Journal of Advanced Zoology

ISSN: 0253-7214

Volume ***45*** *Issue* ***4*** ***Year 2024*** *Page* ***25-33***

Probiotic Administration of *Bacillus Subtilis* on Growth Performance and Biochemical Parameters in Broilers Chicken

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Abstract

Problem statement: As the intestinal function is intimately affected by fed diets, many kinds of natural substances, prebiotics and probiotics have been supplemented to broilers to increase poultry production by activating intestinal function. The aim of this study was to investigate whether *Bacillus subtilis* could improve the growth performance and serum biochemistry in broiler chicken.

Approach: A total of 200 broiler chicks were randomly assigned to 4 treatment groups, consisting of 2 replicates of 25 birds each. Commercial mash pre-starter, starter and finisher diets were supplemented with 50, 100 and 150 ppm of *B. subtilis*.

Results: Body weight gain was better in all the experimental groups than the control. The growth performance was increased in 150 ppm of *B. subtilis*. Total cholesterol and Triglycerides were decreased, HDL, Serum Glutamine Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT) were increased.

Conclusion: This study shows that adding *B. subtilis* to the broiler diet could improve the growth performance, increase feed efficiency and regulate serum biochemical parameters. Based on our study, it could be recommended that addition of *B. subtilis* at 150 ppm (150 mg/kg) could improve the growth performance of broiler chicken.

Keywords: Probiotic, Chicken, Performance, SGOT and SGPT.

Introduction:

The growth of the poultry industry average ranges between 5% - 6% in layers and 10% - 12% in the broiler segment. The industry is evolving and consolidating its position in Indian Economy (Linge, 2005). Probiotics are live bacteria, fungi, or yeasts that supplement the gastrointestinal flora and help to maintain a healthy digestive system. The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) working group have defined probiotics as "live microorganisms that, when administered in adequate amounts, confers a health benefit on the host" [FAO 2006].

B. subtilis are found in soil and the gastrointestinal tract of ruminants and humans. It enhances laying performance and helps the immune system and gut health. [Mazanko *et al.*, 2018; Cheng *et al.*, 2017; Sadeghi *et al.*, 2015; Forte, *et al.*, 2016; Abd El-Hack, *et al.*, 2016; The genera of probiotic microorganisms commonly used for poultry include Bifidobacterium, Lactococcus, Lactobacillus, Bacillus, Streptococcus, and yeast such as Candida. The standard criteria for selecting probiotic strains include tolerance to gastrointestinal conditions, the ability to adhere to the gastrointestinal mucosa, and the competitive exclusion of pathogens [Gadde *et al.*, 2017, Klaenhammer,1999]. Additionally, probiotics are selected based on their survival in manufacturing, transportation, storage, application processes, and their ability to maintain viability and desirable characteristics [Bajagai *et al.*, 2016] and the modulation of the immune system [Bai *et al.*, 2013, Broom, L.J. and Kogut, 2018].

B. subtilis can form spores in adverse environment that has some unique biological characters such as resistance to acid, alkali, and heat. They also grow fast. Thus, the spores can still plant in intestinal tracts to grow and breed on arrival after the extrusion process for granulating in feed processing and the expose to strong acidic environment in animals' stomach. Moreover, *B. subtilis* is aerobic bacteria, it takes a large amount of free oxygen while reproducing in the intestinal tract thus it can strongly restrain the growth of the majority of aerobic pathogen bacteria, enhance the growth of anaerobic probiotics such as Lactobacillus, yeast and Bifidobacterium (Wang *et al*., 2006). Therefore, it is useful to restore and maintain the intestinal flora balance of animal, improve immune function, enhance animals' resistance to disease, and promote their growth (Gao *et al.*, 2012; Zhenhua Gao *et al.*, 2017). Now, *B. subtilis* has become an advanced research hotspot on animal probiotics study and it was one of bacteria approved by the Ministry of Agriculture, Govt. of India that can be applied in animal husbandry.

