

Research Article

Growth Performance, Nutrient Digestibility, and Gut Micro Flora of Japanese Quail Fed Diet Supplemented with *Bacillus megaterium*

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Abstract

The study was carried out to explore the effect of *Bacillus megaterium* (BM) on the growth performance, nutrient digestibility, immune status and gut microflora of Japanese quail. A total of 200 one-day-old healthy quail birds with similar average weight (8.00±2g) were randomly allocated in four dietary treatments with five replicates having 10 chicks each. The dietary treatments were followed as control (Ctrl) fed a basal diet with no probiotic addition, while BM-500 (500 mg/100Kg), BM-750 (750 mg/100Kg) and BM, 1000 (1000 mg/100Kg) fed basal diet supplemented with *B. megaterium* at the dose 500, 750 and 1000 mg/100 kg respectively. The current results demonstrated that birds fed with BM-750 showed an increase in weight gain during starter, finisher, and whole period (P≤0.01). During the starter period no difference was found in feed intake, while in finisher and overall period significant reduced feed intake observed in *B. megaterium* supplemented treatments. Moreover, feed efficiency significant improved in starter (P≤0.05), finisher (P≤0.01) and overall (P≤0.01) periods in BM treatments than Ctrl. The percentage of DM (P≤0.01), CP (P≤0.01) and EE (P≤0.05) digestibility were improved significantly in BM supplemented groups than Ctrl. The highest serum level of Ig A, IgG and IgM was recorded in BM-750 than control. No effect was observed among treatment groups for immune indicators. Significant (P≤0.01) reduction in pathogenic bacteria *E. coli* and *Salmonella* were observed in BM -750, BM-1000, and BM-500 than Ctrl. However, compared with the Ctrl treatment the *Lactobacillus count* was increased in BM treatments (P≤0.01). Conclusively, novel *B. megaterium* probiotics had beneficial effect on growth performance, nutrient digestibility, immunoglobulins and micro biome of J. quail.

Keywords: *Bacillus megaterium*; Growth Performance; Immunoglobulin gut microflora; Japanese quail; Nutrient digestibility



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Introduction

Quail farming is gaining more attention in developing countries due to its nutritious meat and egg production, and it could help overcome the existing gap in meat supply (Sarkar *et al.*, 2023). However, quail production in the poultry industry in Pakistan has

not yet achieved its maximum potential. The growth performance and rearing period of quail is an important and challenging phase for nutritionists.

The poultry industry consistently seeks effective nutritional approaches to enhance the efficiency of quail production (Tan *et al.*, 2012). Additionally, poultry producers are highly concerned about the growth performance and health indicators of quail, given their impact on average weight gain and the overall health benefits. Assessing the growth rate and quality of marketable meat through subjective determination is essential for providing crucial information to sustain this vital industry (Fikry *et al.*, 2021; Ebeid *et al.*, 2021).

The development of the poultry sector has coincided with the intensification of production systems, thereby increasing the exposure of quail to stressors. This heightened exposure may result in the occurrence of diseases and subsequent losses in productivity (Fouladi *et al.*, 2018). The use of antibiotics for prophylaxis and disease control in poultry is even frequently applied as a main source of disease control and prevention (Ahmad *et al.*, 2022; Neveling and Dicks, 2021). Though, investigation of eccentric and more sustainable substitutes, such as use of organic acids, essential oils, probiotics, prebiotics, trace minerals, acidifiers and phytobiotics, for the treatment of disease, as well as growth promoters, is becoming increasingly evident in poultry production (Ahmad *et al.*, 2023; Su *et al.*, 2021; Pirzado *et al.*, 2021; Hazrati *et al.*, 2020).

Classically, in poultry production, probiotics are live microorganism which when adequately administered confer health benefits to host (FAO/WHO, 2002). Probiotics are administered to the animal diet through water or sprays. They are viewed as enhancers of production and growth, as they affect gut microflora positively to improve digestive efficiency by increasing beneficial biome, boosting immune functions and safeguarding against harmful bacteria (Hume, 2011). Therefore, various species of beneficial bacteria have been explored to elucidate and better understand their application in the poultry sector (Pirzado *et al.*, 2021; Zheng *et al.*, 2019). Among them, the *Bacillus* based probiotics *Bacillus megaterium* used frequently in aquaculture (Luo *et al.*, 2020), but still not explored in poultry production.

