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EFFECT OF LOW PROTEIN DIETS WITH SUPPLEMENTATION OF *BACILLUS PUMILLUS* ON GROWTH AND CARCASSES PERFORMANCE, HEMATOLOGICAL AND BIOCHEMICAL INDICES OF BROILER CHICKEN

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ABSTRACT

The research objective was to determine the effect of *Bacillus pumilus* on growth performance hematological and biochemical indices of broilers chicken fed a low-protein diet. A total number of 180-day-old chicks were brought and placed into three groups, and each treatment was divided into six replicates (10 birds per replicate). Experimental diets included control (CO) having 22% crude protein (CP) for the starter and 20.0% CP for the finisher. However, the low protein (LP) contain 20% CP for the starter, 18.0% CP for the finisher, and *Bacillus pumilus* (*BP-2000)* treatment (LP+0.01% BP for starter and finisher). The birds fed a diet with *BP-2000* improved the live body weight (LBW), and average daily gain (ADG) in the starter and overall period. In contrast, the feed conservation ratio (FCR) was reduced in the starter and overall phases ($P<0.05$). Dressing, breast, and leg muscle percentage significantly improved in *BP-2000* ($P< 0.05$). Abdominal fat percentage was significantly reduced in *BP-2000*. The hemoglobin (HB), red blood cells (RBC), packed cell volume (PCV) showed no difference between all treatments, but the white blood cell (WBC) count was higher in *BP-2000*. Total protein (TP), albumin (ALB), globulin (GLB), and creatinine (CRAET) were not affected by dietary supplementation of *BP-2000*. High-density lipoprotein (HDL) was significantly higher in *BP-2000* than control and LP group ($P<0.05$). A lower serum level of total cholesterol (TC) and low-density lipoproteins (LDL) was observed in BP-2000 than LP. In conclusion, the current study showed that birds fed diet low in proteins with supplementation of *BP-2000* improve growth performance, carcasses, and some blood biochemical indicators.

Key-words: Probiotics, *Bacillus species*, Gut microflora, Bacterial supplementation, Growth performance, Carcass characteristics, Gastro intestinal tract, Blood biochemical indices, Broiler chickens.

List of Abbreviations:
ALB = Albumin; ADG = Average daily gain; ADFI = Average daily feed intake; AF% = Abdominal fat percentage; AWG = Average weight gain; BM% = Breast muscle percentage; *BP* = *Bacillus pumilus*; BP 0.01 = Low energy + *Bacillus pumilus* 0.01%; CO = Control; CP = Crude protein; CREAT = Creatinine; Dressing % = Dressing percentage; DM = Dry matter; FCR = Feed conversion ratio; GLB = Globulin; GIT = Gastro Intestinal Tract; HB = Hemoglobin; LBW = live body weight; LDL = low-density lipoproteins; LE = Low energy; LM% = Leg muscle percentage; ME = Metabolizable energy; PCV = Packed cell volume; RBC = Red blood cells; SBM = soybean meal; TC = Total cholesterol; TP = Total protein.

