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Title: Supplementation of Probiotics in Broiler Rations as an Alternative to Antibiotics

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ABSTRACT

Routine use of antibiotics in poultry production leads to its residues in meats and eggs and multi-drug resistance develops in human gut due to consumption of contaminated products. The study was conducted with a view to investigate the effect of probiotic supplementation to the poultry ration as an alternative to antibiotics on the performance of broilers. The experimental broilers were divided in four groups according to the treatments as control (no antibiotics or probiotics supplementation), AS20 (antibiotics supplementation @20g 100kg^{-1} of feed), PS10 (probiotics supplementation @10g 100kg^{-1} of feed) and PS20 (probiotics supplementation @20g 100kg^{-1} of feed). The experiment was laid out at Dr. Purnendu Gain Field Laboratory of Agrotechnology Discipline, Khulna University, Khulna by following Completely Randomized Design (CRD) with 4 replications for the treatments and 15 birds were included in each replication. The generic name of antibiotic used was oxytetracycline and trade name of probiotic was probiolac which contains a combination of several microorganisms of 32 billion CFU100g^{-1}. Results revealed that the performances of broilers in terms of body weight gain, feed intake, feed conversion ratio, feed cost and mortality rate varied insignificantly due to antibiotics and probiotics supplementation. Numerically, highest mortality was recorded in antibiotics supplemented group and no mortality observed in control and probiotic (@20g 100kg^{-1} of feed) fed group. Significant positive correlation was observed among feed intake, body weight, feed conversion ratio and growth rate of the chicks. It could be concluded that probiotic can be supplemented as an alternative to antibiotic in broiler ration.

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INTRODUCTION

Dietary antibiotics have been used in poultry industry since long as a means to control infectious diseases and also to improve growth performance and feed efficiency (Gaddeet al., 2018). Antibiotics induce their effect by stabilizing the intestinal microbial flora thereby preventing proliferation of specific intestinal pathogens (Shane, 2005). 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 (Van Boeckel et al., 2017). Due to increasing concerns about antibiotic resistance and potentials for being banned, there is an increasing interest in finding alternatives to antibiotics in poultry production in many countries of the world.

In view of the severe restrictions or total ban on the use of antibiotics as growth promoters in poultry production, probiotics have been suggested as an alternative to antibiotics. Alam and Ferdaoushi (2018) stated that body weight, growth rate and feed conversion efficiency improved significantly in Hubbard Isa Starbro broilers those fed with probiotics supplemented feeds. An overall increase of body weight in chicken fed with multi-strain probiotics was also reported by Zhang and Kim (2014).Probiotics containing *Lactobacillus*, *Bifidobacterium*, coliforms, and *Clostridium* species were found significantly effective in gaining the body weight of broilers (Song et al.,2014). Study reports from Bansal et al. (2011) also highlighted the better performances of broilers in terms of growth, feed efficiency and meat quality due to supplementation of probiotics in feed. However, Babazadeh et al. (2011) stated no significant positive effect of probiotics on feed intake, body weight gain and feed efficiency of broilers. Considering the view in mind the present study was undertaken to investigate the effects of different levels of antibiotics and probiotics supplemented to the feed on the performances of broilers in Bangladesh conditions.

MATERIALS AND METHODS

Locale of the Study and Design of Experiment

The experiment was conducted at Dr. Purnendu Gain Field Laboratory, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh. Two hundred and forty Cobb*500*™hybrid broiler chicks (day-old) were collected from Kazi Farms Ltd., Bangladesh to conduct the experiment. The experimental birds were divided into four treatment (experimental diets) groups with four replications by following Completely Randomized Design (CRD). The feeding trial was continued till 5thweek of broiler age.

Management Practices

The experimental birds were kept in a shed having slate floor. The shade was well ventilated and 24 hours of lighting period was maintained for first 7 days and gradually reduced to 20 hours at 5th week of the experiment. Vaccinations and proper bio-security measures were maintained according to the hatchery supplied guideline brochure. All birds were kept in the similar environment and uniform management was allowed to all the birds.