B. megaterium is a nonpathogenic gram positive, spore forming organism with unique qualities like powerful temperature resistance, stress resistance, feasible storage and enhance the endogenous enzymes. Owing to its rapid weight gain rate in a defined medium, it is one of the most used beneficial organisms in experimental tests in several scientific areas, such as combined genetic analysis and physiology, and immune status of animals (Sayah *et al.*, 2022). In agriculture, it can be used for special purpose wastewater treatment (Luo *et al.*, 2016). In animal feed it can be used as feed supplements for cattle (Deng *et al.*, 2021; Wang *et al.*, 2018), laying hens (Ding *et al.*, 2016) and some species of fishes (Yao *et al.*, 2020).

In poultry production, the understanding that nutritional strategies are important to reduce disease and boost the growth rates has prompted much research (Korver, 2023), meanwhile nutritional aspects are closely associated to the health, welfare, and growth of quail (Manning *et al.*, 2007). Recognizing the imperative to enhance sustainability in poultry production while maintaining economic viability, the primary objective of the current research was to assess the impact of dietary supplementation with *B. megaterium* on the growing period of quail. The study aimed to comprehensively evaluate the

growth performance, digestibility, immune indexes, and microflora, to provide insights into the potential benefits of *B. megaterium* supplementation.

Methodology

Housing management

The research study was conducted at Animal Nutrition Experimental Station, Sindh Agriculture University Tandojam, Pakistan (latitude: 25.4167° N, longitude: 68.5333° E). A total of 200 one-day-old quail chicks was purchased from local hatchery, Karachi, Pakistan, and randomly distributed into four treatments with five replicates having 10 birds in each. Four diets were prepared such as, Control (Ctrl) fed as basal diet with no probiotic addition, BM-500 (BD added with 500mg/100kg feed), BM750 (BD added with 750mg/100kg feed), and BM-1000 (BD added with 1000mg/100kg feed). The house was washed and disinfected accordingly. Rice husk was used as bedding material. Feed and water were provided *adlib*. The experimental facility was equipped with stainless steel cages measuring 25 x 15 x 25 cm replicate, and each bird was allocated a stocking density of 75 cm² /bird. During the first week, the temperature was maintained at 34°C, and in the final week, it was maintained at 24°C. The humidity was maintained within the range of 50% to 70% throughout the entire experiment. Lighting was provided continuously for 24 hours during the first week, followed by 23 hours of lighting throughout the entire experiment. The experiment lasted for 42 days. The *B. megaterium* was provided by Jinan Rentai Company Pvt, Ltd, China.

Table 1. Basal diet formulation for Japanese quail.

Materials	Starter Phase (1-3 weeks)	Finisher Phase (4-6 weeks)
Maize	50.656	56.549
Soya Bean Meal	37.136	32.78
Soya Oil	3.937	3.61
Canola Meal	3.000	3.00
Poultry Meal	1.500	1.50
DCP	1.124	1.03
Limestone	1.110	0.583
Lysine Sulphate 55%	0.838	0.500
Supplement (vit. & mineral)	0.500	0.239
DL Methionine	0.200	0.210
Total	100.001	100.001
Calculated nutrients analysis		
CP	23.00	21.50
ME	2950.00	3050.00
DM	89.32	89.32
EE	6.08	6.08
CF	3.24	3.24
Ash	3.84	3.48
CA	0.60	0.60
Ph(Av)	0.30	0.30

* The premixture providing (one kg of diet) VA 10000 IU, VB1 1.8 mg, VB2 40 mg VB12 0.71 mg, VB3 2000IU, VE 10IU, VK3 2.5 mg, biotin 0.12mg, folic acid 0.5mg, D-PA 11mg, cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 18 mg, Mn (as manganese sulfate) 16 mg, Zn (as zinc sulfate) 40 mg, I (as podetium iodide) 0.35 mg & Se (as sodium selenite) 0.15 mg.