INTRODUCTION

The poultry industry is a significant and growing contributor to the country's gross domestic product (1.3%), with 26.8% of the total meat production, 5.76% of the agricultural sector, and 1.26% of GDP. Poultry production is one of Pakistan's most dynamic and long-standing economic pillars. Over 1.5 million individuals have found stable work in the poultry industry in the last few years (GOP, 2014). The population is expected to increase worldwide from 7.7 billion in 2018 to nearly 10 billion in 2050. So, with the increase in world population, the demand for meat is expected to increase by 2.4% annually between 2015 and 2030 (FAO, 2017; FAO, 2020). Due to its importance as an animal protein source for human development, poultry is in high global demand. Although protein is the second most crucial component of the diet, it plays an essential role in tissue production, repairing and maintenance and the structural component of the body, enzymes, and hormones (Beski *et al*., 2015). The industry grew by 20-30% annually in 1970s, and by another 10-15% annually in 1980s. However, chicken meat consumption has increased by more than 4% annually due to a robust local market (Hussain *et al*., 2015). Foodstuff made from poultry provide around 1.1% of the national GDP, and about 40% of the agricultural GDP. Furthermore, modern chicken farming practices use both plant and animal protein sources in their diets because of its high crude protein concentration (40 to 48 percent), balanced amino acid composition, and digestibility, in broiler diets, soybean meal (SBM) is the preferred protein source. Around 80% of broiler chicks' protein and amino acid requirements may be met by SBM. SBM indeed contains hulls and anti-nutritional components like trypsin inhibitor (Abdel-Hafeez *et al*., 2017). The probiotic is defined as a living organism that confers the health benefit of the host when given in the correct amount. A probiotic is an inactivated microorganism that gives a beneficial effect on host. Probiotic support a healthy GIT because they have different modes of action, including enhancing gut barrier function, controlling intestinal microflora, and preventing GIT disease (Gaggìa *et al*., 2010; Kabir, 2009; Edens, 2003). Probiotics also improve broiler chicken health and productivity. In addition, probiotics have been linked to numerous health benefits, including increased resistance to infection, better nutrient absorption and digestion, and suppression of potentially harmful bacteria (Ding *et al*., 2021; Savitri, 2021).

Furthermore, several *Bacillus* species are used as probiotics in the diet of livestock and poultry because of their spore-forming abilities (Zhang *et al*., 2014; Park and Kim,2014). Lactobacillus spp. is the most common probiotic used in poultry diets because it is present in the gut and improves health (Kabir *et al*., 2009). Enterococcus probiotics are used to increase growth and health (Franz *et al*., 2011). Probiotics such as *Bifidobacterium* and *Aspergillus oryza*, which belong to the genera *Bacillus, Lactobacillus, Streptococcus*, and *Enterococcus* are often used in poultry diet (Khaksefidi and Rehimi, 2005). The poultry business has made use of probiotics due to the advantage they bring; commonly utilized probiotic *Aspergillus, Candida, Lactobacillus, Bacillus, Bifidobacterium, Streptococcus, Enterococcus, Enterococcus, and Saccharomyces,* among others (Ahmad, 2006). Probiotic regulate the gut microorganisms and inhibit the growth of pathogens (Almada *et al*., 2015). The Bacillus strains produce at high efficiency, and hence, they have the key advantage of resisting the changing conditions in GIT of chickens (Ramlucken *et al*., 2020). However, Bacillus probiotics are utilized as a biocontrol agent to suppress vibrio infections by inhibiting the development of competing pathogens and stimulating growth in shrimps (Nakayama and Nomura, 2009). Probiotic which enhance growth rate, nutrient uptake, gut health and immunity in broiler chickens (Manafi *et al*., 2018; Wang *et al*.,2018; Ramlucken *et al*., 2020; Bilal *et al*., 2021). Probiotics are defined as a group of microorganisms producing a favorable impact when given sufficiently to the host (Reid *et al*., 2003). According to a different study (Hong *et al*., 2005), different Bacillus species can be used as an alternative to antibiotics for humans and animals. The *B. pumilus* promotes intestinal growth and protects the digestive system's health by encouraging the growth of beneficial microorganisms (Abd El-Hack *et al*., 2021). The BW of gigantic freshwater prawns (Zhao *et al*., 2019) and striped catfish (Thy *et al*., 2017) was improved by *B. pumilus*; on the other hand, it also improves the health and immunological function of catfish and Nile tilapia by increasing immunoglobulin's without affecting hematological and biochemical parameters (Munglue *et al*., 2019). In addition to having a detrimental effect on protein metabolism, a low-protein diet reduces digestibility and absorption (Temim *et al*., 1999). Analysis of broiler chicks' hematological and serum biochemical profiles between the ages of 1 and 42 after exposure to probiotic isolates of *Lactobacillus lactis and Lactobacillus plantarum* (Al-Sultan *et al*., 2016).

