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Original Article

Comparative analysis of body weight and serum biochemistry in broilers supplemented with some selected probiotics and antibiotic growth promoters

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ABSTRACT

Objective: The present work was conducted on Cobb-500 broilers fed with either probiotics (Yogurt, Promax®) or antibiotic growth promoters (Ciproflox®, Renamycin®) to assess the effects on body weight and biochemical parameters.
Materials and methods: A total of 30 day-old broiler chicks were purchased and acclimatized for 7 days in the experimental shed. After initial acclimatization, the broilers were allotted into five equal groups (n=6). Group A was considered as control and was fed commercial ration and fresh drinking water. Groups B and C were fed commercial ration and probiotics (Yogurt, Promax®) respectively mixed in water. Groups D and E received commercial ration and antibiotic growth promoters (Ciproflox®, Renamycin®) respectively with water. Body weight of each bird was recorded on day 7, 14, 21, 28 and 35. At the end of trial period, the birds were sacrificed to collect blood in order to prepare serum samples for biochemical analyses considering total cholesterol, triglyceride, high density lipids (HDL), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST).
Results: Body weight in all treatment groups (B, C, D and E) was significantly ($P<0.05$) higher as compared to control (A) group. Total cholesterol and triglyceride levels significantly ($P<0.05$) decreased in probiotics treated groups as compared to control and antibiotic growth promoters treated groups. AST and ALT values increased significantly ($P<0.05$) in antibiotic growth promoters treated groups as compared to control whereas, these values decreased in probiotics treated groups. Creatinine levels were significantly ($P<0.05$) higher in antibiotic growth promoters treated groups as compared to all others groups.
Conclusion: Significantly increased body weight is observed in probiotics and antibiotic growth promoters supplemented broilers. Probiotics also improve the lipid profile and other biochemical parameters as compared to growth promoter. Probiotics (like Yogurt and Promax®) seem to be better choice than antibiotic growth promoters as feed supplements.

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KEYWORDS

Biochemical parameters; Body weight; Broilers; Growth promoters; Probiotics

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INTRODUCTION

Bangladesh is an agriculture oriented country where about 80 percent of the population depends on agriculture. Poultry plays one of the most prominent roles in the income oriented activities of the rural people of Bangladesh (Kabir, 2009). There is a tremendous demand of animal protein source that is free from infectious agents (Alkhalf et al., 2010). To ensure maximum production, feeding and management practices are very important. Modern feeding practice involves the use of probiotics, growth promoters, balanced diet, antibiotics, steroids and so many new concepts (Rahman et al., 2013).

The term probiotic came out with this concept in the early 70s to describe the activity of adding (pro) life (biotic) to a system instead of destroying it (antibiotic). Probiotics are live microorganisms which are nonpathogenic and nontoxic and when administered through the digestive route, they favor host's overall health (Guilliot, 1998). The possible mode of actions of probiotics is: Inhibition of viable bacterial count (antimicrobial effect), alteration of microbial metabolism (improvement in digestion), and stimulation of immunity (immunological compromise and improvement in immune response) (Fuller, 2001).

The term 'antibiotic growth promoter' is used to define any agent that either destroys or inhibits bacteria when administered at a small, sub-therapeutic dose (Hao et al., 2014). The greater use of several antibiotics for growth promotion purpose has arisen due to the popularity and necessity of poultry farming. The positive effect of antibiotics on growth was first discovered in the early 1940s, when it was found that dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues improved overall growth of subjected animals. Antimicrobial agents could be used and as feed additives as well as with drinking water for not only therapeutic but also prophylactic purposes (Fabrega et al., 2008). Commonly, antibiotics promote growth by interacting with intestinal microbial population and stimulating feed intake (Dibner and Richards, 2005). Another important benefit of antibiotic growth promoters lies in their ability to control important zoonotic pathogens such as *Salmonella* spp., *Campylobacter* spp. and *E. coli* (Engberg et al., 2000).

