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Improving the Growth Performance and Nutrient Utilization in Broilers Fed Low Protein Diet: The Effect of *Bacillus pumilus* on Digestibility and Microflora Dynamics

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SAP and SAS conceptualized and designed the study. SAS and MA performed the data curation. MBA and MAF formal analysis and methodology. SAS and GR wrote the manuscript. SAP and KMA-A critical revision and editing of the manuscript.

Key words
Low protein, Broilers, Growth, Microflora, *Bacillus pumillus*, Digestibility

ABSTRACT

The main purpose of this experiment was to evaluate the effect of dietary addition of *Bacillus pumilus* on growth performance, nutrient digestibility and microflora of broiler chickens fed low protein diet. A total of 180 one-day-old male chicks were randomly distributed into three treatment groups having 60 birds each (six replicates of 10 birds). Experimental diets were (i) a basal (CON) diet (22% crude protein (CP) in the starter phase and 20% CP in the finisher phase), (ii) a low protein (LP) diet with 20 and 18% CP in each starter and finisher phases, respectively, and (iii) a LP-0.01 diet supplemented with *Bacillus pumillus* @ 0.01% of diet. The diets were offered according to a statistical design for how many days. The diets were offered using the automatic feeding and watering system. The birds were kept in an environment-controlled shed. The birds consuming the LP-0.01 diets significantly increased their live body weight (LBW) and average daily gain (ADG) whereas reduced feed conversion ratio ($P<0.05$) compared with control. Carcass, breast- and leg-muscle percentages were ($P<0.05$) higher in the birds consuming LP-0.01 than LP but not different from the control. The LP-0.01 diets had the highest availability of dry matter (DM), CP, fat and ash ($P<0.05$) and villus height of jejunum ($P<0.05$). The LP-0.01 diets reduced the count of pathogenic bacteria, *Salmonella* and *E-Coli*, while increased the *Lactobacillus* count ($P<0.05$). It is concluded that supplementation of *Bacillus pumilus* in low protein broiler diets counterbalances the effects arising from low protein and is equally beneficial to traditional broiler diets in terms of growth performance, nutrient digestibility, and microflora.

INTRODUCTION

Due to the shortage and high prices of red meat, chicken meat is the alternative economical source of good quality animal protein in Pakistan, and this sector is rarely bringing the gap between supply and demand in the meat protein market. Poultry ration formulation is challenging because it uses protein and energy sources of very high quality and cost. A balance between the cost and quality of the ration ingredients forces a farmer either to reduce the content of protein in the ration or try to find cheap alternatives (Davies *et al*., 2010). Therefore, the use of cheap protein ingredients at optimum levels will not only reduce the cost of production but also environmental pollution by decreasing the excretion of undigested nitrogen into the environment. Variable results have been mentioned in literature regarding the use of low dietary protein levels in poultry without a certain conclusion for

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the reader (Rehman *et al*., 2017; Yang *et al*., 2015). Some studies reported that growth performance and carcass quality traits were negatively affected by lowering the dietary protein levels by 2 to 3%. Others reported that low protein broiler diets impair digestion, structure of intestinal villi, and protein metabolism (Pirzado *et al*., 2021).

Probiotics based on bacterial products have been successfully used in broiler diets leaving beneficial effects on the host. All bacterial-based probiotics are not the same in their effects, however, commonly, they are known for increasing gut and beneficial bacterial enzyme activities, gut integrity and maturation and immune function, and decreasing the unwanted bacterial enzyme activity and inflammation (Laudadio *et al*., 2012; Awad *et al*., 2017). *Bacillus* species are the most commonly used probiotics in chicken diets, however, other species including *Bacillus pumilus, Lactobacillus, Streptococcus, Bifidobacterium* and *Aspergillus* have also been used (Alagawany *et al*., 2016; Bughio *et al*., 2021).

