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Article

Serum biochemical changes and growth response study following probiotic and phytobiotic supplementation in broiler chickens

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Abstract: Many alternative substances have been investigated for their potential to replace antibiotics as growth promoters. Probiotics and phytobiotics are some of the products that can be used as growth promoters in broiler. The present study was designed to investigate either single or combined effect of a probiotic and phytobiotic on serum bio-chemistry and growth performances in broilers. A total of 50 Cobb-500 day old chicks were divided into five groups (10 birds each). The birds of Group A were offered a basal diet (corn-soya based), Group B basal diet + 0.10% Renamycin 100®, Group C basal diet + 0.15% probiotic (Bio-Top®), Group D basal diet + 0.10% phytobiotic (Galibiotic) and Group E basal diet + 0.15% Bio-Top® + 0.10% Galibiotic. Body weight, feed intake, weight gain, feed conversion ratio (FCR) and serum bio-chemistry (Triglyceride, cholesterol, LDL, HDL, ALT, AST, creatinine) were recorded. Serum biochemical values differed significantly (P<0.05) among the groups. The average final live weight gain was 665grams, 686grams, 1095grams, 780grams and 1065grams in groups A, B, C, D and E, respectively. The feed conversion ratio was 2.1, 1.96, 1.72, 1.83, and 1.75 in A, B, C, D and E group, respectively. The present study revealed that supplementation of probiotic and phytobiotic in feed significantly reduced triglyceride, cholesterol and HDL values compared to value of control group (P<0.05). Probiotic or its' combination with phytobiotic has the potential to be exemplary alternatives to antibiotic as growth promoters.

Keywords: phytobiotic; probiotic; serum bio-chemistry; growth performance; broiler

1. Introduction

The presence of antibiotic residues in poultry meat and eggs may have deleterious effects on human health due to increased and indiscriminate use of antibiotics in the poultry industry for therapeutic, prophylactic and growth promotion purposes. The residues of antibiotics can cause resistance of human flora and pathogenic microbes to those groups of antibiotics. Moreover, cross-resistance to antibiotics used in the therapy of humans and other animals could also result (Van den Bogaard and Stobberingh, 2000; Caprioli *et al.*, 2000; Edens, 2003; Pelicano *et al.*, 2004). Thus efforts have been made in different parts of the world to limit the use of antibiotics as growth promoter (AGPs) in livestock production. Because of the ban on the use of AGPs, there is growing demand for natural alternative substances, which can sustain or promote growth performance and prevent disease.

Probiotics are feed additives that contain live microorganisms and promote beneficial effects to the host by favoring the balance of the intestinal microbiota (Huang *et al.*, 2004). These products along with many herbal products are of great importance as possible candidates to replace antibiotic use as growth promoters (Landy and Kavyani, 2014; Landy *et al.*, 2011). Another option of alternative to antibiotics are the aromatic plant extracts (Angel *et al.*, 2005). Probiotics have been shown to bring down egg yolk cholesterol levels (Abdulrahim *et al.*, 1996; Haddadin *et al.*, 1996) and serum cholesterol in chicken (Jin *et al.*, 1998). Phytogenic feed additives or phytobiotics are also used in animal feeding to improve performance by complementing feed properties and improving the quality of animal sourced food (Windisch *et al.*, 2008).
In Bangladesh, many probiotics and phytobiotics are commercially available in the markets and their indiscriminate use without experimental backing is not in line with best practice. One of such products available in the market of Bangladesh is a probiotic commercially called Bio-Top® which is claimed to have some beneficial properties. Galibiotic is also available as a phytobiotic containing medium chain fatty acids (extraction of coconut oil and palm kernel oil), enzymes and carriers in the commercial market for use in poultry production. However, their uses in broiler production as growth promoter alternative to antibiotics are unknown. In addition, the combined effects of probiotic and phytobiotic have not yet been studied in Bangladesh. Thus the present study was undertaken to determine the single or combined efficacy of probiotic and phytobiotic supplementation in feed on growth performance and serum bio-chemistry of broilers as growth promoter alternative to antibiotic.