Growth performance

The parameters related to growth performance, including live body weight, feed intake,

and feed conversion ratio, were measured at 21 and 42 days of age. The determination and calculation of these parameters were conducted following the approach outlined by El-Kholy *et al.* (2018). Below mentioned formula was used to determine feed conversion ratio.

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

Nutrient digestibility

Three birds from each replicate were selected and shifted to metabolic cages for excreta collection. Digesta excreta samples were collected for three consecutive days (39-41). The samples were dried in a hot air oven at 65 °C for 2 days. Furthermore, excreta and feed samples were grinded and homogenized for the analysis of dry matter (DM), crude protein (CP), and fat according to reported methods (AOAC, 2005). The following formula was used to determine digestibility.

$$\text{Nutrient digestibility \%} = \frac{(\text{Nutrient in diet} - \text{Nutrient in excreta})}{\text{Nutreint in diet}} \times 100$$

Immunoglobulin

Two birds were chosen from each replicate and slaughtered using the Halal method as outlined in the procedure documented by Mughal (2020). 5 ml blood was collected in sterile vacutainers and left to clot for 04 hours. The serum was centrifuged at 2500 rpm for 15 minutes at 4 °C. The samples were immediately frozen into -20 °C for analysis of immunoglobulin. The immune response including IgA, IgG and IgM were evaluated by chicken specific ELISA kits instruction (Shanghai Lengton Bioscience Co, LTD, Shanghai China).

Intestinal microbiota

On the 42 day of experiment, two healthy birds from each replicate were selected for collection of intestinal contents. The samples were placed in sterile tubes and immediately stored in the deep freezer for further analysis. One gram of intestinal content was poured in sterilized tubes and tenfold dilution was prepared by using buffer peptone water on MRS Agar, SS Agar, and MacConkey Agar for identification *E. coli*, *Salmonella* and *Lactobacillus* respectively. Furthermore, plates were incubated for 24 hours at 37 °C for bacterial growth. The bacterial count of *Lactobacillus* *E. coli* and *Salmonella* were counted by using Colony counter according to the described method (Cao *et al.*, 2013).

Statistical analysis

The data were tabulated in excel and analyzed by one way ANOVA using SPSS 19. The means and means of standard errors were expressed. For significance of result the Tukey test was applied. The P value is set at ≤ 0.05.

Results

Effect of different treatments on the growth performance of Japanese quail

As described in table 2 the weight gain was increased ($P \leq 0.05$) in experimental treatments BM-750, BM-1000 and BM-500, during starter phase (1-21 days), finisher phase (22-42 days) and overall period (1-42 days). No difference was observed in feed intake during starter phase, while the highest feed intake recorded in Ctrl than BM-500, BM-750, and BM-1000 during finisher and overall period ($P \leq 0.05$) compared to the Ctrl. The better FCR was observed in *B. megaterium* supplemented treatments than Ctrl during starter period, however in finisher and overall periods FCR was improved in birds received diet

with BM-750 than other groups ($P \leq 0.05$).

Table 2. Effect of dietetic addition of *Bacillus megaterium* on growth performance of Japanese quail.

Treatment	Ctrl	BM-500	BM-750	BM-1000	SEM	P-Value
Weight Gain (WG)						
1-21 days	69.91 ^b	74.94 ^a	76.80 ^a	74.96 ^a	5.951	0.034
22-42 days	96.41 ^c	103.39 ^b	116.36 ^a	109.30 ^b	6.384	0.011
1-42 days	166.33 ^c	178.33 ^b	193.67 ^a	184.26 ^b	8.190	0.001
Feed Intake (FI)						
1-21 days	132.10 ^a	132.76 ^a	131.86 ^a	132.10 ^a	6.555	0.963
22-42 days	327.11 ^a	319.00 ^b	315.00 ^b	317.96 ^b	9.061	0.001
1-42 days	459.21 ^a	451.76 ^b	444.73 ^b	450.06 ^b	13.07	0.002
Feed Conversion Ratio (FCR)						
1-21 days	1.88 ^a	1.77 ^b	1.71 ^b	1.76 ^b	0.241	0.049
22-42 days	3.39 ^a	3.09 ^b	2.69 ^c	2.92 ^b	0.887	0.007
1-42 days	2.76 ^a	2.53 ^b	2.29 ^c	2.44 ^b	0.553	0.0001

