

Original Research Article

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Effect of Supplementation of Probiotic (*Bacillus subtilis*) on Growth Performance and Carcass Traits of Broiler Chickens

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ABSTRACT

This study was conducted to find out the efficacy of probiotic (*Bacillus subtilis*) on growth performance and carcass traits of broiler birds. The experiment followed completely randomized design in which the day old broiler chicks (n=180) were divided into 4 dietary treatment groups (T₁-T₄) as 3 replicates of 15 chicks in each. The chicks of four different groups were fed with basal diet, antibiotic growth promoter (Enramycin @ 250 mg/kg feed) and commercial probiotic containing 3 strains of *Bacillus subtilis* (DM 03, TAM 4 and IQB 350) spores at the concentration of ½ million/g and 1 million/g of finished feed, respectively. The productive indicators evaluated were: body weight, feed consumption and feed conversion ratio (FCR). The carcass quality traits were also determined. The supplementation of *Bacillus subtilis* (1million/g of finished feed) resulted in highly significant (P<0.01) increase in the body weight of birds as compare to control (T₁) during 4th and 5th weeks of experiment. The feed consumption recorded lower in T₄. The addition of *Bacillus subtilis* based probiotic and AGP showed highly significant (p<0.01) variation regarding weekly FCR during 3th and 4th week. The weight of liver, heart and intestine and the weight of different cuts (thigh, wing, and back) as percent of live weight accounted non-significant variations among different groups. However the weight of breast as percent of live weight was highly significant (P<0.01) between the groups and found maximum in T₄ group.

Keywords

Probiotic, *Bacillus subtilis*, growth performance, carcass traits, broiler

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Introduction

Poultry are the cheapest source of animal protein, contributing significantly to supply the growing demand for animal food products around the world (Farrell, 2013). The consumption and trade in poultry products is increasing rapidly as the human population increases, making it the second largest source of meat after pork (FAO, 2014). The biggest challenge of commercial poultry production is

the availability of quality feed on sustainable basis at stable prices. Probiotics (or direct fed microbials) are increasingly being popular as one of the alternatives to Antibiotic Growth Promoters (AGP). Probiotics can improve broiler chicken growth rates (Afsharmanesh and Sadaghi, 2014; Lei *et al.*, 2015), it also helps in maintenance and establishment of intestinal micro biota beneficially that may enhance beneficial colonization in the GIT against pathogens. Supplementation of

probiotics enhanced the growth rate in broilers better than AGP (Zhang and Kim, 2014) and other substitutes for AGP, such as phytochemicals e.g. essential oils (Khaksar *et al.*, 2012). Probiotics are active against enteropathogens in several ways, including improved immunity-based elimination, competing for mucosal attachment and crucial nutrients, and producing antimicrobial complexes (Patel *et al.*, 2015).

In broiler nutrition, probiotic species such as *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* are widely used to prevent poultry pathogens and diseases and improve broiler's growth Performance. *Bacillus* species are superior probiotic feed-additives for poultry and pigs due to their big genomes with relevant features; they are spore producers which makes the product stable for long time and enhancing the bird's intestinal integrity and growth performance (Vazquez, 2016)). As a widely used probiotic strain, combination of *Bacillus subtilis* and *Bacillus licheniformis* are considered one of the most health-boosting bacteria because they have demonstrated a positive effect in aiding nutrient digestion and absorption in the host's body (Scgarrrd and Demark, 1990).

In recent times, there has been significant progress in scientific evaluation and studies on probiotic *Bacillus subtilis*, revealing possible mechanisms of action like antimicrobial effect by synthesis of antimicrobial substances, antidiarrheal effect, immunostimulatory effect, competitive exclusion of pathogens, prevention of intestinal inflammation, and normalization of intestinal flora (Suva *et al.*, 2016). Blanch *et al.*, (2017) observed the addition of *Bacillus subtilis* DSM 17299 may efficiently compensate certain reductions of ME, CP and amino acid in broiler diets supplemented with NSP-enzymes and phytase.