Bacillus pumilus is increasingly being employed as a feed additive in fish diets. Fish and mice fed *B. pumilus* had higher body weights than those fed on a control diet. Supplementation of *B. pumilus* in fish's diet prevents the growth of pathogenic bacteria. Although a large number of microorganisms are utilized as probiotics in chicken production, the gastrointestinal tract has a low pH value and a high bile salt concentration (Soomro *et al*., 2019). *Bacillus species*, which survives in extreme gastrointestinal environments through spore formation, is gaining more attention as a probiotic in animal feed (Aslam *et al*., 2024). With its longer shelf life and greater stability, *Bacillus strains* has a significant advantage over its competitors in the feed industry (Purba *et al*., 2020; Li *et al*., 2020). Nutritional digestibility, immune modulation, and increased nutrient absorption are just a few of the advantages of supplementing *B. subtilis* in chicken feed. Different types of probiotic bacteria have distinct characteristics. The effects of *B. subtilis* strain-specific, as well as the mechanisms by which they function, remain unknown. Research on *B. subtilis* strain-specific impacts on broiler performance and intestinal physiology underscores the need for further investigation on the different *B. subtilis* strains to better understand their mechanisms of action. Anti-clostridium spp. activity in *B. subtilis* is often beneficial to broiler performance. The prospect of reducing the cost of feed by using a *Bacillus*-based probiotic is intriguing enough to warrant further investigation. However, no research has been conducted on broilers supplemented with *B. pumilus* in a low-protein diet. Therefore, this study has been designed to examine whether the dietary addition of *B. pumilus* influences growth performance, nutrient digestibility, and intestinal microflora of broilers when fed a low-protein diet.

MATERIALS AND METHODS

Chicks and their maintenance

A total of 180 healthy day-old (Arber Acres) chicks were purchase from a local hatchery and maintained in brooders at 32°C during 1st week and it was gradually decreased by 2°C per week till it reached 22°C, the relative humidity was maintained between 55 to 65% and the light for 24 h during the whole experiment. All birds were vaccinated according to the schedule recommended by Pakistan Poultry Association (PPA).

Dietary treatments

The chicks were randomly distributed into three treatment groups having 60 birds each (six replicates of 10 birds). Experimental diets were (i) a basal (CON) diet (22% crude protein (CP) in the starter phase (0-21 days) and 20% CP in the finisher phase (22-42 days), (ii) a low protein (LP) diet with 20 and 18% CP in each starter and finisher phases, respectively, and (iii) a LP diet supplemented with *Bacillus pumillus* (Jinan Rentai import export co. China) 0.01% of diet (LP-0.01) as shown in Table I. The diets and water were offered *ad libitum*.

Sampling and feed analysis

Feed samples were collected bi-weekly and subjected to proximate analyses to determine DM, CP, crude fat and ash content following the procedures (AOAC, 2000). Live body weight (LBW) and feed intake were recorded every week. The feed conversion ratio (FCR) was calculated according to the formula FCR = Feed intake/weight gain

Carcass characteristics were determined by slaughtering two birds per replicate at the end of the experiment. The LBW and carcass weight were recorded. The dressed percentage was calculated by following formula:

Dressing percentage = (Carcass yield)/ (Live body weight) ×100

Relative weight of breast muscle, leg meat and abdominal fat were calculated by using following formula:

Relative weight of each part = (Weight of each part)/ (Carcass weight) ×100

Apparent nutrient availability

For the determination of nutrient availability, three birds per replicate were transferred to a separated pen on 39th day of the experiment for feces collection. Fecal samples were collected for 3 consecutive days and compose of each treatment. The fecal samples were then dried in an oven at 65°C and ground thereafter. The dried and ground samples were analyzed for proximate composition as described previously. The following formula was used for determination of availability of nutrients.

$$\text{Availability of nutrients } \% = \frac{\text{Nutrient in feed} - \text{Nutrient in feces}}{\text{Nutrient in feed}} \text{ X } 100$$

Table I. Feed formulation and nutrient composition of basal diets.

Ingredients%	Control		Low protein[1]	
	Starter	Finisher	Starter	Finisher
Corn	57.47	58.98	63.94	65.73
Soybean oil	1.50	4.32	0.52	3.45
Soybean	30.96	25.05	25.51	19.1
CSM	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 (%)	1.200	1.050	1.050	0.950
Meth (%)	0.450	0.440	0.400	0.400
Ca (%)	0.990	0.904	0.990	0.906
Total P (%)	0.679	0.669	0.661	0.61
Avail P (%)	0.450	0.42	0.450	0.42

*The pre-mixture 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 and Se (as sodium selenite) 0.15 mg. [1]The low protein diet was supplemented with *Bacillus pumillus* @ 0.01% of diet as a third treatment.

