



Use of probiotics instead of antibiotics in broiler production

MJ Alam^{1*}, Z Ferdaushi²

¹Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka1207;

²Combined Military Hospital, Bangladesh Army, Bangladesh.

Abstract

Effects of different available commercial probiotics on growth performance and blood parameters were evaluated. To achieve the objectives, a total of 198 one-day-old Hubbard Isa Starbro broiler chicks were raised over 28 days. Chicks were wing-banded, weighed individually and randomly allocated into six equally major groups each having three replicates. Broilers of group T1 (control group) were fed the starter and finisher diets. The broilers of groups T2 was fed the control starter and finisher diets supplemented with antibiotic and groups T3-T5 were fed 4 different commercially available probiotics (T3-Guardizen-M, T4-Protexin and T5-Poultry star sol in drinking water. Weekly body weight, feed consumption and feed conversion were recorded during experimental period. Blood parameters at 4 weeks of age including packed cell volume (PCV), haemoglobin (Hb), total protein, albumin, triglycerides, high density lipoprotein (HDL), uric acid, cholesterol, glucose, intestinal microflora, pH and color properties of meat were determined. All birds were kept under similar environmental, managerial and hygienic conditions. Probiotic supplementation significantly increased the body weight and daily weight gain of broiler chicks at 28 days ($p < 0.05$). Improved feed conversion was noticed in birds fed a diet supplemented with probiotic. The effects of probiotics on carcass and some internal organs were measured and results shows that feeding broilers with probiotics have significant effects ($P < 0.05$) on dressed carcass weight, abdominal fat, breast, thigh and liver while it appeared insignificant on gizzard ($P > 0.05$). The lower percent of abdominal fat and the higher percent of dressed carcass, breast and thigh were observed in experimental probiotic(s) groups. pH and meat color did not affect among treatments. There was lower mortality rate in probiotics among groups. Moreover, there was no significant change for Hb, PCV, total protein and albumin concentrations among different groups. Also, total protein, lipids and albumin concentrations were not affected by probiotic(s) supplementation. In addition, different probiotics showed a significant decrease ($p < 0.05$) in triglycerides, cholesterol and uric acid concentration compared to control group. Birds supplemented with probiotics had higher number of lactobacilli but lower number of colibacilli compared to the control. It can be concluded that use of selected commercial probiotic(s) resulted in improved growth performance and carcass yields, and reduced serum cholesterol and uric acid in broiler chickens. Moreover, supplementation of the probiotic(s) to broilers had no detrimental effect on their growth performance and blood parameters. Therefore, usage of these probiotic(s) bacteria as antibiotic alternatives in poultry nutrition can be recommended.

Key words: Blood parameters, broiler, growth performance, probiotic (s)

Introduction

Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics of broiler industry. Therefore, it is highly essential to improve feed efficiency of poultry to produce meat economically and also food safety is more seriously considered than before. On the other hand, economy of food production is also a factor that cannot be ignored. A huge amount of antibiotics has been used to control diseases and improve performances in livestock. However, due to growing concerns about antibiotic resistance and the potential for a ban for antibiotic growth promoters in many countries in the world, there is an increasing interest in finding effective alternatives to antibiotics in poultry production. Poultry feed influences the production cost of chicken. Recently, it is believed that Probiotics have beneficial effects to improve the productive performance of poultry. Probiotics are specific agents produced by micro-organism containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae* and *Torulopsis* (Mohan et al. 1995). However, according to the currently adopted definition by Food and Agriculture Organization and World Health Organization (2001), probiotics are: live microorganisms which when administered in adequate amounts confer a health benefit on the host. The most important advantage of a probiotic is that it neither has any residues in animal production nor exerts any antibiotic resistance by consumption. Therefore, a lot of researchers have partially replaced antibiotics with probiotics as therapeutic and growth promoting agents. It was reported that probiotics have a good impact on the poultry performance (Mountzouris et al., 2007; Koenen et al., 2004), improve microbial balance, synthesize vitamins (Fuller, 1989), decrease pH and release bacteriocins (Rolfe, 2000), improve feed consumption in layers and broilers (Nahashon et al., 1994).

The broiler industry is constantly searching for ways to improve its product and quality in order to meet the