Materials and Methods

For this experiment the total growth period (0 to 6wks) of broilers was divided into 3 phases pre starter (0-14 d), starter (14-21 d) and finisher (21-42 d). The pre starter diets contained 22% CP and 3000 kcal ME, starter 21.5% CP and 3050 kcal ME and finisher diet contained 19.5% CP and 3100 kcal ME/kg feed. The diets were formulated using maize, deoiled soybean cake meal, dicalcium phosphate (DCP), limestone powder (LSP), soy oil, mineral and premixes containing trace minerals, vitamins and feed additives. The experiment was conducted to having completely randomized design. The day old broiler chicks (n=180) were randomly allotted to 4 dietary treatment groups (T₁-T₄). Each group had 3 replicates of 15 chicks in each i.e 3*15=45 chicks/group. The chicks of group T₁ (NC) were fed diet without any growth promoter (control), in T₂ the chicks fed control diet supplemented with antibiotic growth promoter (Enramycin @ 250 mg/kg feed).

Chicks in group T₃ and T₄ were given control diet supplemented with commercial probiotic containing three strains of *Bacillus subtilis* (DM 03, TAM 4 and IQB 350). @ 11.5 g and 22.5 g/Q feed respectively so as to get concentration of *Bacillus subtilis* spores will be 0.5 million/g and 1 million/g of finished feed in diets T₃ and T₄. The body weights of individual birds were recorded at weekly interval, and average body weight gain was calculated. Feed consumption of birds of each replicate was recorded at weekly intervals and feed consumption per bird per week and FCR were calculated. At the end of 6th week of age, three birds from each replicate were taken randomly for the recording of carcass characteristics. Birds were dressed, eviscerated and the dressed, eviscerated ready-to-cook and cut up yields were estimated.

Results and Discussion

Growth Performance

The average body weight, weekly and cumulative weight gain of broiler chicks of different treatment groups is presented in the table 1 and 2, respectively. Results of the study have been grouped into three phases prestarter (0-14 days), starter (14-21days) and finisher (21-42 days).

Pre – starter Phase (0-14 days)

The supplementation of *Bacillus subtilis* based probiotic did not resulted in any significant variation in the weekly weight gain as compared to control and groups fed AGP supplemented diet during first 14 d of experiment. Similarly the cumulative body weight gains of birds of different dietary treatments at the end of pre starter phase were very close and did not vary significantly amongst the group (Table 2).

Starter phase (14-21 days)

The difference in average weekly body weight gain was highly significantly ($P<0.01$) amongst the treatment groups during the starter phase. Higher weight gain was recorded in broiler chicks received higher concentration of *Bacillus subtilis* spores (T4) as compare to other treatment groups.

Similarly the difference in cumulative weight gain was found to be highly significant ($P<0.01$) between treatment groups and control. Highest cumulative weight gain (844g) was recorded in the birds of group T4 and it was significantly ($P<0.01$) higher as compare to other groups.

The difference in cumulative weight gain was not significant between birds of groups T2 and T3 at the end of starter phase (21d).

Finisher phase (21-42 days)

A highly significant variation ($P<0.01$) were recorded with respect to weekly gain in body weight in the birds of all the three treatment groups as compared with the control group during 4th week. However, there was non-significant ($P>0.05$) increase in the weight gain of experimental birds in treatment groups as compare to control during 5th week of feeding trial. The highest weight gain was recorded in the birds T4 group (421.13g) followed by birds of group T3, T2 and T1 at the end of 42 day of study.

The difference in the cumulative weight gain of birds in group T2, T3 and T4 was significantly higher ($P<0.01$) during 4th, 5th and 6th week.as compare to birds of control (T1) group. The cumulative weight gain of birds of group T4 was significantly ($P<0.01$) higher as compare to all other groups however CWG was comparable in birds of group T2 and T3 during 4th and 5th wk period. Cumulative weight gain of birds of T4 group at the end of 6th week was significantly ($P<0.05$) higher as compare to birds of T1 and T2 groups.