However, the use of antibiotic growth promoters has been under the radar for many years. Many countries are questioning their utility and withdrawing them from markets (Kabir, 2009) Antibiotics as a growth promoter have been widely used extensively in poultry feed for more than 50 years but it is banned in some parts of the world. At present, there is a huge controversy regarding the use of growth promoters for animals targeted for meat production. However, many local farms are still neglecting the harmful effects and using antibiotic growth promoters in Bangladesh. Such wide spread use of antibiotics for promoting growth could easily contribute to the already alarming pool of antibiotic resistant bacteria. Residues of antibiotics in meat and other products can directly harm consumer's health and at the same time an indirect effect could be their role in producing resistance in several human pathogens (Emborg et al., 2004). Antibiotics also cause imbalance in intestinal normal flora (Andremont, 2000). Consumers are looking for a better alternative and probiotics could be an amazing safe choice as many countries are already using them (Trafalska and Grzybowska, 2004; Griggs and Jacob, 2005; Nava et al., 2005). Therefore, this study was undertaken with a view to compare the effects of some selected probiotics (Yogurt, Promax®) with antibiotic growth promoters (Ciproflox®, Renamycin®) on body weight and biochemical parameters of broilers.

MATERIALS AND METHODS

Ethical approval: For this research, ethical approval was issued by the Animal Welfare and Ethical Committee, Faculty of Veterinary Science, Bangladesh Agricultural University. The approval number is 04/AWEC/2017, Date: 28.8.17.

Experimental birds: The research work was carried out from April, 2016 to May, 2016 in the experimental Physiology shed, Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202. A total of 30, day old broiler chicks were purchased from Nourish Hatchery, Mymensingh. Initially, they were reared for 7 days in a brooding house with same feed and drinking water for acclimatization. Later on the birds were transferred into the experimental shed and divided into groups with different treatments.

Experimental design: After the initial rearing period (7 days), the broiler chicks were randomly distributed into the following groups:
Group A: Commercial broiler feed and drinking water.
Group B: Commercial broiler feed and supplementation of Yogurt at 5 gm/L drinking water.
Group C: Commercial broiler feed and supplementation of Promax® at 0.5 gm/L drinking water.
Group D: Commercial broiler feed and supplementation of Ciproflox® at 0.25 mL/L drinking water.
Group E: Commercial broiler feed and supplementation of Renamycin® at 1 gm/L drinking water.

Management practices: The day old broiler chicks were provided with vitamin C (1 g/5 L water) to overcome transportation stress. The experimental shed was washed using clean water and disinfected with Iosan® and Virkon-S®. Then it was kept completely empty for two days before placing the chicks. All necessary equipment was set properly and optimum hygiene was ensured. The experimental physiology shed was partitioned into five pens using wire-net. A group of 6 broiler chicks were randomly allocated to each pen. Each pen was 3 ft x 2 ft. Therefore, average floor space allocated for each bird was 1 ft^2. Fresh, dry rice husk was the choice of litter material and the depth achieved was about 3 cm. Already used up litter materials were changed regularly with new materials to prevent birds from fungal or notorious coccidial attack. The birds were regularly exposed to natural lighting for about 12 h a day. At night, electric bulbs were the source of light and heat. In order to ensure optimum humidity and temperature, all the windows were kept open at day and ceiling fans were used. Feeders and water pots were properly cleaned and dried daily before use. Strict sanitary measures were ensured during whole experimental period.

Diet: Commercial poultry feed (Quality Feeds Ltd., Dhaka) was used throughout the experiment. Feed was collected from local markets of Mymensingh. The broiler chicks up to14 days old were supplied standard broiler starter and later on broiler grower from 15-35 days of age, as recommended by Quality Feeds Ltd., Dhaka.

Yogurt: It was collected from the Dairy Farm, Bangladesh Agricultural University, Mymensingh-2202.

Promax®: It is a popular commercial probiotic. Promax® is marketed by Eskayef Bangladesh Limited and manufactured in India by Sanzyme (P) Ltd. Each gram contains: *Bacillus subtilis*, *Bacillus coagulans, Saccharomyces boulardii* 4.5 x 108 cfu.