demands of an increasingly demand of consuming public. In this regard, numerous references exist on increasing poultry meat yields and improving carcass quality. For this reason, many ingredients have been using in broiler diets, in recent years. Moreover, there is currently a world trend to reduce the use of antibiotics in animal food due to the contamination of meat products with antibiotic residues (Menten, 2001), as well as the concern that some therapeutic treatments for human diseases might be jeopardized due to the appearance of resistant bacteria (Dale, 1992). It is also reported that additional benefits can be gained by supplementing probiotics in broiler diets as feed additives. Probiotics are used to get rid of abnormalities in the gastrointestinal tract produced by stress and therefore normalize the gut activity (Kutlu and Görgülü, 2001). Studies on the beneficial impact on poultry performance have indicated that probiotic supplementation can have positive effects. Probiotics are reported to prevent colonization gut by pathogens like *Escherichia coli* and *Salmonella*. They also prevent contamination of carcasses by intestinal pathogens during processing and promote higher growth rate and feed conversion efficiency in growing chickens (Hose and Sozzi, 1991; Juven et al., 1991). The use of probiotics for meat and carcass quality improvement has been questioned and many unclear results have been shown. Some authors reported advantages of probiotic administration (Burkett et al., 1977; Jensen and Jensen, 1992; Maruta, 1993; Corrêa et al., 2000; Vargas et al., 2002), whereas others did not observe improvement when probiotics were used (Owings et al., 1990; Quadros et al., 2001). There has been others research by scientists to evaluate probiotics on broilers; however, to date, the data is inconclusive.

There is therefore a need for research on comparison effect of available probiotics. This study was carried out to evaluate effects of four probiotics include; Guardizen-M (trade name), Protexin (trade name) and

Poultry star sol (trade name) in comparison of probiotics less and antibiotics on broilers performance.

Materials and Methods

Experimental design and basal diet: A total of 198-day old broiler chicks of “Hubbard Isa Starbro” strain were purchased from Kazi Farms Ltd., Dhaka, Bangladesh. The birds were randomly assigned to six groups as T1 (Control group-no antibiotics or probiotics), T2 (antibiotics fed group), T3 (Guardizen-M probiotics fed group), T4 (Protexin Boost probiotics fed group) and T5 (Poultry star sol probiotics fed group) each consisting of 33 chickens in which 11 in each replication (3 replications in each group) and were reared in well partitioned area in a room under strict hygienic condition in the experimental poultry shed in the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The experimental groups were fed with commercial ration, but the group T2 supplemented with antibiotics (2.5ml Ciprocin vet® from Square Pharmaceuticals Ltd. whose generic name is Ciprofloxacin Hydrochloride USP/5litre water) and T3, T4 and T5 were fed with commercial ration plus 2.5 g of different probiotics [Guardizen-M®, Dong Bang Co. Ltd., Korea which contains *Lactobacillus plantarum*, *L. bulgaricus*, *L. casei*, *L. acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *S. faecium*, *Aspergillus oryzae* and *Torulopsis bovina*; Protexin® Boost, manufacturer Protexin Animal Health and marketed by Elanco (Bangladesh) Ltd.; Poultry star sol®, manufacturer Biomin and marketed by Renata Ltd., Animal Health Division]/5 L drinking water up to 4th week of age. It is worth to mention that no any antibiotic was supplemented to or injected in the broilers from the first day until the end of the experiment whose were selected for probiotics based. The basal diet was formulated to meet or exceed nutrients recommended by the NRC (1994) for broiler chickens based on soybean meal and corn (without supplementing antibiotics, coccidiostats or growth promoters). Chemical composition of feed ingredients

and experimental diets for dry matter (DM), crude protein (CP), crude fiber (CF) and crude fat (EE) were determined by AOAC, 1990), whereas starter and finisher diet contained 94% and 93% DM, 22% and 20% CP, and 3000kcal/kg and 3100kcal/kg ME, respectively (Table 1).

Table 1. Composition of basal diets and calculated and determined nutrient analysis (AOAC, 1990) (as-fed basis)

Ingredients and composition (%)	Starter diet	Finisher diet
Ground corn	46.29	46.23
Soybean meal (48% CP)	22.14	21.00
Full fat soy	12.50	10.00
Ground wheat	10.00	10.00
Fish meal	4.00	2.50
DCP	1.67	1.73
Ground limestone	0.59	1.30
Salt (NaCl)	0.25	0.26
Soya oil	1.58	3.31
Poultry fat	-	1.50
Lysine	-	0.08
DL-methionine	0.24	0.25
Choline chloride	0.04	0.04
Trace mineral premix ¹	0.30	0.30
Vitamin premix ²	0.50	0.50
Coccidiostat	0.10	0.10
Lasolocyde	-	0.10
Analysis (%)³	94.00	93.00
Dry matter (%)	22.00	20.00
Crude protein (%)	3000	3100
ME kcal/kg		

¹Trace mineral mixture provides in milligrams per kg of diet: Mn, 70; Zn, 50; Fe, 30; Cu, 5, Se, 0.3.; ²Vitamin mixture provides per kg of diet: vitamin A 8000 IU; cholecalciferol 1000 IU; riboflavin 5 mg/kg; niacin 40 mg/kg; thiamin 2 mg/kg; folic acid 0.6 mg/kg; vitamin B12 15 µg/kg;

³Calculated by AOAC (1990).

Vaccination schedule for newcastle and gumboro diseases was maintained properly according the recommended manual of Hubbard Isa Starbro strain.