MATERIALS AND METHODS

Birds, Diet and Experimental Period

Two hundred day-old male broiler chicks (Ross 308) assigned to 25 chicks of 2 treatment groups, randomly. The experimental design was completely random, consisting of three dietary levels (50, 100 and 150 ppm) of each two forms (powdery and granular) of *Bacills substills* and a control group (without probiotics) were formulated (Table 1, 2, and 4). Each treatment had two replicates of 25 birds. Chicks fed three basal diets of Maize-soybean during four periods of 0-10 days birds fed with Pre-Broiler Starter, 11-20 days birds fed with Broiler Starter I, 21–30 days birds fed with Broiler Starter II, 31-36 days birds fed with Broiler Finisher diets. The diets supplemented with amino-acids, minerals, and vitamins to meet all the Ross 308 requirements (Ross 2019). The *Bacills subtilis* (containing 1×10^9 CFU/g) was provided by Pucheng Lifecome Biochemistry Co. Ltd., Fujian, China.

Body weight and Feed Intake Measurement:

Birds were group weighed at 1, 10, 20, 30 and 36 days of age. Feed intake was recorded replicate wise at 10, 20, 30 and 36 days of age. The data on feed intake and bodyweight gain were used to calculate feed/gain ratios or feed conversion ratio (FCR).

Table 1: Ingredients composition (%) of experimental Pre-Broiler Starter rations

Ingredients	Control (without probiotic)	Probiotics@ 50 ppm	Probiotics@ 100 ppm	Probiotics@ 150 ppm
Maize	48.33	48.33	48.33	48.33
Soya DOC 48%	35.45	35.45	35.45	35.45
Crushed fish 45%	6.00	6.00	6.00	6.00
Meat-cum-Bone Meal	4.00	4.00	4.00	4.00
Ricebran oil	3.60	3.60	3.60	3.60
Di-calcium Phosphate	0.05	0.05	0.05	0.05
DL-Methionine	0.29	0.29	0.29	0.29
L-Lysine HCl	0.90	0.90	0.90	0.90

L-Threonine	0.10	0.10	0.10	0.10
Sodium bi-carbonate	0.10	0.10	0.10	0.10
Salt	0.07	0.07	0.07	0.07
Choline chloride 60%	0.15	0.15	0.15	0.15
Additives	0.96	0.96	0.96	0.96
B. subtilis	Nil	0.005	0.010	0.015
Total	**100**	**100**	**100**	**100**

Table 2: Ingredients composition (%) of experimental Broiler Starter-I rations

Ingredients	**Control (without probiotic)**	**Probiotics@ 50 ppm**	**Probiotics@ 100 ppm**	**Probiotics@ 150 ppm**
Maize	55.40	55.40	55.40	55.40
Hypo Soya DOC	29.52	29.52	29.52	29.52
Crushed fish 45%	6.00	6.00	6.00	6.00
Meat-cum-Bone Meal	4.00	4.00	4.00	4.00
Ricebran oil	2.90	2.90	2.90	2.90
Di-calcium Phosphate	0.40	0.40	0.40	0.40
DL-Methionine	0.26	0.26	0.26	0.26
L-Lysine HCl	0.10	0.10	0.10	0.10
L-Threonine	0.03	0.03	0.03	0.03
Sodium bi-carbonate	0.20	0.20	0.20	0.20
Choline chloride 60%	0.17	0.17	0.17	0.17
Salt	0.09	0.09	0.09	0.09
Additives	0.91	0.91	0.91	0.91
B. subtilis	Nil	0.005	0.010	0.015
Total	**100**	**100**	**100**	**100**

