African Journal of Biotechnology Vol. 8 (10), pp. 2329-2334, 18 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effects of dietary probiotic, prebiotic and butyric acid glycerides on performance and serum composition in broiler chickens

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Accepted 5 March, 2009

An experiment on ROSS 308 male broilers was conducted to evaluate probiotic (Primalac), prebiotic (Fermacto) and butyric acid glycerides (Baby C_4) on broiler performance and serum composition. Seven hundred and four day-old broilers were randomly distributed in a 2×2×2 factorial arrangement with two levels of probiotic, prebiotic and butyric acid glycerides. Eight treatments with four replicates each with 22 birds per pen were used. Three-way interaction between dietary treatments were observed for final body weight (P<0.05), feed intake (P<0.01) and feed conversion ratio (P<0.01) in the experiment. Body weight, feed intake and feed conversion ratio between supplementary treatments and control group were significantly different (P<0.01). Three-way interaction between dietary treatments were observed for total cholesterol (P<0.05), LDL (P<0.01), cholesterol/HDL ratio and HDL/LDL ratio (P<0.01) in the experiment. Total cholesterol concentration, LDL, HDL/LDL ratio and cholesterol/HDL ratio between supplementary treatments and control group were significantly different (P<0.05 and P<0.01). Serum triglyceride, HDL and VLDL cholesterol concentrations were not significantly different among dietary treatment when compared to control group (P>0.05). In conclusion, dietary supplementation improved the body weight, feed conversion ratio, HDL/LDL ratio and decreased feed intake, total cholesterol, LDL cholesterol and cholesterol/HDL ratio in the serum of broiler chickens.

Key words: Probiotic, prebiotic, butyric acid glycerides, serum cholesterol, broiler.

INTRODUCTION

Due to growing concerns about antibiotic resistance and the potential for a ban for antibiotic growth promoters in many countries, there is an increasing interest in finding alternatives to antibiotics in poultry production (Patterson and Burkholder, 2003; Chichlowski et al., 2007). Some possible alternatives to antibiotics for growth promotion and improvement of feed efficiency in domestic avian species are dietary supplementation of probiotic, prebiotic and organic acids (Jin et al., 1997; Biggs et al., 2007; Xu et al., 2006). Probiotic, which means "for life" in Greek (Gibson and Fuller, 2000), has been defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance, stimulating synthesis vitamins of the B-group, improving immunity system, preventing harmful microorganism, providing digestive enzymes and increasing of production of volatile fatty acids (Fuller, 1989; Rolf, 2000). In broiler nutrition, probiotics have a beneficial effect on broiler performance (Zulkflia et al., 2000; Kabir et al., 2004; Gil et al., 2005), improvement in feed conversion (Yeo and Kim, 1997), reduction in mortality (Kumprecht, 1998) and depress serum cholesterol (Mohan et al., 1996; Kurtoglu et al., 2004). There are also conflicting studies; probiotic have been shown not to improve weight gain, feed conversion ratio and to reduce mortality (Jin et al., 1997).

Prebiotics are food ingredients that selectively stimulate the activity and/or growth of endogenous bacteria such as Bifidobacteria and Lactobacilli, which benefit the host (Gibson and Roberfroid., 1995). They are short chain carbohydrate that is non-digestible by animal enzyme

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(Quigly et al., 1999). Several studies have shown that administering prebiotics can improve weight gain, feed intake and feed convention in broiler (Waldroup et al., 1993; Grimes et al., 1997; Wu et al., 1999; Xu et al., 2003; Pelicano et al.., 2004; Rodrigues et al., 2005). However, some reports indicate that prebiotic supplementation did not effect in body weight gain, feed intake or feed convention (Stanczuk et al., 2005). Prebiotic supple-mentation may also depress choles-terol concen-tration in blood and egg yolk (Mohan et al., 1995; Jin et al., 1998; Li et al., 2007).