Growth performance and mortality: Measurements of broiler performance including body weight, daily weight gain, daily feed consumption and mortality rate were determined. All birds in each group were weighed individually at hatch, 1, 2, 3 and 4 weeks of age. Daily weight gains were then calculated for the periods: day-old, 1, 2, 3 and 4 weeks. The feed offered to each room was recorded daily with an automatic weighing machine. At the end of each week feed residues were weighed, feed consumption was therefore recorded on a weekly basis and then calculated as feed consumed per day over the periods: day-old, 1, 2, 3 and 4 weeks. The feed conversion ratios could then be calculated for the time periods of 1st, 2nd, 3rd, and 4th weeks. The feed conversion ratio is feed consumed/weight gain. Mortality rate was weekly determined as a cumulative percentage.

Carcass characteristics: At the end of the experiment, three chickens per replicate were randomly selected and slaughtered for carcass analysis. The birds were dissected at the end of the 4th week feeding trial according to the procedure of Jones (1984). After removing the skin, head and viscera, final processing was performed and the dressed broilers were swept using absorbent paper (AP). The heads, feathers, feet and viscera were removed after slaughter. Then, abdominal fat pad was removed and weighed. Dressed carcass weight was calculated as the percentage of body weight (Petek *et al.* 2005). Thigh, breast, liver and gizzard were weighed individually. All of these traits were calculated in relation to live BW.

Color measurement: The colour measurements were carried out using a tristimulus colorimeter (Minolta Chroma Meter Measuring Head CR-200, Minolta, Osaka, Japan) and this was used to objectively measure CIE Lab values (L* measures relative lightness, a* relative redness and b* relative yellowness). Before each measurement, the apparatus was standardized

against a white tile. The colour values were measured four times on the surface of each carcasses. Colorimeters readings (L*, a* and b*) were always measured from the same points on carcass surfaces (back, breast, leg) for all the carcasses.

pH analyses: The pH value was measured by direct probe of pH meter (SCHOTT L 6880, Lab Star pH). pH measurements were determined by thrusting probe pH meter into breast and leg muscles.

Hematological and biochemical analyses: At the end of the experiment, fresh blood samples were collected from chickens of different groups to measure packed cell volume (PCV) and Hemoglobin (Hb) concentrations. PCV was estimated by the microhematocrit method using capillary glass tubes. Hb concentration was determined according to Coles (1986). On the other hand, at 28 days of age, 4 ml of blood was collected from wing vein from 6 birds in each treatment. In order to prevent clotting, blood was collected in heparinized test tubes and centrifuged (at 2,000 rpm for 10 min), and the serum was separated, then stored at -20°C until assayed to measuring blood parameters (cholesterol, triglycerides and high density lipoprotein (HDL)) using commercial kits (Pars Azmoon) and for total protein, albumin, uric acid and glucose concentrations by using commercial diagnostic kits (Stat Fax 3200, Awareness Technology Inc. Palm City, FL, USA) according to the manufacturer's protocols. The experimental analyses were conducted at the laboratory of the Sher-e-Bangla Agricultural University and Advanced Animal Science, Bangladesh.

Bacterial enumeration: Conventional microbiological techniques using selective agar media were used for analysis. Samples of ileal contents from the bled birds were homogenized in buffered peptone water, and serial of decimal dilution were prepared. Following selective agar media were used for enumeration of target bacterial groups - total aerobes (plate count agar - Merck), lactic acid bacteria (MRS agar - Merck), and coli bacilli (Mac Conkey agar - Merck). Results were

expressed as log10 colony forming units per gram of ileum digesta (log10 cfu/g).

Statistical analysis: All data were analyzed using the One-Way analysis of variance using GLM procedure (SAS Institute Inc., 2003). Significant differences among treatments were identified at 5% level ($P < 0.05$) by Duncan's multiple range tests (Duncan, 1955).

Results and Discussion

Growth performance: Table 2 shows the effect of dietary antibiotic and different probiotics on performance of boiler chickens. According to comparisons of this table it has been proven that the higher amount of body weight gain and the lower level of FCR were observed in the probiotic(s) groups. The improvement in the body weight, daily weight gain, feed consumption and feed conversion ratio in this study may be due to the increased efficiency of digestion and nutrient absorption processes due to presence of the probiotic bacteria. Edens (2003)

reported that 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. As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes. Edens et al. (1997) showed that in vivo and ex vivo administration of *Lactobacillus reuteri* resulted in an increased villus height, indicating that probiotics are potentially able to enhance nutrient absorption and thereby improve growth performance and feed efficiency. On the other hand, the beneficial effect of growth promoting feed additives on animals arises from stabilizing feed hygiene and beneficially modulating the gut ecosystem by controlling potential pathogens. Kabir et al. (2004), for example, conducted a 6-week growth performance study with broilers and found that live weight gain and carcass yields were significantly higher in broilers fed probiotic supplementation.

