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Research Article
Effects of Dietary Supplementation of a Single-and a Multi-Strain Probiotic on Growth Performance and Intestinal Histomorphology of Commercial Broiler Chickens

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Abstract

Background and Objective: The effects of dietary supplementation of a multi-strain probiotic (MSP) (*Bacillus subtilis*, *B. coagulans*, *B. licheniformis* and *Clostridium butyricum* HJCB998) on growth performance and intestinal mucosal architecture of broiler chickens were compared with a *Bacillus subtilis* PB6 (BSPB6) based single strain probiotic through an experiment which lasted for 42 days involving 400 straight run flock of Vencobb chickens. **Materials and Methods:** The chicks having an initial mean body weight (BW) of 42.6±0.5 g were randomly allocated to four dietary treatments each consisting of ten replicates (n = 10 chicks/replicate) and were fed with a basal diet devoid of any growth promoter (negative control, NC), the basal diet supplemented with 0.2 g kg^{-1} BSPB6 (BSPB6) and the basal diet supplemented with MSP either at 0.5 g kg^{-1} (MSP 0.5) or 1.0 g kg^{-1} (MSP 1.0). **Results:** The birds fed with the MSP 0.5 and MSP 1.0 diet were found to have numerically better ($p = 0.230$) BW and average daily body weight gain (ADG) vis-α-vis the BSPB6 and NC group. It was also observed that a higher dietary inclusion of MSP (MSP 1.0) did not yield any additional benefit. Supplementation of the BSPB6 or MSP either at 0.5 or 1 g kg^{-1} feed numerically improved ($p = 0.638$) villus height (VH). However, the effects of these dietary treatments on crypt depth (CD) and VH/CD ratio was not conspicuous ($p>0.05$). Analysis of economics of feeding different diets indicated that MSP 0.5 group had superior return on feed cost (INR) per kg BW due to numerical improvement ($p>0.05$) in growth performance than other dietary treatments and control. **Conclusion:** It was concluded that dietary supplementation with MSP yielded better return on investment than BSPB6 and the effects might be mediated through a better BW and moderate positive impacts on intestinal mucosal architecture.

Key words: Broiler chickens, multi-strain, probiotic, growth performance, histology of gut

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The restriction on use of antibiotic as growth promoter in commercial poultry farming has instigated search for newer and feasible alternatives for maintaining poultry health[1]. Probiotics are one such alternative and can be defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[2].

Many probiotic products are available for commercial use in poultry where use of *Bacillus subtilis* spores have been predominant due to their capacity to resist harsh environmental conditions, thriving pelletizing process, tolerance to wide pH variation, dehydration, bile salts and long shelf life[3-5]. Their use primarily targets displacement of enteric pathogen like *Clostridium perfringens*, the causative organism of necrotic enteritis in chicken[6].

Earlier reports of probiotic application in poultry has shown that multi-strain probiotics enhanced performance more than the single strain probiotic[7-9]. In search of other potential alternative strains for probiotic application in poultry, it has shown that spore forming obligatory anaerobe like *C. butyricum* promotes growth performance, immune function and benefits the balance of the intestinal microflora in broiler chickens[10].

With this background, the present study was conducted to compare the effects of dietary supplementation of a single and a multi-strain probiotic, the latter at two different levels of inclusion, on intestinal histomorphology and growth performance of broiler chickens.

MATERIALS AND METHODS

Ethical approval: Experiments were carried out in accordance with the guidelines laid down by the Institute of Animal Ethics Committee for the use of poultry birds.

Probiotic: The multi-strain probiotic (MSP) (Improval™ BFS) used in the present study, is a proprietary preparation manufactured by Zydus Animal Health (A Div. of Cadila Healthcare Ltd., Ahmedabad, India). The preparation is composed of spore-forming bacteria, containing 0.5×10^9 viable spores g^{-1} of *B. subtilis*, *B. coagulans*, *B. licheniformis* and *Clostridium butyricum* HJCB998. The single strain probiotic, a commercially available preparation (the name of which is kept masked to avoid commercial complications) contains 2×10^9 viable spores g^{-1} of *Bacillus subtilis* PB6 (BSPB6).

