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ARTICLE

Growth Performance, Physicochemical Properties of Meat of Broiler Chicken's Supplemented with *Bacillus pumilus*

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ABSTRACT

This study examined that the efficiency of Bacillus pumilus (BP) on growth performance and meat quality of broiler chickens. A total of day old 240 male broiler chicks were purchased from local hatchery Hyderabad. The chicks were allocated into four groups with six replicates of 10 birds in each replicate. Four diets were prepared for experiment, which included control as basal diet, BP- 0.1 (CONT + 0.1% BP), BP-0.2 (CONT + 0.2% BP) and AGP (CONT + zinc bacitracin 20 ppm) as antibiotic growth promotor. The results showed that live body weight (LBW), average daily gain was recorded higher (P<0.01) in birds fed diet with BP-0.1 than control and BP-0.2 groups, as well as ADFI also increased in BP-0.1 than control group. BP-0.1 supplementation significantly reduced feed conversion ratio (FCR) than other groups(P<0.01). Both moisture and crude protein (CP) content influenced by supplementation of BP-0.1 than other groups. However, birds fed diet with BP-0.1 had least fat content in breast meat compared with the other groups (P<0.01). In addition, BP-0.1probiotic- fed birds had the lowest (P<0.01) pH, cooking loss (CL) and drip loss (DL) as compared to control, BP-0.2 and AGP groups. The water holding capacity (WHC) were found higher in BP-0.1 as compared to other groups (P<0.01). In conclusion, Bacillus pumilus probiotic supplementation is a promising approach for substitution of antibiotics in broiler diet for enhancement of growth performance and physiochemical properties of meat.

1. Introduction

Nowadays the partial or total restrictions to use of antibiotic growth promotors (AGPs) in poultry industry throughout globe due to accumulation of antibiotic resistant bacteria in meat that may cause serious threats to the health security of human beings [1-3]. In addition, immerging the demand from consumers for organic and antibiotics free chicken meat [4]. In this regard, the European union and USA made strict rules and regulation to

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regulate the use of antibiotics [5,6] Therefore, the poultry researchers and scientists feel pressure to looking for substitutes of antibiotics that maintain the production performance, health status of animals and fulfill the demands of consumers for antibiotic free meat [7].

Recently, numerous studies have identified that probiotics supplementation in poultry feeds are possible to improve the production performance, health status, intestinal integrity and meat quality [4,8]. Various bacterial strains are adopted by feed industry as a probiotic, among them *bacillus spp*. frequently used in poultry diet because it can store at ambient temperature for long time due to its heat resistant capacity [5]. *Bacillus pumilus* is produced by advanced production process of liquefaction fermentation and centrifugal spray. *B. pumilus* is a nonpathogenic spore forming bacterium, widely used in fish nutrition and livestock [9]. Supplementation of *B. pumilus* has positive effects that enhance the production performance by inhibition the growth of harmful bacteria while promote the beneficial bacterial breeding and creating the favorable gut environment for maintaining the dynamic balance of intestinal microflora in the host [10,11]. Moreover, *B. pumilus* plays an important role in protection of intestinal tract which translates into better feed efficiency and promotes the digestion and absorption of nutrients in feed [12].

B. pumilus has a growth promoting properties and can be used as an alternatives of feed additives in feed industry. Supplementation of *B. pumilus* in broiler diet improve the body weight, reduction in feed intake and improves the feed efficiency [8]. Furthermore, supplementation of *B. pumilus* probiotic improves the weight gain, FCR, specific growth rate in giant freshwater prawns [12], catfish [13], mice [11] and T. ovatus [14]. There were limited studies reported on broiler chickens with supplementation of *B. pumilus* in diet. Therefore, t1e main purpose of study was chosen to examine the impact of *B. pumilus* on growth performance and physiochemical properties of meat in broiler chickens.

2. Materials and Methods

The broiler experiment was carried out in accordance with the recommended guidelines for animal welfare and approved by the of ethical committee of Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University, Tandojam, Pakistan.

2.1 Dietary Treatments and Bird's Management

A total of day old 240 male broiler chicks were purchased from local hatchery Hyderabad. The chicks were allocated into four groups with six replicates of 10 birds in each replicate. Four diets were prepared for experiment, which included control as basal diet, BP- 0.1 (CONT + 0.1% BP), BP-0.2 (CONT + 0.2% BP) and (CONT + zinc bacitracin 20 ppm) as antibiotic growth promotor (Table-1). Before arrival of chicks, the shed was cleaned and disinfected and birds were kept in floor pens covered with wood dust. The initial room temperature was set at 35°C in 1st week and gradually decreased by 2°C each week until reached the 24°C. The relative humidity was maintained at 55 to 60% and lighting procedure was subjected to 23h light and one hour darkness provided in 1st week. From 2nd week the birds were subjected to gradually decrease in light schedule one hour per week and fixed at 19 hours. The *ad libitum* feed and water provided to birds. The *Bacillus pumilus* probiotic was purchased from Jinan Rentai Import and Export Co. Ltd., China. *Bacillus pumilus* containing at minimum $2.0x10^{11}$ viable spores/g.