Morphometry of jejunum

The whole small intestine was dissected from the slaughtered birds carefully, the jejunum was separated and flushed with 0.9% saline to remove the content and then fixed in 10% formalin solution for further analysis. Specimens were rinsed in running tap water for a few hours after being fixed for 12 to 24 h, then dehydrated in alcohol and xylene, embedded in paraffin, cut into 2 to 5 mm thick slices, mounted on glass slides, and stained with hematoxylin and eosin. Stereoscopic microscope at a 4X was used to determine the villus height and crypt depth, using Opticam vision lite 2.1 software.

Intestinal microflora

The intestinal content from whole small intestine was taken in test tubes from slaughtered birds and stored in a deep freezer. The count of *Lacto bacillus*, *E. coli* and *Salmonella* was conducted in lab (Khan *et al*., 2022). Standard plate count method was used to figure out the total number of surface-viable bacteria cells. The number of colony-forming units per gram (cfu/g) was used to show how many bacteria were in the intestinal content.

Statistical analysis

The data were statistically analyzed according to one-way ANOVA using SPSS® 19.0 software. Duncan's novel multiple-range test was used to compare the significant variations in treatment means. The data are presented as least square means with standard errors of the mean.

RESULTS

Significant effect of dietary treatments was found on all parameters of growth performance except FCR during starter phase (Table II). Significantly higher LBW was observed in LP-0.01 than LP but non-significant with Control ($P<0.05$). In starter phase, ADG and FCR were significantly better in groups LP-0.01 and Control than group LP ($P<0.05$). A similar trend was observed during the overall period of experiment. LBW and ADG were significantly higher in Control and LP-0.01 than LP while FCR of both groups statistically decreased than LP. During overall experimental period, highest LBW and ADG with better FCR were found in LP-0.01 ($P<0.05$).

Table II. Effect of dietary treatments on growth performance in broilers (g).

Parameters	Control	LP	LP-0.01	P. value
Starter (1-21 day)				
LBW	810.67±21.41^{ab}	755.67±25.33^{b}	823.67±31.09^{a}	0.023
ADG	38±0.04^{a}	35±0.08^{b}	39±0.06^{a}	0.010
ADFI	47±0.19^{a}	46±0.27^{a}	45±0.15^{a}	0.266
FCR	1.22±0.01^{a}	1.31±0.01^{b}	1.16±0.07^{a}	0.001
Overall (1-42 day)				
LBW	2517.3±42.06^{a}	2296.8±35.05^{b}	2611.9±39.09^{a}	0.008
ADG	58.897±1.56^{a}	53.622±1.29^{b}	61.155±1.05^{a}	0.008
ADFI	91.587±2.20^{a}	87.037±2.95^{a}	92.460±1.76^{a}	0.269
FCR	1.55±0.03^{b}	1.62±0.01^{a}	1.51±0.08^{b}	0.001

[a,b,c]Superscripts with different letters in rows and columns varied significant ($P<0.05$). Control, basal diet; LP, low protein; LP-0.01, low protein +0.01% *B. pumilus.* LBW, live body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Table III. Effect of dietary treatments on carcass characteristics (%) in broilers.

Param-eters	Control	LP	LP-0.01	P. value
DP	71.126±0.983a	66.545±0.328b	72.794±0.726a	0.002
BM	25.027±0.509^{a}	21.729±0.579^{b}	24.554±0.491^{a}	0.009
TM	17.077±0.187^{a}	14.676±0.571^{b}	17.543±0.198^{a}	0.003
AF	1.8479±0.096^{b}	2.3347±0.067^{a}	1.3870±0.101^{c}	0.001

For abbreviation and statistical details, see Table II. DP, dressing percentage; BM, breast muscle; TM, thigh muscle; AF, abdominal fat.

Table III shows data regarding carcass characteristics of dietary treatment. The highest dressing percentage was observed in LP-0.01 ($P<0.05$) followed by control. Similarly, the highest relative weight of LM was found in LP-0.01 and lowest in LP ($P<0.05$). While highest relative weight of BM and abdominal fat were observed in Control and LP respectively ($P<0.05$).

Table IV shows the significant effect of dietary treatments on nutrient availability. The highest DM, crude protein, crude fat and ash availability were recorded in LP-0.01 than LP and control respectively ($P<0.05$). Availability of all nutrients studied was significantly increased with supplementation of *B. pumilus* in LP-0.01 ($P<0.05$).