Short- chain fatty acids such as butyrate are also con-sidered as potential alternatives to antibiotic growth promoter (Dierick et al., 2002; Lesson et al., 2005; Van Immerseel, et al., 2005). Dietary butyric acid had no effect on body weight or weight gain in starter, grower and finisher periods, but birds consumed less feed intake when diets were supplementation with butyrate relative to the control birds (Lesson, et al., 2005).

There are similar effects of probiotic, prebiotic and butyric acid glycerides on the performance of broiler chickens and there are a commercial varieties of these supplementation available and producers are presented with a challenge to choose the most suitable supple-mentation to reduce their costs of diet and production. Therefore, the objective of this study was to investigate the interaction effects of supplementation of probiotic (premalac), prebiotic (Fermacto) and butyric acid glyce-rides (Baby c4) on the performance and blood chemistry of broiler chickens under commercial conditions.

MATERIAS AND METHODS

Experimental animals

Seven hundred and four day-old male broilers chicks of commercial strain (Ross 308) were randomly divided into 8 treatments each composed of 88 birds. Birds in each treatment were placed in 4 pens, each containing 22 birds. All birds were raised on floored pen (2.5 ×1.25 M), in an environmentally controlled house with continuous light (10 to 20 lux) and had access to feed and water *ad libitum*. The house temperature was maintained at 33°C for the first 3 d, after that temperature was gradually reduced by 3°C per week until reaching 24°C; this temperature was maintained until the end of the 42 d of experiment. The trial was conducted in 3 periods consisting of the starter period (1 to 10 day), grower period (11 to 28 day) and finisher period (29 to 42 day).

Experimental design and diets

Birds were distributed in a completely randomized block design with 2×2×2 factorial arrangement. The composition and nutrient analysis of basal diet are shown in Table 1. The basal diet was a typical corn- soybean diet as mash form that was formulated to meet Ross nutrient requirements for starter grower, and finisher growth periods (Aviagen, 2004). Commercial probiotic (Primalac), Prebiotic (Fermacto), and Butyric acid glycerides (Baby C4) were used according to manufacture's instruction. Probiotic was added to the diet at 0.9 kg/ton in the starter, 0.45 kg/ton in the grower and 0.225 kg/ton in the finisher periods. Prebiotic was added to the diet at 1.8 kg/ton in the starter, and 1 kg/ton until slaughter age (42 d). Butyric acid glycerides were added to the diet at 3 and 2 kg/ton in the starter and grower periods respectively (1 to 28 d). Thus, the experimental diets consisted of:

Basal diet unsupplemented or control diet (T_1)
Basal diet + Probiotic (T_2 or Pro)
Basal diet + Prebiotic (T_3 or Pre)
Basal diet + butyric acid glycerides (T_4 or But)
Basal diet + Probiotic + Prebiotic (T_5 or Pro×Pre)
Basal diet + Probiotic+ butyric acid glycerides (T_6 or Pro× But)
Basal diet + Prebiotic + butyric acid glycerides (T_7 or Pre× But)
Basal diet + Probiotic + Prebiotic + butyric acid glycerieds (T_8 or Pro× Pre× But)

Performance parameters

Chickens were weighed at 1, 10, 28 and 42 d on Pen basis to determine weight gain. Feed consumed was recorded daily, the uneaten discarded, and feed conversion ratio (FCR) was calculated (total feed : total gain). Mortality was recorded as it occurred and percentage mortality was determined at the end of the study.

Measurement of serum indices

On 42 day of experimental period, 3 ml of blood was collected from brachial vein from one bird of each pen (from four birds of each treatment). Serum was isolated by centrifugation at 3,000×g for 10 min. The serum concentrations of total triglyceride, cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL), cholesterol/ HDL and HDL/ LDL ratio in serum samples were analyzed by an automatic biochemical analyzer (Clima, Ral. Co, Espain), following the instructions of the corresponding reagent kit (Pars Azmon Co., Iran). VLDL cholesterol was calculated from triglycerides by divided the factor 5 (Arun et al., 2006). The LDL cholesterol was calculated by using the formula: LDL cholesterol = Total cholesterol - HDL cholesterol - VLDL cholesterol.