Ration Formulation and Feeding System

The birds were assigned at random to four experimental diets supplemented with four levels of antibiotics and probiotics. These were control (no antibiotics or probiotics supplementation), AS20 (antibiotics supplementation @20g100kg^{-1}of feed), PS10 (probiotics supplementation @10g100kg^{-1}of feed) and PS20 (probiotics supplementation @20g100kg^{-1}of feed). The generic name of antibiotic used was oxytetracycline and the trade name of probiotic was 'probiolac' which was in powder form. Probiolac contains 32 billion CFU (in 100 gm) of following organisms in equal proportions: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus salvaricus, Bifidobacterium animalis, Bifidus, Streptococcus faecium* and *Aspergillus oryzae*. Other ingredients of four experimental rations were kept in constant proportions (Table 1). Feeds and water were supplied *ad libitum* to the broilers two times daily at 7.30 am and at 4.00 pm.

Table 1. Composition of experimental diets (kg 100kg^{-1} of diets)

Ingredient	Levels of antibiotics or probiotics supplementation							
	Starter rations (0 to 14 days)				Finisher rations (15 to 35 days)			
	Control	AS20	PS10	PS20	Control	AS20	PS10	PS20
Maize (*Zea mays*)	50.00	50.00	50.00	50.00	55.00	55.00	55.00	55.00
Rice polish (*Oryza sativa*)	10.00	10.00	10.00	10.00	9.50	9.50	9.50	9.50
Soybean meal	32.00	32.00	32.00	32.00	27.00	27.00	27.00	27.00
Soybean oil	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00
Meat and bone meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Di calcium phosphate	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Common salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
ADM - Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Probiotic (Probaiolac)	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.02
Antibiotics (Oxytetracycline)	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00
Total amount (kg)	100.00	100.02	100.01	100.02	100.00	100.02	100.01	100.02
Energy content (Kcal/kg)	3000.00	3000.00	3000.00	3000.00	3086.00	3086.00	3086.00	3086.00
Crude protein content (g/100g)	23.00	23.00	23.00	23.00	21.00	21.00	21.00	21.00

Control: no antibiotics or probiotics supplementation; AS20: antibiotics supplementation @20g 100kg^{-1} of feed; PS10: probiotics supplementation @10g 100kg^{-1} of feed; PS20: probiotics supplementation @20g 100kg^{-1} of feed.

Data Collection and Statistical Analysis

Feed intake and refusal as well as mortality data were collected daily from each pen. Body weight data were recorded weekly from each pen. Feed conversion ratio was determined pen wise on weekly basis during the experimental period. It was calculated by using the following formula:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Analysis of variance was done with the help of computer program 'MSTAT-C'. Least significant difference (LSD) was done to compare the treatment means for different parameters. Correlation analysis was done by computer package program SPSS, version 16.5.

RESULTS AND DISCUSSION

Body weight of broilers did not vary significantly ($p>0.05$) due to probiotic and antibiotic supplementation except 1st and 5th weeks of age (Table 2). At 5th week of age the body weight () of AS20 (antibiotics supplementation @20g100kg^{-1}of feed) group was highest (1766.79g bird^{-1}) and lowest in PS10 group (probiotics supplementation @10g100kg^{-1}of feed) (1585.95g bird $^{-1}$) where the mean difference was significant ($p<0.01$). However, no significant variation ($p>0.05$) in body weight gain of broilers due to antibiotic and probiotics supplementation at most of the age categories coincides with the findings of Kral et al. (2012) and Amerah et al. (2013). Kral et al. (2012) worked on same broiler hybrid (Cobb*500*™) and obtained average weight in control group as 1503 g and in the probiotic group it was 1190 g bird^{-1} at the age of 5th week. The findings from the current study also differ from the findings of Amer and Khan (2012) and Tabidi et al. (2013). The body weight of the experimental broilers during first three weeks was almost similar to those as reported by Aluwong et al. (2012). However, body weight of broiler in the present study at the age of 4th and 5th weeks differed from their findings. Significantly increased body weight of broilers due to probiotics supplementations were also reported by other researchers (Bansal et al., 2011; Song et al., 2014; Alam and Ferdaushi, 2018).