General bird husbandry and treatments: A total of 400 one-day-old Vencobb broiler chicks (initial mean body weight 42.6±0.5 g) of mixed sex were distributed into 4 treatment groups according to the experimental design described in Table 1. Distribution of the chicks between the groups and within the groups between the pens was done following a completely randomized block design. Each treatment group consisted of 10 replicate pens (each pen measured 1.2 m×1.2 m) and there were 10 chicks in a single pen (n = 100 in a group). The chicks were raised on litter composed of saw dust and paddy straw. The birds were vaccinated at 5 and 20 day against Newcastle disease (Nobilis® ND Clone 30, MSD Animal Health, Kenilworth, NJ, USA) and infectious bursal disease (Nobilis® Gumboro 228E, MSD Animal Health, Kenilworth, NJ, USA) at 12 day of age. The lighting schedule involved 24 h light during the first week and 20 h of light up to 5th week and 24 h light for 6th week. The test facility, pens and birds were observed twice daily for general flock condition, lighting, water, feed, ventilation and unanticipated events and records were maintained from the beginning whenever any bird was found dead, culled or sacrificed due to any reason. All the mortalities were subjected to necropsy to determine the probable cause of death.

Diets and chemical composition: Diet and drinking water were offered *ad libitum*. The birds were fed with a pre-starter (1-14 day), starter (15-28 day) and a finisher diet prepared fresh at the beginning of each feeding period with raw materials of same lot. All diets were formulated following the ideal protein ratio using standardized ileal digestible (SID) amino acid requirement for broiler chickens[11]. Accordingly, the digestible lysine content was maintained at 1.22, 1.10 and 1.00% and the rest of the amino acids were fixed using

Table 1: Experimental design

Description	
NC (Negative control)	Corn soybean-based diet which was devoid of any antibiotic growth promoter or probiotic feed additive
BSPB6	The NC diet was supplemented with 2×10^9 viable spores g^{-1} *Bacillus subtilis* PB6 at the rate of 0.2 g kg^{-1} diet
MSP 0.5	The NC diet was supplemented with 0.5×10^9 viable spores g^{-1} of multi-strain probiotic (*Bacillus subtilis*, *B. coagulans*, *B. licheniformis* and *Clostridium butyricum* HJCB998) at the rate of 0.5 g kg^{-2} diet
MSP 1.0	The NC diet was supplemented with 0.5×10^9 viable spores g^{-1} of multi-strain probiotic (*Bacillus subtilis*, *B. coagulans*, *B. licheniformis* and *Clostridium butyricum* HJCB998) at the rate of 1 g kg^{-3} diet

[1]Inclusion level was based on previous study by Murshed and Abudabos[12]. [2]Inclusion level was based on the commercial proprietary preparation, Improval™ BFS (Zydus AH, A div. of Cadila Healthcare Ltd., Ahmedabad, India). [3]The objective was to study the effect of multi-strain probiotic on growth performance and intestinal histomorphology of commercial broiler chicken at higher inclusion level

Table 2: Composition of the proposed basal diet and the calculated chemical composition

Items	Starter	Grower	Finisher
Ingredients (g kg^{-1})			
Maize	582.5	616.5	640.5
Soybean meal, 460 g of crude protein kg^{-1}	320.0	275.0	240.0
De-oiled rice bran	10.0	10.0	10.0
De-oiled mustard cake, 350 g of crude protein kg^{-1}	25.0	30.0	35.0
Rice bran oil	25.0	35.0	45.0
Di-calcium phosphate	13.0	11.5	10.0
Limestone powder	10.1	8.2	7.1
Salt	3.0	2.5	2.0
Sodium bi carbonate	2.0	2.0	2.0
L- lysine HCl, 780 g kg^{-1}	2.6	2.5	2.0
DL-methionine, 998 g kg^{-1}	2.5	2.3	2.0
L-threonine, 990 g kg^{-1}	1.0	1.2	1.1
Toxin binder[1]	1.0	1.0	1.0
Organic trace mineral premix[2]	0.5	0.5	0.5
Vitamin premix[3]	0.5	0.5	0.5
Choline chloride 60%	0.5	0.5	0.5
Coccidiostat (Salinomycin 12%)	0.5	0.5	0.5
Antioxidant	0.1	0.1	0.1
Phytase 5000 ftu g^{-1}	0.1	0.1	0.1
NSPase enzyme	0.1	0.1	0.1

[1]AntaFerm MT80, Dr Eckel Animal Nutrition GmbH and Co. KG; [2]Organic yeast protein complex of trace minerals was used; [3]The vitamin premix contained (per kg premix) retinyl acetate 3.75 mg, 1,25-hydroxy-cholecalciferol 4 mg, DL-α-tochopheryl acetate 30 mg, menadione 4 mg, thiamine propyl disulphide 3 mg, riboflavin tetrabutyrate 8 mg, riboflavin tetrabutyrate 8 mg, methylcobalamin 0.025 mg, sodium pantothenate 15 mg, pyridoxine 5 mg, niacin 60 mg, biotin 0.2 mg, folic acid 2 mg; Probiotic strains were added to the respective treatment diets by replacing an equivalent volume of de-oiled rice bran.