B. pumilus improves the growth performance and immunological indicators in black goats (Zhang *et al*., 2020). Several research findings reported that *B. pumilus* can enhance growth performance, hematological, biochemical, and immunological indices in aquatic spp., Therefore, this research aims to calculate the impact of a novel strain of *Bacillus* on many key indices of bird's health and performance, including their overall size, blood count, and biochemical profiles.

MATERIALS AND METHODS

The trial was held at the farm of the Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University Tando Jam.

Housing management

The Poultry Experimental Station, Faculty of Animal Husbandry and Veterinary Sciences, Department of Poultry Husbandry, Sindh Agriculture University Tandojam received 180-day-old Cobb chicks in the shed. The house was cleaned with water and sanitized with a lime spray before the chicks arrived to eliminate all the bacteria. The wood shaving litter was spread, and brooders were set up and running to maintain the experimental house's required temperature. The humidity level was maintained between 55-65%, and the house temperature was initially kept at 32°C for the first week before gradually dropping by 2°C each week until it reached 22°C. During the experimental trial, water and food were available as needed. The light was accessible for the duration of the experiment for 24 hours. Regularly examine the health of the chicks. The Pakistan Poultry Association (PPA 2020) states that all chicks received timely immunization.

Dietary Treatments

One hundred eighty (n=180) Cobb chicks were randomly chosen and divided into three treatment groups, each with six replicates and ten birds. Three experimental diets were formulated, including a control diet (CO) fed normal protein (22%), LP (birds fed 2% low protein diet supplemented with 0.01% *B. pumilus*), and BP-2000 (birds fed 2% low protein diet). Forty-two days comprised the experimental period (starter phase: 1–21 days; finisher phase: 22–42 days). Table-1 describes the experimental design, ingredient composition, and analyzed value. Jinan Rentai Import and Export Co., Ltd, a Chinese company, provided the *B. pumilus* for purchase.

Data Collection

Feed analysis

Proximate analysis of the feed sample was performed to assess dry matter, crude protein, crude fat, and ash contents according to the procedure of the Association of Official Analytical Chemists (AOAC, 2005).

Growth performance

The feed was stopped for approximately six hours before recording the weight of the broilers. The weight was recorded at 1-21 and 22-42 days on a replicate basis. The live body weight, average daily gain, and feed intake were recorded. The formulas were used to calculate the FCR (feed conservation ratio) and the average feed intake.

Feed intake: Total feed offered – Total feed refused/ Total number of Birds.
F.C.R: Feed intake / Weight gain.

Carcass Characteristics (%)

Two birds from each replicate were chosen, weighed, and slaughtered at the experiment's conclusion (day 42) to assess the dressing, breast muscle, leg muscle, and abdominal fat percentage. The following formulas were used to determine carcass characteristics.

Dressing % = carcass yield/ live body weight×100
Breast muscle % =breast muscle/ carcass yield weight×100
Leg muscle % =leg muscle/ carcass yield weight×100
Abdominal fat % =abdominal fat/ Carcass yield ×100

Blood biochemistry and hematology

Sample collection

On the last day of the study, 5 ml of blood was collected from the wing vein and placed in vacutainers containing EDTA as an anti-coagulant to prevent clotting. The automated hematological analyzer analyzed the HB, PCV, RBC, and WBC count (Model BIOCELL-86, Biogen GmbH, Berlin, and Germany). Additionally, 5 ml of blood was drawn, and the serum was collected by centrifuging at 2500 rpm for 15 minutes at 4 °C. Then, it was put in storage at -20 °C pending analysis. Using a fully automated biochemical analyzer (Model SMT-120V, Quadratech Diagnostics LTD, East Sussex, UK), the Total Protein (TP), Albumin (ALB), Globulin (GLB), Creatinine (CREAT), Total Cholesterol (TC), High-density lipoprotein (HDL), and Low-density lipoprotein (LDL) were all determined.

Statistical analysis

With SPSS 19.0, the data were statistically analyzed using ANOVA. The Duncan's multiple-range test was used to compare the significant variation in treatment means. They presented the means and the standard error mean.