^{a,b,c}, different superscripts are significantly different; Ctrl=control; BM-500= *B. megaterium* 500 mg/100 kg feed; BM-750= *B. megaterium* 750 mg/100 kg feed; BM-1000= *B. megaterium* 1000 mg/100 kg feed; SEM= standard error mean; WG=weight gain; FI= feed intake; FCR= feed conversion rate.

Effect of *Bacillus megaterium* on digestibility of Japanese quail

Table 3 showed the effect of *B. megaterium* on the digestibility percentage of Japanese quail. The results showed that DM, CP, and EE digestibility percentage were significantly ($P \leq 0.05$) higher in BM-750 followed by BM-500 and Ctrl treatments, while no significant effect observed with BM-1000. Moreover, Ctrl and BM-500 group showed no significant difference for fat digestibility.

Table 3. Effect of nutritional addition of *Bacillus megaterium* on nutrient digestibility of Japanese quail.

Treatment	Ctrl	BM-500	BM-750	BM-1000	SEM	P-Value
DM %	66.17 ^c	68.21 ^b	71.09 ^a	70.48 ^a	4.88	0.001
CP %	63.05 ^c	64.27 ^b	66.98 ^a	66.14 ^a	3.47	0.003
E.E %	58.62 ^b	59.12 ^b	61.94 ^a	61.34 ^a	2.43	0.015

^{a,b,c}, different superscripts are significantly different; Ctrl=control; BM-500= *B. megaterium* 500 mg/100 kg feed; BM-750= *B. megaterium* 750 mg/100 kg feed; BM-1000= *B. megaterium* 1000 mg/100 kg feed; SEM= standard error mean; DM= dry matter; CP= crude protein; EE= ether extract.

Effect of *Bacillus megaterium* on immunity of Japanese quail

Immune indexes of Japanese quails are described in Table 4. The highest serum level of Ig A, IgG and IgM was recorded in BM-750 than control ($P \leq 0.05$). No effect was observed among treatment groups for immune indicators.