Ciproflox® Oral solution: It is a commonly used poultry drug manufactured by Eskayef Bangladesh Ltd. Each ml solution contains: Ciprofloxacin Hydrochloride USP (equivalent to 100 mg Ciprofloxacin).

Renamycin® Soluble powder: It is one of the commonest and most widely used drugs in poultry industry. It is manufactured by Renata Ltd. (Animal Health Division). Each gram powder contains Oxytetracycline hydrochloride 200 mg.

Measurement of body weight: The body weight of each bird was measured in a weekly basis. An electrical balance was used to serve this purpose from 7^{th} day of experiment up to 35^{th} day of experiment.

Blood collection: After completion of 5 weeks experimental period, blood samples were collected at slaughter. About 10 mL blood was collected from each bird in a container without any anticoagulant for preparing serum.

Preparation of serum: About 10 mL of blood was collected in a sterile test tube and kept in a slanting position at room temperature. These samples were refrigerated overnight at 4°C. The separation of serum from the clotted blood was achieved following centrifugation at 1000 rpm for 15 min. These cell free serum samples were preserved at –20°C for further biochemical analysis.

Biochemical parameters: The biochemical tests were performed in collaboration with Professor Muhammad Hossain Central Laboratory, Bangladesh Agricultural University, Mymensingh. In the separated serum the total cholesterol, triglycerides, high density lipoproteins (HDL), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were studied with a UV spectrophotometer T 80, PG instruments, Great Britain. Specific reagents from High Tenchnology Incorporation (HTI), USA were used for each test.

Statistical analysis: Data collection was carried out carefully and presented as mean±SEM. For the comparison of data among the groups One Way ANOVA (Analysis of Variance) was chosen. These data were analyzed with the help of computer package SPSS statistics 20.0 software, considering probability $P<0.05$ statistically significant.

RESULTS AND DISCUSSION

The exclusive reason of this experiment was to compare the effects of some selected probiotics (yogurt and Promax®) and antibiotic growth promoters (Ciproflox®, Renamycin®) on body weight and biochemical parameters of broilers. Body weights of different birds were studied to compare growth in different groups. Total cholesterol, triglyceride, HDL, creatinine, AST & ALT values were studied among the biochemical parameters to compare lipid profiles, liver and kidney functions.

Body weight: Body weight was measured on a weekly basis. On day 7, almost similar body weights were observed in all groups (**Table 1**). On day 14, the highest body weight was observed in group E (Renamycin®) and

Table 1: Effects probiotics (Yogurt, Promax®) and antibiotic growth promoters (Ciproflox®, Renamycin®) on body weight

Groups	Pre-treatment body weight (gm)	Post-treatment body weight (gm)			
	Day 7	Day 14	Day 21	Day 28	Day 35
A (Control)	265.00±2.44	552.16± 4.28^{b}	1083.33±11.85^{c}	1443.33±11.73^{c}	1895.50±10.50^{c}
B (Yogurt)	266.66±3.33	638.83±5.23^{a}	1199.50±5.79^{b}	1603.67±5.86^{b}	2160.33±14.65^{a}
C (Promax®)	265.83±3.00	657.17± 9.17^{a}	1268.33±7.49^{a}	1641.66±13.01^{a}	2203.33±12.56^{a}
D (Ciproflox®)	264.33±1.56	651.33± 7.25^{a}	1168.33±14.00^{b}	1602.00±6.11^{b}	2090.00±9.66^{b}
E (Renamycin®)	266.00±1.52	657.33± 6.55^{a}	1168.67±12.25^{b}	1622.00±7.83^{ab}	2148.33±18.86^{a}

Values with different superscript letter(s) in the same column differ significantly (P<0.05).

Table 2: Effects probiotics (Yogurt, Promax®) and antibiotic growth promoters (Ciproflox®, Renamycin®) on lipid profile of broilers.

Groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)
A (Control)	213.00±1.78^{a}	120.16±1.51^{a}	83.16±1.75^{a}
B (Yogurt)	191.00±1.84^{c}	99.00±0.96^{c}	70.00±1.23^{b}
C (Promax®)	176.50±1.99^{d}	96.50±1.83^{c}	69.67±2.10^{b}
D (Ciproflox®)	199.50±1.40^{b}	110.33±1.11^{b}	66.33±1.56^{c}
E (Renamycin®)	209.33±1.08^{a}	116.50±1.89^{a}	71.83±1.10^{b}

Values with different superscript letter(s) in the same column differ significantly (P<0.05).

Table 3: Effects probiotics (Yogurt, Promax®) and antibiotic growth promoters (Ciproflox®, Renamycin®) on serum creatinine, AST and ALT values in broilers.

Groups	Creatinine (mg/dL)	AST (U/L)	ALT (U/L)
A (Control)	1.06±0.05^{c}	136.50±1.60^{b}	12.60±0.26^{c}
B (Yogurt)	0.70±0.01^{d}	119.00±2.10^{c}	6.11±0.70^{d}
C (Promax®)	0.72±0.05^{d}	115.00±2.30^{c}	5.36±0.38^{d}
D (Ciproflox®)	2.47±0.15^{b}	145.66±2.31^{a}	21.16±1.16^{a}
E (Renamycin®)	3.05±0.06^{a}	143.50±1.23^{a}	18.50±1.05^{b}

Values with different superscript letter(s) in the same column differ significantly (P<0.05).

lowest in group A (Control) (**Table 1**). No significant (P>0.05) differences among Probiotics treated (B, C) groups and antibiotic growth promoters treated (D, E) groups were observed. However, body weight enhanced significantly (P<0.05) in all treatment groups (B, C, D and E) compared to control (A) group (**Table 1**).

On day 21, 28 and 35, the highest body weight was recorded in group C (Promax®), and lowest in group A (Control)(**Table 1**). Body weight increased significantly (P<0.05) in all treatment groups (B, C, D and E) compared to control (A) group and on day 35, the highest average weight was found in group C (Promax®)(**Table 1**). Probiotic supplementation improves live wait gain (Zhang and Kim, 2014).

Effectuation of probiotics results in betterment in digestibility and convenience of many nutrients such as fats, amino acids and carbohydrates, as well as, several mineral elements and vitamins (Yeo and Kim, 1997). It is well known that many of the beneficial bacteria enhance health and growth by competitive exclusion and antagonism (Fuller, 2001; Roberto et al., 2003). Probiotic renders nutrients, effectively favors the growth of beneficial microorganisms in the intestines providing better arrangement of those bacteria population (Capcarová et al., 2011).

Effect on biochemical parameters: The highest total cholesterol level (213.00±1.78 mg/dL) was found in group A (control) and the lowest (191.00±1.84 mg/dL) was in group B (Yogurt) (**Table 2**). The total cholesterol level faded significantly (P<0.05) in probiotics treated groups (B and C) compared to control (**Table 2**).

The highest triglyceride level (120.16±1.51 mg/dL) was observed in group A (control) and lowest (96.50±1.83 mg/dL) in group C (Promax) (**Table 2**). Significantly (P<0.05) higher amount of triglyceride was measured in group A (Control) and group E (Renamycin) compared to all other groups (**Table 2**). The highest HDL level (83.16±1.75 mg/dL) was found in Group A (Control) and lowest (66.33±1.56 mg/dL) in Group D (Ciproflox®) (**Table 2**). The HDL value was significantly (P<0.05) high in Group A (Control) compared to all other groups. The statistical difference among treated groups were insignificant (P>0.05) (**Table 2**).

Probiotic supplementation results in reduction of the total serum cholesterol level in White Leghorn layers from 176.5 to 114.3 mg/dL serum (Mohan et al., 1995). Also, Mohan et al. (1996) noted, chickens that had 75, 100, and 125 mg probiotic/Kg diets exhibited lower serum cholesterol content (93.3 mg/dL) compared to the control birds (132.2 mg/dL). Serum cholesterol and triglycerides are reduced significantly by the implementation of probiotics (Mansoub, 2010; Amer and Khan, 2012).