Table 3: Ingredients composition (%) of experimental Broiler Starter-II rations

Ingredients	**Control (without probiotic)**	**Probiotics@ 50 ppm**	**Probiotics@ 100 ppm**	**Probiotics@ 150 ppm**
Maize	59.54	59.54	59.54	59.54
Hypo Soya DOC	25.30	25.30	25.30	25.30
Crushed fish 45%	4.80	4.80	4.80	4.80
Meat-cum-Bone Meal	4.50	4.50	4.50	4.50
Ricebran oil	3.86	3.86	3.86	3.86
Di-calcium Phosphate	0.20	0.20	0.20	0.20
DL-Methionine	0.30	0.30	0.30	0.30
L-Lysine HCl	0.11	0.11	0.11	0.11
L-Threonine	0.03	0.03	0.03	0.03
Sodium bi-carbonate	0.20	0.20	0.20	0.20
Choline chloride 60%	0.17	0.17	0.17	0.17
Salt	0.08	0.08	0.08	0.08
Additives	0.91	0.91	0.91	0.91
B. subtilis	Nil	0.005	0.010	0.015
Total	**100**	**100**	**100**	**100**

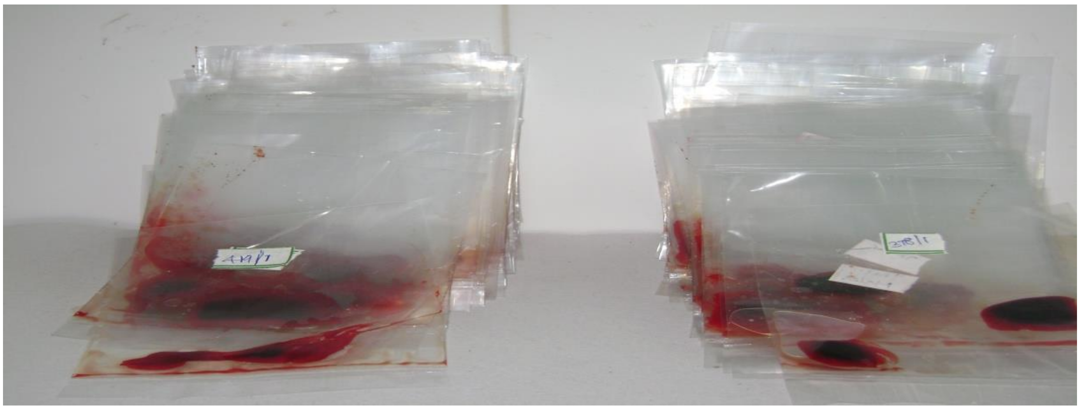
Table 4: Ingredients composition (%) of experimental Broiler Finisher rations

Incredients	Control (without probiotic)	Probiotics@ 50 ppm	Probiotics@ 100 ppm	Probiotics@ 150 ppm
Maize	57.00	57.00	57.00	57.00
Ricebran oil	5.90	5.90	5.90	5.90
Soya DOC 48%	20.50	20.50	20.50	20.50
Meat-cum-Bone Meal	5.00	5.00	5.00	5.00
Crushed fish 45%	10.00	10.00	10.00	10.00
DL-Methionine	0.26	0.26	0.26	0.26
L-Lysine HCl	0.12	0.12	0.12	0.12
L-Threonine	0.03	0.03	0.03	0.03
Sodium bi-carbonate	0.20	0.20	0.20	0.20
Salt	0.19	0.19	0.19	0.19
Choline Chloride 60%	0.17	0.17	0.17	0.17
Additives	0.62	0.62	0.62	0.62
B. subtilis	Nil	0.005	0.010	0.015
Total	100	100	100	100

BODY WEIGHT AND FEED CONSUMPTION

Chicks were weighed individually and the feed consumption was measured 10 days once of experimental period. Cumulative Weight Gain and Feed Consumption were determined, from which, once in 10 days and cumulative Feed Conversion Ratio was calculated.