Table 2. Effect of treatments on growth performance of broilers

Experiment/ Treatment	Weigh improvement (Av. g/day)	Feed intake (Av. g/d)	Average of FCR	Average of wt. (g)
T1	40.9 ^b	84.9 ^c	1.81 ^a	1501.9 ^c
T2	41.1 ^{ab}	85.3 ^b	1.80 ^a	1550.2 ^b
T3	41.3 ^{ab}	86.6 ^a	1.70 ^{ab}	1610.3 ^a
T4	41.8 ^a	86.1 ^a	1.61 ^b	1590.8 ^{ab}
T5	41.6 ^a	86.0 ^a	1.65 ^b	1605.6 ^a
SEM	0.98	1.12	0.02	12.6
P-value	0.02	0.006	0.003	0.02

a-b means with in columns with different superscript differ significantly; T1 (Control group-no antibiotics or probiotics), T2 (antibiotic fed group), T3 (Guardizen- M probiotics fed group), T4 (Protexin probiotics fed group) and T5 (Poultry star sol probiotics fed group).

Carcass characteristics: Table 3 shows the effect of antibiotics and different probiotics on carcass and its parameters. According to the data, there are significant differences in the carcass characters ($p < 0.05$). The results revealed that the treatments had significant

effects in dressed carcass weight, abdominal fat, breast, thigh and liver ($p < 0.01$), but no difference in gizzard ($p > 0.05$). However, the broilers with probiotics, resulted in the most favorable carcass weight while broilers fed with control was comparatively lower. The

lower percent of abdominal fat and the higher percent of breast were observed in experimental probiotics groups. Probiotics have been used as alternatives to antibiotics. For this reason, these probiotics are becoming more important due to their antimicrobial effects and the stimulating effect on animal digestive system (Osman et al., 2005). The active principles of

probiotics act as a digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (Buzuk et al., 2001). According to the results, it can be decided that feeding with these probiotics have positive effects on growth and carcass traits of chicken broilers.

Table 3. The effect of different probiotics on carcass traits of broilers

Parameter(g)	T1	T2	T3	T4	T5	SEM	P-value
Dressed carcass wt.	1126.43 ^c	1162.65 ^b	1207.73 ^a	1193.10 ^{ab}	1204.2 ^a	6.91	0.0001
Abdominal fat	3.86 ^a	3.74 ^a	3.53 ^{ab}	3.60 ^{ab}	3.58 ^{ab}	0.15	0.01
Gizzard	3.08	3.10	3.15	3.25	3.20	0.11	0.21
Breast	33.18 ^c	33.15 ^c	34.20 ^b	35.24 ^a	35.21 ^a	1.52	0.005
Thigh	26.11 ^b	25.48 ^c	26.43 ^b	26.50 ^b	27.11 ^a	1.02	0.006
Liver	3.27 ^c	3.29 ^c	3.62 ^b	4.14 ^a	3.98 ^{ab}	0.33	0.001

Means with different superscripts in the same row differ significantly (p<0.05).

Mortality: Concerning mortalities, cumulative mortality rates were lower in the birds fed the probiotic level than the other groups over the period 0–4 weeks of age (Table 4). With similar trials with broilers given different probiotic(s) preparations, the effects on

mortality were inconsistent (Jin et al., 1998a, b; Zulkifli et al., 2000). O’Dea et al. (2006) reported that there were no significant differences in broiler mortality between the probiotic treatment groups in any of the trials.

Table 4. Mortality % of chickens fed on different treatment containing probiotics

Age in wk	<u>Mortality % of treatment groups</u>					SEM	Level of Significance
	T1	T2	T3	T4	T5		
1	1.5	0	0	0	0	0.04	NS
2	0	0.5	1	0	0	0.04	NS
3	0.5	1	0	1	0	0.07	NS
4	1.0	0	0	0	0	0.02	NS

Means within rows with no common letters are significantly different (p<0.05).

pH and meat color: The results of the pH and L*, a* and b* values determined in this study were shown in Table 5. The use of probiotic didn’t influence (p>0.05) on pH values, L*, a* and b* values during the slaughter ages at 28 days. Karaoğlu et al. (2004) and

Yang and Chen (1993) reported that pH values were low in broiler carcasses during post-mortem and increased by storage, and L*, a* and b* values were also affected by storage, and meat colour traits were highly correlated with pH values. There is high

correlation between muscle ultimate pH and meat colour, and particularly for lightness. It is also well known that darkly coloured muscle is associated with high muscle pH (Livingston and Brown, 1981). Therefore, a high-pH of muscles have darker colour than those of a low-pH (Allen et al., 1997; Fletcher et al., 2000). Castellini et al. (2002) reported that pH value can vary between 5.96 and 6.18 in fresh broiler muscle. Qiao et al. (2002) also determined that mean

pH values of broiler breast meat as 5.96 ± 0.03 . No significant differences were found among the different probiotics, antibiotics and control groups ($p > 0.05$). Results of our experiment show clearly that the use of the probiotic(s) in broiler diets had no adverse affect pH, L*, a* and b* value. Therefore, the effectiveness of probiotic(s) supplements to broiler diets has similar quality on carcass color traits after slaughter which is acceptable to the consumer.