2. Materials and Methods

2.1. Experimental set up

The experiment was carried out in the experimental broiler rearing shed, Department of Pharmacology, Bangladesh Agricultural University (BAU) that was well cleaned and disinfected. Fifty day-old Cobb-500 commercial broiler chicks were used for the experiment and supplied with dextrose water and Vitamin C as anti-stress during transportation. After 5 days of brooding, chicks were randomly divided into five treatment groups. Birds of group A (control) were provided Corn-soya based basal diet, group B (antibiotic) with Corn-soya based diet with 0.10% Oxytetracycline Hydrochloride (Renamycin 100®, Renata Ltd., Bangladesh), group C (probiotic) with corn-soya based diet with 0.15% probiotic (Bio-Top®, Shinil Biogen Co. Ltd. ,Korea), group D (phytobiotic) with corn-soya based diet with 0.10% phytobiotic (Galibiotic®, Square Pharmaceuticals Ltd., Bangladesh), group E (combination) corn-soya based diet plus 0.15% probiotic (Bio-Top®) and 0.10% phytobiotic (Galibiotic®) as combination (Table 1). The composition of the probiotic (Bio-Top®) is as such; *Bacillus licheniformis* ($4x10^{10}$ CFU/g), *Bacillus subtilis* ($4x10^{10}$ CFU/g) and Zinc oxide (20g). While that of the galibiotic is a combination of active medium chain fatty acids with carrier. The trade name of the commercial phytobiotic used in this experiment was Galibiotic. The inclusion rate of both the probiotic and galibiotic was used as suggested by the manufactures (250g/100kg feed in commercial broiler).

Table1. Ingredients composition of broiler ration.

Ingredients (%)	Groups				
	A	B	C	D	E
Corn	62.67	62.67	62.67	62.67	62.67
Soya meal	26.67	26.67	26.67	26.67	26.67
Pro-pack	8	8	8	8	8
DCP	1.67	1.67	1.67	1.67	1.67
Lysine	0.13	0.13	0.13	0.13	0.13
Methionine	0.2	0.2	0.2	0.2	0.2
Vit., Min., Premix	0.26	0.26	0.26	0.26	0.26
Lime stone	0.33	0.33	0.33	0.33	0.33
$NaHCO_3$	0.16	0.16	0.16	0.16	0.16
Oil	3	3	3	3	3
Bio-Top®	-	-	0.15	-	0.15
Galibiotic®	-	-	-	0.10	0.10
Renamycin 100®	-	0.10	-	-	-

ME=3000 Kcal/Kg, CP=22%

2.2. Data collection and record keeping

Body weights of birds were recorded weekly with the help of electric balance. The average body weight gain of broilers in each group was calculated by deducting initial body weight from the final body weight. Feed consumption was calculated as the total feed consumed in each group divided by the number of birds. The amount of feed consumed per unit of weight gain was calculated and shown as feed conversion ratio (FCR).

2.3. Hemato-biochemical parameters

At the end of the experiment about 3 ml of blood were collected from wing vein of three birds of each group in sterile test tubes without any anticoagulant and kept in a slanting position at room temperature after which the serum was separated from the clotted blood following centrifugation at1000 rpm for 15 minutes. The serum was preserved at –20°C for further use. All the tests were performed using HumaLyzer-2000 (Human, Germany).

2.4. Statistical analysis

The data regarding weight gain, feed consumption, feed conversion ratio and different biochemical parameters of the control and treated groups were analyzed statistically by Graph Pad Prism 6. The mean differences among the treatment groups were determined using one way ANOVA followed by Bonfferoni post-hoc test.

3. Results

The highest live weight was found in broilers of group C followed by group E then group D (Table 2). Broilers receiving probiotic and combination of probiotic and phytobiotic supplements weighed significantly higher than those of control (P<0.05). Differences in cumulative feed conversion ratio (FCR) in broilers of different dietary groups differed significantly (P<0.05). The lowest FCR value was obtained in birds of group C (probiotic) followed by group E (combination) but an improved efficiency that differed from control group (P<0.05). The results clearly shows that the broilers receiving probiotic and combination of probiotic and phytobiotic were the best converters of feed into live weight and the effect of probiotic was more prominent.

Table 2.Comparison of growth performance in different groups of broiler from day 1-28.

