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Full Length Research Paper

Impact of Probiotic and Prebiotic Supplementation on Broiler Chicken Performance and Blood Biochemistry

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This experiment was conducted to compare the effects of antibiotic, probiotic, prebiotic and mixture of probiotic and prebiotic as dietary growth promoter on serum biochemical parameters, energy and protein efficiency of broiler chickens. Three hundred day old Ross 308 broilers were equally distributed into 30 floor pens and reared for 42 days. A basal diet was formulated according to the recommendations of NRC for starter (1 to 21 days) and grower (22 to 42 days) periods and considered as control diet. Four tested diets were formulated by supplementing the basal control diet with antibiotic, Flavomycin; probiotic, Primalac; prebiotic, Biolex-MB and mixture of probiotic plus prebiotic (synbiotic). Six replicates were used for each treatment. Specific growth rate and growth efficiency were highest in birds under prebiotic and synbiotic treatments in starter and total rearing period, respectively. Broilers that were fed diet containing synbiotic had the highest energy efficiency ratio which was significant as compared to control group. Protein efficiency ratio followed the same trend and improved when synbiotic was used in the diet ($p < 0.05$). At 21 and 42 day of age, dietary supplementation with probiotic and synbiotic decreased ($p < 0.05$) serum cholesterol concentration, when compared with birds fed Flavomycin diet. The results suggested that the mixture of probiotic and prebiotic could be effective as antibiotic to improve the health and performance of broiler chickens.

Key words: Broiler, performance, cholesterol, probiotic, prebiotic

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 can not be ignored. A huge amount of antibiotics have 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 alternatives to antibiotics in poultry production.

One choice could be directed microbials (DFM), also called probiotics, which are live microbial feed supple-ments that beneficially affect the host animal by improving its intestinal health (Fuller, 1989). Primalac is a kind of commercial probiotic that contains at least 1×10^8 CFU g^{-1} *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* (Chichlowski et al., 2007a,b). Prebiotics are non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Biolex-MB is a commercial prebiotic of the mannan-oligosaccharides family, which is obtained by extraction from the outer cell wall of the yeast *Saccharomyces cerevisiae*.

Various findings on the effect of different probiotics and prebiotics on the health and growth responses of broiler chickens was reported (Kabir et al., 2004; Piray et al.,

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2007). Most recently, considerable attention has been paid to test the potency of growth promotants on altering lipid metabolism. Probiotic supplementation has been shown to reduce the cholesterol concentration in egg yolk (Abdulrahim et al., 1996; Haddadin et al., 1996) and serum in chicken (Mohan et al., 1996; Jin et al., 1998). Recent report suggested that feeding of chicory beta fructans, a prebiotic, reduced the serum cholesterol and abdominal fat of broiler chicken (Yusrizal and Chen, 2003). One of the most important compounds of feeds for farm animals is the protein source. It is reported that protein efficiency could be affected by intestinal microflora. Pathogenic bacteria increase the breakdown of proteins to nitrogen and reduce the efficiency of dietary protein (Mikulec et al., 1999). The aim of this study was comparing the effects of the antibiotic, probiotic, prebiotic and the probiotic plus prebiotic mixture on blood biochemical parameters and efficiency of dietary energy and protein of broiler chickens.

MATERIALS AND METHODS

Birds and housing

In this study, 300 broiler chickens of the commercial Ross 308 strain were used in a randomized completely design with 5 treatment (6 replicates in each treatment and 10 birds/replicates) and reared on the floor pens for 42 days. A basal diet was formulated and considered as control according to recommendation of NRC, 1994 for starter (1 to 21 days), and grower (22 to 42 days) diets. Four tested diets were formulated by supplementing the basal control diet with antibiotic (Flavomycin, 650 g ton^{-1}), probiotic (Primalac, 900 g ton^{-1}), prebiotic (Biolex-MB, 2000 g ton^{-1}), and mixture of probiotic (900 g ton^{-1}) plus prebiotic (2000 g ton^{-1}), respectively (Table 1). From day 1 to 42 of the study, water and experimental diets were given to the birds, *ad-libitum*. Broilers and feed intake were weighed weekly. Energy and protein efficiency ratios and specific growth rate were calculated as follows:

Specific growth rate (SGR) = 100 × (ln FBW - ln IBW) / *t*

Where, FBW is final body weight (g), IBW is initial body weight (g) and *t* is time in days.

GE=WG/LW

Where, GE is growth efficiency for time period, WG is weight gain for specific time period and LW is initial weight as a covariate.

Protein intake (PI) = total feed intake × (CP% of diet / 100)

Protein efficiency ratio (PER) = weight gain/total protein intake

Energy intake (EI) = (kcal ME of per kg diet × feed intake) /1000

Energy efficiency ratio (EER) = weight gain × 100 / total ME intake

Biochemical serum data collection and analysis

At 21 and 42 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) cholesterol) using commercial kits (Pars Azmoon) according to the manufacturer's protocols. Very low density lipoprotein (VLDL) cholesterol was calculated from triglycerides by dividing the factor 2.2. The low density lipoprotein (LDL) cholesterol was calculated by using the formula:

LDL cholesterol = Total cholesterol – HDL cholesterol – VLDL cholesterol.