RESULTS

Growth performance

The supplementation of *B. pumilus* on live body weight is shown in Table-2. Starting from days 1 to 21, Significantly higher LBW was observed in BP-2000 than LP but non-significant with CO ($P<0.05$). In the starter phase, ADG was significantly better in groups BP-2000 than CO and groups LP ($P<0.05$) and FCR was better in LP groups than CO and BP-2000. The experiment will last 22 to 42 days during the second phase. LBW was significantly higher in LP group than CO and BP-2000 and ADG was better in BP-2000 than CO than LP. While FCR of the second phase is higher in LP than CO and BP-2000. The highest LBW and ADG were in the BP-2000 group during the overall experimental period, but FCR was better in LP groups.

Carcass characteristics

Table (3) shows the effects of *B. pumilus* on carcass performance of broiler chickens. Dressing and BM% was significantly different ($P < 0.05$) influenced in BP 2000 in contrast with LP, while no statistical difference was noted with CO. Moreover, the LM was higher in CO group, and AF% was higher in LP group.

Table 1. Feed formulation and nutrient value of basal diets

	Control		**Low Protein (LP)**	
Ingredients%	**Starter**	**Finisher**	**Starter**	**Finisher**
Corn	57.47	58.98	63.94	65.73
Soybean Oil	1.50	4.32	0.52	3.45
Soybean Meal	30.96	25.05	25.51	19.1
C.S.M	5.00	7.00	5.00	7.00
Salt	0.35	0.35	0.35	0.35
$CaPO_4$,	1.53	1.39	1.50	1.40
Limestone	1.54	1.40	1.61	1.44
Lys,	0.24	0.22	0.21	0.26
Meth,	0.14	0.15	0.11	0.14
Cyst,	0.07	0.04	0.05	0.02
Choline,	0.20	0.10	0.20	0.10
Premix,	0.50	0.50	0.50	0.50
Zeolite,	0.50	0.50	0.50	0.50
Total,	100	100	100	100
Nutritional value of diet				
AME (kcal/kg)	2950	3050	2950	3050
Crude Protein (%)	22.00	20.00	20.00	18.00
Lys (%)	1.20	1.05	1.05	0.95
Meth (%)	0.45	0.44	0.40	0.40
Ca (%)	**0.99**	**0.90**	**0.99**	**0.90**
Total P (%)	**0.67**	**0.66**	**0.66**	**0.61**
Avail P (%)	0.45	0.42	0.45	0.42

Pre-mixture offers the following amounts of calories and nutrients per kilogram of food: Pyridoxal 5-Phosphate (D-PA) 11 mg, Folic acid 0.5 mg, biotin 0.12 mg, Vitamin K32.5 mg, Vitamin E 10 IU, Vitamin C 10 IU, Vitamin E 10 IU, Vitamin A 10,000 IU, Vitamin B1 1.8 mg, Vitamin B2 40 mg, Vitamin B12 0.71 mg, Vitamin B3 2000 IU, Vitamin B3 2000 IU, Vitamin V 10 IU, and Vitamin K3 18 mg of ferrous sulfate, manganese sulfate 8 mg of copper sulfate, and 16 mg of make up the iron.

Hematological parameters

Hematological parameters are shown in Table 4, which indicates HB, RBCs, WBCs and PCV%. The results of all treatments did not significantly differ from one another. However, WBCs count was greater in BP 2000-fed birds than CO or LP-fed birds.

Biochemical indices

Biochemical indices were also affected by low proteins, which is shown in Table 5, which shows that serum TP, ALB, and GLB were not affected by dietary supplementation of BP- 2000. Moreover, creatinine also not affected by the addition of BP- 2000. The highest concentration of HDL was observed in BP-2000 followed by control and low protein ($P<0.05$). Furthermore, the lowest concentration of LDL and cholesterol in serum was noted in BP-2000 than LP, no significant difference was recorded with CO.