SAMPLE COLLECTION



BIOCHEMICAL PARAMETERS

At the period of (day 36), 6 broilers were randomly selected from each replicate of each treatment group and blood samples were collected from the bronchial vein during slaughter. The collected blood samples were centrifuged at 2000 rpm for 10 min and the serum samples were decanted into aseptically treated vials and stored at –20 ºC until further analysis of Biochemical values (Triglycerides, HDL, SGOT and SGPT) with commercial kit (Merck, Bangalore).

RESULTS

Growth performance:

Table 5, 6 and 7 represents the mean body weight gain, feed intake and feed conversion ratio of broiler chicks fed different levels of *B. subtilis* at 36 days of age, respectively. Results showed that chicks fed 150 ppm of *B. subtilis* had the higher body weight gain and improved feed efficiency compared with the control group and other dietary treatment groups. Meanwhile, chicks fed 150 ppm *B. subtilis* had higher feed consumption compared with the other dietary treatment groups (control, 50 ppm and 100 ppm of *B. subtilis)*. Results of the present study showed that the inclusion of 150 ppm of *B. subtilis* in broilers ration improved body weight gain, feed intake and feed efficiency.

Table 5: Effect of supplementation of different levels of *B. subtilis* on body weight (gm) of broiler chicks from 0-36 day.

	10th day	20th day	30th day	36th day
Control	254.20 ± 11.96a	746.66 ± 39.39a	1459.38 ± 75.34b	1916.64 ± 99.86b
50 ppm	282.44 ± 4.95a	823.34 ± 7.17a	1583.04 ± 35.30a	2080.44 ± 41.90a
100 ppm	288.80 ± 3.34a	836.4 ± 8.28a	1625.22 ± 8.82a	2003.36 ± 84.16ab
150 ppm	306.88 ± 3.43a	873.7 ± 5.87a	1670.78 ± 5.92a	2123.16 ± 12.32a
Treatment			1390.65**	
Days			7.35**	
Treatment X Days			1.05ns	

** Significant at P< 0.01.Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

Table 6: Effect of supplementation of different levels of *B. substillus* on feed intake (gm) of broiler chicks from 0-36 day.

	10th day	20th day	30th day	36th day
Control	347.00± 4.83a	1245.00 ± 17.07a	2752.00 ± 19.68a	3566.00 ± 36.87a
50 ppm	338.00 ± 5.79a	1234.00 ± 16.5a	2645.00 ± 23.83a	3526.04 ± 26.58ab
100 ppm	330.00 ± 5.47a	1121.00 ± 14.97ab	2547.00 ± 61.26ab	3323.04 ±104.97ab
150 ppm	266.80 ± 5.72a	960.48 ± 16.28b	2381.10 ± 29.90b	3289.00 ± 37.05b
Treatment			1105**	
Days			728**	
Treatment X Days			<1	

** Significant at P< 0.01, Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

Table 7: Effect of supplementation of different levels of *B. substillus* on feed conversion ratio of broiler chicks from 0-36 day.

	10th day	20th day	30th day	36th day
Control	1.171 ± 0.026a	1.47 ± 0.019a	1.640 ± 19.68a	1.975 ± 0.012a
50 ppm	1.170 ± 0.026a	1.42 ± 17.070a	1.623 ± 23.83a	1.858 ± 0.015a
100 ppm	1.126 ± 0.013ab	1.357 ± 0.013ab	1.612 ± 61.26a	1.864 ± 0.087a
150 ppm	1.004 ± 0.040b	1.271 ± 0.079b	1.600 ± 29.90a	1.773 ± 0.036a
Treatment			99.01**	
Days			4.63**	
Treatment X Days			<1	

** Significant at P< 0.01, Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

BIOCHEMICAL PARAMETERS

The summary of the results on the effects of varying levels of *B. subtilis* on the biochemical changes of broiler on 15th, 22nd and 36th day of age are presented in table no 8 to12.

TOTAL CHOLESTEROL (md/dL)

The total cholesterol levels of broiler chicken that were fed with *B. subtilis* under different levels were presented in the table no. 8. The influence of *B. subtilis* treatment clearly indicated that the broiler fed 150 ppm of *B. subtilis* recorded the significantly (128.67 ± 3.16) lower cholesterol levels than control (141.33 ± 6.44) groups.