Statistical analysis

All data were analyzed using the One-Way Anova procedure of SAS® (SAS, 1998) for analysis of variance. Significant differences among treatments were identified at 5% level by Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance

The results of feed additives on broilers performance are presented in the Table 2. Supplementation of prebiotic significantly increased the SGR and GE as compared to the control and probiotic treatments during the starter period ($p < 0.05$). Moreover, these indices were highest in broilers fed synbiotic than those of control and probiotic groups from 1-42 d of experiment ($p < 0.05$). At the starter (1 to 21 d) and total (1 to 42 d) periods of the experiment, the energy intake and protein intake were not affected by dietary treatments ($p > 0.05$). Energy efficiency ratio (EER) and protein efficiency ratio (PER) followed the same trend. All the growth promoting additive treatments had better PER and EER than control birds ($p < 0.05$). The highest value of PER and EER was shown by broilers under synbiotic treatment.

In the present study, the beneficial effects of a probiotic, prebiotic and synbiotic products on broiler performance are in agreement with previous studies (Zulkifli et al., 2000; Thitaram et al., 2005; Nayebpor et al., 2007; Falaki et al., 2010). In contrast, Gunal et al. (2006), Zhang et al. (2005) and Willis et al. (2007) reported that using these additives shed in the broiler ration had no significant effects on growth performance of broiler chickens. It is reported that the use of mixture of probiotic plus prebiotic because of their synergistic effect could reduce the count of pathogenic bacteria and increase the population of useful microflora in gut (Fairchild et al., 2001; Rada et al., 1995). So, it could be concluded by removing pathogenic bacteria that can adhere to the gastrointestinal track wall, Immune system may be less stimulated and a favorable medium is provided for the use of energy and nutrients by birds (Savage and Zakrzewska, 1996; Fairchild et al., 2001). Also, the use of prebiotics by increasing in length of the intestinal mucosa, increases the absorption areas and improves the birds energy and protein efficiency ratio

Table 1. Ingredient composition (as percent of dry matter) and calculated analysis of the basal diets.

Grower (22 – 42 days)	Starter (1 – 21 days)	Ingredient
Corn	61	58.7
Soybian meal	29	30
Wheat bran	5	5
Fish meal	0	2
Soybian oil	2	1
Oister shell meal	1	1.2
DCP	1.07	1
Vitamin and mineral perimix	0.5	0.5
DL- Methionine	0.13	0.1
L-lysine	0.15	0.25
Salt	0.25	0.1
Coccidiostat	0	0.05
Total	100	100
Nutrient content		
ME(Kcal/Kg)	2850	2950
Crude protein (%)	20.48	18.44
Crude fiber (%)	3.89	3.81

Vitamin and mineral provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; choline chloraid, 200000 mg, manganeze, 40000 mg, iron, 20000 mg; zinc, 40000 mg, copper, 4000 mg; iodine, 400 mg; selenium, 80 mg

(Santin et al., 2001). Furthermore, the effect of probiotics and prebiotics on reduction of pathogenic bacteria could reduce the breakdown of proteins to nitrogen. In this way, the utilization of proteins (amino acids) is improved, particularly from food that does not contain them in optimum quantities (Mikulec et al., 1999). Finally, each of the above mentioned reasons may lead to better growth responses of broiler chickens.

Serum lipid concentrations

The effect of feed additives on blood constituents is presented in Table 3. At 21 day of age, no significant differences were observed in triglycerides, HDL, LDL and VLDL levels between treatments ($p > 0.05$). In 21 day old brids, dietary supplementation with probiotic decrease cholesterol concentration ($p < 0.05$), when compared with birds fed the control, prebiotic and antibiotic diets. Also, at 42 day of age, HDL and LDL levels were not affected by dietary treatments, while the synbiotic and probiotic supplemented groups had a lower cholesterol and triglycerides concentrations ($p < 0.05$) compared with those of control and antibiotic supplemented groups, respectively. However, the cholesterol and triglycerides concentration did not show significant difference between the non-antibiotic additives groups. Moreover, in the birds under probiotic treatment the serum VLDL was lower than those under the control and antibiotic treatments ($p < 0.05$).