Table 3: Calculated nutrient values (minimum) of the basal diets

	Starter	Grower	Finisher
ME (MJ kg^{-1})	2950.00	3050.00	3150.00
Standard ileal digestible amino acid (g kg^{-1})			
Lysine	1.22	1.10	0.98
Methionine	0.45	0.42	0.39
Met+Cys	0.85	0.80	0.74
Threonine	0.78	0.73	0.69
Tryptophan	0.20	0.19	0.18
Arginine	1.28	1.20	1.08
Isoleucine	0.82	0.76	0.69
Valine	0.94	0.86	0.77
Protein (g kg^{-1}))	21.50	20.00	18.50
Calcium (g kg^{-1})	0.90	0.80	0.70
Av P (g kg^{-1})	0.45	0.42	0.40
Sodium (g kg^{-1})	0.22	0.20	0.18
Potassium (g kg^{-1})	0.86	0.80	0.75
Chloride (g kg^{-1})	0.24	0.20	0.18

the SID ratio in the starter, grower and finisher diets respectively. The metabolizable energy values were maintained at 2900, 3000 and 3150 kcal kg^{-1} respectively in the starter, grower and finisher diets. The ingredients and calculated chemical composition of the experimental diets were as per the breed standard and are presented in Table 2 and 3 respectively.

Growth performance parameters: The body weights (BW) of individual bird were recorded at weekly interval and average daily body weight gain (ADG) was calculated during 1-14, 15-28, 29-42 and 1-42 day. Feed consumption of birds of each replicate was recorded at weekly intervals and feed consumption per bird per week was calculated which were used to derive data on average daily feed intake (ADFI) during 1-14, 15-28, 29-42 and 1-42 day. Feed conversion ratio (FCR) was calculated as a ratio between feed intake over body weight during corresponding growth periods as detailed above. Mortality, if any, was recorded as it occurred and the data was used to adjust subsequent measurements. Finally, European performance efficiency factor (EPEF), as suggested by Huff *et al.*[13], was calculated using following formula:

$$EPEF = BW\ (kg) \times \%\ liveability \times 100/FCR \times trial\ duration\ (day)$$

Histology of the small intestine: The histological study of the small intestine (SI) was performed to evaluate the effects of the trial diets on the histomorphology and integrity of gut. At 42 day of age one male bird was taken from each pen at random (10 birds from each dietary group, 40 birds in total) and they were slaughtered by exsanguinations. The SI was removed and washed with sterile phosphate buffered saline (PBS) and the contents were removed. Segments measuring 2 cm in length from the mid-points of the jejunum were cut and fixed in 10% buffered formalin. The tissue samples were later embedded in paraffin and a 2 µm section of each sample was placed on a glass slide and stained with haematoxylin and eosin. Histological sections were examined with a phase contrast microscope coupled with an integrated digital imaging analysis system (Olympus Corporation, Tokyo, Japan). The variables measured were villus height, crypt depth and thickness of the lamina propria, tunica muscularis and tunica serosa. Villus height was measured from the tip of the villus to the top of the lamina propria and the crypt depth was measured from the base up to the region of transition between the crypt and villus. Ten measurements were taken per bird for each variable and the average of these values was used for statistical analysis[14].

Statistical analysis: Data was analysed according to one-way analyses of variance using the diets as the grouping factor in a SPSS (version 17.0) processor. The pens were the experimental units for all the parameters except for histology of small intestine where it was the single observations which were used as experimental units. The results were expressed as means and pooled standard error of means. Probability

values of $p<0.05$ were expressed as statistically significant and in case the means were found to be significantly different then the means were separated by Turkey's B test.