Table 5. The effect of probiotic on the pH, L*, a* and b* values of broiler carcasses at 28 days of age.

Treatments	pH	L*	a*	b*
T1	6.03	65.19	2.48	10.09
T2	6.02	65.16	2.86	10.31
T3	6.01	65.11	2.38	10.49
T4	6.05	64.61	2.55	10.60
T5	6.06	64.57	2.60	10.58
SEM	0.05	0.84	0.45	0.31
Level of Significance	NS	NS	NS	NS

Any three means in the same column having the same sections are not significantly different at $p < 0.05$.

Haematological and biochemical parameters: The result of this experiment for cholesterol, high-density lipoproteins (HDL), triglyceride and glucose are presented in Table 6. Concerning the effect of probiotic(s) supplemented on serum cholesterol, in samples tested illustrated in Table 6 which indicate(s) that the probiotic(s) has cholesterol decreasing effect on broilers. At 28 days of age, chicken groups fed with various probiotic(s) showed a significant decrease in cholesterol concentrations when compared to the antibiotic and control group. Reduction in circulating cholesterol with supplemental probiotic(s) agrees with the results of other researchers (Onifade et al., 1999; Jouybari et al., 2009). Also, blood cholesterol levels of layers fed yeast supplemented diets were low compared to the control (Saadia et al., 2010). Mohan et al. (1996) mentioned that chickens that received probiotics in diets had lower serum cholesterol content compared to the control birds. Similar results were reported by Arun et al. (2006) who found that serum total cholesterol and triglycerides were reduced significantly by dietary

supplementation of probiotic(s). The significant reduction in serum cholesterol of broiler chickens fed probiotic(s) supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic(s) supplementation (Mohan et al., 1995, 1996). Probiotics could contribute to the regulation of serum cholesterol concentrations by deconjugation of bile acids. Since the excretion of deconjugated bile acids is enhanced and cholesterol is its precursor, more cholesterol molecules are spent for the recovery of bile acids (De Smet et al., 1994). Also, it was speculated that *Lactobacillus acidophilus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim et al., 1996). In addition, usage of probiotic had enhancing effect on HDL; all groups that received probiotic(s) bacteria in drinking water had the highest concentration of HDL in their blood. The effect of treatments also observed significant different in blood glucose. A significant

increase in glucose level was recorded in antibiotic treatment which had no significant difference with other groups except control ($P < 0.01$). Using of antibiotic or probiotic has enhanced the serum glucose level in broilers; which is agreeing with results of Azza et al. (2012). With regard to the influence of probiotic on haematological and biochemical parameters

investigated in the experimental, no significant changes on haemoglobin and PCV among groups at all times of this trial as illustrated in Table 7. This agrees with the study done by Dimcho et al. (2005) who found that the probiotic supplementation did not affect the blood constituents comprising, haemoglobin concentrations.

Table 6. Effect of antibiotic and probiotics on cholesterol, HDL, triglycerides and glucose levels of broiler.

Treatments	Cholesterol (mg/dl)	HDL	Triglycerides (mg/dl)	Glucose (mg/dl)
T1	159 ^a	25.50 ^b	55 ^{ab}	77.50 ^b
T2	159 ^a	23.30 ^c	58 ^a	82.90 ^a
T3	136 ^b	29.70 ^a	50 ^b	79.50 ^{ab}
T4	135 ^b	31.50 ^a	51 ^b	81.50 ^{ab}
T5	140 ^b	30.70 ^a	52 ^b	80.70 ^{ab}
SEM	2.72	1.71	0.44	0.67
P-value	0.0001	0.0001	0.001	0.009

^{a-c} Means with different superscripts are statistically different ($P < 0.01$).

The amount of serum total protein, albumin and uric acid levels are shown in Table 7. The treatments had no significant effect on total protein and albumin in serum ($P > 0.05$), but significant effect was observed in uric

acid. The serum concentrations of total protein and albumin were not affected by any of the probiotic(s) supplementation in this study.

Table 7. Effect of antibiotic and probiotics on total protein, albumin, Hb, uric acid and glucose levels of broiler

Treatments	Total protein (g/dl)	Albumin (g/dl)	Hb (g/L)	Uric acid (mg/dl)	Glucose (mg/dl)	PCV%
T1	4.89	3.91	85.12	4.28 ^{ab}	77.5 ^b	28.6
T2	5.06	3.94	82.9	4.56 ^a	82.92 ^a	29.01
T3	4.96	3.98	81.4	4.53 ^a	79.0 ^{ab}	29.6
T4	4.85	3.93	83.4	4.37 ^{ab}	81.5 ^{ab}	29.4
T5	4.72	3.86	82.9	4.60 ^a	80.72 ^{ab}	29.8
SEM	0.05	0.02	0.89	0.04	0.67	0.09
P-value	0.37	0.22	0.02	0.05	0.009	0.35

^{ab} Means with different superscripts are statistically different ($P < 0.05$).

