate blood collection tubes. Moreover, the vital organs of slaughtered chicks, i.e., heart, liver, gall bladder, spleen, and intestine were collected in sterile falcon tubes. All the samples were transported to the University of Veterinary and Animal Science Lahore, Pakistan while maintaining lower temperature via ice kits in sample container. The histopathological and haematological parameter of samples were performed. All vital organs of the birds in each group were weighed and compared. The length and weight of the intestines were also measured.

Hematological Parameters

Venous blood of birds was collected in EDTA tubes to prevent blood clotting. Hematology of blood samples was performed manually, as previously described by [17]. The hematological parameters included WBC count, RBC count, platelet count, hemoglobin, and hematocrip. Cell counting was done using hemocytometer while hematocrit was measured, after the centrifugation of blood, in heparinized capillary tubes. Hemoglobin levels was evaluated using Sahli's approach. For serum separation, blood samples in non-EDTA tubes were allowed to stand for an hour that

resulted in the formation of clots, followed by centrifugation at 3,000 rpm for 20 min. The obtained serum was preserved at − 20 °C. Furthermore, the biochemical parameters such as alanine aminotransferase (ALT), creatinine, serum aspartate aminotransferase (AST), and uric acid were evaluated via commercial kits.

Internal Organs and Carcass Weight

In order to determine relative weight of organs, the slaughtered chicks were briefly exposed to the temperature of 50–60 °C for 30 s, followed by feather plucking and eviscerated in a tank. The intestines were then extracted through the vent. The vital organs of chickens were harvested and calculated as body weight percentage. For the carcass weight determination, birds were slaughtered via cervical dislocation. Carcass weight measurements were carried out after de-feathering and removal of feet, head, and viscera. The dressing percent was calculated by dividing the carcass weight by live body weight.

$$Yield\ or\ dressing\ percentage = \frac{Hot\ carcass\ weight}{Live\ body\ weight} \times 100$$

Evaluation of Meat Quality

The meat quality was evaluated on the basis of pH and temperature parameters, specifically in the major pectoral muscles of broiler breast. For this purpose, preliminary pH and temperature of breast meat was noted after 20 min of slaughtering. The pH of meat was determined by penetrating the tip of pH meter in a 1 cm incision of the muscular tissues [18]. Minolta Colorimeter was further used to accurately determine the meat color which captures multiple light wavelengths at same spot. It indicates $L*$ value as a measure of lightness, $a*$ as a measure of green to red color, and $b*$ as a measure of blue to yellow color. Higher values indicate the intensity of specific color.

Histological Evaluation of Meat

Muscle histomorphometry was conducted via the technique described by [19]. An AEM-450 semiautomatic rotary microtome was used to process and section the samples up to 5 µm, followed by their staining with hematoxylin and eosin (H&E) stain. The stained sections of muscle tissues were observed under × 4 and × 10 objective lens (total magnification of × 40 and × 100, respectively) to locate the muscle fibers in the per unit count area and determine muscle fibers diameter. Muscle fibers per mm^2 was calculated using the mathematical formula: muscle fibers per $mm^2 = (1/r^2)*$ muscle fiber counted in a 0.5-mm radius circle.

Histological Examination of Intestine

The histological examination of different sections of chicken's intestine (duodenum, jejunum, and ileum) was carried out after proper cleaning with saline. About 1 cm long sections were cut from the middle of duodenum, jejunum, and ileum, respectively, washed with normal saline and placed in 10% formalin fixative for further examination. Intestinal segments were prepared via continuous embedding of paraffin sectioning (Amos Scientific AEM-450, Austria) to get a 5-µm section, and H&E staining. The villus height, villus width, and crypt depth of small intestine sections stained with hematoxylin and eosin (H&E) were identified. A total of 10 villi per sample were measured using light microscope with some modifications.A villus height ratio crypt depth (VH:CD) were estimated as well. Villi were chosen in one step completely based on unbroken lamina propria, and all observations were made on five well-oriented villus crypt devices. The crypt depth was measured from foundation to base, and the width of the villus was measured from the villus's center. All morphometric parameter measurements were made in micrometers (µm). Microscope (Labomed, Lx 400, Fremont, Calif, 9453, USA) was used for slide imaging. Villi surface were calculated as per [20].

NMR Analysis of Meat

Meat samples were processed for NMR analysis. Using liquid nitrogen, all frozen meat samples were crushed one by one into quality powder using a mortar and pestle. Then, 0.12 g (120 mg) of powdered meat from each sample was weighed and kept in sterile Eppendorf tubes. 0.5 mL (500 µL) ice cold perchloric acid was added into the tube and vortex for 30 s. For 10 min, the samples were incubated in the ice box followed by centrifugation of samples at 12,000 rpm at 4 °C for 15 min to obtain the supernatant. During the process, K_2CO_3 was used to maintain the pH in range of 7 to 8. All the samples were again incubated on ice for 30 min and centrifuged at 12,000 rpm. The supernatant was collected and pellet of $KClO_4$ precipitates was discarded. Finally, the supernatants of samples were lyophilized into a fine powder and stored at − 80 °C for further NMR evaluation. A solution of 50 µl trimethylsilylpropanoic solution (TSP) in 500 µl D2O (chemical shift standard solution) was used to dissolve the lyophilized samples while adjusting the pH 7.4 for NMR spectroscopy. The collection of 1H-NMR data was conducted using a 600 MHz (Advance neo) NMR

spectrometer. The studies were carried out using Bruker's pulse utility "ZG" without solvent suppression with 128 scans and 64 K information elements. At the same time as processing, line broadening (lb = 0.3) is employed, but no filling is given.

Statistical Analysis

All spectrum captured was evaluated using the MestReNova (14.1.2) software. One-way ANOVA was applied on values of each set and p value of less than 0.05 was considered statistically significant. On each spectrum, phase correction and post-analytical correction were conducted manually prior to identification and targeted profiling. According to accessible databases such as HMDB or already publications, each peak was assigned to a metabolite. An in-house standard database and published literature were used to assign signals and identify metabolites. For the identification of metabolites, spectral libraries such as Human Metabonome Database (HMDB) and already published literature was consulted.

Results

In this study, the effect of *Bacillus licheniformis* as a probiotic supplementation in broiler feed was evaluated on the basis of broiler's growth performance (FCR, body weight, feed intake, weight increase, and mortality rate), hematology, meat quality, and inner organs weight in comparison to the antibiotic and control group in 35-day long experimental trial.

Broiler's Growth Performance

The bodyweight of the broiler birds of each group was measured on weekly basis. Initially, the body weight was almost same for all groups. As the trial proceeded the bodyweight of broilers in antibiotic group were recorded as higher, in first 2 weeks, compared to the other two groups as shown in Fig. 1. Then, the average body weight of birds in week 3 was 901 ± 47.29 g for control group, 930 ± 35.25 g for the antibiotic group, and 965.4 ± 43 g for probiotics group. The average body weight of probiotics groups was higher (2002.6 ± 84.12 g) compared to the antibiotic group (1960 ± 21.10 g) and control group chickens (1823.3 ± 62.35 g). The data suggested that birds in probiotic group gained more body weight compared to antibiotic and control group birds. Thus, the probiotic supplementation remained more efficient than supplementation of antibiotic in poultry feed.