RESULTS

Growth performance: Data on BW and ADG of the birds in different experimental groups are presented in Table 4. At 42 day, the birds fed with MSP 0.5 and MSP 1.0 diets had numerically greater ($p = 0.230$) BW and ADG as compared with the NC and the BSPB6 groups of birds. There were non-significant ($p>0.05$) variations between the dietary groups with regard to BW and ADG of the birds at different period of experiment. Numerical difference ($p>0.05$) in BW between groups were observed on 21, 28, 35 and 42 day where highest BW was recorded in BSPB6 group (909 g) on 21 day, MSP 0.5 group (1245.3 g) on 28 day, MSP 1.0 group (1687.9 g) on 35 day and MSP 0.5 group (2252.5 g) on 42 day. The NC group had numerically lesser BW ($p>0.05$) than either of the treatment groups (BSPB6, MSP 0.5 and MSP 1.0) during these periods.

Data on FCR and EPEF are presented in Table 5. Overall, there was no effect of dietary treatments on the FCR of the experimental birds ($p>0.05$). Feed conversion ratio (FCR) during 1-14 day of age was numerically better ($p = 0.431$) in broiler fed MSP 1.0 diet as compared with the rest of the dietary treatments. During 15-28 and 1-28 day period, FCR was numerically poorer ($p = 0.501$) in the NC group and was relatively better ($p = 0.501$) in the MSP 1.0 group. Similar trend in FCR was observed when data was pooled from 1-42 day of period where it was superior in MSP 1.0 group (1.655) and poorer in NC group (1.674). EPEF values ($p = 0.340$) were found numerically better in treatment groups as compared with the NC group where highest value was recorded in the MSP 1.0 group (316.01).

Differences with regard to feed intake were observed between the groups ($p>0.05$) only when measured at different periods of the experiment (data not shown). Mortality was negligible and was non-specific in nature (data not shown). Hence, liveability was not affected by dietary treatments.

Intestinal histomorphology: The data related to the height of the villus, depth of the crypts and villus height to crypt depth ratio (VH/CDare presented in Table 6. No significant differences ($p = 0.773$) were observed between the groups supplemented with different dietary treatments. Numerically, the villi were longer ($p = 0.638$) in birds fed BSPB6, MSP 0.5 and MSP 1.0 diets as compared with the NC group where it was superior in birds fed MSP 0.5 diet. Interestingly, crypts were comparatively deeper ($p = 0.466$) in birds fed BSPB6 diet

Table 4: Body weight (BW) and average daily body weight gain (ADG) at different periods of the experiment

	Diet					
Item	NC	BSPB6	MSP 0.5	MSP 1.0	Pooled SE[2]	p-value[2]
BW (g)						
14 day	471.30	472.60	471.80	474.50	2.180	0.959
21 day	893.60	909.00	905.10	908.50	4.820	0.664
28 day	1231.20	1235.90	1245.30	1236.60	4.930	0.799
35 day	1659.40	1687.40	1679.30	1687.90	9.130	0.676
42 day	2218.30	2248.30	2252.50	2249.90	6.680	0.230
ADG (g bird^{-1} day^{-1})						
1-14 day	30.62	30.72	30.65	30.85	0.156	0.959
15-28 day	54.28	54.52	55.26	54.44	0.392	0.834
29-42 day	70.50	72.32	71.94	72.37	0.484	0.499
1-42 day	51.80	52.52	52.62	52.55	0.159	0.230

[1]NC: Negative control devoid of antibiotic or probiotic feed additive; BSPB6: NC +0.2 g kg^{-1} *Bacillus subtilis* PB6 (2×10^9 spores g^{-1}); MSP 0.5: NC +0.5 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1}); MSP 1.0: NC +1 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1}). [2]Each mean represents 10 replicate pens with 10 birds per pen

Table 5: Feed conversion ratio (FCR) at different periods of the experiment and European performance efficiency factor (EPEF) at day 42

Diets[1]	1-14 day	15-28 day	1-28 day	29-42 day	15-42 day	1-42 day	EPEF[3]
NC	1.189	1.640	1.476	1.916	1.794	1.674	305.530
BSPB6	1.190	1.591	1.445	1.913	1.772	1.659	315.090
MSP 0.5	1.202	1.574	1.440	1.924	1.769	1.659	314.450
MSP 1.0	1.183	1.580	1.436	1.919	1.770	1.655	316.010
Pooled SE[2]	0.004	0.017	0.010	0.015	0.006	0.004	2.278
p-value[2]	0.431	0.501	0.501	0.994	0.450	0.415	0.340