Table IV. Effect of dietary treatments on availability (%) broilers.

Param-eters	Control	LP	LP-0.01	P. value
DM	72.373±0.8^{8a}b	70.730±0.52^{b}	74.540±0.37^{a}	0.015
CP	65.240±0.34^{ab}	63.810±0.29^{b}	65.966±0.5^{2}a	0.022
CF	61.278±1.20^{ab}	60.000±0.96^{b}	64.772±0.99^{a}	0.045
Ash	62.073±0.55^{a}	59.366±0.43^{b}	63.123±0.33^{a}	0.003

For abbreviation and statistical details, see Table II. DM, dry matter; CP, crude protein; CF, crude fat.

Table V. Effect of dietary treatments on intestinal (jejunum) morphology (µm) broilers.

Parameters	Control	LP	LP-0.01	P. value
Villus height	604.42± 55.65^{ab}	582.58± 81.80^{b}	837.79± 36.33^{a}	0.044
Crypt depth	168.20± 7.40^{a}	149.28± 14.90^{a}	163.45± 6.43^{a}	0.451

For abbreviation and statistical details, see Table II.

Table V showed results of jejunum morphology, related parameters showed results on villus height and crypt depth in broilers. The highest villus height was recorded in LP-0.01 in comparison with LP, while no significant difference was observed in control ($P<0.05$). Moreover, no significant difference was found in crypt depth among all treatments.

Table VI showed data regarding gut microbiology that indicated pathogenic bacteria counts were decreased and *Lactobacillus* were increasing in LP-0.01 while lowest in Control. The highest population of *Salmonella* was observed in Control followed by LP and lowest in LP-0.01. A similar trend was observed for a population of *E. coli*.

Table VI. Effect of dietary treatments on gut microbiology (cfu/gm) broilers.

Parameters	Control	LP	LP-0.01	P. value
Salmonella	4.33±0.39^{a}	4.00±0.22^{a}	1.00±0.17^{b}	0.027
E. coli	5.33±0.39^{a}	4.67±0.43^{a}	2.33±0.33^{b}	0.002
Lactobacillus	6.53±0.66^{b}	5.47±0.88^{c}	9.00±0.57^{a}	0.003

For abbreviation and statistical details, see Table II.

DISCUSSION

Due to continuous inflation commodity prices are increasing day by day which increases the cost of production of broiler meat. Recently it has been observed that major feed ingredients like soybean meal, canola meal, sunflower meal, and maize grain prices have increased to almost double within a span of just 3 to 4 years. Protein is a major component of a broiler diet and an increase in prices of protein sources will directly impact on cost of production. Various substitution methods and feed supplementation are being tried worldwide to reduce the proportion of protein in feed like different feed additives, enzymes and probiotics. *Bacillus* species are helpful for the growth of lactic acid-producing bacteria (Tsai *et al*., 2019) and reduce the pH of intestine. Intestinal environment acidification was significantly observed to inhibit the growth of harmful bacteria e.g. *Salmonella* (Aron-Wisnewsky *et al*., 2021).

The effectiveness of probiotics is influenced by a wide range of factors, including strain selection, administration level, application method, ability of the selected strain to survive at ambient temperature, long-term storage and viability, fermentation method, and type of substrate used for probiotic microbe growth (Zhao *et al*., 2019). Susceptible to high temperatures and challenging storage conditions, *B. pumilus* thrives in these environments. As a probiotic strain for chickens, *B. pumilus* is universally accepted as safe (Zhang *et al*., 2021). *Bacillus* strains were shown to be beneficial to broilers, layers, and turkeys in various studies (Hassan *et al*., 2021). In this study, the effects of *B. pumilus* on broiler performance were examined. Despite extensive research on *B. subtilis* strains, the impact of *B. pumilus* on broilers has only been documented a handful of times. Probiotics have been found to have a long-term effect on growth in other studies. The BW of huge freshwater prawns and striped catfish was shown to be increased by *B. pumilus* (Jayaraman *et al*., 2013). The disparities in findings may be due to differences in strains investigated, probiotic dosage, feed mix, and rearing environment.