Statistical analysis

All data were analyzed by ANOVA using the SAS (SAS Institute, 2000) GLM program. Treatment means were portioned by LSMEAN analy-sis. The model is:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + Block_L + e_{ijkl}$$

Where Y is the observed response, μ is the overall mean, A_i is the effect of Probiotic, B_j is the effect of Prebiotic, C_K is the effect of butyric acid glycerides AB_{ij}, AC_{ik} ,and ABC_{ijk} interaction between the effect of two or three factors, block_L is the effect of block e_{ijkl} and is the error.

RESULTS AND DISCUSSION

The final body weight, feed intake and feed conversion ratio of chicks is shown in Table 2. Three-way interaction between dietary treatments were observed for body weight (P<0.05), feed intake (P<0.01) and feed conver-sion ratio (P<0.01) in the experiment. The result showed that all the treatments except the treatment containing only prebiotic have better final body weight in compare with control treatment. These results are in agreement with those of Runho et al. (1997), Jin et al. (1998) and

Table 1. Composition of the basal diet fed to broilers.

Ingredient and Composition	Starter (0-10d)	Grower (11-28d)	Finisher (29-42d)
Ingredient % as fed			
Corn	49.03	59.6	65.99
Wheat	5.58	5.00	5.00
Soybean meal (44% CP)	26.86	16.05	10.12
Gluten	10.00	11.48	11.50
Soybean oil	3.50	3.34	3.09
Limestone	1.45	1.23	1
Dicalcum phosphate	1.95	1.8	1.83
Salt (NaCl)	0.36	0.36	0.36
Vitamin Permix[1]	0.25	0.25	0.25
Mineral Permix[2]	0.25	0.25	0.25
L-Lysine	0.52	0.58	0.57
DL-Methionine	0.25	0.06	0.04
Nutritional content (calculated)			
ME, Kcal/kg	3010	3150	3200
CP	23.00	20.00	18.00
Calcium	1.00	0.90	0.90
Available phosphorus	0.50	0.45	0.45
Lys	1.41	1.16	1.05
Met and Cystine	1.09	0.81	0.78

vitamin premix the following per kilogram of complete feed: Vitamin A, 4,500 IU (retinyl acetate); cholecalciferol, 1000 IU; vitamin E, 25 IU; vitamin B_{12}, 0.02 mg; menadione, 1.5 mg; riboflavin, 3 mg; thiamin 1.5 mg; pantothenic acid, 5 mg; niacin, 20 mg; choline, 150 mg; folic acid, 0.5 mg; biotin, 0.5 mg; and pyriodoxine, 2.5 mg.

The mineral premix supplied the following per kilogram of complete feed: Manganese, 60 g; zinc, 40 mg; iron, 80 mg; copper, 8 mg; selenium, 0.2 mg; iodine, 0.8 mg; and cobalt, 0.4 mg.

Table 2. Effect of dietary treatment on the performance of broiler chickens at 42 d age.

Treatment	Levels			Body weight	Feed intake	Feed conversion ratio
	Pro	Pre	But			
Pro× Pre× But	0	0	0	1910.01[b]	3824.90[a]	2.00[a]
	1	0	0	2037.89[a]	3464.12[bc]	1.70[b]
	0	1	0	1971.61[ab]	3386.38[bc]	1.72[b]
	0	0	1	2063.83[a]	3404.92[bc]	1.65[b]
	1	1	0	2037.80[a]	3506.53[bc]	1.72[b]
	1	0	1	2009.10[a]	3345.11[c]	1.66[b]
	0	1	1	2027.26[a]	3595.50[b]	1.77[b]
	1	1	1	2015.30[a]	3361.92[bc]	1.68[b]
	SE			31.35	71.6	0.03
	P- value			0.05	0.004	0.0003

[a-c] Means with in columns with different superscript differ significantly (P<0.05; P<0.001). [1]PRO= Probiotic; PRE= Prebiotic; and BUT= Butyric acid glycerides.