Table 1. Feed formulation and nutrient composition of basal diet

Ingredients	Starter 0-21 days	Finisher 22-35 days
Corn	52	58.3
Rice polish	5	5
Canola meal	12	5
Rape seed meal	4	4.5
Soy bean	14	12
PBM	6.3	8
Poultry oil	3.5	4
DCP	0.5	1
Limestone	0.3	0.32
HCL-Lys	1	0.5
DL-Met	0.36	0.4
L- Thr	0.23	0.21
L- Isoleuc	0.11	0.08
L- Val	0.08	0.08
Salt	0.06	0.03
Sodium	0.04	0.05
Premix	0.02	0.03
BP	0.5	0.5
Total	100	100
Calculated composition		
CP	22	21
ME (kcal/kg)	2900	3050
DM %	89.5	89.5
Fat %	4.8	6.4
Fiber %	3.9	4.5
Calcium %	0.99	0.75
Available P %	0.45	0.45

The premix provided the following per kg of diets:VA 10000IU,VB1 1.8mg,VB2 40mg,VB12 0.71mg,VD3 2000IU,VE 10IU,VK3 2.5mg, biotin 0.12mg, folic acid 0.5mg,D-pantothenic acid 11mg,Cu (as copper sulfate) 8mg,Fe (as ferrous sulfate) 80 mg,Mn (as manganese sulfate) 60 mg, Zn (as zinc sulfate) 40mg,I (as potassium iodide) 0.0.35 mg,Se (as sodium selenite) 0.15 mg.

2.2 Growth Performance

At the 35 day of the experiment live body weight and feed intake was recorded on pen basis using digital weighing balance. The average feed intake, average daily gain and feed conversion ratio was calculated. The FCR was corrected by dead birds.

2.3 Meat Quality Analysis

2.3.1 pH value

The pH value of breast meat samples was recorded immediately after slaughter. 10 gram of breast meat sample was mixed in 90ml deionized water, after 60minutes the pH value of homogenized sample was analyzed by using portable pH meter (HANNA ITALIA, made in Mauritius) calibrated at 4.0 and 7.0 pH [15]. The electrode attached with pH meter was used to calculate the pH of homogenized meat sample.

2.3.2 Drip loss

After the slaughtering drip loss was determined by using previously described method [16]. 50 grams of fresh meat samples placed in polyethene bags, sealed tightly and kept in refrigerator at 4°C for 24 hours. After refrigeration samples removed from polyethene bags and gently soaked, dried and weighed again. Following formula was used to calculate drip loss.

$$\text{Drip loss (\%)} = \{(D1\text{-}D2) \div D1\} \times 100$$

2.3.3 Cooking loss (CL)

Cooking loss was analyzed by using previous reported procedure [17]. 20 grams of meat sample was weighed and enclosed in plastic bags with sealed lock and cooked for one hour in water bath at 80°C until inner temperature reached at 72°C. The cooked meat samples were removed from polythene bags then cooled at room temperature. Cooking loss was measured by using following formula.

$$\text{Cooking loss (\%)} = \{(C1\text{-}C2) \div C1\} \times 100$$

2.3.4 Water holding capacity (WHC)

Breast meat water holding capacity was estimated according to the method described by [18]. 10g of crushed meat sample and 12ml NaCl solution was added in centrifuge tube. The meat sample was centrifuged at 10,000 rpm for 15 minutes at 4°C and supernatant was collected in measuring cylinder for calculation. The difference connecting the amount of NaCl solution having 0.6M consumed and supernatant was water holding capacity. Following formula was used for calculation.

$$\text{Water holding capacity \%} = \frac{\text{Actual weight -Supernatant}}{\text{Actual weight}} \times 100$$

2.3.5 Proximate analysis of meat

Nutritive value of breast meat muscle analyzed according to the standards methods described by AOAC [19] standard methods. Moisture content analyzed by 2 g of breast meat sample drying at 105°C in electric oven for one hour. Crude protein was determined by kjeldhal method and CP was obtained as N% x 6.25. Fat content of breast meat estimated by Soxhlet apparatus by extraction using N-hexane. Meat samples were ignited at 550°C in muffle furnace to estimate the ash content.

3. Results

Table 2. Effect of *Bacillus pumilus* on growth performance of broiler chickens.