For the measurements of body weight gain, the final body weight of chicks was deducted from the bodyweight measured in the previous week of trial. The results indicated that the body weight gain in chicks was comparatively higher in antibiotics group (198.22 ± 11.94 g, 282.6.8 ± 2.41 g) during the first and second week of trial compared to that of probiotics (185.6 ± 10.36 g, 257.6 ± 8.63 g) and control group (134.6 ± 5.59 g, 237.2 ± 8.59 g). After third, fourth, and fifth week, the body weight rise of probiotics showed significant results compared to the other groups. Thus, the overall body weight gain of birds in probiotic group (1950.4 ± 32.74 g) was higher compared to antibiotic (1906.2 ± 29.58 g) and control group (1772.2 ± 32.19 g) as shown in Fig. 2. Hence a significant increase in percentage of body weight gain was

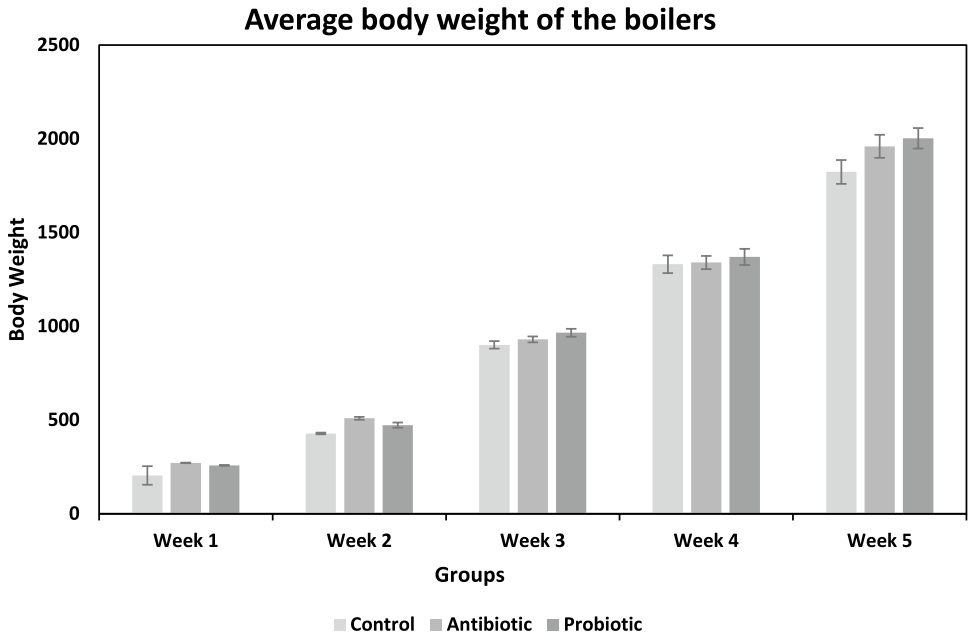


Fig. 1 Average body weight per week of the boilers among different treatment groups. *The y- bars represent the standard deviation (± SEM) of *body weight* across in five parallel replicates

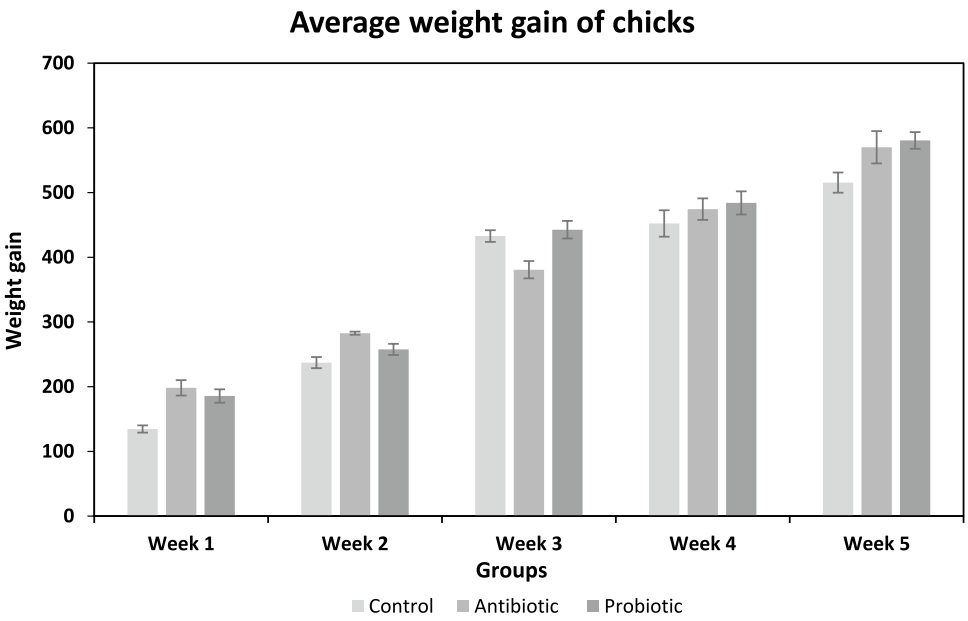


Fig. 2 Average body weight gain per week of the among different treatment groups. *The y- bars represent the standard deviation (± SEM) of *average body weight gain* across in five parallel replicates

Table 2 Percentage increase in broiler's body weights among different treatment groups

Parameters	Antibiotic to control group	Probiotic to antibiotic group	Probiotic to control group
%Age	7.51%	2.10%	9.80%

observed in probiotics (9.80%) and antibiotics group (7.51%) compared to the control group (Table 2). However, while comparing the two treatments groups, the probiotic group proves to be more efficient than the antibiotic, resulting in a 2.10% increase in weight gain, determined by the formula.

$$Percent\ increase = \frac{New\ value - Original\ value}{Original\ value}(100)$$

The difference of feed intake among the groups was also recorded on weekly basis throughout the trial. The feed intake of the broilers in antibiotic group was higher in the first and second week than that of the control and probiotic groups; however, in the third, fourth, and fifth weeks, the control group's feed intake (786.66 ± 4.81 g, 929.3 ± 9.60 g, and 1138.23 ± 6.91 g) was higher than that of probiotic (741.28 ± 6.29 g, 837.3 ± 8.09 g, and 1038.09 ± 5.19 g) and the antibiotic (728.91 ± 5.25 g, 908.52 ± 12.20 g, and 1125.7 ± 4.67 g). It indicated that the chicks fed with probiotic supplemeted feed gained more weight while ingesting comparatively less quantity of feed (Fig. 3). The performance of broilers, in each group, was also accessed on the basis of Feed conversion ratio (FCR). It is the ratio of total intake of feed to the

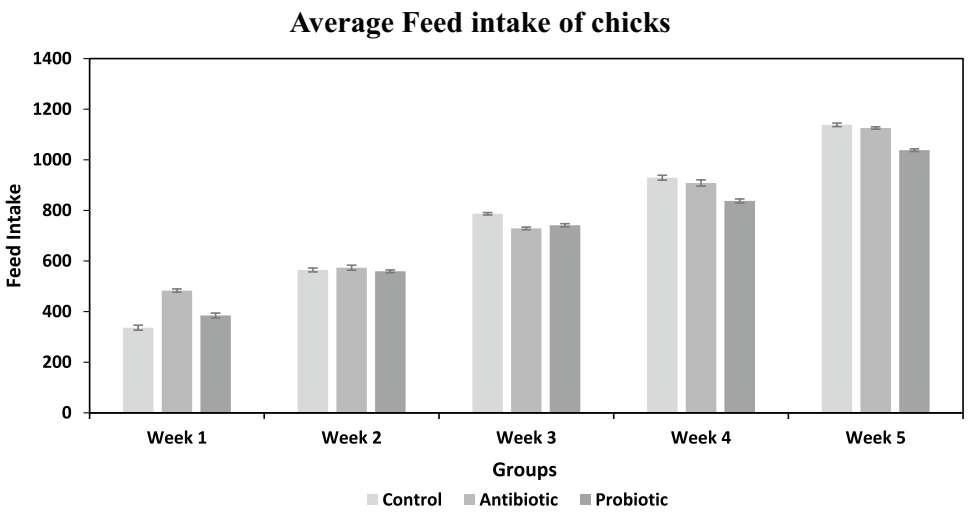


Fig. 3 Average feed intake per week of boilers among different treatment groups. *The y- bars represent the standard deviation (± SEM) of *feed intake* across five parallel replicates

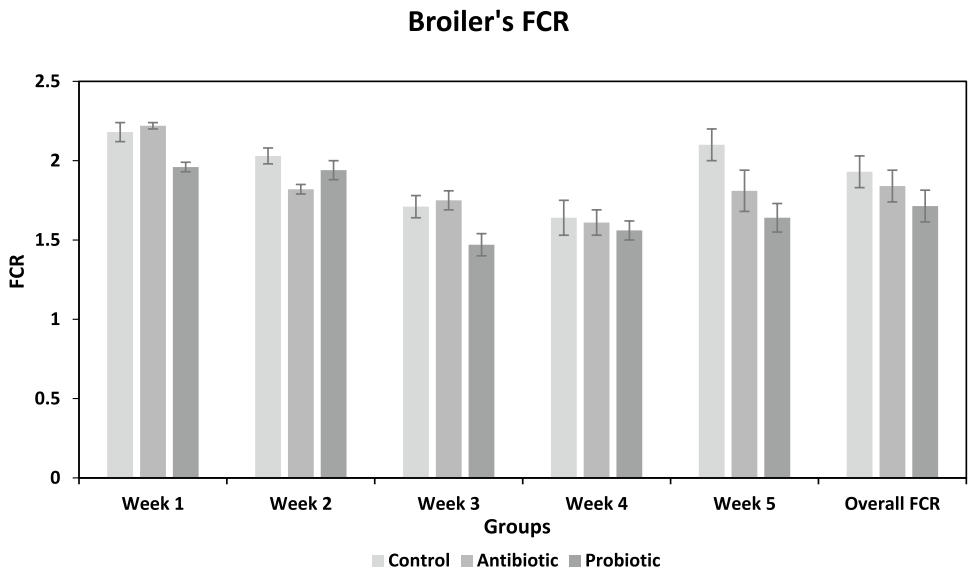


Fig. 4 Feed conversion ratio (FCR) per week of boilers among different treatment groups. *The y- bars represent the standard deviation (±SEM) of *broiler's feed conversion rate* in five parallel replicates

