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The Viability of Dietary Probiotics (Bactosac®) Influencing Growth Parameters, Cellular Alteration in Intestinal Wall and Immune Response of Broilers

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Abstract: The present study aimed to find out the efficacy of a commercial probiotics supplementation to the diet on the growth performance, histological changes of the jejunal tissue and humoral immune response of broiler chickens. One hundred day old broiler chicks were randomly divided into four groups as group A (Vaccinated probiotics fed group), B (Non-vaccinated probiotics fed group), C (Vaccinated conventional fed group) and D (Non-vaccinated conventional fed group). Groups A and B were taken as experimental birds fed with commercially available feed with the addition of probiotics as per schedule whereas groups C and D were taken as control birds fed with commercial ration. At 30 days of age the birds were immunized with 0.5% of sheep RBC to determine the level of antibody production. The result revealed that the live weight gains obtained were significantly ($p<0.01$) increased more in experimental birds during the periods of 2nd, 3rd, 4th, 5th and 6th week of age compare to control ones at all levels in both vaccinated and non-vaccinated birds. The broiler fed with probiotics gave a strong evidence of increasing the length of jejunal villi compared to conventional fed broiler observed. The antibody production was found significantly ($p<0.01$) higher in probiotics fed broiler than the control ones. Significant variations were observed in the weight of bursa and spleen due to probiotics supplementation. It was concluded that probiotics supplementation have a significant influence on growth performance, meat yield, morphological changes of intestinal wall and immune response of broiler chickens.

Keywords: Broilers, cellular alteration, growth performance, immune response, probiotics

INTRODUCTION

Enteric diseases become an important concern to the poultry industry due to low productivity, increased mortality and public health hazard associated with contamination of poultry products destined for human consumption. With increasing risk of antibiotic resistance and thereby ban on sub-therapeutic antibiotic usage in many countries, it has become imperative to find out alternatives to antibiotics for poultry production. Probiotics are being brought under consideration to fill this gap and already some farmers are using them in preference to antibiotics (Fuller, 1989). Probiotics are defined as feed additives that contain live microorganisms and promote beneficial effects to the host by favoring the balance of the intestinal microbiota (Fuller, 1989; Kabir, 2009). Probiotic supplementation has been shown to improve production parameters-body weight, FCR, mortality (Mohan *et al*., 1996; Huang *et al*., 2004; Kabir *et al*., 2004; Kabir, 2009) and the probiotics have positive effect on humoral and cellular immune responses (Huang *et al*., 2004; Kabir *et al*., 2004; Kabir, 2009) too. In Bangladesh, there are many probiotics are commercially available in the market and their indiscriminate use without experimental evidenced cannot be acceptable. According to the information level of the manufacturer, probiotics compare the following characteristics:

- Microorganism of probiotic can requisite and adjusts within a shortest possible time.
- The stability of microflora may be affected by many factors like change of feed, vaccination, intestinal p^H, bile salt concentration in the gut and use of antibiotics.

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- Many strains of lactic acid producing bacteria are resistant to antibiotics.

It must have rapid colonizing ability and strong foothold in the gut. One of such product available in the market of Bangladesh is probiotics commercially called BACTOSAC® claimed to have all the aforesaid properties. Although a thorough study is lacking probiotics are used in many poultry farms. The present study is therefore under taken to determine the efficacy of probiotics brand BACTOSAC® on the growth performance, histological changes of the jejunal tissue and humoral immune response of broiler chickens from day old to 42 days of age.

MATERIALS AND METHODS

Experimental birds: A total number of one hundred day-old-broiler chicks (Cobb 500 strain) were obtained from the local sale centre of Kazi Farms Limited, Mymensingh, Bangladesh. At the beginning of the experimental study, the broiler chicks were equally divided primarily into two main groups- group I and group II. Group I comprised 50 broiler chicks which belonged to Probiotics Fed Group (PFG) and the remaining in group II comprised 50 broiler chicks which belonged to Conventional Fed Group (CFG). Initial body weight of each bird from each group was recorded just prior to keeping them in two well separated blocks. A total of 25 birds out of 50 from both groups I and II were targeted and selected for vaccination on 6th day of age and the remaining 25 birds from both groups were tagged as non-vaccinated group. In term of the schedule of the experiment as mentioned all birds belonging to group I and II were again divided into four subgroups as group A, B, C and D on 6th day of age.