In present experiment the cumulative weight gain was significantly high in the birds fed higher concentration of *Bacillus subtilis* spores as probiotics during starter and finisher stage and the findings are in agreement with Tournut (1998) who stated that the efficacy of probiotics depend on the quantitative and qualitative characteristics of microorganisms used in the production of probiotic growth promoters. Previous researcher Sabatkova *et al.*, (2008) and Ahmad and Taghi (2006) also reported improvement in weight gain when broiler diet was supplemented with probiotics (*Bacillus subtilis* and *Bacillus licheniformis*) during 21-42 days period. The improvement in body weight gain due to supplementation of different starins of *Bacillus subtilis* based

probiotics, in present experiment is associated with significantly better feed conversion ratio and also with significant increase in the height of villus and depth of crypt in duodenum of birds in these groups. In contrast to our findings Edens (2003) reported that the addition of a probiotic, with a predominance of *Bacillus subtilis* (Calsporin; Calpis Corporation, Tokyo, Japan) did not improve body weight (Calsporin 2416 g vs. control 2407 g) at 42 days of age.

Weekly and Cumulative Feed consumption

The effect of supplementation of *Bacillus subtilis* based probiotic at two levels and AGP on average weekly and cumulative feed intake has been presented in table 3 & 4, respectively.

The average weekly feed intake due to supplementation of probiotics or AGP did not vary significantly amongst the treatment groups till 5th week of experiment, though cumulative feed intake was significantly ($P<0.05$) low in group T3 (1918.11 g) as compare to group fed control and AGP supplemented diets (1971.44 & 1985.36 g, respectively). During 5th and 6th week of experiment the effect of supplementation of probiotics or AGP on weekly and cumulative feed intake was highly significant. The weekly feed intake was significantly ($P<0.01$) low in group fed probiotics in higher concentration (T4) as compare to other groups. Also the intake was significantly ($P<0.01$) low in group T3 as compare to T2 and T1, and also between groups T1 and T2 during 5th and 6th week of experiment. The cumulative feed intake at the end of finisher phase was significantly ($P<0.01$) low in groups fed probiotics supplemented diet irrespective of levels used as compare to control and AGP supplemented groups. Though the difference in cumulative feed intake between T2 and T1 was statistically

comparable, it was numerically higher in T1 as compared to T2 (3719g v/s 3698 g)

Comparatively lower feed consumption observed in probiotic (*Bacillus subtilis* spores) supplemented group in present experiment is in agreement with the results reported by earlier researchers (Shim *et al.*, 2012; Eseceli and Demir, 2010 and Erdogan, 2007) that supplementation of probiotic decreased the feed intake significantly ($P<0.05$) as compared to control group. Increased villus height and crypt depth in the birds of probiotics supplemented group improved the nutrient absorption and this may be the possibly reason for lower feed intake with improved growth performance in the birds of these groups. In contrast to our findings some researcher (Panda *et al.*, 2008 and Rada *et al.*, 2013) did not found significant difference in feed intake between control and probiotic supplemented groups.

Weekly and cumulative Feed Conversion Ratio (FCR)

The weekly FCR of birds of different treatment groups was statistically comparable during pre-starter phase. Cumulative feed conversion ratio was also comparable at the end of pre starter phase (0-14 day period). The effect of supplementation of probiotics on weekly feed conversion ratio and cumulative FCR at the end of starter phase was significantly ($P<0.01$) better in groups fed diet supplemented with probiotics irrespective of concentration as compare to birds fed control and AGP supplemented groups.

During 4th week of experiment weekly and cumulative FCR was highly significantly ($P<0.01$) better in group fed higher concentration of *Bacillus subtilis* based probiotics (T4) amongst the treatment groups in which better weekly and cumulative FCR

was also observed in birds of group T3 as compare to birds of T2 and T1 groups, however weekly and cumulative FCR between birds of group T1 and T2 was statistically comparable. During 5th and 6th week no significant effect of probiotics or AGP supplementation on weekly FCR was noticed (Table 5), however the difference in cumulative FCR at 4th wk was highly significant amongst dietary groups (Table 6). At the end of 5th week also the cumulative

FCR was significantly (P<0.01) better in birds fed higher concentration of *Bacillus subtilis* based probiotics (T4) (1.44) as compare to other groups; whereas at 6th wk significantly (P<0.01) better cumulative FCR was reported in groups (T3, 1.59 and T4, 1.55) fed probiotics as compare to control and AGP supplemented groups. The difference in cumulative FCR in birds of T1 and T2 groups was statistically comparable during 5th and 6th week of age.

