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Original Article

Effects of Selected Probiotics and Synbiotics on Growth Performance and Blood-biochemical Changes in Broiler Chickens

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Abstract

In broiler production, probiotics and synbiotics have been introduced as a replacement for antibiotic growth promoter. The study investigated the effects of selected probiotics and synbiotics on body weight and blood-biochemical parameters in broiler chickens. A total of 48, day-old "Lohman" broiler chicks were reared for 28 days. At day 13, the chicks were randomly divided into four equal groups: A, B, C, and D (n=12 in each group). The group A was considered as non-treated control. Group B was supplemented antibiotic (ciprofloxacin) with water. Group C and D were supplied with probiotics at a dose rate of 1 g/L and synbiotics 1.2 g/L, respectively through drinking water. Synbiotics treated broilers had significantly ($p<0.05$) higher live body weight and lower feed conversion ratio (FCR). Total erythrocyte count (TEC), hemoglobin concentration (Hb), and packed cell volume (PCV) were significantly ($p<0.05$) higher in the probiotics and synbiotics treated groups than in the control group. Broilers supplemented with probiotics and synbiotics had higher levels ($p<0.01$) of serum alanine transaminase (ALT) and aspartate transaminase (AST). Serum total cholesterol (TC) and triglycerides (TG) were significantly lower in the probiotics and synbiotics supplemented group than in the control group, while HDL cholesterol was significantly ($p<0.05$) higher in the treated group. It can be concluded that this study aids to understand of the use of probiotics and synbiotics those have a positive effect on body weight and the hemato-biochemical profile in broiler chickens.

Introduction

Now-a-days broiler industry is facing several constraints which cannot be overlooked. Broiler chickens are reared in intensive condition which induces a stressful condition that predisposes the chickens to various pathogenic diseases (Dawkins et al., 2004). Feed additives such as antibiotics or growth promoters and anti-coccidial drugs are routinely used to improve performance and minimize losses due to disease causing microorganisms within the poultry industry (Castanon, 2007). Antibiotics as additive stuff have adverse effects on human, animal health and are risky due to cross-resistance amongst pathogens and residues in tissues. Therefore, the use of antibiotic as growth promoters has been banned in many countries and these led to investigations of alternative feed additives in animal production. Recent restrictions on the use of antibiotics as feed additives have prompted poultry farmers to look for safe and healthy alternatives to produce food (De Vrese and Schrezenmeir, 2008). Consequently, there have been increasing concerns about the impact of antimicrobial resistance on animals and humans. As a result, probiotics and combinations of probiotics and prebiotics (synbiotics) have been introduced in poultry production as an alternative to antibiotics and growth promoters (Mountzouris et al., 2010).

A probiotic is a live microorganism culture that can manipulate and maintain a healthy microflora in the gut. They balance the intestinal microbial flora, decrease the population of pathogenic microorganisms,

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stimulate the immune system, increase nutrient availability to the host, and reduce stress-related losses and poor performance (Jiang et al., 2020). Probiotics and antibiotic growth promoters supplemented broilers had increased body weight and improved the lipid profile and other biochemical parameters (Miah et al., 2014). Prebiotics are indigestible substances to the host animal, primarily oligosaccharides, which promote the growth of health-promoting bacteria in the gastrointestinal tract, particularly lactobacilli and bifidobacteria and pathogenic bacteria are reduced by competing for binding sites on the intestinal mucosa (Davani-Davari et al.,2019). Combination of prebiotic and probiotics are known as synbiotics that beneficially affect the host by improving the survival and implantation of newly added strains in the intestine by activating the metabolism of health-promoting bacteria and/or selectively stimulating their growth (Gibson and Roberfroid, 1995). Synbiotics includes both prebiotics and probiotics part, of which prebiotics part provides specific substrates such as fructo-oligosaccharides, inulin, and galacto-oligosaccharides which support selective growth of probiotic bacteria like lactobacilli and bifidobacteria (Huebner et al., 2007). Synbiotics are more beneficial for growth of broiler chickens due to the improvement of intestinal microflora, cecal volatile fatty acid concentration, and intestinal histomorphological parameters, when compared to probiotics alone (Awad et al., 2009; Mookiah et al., 2014). Synbiotics, synergistic mixtures of probiotic and prebiotics, may be more efficient than prebiotics and probiotics. The study was designed to know the comparison of synbiotics and probiotics on growth performance, hemato-biochemical profile and liver and kidney functions of broiler chickens.