B. pumilus strains did not influence growth performance during the first three weeks of life (days 1–21). Even so, LBW and ADG were high in LP-0.01 groups and slightly lower in control and LP groups from day 21 to 42, while ADFI and FCR were significantly higher at day 21 in the control group and lower in the LP-0.01 groups overall, while FCR was significantly higher in the control group than the LP-0.01.

There have been limited studies on supplementation of *B. pumilus* in poultry. Our study provided a new way of finding an effect of *B. pumilus* with low protein. In our research, low dietary CP showed negative impact on LBW, ADG and FCR in broiler while *B. pumilus* supplementation in feed of broiler showed growth performance results similar to basal diets. Similar results revealed by (Saied *et al*., 2022) supplementation with reduced dietary CP thus supplementation of *B. pumilus* may have a positive impact on these parameters. This results also by (Hassaan *et al*., 2021) verified that *B. pumilus* addition noticeably augmented the BW, ADG, and reduced FCR in broilers. It is speculated that improvement in growth performance is possibly ascribed to triggering the secretion of numerous digestive enzyme activities and additional secreted active substances by *B. pumilus* probiotics. The abovementioned evidence is taken together that addition of *B. pumilus* in broiler diet had beneficial results on growth performance.

Dressing percentage EV, BM and LM percentage were decreased in broiler chickens fed with a low CP diet, while abdominal fat was higher in birds fed with a low CP diet line with our study this may be due to an imbalance of energy and protein. The significant effect of dietary treatment on all parameters of carcass characteristics in this study with supplementation of *B. pumilus* in low CP diet. The highest dressing percentage was observed in LP-0.01 followed by Control. Similarly, the highest relative weight of the leg was found in LP-0.01 and lowest in LP. While the highest relative weight of breast and abdominal fat were observed in Control and LP, respectively. Our findings agree with (Yadav *et al*., 2018) noted that supplementation of probiotics efficiently amplified the carcass percentage and depressed the AF rate in broilers. Furthermore, (Ghasemi-Sadabadi *et al*., 2019; Khan *et al*., 2022) noticed that dietary addition of *B. subtilis* (DM 03, TAM 04 and IQB 350) had a positive effect on breast muscle yield. Authors also found that poultry diet incorporation with probiotics may improve the thigh muscles weight and reduce the AF content (Tsai *et al*., 2019). Supplementation of *B. pumilus* in diet can improve digestion and absorption due to greater intestinal microflora and activity, increased nutrients intake and decreased abdominal fat content. The lower abdominal fat content indicated that *B. pumilus* may led to greater economic productivity and the public wants to lower fat meat.

Nutrient digestibility plays a significant role in the animal feed industry as a higher digestibility rate increases feed efficiency and overall farm economics. While DM, CP and ME digestibility compromise in broilers fed a low CP diet. *B. pumilus* supplementation neutralizes the negative effect of a low protein diet. In our study, highest DM availability was found with the supplementation of *B. pumilus*. Similarly, the highest availability of crude protein, crude fat and ash. This is because probiotics play a key role in maintaining a favorable environment in the digestive system of chicken. Our findings are well supported by the study carried out by (Pirzado *et al*., 2020) which proves feed suppliments can enhance the secretion of digestive enzymes of poultry birds and increase enzymatic activity. *Bacillus* can fragment a huge of proteins, lipids, and carbohydrates into smaller pieces (Park *et al*., 2016; Xia *et al*., 2018). The increased activity of enzymes amylase and protease may led to higher digestion and absorption of nutrients (Bonos *et al*., 2021).

The intestine plays a pivotal role in absorption of basic nutrients, and improvement in digestibility plays an important role in morphology of intestine. The villus structure plays a key role as villus is the basic unit to absorb nutrients. Whereas, crypt depth is considered a villus factory, and deep crypt indicates rapid tissue turnover and high demand for new tissues (Yang *et al*., 2016; Mushtaq *et al*., 2023).

Our study results showed a significant effect with supplementation of *B. pumilus* on villus height and crypt depth in broilers. The highest villus height with numerically lowest crypt depth was observed through supplementation of *B. pumilus*. The positive effects of some dietary *Bacillus* spp. probiotics on intestinal morphology of broilers have been previously documented and it has been reported that *Bacillus* probiotic supplementation can interfere positively with influence on morphology of intestine of broilers, enhancing feed efficiency and growth (Xiao *et al*., 2018). Our results are well supported by research conducted which indicates that villus height, villus width and muscle thickness of fish fed on probiotic supplemented diets were significantly higher than that of the fish without treatment. (Aron-Wisnewsky *et al*., 2021) disclosed that Nile tilapia kept in the cage fed *B. amyloliquefaciens* showed increase in the number of goblet cells per villus in comparison with the control.