Table.1 Effect of supplementation of probiotic (*Bacillus subtilis*) and AGP (g/bird) on average weekly gain in body weight (g) (Mean±SE)

Week	Groups / Treatments				Sig.
	T1	T2	T3	T4	
1 st	137.81±2.04	137.40±1.23	139.74±2.02	140.33±0.87	NS
2 nd	292.03±2.05	292.81±1.88	292.70±3.80	290.67±3.43	NS
3 rd	364.44±5.6 ^a	406.66±3.00 ^b	405.67±5.48 ^b	423.56±4.23 ^c	**
4 th	587.58±3.72 ^a	581.47±2.02 ^a	582.83±4.46 ^a	625.43±12.19 ^b	**
5 th	406.74±3.10	416.54±2.61	414.68±6.69	432.87±9.46	NS
6 th	309.86±32.72	319.06±28.45	392.94±38.22	421.13±23.53	NS

Superscripts are read row wise for comparison of means. Means in the same row with different superscripts a, b, c are significantly different * (P<0.05), ** (P<0.01), NS= Non Significant.

Table.2 Effect of supplementation of probiotic (*Bacillus subtilis*) and AGP on cumulative gain in body weight (g)

Days of Observation	Groups / Treatments				Sig.
	T1	T2	T3	T4	
0-7	137.81±2.04	137.40±1.23	139.74±2.02	140.33±0.87	NS
0-14	429.84±2.52	430.21±2.54	432.44±1.77	431.00±3.01	NS
0-21	794.28±3.47 ^a	836.88±3.05 ^b	838.11±4.70 ^b	844.57±2.45 ^c	**
0-28	1381.86±5.84 ^a	1418.35±5.07 ^b	1420.94±4.10 ^b	1480.00±9.00 ^c	**
0-35	1788.61±3.95 ^a	1834.89±4.49 ^b	1835.63±2.66 ^b	1912.88±9.19 ^c	**
0-42	2107.68±21.55 ^a	2144.76±26.57 ^{ab}	2256.76±35.57 ^{bc}	2305.82±32.37 ^c	*

Superscripts are read row wise for comparison of means. Means in the same row with different superscripts a, b, c are significantly different * (P<0.05), ** (P<0.01), NS= Non Significant.

Table.3 Effect of supplementation of probiotic (*Bacillus subtilis*) and AGP on average weekly feed consumption (g/bird)

Week	Groups / Treatments				Sig.
	T1	T2	T3	T4	
1 st	124.01±1.19	130.56±3.36	121.07±3.87	129.12±2.41	NS
2 nd	336.74±7.68	328.02±8.62	323.97±6.51	322.79±6.62	NS
3 rd	619.86±7.38	657.59±6.83	629.28±12.45	630.51±8.03	NS
4 th	890.81±3.05	869.18±2.72	843.77±15.23	862.47±11.87	NS
5 th	849.09±1.86 ^d	835.09±1.85 ^c	826.98±1.69 ^b	811.30±3.74 ^a	**
6 th	898.71±2.88 ^d	877.82±2.44 ^c	861.73±5.06 ^b	837.87±3.21 ^a	**

Superscripts are read row wise for comparison of means. Means in the same row with different superscripts a, b, c, d are significantly different * (P<0.05), ** (P<0.01), NS= Non Significant.