These findings agree with those of Dimcho et al. (2005) who found that probiotic(s) supplementation did not affect the total proteins concentrations of chickens. Djouvinov et al. (2005) and Aluwong et al. (2012) also

didn't observed significant effect on total protein and albumin level with supplementing rations with probiotics. The highest level of uric acid was observed in control group than the probiotic(s) groups.

Decreased uric acid in probiotics received groups agrees with Kamgar et al. (2013) and Newaj-Fyzul et al. (2007) findings. Results of this experiment revealed that there was a significant decrease in uric acid level in probiotic(s) groups, indicating beneficial effect of the probiotic(s) on the kidney function. On the other hand, certain probiotic(s) microorganisms can utilize urea, uric acid and creatinine and other toxins as its nutrients for growth (Salim et al., 2011).

Intestinal bacterial status: Influence of various experimental groups on the microbial population in the GI tract is reported in Table 8. Treatments had significant effect on microbial population of the selected bacteria in GI tract.

In the case of total aerobic bacteria, the order of counts was observed in T1, T5, T2, T3 and T4 groups, respectively. The lowest number of total aerobic bacteria was recovered in T4 group ($P < 0.01$). The higher population of Lactobacillus was observed in the groups that consumed probiotics and also antibiotics in drinking water whose had significant difference with control group ($P < 0.01$). The lower number of Coli bacilli bacteria was observed in probiotics and antibiotic groups those had significant difference with control group. Probably reduction of Coli bacilli occurs due to activity of the used beneficiary bacteria in the probiotics. The maximum population of Coli bacilli was observed in control group ($P < 0.01$).

Table 8. Effect of antibiotic and probiotics on population of intestine micro flora in broiler chickens ($\log_{10}6$ CFU/g)

Treatments	Total aerobic count	Lactic acid bacteria	Coli bacilli
T1	8.83 ^a	9.50 ^b	8.08 ^{ab}
T2	8.61 ^a	10.45 ^a	7.60 ^{bc}
T3	8.60 ^a	10.28 ^a	7.16 ^c
T4	7.79 ^b	10.32 ^a	7.32 ^c
T5	8.62 ^a	10.67 ^a	7.67 ^{bc}
SEM	0.10	0.13	0.14
P-value	0.0001	0.0001	0.0009

^{a-c} Means within same column with different superscripts are significantly different ($P < 0.05$).

Gut microflora has significant effects on host nutrition, health, and growth performance of chickens (Barrow, 1992) by interacting with nutrient utilization and the development of gut system of the host. Reduced ileal colibacilli populations were noticed in chickens given a diet supplemented with lactobacilli strains, but the populations of other kinds of bacteria were not affected (Watkins and Kratzer, 1984).

Conclusion

According to results of this experiment it could be concluded that different probiotic performed positively more or similar to antibiotic and better than control on growth performance, carcass characteristics,

biochemical parameters in serum-blood and intestinal microflora of broiler chickens without detrimental effect. Therefore, usage of these probiotic(s) bacteria as antibiotic alternative in poultry nutrition can be recommended to avoid the human health hazard.

Acknowledgments

The authors appreciate thanks to Ministry of Science & Technology, Govt. of the People's Republic of Bangladesh for funding the research project.

References

Abdulrahim SM, Haddadin MSY, Hashlamoun EAR, Robinson RK (1996). The influence of