Feeding and management: Commercially available poultry feed (Narish Feeds Ltd., Dhaka) was used throughout the experimental study. The broiler chicks were fed with standard broiler starter for 14 days and broiler grower for 15-28 days and broiler finisher ration for 29-42 days of age, as formulated by Narish Feeds Ltd., Dhaka. Probiotics as per instruction was added to drinking water at a level of 1cc/5-litres water every day from 0 day to 2nd week of age and 1cc/5-liter water 3 days in a week in 3rd week of age and 1cc/10-liter water from 4th week to rest given to birds belonging to group A and B. The remaining two groups such as group C and group D were kept as control without adding probiotics in drinking water.

Bactosac®: Bactosac® marketed by PVF Agro Limited (Bangladesh) and manufactured by K.M.P Biotech Co. Limited, Thailand was used in this study and containing six strains of various organisms, those are *Lactobacillus acidophilus, Lactobacillus plantarum, Bacillus subtilis, Bacillus licheniformis, Pediococcus pentosaceus, Saccharomyces cerevisiae.*

Immunization: Birds were vaccinated against Baby chick Ranikhet disease by BCRDV following the recommendation of the vaccine manufacturer at the age of 6th day followed by a booster dose at 24th day intraoccularly. The birds were also vaccinated against Gumboro disease by Gumbo-L vaccine (ACI Limited, Bangladesh) following the instruction of the manufacturer. One ampoule Gumbo-L was diluted with 30 mL of diluents and the birds were vaccinated at the age of 11th day followed by a booster dose at 21st day intraoccularly.

Body weight of birds: The live body weight of five birds from each subgroup was taken with the help of standard balance on day old age and sequentially at 7 days interval up to the end period of the experiment.

Carcass yield and cut up meat parts: To study the carcass yield and cut up meat parts of birds, five birds from each group were sacrificed randomly on the 2nd, 4th and 6th week of age. Birds were dissected according to the procedure of Jones (1984). After removing the skin, head and viscera, then final processing was performed and carcass yield was recorded by using electrical weighing machine. Weight of cut up meat parts such as leg and breast was calculated separately.

Preparation of samples for histological studies: For histological studies, the portions of jejunum were collected and fixed in the Bouin's fluid for fixation of tissues. The tissues were then dehydrated in the graded alcohol, cleared in xylene, embedded in paraffin and finally the sections were cut at 6 micron thickness by rotary microtome (Model 820, USA). The sections so prepared were stained with standard Hematoxylin and Eosin (H and E) method (Gridley, 1960).

Experimental immunization: Five birds from each group were injected intravenously (brachial vein) with 0.1 mL of 0.5% Sheep Red Blood Cells (SRBC) at 30 days of age for experimental immunization. Antibody responses to the sheep red blood cells inoculation was measured using a microtiter hemagglutination assay (Wegmann and Smithies, 1966).

Statistical analysis: The data of experimental study on live weight, carcass yield, cut up meat parts, antibody titre, spleen and bursa weights were analyzed using MSTAT computer package programme in a Completely Randomized Design (Steel and Torrie, 1980) and means were compared for significant differences using Duncan's multiple range test (Kramer, 1956).

RESULTS AND DISCUSSION

The results presented in Table 1a and b revealed that the live weight gains were significantly ($p<0.01$) higher in experimental birds as compared to control ones at all levels during the period of 2nd, 3rd, 4th,5th and 6th weeks of age, both in vaccinated and non vaccinated birds. It is however remarkable that a significantly ($p<0.05$) higher live weight gain was constantly attained by non-vaccinated broiler chicks fed with the probiotics from the 1st week of age. The analysis of data clearly evidenced that the average live weight gains were found always on the increase in non-vaccinated birds as compared to vaccinated birds on the 2nd, 3rd, 4th, 5th and 6th week of age. This result is in agreement with many investigators. Higher body weight gains for probiotic fed broilers were also reported by Kabir *et al*. (2004), Islam *et al*. (2004), Kamruzzaman *et al*. (2004), Celik *et al*. (2007), Kabir (2009), Toghyani *et al*. (2011) and Kral *et al*. (2012).

The data presented in Table 1a and b reflects the occurrence of significantly ($p<0.01$) higher carcass yield in broiler chicks fed with the probiotics on the 2nd, 4th and 6th week of age both in vaccinated and non-vaccinated birds. It is interesting to note that the average carcass weight was estimated to have yielded more in non-vaccinated birds than the vaccinated birds on the 2nd, 4th and 6th weeks of age. Although Mahajan *et al*. (1999) recorded in their study that mean values of giblets, hot dress weight, cold dress weight and dressing percentage were significantly ($p<0.05$) higher for probiotic (Lacto-Sacc) fed broilers during summer and winter. Edens (2003) observed the use of probiotics has many potential benefits and include modified host metabolism and killing of pathogens in the intestinal tract, reduced bacterial contamination on processed broiler carcasses, enhanced nutrient absorption and performance. Kabir *et al*. (2004) showed that the probiotics supplementation promoted significant influence on live weight gain, high carcass yield and prominent cut up meat parts.