The mechanisms by which probiotics decreases total cholesterol and triglyceride may include their property to deconjugate bile acids enzymatically using bile-salt hydrolase (Surono, 2003). Probiotic microorganisms suppress hydroxy-methyl-glutaryl-coenzyme A, which is an enzyme necessary for cholesterol synthesis pathway thereby, lessen cholesterol synthesis (Fukushima and Nakano, 1995). However in antibiotic growth promoter treated groups, total cholesterol and triglyceride levels significantly (P<0.05) increased compared to the probiotic treated groups (**Table 2**). Addition of antibiotics shows a higher blood triglyceride and cholesterol level (Li et al., 2007). These increased levels are attributed to the degraded effect of such antibiotics on the absorption of fat in the GI tract (Mansoub, 2011).

The highest creatinine value (3.05±0.06 mg/dL) was measured in group E (Renamycin®) and lowest (0.70±0.01 mg/dL) in group B (Yogurt) (**Table 3**). The creatinine level was significantly (P<0.05) increased in antibiotic growth promoter treated groups (D and E) compared to all other groups. Increased level of creatinine in serum can be indicative of kidney damage (Yalcin et al., 2012). However, significantly decreased creatinine level was detected in probiotics treated groups (B and C). Certain probiotic microorganisms have the ability to use urea, creatinine and other toxic chemicals as its nutrients for growth (Salim et al., 2011).This might be the reason for low creatinine level in the present experiment. Therefore, relatively low level creatinine may be an indication of the renal protective effects of the probiotics.

The highest AST value (145.66±2.31 U/L) was measured in group D (Ciproflox®) and lowest (115.00±2.30 U/L) in group C (Promax®) (**Table 3**).Whereas, ALT value (21.16±1.16 U/L) was highest in group D (Ciproflox®) and lowest (5.36±0.38 U/L) in group C (Promax®) (**Table 3**). Both AST and ALT values decreased significantly (P<0.05) in probiotics treated groups (B and C) compared to control group A. Implementation of probiotics lowers AST and ALT level (Santoso et al., 1995) . AST is well distributed in several organs such as skeletal muscles, the heart, liver, whereas primary source of ALT is mainly liver. Decreased levels of these enzymes may be expressed less liver and skeletal muscle damage. However, a significant increased (P<0.05) values were observed in antibiotic growth promoters treated groups (D and E). Use of ciprofloxacin may lead to elevated levels of AST and ALT (Agbafor et al., 2015). Hepatotoxicity is indicated by the rising activities of the enzymes AST, ALT and ALP as a result of the malfunction of the sites of their production (Yalcin et al., 2012; Agbafor et al., 2015). Some antibiotics have the tendency to cause lipid peroxidation, which is responsible for cellular toxicity and initiating tissue damage (Farombi, 2001). These mechanisms may be the cause of increased AST and ALT values in our study.

CONCLUSION

Probiotics like Yogurt and Promax® can be perfect alternatives of antibiotic growth promoters. Both of the probiotics showed amazing positive influence on body weight, lipid profile and other selected biochemical parameters in broilers. Although antibiotic growth promoters improved growth but their role in increased creatinine, AST and ALT level could be an indication of nephrotoxicity and hepatotoxicity respectively. Further investigations are necessary to compare the dose dependent response of probiotics and antibiotic growth promoters on a much larger group of broilers and other popular poultry species. A histopathological study of the livers and kidneys will strengthen the findings of the present investigation.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

AUTHORS' CONTRIBUTION

MIH designed and carried out the experiment and contributed in sample collection, biochemical analysis, data colletion , statistical analysis and also drafted the manuscript. MAM contributed in critical checking of this manuscript. NA supervised the research work. All the authors read and approved the manuscript before submission.

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