Parameters	Treatment groups				
	Control (A)	Antibiotic (B)	Probiotic (C)	Phytobiotic (D)	Combination (E)
ILW (gm/bird)	41.25±1.25	41.75±1.79	42±1.58	41±1.87	40.50±2.21
FLW (gm/bird)	665±4.16^{ab}	686±2.08^{ab}	1095±6.03^{a}	780±27.79^{a}	1065.0±18.93^{a}
LWG (gm/bird)	623.75±2.91^{ab}	644.25±1.39^{ab}	1053±4.90^{a}	739±25.79^{a}	1024.50±21.25^{a}
FI(gm)	1310	1263	1812	1352	1793
FCR	2.1^{ab}	1.96^{ab}	1.72^{a}	1.83	1.75

Values with different superscripts in same row differ significantly (P<0.05).^{a,b} Means bearing dissimilar superscript in a row differ significantly. Values are in grams (Mean ± SE). Initial live weight (ILW), Final live weight (FLW), Live weight gain (LWG), Feed conversion ratio (FCR), Feed intake (FI).

The total cholesterol level decreased significantly (P<0.05) in probiotics treated (group C) and combination (group E) compared to the control (group A) (Table 3). The highest triglyceride level (136.0±1.38 mg/dL) was observed in group A (control) and lowest (53.00±1.11 mg/dL) in group C (probiotic). Significantly (P<0.05) reduced triglyceride level was recorded in group C (probiotic), followed by group D (phytobiotic) and then group E (combination) compared to group A (control). The highest HDL level (84.16±0.89 mg/dL) was recorded in group A (control) and lowest (62.55±2.01 mg/dL) in group E (combination). The HDL value was significantly (P<0.05) increased in group A (control) compared to all other groups. The highest LDL level (72.25±0.80 mg/dL) was observed in group A (control) and lowest (62.55±2.01 mg/dL) in group E (combination). LDL level was significantly (P<0.05) reduced in group E (combination) and group C (probiotic) compared to group A (control). The statistical difference among treated groups were significant (P<0.05). This data clearly suggest that probiotic or combination of probiotic and phytobiotic have a hypolipedemic effect on broilers.

Table 3. Serum biochemistry of broilers in different groups.

Groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Creatinine (mg/dL)	AST (U/L)	ALT (U/L)
A	182.4±1.43[abc]	136.0±1.38[abc]	84.16±0.89[abc]	72.25±0.80[c]	2.070±0.009[ab]	240.0±2.81[ab]	14.43±0.48[ab]
B	172.0±1.53[abc]	121.5±2.11[abc]	80.68±1.25[a]	67.00±0.54	2.813±0.014[ab]	260.10±3.96	18.30±0.34[b]
C	146.0±1.43[a]	53.00±1.11[a]	72.78±0.70[a]	69.04±1.43	0.835±0.006[a]	237.10±3.21[a]	18.41±0.40[a]
D	158.0±1.24[b]	65.40±1.55[b]	72.28±1.75[b]	70.60±2.55[c]	1.703±0.008	250.50±3.30	17.52±0.45[b]
E	149.5±1.27[c]	72.41±1.84[c]	65.94±0.57[c]	62.55±2.01	0.462±0.008[b]	228.40±1.58[b]	13.12±0.44[b]

Serum biochemical (Mean±SE) parameters in different groups of broilers (n=10). Group A=Control, Group B=Antibiotic, Group C=Probiotic, Group D=Phytobiotic and Group E=Combination. Total cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), Aspartate amino transferase (AST) and Alanine amino transferase (ALT)

The highest AST value (260.1±3.96U/L) was measured in group B (antibiotic) and lowest (228.4±1.58U/L) in group E (combination) (Table 3). Whereas, ALT values (18.41±0.40U/L) was highest in group C (probiotic) and lowest (4.60±0.26 U/L) in group E (combination) (Table 3). Both AST and ALT values were significantly decreased in combination group (E) compared to group A (control). The highest creatinine value (2.813±0.014mg/dL) was seen in group B (antibiotic) and lowest (0.4625±0.008mg/dL) in group E (combination). The creatinine level was significantly (P<0.05) increased in antibiotic compared to all other groups.