Table 8: Effect of supplementation different levels of *B. subtilis* on total cholesterol level (mg/dl) of broiler chicks on day 15, 22 and 36.

	15^{th} day	22nd day	36^{th} day
Control	137.67 ± 2.44a	132.67 ± 2.33a	141.33 ± 6.44a
50 ppm	120.67 ± 12.09b	138.50 ± 8.11a	135.00 ± 7.54a
100 ppm	112.67 ± 8.47b	132.00 ± 2.19a	130.67 ± 5.10a
150 ppm	105.17 ± 5.61b	117.33 ± 9.43a	128.67 ± 3.16b
Treatment		4.50*	
Days		4.11*	
Treatment X days		1.44ns	

** Significant at P< 0.01. Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

TRIGLYCERIDES (mg/dL)

The results of triglycerides are represented in Table no 9. At the end of the experiment (36 days old), the influence of *B. substillus* clearly indicate that the broiler fed with 150 ppm of *B. substillus* had significantly (P<0.01) lower (111 ±5.40) Triglycerides levels than the broiler fed control (131.67 ± 4.76) groups.

Table 9: Effect of supplementation of different levels of *B. subtilis* on Triglyceride level (mg/dl) of broiler chicks on day 15, 22 and 36.

	15^{th} day	22nd day	36^{th} day
Control	103.50 ± 14.78a	138.50 ± 9.54a	131.67 ± 4.76a
50 ppm	99.67 ± 10.96a	118.83 ± 7.62ab	124.17 ± 7.31a
100 ppm	100.67 ± 10.69a	111.50 ± 4.06ab	115.00 ± 9.98a
150 ppm	84.83 ± 11.82a	106.50 ± 12.42b	111.00 ± 5.40a
Treatment		8.30**	
Days		<1	
Treatment X days		1.84ns	

** Significant at P< 0.01, Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

HIGH DENSITY LIPOPROTEIN (md/dL)

The influence of *B. subtilis* treatment clearly indicate that the broiler fed with 150 ppm of *Bacills sbstills* (36.67 ± 3.22) recorded significantly (P<0.01) higher HDL levels than broiler fed control (24 ± 2.87) groups (Table no 10).

Table 10: Effect of supplementation of different levels of *B. subtilis* on High Density Lipoprotein level (mg/dl) of broiler chicks on day 15, 22 and 36.

	15^{th} day	22nd day	36^{th} day
Control	10.67 ± 0.99b	12.17 ± 0.54b	24.00 ± 2.87b
50 ppm	16.50 ± 1.38ab	16.50 ± 1.61ab	30.00 ± 2.47a
100 ppm	14.67 ± 1.84ab	18.33 ± 3.34a	32.83 ± 4.10a
150 ppm	19.17 ± 1.01a	20.33 ± 3.34a	36.67 ± 3.22a
Treatment		68.95**	
Days		8.16**	
Treatment X days		1.14ns	

** Significant at P< 0.01. Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (IU/L)

The mean serum glutamic oxaloacetic transaminase of broilers on 36 days of age was significantly lower in 150 ppm of *B. substillus* (98.33± 6.57) supplemented group than the control group (134.33 ± 4.10). (Table no 11).

Table 11: Effect of supplementation of different levels of *B. subtilis* on Serum Glutamic Oxaloacetic Transaminase level (IU/L) of broiler chicks on day 15, 22 and 36.