Materials and Methods

Statement of the experiment

The study was done at the experimental shed of the Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, from November 1st to November 28th, 2018. A total of forty-eight healthy day-old Lohman broiler chicks were purchased from Agro Industrial Trust Hatchery Ltd., via dealership of Ms. Guru Poultry Farm, Digharkanda, Mymensingh, Bangladesh. The current study and experimental protocols were approved and carried out following the guidelines for animal care and use established by the Animal Welfare and Experimentation Ethics Committee at Bangladesh Agricultural University in Mymensingh, Bangladesh [AWEEC/BAU/2020-15].

Experimental design

Experimental Lohman broiler chicks were reared in a single shed with same ration and management during 0-12 days of age when the chicks did not receive any supplement. At 13th day of age, total 48 chickens were randomly divided into four equal groups (namely A, B, C and D), each group containing 12 chickens. From 13th day of age of each bird, the experimental supplementation was started. Chickens of group A was kept as untreated control and received non-medicated water, group B was supplemented with Ciprofloxacin (100 mg/ml) at the dose of 1 ml /L, group C with probiotics (Promax) at 1g/L and group D with synbiotics (Bio-lux) at 1.2g/L, through drinking water. The commercially available antibiotic Ciprofloxacin- Ciprocin Vet (100mg/ml), probiotics (Promax) and synbiotics (Bio-lux) were used in accordance with the manufacturer's instructions. Probiotics (Promax) and synbiotics (Bio-lux) were purchased from ACI Animal Health, Bangladesh (Table 1 and Table 2). Ciprofloxacin Hydrochloride USP (100 mg/ml) oral solution "Ciprocin Vet"-100 ml liquid solution was obtained from SQUARE Pharmaceuticals Limited, Bangladesh. Chickens received their freshly prepared daily medication at 08.00 am in the morning each day. Treatment was continued up to the age of 28 days.

Table 1. Composition of supplemented probiotics (Promax ®)

Lactobacillus acidophilus	*Bacillus Licheniformis*
Lactobacillus bulgaricus	*Bacillus Megaterum*
Lactobacillusplantarum	*Bacillus Mesentricus*
Streptococcus faecium	*Bacillus polymyxa*
Bifidobacterium bifidus	*Saccharomyces bourlrdii*
Bacillus subtilis	*Bacillus Licheniformis*
Total viable count 5000 billion spores	

Table 2. Composition of supplemented synbiotics (Bio-Lux ®)

Lactobacillus acidophilus	5×10^6 CFU
Lactobacillus plantarum	5×10^6 CFU
Saccharomyces cerevisiae	1×10^8 CFU
Bacillus subtilis	1×10^9 CFU
d l methionine	100 mg
l lysine	100 mg
Vtamin A	1500 IU
Vtamin D_3	300 IU
Vtamin E	15 IU

The broiler chickens were immunized against common infectious diseases by vaccination as per manufacturers' instructions and following schedule was followed.

Table 3. Vaccination of experimental broiler chickens

Age of Birds (days)	Name of the Vaccines	Name of the company	Dose	Route
07	BCRDV (Newcastle L-63)	Square Pharmaceuticals Ltd., Agrovet Division, Dhaka, Bangladesh	One drop	Eye
12	Gumboro (Bangla GUMBORO VAC)	FnF Pharmaceuticals Ltd., Dhaka, Bangladesh	One drop	Eye
17	Gumboro booster (Bangla GUMBORO VAC)	FnF Pharmaceuticals Ltd., Dhaka, Bangladesh	One drop	Eye

Broiler feed including starter, grower and finisher were purchased from ACI Godrej Agrovet Feeds Ltd, Dhaka, Bangladesh. The chickens were fed grower feed for 12 to 23 days and finisher feed for 23 to 30 days twice a day. During the experimental periods, strict biosecurity measures were implemented.