Table 1a: Effect of probiotics feeding on live weight, carcass yield and cut up meat parts of broilers

		Probiotic fed group (PFG)						
			7 days		14 days		21 days	
Parameters		0 day	A	B	A	B	A	B
Live weight (in gm)		33.24±1.13^{a}	151.0±1.87^{a}	153.0±2.55*^{a}	410.0±2.24^{a}	409.0±3.32^{a}	880.0±12.25^{a}	888.0±5.83^{a}
Carcass yield (in gm)		-	-		251.0±1.0^{a}	241.6±4.0^{a}	-	-
Cut up meat parts (in gm)	Breast	-	-	-	71.72±0.86^{a}	80.94±0.68^{a}	-	-
	Leg weight	-	-	-	91.10±1.01*^{a}	96.38±1.21*^{a}	-	-

		Probiotic fed group (PFG)					
		28 days		35 days		42 days	
Parameters		A	B	A	B	A	B
Live weight (in gm)		1460.0±7.75^{a}	1496.0±16.31^{a}	1870.0±37.42^{a}	1906.0±42.26^{a}	2290.0±36.74^{a}	2360.50±4.65^{a}
Carcass yield (in gm)		989.8± 0.49^{a}	1013.1± 1.50^{a}	-	-	1635.43±3.35^{a}	1708.45±3.24^{a}
Cut up meat parts (in gm)	Breast	332.8± 1.18^{a}	350.0± 0.510^{a}	-	-	529.0±0.40^{a}	553.4± 1.14^{a}
	Leg weight	230.8±1.21^{a}	222.2± 0.254^{a}	-	-	463.6±0.53^{a}	482.8± 0.784^{a}

Table 1b: Effect of conventional feeding on live weight, carcass yield and cut up meat parts of broilers

		Conventional fed group (CFG)						
			7 days		14 days		21 days	
Parameters		0 day	C	D	C	D	C	D
Live weight (in gm)		33.14±0.9^{a}	134.0±5.78^{a}	144.0±5.33*^{b}	395.0±4.47^{b}	398.0±4.81^{b}	830.0±6.51^{b}	844.0±4.0^{b}
Carcass yield (in gm)		-	-	-	176.0±1.0^{b}	173.6±0.509^{b}	-	-
Cut up meat parts (in gm)	Breast	-	-	-	59.20±0.335^{b}	70.46±0.31^{b}	-	-
	Leg weight	-	-	-	69.56±0.27*^{b}	75.50±0.45*^{b}	-	-

		Conventional fed group (CFG)					
		28 days		35 days		42 days	
Parameters		C	D	C	D	C	D
Live weight (in gm)		1400.0±15.81^{b}	1416.0±10.29^{b}	1650±13.54^{b}	1740.5±19.24^{b}	1930.5±33.91^{b}	2010±53.38^{b}
Carcass yield (in gm)		951.6±0.979^{b}	961.0±0.707^{b}	-	-	1302±3.35^{b}	1350.5±3.34^{b}
Cut up meat parts (in gm)	Breast	270.4±0.81^{b}	282.2±0.663^{b}	-	-	445.4±0.503^{b}	461.4±0.62^{b}
	Leg weight	225.3±0.547^{b}	215.9±0.631^{b}	-	-	411.5±0.210^{b}	421.6±0.43^{b}

Means with different superscripts column wise differ significantly at **: $p<0.01$ but means with different superscripts column wise differ significantly at *: $p<0.05$; A and C: Vaccinated birds; B and D: Non-vaccinated birds

Table 2: Mean (±SE) antibody titre (log_2) and mean (±SE) weight of spleen and bursa of broilers at the 5th week of age

	Probiotics fed group (PFG)		Conventional fed group (CFG)	
Parameters	A	B	C	D
Antibody titer (log_2) to SRBC antigen	6.3± 0.04^{a}	5.1±0.033^{a}	3.6±0.21^{b}	2.3±0.77^{b}
Spleen (weight in gm)	0.47±0.053*	0.79±0.014^{a}	0.367±0.018*^{b}	0.47±0.015^{b}
Bursa (weight in gm)	1.37±0.037^{a}	1.83±0.038*^{a}	0.80±0.052^{b}	1.42±0.024*^{b}