Parameters	CONT	BP-0.1	BP-0.2	AGP	SEM	*P Value*
iBW	41.39[a]	41.33	41.43	41.36[a]	0.03	0.987
LBW	1930.8[c]	2141.0[a]	2120.4[b]	2113.4[a]	19.7	0.000
ADG	55.16[c]	61.17[a]	57.75[b]	60.38[a]	2.51	0.000
ADFI	90.27[b]	92.28[a]	91.60[ab]	92.68[a]	0.56	0.014
FCR	1.63[a]	1.51[d]	1.58[b]	1.53[c]	0.01	0.000

[a,b,c] Means in same row with no common superscripts differ significantly (P<0.05). CONT= control, BP-0.1=Bacillus *pumilus* 0.1, BP-0.2=Bacillus *pumilus* 0.2, AGP=Antibiotic growth promotor, SEM=Standard error management, iBW=initial body weight, ADG=average daily gain, ADFI=average daily feed intake, FCR=feed conversion ratio.

The birds fed diet with BP-0.1 significantly (P<0.05) improved the LBW, ADG than CONT and BP-0.2 diets, while no significant (P>0.05) difference observed between BP-0.1 and AGP groups in Table 2. ADFI was significantly increased in BP-0.1 as compared to CONT groups. Moreover, FCR was reduced (P<0.05) in birds fed diet with BP-0.1 in comparison with CONT, BP-0.2 and AGP groups.

Table 3. Effect of *Bacillus pumilus* on chemical properties of meat in broiler chickens.

Parameters	CONT	BP-0.1	BP-0.2	AGP	SEM	*P Value*
Moisture%	74.94[a]	72.26[c]	73.98[b]	73.78[b]	0.29	0.000
CP%	21.27[c]	23.85[a]	22.68[b]	22.77[b]	0.27	0.000
Fat%	3.18[a]	1.75[d]	2.43[b]	2.88[c]	0.16	0.000
Ash%	1.05[c]	1.41[a]	1.15[ab]	1.19[b]	0.04	0.000

[a,b,c] Means in same row with no common superscripts differ significantly (P<0.05). CONT= control, BP-0.1=*Bacillus pumilus* 0.1, BP-0.2=*Bacillus pumilus* 0.2, AGP=Antibiotic growth promotor, SEM=Standard error management, CP= Crude protein.

Table 3 presented that moisture and CP percentage in meat was significantly (P<0.05) increased in CONT in comparison to BP-0.1, BP-0.2 and AGT diets. However, Ash percentage in breast meat was recorded higher in BP-0.1 than CONT and AGP groups (P<0.05). Fat percentage

was significantly (P<0.05) reduced in BP-0.1 as compared to CONT, BP-0.2 and AGP diets.

Table 4. Effect of *Bacillus pumilus* on physical properties of meat in broiler chickens.

Parameters	CONT	BP-0.1	BP-0.2	AGP	SEM	*P Value*
pH%	6.05^{a}	5.54^{d}	5.93^{b}	5.72^{c}	0.06	0.000
WHC%	65.16^{c}	71.23^{a}	68.66^{b}	70.20^{ab}	0.72	0.000
DL%	3.60^{a}	1.97^{c}	2.64^{b}	2.57^{b}	0.17	0.000
CL%	28.02^{a}	21.68^{d}	24.91^{b}	23.49^{c}	0.70	0.000

^{a,b,c} Means in same row with no common superscripts differ significantly (P<0.05). CONT= control, BP-0.1=*Bacillus pumilus* 0.1, BP-0.2=*Bacillus pumilus* 0.2, AGP=Antibiotic growth promotor, SEM=Standard error management, WHC= Water holding capacity, DL=Drip loss, CL= Cooking loss.

In Table 4 highest values of CL and pH were observed in CONT than BP-0.1, BP-0.2 and AGP groups (P<0.05). The higher WHC were found in BP-0.1 supplemented group as compared to CONT and BP-0.2 groups, however no difference was recorded between BP-0.1 and AGP groups (P<0.05). DL was recorded higher (P<0.05) in CONT as compared to BP-0.1, BP-0.2 and AGP groups.

4. Discussion

The present study may provide new insights in poultry feed industry to use of *Bacillus pumilus* probiotic in broiler diet. To the best of our knowledge there is limited literature available regarding the use of *B. pumilus* in poultry diet. However, *B. pumilus* showed better performance on fishes and pigs. In our study the administration of *B. pumilus* improved the LBW, ADG and FCR, which was in agreement with Zhang et al. [11], who reported that growth performance of mice was improved by supplementation of *B. pumilus* strain fsznc-09. Similar findings were reported by Munglue et al. [13] and Liu et al. [14], which shows that *B. pumilus* had a beneficial effect on growth performance and FCR catfish and Trachinotus ovatus respectively. The mechanism of stimulatory effect of *B. pumilus* probiotics on broilers still not clear. However, some previous research findings have observed that *B. pumilus* may increase the activity of digestive enzymes i.e., protease, cellulase and amylase that enhance the digestibility and absorbability of nutrients in GI tract of Rohu fingerlings and Giant freshwater prawns [20] and [12]. The abovementioned reports verified our results.