Table 3 Effect of different diets on the growth performance of broilers of different treatment groups in the duration of 5 weeks

Parameters	*Control*	*Antibiotic*	*Probiotic*	*p value*
Average body weight (g)	1823.4 ± 62.34	1960 ± 21.10	2002.6 ± 84.12	0.142
Body weight gain (g)	1772.2 ± 32.19	1906.2 ± 29.58	1950.4 ± 32.74	0.142
Mortality rate (%)	0	0	0	0
Feed intake (g)	3655.3 ± 126.51	3820.05 ± 115.75	3560.69 ± 112.55	0.951
Feed conversion rate (FCR)	1.933 ± 0.11	1.85 ± 0.11	1.715 ± 0.11	0.357

*Values shows the mean ± SEM of five replicates

*One-way ANOVA indicates that the values in one set are significantly different ($p < 0.05$) from each other

total gain in body weight. On week 5, the FCR of Probiotics group remained significantly low ($p < 0.05$) compared to the antibiotics and control group as indicated in Fig. 4. The control group overall FCR (1.933 ± 0.11) remained higher than the probiotic group (1.715 ± 0.10) and antibiotic group (1.85 ± 0.11). Figure 4 indicates that the chicks fed on probiotics supplemented feed gained more weight and consumed less feed.

The mortality rate was estimated by the ratio of the total number of broilers per group to total number of dead broilers per group, during the whole duration of trial. No mortality was recorded in any of the group in our five week experimental trial as shown in Table 3.

Slaughtering Characteristics of Broilers

After the completion of the trial, five birds from each experimental group were slaughtered and their organ's weight was recorded as indicated in Table 4. The results show that there is no significant difference in the weights of hearts, livers, spleens, and gallbladders of the birds of all groups. The filled intestine weights of probiotic group (117.96 ± 5.12 g) was greater compared to the antibiotic (110.78 ± 12.71 g) and control group (104.8 ± 10.31 g). Similarly, the empty intestines of probiotics group chicken were heavier (79.46 ± 3.49 g) compared to that of antibiotic and control group, i.e., 71.3 ± 6.30 g and 72.48 ± 6.52 g, respectively. The control group had the maximum intestinal length, measuring 213.5 ± 15.22 cm, while the probiotic group had the lowest intestinal length, measuring 194 ± 5.51 cm, as shown in Fig. 5.

Table 4 Comparison of different organ weights among the broilers of different treatment groups

Organs	*Control*	*Antibiotic*	*Probiotic*	*p value*
Broiler heart	10.05 ± 0.37	11.49 ± 0.45	10.07 ± 0.72	0.502
Broiler spleen	2.775 ± 0.52	2.57 ± 0.11	2.753 ± 0.30	0.239
Broiler liver	49.23 ± 4.94	49.87 ± 4.37	49.03 ± 1.15	0.071
Broiler gall bladder	1.07 ± 0.13	1.76 ± 0.23	1.68 ± 0.18	0.135

*Values shows the mean ± SEM of five replicates

*One-way ANOVA indicates that the values in one set are significantly different ($p < 0.05$) from each other

Moreover, carcass characteristics of the slaughtered chickens were also determined as presented in Fig. 6. The

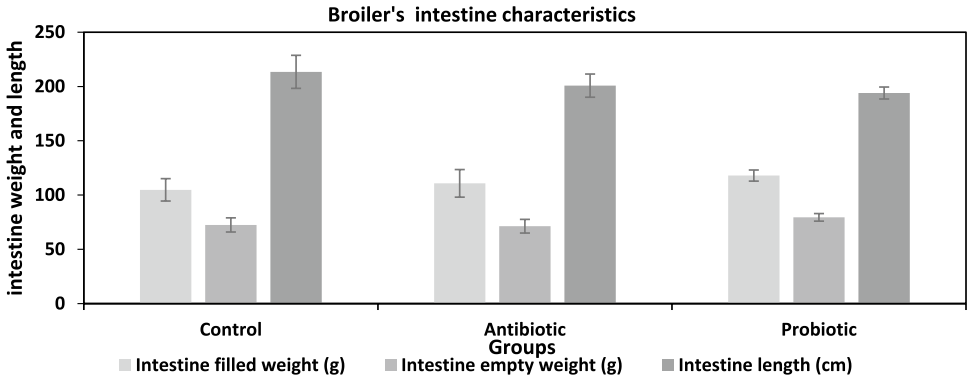


Fig. 5 Comparison of broiler's intestine characteristics among different treatment groups. *The y- bars represent the standard deviation (±SEM) of *broiler's intestine characteristics* in five parallel replicates

overall body weight of the birds remained higher in the probiotic and antibiotic group compared to the control. The hot carcass weight of probiotic and antibiotics group broilers was remained 1335.4 ± 42.69 g and 1270 ± 19.50 g, respectively, compared to that of control (1162.6 ± 39.58 g). In 35 days of trial, the dressing percentage (%)/yield varied from 62.89 ± 0.62 to 66.89 ± 0.69%. The carcass percentage of birds the probiotic-supplemented group was greater (68.01 ± 0.69%) than birds of the antibiotic-supplemented group (65.45 ± 0.45%) and birds in control group (63.77 ± 0.61%).

Meat Quality

The meat quality of slaughtered broiler chicken was evaluated on the basis of different parameters such as meat color, meat pH, extent of cooking loss, and breast muscle sheer force (N). The temperature and pH of meat was measured after 20 min of slaughter. The results indicate that consumption of *B. licheniformis*-supplemented feed increased the temperature and pH (30.8 ± 1.8 and 5.99 ± 0.06) compared to the antibiotic (28.9 ± 0.91 and 5.66 ± 0.12) and the control group (28.91 ± 0.4 and 5.9 ± 0.29) as indicated in Fig. 7. While the shear force of meat in probiotics group was recorded lesser (17.265 ± 0.82) compared to the control (21.455 ± 3.14) and antibiotic group (21.085 ± 6.78). The meat color of chickens fed on *B. licheniformis*-supplemented feed showed higher yellowness (*b**) (53.9 ± 1.6) compared to the antibiotics (50.91 ± 0.5) and control group (50.89 ± 0.96). Moreover, in terms of cooking loss, all groups exhibited similar in cooking loss. Conclusively, the dietary supplementation with *B. licheniformis* resulted in higher lightness (*L**) and yellowness (*b**), with reduced shear force and redness (*a**) compared to that of antibiotic and control groups, as indicated in Table 5.

Muscles histological characteristics were also assessed to compare the effects of different diets in different broiler groups. The histological examinations of muscle indicates higher muscle fiber diameter (56.77 ± 1.58 μm), fiber density (29.26 ± 0.65 n/μm) and fascicle diameter (654.08 ± 5.55 μm) in the meat of probiotics group broilers in comparison to antibiotics and control groups, as shown in Fig. 8 and Table 6.