Table 2. Effect of dietary supplementation of *B. pumilus* on growth performance (gm)

Parameter	Treatments			P.Value
	CO	**LP**	**BP-2000**	
From (1- 21) days				
LBW	810.66±18.03[a]	735.33±31.78[b]	823.66±22.67[a]	0.010
ADG	38.60±0.85[a]	35.01±1.51[b]	39.22±1.07[a]	0.010
ADFI	47.36±1.26[a]	46.12±1.32[a]	45.63±0.96[a]	0.266
FCR	1.22±0.026[b]	1.31±0 .018[a]	1.16±0.030[b]	0.001
From (22- 42) days				
LBW	1705.44±24.32[a]	1677.42±14.16[a]	1705.66±27.67[a]	0.292
ADG	77.52±1.10[a]	76.24±0.64[a]	77.53±1.25[a]	0.292
ADF1	137.96±2.17[b]	143.48±2.13[a]	133.21±2.08[b]	0.003
FCR	1.77±0.09[b]	1.88±0.041[a]	1.71±0.031[b]	0.002
Overall (1- 42)				
LBW	2438.58±27.32[a]	2336.51±30.94[b]	2451.79±7.35[a]	0.002
ADG	58.63±0.65[a]	55.63±0.73[b]	58.37±0.17[a]	0.002
ADF1	92.66±0.48[a]	94.80±1.03[a]	89.42±1.47[b]	0.003
FCR	1.59±0.01[b]	1.70±0.03[a]	1.53±0.02[c]	0.000

Rows of superscripts with various letters varied significantly (P 0.05). LP = low protein, BP-2000 = low protein, ADG = average daily gain, ADFI = average daily feed intake, and FCR = feed conversion ration control +0.01% Bacillus pumillus.

Table 3. Effect of dietary supplementation of *B. pumilus* on carcass characteristics (%)

Rows of superscripts with various letters varied significantly ($P<0.05$). Basal diet is the control. Low protein is

Parameter	Treatments			P.Value
	CO	**LP**	**BP-2000**	
Dressing%	70.91±2.92[a]	69.27±2.17[a]	71.83±5.30a	0.047
BM%	26.83±1.08[a]	24.42±0.96[a]	26.53±2.01[a]	0.049
LM%	19.08±2.59[a]	18.55±3.42[a]	18.71±4.53[a]	0.977
AF%	2.65±1.38[a]	3.83±1.77[a]	3.12±1.09[a]	0.382

denoted as LP or BP-2000. +0.01% Bacillus pumillus. LM = Leg muscles, AF = Abdominal Fat, BM = Breast muscle.

Table 4. Effect of dietary supplementation of *B. pumilus* on Hematology

Parameter	Treatments			P.Value
	CO	**LP**	**BP-2000**	
HB (gm/dl)	10.98±1.34[a]	11.23±1.27[a]	11.46±0.50[a]	0.828
RBCs (106/ul)	3.48±0.61[a]	3.41±0.47[a]	3.6±0.34[a]	0.791
PCV (%)	32.14±5.38[a]	30.62±3.54[a]	32.52±4.68[a]	0.699
WBCs (103/ul)	16275.00±320.15[b]	15300.00±852.4[b]	18175.00±939.41[a]	0.001

Hb = Hemoglobin, RBC = Red Blood Cells, WBC = White Blood Cells, PCV = Packed Cell Volume, CO = basal diet, LP = Low protein, BP-2000 = low-protein +0.01% Bacillus pumillus.