Body weight and feed conversion ratio (FCR)

The bodyweight of each chicken was measured at weekly interval (day 14, 21 and 28) and total body weight gain were measured. FCR was determined from total feed consumed by birds divided total body weight gain. Feed intake was determined by subtracting the amount of feed given to the chickens from the amount of feed left at the end of each feeding period.

Blood sampling and serum preparation

Following the completion of the experimental period, blood samples were collected after slaughter. 10-12 mL of blood was collected from each chicken from the flow of blood at the time of slaughter. Half of the blood was kept in a sterile vacutainer containing heparin, and the other half was taken in another sterile test tube without any anticoagulant for serum preparation. Blood samples without anticoagulant were kept in the refrigerator overnight at 4°C in a slanting position. Following a 15-minute centrifugation at 1000 rpm, the serum was separated from the clotted blood. Cell-free serum samples were kept at –20°C for further biochemical analysis.

Analytical procedure of blood and serum sample

The Hb, TEC and PCV were measured within two hours of the collection as per methods described by Ghai, 2012. The biochemical tests were conducted at Professor Mohammad Hossain Central Laboratory, Bangladesh Agricultural University, Mymensingh-2202. The serum total cholesterol (TC), triglycerides (TG), high density lipoproteins cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured (Haque et al., 2017), using a UV spectrophotometer T 80, PG instruments, Great Britain.

Statistical analysis

Data were inserted in Microsoft Excel- 2013 and exported to the software Graph Pad Prism Version-8 for analysis. One-way analysis of variance (ANOVA) was performed to determine the effects of antibiotics, probiotics and synbiotics on body weight and different hematological and biochemical parameters of chickens. Descriptive statistical analysis was done to measure the mean, standard deviation and standard error mean (SEM) and p value of different parameters. Results are expressed as the mean + SEM. Because of using multiple comparisons, the corrected p value was calculated and $p<0.05$ was considered as statistically significant.

Results

Effects of synbiotics and probiotics on growth performance

Effects of synbiotics and probiotics on growth performance traits are presented in Table 4. At 14th and 21st day of age, the body weight variation was not statistically significant ($p>0.05$) among four groups (Table 4). On the 28th day of age, body weights were significantly higher in the treated groups than in the control. The highest body weights were in group D (1872.50 ± 72.6 g) and group C (1797.50 ± 48.52 g) and the lowest (1615.5 ± 43.6 g) in group A (control). The feed conversion ratio (FCR) was 1.53, 1.36, 1.35 and 1.29 in groups A, B, C and D, respectively. Synbiotics group showed a lower feed conversion ratio than the control group and the difference was found significant ($p<0.05$) (Table 4).

Effects of synbiotics and probiotics on hemato-biochemical parameters

Selected hematological parameters (Hb, TEC and PCV values) (Mulatu et al., 2019) are presented in Table 4. The highest Hb (10.34 ± 0.30 g %) concentration was recorded in group D (synbiotics), the second-highest Hb concentration (9.6 ± 0.15 g %) in group B (antibiotic) and the lowest in group A (7.98 ± 0.09 g %). Synbiotic supplementation increased the Hb concentration (10.34 ± 0.34 g %) significantly. The highest TEC was recorded in group D (3.11 ± 0.07 x10^6/μL) followed by group C (3.05 ± 0.12 x10^6/μL) and the lowest in group A (2.33 ± 0.09 x10^6/μL). The highest PCV was in group D (39.16 ± 0.57 %) and group C (38.8 ± 1.64 %) and the lowest was in group A (32.0 ± 0.04 %). The differences between control, probiotics and synbiotics groups were found significant ($p<0.05$).