Hematological Parameters

Blood profile of chickens of all groups was also determined for each treatment group. Haematological paramters such as blood cell count, hemoglobin concentration, packed cell volume, and other serological components were determined. The RBC

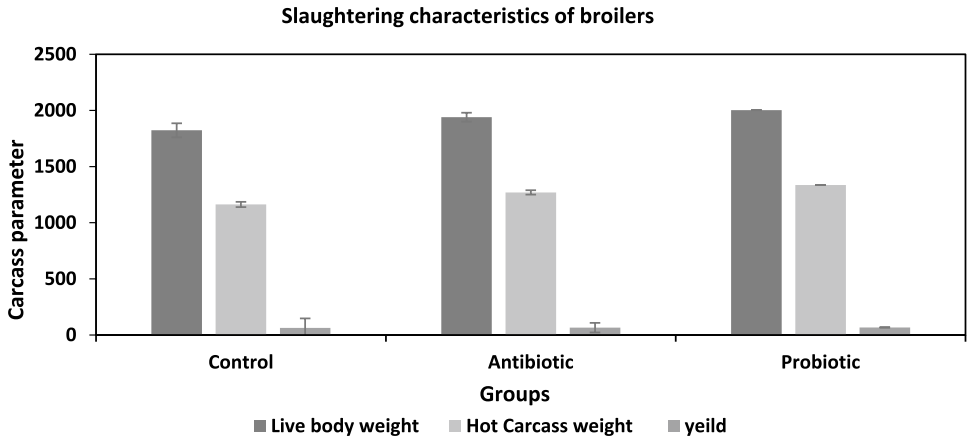


Fig. 6 Comparison of slaughtering characteristics of broilers among different treatment groups in terms of body weight, carcass weight, and mass yield. *The y- bars represent the standard deviation (±SEM) of *slaughtering characteristics of broilers* in five parallel replicates

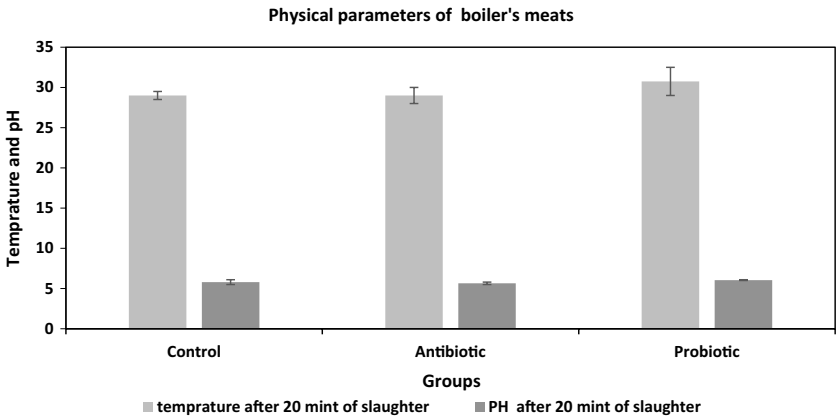


Fig. 7 Physical parameters of slaughtered broiler chicken in terms of temperature and pH. *The y- bars represent the standard deviation (± SEM) of *physical parameters of broilers* in five parallel replicates

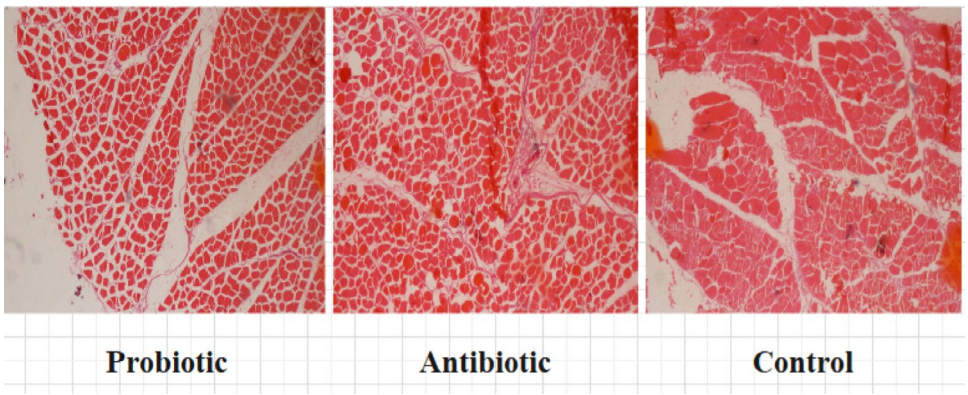
Table 5 Comparative analysis of meat quality of broilers of different treatment groups

Groups	Meat color					Meat Tenderness	Cooking loss (%)		
	*L**	*a**	*b**	*c**	*Hue*	*Shear force (N)*	*Sample Initial wt (g) (w1)*	*Sample Final wt (g) (w2)*	*(w1 − w2)*100 w1*
Control	50.89 ± 0.96	13.78 ± 0.8	13.3 ± 0.46	19.155 ± 0.89	44.02 ± 0.67	21.455 ± 3.14	193 ± 5	173 ± 1	10.31 ± 1.08
Antibiotic	50.91 ± 0.5	14.045 ± 0.07	12.71 ± 0.04	18.94 ± 0.08	42.145 ± 0.05	21.085 ± 6.78	214 ± 2	192 ± 6	10.29 ± 1.95
Probiotic	53.9 ± 1.6	12.305 ± 1.35	13.15 ± 2.23	18.035 ± 2.51	46.73 ± 1.71	17.265 ± 0.82	200 ± 8	179 ± 5	10.45 ± 1.08

*Here, *L**, lightness; *a**, redness; *b**, yellowness; *c**, intensity of color; Hue, dominant wavelength of light giving a color its specific identity, Shear force, The Force required to cut the meat; Cooking loss, the moisture loss during cooking

*Values shows the mean ± SEM of five replicates

*One-way ANOVA indicates that the values in one set are significantly different ($p < 0.05$) from each other



(A) (B) (C)

Fig. 8 Morphological examination of pectoral muscle of **A** broilers fed with probiotics supplemented feed, **B** broilers fed with antibiotics supplemented feed, and **C** broilers fed with simple feed

Table 6 Histological parameters of pectoral muscles of broilers of different treatment groups

Parameter	*Control*	*Antibiotic*	*Probiotic*	*p value*
Fascicle diameter (um)	598.39 ± 6.85	605.66 ± 3.51	654.08 ± 5.55	0.012
Fiber density (n/um)	21.50 ± 0.57	25.35 ± 0.60	29.26 ± 0.65	0.006
Fiber diameter (μm)	50.09 ± 0.80	51.33 ± 0.82	56.77 ± 1.58	0.108

*Values shows the mean ± SEM of five replicates

*One-way ANOVA indicates that the values in one set are significantly different ($p < 0.05$) from each other

count remained highest for probiotics group ($1.92 \pm 0.07 \times 10^6$/µL) compared to antibiotic ($1.857 \pm 0.05 \times 10^6$/µL) and control group ($1.72 \pm 0.07 \times 10^6$/µL). The WBC count also remained higher ($2.05 \pm 0.07 \times 10^6$/µL) compared to the antibiotics and control group, i.e., $1.927 \pm 0.08 \times 10^6$/µL and $1.58 \pm 0.04 \times 10^6$/µL, respectively. The platelet count was recorded as $36.38 \pm 0.46 \times 10^7$/µL in bird of probiotics group, higher than the birds of antibiotics ($33.08 \pm 0.58 \times 10^7$/µL) and control group ($30.45 \pm 0.54 \times 10^7$/µL) as mentioned in Table 7.

Similarly, higher level of hemoglobin (110.21 ± 1.38 g/L) was observed in the probiotics group. The serological investigation of serum indicated lower cholesterol (134.9 ± 20.95 mg/dL) in birds of probiotics group. The cholesterol level in the birds of antibiotics and control group remained higher, i.e., 157.7 ± 14.64 mg/dL and 149.7 ± 5.75 mg/dL, respectively. The glucose concentration in probiotics group was calculated to be higher (268.5 ± 22.93 mg/dL) among all groups while the urea concentration in serums of probiotics group chickens remained lower (66.03 ± 6.17 mg/dL) as indicated in Table 8.

Gut Morphometry

The histological examination of gut morphology of slaughtered broilers' intestine was performed to evaluate different parameters. Figure 9 indicates a general morphology of different parts of broiler's intestine. Results indicated that the probiotic ingestion, in birds, induced better intestinal morphological development than the birds of control and antibiotic group. For duodenum, the birds in probiotic group exhibited significantly greater measurements in villus height (1533.10 ± 40.50 µm), villus width (155.67 ± 15.00 µm), villus surface area ($739{,}314.49 \pm 102{,}042.82$ µm^2), crypt depth (268.22 ± 13.81 µm), villus to crypt ratio (125.73 ± 15.23), Lamina propria thickness (222.07 ± 7.83 µm), and muscularis externa thickness (5.59 ± 0.04 µm) compared to the measurements of these parameters in birds of antibiotic and control groups as indicated in Fig. 10 and Table 9.