In agreement with our findings, it is reported that the probiotic supplementation significantly reduces the serum cholesterol level of the chickens (Panda et al., 2001; Kalavathy et al., 2003; Jin et al., 1998). Kannan et al. (2005) have reported that the use of 0.5 g kg^{-1} mannanoligosaccharide obtained from yeast in the ration of broiler chickens, significantly reduced the serum cholesterol level on day 35 as compared with the control ($p < 0.05$). Tizard et al. (1989) reported that mannans and other similar carbohydrates (such as fructans) prevent cholesterol absorption in gastrointestinal tract. In contrast, Yalcinkaya et al. (2008) reported that the use of MOS in broilers diet could not significantly reduce the serum cholesterol and triglycerides levels as compared with the control group. Synthesis of bile acids from cholesterol in the liver is the most important way of cholesterol excretion (Wilson et al., 1998). The use of probiotics and prebiotics can disintegrating bile salts and de-conjugate production of enzymes by the activity of lactic acid bacteria, as well as reduction of the pH in the intestinal tract can be effective in reducing the cholesterol concentration. Solvability of non-conjugate bile acids is lowered at a low pH and consequently, they are absorbed less from the intestine and are excreted more in the feaces (Klaver and Van der Meer, 1993). Consequently, the liver, for re-establishment of the hepatic cycle of bile

Table 2. The main effects of treatments on growth performance broiler chickens.

SGR (% d^{-1})	Control	Antibiotic	Probiotic	Prebiotic	Synbiotic
1-21	12.30^{c}	12.44^{abc}	12.39^{bc}	12.67^{a}	12.64^{ab}
1-42	18.52^{b}	18.76^{a}	18.64^{ab}	18.69^{ab}	18.78^{a}
GE (g/g)					
1-21	12.27^{c}	12.66^{abc}	12.51^{bc}	13.33^{a}	13.24^{ab}
1-42	47.92^{b}	50.40^{a}	49.23^{ab}	49.69^{ab}	50.64^{a}
Energy intake (Kcal)					
1-21	2986.09	2916.37	2932.89	2973.58	2973.76
1-42	13149.5	13163.5	13154.2	13067.4	13165.6
EER (g/g)					
1-21	17.12^{b}	18.34^{a}	17.95^{ab}	18.80^{a}	18.86^{a}
1-42	15.18^{b}	16.17^{a}	15.76^{ab}	15.95^{a}	16.30^{a}
Protein intake (g)					
1-21	214.57	209.56	210.75	213.68	213.69
1-42	821.96	822.83	822.25	816.82	822.96
PER (g/g)					
1-21	2.38^{b}	2.55^{a}	2.49^{ab}	2.61^{a}	2.62^{a}
1-42	2.42^{b}	2.58^{a}	2.52^{ab}	2.55^{a}	2.60^{a}

^{a,b,c} Means in each row with different superscripts are significantly different ($p < 0.05$). EER = energy efficiency ratio, PER = protein efficiency ratio, SGR = specific growth rate, GE = growth efficiency.

Table 3. The main effects of treatments on serum lipid concentratios (as mmol/l) of broiler chickens.

21 day of age	Control	Antibiotic	Probiotic	Prebiotic	Synbiotic
Cholesterol	3.89^{a}	4.00^{a}	3.32^{c}	3.76^{ab}	3.47^{bc}
Triglycerid	1.04	1.06	0.91	1.12	1.03
HDL	1.87	1.99	2.02	2.11	2.28
LDL	1.54	1.53	0.88	1.13	0.71
VLDL	0.47	0.48	0.42	0.51	0.47
42 day of age					
Cholesterol	4.15^{ab}	4.32^{a}	3.71^{bc}	3.77^{abc}	3.58^{c}
Triglycerid	0.97^{a}	0.91^{a}	0.62^{b}	0.76^{ab}	0.72^{ab}
HDL	2.02	2.16	1.67	1.81	1.93
LDL	1.68	1.74	1.75	1.60	1.31
VLDL	0.44^{a}	0.41^{a}	0.28^{b}	0.34^{ab}	0.33^{ab}

^{a,b,c} Means in each row with different superscripts are significantly different ($p < 0.05$).

acids, coverts more cholesterol concentration into the tissues and therefore their concentrations in the blood is reduced (Ros, 2000). In the growing birds, VLDL is the most important triglycerides carrier. A reduction in the serum triglycerides level may be due to an increase in the population of lactic acid bacteria in the gastrointestinal tract. Santose et al. (1995) have reported that supplementation of *Bacillus subtilis* to the ration of broiler chickens, in addition to reducing the carcass fat, reduces the triglycerides concentration in the serum, the liver and the carcass and suggest that this bacterium can be effective in reducing the activity of acetyl coenzyme A carboxylase (the enzyme limiting the synthesis rate of fatty acids).

Conclusion

The use of probiotic plus prebiotic due to the improve-ment in growth indices; EER, PER and reduction in serum cholesterol was more effective than separately probiotic or prebiotic supplementation to improve the gastrointestinal health and performance of boilers. Thus, this plays an important role in increasing the economic efficiency and conserving the health of consumers. Furthermore, according to the results, synbiotic could be introduced as a safe and natural alternative to antibiotic growth promoters in broiler diets.

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