Microorganisms play a key role in GIT as these create favorable and unfavorable environments in the intestine for digestion, secretion, and absorption. *Bacillus* and *Lactobacillus* species play a favorable role in the gut of the chickens. Our results based on the experiment show that counts of pathogenic bacteria were decreased, and *Lactobacillus* were increasing through supplementation of *B. pumilus*. The highest population of *Salmonella* was observed in Control followed by LP and lowest in LP-0.01. A similar trend was observed for a population of *E. coli*. However, the population of *Lactobacillus* was lowest in Control (6.33 cfu/g) and highest in LP-0.01. Supplementing broiler feed with *B. pumilus* improves growth of birds, modulates intestinal microflora, improves morphometry of intestine, and improves meat-oxidative stability. Qin *et al*. (2024) reported that positive effects of *B. pumilus* addition are co-related to the alterations in the intestinal microflora mechanism and nutrients absorbed more efficiently in the gut. Hence, it may be suggested that *B. pumilus* has probiotic potential in chicken feed and can be tried in large-scale studies.

CONCLUSION

It is concluded that supplementation of *Bacillus pumilus* in low-protein broiler diets counter-balance the effects arising from low protein and is equally beneficial to the traditional broiler diets in terms of growth performance, nutrient digestibility and intestinal microflora. It is further added that dietary protein levels of boiler diets could be reduced if *B. pumilus* is included in feed at commercial levels.

DECLARATIONS

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IRB approval

The research study was approved by Departmental Board of Studies, Department of Animal Nutrition (approval code: No. NUTR-245-2023), Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam.

Ethical statement

All the methods and experimental protocols were approved by the Animal Care and Ethics Committee (AEC-FAHVS-59-2023) of Sindh Agriculture University, Tandojam.

Statement of conflict of interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

REFERENCES

Alagawany, M., Abd El-Hack, M.E., Arif, M. and Ashour, E.A., 2016. Individual and combined effects of crude protein, methionine, and probiotic levels on laying hen productive performance and nitrogen pollution in the manure. *Environ. Sci. Pollut. Res*., **23**: 22906-22913. https://doi.org/10.1007/s11356-016-7511-6

Aron-Wisnewsky, J., Warmbrunn, M.V., Nieuwdorp, M. and Clément, K., 2021. Metabolism and metabolic disorders and the microbiome: The intestinal microbiota associated with obesity, lipid metabolism, and metabolic health pathophysiology and therapeutic strategies. *Gastroenterology*, **160**: 573599. https://doi.org/10.1053/j.gastro.2020.10.057

Aslam, M., Abro, R., Umer, M., Hassan, A.U., Rizwana, H. and Pirzado, S.A., 2024. Growth performance, nutrient digestibility, and gut micro flora of Japanese quail fed diet supplemented with *Bacillus megaterium*. *J. Agric. Vet. Sci.,* **3**: 1-12.

Association of Official Analytical Chemists (AOAC), 2000. *Official methods of analysis,* 17th ed., Washington, DC, USA.

Awad, E.A., Zulkifli, I., Soleimani, A.F. and Aljuobori, A., 2017. Effects of feeding male and female broiler chickens on low-protein diets fortified with different dietary glycine levels under the hot and humid tropical climate. *Ital. J. Anim. Sci*., **16**: 453-461. https://doi.org/10.1080/1828051X.2017.1291288

Bonos, E., Giannenas, I., Sidiropoulou, E., Stylianaki, I., Tzora, A., Skoufos, I. and Christaki, E., 2021.

Effect of *Bacillus pumilus* supplementation on performance, intestinal morphology, gut microflora and meat quality of broilers fed different energy concentrations. *Anim. Feed Sci. Tech*., **274**: 114859. https://doi.org/10.1016/j.anifeedsci.2021.114859

Bughio, M.A., Uroosa, M., Gulfam, A.M., Ghulam, S.B., Dildar, H.K., Mansoor, T.S. and Shoaib, A.P., 2021. Growth performance, physicochemical properties of meat of broiler chicken's supplemented with *Bacillus pumilus*. *J. Zoo. Res*., **3**: 1-18.