In the jejunum, the probiotic group demonstrated significantly higher values for villus height (VH), villus width (VW), villus surface area (VSA), villus height: crypt ratio (VH:CD), Lamina propria thickness (LPT), and muscularis externa thickness (MET) compared to the measurement of these parameters in birds of antibiotic and control groups. Notably, crypt depth was greater in the birds of control group (253.01 ± 23.9 µm) than the birds of antibiotic (225.02 ± 6.01 µm) and probiotic group (218.91 ± 15.9 µm) (Fig. 11, Table 9).

The probiotic group exhibited higher Ileum VH, LPT, and VH:CD (955.74 ± 28.55 µm, 89.07 ± 15.21 µm, and 5.14 ± 0.27, respectively) in comparison to the birds of antibiotic (869.81 ± 39.01 µm, 87.73 ± 10.8 µm, and 4.29 ± 0.1) and control group (930.01 ± 59.89 µm, 69.3 ± 2.7 µm, and 4.19 ± 0.4). However, compared to the probiotic group (103.01 ± 7.01 µm and $306{,}701 \pm 21{,}491.26$ µm^2) and antibiotic group (85.02 ± 2.01 µm and $245{,}675.3 \pm 17{,}018.2$ µm^2), the villus width and surface area were higher in the antibiotic group (113.9 ± 10.89 µm and $314{,}774.3 \pm 42{,}984.09$ µm^2). In comparison to the probiotic group (185.9 ± 4.7 µm and 197.92 ± 21.86 µm) and antibiotic group (202.08 ± 5.92 µm and 166.91 ± 26.01 µm), the control group's crypt depth

Table 7 Analysis of hematological parameters of broiler's blood of different treatment groups

Parameter	*Control*	*Antibiotic*	*Probiotic*	*p value*
RBCs (×10⁶/µL)	1.72 ± 0.07	1.857 ± 0.05	1.92 ± 0.07	0.089
HB (g/L)	90.13 ± 1.03	102.77 ± 1.05	110.21 ± 1.38	0.001
WBCs (×10⁶/µL)	1.58 ± 0.04	1.927 ± 0.08	2.05 ± 0.07	0.001
Hematocrip (%)	29.18 ± 0.35	29.47 ± 0.49	30.94 ± 0.78	0.107
MCV (fl)	11.31 ± 0.36	11.32 ± 0.39	11.58 ± 0.36	0.842
Platelets (×10⁷/µL)	30.45 ± 0.59	33.08 ± 0.58	36.38 ± 0.46	0.001

*Here, HB, hemoglobin; RBC, Red blood cells; WBC, White blood cells; MCV, Mean corpuscular volume

*Values shows the mean ± SEM of five replicates

*One-way ANOVA indicates that the values in one set are significantly different ($p < 0.05$) from each other

Table 8 Serum biochemical parameters of broiler chickens of different treatment groups

Parameter	*Control*	*Antibiotic*	*Probiotic*	*p value*
TG (mg/dL)	202.3 ± 125.46	72.5 ± 4.93	96.01 ± 32.82	0.438
TC (mg/dL)	149.7 ± 5.75	157.7 ± 14.64	134.9 ± 20.95	0.572
HDL(mg/dL)	77.02 ± 9.02	70.3 ± 6.05	66.03 ± 3.48	0.511
TP (g/L)	171.07 ± 21.78	171.9 ± 16.69	107.9 ± 27.07	0.106
Creatinin (mg/dL)	64.15 ± 2.79	40.6 ± 3.81	42.09 ± 3.98	0.001
AST (U/L)	166.3 ± 57.73	168.7 ± 54.97	62.9 ± 9.33	0.224
ALT (U/L)	31.3 ± 2.68	42.7 ± 7.01	33.7 ± 3.42	0.242
Urea (mg/dL)	83.7 ± 7.29	76.02 ± 7.35	66.03 ± 6.17	0.241
Glucose (mg/dL)	241.5 ± 13.9	214.3 ± 8.01	268.5 ± 22.93	0.099

*Here, *TG*, triglycerides; *TC*, total cholesterol; *HDL*, high density lipoprotein; *TP*, total protein; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase

*Values shows the mean ± SEM of five replicates

*One-way ANOVA indicates that the values in one set are significantly different ($p < 0.05$) from each other

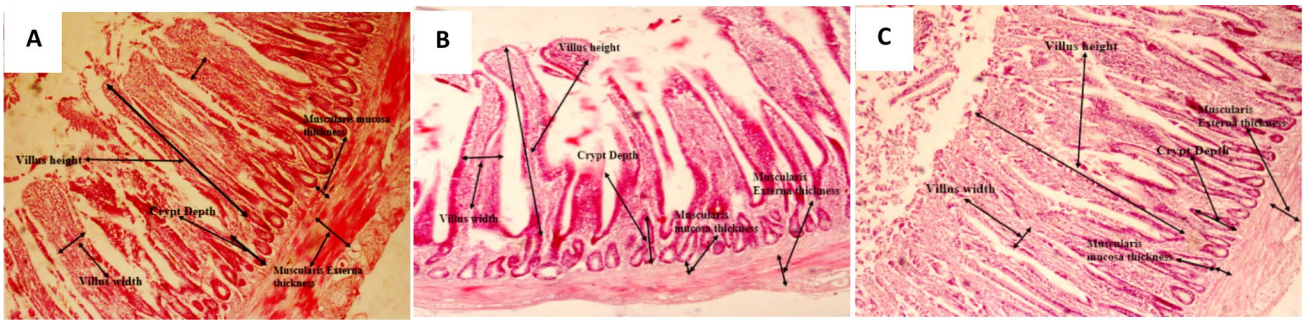


Fig. 9 General histological demonstration of intestine morphology **A** duodenum, **B** jejunum, and **C** ileum

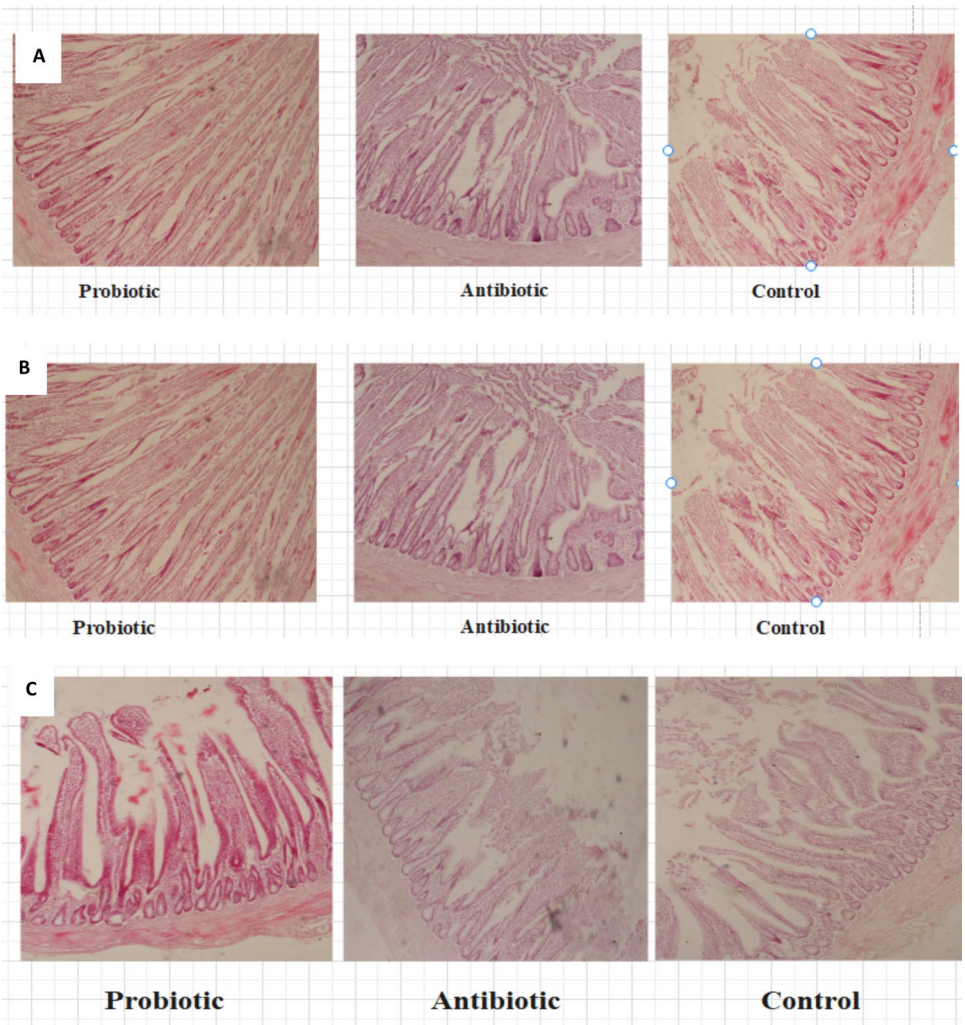


Fig. 10 Morphological examination of intestine (**A**) duodenum (**B**) jejunum and (**C**) ileum

and Lamina propria thickness (220.01 ± 4.62 µm and 223.07 ± 21.07 µm) were higher as shown in Table 9.