Broilers receiving synbiotics and probiotics had a lower concentration of TC (139.5 ± 6.8 mg/dl and 153.0 ± 2.8 mg/dl), TG (75.11 ± 1.4 mg/dl and 82.13 ± 1.2mg/dl) and higher concentration of HDL-c (42.65 ± 1.22 mg/dl and 41.43 ± 1.43 mg/dl) (Table 5) compared to the control group.

The mean values of creatinine were similar in all groups (Table 5). In the case of AST, the values were higher in group C (10.85 ± 0.72 U/L) followed by group B (10.03 ± 0.92 u/L) and group D (8.76 ± 0.93 U/L) than in control group (7.76 ± 0.59 U/L). ALT concentrations were significantly higher in synbiotics supplemented groups (7.69 ± 0.22 U/L)) than in other groups.

Table 4. Effects of synbiotics and probiotics on live body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR) in broiler chickens

Groups	BW (g) (Mean ± SEM)			BWG (g)	FCR
	14th day	21st day	28th day		
Group A (Control)	526.25 ± 16.5[a]	1115.63 ± 57.2[a]	1615.00 ± 43.60[a]	1088.75 ± 40.9[a]	1.53 ± 0.1[a]
Group B (Antibiotics)	556.88 ± 21.8[a]	1081.25 ± 35.12[a]	1752.50 ± 54.51[b]	1195.62 ± 62.5[b]	1.36 ± 0.0[b]
Group C (Probiotics)	562.50 ± 25.6[a]	1144.38 ± 25.6[a]	1797.50 ± 48.52[b]	1205.00 ± 51.2[b]	1.35 ± 0.1[b]
Group D (Synbiotics)	560.63 ± 29.3[a]	1133.13 ± 47.3[a]	1872.50 ± 72.6[c]	1291.87 ± 58.9[c]	1.29 ± 0.0[b]

[a,b,c] values with different superscript letters in a column differs significantly (p<0.05)

Table 5. Effects of synbiotics and probiotics on hematological and biochemical parameters (Mean ±SEM) in broiler chickens

Parameters	Group A (Control)	Group B (Antibiotics)	Group C (Probiotics)	Group D (Synbiotics)
Hb (g %)	7.98 ± 0.09[a]	8.08 ± 0.14[a]	9.63 ± 0.15[b]	10.34 ± 0.30[b]
TEC ($x10^6$/µL)	2.33 ± 0.09[a]	2.37 ± 0.04[a]	3.05 ± 0.12[b]	3.11 ± 0.07[b]
PCV (%)	32.04 ± 0.73[a]	35.20 ± 1.43[a]	38.80 ±1.64[b]	39.16 ± 0.57[b]
TC (mg/dl)	161.3 ± 8.6[a]	162.7 ± 9.3[a]	153.0 ± 2.8[b]	139.5 ± 6.8[b]
TG (mg/dl)	99.07 ± 7.8[a]	92.67 ± 3.7[a]	82.13 ± 1.2[b]	75.11 ± 1.4[b]
HDL-c (mg/dl)	37.58 ± 0.57[a]	37.76 ± 0.86[a]	41.43 ± 1.43[b]	42.65 ± 1.22[b]
LDL-c (mg/dl)	95.32 ± 10.6[a]	101.22 ± 8.6[a]	95.42 ± 3.2[a]	90.34 ± 7.25[a]
Creatinine (mg/dL)	0.83±0.05[a]	0.86±0.05[a]	0.82±0.04[a]	0.95±0.05[a]
ALT (U/L)	3.96±0.38[a]	4.31±0.54[a]	3.85±0.43[a]	7.69±0.22[b]
AST (U/L)	7.76±0.59[a]	10.03±0.92[a]	10.85±0.72[a]	8.76±0.93[a]

[a,b,c] values with different superscript letters in a row differs significantly (p<0.05)