Means with different superscripts row wise differ significantly at **: p<0.01 but means with different superscripts row wise differ significantly at *: p<0.05; A and C: Vaccinated birds; B and D: Non-vaccinated birds

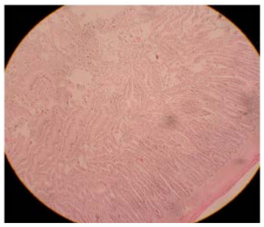

Fig. 1a: Photomicrograph of jejunal tissue of probiotics fed broilers showing longer and larger villus growth (×10, H&E)

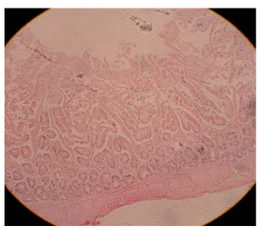

Fig. 1b: Photomicrograph of jejunal tissue of conventional fed broilers showing shorter and smaller villus growth (×10, H&E)

The analysis of data as presented in Table 1a and b focuses that the weight of shank was found significantly (p<0.01) greater for experimental birds as compared to control ones on the 2nd,4th and 6th week of age. While a significantly (p<0.01) higher breast weight in broiler chicks fed with the probiotics was observed on the 4th and 6th week of age. It is remarkable that an analogously and significantly (p<0.05) higher breast portion weight was found in experimental birds as compared to control ones during 2nd week of age. The present findings indicated that in BACTOSAC® fed broilers, the yield of cut up meat parts was better than the control ones, which might be due to the higher body weight and better conformation and finish. Similar findings were also demonstrated by Mahajan *et al*. (1999) and Kabir *et al*. (2004). On the contrary Toghyani *et al*. (2011) recently demonstrated that Carcass yield and relative organ weights were not influenced by dietary treatment of probiotic.

The length of the jejunal villi of probiotics fed broilers as recorded was found to be greater than the conventional fed broilers (Fig. 1a and b). It is clearly demonstrated from the histomicrography of jejunum that the total length of jejunal wall is much longer and larger in both of vaccinated and nonvaccinated probiotics fed broilers than the vaccinated and nonvaccinated conventional fed broilers (Fig. 1a and b). The jejunal glands are elongated and larger in probiotics groups whereas the jejunal glands remain always round and smaller in conventional fed groups (Fig. 1a and b). The present result supports the findings of Samanya and Yamauchi (2002). They indicated that birds that were fed dietary *B. subtilis* var. natto for 28 days had a tendency to display greater growth performance and pronounced intestinal histologies, such as prominent villus height than the controls. This findings also supported by Kabir *et al*. (2005), Samli *et al*. (2007) and Awad *et al*. (2008, 2009).

The evidence of antibody production in response to SRBC and weight gain of spleen and bursal of broilers are presented in Table 2. The antibody production was found significantly (p<0.01) higher in experimental birds as compared to control ones. Panda *et al*. (2000) similarly reported that there was significantly higher antibody production in the 100 mg probiotic supplementation groups at 10 days and 5 days post inoculation in response to SRBC antigen when injected at 14 days and 21 days of age respectively, compared to control. Cross (2002) indicated that some probiotics could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens. Kabir *et al*. (2004) revealed that probiotics supplementation promoted significant influence on immune response, this statement also supported by Haghighi *et al*. (2005), Altaf-Ur-Rahman *et al*. (2009), Taklimi *et al*. (2012) and Apata (2012).

In non-vaccinated birds fed with the probiotics there is a slight increase in weights of spleen (p<0.01). Higher spleen weights were found in vaccinated birds fed with the probiotics. Analogously the weights of bursa were found significantly (p<0.01) higher for vaccinated birds fed with the probiotics as compared to control ones. However a significantly (p<0.05) higher bursal weights were obtained in non-vaccinated birds fed with the probiotics. Kabir *et al*. (2004) observed the significacant increase in the weight of spleen and bursa due to probiotics supplementation. The present findings differed from Mohan *et al*. (1996) and Panda *et al*. (2000) who found that supplementation of probiotic had no effect on weight of internal organs like spleen and bursa. The present results demonstrated that the differences in the weight of spleen and bursa of probiotics and conventional fed broilers could be attributed to different level of antibody production in response to SRBC. It is interesting to note that the weights of spleen and bursa were found higher in non-vaccinated broilers as compared to vaccinated broilers.

CONCLUSION

From the experimental results it was concluded that supplementation of probiotics had significant effect on growth performance, histological changes of the jejunal tissue and immune response in broilers.

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