Effect of *Bacillus megaterium* on gut microflora of Japanese quail

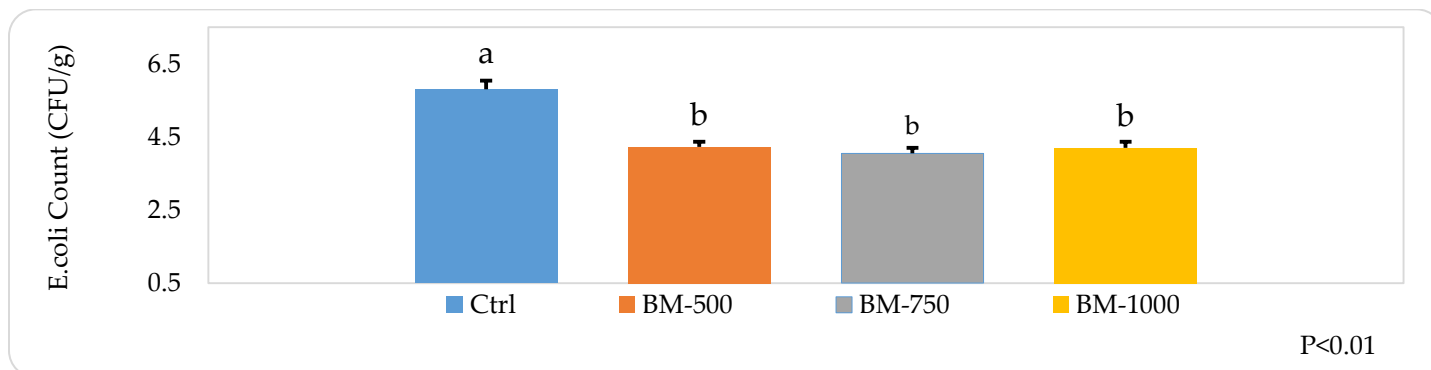
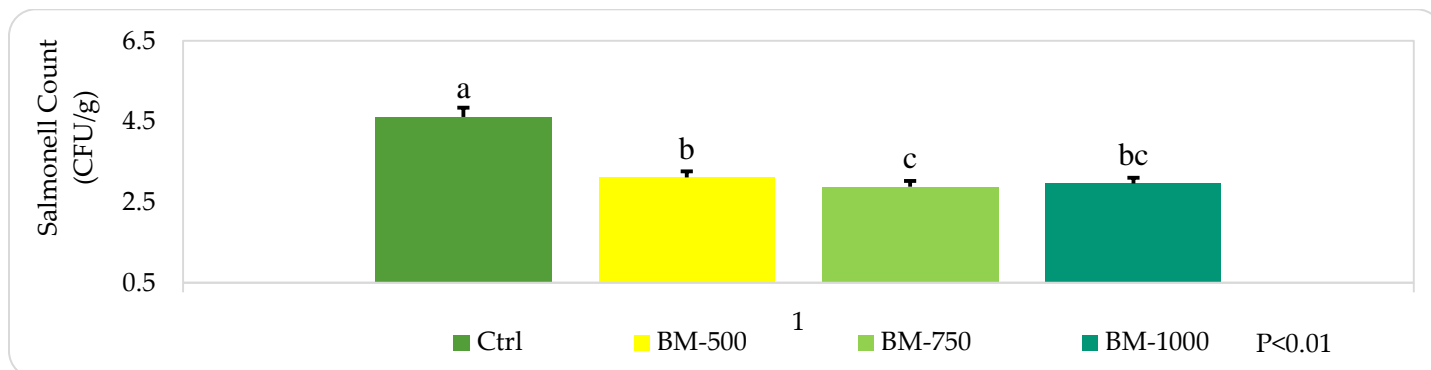
The *E. coli* count as induced by dietetic doses of *B. megaterium* remains shown in figure 1. Significant ($P < 0.01$) reduction of *E. coli* count was noted in quail birds supplemented with all dietary treatments (BM-500, BM-750, and BM-1000) compared Ctrl treatment, while no significant difference was found among all groups added with *B. megaterium*.

The dietary addition of *B. megaterium* significant reduction ($P < 0.05$) in *salmonella* count in BM-750 followed by BM-1000 and BM-500 (Figure 2). However, birds of Ctrl treatment recorded the highest *salmonella* count.

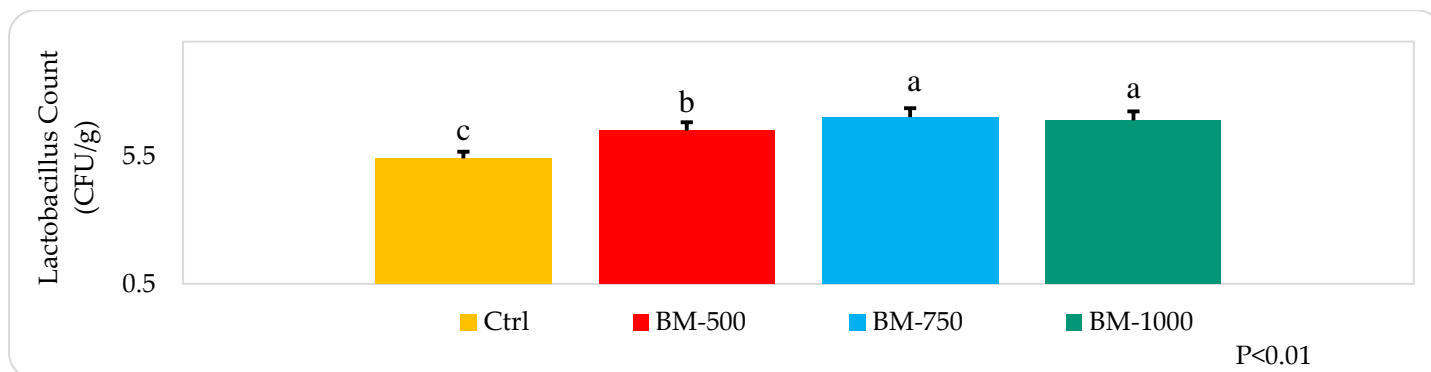
Table 4. Effect of nutritional addition of *Bacillus megaterium* on serum immunoglobulin of Japanese quail (mg/ml).