[1]NC: Negative control devoid of antibiotic or probiotic feed additive; BSPB6: NC +0.2 g kg^{-1} *Bacillus subtilis* PB6 (2×10^9 spores g^{-1}); MSP 0.5: NC +0.5 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1}); MSP 1.0: NC +1 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1}). [2]Each mean represents 10 replicate pens with 10 birds per pen

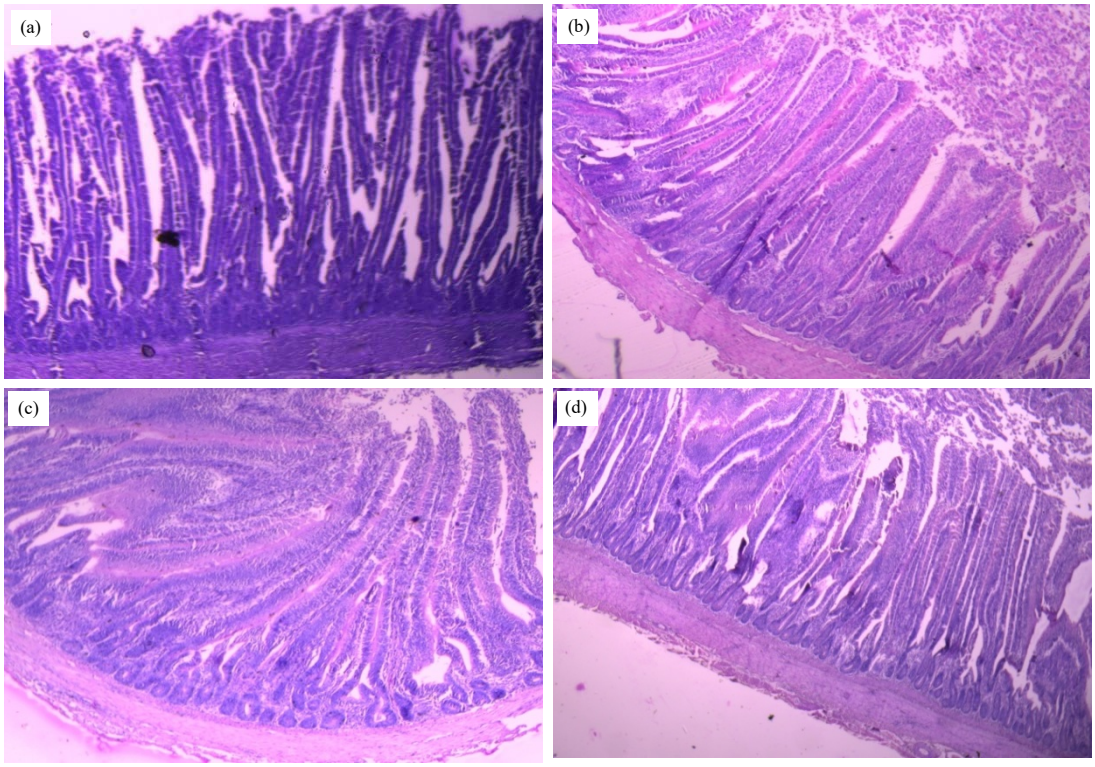

Fig. 1(a-d): Cross section of small intestine (jejunum) of experimental birds (male only) at 42 day of age. The NC group showed distorted villi, indicating relatively poor gut integrity, as compared with the treatment groups

[1]NC: Negative control devoid of antibiotic or probiotic feed additive; BSPB6: NC +0.2 g kg^{-1} *Bacillus subtilis* PB6 (2×10^9 spores g^{-1}); MSP 0.5: NC +0.5 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1}); MSP 1.0: NC +1 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1})

Table 6: Villus height and crypt depth in the experimental birds at 42 day of age (male birds, jejunum only)

Parameters	NC[1]	BSPB6	MSP 0.5	MSP 1.0	SEM[2]	p-value[2]
Villus height µm	634.10	657.60	659.10	648.40	7.47	0.638
Crypt depth µm	187.40	198.80	189.70	189.80	2.71	0.466
Villus height: Crypt depth	3.39	3.32	3.49	3.47	0.06	0.773