Table 2. Cumulative body weight (g bird^{-1}) of broiler birds fed different levels of antibiotics and probiotics at different age categories

Age of broiler (week)	Levels of antibiotics and probiotics supplementation				LSD	Level of significance
	Control	AS20	PS10	PS20		
1st	152.99^{a}	141.99^{a}	137.34^{ab}	133.99^{b}	11.28	**
2nd	446.16	434.71	445.885	438.46	25.11	NS
3rd	896.25	840.25	837.75	864.3	44.25	NS
4th	1241.67	1230.17	1195.83	1227.42	84.21	NS
5th	1747.40^{a}	1766.79^{a}	1585.95^{b}	1631.67^{ab}	148.40	**

Control: no antibiotics or probiotics supplementation; AS20: antibiotics supplementation @20g100kg^{-1}of feed; PS10: probiotics supplementation @10g100kg^{-1}of feed; PS20: probiotics supplementation @20g100kg^{-1}of feed. NS = Non significant ($P>0.05$); ** $P<0.01$; Mean values followed by same letter within a row are not significantly different at $P\leq0.05$ by the Least significant difference (LSD) test.

Feed intake of broiler birds varied insignificantly ($p>0.05$) among the groups fed with different levels of antibiotics or probiotics (Table 3). Numerically highest cumulative feed intake was recorded as 3058.28g bird^{-1} at 5th week of age in broiler group fed with probiotics at the rate of 10g100kg^{-1}of feed (PS10). Feed conversion ratio (feed intake/body weight gain) also showed insignificant ($p>0.05$) variation among broiler groups fed with different levels of antibiotic or probiotic (Table 4). The data of the Table 5 revealed no significant mean difference ($p>0.05$) in feed cost of broiler (BDTbird^{-1}) among different treatment groups. Mean difference of mortality rate (%) also showed insignificant ($p>0.05$) variations among different treatment groups (Table 6). No significant variation ($p>0.05$) in feed intake of broilers in the present study is supported by the findings of Jouybari et al. (2009), Amerah et al. (2013), Yousefi and Karkoodi (2007) and Babazadehet al. (2011). However, the present findings differed from other findings (Khaksefidi and Rahimi, 2005; Amer and Khan, 2012; Tabidi et al., 2013) in respect of feed intake where the researchers observed positive significant effects of probiotics. Significant improved in feed intake due to probiotics supplementation were also reported by Shareef and Al-Dabbagh (2009) in broilers.

Table 3. Cumulative feed intake (gbird^{-1}) of broiler birds fed different levels of antibiotics and probiotics at different age categories

Age of broiler (week)	Levels of antibiotics and probiotics supplementation				LSD	Level of significance
	Control	AS20	PS10	PS20		
1st	153.97	154.42	151.81	149.98	6.11	NS
2nd	570.66	575.78	582.89	569.12	21.23	NS
3rd	1240.33	1305.21	1315.00	1279.84	55.32	NS
4th	2109.00	2097.14	2162.01	2110.14	110.70	NS
5th	2987.24	2993.99	3058.28	2971.01	152.10	NS

Control: no antibiotics or probiotics supplementation; AS20: antibiotics supplementation @20g100kg^{-1}of feed; PS10: probiotics supplementation @10g100kg^{-1}of feed; PS20: probiotics supplementation @20g100kg^{-1}of feed. NS = Non significant ($P>0.05$); LSD= Least significant difference.

Table 4. Feed conversion ratio (feed intake/weight gain) of broiler birds fed different levels of antibiotics and probiotics at different age categories

Age of broiler (week)	Levels of antibiotics and probiotics supplementation				LSD	Level of significance
	Control	AS20	PS10	PS20		
1st	0.94	0.94	0.94	1.04	0.08	NS
2nd	1.28	1.33	1.31	1.30	0.09	NS
3rd	1.43	1.55	1.58	1.48	0.13	NS
4th	1.70	1.71	1.82	1.73	0.20	NS
5th	1.71	1.70	1.93	1.82	0.16	NS

Control: no antibiotics or probiotics supplementation; AS20: antibiotics supplementation @20g100kg^{-1}of feed; PS10: probiotics supplementation @10g100kg^{-1}of feed; PS20: probiotics supplementation @20g100kg^{-1}of feed. NS = Non significant (P>0.05); LSD= Least significant difference.

Table 5. Cumulative feed cost (BDTbird^{-1}) of broiler birds fed different levels of antibiotics and probiotics at different age categories

Age of broiler (week)	Levels of antibiotics and probiotics supplementation				LSD	Level of significance
	Control	AS20	PS10	PS20		
1st	4.465	4.478	4.2	4.349	0.18	NS
2nd	16.55	16.70	16.90	16.50	0.62	NS
3rd	37.13	37.85	38.14	37.12	1.60	NS
4th	61.16	60.82	62.70	61.19	3.21	NS
5th	86.63	86.83	88.69	86.16	4.41	NS

Control: no antibiotics or probiotics supplementation; AS20: antibiotics supplementation @20g100kg^{-1}of feed; PS10: probiotics supplementation @10g100kg^{-1}of feed; PS20: probiotics supplementation @20g100kg^{-1}of feed. NS = Non significant (P>0.05); LSD= Least significant difference; BDT: Bangladeshi currency (1 US$ = 85.00 BDT approx.).