	15th day	22nd day	36th day
Control	101.17 ± 11.8a	125.17 ± 3.69a	134.33 ± 4.10a
50 ppm	89.50 ± 14.80a	103.83 ± 5.03ab	117.17 ± 9.40ab
100 ppm	97.17 ± 5.98a	93.00 ± 10.51b	126.17 ± 30.21a
150 ppm	87.00 ± 12.50a	89.33 ± 5.95ab	98.33 ± 6.57b
Treatment		6.57**	
Days		3.09*	
Treatment X days		1.73ns	

** Significant at P< 0.01. Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

SERUM GLUTAMIC-PYRUVIC TRANSAMINASE (IU/L)

The mean serum glutamic pyruvic transaminase was significantly lower in the 150 ppm of *B. subtilis* supplemented group (11.77 ± 1.14) than the control (19.5 ± 4.08) group (Table no 12).

Table 12: Effect of supplementation of different levels of *B. subtilis* on Serum Glutamic Pyruvic Transaminase (IU/L) of broiler chicks on day 15, 22 and 36.

	15th day	22nd day	36th day
Control	15.50 ± 1.77a	17.00 ± 1.93a	19.5 ± 4.08a
50 ppm	13.00 ± 1.00a	17.50 ± 2.57a	15.83 ± 1.58b
100 ppm	14.33 ± 3.72a	10.17 ± 0.94b	13.00 ± 2.68b
150 ppm	12.17 ± 4.58a	14.17 ± 2.63b	11.77 ± 1.14b
Treatment		<1	
Days		1.75 ns	
Treatment X days		<1	

** Significant at P< 0.01. Mean in a column followed by a same letter (s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test. # Mean ±S.E

The present results showed that chicks fed 150 ppm of *B. subtilis* reduced the plasma cholesterol and triglycerides levels compared with broiler chicks fed with control, 50 and 100 ppm of *B. subtilis*. Chicks fed ration containing 150 ppm of *B. subtilis* increased the high density lipoproteins.

DISCUSSION

Results of the present study showed that the inclusion of 150 ppm of *B. subtilis* in broilers ration improved body weight gain, feed intake and feed efficiency. The obtained results confirmed the previous findings of several researchers (Zhang *et al.,* 2005; Nilson *et al.,* 2004;). Also in agreement with our study, Onifade *et al.* (1999) reported that probiotics improved feed/gain ratio and BW gain. These results suggest that *B. subtilis* (Probiotics) increased these parameters at an optimum level which probably refer to increased digestive tract activity. It seems that the feed digestion will be altered by adding more *B. subtilis* and the bird growth performance will also alter too.

Our biochemical observations corroborated the data published by Kannan *et al.*, 2005, De Smet *et al.*, 1994 and Savage *et al.*, 1992 who stated that there was a decrease in plasma cholesterol for chicks fed diets contains *B. subtilis* and different probiotics**.** Liver functioning test (LFT) is mainly based on enzymatic level of SGOT, SGPT and alkaline phosphatase. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) catalyze the aspartate into oxaloacetate and alanine into pyruvate, respectively. The mean value of SGOT and SGPT was found to be increased significantly similar to other studies. Probiotics could contribute to the regulation of serum cholesterol concentrations by deconjunction of bile acids (Park, *et al.*, 2018). Since, the excretion of deconjugated bile acids is enhanced and cholesterol is its precursor, more molecules are spent for recovery of bile acids (Pelicano *et al.*, 2005).

CONCLUSION

Broiler chicks fed with 150 ppm of probiotics (*Bacills sustillus*) had the higher body weight gain, feed intake and lower in total plasma cholesterol and triglycerides compared with the control group or other dietary treatments. HDL, SGOT and SGPT were also increased with the inclusion of 150 ppm of probiotics (*Bacills sustillus*) of broiler chicken. Based on the results of this study, it could be concluded that probiotics (*Bacills sustillus*) at a level of 150 ppm (150 mg/kg) is recommended to be the best dietary treatment throughout improvement of performance of broilers and stimulation of biochemical parameters.

Declaration:
The growth performance and biochemical values were increased with supplementation of 150 ppm (150mg/kg) of *B. subtilis* in broiler chicken.

Acknowledgement

The authors are really grateful to Suguna Foods Private Limited, Coimbatore to afford amenities to carry out the work.

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