[1]NC: Negative control devoid of antibiotic or probiotic feed additive; BSPB6: NC + 0.2 g kg^{-2} Bacillus subtilis PB6 (2 X 10^9 spores g^{-1}); MSP 0.5: NC + 0.5 g kg^{-1} multi-strain probiotic (0.5 X 10^9 spores g^{-1}); MSP 1.0: NC + 1 g kg^{-1} multi-strain probiotic (0.5 X 10^9 spores g^{-1}). [2]Each mean represents 10 replicate pens with one bird randomly selected per pen

as compared with rest of the groups leading to poorer VH/CD ratio in former (BSPB6) while superior (p = 0.773) value in birds fed MSP 0.5 diet. The villus architecture of representative birds from each group is shown in Fig. 1. The NC group showed distorted villi, indicating relatively poor gut integrity, as compared with the treatment groups.

Economics of feeding: Data on economics of feeding different diets is presented in Table 7. The data indicates that feeding the NC diet resulted in highest feed cost per kg BW (36.01 INR). Feeding of the MSP 0.5 diet resulted in reduced feed cost per kg BW (35.87 INR) as compared with the BSPB6 (35.89 INR) and the MSP 1.0 (35.96 INR) group. Increasing the dose of probiotic in MSP 1.0 diet did not yield any additional benefit.

DISCUSSION

The objective of the present study was to compare the effect of the single-strain BSPB6 based probiotic and the MSP on growth performance and intestinal histomorphology of commercial broiler. Our results demonstrated that although BW, ADG and FCR were non-significantly affected by the probiotic treatments, birds fed with the MSP 0.5 diet

Table 7: Economics of feeding different diets in the experiment

Cost of feed (INR kg^{-1})	NC[1]	BSPB6	MSP 0.5	MSP 1.0
Prestarter	22.260	22.380	22.370	22.480
Starter	22.010	22.130	22.120	22.230
Finisher	21.800	21.920	21.910	22.020
Feed intake (kg)				
Prestarter	0.510	0.511	0.516	0.511
Starter	1.244	1.211	1.215	1.201
Finisher	1.888	1.935	1.935	1.940
Total kg	3.641	3.658	3.666	3.652
Feed cost (INR)				
Prestarter	11.350	11.450	11.530	11.480
Starter	27.370	26.810	26.880	26.710
Finisher	41.160	42.430	42.410	42.730
Total (INR)	79.870	80.680	80.820	80.920
Body weight (kg)	2.218	2.248	2.253	2.250
Feed cost (per kg BW)	36.010	35.890	35.870	35.960

[1]NC: Negative control devoid of antibiotic or probiotic feed additive; BSPB6: NC +0.2 g kg^{-1} *Bacillus subtilis* PB6 (2×10^9 spores g^{-1}); MSP 0.5: NC +0.5 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1}); MSP 1.0: NC +1 g kg^{-1} multi-strain probiotic (0.5×10^9 spores g^{-1})

yielded superior return on investment based on analysis of economics of feeding different diets. It has happened largely due to the improvement in growth performance of probiotic supplemented birds compared with the NC group.

Earlier studies on the effects of probiotics on broiler growth performance have been inconsistent. Indeed, there are numerous studies that report positive effects of various probiotics on bird performance[10,15-18], while others have found no or minimal effect[19-23]. Our results tend to support the latter group of studies, although the improvement in BW and ADG of birds fed the MSP 0.5 diet (2252.5 g vs. 2218.3 g of NC group) may require to undertake the experiment on large scale simulating a range of environments relevant to commercial production practices to achieve statistical significance[24].