Davies, B.S., Beigneux, A.P., Barnes, R.H., Tu, Y., Gin, P., Weinstein, M.M. and Fong, L.G., 2010. GPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. *Cell. Metab*., **12**: 42-52. https://doi.org/10.1016/j.cmet.2010.04.016

Ghasemi-Sadabadi, M., Ebrahimnezhad, Y., Shaddel-Tili, A., Bannapour-Ghaffari, V., Kozehgari, H. and Didehvar, M., 2019. The effects of fermented milk products (kefir and yogurt) and probiotic on performance, carcass characteristics, blood parameters, and gut microbial population in broiler chickens. *Arch. Anim. Breed*., **62**: 361-374. https://doi.org/10.5194/aab-62-361-2019

Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., Elashry, M.A., Moustafa, M.M., Wassel, M.A. and Elsaied, H.E., 2021. Synergistic effects of *Bacillus pumilus* and exogenous protease on Nile tilapia (*Oreochromis niloticus*) growth, gut microbes, immune response and gene expression fed plant protein diet. *Anim. Feed Sci. Tech*., **275**: 114892. https://doi.org/10.1016/j.anifeedsci.2021.114892

Jayaraman, S., Thangavel, G., Kurian, H., Mani, R., Mukkalil, R. and Chirakkal, H., 2013. *Bacillus subtilis* PB6 improves intestinal health of broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis. *Poult. Sci*., **92**: 370-374. https://doi.org/10.3382/ps.2012-02528

Khan, S., Chand, N., Hafeez, A. and Ahmad, N., 2022. Effect of gum arabic on overall growth performance, visceral and lymphoid organs along with intestinal histomorphology and selected pathogenic bacteria in broiler chickens. *J. Anim. Hlth. Prod.,* 10:73-80. https://doi.org/10.17582/journal.jahp/2022/10.1.73.80

Laudadio, V., Passantino, L., Perillo, A., Lopresti, G., Passantino, A., Khan, R.U. and Tufarelli, V., 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. *Poult. Sci*., **91**: 265-270. https://doi.org/10.3382/ps.2011-01675

Li, J., Li, L., Guo, D., Li, S., Zeng, Y., Liu, C. and Xie, W., 2020. Triglyceride metabolism and angiopoietin-like proteins in lipoprotein lipase regulation. *Clin. Chim. Acta*, **503**: 19-34. https://doi.org/10.1016/j.cca.2019.12.029

Mushtaq, M., Khan, I.U., Shuaib, M., Chand, N., Sufyan, A., Shah, M., Islam, Z., Uzair, M.S., Khan, A., Ullah, Q. and Zeb, U., 2023. Effect of probiotic *Bacillus clausii* on production parameters and intestinal histomorphology of meat-type chicken. *Pakistan J. Zool*., pages. 1-8. https://doi.org/10.17582/journal.pjz/20230228080217

Park, J.H., Kotani, T., Konno, T., Setiawan, J., Kitamura, Y., Imada, S. and Matozaki, T., 2016. Promotion of intestinal epithelial cell turnover by commensal bacteria: Role of short-chain fatty acids. *PLoS One*, **11**: e0156334. https://doi.org/10.1371/journal.pone.0156334

Pirzado, S.A., Arain, M.A., Huiyi, C., Fazlani, S.A., Alagawany, M. and Gouhua, L., 2021. Effect of Azomite on growth performance: Immune function and tibia breaking strength of broiler chickens during starter period. *Anim. Biotech*., pp. 1-6. https://doi.org/10.1080/10495398.2021.1914644

Pirzado, S.A., Purba, A., Huiyi, C., Guilin, C. and Gouhua, L., 2020. Effect of azomite with low energy diet on growth performance, nutrient digestibility and bone mineralization of broiler chickens. *Int. J. Agric. Biol*., **24**: 505-510.