Discussion

The increased body weight gains in the treated group (group B, C and D) might be due to an increased feed utilization and metabolism of supplied feed nutrients by the action of health-promoting bacteria present in the gut. The findings of the present study are similar to that of (Oliva et al., 2016), who investigated that synbiotics and probiotics improved (p<0.05) the final body weight and cumulative weight gain of broiler chickens. Synbiotics appeared to be superior to probiotics in improving growth performance (Table 4). The result obtained coincide with the findings of Pourakbari et al., 2014, who stated that probiotics and synbiotics had been used as a growth promoter due to their richness in microbial metabolism and bodyweight were significantly (p<0.05) higher in synbiotics supplementation. The addition of probiotics and synbiotics (combination of prebiotic and probiotic) to broiler chicken ration has been effective in improving FCR. Improvement in growth performance and feed efficiency in broilers chickens fed probiotics and synbiotics may be attributed to the total effects of probiotic action, including the maintenance of beneficial microbial population improving feed intake and digestion (Fuller, 1989).

Supplementation of probiotics and synbiotics in diet increase blood cellular volume. The present findings coincide with the results of (Beski et al., 2015) who reported that addition of probiotics and synbiotics in feed significantly (p<0.01) increase hemoglobin concentration, packed cell volume of broiler chickens. Al-Kassie et al., (2008) and Karoglu and Drudag (2005) stated that increased Hb concentration, TEC and PCV may be added probiotic diet, could inhibit the nutritional stress in broiler chickens. Also, these results may be attributed to increased iron absorption (Ohta et al., 1998).

The result showed that, the highest TG level was recorded in group A and B whereas the lowest (p<0.05) was in group D (synbiotics). Decrease of serum TC and TG coincides with the findings of Tayeri et al., 2018 and Pourakbari et al., 2014 who reported that supplementation with probiotics and synbiotics decrease the TC and TG compared with control group.

The significant decrease in serum TC in probiotic and symbiotic supplemented broilers may be due to reduced synthesis of cholesterol in the GI tract (Mohan et al., 1996). It had been speculated that *Lactobacillus acidophillus* reduces the cholesterol within the blood by deconjugating bile salts within the intestine, thereby preventing them from acting as precursors in cholesterol synthesis. Lactobacillus has found to possess a high salt hydrolytic activity, which is liable for deconjugation of bile salts (Surono, 2003). Fukushima and Nakano, (1995) demonstrated that probiotic microorganisms inhibit hydroxymethyl-glutaryl-coenzyme A; which involved in the cholesterol synthesis pathway thereby decrease cholesterol synthesis. Prebiotic and synbiotic decreased serum TC levels, as well as decreasing LDL-c and the LDL/HDL ratio. Higher HDL-c values ($p<0.05$) were recorded with the use of synbiotics (Table 5) which proves beneficial effect of its inclusion. This type of effect was also experienced by Cheng et al., 2019 and Tufan et al., 2017, who reported that adding of synbiotics in feed improves the blood HDL-c level

Although the mean values of ALT and AST were found higher in probiotics and synbiotics group (Table 5) than control group of broiler chickens, they are in normal range which indicates non-harmful for consumption of broiler meat. The present findings are differed with the previous findings of Miah et al., 2014, who found that probiotics reduced the AST and ALT values. It may be due to the unknown activity of any component of used synbiotics preparation, for which more investigation is needed with each component separately.

Conclusions

Synbiotics (combining strategies of prebiotic with probiotic) provided additive benefit in growth performance, feed conversion ratio, hematological and biochemical parameters in broiler chickens than that of individual use of these additives or antibiotics. The present findings strengthen the current knowledge regarding the use of synbiotics as an alternative growth promoter in broiler chickens. However, further studies are needed with a large number of broiler chickens to determine the effective dose range of probiotics and synbiotics, their side effects and the benefit cost analysis in the field condition.

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Author's Contribution

MAM designed the experiment. KKIK and MAI performed the experiment and wrote the original draft; KKIK, KMS and MAM editing and finalization of draft, and MAI, MMI and MAM analyzed the data; KMS, MAM and MKI critically revised the manuscript.

Conflicts of Interest

The author declares that no conflict of interest exists.

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