Table 6.Mortality rate (%) of broiler birds fed different levels of antibiotics and probiotics at different age categories

Levels of antibiotics and probiotics supplementation	Mortality rate (%)
Control(no antibiotics or probiotics supplementation)	0.00
AS20 (antibiotics supplementation @20g100kg^{-1} feed)	3.34
PS10 (probiotics supplementation @10g100kg^{-1} feed)	1.67
PS20 (probiotics supplementation @20g100kg^{-1} feed)	0.00
LSD	8.48
Significance level	NS

Control: no antibiotics or probiotics supplementation; AS20: antibiotics supplementation @20g100kg^{-1}of feed; PS10: probiotics supplementation @10g100kg^{-1}of feed; PS20: probiotics supplementation @20g100kg^{-1}of feed. NS = Non significant (P>0.05).

The correlations matrix among the parameters like feed intake, body weight, feed conversion ratio and growth rate have been shown in Table 7. The feed intake (gbird^{-1}) and cumulative body weight (gbird^{-1}) were positively and significantly (p<0.01) correlated (r=0.99). Significant (p<0.01) positive correlations were also observed between feed intake and feed conversion ratio (r = 0.90) as well as feed intake and growth rate (gd^{-1})(r = 0.75). The correlation matrices between body weight and feed conversion ratio (r = 0.86); body weight and growth rate (r = 0.82) and growth rate and feed conversion ratio (r= 0.66) were also found positive and significant (p<0.01).No significant variation indicates that the feed conversion ratio is almost similar due to antibiotic and probiotic supplementation. The results coincide with the findings of Tabidi et al. (2013) who also observed insignificant variation in FCR due to probiotic supplementation. However, the present findings differed from the findings of Amer and Khan (2012) and Khaksefidi and Rahimi (2005) who reported positive effects of probiotic in FCR. Feed efficiency was also improved significantly (p<0.01) due to supplementation of probiotics in broiler feed (Bansal et al., 2011). Improved feed efficiency was also noticed in birds fed with a diet supplemented with probiotic in Hubbard Isa Starbro broiler (Alam and Ferdaushi, 2018). No significant variation indicates that the feed cost was almost similar to the broiler birds under different treatments. The results coincide with the findings of Habibi et al. (2013) who observed no significant (p>0.05) variation for the feed cost due to probiotic supplementation. Numerically highest (p>0.05) mortality rate of AS20 (antibiotics supplementation @20g 100kg^{-1} of feed) group(3.34%) in the present study coincides with the findings of Tabidi et al. (2013) and Amer and Khan (2012). They also found the mortality rate of antibiotic group as higher than the mortality rate of probiotic group.

Table 7. Correlations matrix among the parameters

	Cumulative feed intake (gbird^{-1})	Cumulative body weight (gbird^{-1})	Feed conversion ratio (FCR)
Cumulative feed intake (gbird^{-1})			
Cumulative body weight (gbird^{-1})	0.99**		
Feed conversion ratio (FCR)	0.90**	0.86**	
Growth rate (gd^{-1})	0.75**	0.82**	0.66**

** Correlation is significant at the 1% level of significance.

Significant ($p<0.01$) positive correlation between feed intake (gbird^{-1}) and cumulative body weight (gbird^{-1}) in the present study indicates that with the increase of body weight the feed intake also increased. Correlation study also stated that with the increase or decrease of body weight the feed conversion ratio and the growth rate also be increased or decreased, respectively.

CONCLUSIONS

The supplementation of probiotics to the diet showed statistically similar results to that of antibiotic supplementation for body weight, feed intake, feed conversion ratio and feed cost of broiler birds. But no mortality was recorded in broiler group fed probiotic at the rate of 20g 100kg^{-1} feed and control group. Therefore, it could be concluded that probiotics can be supplemented @20g 100kg^{-1} of the broiler diet as an alternate to antibiotic for better survivability of broilers.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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