Purba, M.A., Pirzado, S.A., Cai, H., Haile, T.H., Zheng, A., Liu, J. and Ginting, N., 2020. A study about protective effect of *Brevibacillus laterosporus texasporus* culture on broiler chickens infected with *Salmonella pullorum*. *Int. J. Sci. Tech. Manage*., **1**: 68-78. https://doi.org/10.46729/ijstm.v1i2.10

Qin, S., Xiao, X., Dai, Z., Zhao, G., Cui, Z., Wu, Y. and Yang, C., 2024. Effects of *Bacillus licheniformis* on growth performance, immune and antioxidant functions, and intestinal microbiota of broilers. *Poult. Sci*., **103**: 103210. https://doi.org/10.1016/j.psj.2023.103210

Rehman, A., Jingdong, L., Chandio, A.A. and Hussain, I., 2017. Livestock production and population census in Pakistan: Determining their relationship with agricultural GDP using econometric analysis. *Inf. Process. Agric*., **4**: 168-177. https://doi.org/10.1016/j.inpa.2017.03.002

Saied, A.M., Attia, A.I., El-Kholy, M.S., Reda, F.M. and Nagar, A.G.E.L., 2022. Effect of cinnamon oil supplementation into broiler chicken diets on growth, carcass traits, haemato-biochemical parameters, immune function, antioxidant status and caecal microbial count. *J. Anim. Feed Sci*., **31**: 21-33. https://doi.org/10.22358/jafs/146921/2022

Soomro, R.N., Abd El-Hack, M.E., Shah, S.S., Taha, A.E., Alagawany, M., Swelum, A.A. and Tufarelli, V., 2019. Impact of restricting feed and probiotic supplementation on growth performance, mortality and carcass traits of meat-type quails. *J. Anim. Sci.*, **90**: 1388-1395. https://doi.org/10.1111/asj.13290

Tsai, C.Y., Wang, K.T., Wu, Y.S., Yeh, S.P. and Wu, T.M., 2019. Secretory expression and characterization of xylanase isolated from *Bacillus subtilis* E20 increase the utilization of plant ingredients in tilapia feed. *Aqua. Res*., **50**: 2240-2250. https://doi.org/10.1111/are.14105

Xia, Y., Lu, M., Chen, G., Cao, J., Gao, F., Wang, M. and Yi, M., 2018. Effects of dietary *Lactobacillus rhamnosus* JCM1136 and *Lactococcus lactis* subsp. *lactis* JCM5805 on the growth, intestinal microbiota, morphology, immune response and disease resistance of juvenile Nile tilapia, *Oreochromis niloticus*. *Fish Shellfish Immunol*., **76**: 368-379. https://doi.org/10.1016/j.fsi.2018.03.020

Xiao, Y., Kong, F., Xiang, Y., Zhou, W., Wang, J., Yang, H. and Zhao, J., 2018. Comparative biogeography of the gut microbiome between Jinhua and Landrace pigs. *Sci. Rep*., **8**: 5985. https://doi.org/10.1038/s41598-018-24289-z

Yadav, P., Korpole, S., Prasad, G.S., Sahni, G., Maharjan, J., Sreerama, L. and Bhattarai, T., 2018. Morphological, enzymatic screening, and phylogenetic analysis of thermophilic bacilli isolated from five hot springs of Myagdi. *Nepal. J. appl. Biol. Biotechnol*., **6**: 1-8. https://doi.org/10.7324/JABB.2018.60301

Yang, H., Huang, X., Fang, S., Xin, W., Huang, L. and Chen, C., 2016. Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. *Sci. Rep*., **6**: 27427. https://doi.org/10.1038/srep27427

Yang, H., Yang, Z., Wang, Z., Wang, W., Huang, K., Fan, W. and Jia, T., 2015. Effects of early dietary energy and protein dilution on growth performance: Nutrient utilization and internal organs of broilers. *Ital. J. Anim. Sci*., **14**: 3729. https://doi.org/10.4081/ijas.2015.3729

Zhang, N., Wang, L. and Wei, Y., 2021. Effects of *Bacillus pumilus* on growth performance, immunological indicators and gut microbiota of mice. *J. Anim. Physiol. Anim. Nutr*., **105**: 797-805. https://doi.org/10.1111/jpn.13505

Zhao, C., Zhu, J., Hu, J., Dong, X., Sun, L., Zhang, X. and Miao, S., 2019. Effects of dietary *Bacillus pumilus* on growth performance, innate immunity and digestive enzymes of giant freshwater prawns (*Macrobrachium rosenbergii*). *Aqua. Nutr*., **25**: 712-720. https://doi.org/10.1111/anu.12894