Table 5. Effect of dietary supplementation of *B. pumilus* on biochemical indices

Parameter	Treatments			P. Value
	CO	LP	BP-2000	
TP g/dl	3.65±0.21[a]	3.05±0.60[a]	3.40±0.36[a]	0.186
ALB g/dl	2.05±0.17[a]	1.64±0.45[a]	1.93±0.16[a]	0.183
GLB g/dl	1.60±0.06[a]	1.40±0.19[a]	1.47±0.19[a]	0.297
CREAT g/dl	0.75±0.06[a]	0.79±0.11[a]	0.73±0.03[a]	0.622
TC mg/dl	152.00±7.34[a]	168.25±17.65[a]	105.86±69.62[a]	0.145
HDL mg/dl	37.00±3.26[ab]	31.00±3.65[b]	38.00±2.51[a]	0.020
LDL mg/dl	93.25±3.77[ab]	100.50±5.80[a]	89.50±4.93[b]	0.032

TP = Total protein, Alb = Albumin, GLO = Globulin, TC = Total cholesterol, HDL = High Density Lipoproteins, LDL = Low Density Lipoproteins, CO = basal diet, LP = low protein, BP-2000 = low-protein+0.01% Bacillus pumilus

DISCUSSION

The productivity of chicken has not been used to its full potential due to a lack of food resources and a failure to use the best low-cost technology for low-yielding poultry. In order to maximize feed utilization and reduce the cost per kilogram of live weight, it is crucial to continue raising the feed price from the available feed resources (Rajput *et al*., 2023). Recently, it has been observed that soybean meal, sunflower meal, and maize grain the primary feed ingredients their prices have increased to almost double within 2 to 3 years. The largest component of a broiler diet is protein. Hence an increase in the cost of protein sources will directly affect production costs. Probiotics have direct and indirect effects on the microbiota composition and the host through their immunomodulatory properties (Hardy *et al*., 2013). Probiotics such as Bacillus spp. Increased growth and are commonly used in livestock and poultry feed (Nayak 2010; Cruz *et al*., 2012; Gong *et al*., 2018; Wang *et al*., 2021) found that Bacillus species are helpful for lactic acid-producing bacteria (Leser *et al*., 2008). The growth of dangerous bacteria, such as salmonella, was considerably observed to be inhibited by intestinal environment acidity (Van Immerseel *et al*., 2006). Zhao *et al*. (2019) examined the effect of *B. Pumilus* on broiler performance. The LBW of huge freshwater prawns and striped catfish was shown to be increased by *B. Pumilus*. However, our study gives new insights to the poultry industry to investigate novel probiotics with low-protein diets that could reduce feed costs. In our research, low dietary CP negatively impacted LBW, ADG, and FCR in broilers while broiler growth performance was comparable to that basal diet when *B. pumilus* was supplemented in the feed. The same findings were made by (Pirzado *et al*., 2021), who found that a low-protein diet reduces growth performance while a low-protein diet supplemented with feed additive may improve growth performance and feed efficiency. Another research finding showed that supplementation of 108 CFU/ml *B. pumilus* strains fsznc-09 improved the growth performance ADG and reduced the FCR in mice (Zhang *et al*., 2021).

Probiotics have been suggested to increase growth performance by promoting the release of digestive enzymes and other active compounds (Aslam *et al*., 2024). The above-mentioned evidence showed that including *B. pumilus* in broiler diets benefited growth performance. *Bacillus* bacteria positively impact digestive enzymes, and the multiplication of gut flora leads to improved LBW and FCR. However, our study showed BP-2000 had the highest dressing and BM%, while no difference was observed in LM and AF%. (Pirzado *et al*., 2021), Reported that dressing and BM% improved significantly in birds fed a diet containing *B. pumilus*. Comparable effects were noticed by Zhang *et al*. (2021), originating from *B. pumilus* enhanced the carcass characteristics in broiler chickens. The enhancement of carcass weight and BM% yield may be due to improvement in the multiplication of intestinal microbiota, absorption, and metabolism of nutrients by supplementation of probiotics (Yadav *et al*., 2018). It may be speculated that *B, pumilus* improved the digestive function and nutrient utilization by enhancement of enzymic activity, which alleviated the negative impact of reduced protein.