Numerous studies have been carried out on *B. subtilis*, especially on its sporulation and germination characteristics in chicken and other monogastric animals. *Bacillus subtilis*, essentially, an aerobe, has been demonstrated to have substantially reduced growth in anaerobic condition compared to oxic condition[25]. The preference of this organism towards aerobic condition is in alignment with its higher sporulation characteristic in hind gut of chicken or mice (anoxic condition) and higher germination in upper gut (crop) of chicken[26-29]. Above observations plausibly question the necessity of an obligatory anaerobic strain as probiotic candidate in leveraging enhanced beneficial effects in chicken gut compared with *B. subtilis* alone. With this background, in present experiment, we had hypothesised that inclusion of *C. butyrcium*, an obligatory anaerobe in MSP supplemented (MSP 0.5 and MSP 1.0) diet might be beneficial for producing enhanced growth performance in broilers compared with *B. subtilis* PB6 supplemented diet (BSPB6). Our study revealed that birds fed the MSP 0.5 and MSP 1.0 diet yielded numerically better FCR and ADG respectively vis-α-vis the BSPB6 group, however, could not achieve statistically significant difference. The possible explanation of this could be the absence of real enteric challenge or stress in experimental flocks as several studies have demonstrated influence of environmental stress on the results of probiotic research. It was reported by Fuller[30] that the beneficial effects of probiotics can be produced only by presence of growth depressing microflora (enteric challenges). Similarly, Montes and Pugh[31] had demonstrated that the better results with the use of probiotics happened when the birds were subjected to stress conditions. de Carvalho *et al.*[32] observed that *B. subtilis* produces bioactive compounds (secondary metabolites) during transition of exponential/growth phase to stationary phase (cessation of growth). Transition to stationary phase and secretion of beneficial bioactive compounds by *B. subtilis* is triggered by various environmental signals, for example, depletion of essential nutrients, competition with other bacteria (challenge) for ecological niche, extreme low pH and anoxic conditions[32-35]. It indicates the possibility of reduced secretion of beneficial bioactive compounds by probiotic strains in conducive environment of experimental pens where chances of enteric challenges are minimal.

Improvement in the growth performance of broilers of probiotics supplemented group are thought to be due to their capabilities to manipulate intestinal mucosal architecture positively which leads to higher villus height coupled with higher villus to crypt depth ratio[36,37]. In the present study,

dietary supplementation of probiotics (BSPB6, MSP 0.5 and MSP 1.0 group) numerically improved villus height compared with the NC group. Interestingly, except birds fed the BSPB6 diet, other treatment groups (MSP 0.5 and MSP 1.0) had shown numerically higher VH/CD ratio than the NC group. Longer villi indicates more mature epithelia and enhanced activities of the digestive enzymes secreted from tips of the villi, resulting in improved digestibility[38]. It aptly explains the numerical improvement of ADG, BW and FCR in probiotic treated birds (BSPB6, MSP 0.5 and MSP 1.0 group) compared with the NC group.

The deeper crypt depth in the BSPB6 group resulting in poorer VH/CD ratio as compared to multi-strain probiotic supplemented group (MSP 0.5 and MSP 1.0) and NC group. The correlation with the growth performance trend of birds in these groups is obscure. Crypt may be regarded as the villus factory where deeper crypts indicate higher efficiency in terms of faster tissue turnover and permitting renewal of the villus during cellular sloughing[38-40]. Alternatively, deeper crypt may also be precipitated by aggravated inflammatory response in gut mucosa that ultimately increase loss of enterocytes from the tip of villi[41,42]. Although, several studies had reported that probiotic induces anti-inflammatory response at gut mucosa[43-46], extensive works are warranted for reaching definitive conclusion.

In present experiment, we have studied the effect of single and multi-strain probiotics on feed cost (INR) per kg BW (Table 7). Numerical improvement in BW and FCR had resulted in reduced feed cost (INR) per kg BW in birds chickens fed MSP 0.5 compared with the other dietary treatments (BSPB6 and MSP 1.0 group) and the NC group.

CONCLUSION

Supplementation of diets with either single and multiple-strain probiotic, only numerically improved BW, ADG and FCR. No significant difference between the dietary treatments could be ascertained in this study. Plausibly absence of a real enteric challenge precluded the probiotics to elicit their full potential effects on broiler performance and further studies involving conditions simulating the situations prevailing in the field are warranted to find out the real efficacy of these supplements. Based on the present findings, it was finally concluded that supplementation of commercial broiler diet with a multi-strain probiotic yielded better return on investment than a single-strain probiotic and the effects might be mediated through a better BW and moderate positive impacts on small intestinal mucosal architecture.

SIGNIFICANCE STATEMENT

This study discovered that supplementation of multi-strain probiotic yielded better return on investment than a single-strain probiotic and the effects might be mediated through a better BW and moderate positive impacts on small intestinal mucosal architecture. This study will help researchers uncover critical information on the real values of probiotics in commercial settings, given the fact that there is paucity of literature on comparative study between spore-forming multi-and-single strain probiotic (*Bacillus subtilis*). Thus, the findings of the study may set the trend for researchers to design new models, simulating farm environment, for studying the effects of these probiotics on chicken.

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