1H-NMR Analysis of Meat Extracts

NMR analysis of breast and leg meat of the broilers of control, antibiotic, and probiotics group was conducted and results were obtained in the form of NMR spectra as shown in Fig. 11. The NMR analysis identified overall 25 metabolites which include amino acids, peptides, organic acids, amino acid derivatives, carboxylic acid, fatty acids, and short chain fatty acids, nucleic acids, sugars, and their derivatives. The NMR analysis of probiotics group broilers' meat indicated the presence of 22 metabolites compared to

Table 9 Histological parameters of different sections of small intestine (deudenum, jejunum, illeum) of the broiler birds among different treatment groups

Small intestine	Groups	Parameter						
		VH (μm)	*VW (μm)*	*VSA (μm^2)*	*CD (μm)*	*LPT (μm)*	*MET (μm)*	*VH:CD (μm)*
Duodenum	Control	1292.39 ± 26.42	106.31 ± 9.71	451,963.2 ± 30,072.47	257.01 ± 14.19	89.13 ± 7.68	168.22 ± 8.39	5.30 ± 0.21
	Antibiotic	1312.73 ± 54.98	122.72 ± 8.75	506,551.92 ± 44,478.92	250.01 ± 8.58	96.27 ± 12.97	219.77 ± 32.19	5.25 ± 0.37
	Probiotic	1533.10 ± 40.50	155.67 ± 15.00	739,314.49 ± 102,042.82	268.22 ± 13.81	125.73 ± 15.23	222.07 ± 7.83	5.59 ± 0.04
	p value	0.111	0.57	0.048	0.607	0.166	0.177	0.656
Jenunum	Control	1184.19 ± 44.55	105.88 ± 7.20	401,942.7 ± 41,693.26	253.01 ± 23.9	86.93 ± 12.61	192.40 ± 7.18	4.9 ± 0.71
	Antibiotic	1128.80 ± 62.98	120.71 ± 5.57	426,298.36 ± 17,152.21	225.02 ± 6.01	96.42 ± 8.08	193.06 ± 23.23	5.03 ± 0.38
	Probiotic	1277.02 ± 38.38	139.73 ± 8.96	560,058.34 ± 36,721.43	218.91 ± 15.9	116.22 ± 15.22	213.62 ± 40.45	5.88 ± 0.42
	p value	0.229	0.047	0.032	0.408	0.303	0.827	0.424
Illeum	Control	930.01 ± 59.89	85.02 ± 2.01	245,675.3 ± 17,018.2	220.01 ± 4.62	69.3 ± 2.7	223.07 ± 21.07	4.19 ± 0.4
	Antibiotic	869.81 ± 39.01	113.9 ± 10.89	314,774.3 ± 42,984.09	202.08 ± 5.92	87.73 ± 110.8	166.91 ± 26.01	4.29 ± 0.1
	Probiotic	955.74 ± 28.55	103.01 ± 7.01	306,701 ± 21,491.26	185.9 ± 4.7	89.07 ± 15.21	197.92 ± 21.86	5.14 ± 0.27
	p value	0.43	0.95	0.27	0.011	0.395	0.304	0.074

*Here, *VH*, villus height; *VW*, villus width; *VSA*, villus surface area; *CD*, crypt depth; *LPT*, lamina propria thickness; *MET*, mucosal epithelial thickness; *VH:CD*, ratio of villus height to crypt depth

*Values shows the mean ± SEM of five replicates

that of antibiotics (16 metabolites) and control group (12 metabolites) as indicated in Table 10. The comparison of metabolites produced among all the three groups (Table 10) indicated that metabolites such as omega-3-fatty acid, DSS, histidine, uridine, and phenylalanine were produced only in probiotics group.

Discussion

The present study evaluated the effect of probiotics (*Bacillus licheniformis*) on the growth pattern and meat quality of broiler birds. In our study, *B. licheniformis* supplementation in broiler feed resulted in an increase in 7.5% and 10% body weight of broilers compared to the antibiotics and control groups, respectively. Additionally, when carcass parameters (size of liver, spleen, coronary heart, gall bladder, and intestine) of slaughtered broilers were evaluated, the broilers of probiotics group showed no significant weight gain ($p > 0.05$) in organs, indicating that the dietary interventions had no adverse effect on the growth and function of organs. Hence, the average weight gain in broilers was because of muscles weight gain rather than vital organ's enlargement. It has been shown in many other recent studies *B. licheniformis* improves chickens' overall performance in broilers [21–23]. Moreover, in week 5, the feed intake and FCR in probiotics group chicken were significantly lower ($p < 0.05$) compared to the antibiotics and control group. It indicates improved feed efficiency, likely due to enhanced nutrients absorption and improved gut health. The probiotic helps in the efficient digestion of the feed, which results in excessive absorption of nutrients along with reduced fecal excretion of feed [24–26].

The spore forming ability of *B. licheniformis* enhances the birds' intestinal integrity and growth performance, as probiotics inhibit non-beneficial pathogens from the intestine via competitive inhibition and microbial antagonism [27]. The probiotics provide enzymes and secondary metabolites in the chicken's gut to inhibit pathogenic microorganisms and also act as a growth promoter [28]. *B. licheniformis* has been reported to produce alkaline proteases, amylases, and lipases [29, 30]. These enzymes increase digestion and facilitates efficient absorption of nutrients. The ingestion of probiotics boosts the digestibility of essential amino acids; therefore, enhanced feed digestion and absorption result in the average body weight gain. These results are consistent with the study of Amoah et al. (2020) who also reported an increase in broiler's body weight after 2 g/kg feed supplementation with probiotic [31]. Similarly, Zhang and Kim (2014) also reported a 5% increase in the body weight of chicken [32]. Rehman et al. (2020) also reported no weight gain of organs (heart, gizzard, and liver) in broilers supplemented with Protexin [33].