Treatment	Ctrl	BM-500	BM-750	BM-1000	SEM	P-Value
IgA	0.52 ^b	0.70 ^a	0.74 ^a	0.71 ^a	0.022	0.038
IgG	0.96 ^b	1.43 ^a	1.61 ^a	1.40 ^a	0.050	0.017
IgM	1.35 ^b	2.66 ^a	2.78 ^a	2.73 ^a	0.099	0.001

^{a,b,c}, different superscripts are significantly different; Ctrl=control; BM-500= *B. megaterium* 500 mg/100 kg feed; BM-750= *B. megaterium* 750 mg/100 kg feed; BM-1000= *B. megaterium* 1000 mg/100 kg feed; SEM= standard error mean; IgA=Immunoglobulin A, IgG=Immunoglobulin G, IgM=Immunoglobulin M.

Figure 1. Effect of dietary addition of *Bacillus megaterium* on intestinal *E. coli* count of Japanese quail (CFU/g).Figure 2. Effect of dietetic addition of *Bacillus megaterium* on intestinal *Salmonella* count of Japanese quail (CFU/g).

The data depicted in Figure 3 reveal that all treatment groups significantly improve the *Lactobacillus* count than Ctrl treatment. The maximum count of *Lactobacillus* was experiential in BM-750 follow by BM-1000 and BM-500.

Figure 3. Effect of nutritional addition of *Bacillus megaterium* on intestinal *Lactobacillus* count of Japanese quail (CFU/g).

Discussion

Probiotics are feed supplements that balance the gut microflora, increases intestinal digestion and absorption, enhances production performance and FCR of Animals (He *et al.*, 2017; Zaghari *et al.*, 2020). Few studies revealed that *B. subtilis* improved growth performance of broilers (Zhang *et al.*, 2014). A research study revealed that the addition of probiotics in quail's diet with dose of 100, 150, 200 and 250 mg/kg influence growth and feed efficiency (Gao *et al.*, 2017). *B. megaterium* has great influence on growth performance and feed efficiency of animals. There are very limited studies carried out on supplementation of *B. megaterium* on Japanese quail and other poultry birds. In our research study the supplementation of *B. megaterium* probiotic improves weight gain and feed efficiency, while feed intake was reduced significantly in quail birds. Similar results were reported that *B. megaterium* coated feed improved the production performance and decreased FCR in *Songpu mirror carp* (Afrilasari and Meryandini, 2016). Moreover, Parthasarathy and Ravi (2011) also noted that inclusion of *B. megaterium* in the diet of *Catfish* significantly increases weight gain and reduced FCR. Tan *et al.* (2012) also noted improvement in live body weight gain in quails diet fed with different doses 50,100,150 and 200 mg/kg of lactobacillus strains. Probiotics improve intestinal structure, produces immunity to shield pathogenic bacteria and improving the growth and performance (Li *et al.*, 2011). It is assumed that supplementation of *B. megaterium* probiotic increase in growth performance may be due to triggering secretion of digestive activity of enzymes, enhance the nutrient digestibility which ultimate improves the growth performance. The above discussion gave evidence that supplementation of *B. megaterium* in quail's diet has beneficial outcomes on growth performance. Nutrient digestibility is the main indicator to determine the digestive efficiency and growth performance of animals. High digestibility rate improves the feed efficiency and increases farm economics. In our research study the highest digestibility percentage of DM, CP and EE was recorded in birds diet supplemented with *B. megaterium* than Ctrl. In agreement with our findings, Abdel-Moneim *et al.* (2020), demonstrated that a significant increase in nutrient digestibility of quails fed diet with probiotics containing *B. subtilis* spore. Additionally, dietary addition of *B. subtilis*, *Clostridium butyricum* and *Lactobacillus acidophilus* endospores enhanced the digestibility coefficient of DM, CP, and EE (Hossain *et al.*, 2015; Gao *et al.*, 2017). The increase in nutrient digestibility might be justified by the improvement in digestive enzymic activity such as protease, amylase, and lipase, higher activity of digestive enzymes enhances the digestion of protein, CHO and fats, which could reflect the improvement in growth performance in our study (Li *et al.*, 2014; Manafi *et al.*, 2016; Luo *et al.*, 2020). The possible mechanism for improved digestibility of quails by *B. megaterium* are the supplementation of *B. megaterium* in the feed can increased the activity of digestive enzymes which supports the digestion and degradation of nutrients in feed, make up it easier for quail bird to absorb the nutrients in the feed. The main reason for the addition of beneficial bacteria to the quail birds is to decrease infections triggered by pathogens by the process of inevitable elimination, in which microbes fight for space and food. Therefore, the early supplementation of beneficial microbes for inevitable elimination has been suggested as a method to inhibit the deleterious