Table.4 Effect of supplementation of probiotic (*Bacillus subtilis*) on cumulative feed consumption (g)

Days of Observation	Groups / Treatments				Sig.
	T1	T2	T3	T4	
0-7	124.01±1.19	130.56±3.36	121.07±3.87	129.12±2.41	NS
0-14	460.76±8.84	458.58±10.41	445.05±6.42	451.83±5.08	NS
0-21	1080.62±10.64	1116.17±13.84	1074.33±11.83	1082.35±5.86	NS
0-28	1971.44±7.85 ^b	1985.36±12.51 ^b	1918.11±10.83 ^a	1944.82±6.01 ^{ab}	*
0-35	2820.50±5.99 ^b	2820.46±15.30 ^b	2745.10±16.83 ^a	2756.13±9.72 ^a	**
0-42	3719.25±3.22 ^b	3698.28±13.97 ^b	3606.83±14.99 ^a	3594.00±12.86 ^a	**

Superscripts are read row wise for comparison of means. Means in the same row with different superscripts a, b are significantly different * (P<0.05), ** (P<0.01), NS= Non Significant

Table.5 Effect of supplementation of probiotic (*Bacillus subtilis*) and AGP on weekly feed conversion ratio

Week	Groups / Treatments				Sig.
	T1	T2	T3	T4	
1 st	0.90±0.005	0.95±0.017	0.86±0.029	0.92±0.011	NS
2 nd	1.15±0.02	1.12±0.03	1.10±0.08	1.11±0.01	NS
3 rd	1.70±0.05 ^c	1.61±0.02 ^b	1.55±0.03 ^{ab}	1.48±0.08 ^a	**
4 th	1.51±0.01 ^c	1.49±0.05 ^c	1.44±0.01 ^b	1.37±0.01 ^a	**
5 th	2.08±0.01	2.00±0.02	1.99±0.03	1.88±0.08	NS
6 th	2.82±0.6	2.83±0.02	2.05±0.1	2.13±0.2	NS

Superscripts are read row wise for comparison of means. Means in the same row with different superscripts a, b, c are significantly different * (P<0.05), ** (P<0.01), NS= Non Significant

Table.6 Effect of supplementation of probiotic (*Bacillus subtilis*) and AGP on cumulative feed conversion ratio

Days of Observation	Groups / Treatments				Sig.
	T1	T2	T3	T4	
0-7	0.90±0.05	0.95±0.01	0.86±0.02	0.92±0.01	NS
0-14	1.07±0.01	1.06±0.01	1.02±0.03	1.04±0.02	NS
0-21	1.36±0.01 ^b	1.33±0.01 ^b	1.28±0.01 ^a	1.26±0.04 ^a	**
0-28	1.42±0.09 ^c	1.39±0.01 ^c	1.34±0.01 ^b	1.31±0.05 ^a	**
0-35	1.57±0.03 ^c	1.53±0.08 ^c	1.49±0.08 ^b	1.44±0.01 ^a	**
0-42	1.76±0.05 ^b	1.72±0.07 ^b	1.59±0.03 ^a	1.55±0.02 ^a	**

Superscripts are read row wise for comparison of means. Means in the same row with different superscripts a, b, c are significantly different * (P<0.05), ** (P<0.01), NS= Non Significant

Table.7 Effect of supplementation of probiotic (*Bacillus subtilis*) on carcass traits (% of live weight) in broilers

Particulars	Treatments				Sig.
	T1	T2	T3	T4	
Dressed wt.	73.52±2.19	77.49±1.76	79.92±1.73	79.13±1.21	NS
Liver	1.77±0.15	1.95±0.10	1.80±0.11	1.74±0.08	NS
Heart	0.49±0.05	0.50±0.03	0.56±0.05	0.47±0.04	NS
Intestine	4.28±0.44	4.66±0.24	4.15±0.24	4.86±0.26	NS
Breast	11.62±0.34 ^a	12.47±0.09 ^{ab}	13.31±0.33 ^b	15.31±0.72 ^c	**
Thigh	20.36±0.09	20.84±0.97	21.36±0.44	19.47±0.43	NS
Wing	15.84±1.26	17.75±0.46	19.94±1.13	18.85±0.36	NS
Back	14.32±0.23	13.66±0.37	13.52±0.59	12.88±0.27	NS
Giblet	4.72±0.10	5.11±0.24	4.76±0.19	5.04±0.02	NS

Superscripts are read row wise for comparison of means. Means in the same row with different superscripts a, b, c are significantly different * (P<0.05), ** (P<0.01), NS= Non Significant