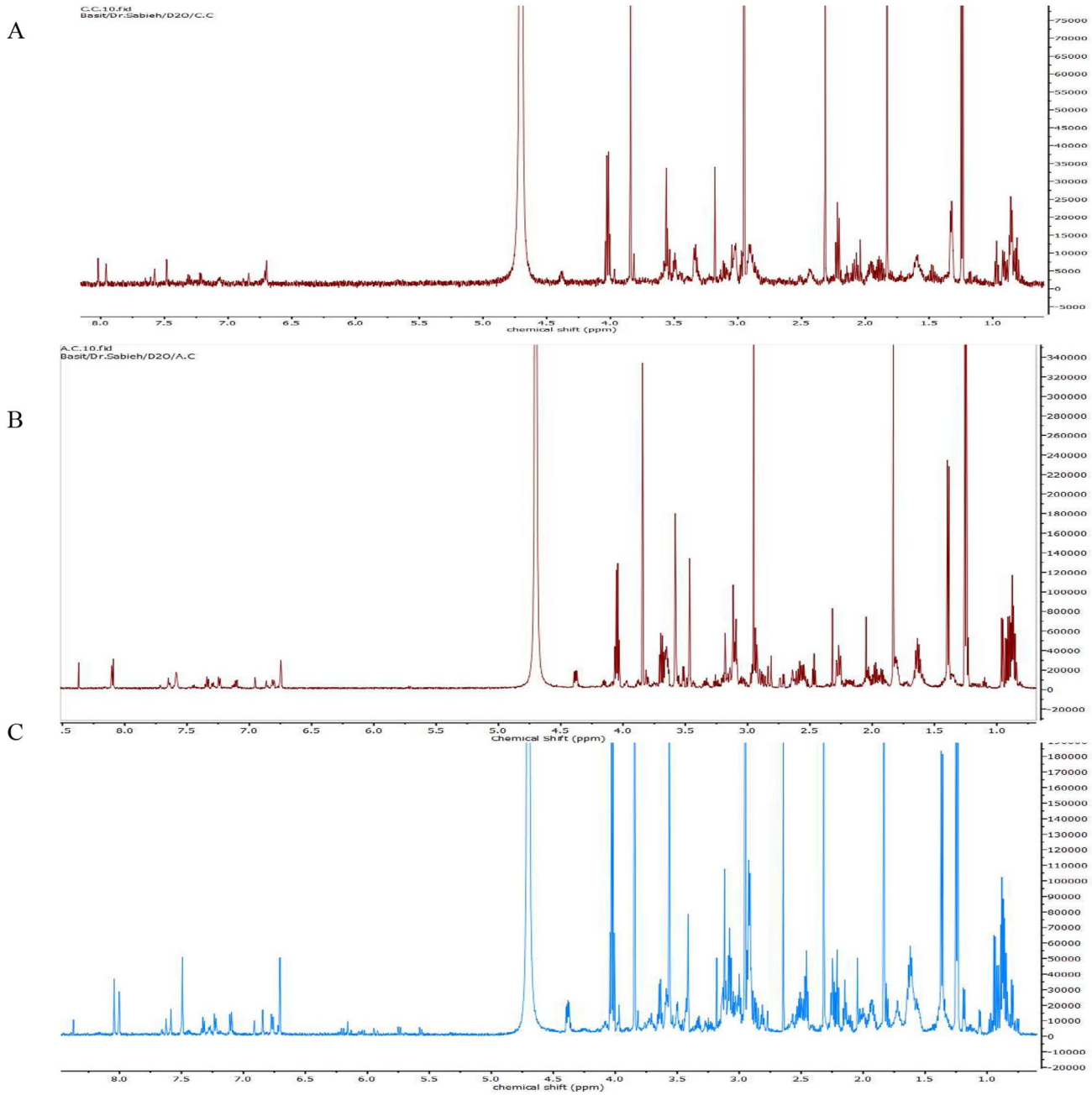


Fig. 11 Comparative analysis of NMR spectra of broiler's breast meat among different treatment groups **A** control, **B** antibiotics, and **C** probiotics

The intake of probiotics also affected the intestinal morphology of the chickens. The histopathological investigations of the probiotic group's chicken intestine indicate an increase in VH, VW, VSA, CD, VH: CD, LPT, and MET (Table 9) compared to those of antibiotic and control group. The enhanced nutrients absorption stimulates active cell mitosis in intestinal cells resulting in all these histological changes [34]. These results of changes in intestine histological morphology supports the findings of Shah et al., 2020 who have also reported similar results with probiotic-supplementation in broilers feed [35]. These intestinal changes are significant because the increase in villi length enhance the surface of gut which ultimately facilitates higher absorption of nutrients leading toward fast growth of chickens [36].

Haematological parameters such as hemoglobin concentration, number of RBCs, and WBCs in blood are clear indications of clinical and nutritional health status of the animal [37, 38]. The results of hematological parameters indicate that chicks fed

Table 10 Comparative NMR analysis of the broilers meat from control, antibiotics, and probiotics group

Metabolite class	Control		Antibiotics		Probiotics	
	Metabolites	^{1}H (ppm) and Multiplicity	Metabolites	^{1}H (ppm) and Multiplicity	Metabolites	^{1}H (ppm) and Multiplicity
Amino acids	Isoleucine	0.94 (t), 1.02 (d), 1.26 (m), 1.46 (dd), 1.97 (m), 3.66 (d)	– – –	– – –	Isoleucine	0.94 (t), 1.02 (d), 1.26 (m), 1.46 (dd), 1.97 (m), 3.66 (d)
	Threonine	1.33 (d)	– – –	– – –	– – –	– – –
	Lysine	1.84 m, 1.43 (m,) 1.49 (m), 1.71 (m), 1.91 (m), 3.01 (t), 3.7 (t)	– – –	– – –	– – –	– – –
	Glycine	3.57 (s), 3.54 (s)	Glycine	3.57 (s), 3.54 (s)	Glycine	3.57 (s), 3.54 (s)
	Arginine	1.64 (m)	– – –	– – –	– – –	– – –
	– – –	– – –	Leucine	0.94 (d), 0.95 (d), 1.66 (m), 1.71 (m), 3.73 (m)	– – –	– – –
	– – –	– – –	Threonine	1.36 (d), 1.32 (d), 3.57 (d), 4.25 (m)	Threonine	1.33 (d)
	– – –	– – –	Valine	2.26 (m), 0.98 (d), 1.03 (d), 3.60 (d)	Valine	1.03 (d), 2.26 (m), 0.98 (d), 2.26 (m), 3.60 (d)
	– – –	– – –	– —	– – –	Valine	3.60 (d), 0.98 (d), 1.03 (d), 2.26 (m)
	– – –	– – –	Glutamine	2.46 (t) 2.15 (m), 2.18 (m), 2.42 (m), 3.76 (t)	Glutamine	2.46 (t), 2.15 (m), 2.18 (m), 2.42 (m), 3.76 (t)
	– – –	– – –	– – –	– – –	Glutamine	2.46 (m), 2.15 (m), 2.18 (m), 2.42 (m), 3.76 (t)
	– – –	– – –	– – –	– – –	Carnitine	2.17–2.25 (t)
	– – –	– – –	– – –	– – –	Phenylalanine	7.32 (d)
Peptides	– – –	– – –	Carnosine	8.12 (d), 3.02(m), 3.21(m), 4.48(m), 7.05(m)	Carnosine	8.06 (s), 2.64 (s)
Organic acids	3-Hydroxybutyrate	1.24 (d), 2.31(dd), 2.38(dd), 4.16(m)	3-Hydroxybutyrate	1.24 (d), 2.31(dd), 2.38(dd), 4.16(m)	3-Hydroxybutyrate	1.24 (d), 2.31(dd), 2.38(dd), 4.16(m)
	– – –	– – –	3-Hydroxyphenylacetate	3.46 (s)	3-Hydroxyphenylacetate	3.46 (s)
Amino acid derivatives	Betaine	3.85 (s), 3.88 (s), 3.27 (s)	Betaine	3.85 (s), 3.88 (s), 3.27 (s)	– – –	– – –
	– – –	– – –	– – –	– – –	Histidine	7.09 (d), 3.16 dd, 3.23 dd, 3.98 dd 7.11 s, 7.86 s
	Choline	3.18 (s), 3.21 (s), 3.50 (m), 4.06 (m)	– – –	– – –	Choline	3.18 (s), 3.21 (s), 3.50 (m), 4.06 (m)
	– – –	– – –	– – –	– – –	O-Acetylcarnitine	2.51 (m)
Dicarboxylic acid	Succinate	2.41 (s), 2.04 (s), 2.39 (s)	Succinate	2.04 (s), 2.39 (s)	Succinate	2.04 (s), 2.41 (s), 2.39 (s)
Fatty acids	– – –	– – –	Butyrate	0.88 (t)		
	– – –	– – –	– – –	– – –	Omega 3 fatty acid	0.99 (dd)
Nutrients	– – –	– – –	– – –	– – –		

Table 10 (continued)

Metabolite class	Control		Antibiotics		Probiotics	
	Metabolites	^{1}H (ppm) and Multiplicity	Metabolites	^{1}H (ppm) and Multiplicity	Metabolites	^{1}H (ppm) and Multiplicity
Nucleic acid	– – –	– – –	Inosine	8.35 (s), 3.84(m), 3.92(m), 4.28(dd), 4.45(dd), 6.10(d). 8.25(s)	Inosine 50-phosphate	4.36 (m)
	– – –	– – –	– – –	– – –	Uridine	8.00 (d), 3.80 dd, 3.90 dd, 4.12 dt, 4.21 dd, 4.34 dd, 5.89 d, 5.90 m
Sugars	Lactate	4.03 (q), 1.31 (d)	Lactate	4.03 (q), 1.31 (d)	Lactate	4.03 (q), 1.31 (d)
	– – –	– – –	– – –	– – –	Lactate	4.03 (q), 1.31 (d)
	– – –	– – –	UDP-glucose	7.94 d	– – –	– – –
Other	Pyruvate	2.35 (s)	Pyruvate	2.35 (s)	Pyruvate	2.35 (s)
	Ornithine	1.93 (m)	Ornithine	1.93 (m)	– – –	– – –

*Multiplicity: *s*, singlet; *d*, doubles; *t*, triples; *m*, multiplets; *q*, quartet; *dd*, double doublet

with probiotics exhibit higher RBC, WBC, and platelet counts as $1.92 \pm 0.07 \times 10^6$/µL, $2.05 \pm 0.07 \times 10^6$/µL, and $36.38 \pm 0.46 \times 10^7$/µL, respectively, along with an increased packed cell volume and hemoglobin concentration (110.21 ± 1.38 g/L). These results are significant because increased RBCs and hemoglobin facilitate effective oxygen transportation to the body tissues [39]. An improved oxygen delivery to body muscles promotes aerobic metabolism and improves ATP production, crucial for protein synthesis. This results in improved health and muscle formation in chickens [40].