- Lactobacillus acidophilus and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. *British Poultry Science*, 34:341-346.
- Allen CD, Russell SM, Fletcher DL (1997). The Relationship of broiler breast meat colour and pH to shelf-life and odor development. *Poult. Sci.*, 76:1042-1046.
- Aluwong T, Raji MA, Hassan BF, Kawu MU, Kobo P, Iaand Ayo JO (2012). Effect of Different Levels of Supplemental Yeast on Performance Indices and Serum Biochemistry of Broiler Chickens. *The Open Conference Proceedings Journal*, 3:41-45.
- AOAC (1990). Official methods of Analysis of the Association of official Analytical Chemists, Washington D. C edited by Kenneth Helrich. 15:69-88.
- Arun K, Panda, RR, Savaram V, Mantena VLN, Sharma R (2006). Dietary supplementation of Lactobacillus Sporogenes on performance and serum biochemico-lipid profile of broiler chickens. *Journal of Poultry Science*, 43:235-240.
- Azza H, Abd-El-Rahman H, Kamel H, Walaa M, Ahmed-Olfat SH, Amira H (2012). Effect of Bactocell® and Revitilyte-Plus as probiotic food supplements on the growth performance, hematological, biochemical parameters and humeral immune response of broiler chickens. *World Applied Sciences Journal*, 18:305-316.
- Barrow PA (1992). Probiotics for chickens, in: R. Fuller (Ed.) *Probiotics: The scientific basis*, (Chapman and Hall, London). pp. 255-257
- Burkett RF, Thayer RH, Morrison RD (1977). Supplementing market broiler diets with Lactobacillus and live yeast cultures. *Animal Science Agricultural Research Report*. Oklahoma State University and USDA. USA.
- Castellini C, Mugnai C, Dal Basco A (2002). Effect of organic production system on broiler carcass and meat quality. *Meat Sci.*, 60:219-225.
- Coles EH (1986). *Veterinary Clinical Pathology* fourth ed. WB Saunders Company, Philadelphia, London, Toronto.
- Corrêa GSS, Gomes AVC, Corrêa AB, Salles AS (2000). Desempenho de frangos de corte alimentados com diferentes promotores de crescimento. In: *Reunião Anual da SBZ, Viçosa*. p. 37.
- Dale N (1992). Probióticos para aves. *Avicultura Profissional*, 10(2): 88-89.
- De Smet P, De Boever S, Verstraete W (1998). Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. *British Journal of Nutrition*, 79:185-194.
- Dimcho D, Svetlana B, Tsvetomira S, Tatiana V (2005). Effect of feeding Lactina_ probiotic on performance, some blood parameters and caecal microflora of mule ducklings. *Trakia Journal of Sciences*, 3:22-28.
- Djouvinov D, Boichera S, Simeonova T, Vlaikova T (2005). Effect of feeding lactina probiotic on performance, some blood parameters and caecal microflora of male ducklings. *Trak Journal Science*, 3:22-28.
- Duncan DB (1955). Multiple range test and multiple F-tests. *Biometrics*, 11: 1-42.
- Edens FW (2003). An alternative for antibiotic use in poultry probiotics. *Brazilian Journal of Poultry Science*, 5:75-97.
- Edens FW, Parkhurst CR, Casas IA, Dobrogosz WJ (1997). Principles of ex vivo competitive exclusion and in vivo administration of Lactobacillus reuteri. *Poultry Science*, 76:179-196.
- FAO/WHO (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina, October 1-4.
- Fletcher DL, Qiao M and Smith DP (2000). The relationship of raw broiler breast meat colour and pH to cooked meat colour and pH. *Poult. Sci.*, 79:784-788.

- Fuller R (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, 66:365–378.
- Hose H, Sozzi T (1991). Probiotics-Facts or Faction. *J. Chem. Tech. Biotech.* 51: 540-544.
- Jensen JF, Jensen MM (1992). The effect of using growth promoting *Bacillus* strains in poultry feed. In: *World's Poultry Congress 18, 1992, Amsterdam. Proc. Amsterdam: WPSA*, 3:398-402.
- Jin LZ, Ho YW, Abdullah N, Ali MA, Jalaludin S (1998b). Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. *Animal Feed Science and Technology*, 70:197–209.
- Jin LZ, Ho YW, Abdullah N, Jalaludin S (1998a). Growth performance, intestinal microbial population, and serum cholesterol of broilers fed diets containing *Lactobacillus* culture. *Poultry Science*, 77:1259–1265.
- Jones R (1984). A standard method of dissection for carcass analysis of poultry. *West of Scotland Agricultural Research. Technical Note*, 222, Ayre, Scotland, pp. 16-20.
- Jouybari M, Rezaei Pour V, Dehpanah N (2009). The effect of novel probiotic on blood parameters and performance in broiler chickens. *Journal of Cell and Animal Biology*, 3:141-144.
- Juven BJ, Meinersmann RJ, Stern NJ (1991). Antagonistic effects of *Lactobacilli* and *Pediococci* to control intestinal colonization by human entero-pathogens in live poultry. *J. Appl. Bacteriol*, 70(2): 95-103.
- Kabir SML, Rahman MM, Rahman MB, Ahmed SU (2004). The dynamics of probiotics on growth performance and immune response in broilers. *Int. J. Poult. Sci.*, 3: 361-364.
- Kamgar M, Pourgholam R, Ghiasi M, Ghane M (2013). Studies on *Bacillus subtilis*, as potential [probiotics, on the biochemical parameters of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to challenge infections. *Advanced Studies in Biology*, 5:37–50.
- Karaoğlu M, Durdağ H (2005). The influence of dietary probiotic (*Saccharomyces Cerevisiae*) supplementation and different slaughter age on the performance, slaughter and carcass properties of broilers. *Int. J. Poult. Sci.*, 4(5):309-316.
- Koenen ME, Karmer J, Van der Hulst R, Heres L, Jeurissen SH, Boersma WJ (2004). Immunomodulation by probiotic *Lactobacilli* in layer and meat-type chickens. *British Poultry Science*, 45:355–366.
- Kutlu HR, Görgülü M (2001). The alternatives for antibiotics-growth factors used in poultry diets as feed additives. *Yem agazin Dergisi (in Turkish)*, 27:45-51.
- Livingston DJ, Brown WD (1981). The chemistry of myoglobin and its reactions. *Food Technol.*, 35:244-252.
- Maruta K (1993). Probióticos e seus benefícios. In: *Conferência APINCO de Ciência e Tecnologia Avícolas; Santos, São Paulo. Brasil*. pp. 203-219.
- Menten JFM (2001). Aditivos alternativos na nutrição de aves: Probióticos e Prebióticos. *Sociedade Brasileira de Zootecnia, A produção animal na visão dos brasileiros, Piracicaba: Fealq*, pp. 141-157.
- Mohan B, Kadrive R, Bhaskaran M, Natarajan A (1995). Effect of probiotic supplementation on serum/yolk cholesterol and on egg cell thickness in layers. *British Poultry Science*, 36(5):799-803.
- Mohan B, Kadirvel R, Natarajan M, Bhaskaran M (1996). Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. *British Poultry Science*, 37: 395–401.
- Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K (2007). Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora

- composition and metabolic activities. *Poultry Science*, 86:309–317.
- Nahashon SN, Nakne HS, Mirosh LW (1994). Production variables and nutrient retention in single comb white leghorn laying pullets fed diets supplemented with direct-fedmicrobials. *Poultry Science*, 73:1699–1711.
- Newaj-Fyzul A, Adesiyun AA, Mutani AA, Ramsubhag J, Austin B (2007). *Bacillus subtilis* AB1 controls an ermine's infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Applied Microbiology*, 103:1699-1706.
- NRC (1994). National Research Council Nutrient Requirements of Poultry. 9th rev. Ed. National Academy Press, Washington, DC.
- O'Dea EE, Fasenko GM, Allison GE, Korver DR, Tannock GW, Guan LL (2006). Investigating the effects of commercial probiotics on broiler chick quality and production efficiency. *Poultry Science*, 85:1855–1863.
- Onifade AA, Odunsi AA, Babatunde, GM, Oloredo BR, Muma E (1999). Comparison of the supplemental effects of *Saccharomyces cerevisiae* and antibiotics in low- protein and high-fiber diets fed to broiler chicken. *Archive of Animal Nutrition*, 52:29-39.
- Osman N, Talat G, Mehmet C, Bestami D, Simsek G (2005). *Intern. J. Poult. Sci.*, 4:879-884.
- Owings WJ, Reynoldas DL, Hasiak RJ, Ferket PR (1990). Influence of dietary supplementation with *Streptococcus faecium* M-74 on broiler body weight, feed conversion, carcass characteristics, and intestinal microbial colonization. *Poult. Sci.*, 69:1257-1264.
- Petek M, Sonmez G, Yildiz H, Baspinar H (2005) Effects of different management factors on broiler performance and incidence of tibial dyschondroplasia. *Br. Poult. Sci.*, 46:16-21.
- Qiao M, Fletcher DL, Northcutt JK, Smith DP (2002). The relationship between raw broiler breast meat color and composition. *Poult. Sci.*, 81:422-427.
- Quadros ARB, Kiefer C, Ribeiro NLC, Zink LA (2001). Características qualitativas da carne de suínos alimentados com rações contendo ou não probióticos. In:XXXVIII Reunião Anual da SBZ, Piracicaba. Anais. Piracicaba, pp. 794-795.
- Rolfe RD (2000). The role of probiotic cultures in the control of gastrointestinal health. *Journal of Nutrient*, 130:3965–4025.
- Saadia M, Nagla KS (2010). Effect of probiotic (*Saccharomyces cerevisiae*) adding to diets on intestinal microflora and performance of Hy-Line layers hens. *Journal of American Science*, 6:159-169.
- Salim HA, Abd-Allah OA, Fararh, KM (2011). Effect of feeding probiotic on hematological, biochemical properties and immune response in broiler. *Benha Veterinary Medical Journal*, 22:35-43.
- SAS Institute (2003). *SAS User's Guide*.Version 9.1 ed. SAS Institute Inc., Cary, NC, USA.
- Vargas Jr JG, Toledo RS, Albino LFT, Rostango HS, Oliveira JE, Carvalho DCO (2002). Características de carcaça de frango de corte, submetidos a rações contendo probióticos, prebióticos e antibióticos. In: XXXIX Reunião Anual da SBZ, Recife. Anais. Recife, CD ROM.
- Watkins BA, Kratzer, FH (1984). Drinking water treatment with commercial preparation of a concentrated *Lactobacillus* culture for broiler chickens. *Poultry Science*, 63:1671-1673.
- WHO (2009). *Cardiovascular Disease*, Fact sheet no. 317, WHO, Geneva, Switzerland.
- Yang CC, Chen TC (1993). Effects of Refrigerated Storage, pH Adjustment, and Marinade on Color of Raw and Microwave Cooked Chicken Meat. *Poult. Sci.*, 72:355-362.
- Zulkifli I, Abdullah N, Mohd Azrin N, Ho YW (2000). Growth performance and immune response of two commercial broiler strains fed diet containing *Lactobacillus* cultures and oxytetracycline under heat stress condition. *Journal of British Poultry Science*, 41:593–597.