4. Discussion

It was observed that broiler chicks fed with probiotic (Bio-Top®) at a level of 0.15%, group (C) showed the significantly (P<0.05) higher final body weight, compared to that of control group (A). On the other hand, the combination group (E) had the second best body weight gain compared to control group (A). The increased body weight recorded in present experiment resembles to that of Kabir (2009), Toghyani *et al.* (2011) and Kral *et al.* (2012) who stated that body weight gain were higher in probiotics fed birds. However, there are conflicting results in the literature concerning the efficacy of probiotics, prebiotics and plant extracts for growth performance in broilers. Angel *et al*., (2005); Botsoglou *et al*., (2002); reported no observable change in body weight gain and feed conversion of broilers by supplementing diets with probiotics, prebiotics, plant extracts and essential oils. However, in the present study data with respect to body weight gain is not as much as higher comparing to previous studies. This may be due to the use of handmade broiler feed instead of using commercial broiler feed and variation in management practices, environmental conditions, age, and sex of birds. The improved body weight gain in combination group may be due to synergistic action of probiotic and phytobiotic.

The differences in feed consumption in relation to body weight of the broilers among different groups, resulted a significant differences in feed conversion ratio (P<0.05). Significant (P<0.05) difference was observed between treatment and control group. Cumulative FCR was 1.72, 1.83, 1.75, 1.96 and 2.1 for probiotic, phytobiotic, combination, antibiotic and control groups, respectively. The significant effect of probiotic on feed conversion ratio (FCR) of broiler was in close agreement with Shim *et al.* (2012); Zhou *et al.* (2010); Hassanein and Soliman (2010). They found that supplementing with *Bacillus subtilis* and *B. licheniformis* improved feed conversion efficiency in broiler. Panda *et al.* (2008) reported the dietary preparation of *Bacillus subtilis* and *B. licheniformis* (at the rate of $6x10^8$ spore per kg of diet) significantly enhanced feed efficiency in White Leghorn Breeders.

Total cholesterol, triglyceride, HDL, LDL values differed significantly (P>0.05) among different groups. Supplementation of probiotic and phytobiotic has a significant effect on lipid metabolism as the present study showed that the values of serum cholesterol, triglyceride HDL was significantly (P<0.05) reduced in either probiotic (C) or phytobiotic (D) group compared to control group (A).

The mechanisms by which probiotics decreased total cholesterol and triglyceride may include enzymatic de-conjugation of bile acids by bile-salt hydrolase of probiotics, assimilation of cholesterol by probiotics, co-precipitation of cholesterol with de-conjugated bile, cholesterol binding to cell walls of probiotics, incorporation of cholesterol into the cellular membranes of probiotics during growth, secretion of inulin which has an inhibitory effect on triglyceride synthesis, inhibition of HMG-Co-A reductase, lowering intestinal p^H and conversion of cholesterol into cloprostanol.

We found higher value of creatinine, AST and ALT in antibiotic group compared to control, probiotic, phytobiotic, and combination groups (P<0.05) may be due to hepatotoxicity and or associated nephrotoxicity of antibiotics. Slightly elevated AST and ALT level in probiotics treated group may be due to an increase in hepatic metabolism since probiotics supplementation is not known to cause liver damage. The total cholesterol level decreased significantly (P<0.05) in probiotics treated (group C) and combination (group E) compared to the control (group A). This finding is contradictory to Kwon *et al.* (2002), who reported cholesterol level being statistically non-significant (P>0.05) after probiotic supplementation. The present study resembles the report of Mohan *et al*. (1996) who reported a significantly (P<0.05) decreased serum cholesterol in probiotics treated groups. Our findings are in agreement with the findings of Kamruzzaman *et al*. (2005) who stated that the values of ALT and AST differed significantly (P<0.05) among the treatment groups. But the present result is contrary to Shareef *et al*. (2009) who found that there was no significant (P>0.05) effect on ALT, AST serum activities, compared with that of control group. Biochemical parameters remained within normal range though there was statistical significance (P<0.05) between the treated and control groups. The biochemical parameters of this study resembles that of Pravbhakaran *et al*. (1996), who reported that the AST and ALT concentration in blood serum decreased with advancement of age. The biochemical parameters probably varied due to the influence of sex, environment, exercise, nutritional status, species variation and climate.

5. Conclusions

Supplementation of probiotic, phytobiotic or combination of both may have beneficial effects and can be used as growth promoter in broiler production as an alternative to antibiotics. Before application of these two products at commercial basis as an alternative to antibiotic growth promoter's further dose dependent trial on a large scale basis is needed.

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Conflict of interest

None to declare.

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