infections. There are numerous studies suggested that probiotics containing beneficial microbes to decrease the multiplication of the GI tract of poultry by disease causing bacteria (Purba *et al.*, 2020; Bashir *et al.*, 2020). In our research experiment intestinal count of *E. coli* and *Salmonella* were reduced, while *Lactobacillus* improved significantly. These findings are in accordance with the findings of Luo *et al.* (2020), who stated that addition of *B. megaterium* in diet of *Songpu mirror carp* increase the *Lactobacillus* population and reduce the pathogenic bacteria in intestine. Additionally, Mirza (2020) found significant rise in *Lactobacillus* count and decrease multiplication of *coliform* bacteria in local quails fed diets with *Lactobacillus animalis* and commercial multi-strain probiotics. Inulin supplementation increased *Lactobacillus* count in ceca and declined *Salmonella* population (Micciche *et al.*, 2018). Moreover, Mohapatra *et al.* (2012) renowned to entire population of harmful microbes decreased in GIT of fish after supplementation of probiotic in feed. Feeding probiotics maintains intestinal microflora and health of animals. Bacilli species are aiding in the multiplication of beneficial bacteria (lactic acid) and helpful in reducing intestinal pH (Knarreborg *et al.*, 2002). It can be hypothesized that *B. megaterium* may immediately utilize the oxygen in intestinal tract, boost the multiplication of beneficial microbes *Lactobacillus* and *Bifidobacterium* and fatty acid production which can effectively decrease the pH on intestine and reduction of harmful bacterial multiplication like *E. coli* and *Salmonella*. The immune status may be evaluated by assessing the serum immunoglobulins (A, G and M) and play vital role in the bird's health status, aiding to protect the bird from pathogens and other toxic substances (Zaghari *et al.*, 2020). The current data indicated that serum immunoglobulins activity was higher in treatments groups within normal ranges than Ctrl.

Conclusion

The outcome of the current research shows that the supplementation of *B. megaterium* in quail diets improves growth performance, nutrient digestibility, and *Lactobacillus* count. *B. megaterium* also had influence on immune indexes. Moreover, the inclusion of *B. megaterium* in quail feed reduced the *E. coli* and *Salmonella* counts in GIT.

Conflict of Interest

All Authors declared no conflict of interest.

Authors Contributions

Rani Abro and Shoaib Ahmed Pirzado; Conceptualize the study, Muhammad Aslam; conducted the experimental trail, Muhammad Umer and Khush Hal Meghwar; sample collection, Abrar Ul Hassan and Huma Rizwana; conducted labortrical analysis, Muhammad Aslam; statistical analysis of data, Muhammad Aslam; wrote the first draft of the manuscript, Shoaib Ahmed Pirzado; revised and edited the manuscript.

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