On the other hand WBCs are responsible for immunity. A sufficient WBC count provides efficient immunity by decreasing the chances of pathogenic infections [41, 42]. Some probiotics, such as *Lactobacillus*, *Lactococcus*, *Enterococcus*, and *Bifidobacteria*, participate in immunity by producing bacteriocins, which penetrate the cell membranes of pathogenic microbes and cause cell leakage [43–45]. Our results are in accordance with the findings of ÇEtïn et al., 2005 who also reported an increase in erythrocyte count, and hemoglobin values by giving probiotic supplemented feed to turkeys [46]. Similarly, Abd El-Hack et al., (2021) reported similar results, i.e., increased hemoglobin (73 g/L), RBC 3.5×10^6/µL, and WBCs 4.10×10^3/µL count by *B. licheniformis* supplementation [47].

The serological investigations of probiotic-fed broiler chickens indicated low cholesterol, triglycerides, HDL, creatinine, and urea compared to those of the antibiotic and control group. However, the blood glucose level for the chicks fed with probiotics remained high. The use of probiotics change the lipoprotein metabolism of bird in different ways [48]. Probiotics assimilate cholesterol in the small intestine by hydrolyzing bile acids and salts through their special enzymes called hydroxysteroid dehydrogenases. The hydrolysis of bile acids and salts results in lower cholesterol absorption in the blood [49]. The other mechanism involves in lowering the cholesterol level is the inhibition of Niemann-Pick C1-like1 (NPC1L1) receptors. The NPC1L1 is widely expressed in small intestine, mostly on the RBCs surface, performing a key role in cholesterol absorption in intestine. However, probiotics downregulate NPC1L1 which ultimately results in the reduction of serum cholesterol level [50]. However, the use of probiotics increases the blood glucose, as reported by Zou et al. (2022) [51]. Probiotics ferment dietary substrates in the gut and produce short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. These SCFAs are beneficial for gut health, but also have a tendency to increase gluconeogenesis in liver, potentially leading to elevated blood glucose levels [52, 53].

The effect of probiotic supplementation on meat quality was evaluated on the basis of meat color, pH, cooking loss, and shear pressure. Probiotics supplementation significantly improved the meat temperature (30.75 ± 1.75 °C) and pH (6.05 ± 0.05) after 20 min of slaughter. Both of these parameters, i.e., temperature and pH are critical for meat quality and shelf life. Lower post-mortem pH and stable temperature is responsible for reduced microbial growth and hence better preservation [54]. Our results indicated that the *B. licheniformis* ingestion increased yellowness (b^*) (53.9 ± 1.6) in breast muscle compared to both the control group and antibiotic-treated group. While cooking loss showed no significant variation among the groups, *B. licheniformis* supplementation led to higher lightness (L^*), yellowness (b^*) along with reduced shear force and redness (a^*) compared to the antibiotic and control groups. However, no significant cooking loss was observed among the groups. The higher lightness L^* and

yellowness b^* of meat indicates the presence of carotenoids in it, which is preferred by the consumers [54]. Lower sheer force attributes the tenderness of meat while low a^* indicates less red color. Usually, the red color of meat comes from myoglobin pigment, and lower a^* values indicate comparatively lower myoglobin in meat [55]. Like hemoglobin, myoglobin is also an oxygen binding protein with only one heam group. On oxygenation, it converts into oxyhemoglobin form, inducing red color in meat. However, chicken's meat is consumed as a white-meat and naturally contain less myoglobin compared to the other red meats [56]. Probiotic supplementation also resulted in increased fiber diameter (56.77 ± 1.58 µm) density (29.26 ± 0.65 n/µm) and fascicle diameter (654.08 ± 5.55 µm), indicating it as a muscle growth promoter [57, 58]. Shah et al. (2019) also reported an increase in muscle fiber diameter (MFD), muscle fiber cross-sectional area (MFCSA), muscle fascicle diameter (MFSD), and muscle fascicle cross-sectional area (MFSCSA) in probiotic group [35].

Representative 600-MHz 1H NMR spectra analyzed the metabolite profile in both breast and leg meat identifying a total of 26 compounds. These included amino acids, peptides, organic acids, amino acid derivatives, dicarboxylic acids, fatty acids, short-chain fatty acids, nutrients, nucleic acids, sugars, and other compounds. Each dietary group—control, antibiotic, and probiotic—exhibited distinct metabolic profiles. The control group exhibited 12 metabolites, the antibiotic group exhibited 16, and the probiotic group gave 22. Moreover, seven of the total 22 metabolites (carnitine, phenylalanine, histidine, O-acetylcarnitine, omega 3 fatty acid, uridine, lactate) were only present in the meat of probiotics supplemeted group. As mentioned previously, probiotics increase enzymatic activity in gut resulting in efficient digestion of carbohydrates, proteins, and fats [59]. Moreover, the ability of probiotics to change the lipoprotein metabolism increases beneficial fatty acids such as omega-3 fatty acid and reduce the saturated fatty acids in chicken meat. Hence, the enhanced utilization of nutrients improve the metabolites concentration and nutritional value of broilers meat. Similarly, Qin et al. (2024) reported an increase in lysine, histidine, arginine, and methionine by 1.66%, 0.03%, 0.38%, and 0.33%, respectively, in probiotics group [60]. Additionally, the work of Xiao et al. (2019) and Le Roy et al. (2016) highlighted the extensive range of metabolites in broiler chick meat and various biological matrices in chickens, respectively [61, 62]. Notably, non-volatile components in fresh meat, including amino acids and nucleotides, were identified as critical taste precursors, influencing the sensory attributes of flavor, tenderness, and juiciness of meat [63]. This comprehensive metabolite analysis provides valuable insights into the chemical composition of meat, contributing to the enhancement of taste and overall quality attributes.

Conclusion

The present study concludes the potential probiotic (*Bacillus licheniformis*) in improving the meat quality of broilers. Findings indicate that supplementing the broiler's feed with *B. licheniformis* can enhance growth performance, carcass yield, organ weight, and meat quality of broiler birds. The supplementation also decreased feed conversion ratio (FCR) without affecting mortality rates. Consequently, incorporating probiotics into feed additives supports weight gain by promoting enhanced digestion and nutrients absorption by increasing villi height. Compared to the control, *B. licheniformis* supplementation resulted in a 7.5% and 10% body weight of broilers compared to the antibiotics and control group, respectively. Thus, *B. licheniformis* exhibits superior performance compared to antibiotics and has the potential to reduce broiler production costs. Therefore, our research concludes that *B. licheniformis*, as a probiotic, can act as a growth promotor for poultry, and has a potential to replace the utilization of antibiotics in poultry feed at both industrial and local farms level.

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Author Contribution B.A.K performed his role in data curation, Investigation, and Methodology. S.S wrote the main manuscript, organized the results, and wrote discussion. A.M performed investigation and Methodology with B.A.K. I.L performed her role in Conceptualization, Validation, and software analysis. S.A, A.R, L.P, and S.A performed their role in conceptualization and validation of the research project. M.N.A conducted Funding acquisition, Project administration and Supervision of the research project.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval Study was approved by the Institutional Review Board. All research practices were conducted in accordance with the International and departmental ethical guidelines.

Competing interests The authors declare no competing interests.

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