Scientist reported that AF% was higher in broiler chicken birds fed a CP diet containing fed with dressing, BM, and LM% were lower in broilers with low CP diet because of imbalances of calories and protein. When feeding on probiotic supplementation, hematological parameters are indicators for health condition in fish (Ahmadifar *et al*., 2014). WBCs indicated the health status of broiler chickens (Reda and Selim, 2015). We observed that hematological parameters had no significant differences in HB, RBCs, and PCV% in BP-2000. While the WBCs were higher in BP-2000 group and lowest in CO and LP groups. However, the research findings are similar to those of Alkhalf *et al*. (2010), who found that adding probiotics to the diet of broilers did not affect HB concentration. Another study (Nakayama *et al*., 2009) found no evidence that adding probiotics to broiler diets had any discernible effects on HB or PCV%, Probiotic supplementation feed for fish improves the RBCs and WBCs count as well as produces good effects on immune-stimulating qualities (Firouzbakhsh *et al*., 2011; Dahiya *et al*., 2012). Improvement in hematological parameters may result from fermentation inside the GIT, which changes the pH to an acidic level, which allows the intestine to absorb more iron salt (Beski and Sardary, 2015). Erythropoietin plays an important role in blood; its production increases with iron uptake. Meanwhile, the vitamin B-complex generated by probiotics in the GIT acidic environment positively impacts blood production (Kander, 2004). Biochemical indices are the most important attributes for broiler life; both TP and serum ALB were unaffected by BP-2000. CREAT levels also not affected by BP-2000. HDL levels, were higher in the BP-2000 group, while LDL and cholesterol levels were lower in BP-2000.

According to Abudabos *et al*., (2019), probiotic dietary supplementation significantly decreased serum TC. Other studies noted that adding probiotics to feed reduced the TC level in serum (Siadati *et al*., 2017; Haque *et al*., 2017). Similar findings were noticed by (Pourakbari *et al*., 2016) regarding the reduction of TC and LDL levels in the blood serum of broilers when fed a diet with multi-strain probiotics. Probiotic bacteria may be able to lower cholesterol levels (Mountzouris *et al*., 2010). According to another research, a diet with probiotic supplements may increase TP and ALB levels (Abudabos *et al*., 2019, Dhanalaxmi *et al*., 2007, Yazhini *et al*., 2018). The benefits of using *Saccharomyces cerevisiae* as a probiotic supplement have been demonstrated to lower chicken serum cholesterol levels (Mohan *et al*., 1996). Another study reported that when compared to the CO, using *Moringa olifera* in broiler diets did not significantly lower serum TC and triglyceride levels (Yalcinkaya *et al*., 2008). TP and ALB are enhanced when feeding on *B. subtillus* supplementation in feed (El-Moneim *et al*., 2020). The enhancement in blood concentration TP and ALB increases the activity of enzymes (trypsin and pepsin) and subsequently enhances the absorption and efficiency of protein utilization (He *et al*., 2019).

Conclusion

In conclusion, the current study showed that feeding on low protein with *B. pumilus* in addition to the broiler diet has a positive impact, enhancing growth performance and carcass characteristics and reducing FCR in broiler chickens. Moreover, *B. pumilus* increases the WBCs in broiler chickens. *B. pumilus* with LP diet increased HDL and lowered LDL and TC levels. Adding *B. pumilus* to broilers diet led to improved growth and decreased mortality.

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Author's Contributions

Conceptualization: Shoaib Ahmed Pirzado and Beshart Ali Tunio, **Conduct experimental trial:** Beshart Ali Tunio, and Abrar Ul Hassan Rajput, **Methodology:** Beshart Ali Tunio, Abrar Ul Hassan Rajput, Muhammad Aslam, Zahid Ali Baloch, **Data Analysis:** Imdad Hussain Leghari and Abrar Ul Hassan Rajput, **Supervision:** Shoaib Ahmed Pirzado

Conflict of interest

The research critically examines the intricate web of conflicts of interest that may arise in animal nutrition, exploring the ethical challenges and potential ramifications for scientific integrity and animal health. The study delves into the multifaceted landscape of probiotics in feed, where the intersection of academic pursuits, corporate interests, and financial incentives poses a significant challenge to the objectivity and credibility of scientific investigations

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