The significant beneficial effect of dietary supplementation of *Bacillus subtilis* spores on feed conversion ratio (FCR) of broiler during starter and finisher phase is in close agreement with the reports of previous researchers [Shim *et al.*, (2012); Zhou *et al.*, (2010) and Sabatkova *et al.*, (2008)] who had reported that supplementation of broiler feed with *Bacillus subtilis* and *B. licheniformis* improved the feed conversion efficiency. Similarly, Panda *et al.*, (2008) reported significantly better feed conversion efficiency in White Leghorn Breeders stock during (25-40 wks of age of birds) with dietary inclusion

of *Bacillus subtilis* and *B. licheniformis* (at the rate of 6×10^8 spores per kg of diet). In present study the cumulative FCR was significantly better in probiotics supplemented group as compare to AGP supplemented group during starter and finisher phase and these findings corroborate with Salim *et al.*, (2013) who has reported better feed conversion ratio in broiler chicken fed diets with probiotic as compared to birds of antibiotic and control groups. The inclusion of desirable microorganisms (probiotics) in the diet allows the rapid development of beneficial bacteria in the digestive tract of the

host, improving its performance (Edens, 2003). As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes (Pelicano *et al.*, 2004), which may explain the improvement in cumulative feed conversion ratio observed in the present study during starter and finisher phase.

Carcass traits

The effect of supplementation of different concentration of *Bacillus subtilis* spores on carcass traits expressed as percentage of pre slaughter body weight of birds is presented in table 7.

The average dressing percentage in slaughter birds, at the end of 42 days of feeding trial was 73.52 ± 2.19 , 77.49 ± 1.76 , 79.92 ± 1.73 and 79.13 ± 1.21 % for the groups T1, T2, T3 and T4 groups, respectively. In the present study a non-significant increase in the dressing percentage was found due to supplementation of probiotics and AGP as compare to control. The effect of dietary supplementation of *Bacillus subtilis* based probiotics and AGP on weight of internal organs *viz.* liver, heart and intestine was non-significant. The weight of different carcass cuts (thigh, wing, and back) as per cent of pre-slaughter weight differ non significantly amongst the group. However the weight of breast as per cent of live weight was significantly ($P < 0.01$) high in the birds of group T4 (15.31%) which were fed on diets supplemented with *Bacillus subtilis* spores @ 1 million/g of finished feed followed by T3 (13.31%), T2 (12.47%) and T1 (11.62%) and difference between T3, T2 groups and T1 was statistically significant and T2 and T3 were comparable in this regard.

The results of present study indicated the beneficial effect of probiotic (*Bacillus*

subtilis) supplementation @ 1 million /g of finished feed on some of the carcass characteristics of broiler, such as yield of breast meat. The findings corroborate with Molnar *et al.*, (2013) who also reported that *Bacillus* species supplemented group had significantly ($P < 0.05$) higher breast yield than the control group. Increased carcass yield, leg and breast weight was also reported by Kabir *et al.*, (2004) and Farhoomand and Dadvend (2007). Whereas Mahmoud *et al.*, (2017) did not found statistically significant difference in carcass yield between birds of probiotic supplemented group and control. In contrast Pelicano *et al.*, (2003) observed that probiotic use in broiler diets lowered the dressed carcass and back yields and increased leg yield while wing and breast yield remained similar across treatment groups. Many reports indicated that the carcass weight increased by increasing the protein content of diet. Adding bacterial probiotic to diet enhanced the protein availability (Nahanshon *et al.*, 1993), the numerical increase in the dressed carcass weight observed in the present study is probably due to increase in nitrogen retention as *B. subtilis* positively affect the ileal CP digestibility.

Probiotics containing different strains of *Bacillus subtilis* spores showed better results in terms of improved growth performance with better FCR as compared to antibiotic growth promoter. The dressing percentage of broiler birds was found non-significant higher due to supplementation of probiotics and AGP as compare to control. It can be concluded that *Bacillus subtilis* spores as probiotics are promising feed additive for growth performance and carcass quality in broilers.

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