Diet and changes in growth performance are major factors which reflects the meat quality traits and most recent studies reported that addition of probiotics in poultry diet exerts potential to improve the meat quality traits in poultry [4,21]. In our results broilers diet supplemented with *B. pumilus* significantly improved the CP and Ash content on other hand moisture and fat content was reduced in meat. This indicates that probiotics supplementation in broiler diet showed better retention of protein and Ash content in meat [22]. Similar findings were observed by others, Liu et al. [23], Kral et al. [24] and Stęczny and Kokoszynski, [20], they revealed that *Bacillus licheniformis, Bacillus subtills* and *EM* probiotics supplementation diet increased the CP and reduction in fat content in broiler meat. Moreover, Hossain et al. [25] and Abdurrahman et al. [26], found lower fat content and higher CP of broilers meat fed diet with probiotic supplementation. Some previous results suggested that addition of probiotics in broiler feed decrease the cholesterol content in meat which has a positive effect on healthy value of meat [27,28]. The cholesterol might be decreased by its integration through bacterial growing cells in the cellular surface of the probiotic bacteria, therefore inhibition of cholesterol absorption back in the body [29,30]. In addition, deconjugation of bile acid by microbial hydrolase and hypolipidemic effect probiotics reported, the short chain fatty acids they produce, particularly propionate, might exhibit controlling effect on decreasing hepatic lipogenesis and inhibition of lipogenesis process related to reduce the fat content in meat [33,32].

Physicochemical properties of meat play an important role in meat quality and attraction of consumers. The pH is an important index of meat quality for further processing and storage, and it is affected after slaughtering [33]. Ivanovic et al. [43] found that birds received 0.05 % *streptococcus faceium cerenelli* in feed significantly reduced the pH in thigh and breast meat. In addition, Castellini et al. [35] and Muhammed et al. [36], they reported that probiotic supplementation in poultry diet reduced the pH of meat muscles after 24 h of slaughter. Abdulla et al, also observed that *Bacillus subtills* probiotic supplementation in broilers reduced the pH of meat during storage. In our study, the pH of meat is decreased in broilers fed diet with *B. pumilus* are similar to above findings after 24 h of slaughter. Current results suggest that dietary *B. pumilus* in broilers reduces the pH range during rigor mortis may be attributed to the *B. pumilus* probiotic functions inhibiting the intramuscular fat degradation by which it affects the meat pH and taste [37]. Water holding capacity (WHC) depends upon two factors i.e., drip loss and cooking loss which serves as main indictors of WHC of meat. WHC has great impact on appearance, cooking characteristics and juiciness of meat [38]. In our results WHC increased significantly, while drip and cooking loss reduced in broilers fed diet with probiotic supplementation. Similar results were obtained by Zhou et al. [39], who found that *bacillus coagulan* supplementation in feed decreased the drip loss and cooking loss in Guanxi yellow chicken.

Pietras, [40] also found that probiotics improved WHC in broiler chickens. In this regard our results were confirmed by Lawson, [41], who stated that water might be expelled out of myofibril because of contraction during rigor mortis and enters into canals made between muscle fiber and the cell membrane due to loss of intracellular matrix by action of calpain; such water may move to the outside as leak or drip. The various feed additives have major effect on cooking and drip losses may be led to increasing the juiciness of the of the meat meanwhile the greater juiciness comes mainly from the meat that exerts less cooking and drip losses.

5. Conclusions

The current study that the birds fed diet supplemented with *Bacillus pumilus* probiotic 0.1g/kg improved the growth performance in terms of LBW, ADG and FCR in comparison with other groups. However, *Bacillus pumilus* had a positive influence on physicochemical properties of meat. Therefore, it is recommended that *Bacillus pumilus* probiotic could be used in broiler diet without any harmful effect on meat quality characteristics.

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Author Contribution

Shoaib Ahmed Pirzado and Masroor Ali contributed idea and design of study, Masroor and Uroosa Majeed performed research trial, Ghulam Shabir and Mansoor Tariq Samoon, determined the physical and chemical properties of meat, Dildar Hussain Kalhoro analyzed the data, Masroor Ali and Gulfam Ali Mughal draft the article and Shoaib Ahmed Pirzado critical review of article.

Disclosures

The all authors declare no potential conflict.

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