Biggs et al. (2007) and on the contrary Leeson et al. (2005); Chichlowski et al. (2007) and Mount et al. (2007). Inconsistent results of using probiotic, prebiotic and organic acid may be due to differences in methods of preparing the supplement, different in dietary nutrient levels, different experimental condition or different sex of birds. Our research demonstrated that dietary treatments decreased feed intake significantly compared with control diet (P<0.05; P<0.01) and the treatments containing probiotic + butyric acid glycerides and Probiotic + Prebiotic have the lowest and highest feed intake among dietary treatments, respectively. Several studies con-

Table 3. Effect of dietary treatment on the blood chemical parameters of broiler chicken at 42 d age (Mg/dL).

Treatment	Levels			Total Cholesterol	HDL	LDL	VLDL	Chol./HDL	HDL/LDL	Triglycerides
	Pro	Pre	But							
But×Pre×Pro	0	0	0	170.50[a]	56.12	77.13[a]	37.25	3.19[a]	0.75[c]	151.25
	1	0	0	128.00[b]	72.21	30.04[bc]	25.75	1.77[b]	2.47[bc]	128.75
	0	1	0	121.25[b]	58.52	35.93[b]	26.80	2.08[b]	2.2[bc]	134.00
	0	0	1	141.50[b]	68.13	43.47[b]	29.90	2.08[b]	1.59[c]	149.5
	1	1	0	92.00[c]	62.13	9.53[d]	20.35	1.49[b]	6.72[a]	101.75
	1	0	1	125.00[b]	63.09	36.81[b]	25.10	2.01[b]	2.25[bc]	125.50
	0	1	1	100.25[c]	58.50	17.40[cd]	24.35	1.75[b]	3.49[b]	121.75
	1	1	1	136.00[b]	70.21	43.20[b]	22.60	1.96[b]	1.78[bc]	113.00
	P-value			0.001	0.19	0.001	0.096	0.001	0.001	0.65
	SE			6.93	4.72	6.02	3.65	0.19	0.55	19.40

[a-d]Means with in columns with different superscript differ significantly (P<0.05; P<0.001).
[1] PRO= Probiotic; PRE= Prebiotic; BUT= Butyric acids glycerides.

ducted to evaluate the feeding of probiotic and butyric acid showed that feed intake decreased in broiler chickens (Zulkifli et al., 2000; Lesson, et al., 2005). Feed conversion ratio was improved significantly for the broilers fed all dietary treatments compared with control diet (P<0.05; P<0.01). Numerous studies showed that feed conversion improved with addition of probiotic, prebiotic and organic acid in broiler diet (Yeo and Kim, 1997; Gil et al., 2005; Rodrigues et al., 2005). Probiotic, prebiotic and organic acids maintained a better microbial environment in digestive tract of birds by reducing the number of pathogenic microbes. This enhanced digestion, absorption and efficiency of utilization of feed (Khaksefidi and Ghoorchi, 2006; Chicholowski and et al., 2007).

The other results of our study demonstrated there was non-significant decrease in feed intake and feed conversion ratio by prebiotic treatment compared with control diet. This results in agreement with those of several studies noted that addition of prebiotic to broiler diet had no significant influence on feed intake and feed conversion (Sohail et al., 2002; Chichlowski et al., 2007; Mountzouris et al., 2007). Mortality for all groups was within the expected range and there was no significant difference in mortality of all treatments. The serum concentrations of triglycerides, total cholesterol, HDL, LDL and VLDL cholesterol, cholesterol/HDL and HDL/LDL ratio, in dietary treatments at the end of the experiment (42d) are presented in Table 3. Three-way interaction between dietary treatments were observed for total cholesterol (P<0.05), LDL (P<0.01), cholesterol/HDL and HDL/LDL ratio (P<0.01). There were not any significant differences on serum triglyceride, HDL and VLDL concentrations of birds fed with all treatments. The serum total cholesterol concentrations were significantly reduced by dietary treatments compared to the control group (P<0.05 or P<0.01), and the treatment containing pro × pre and pre × but showed the lowest cholesterol among all dietary treatments (P<0.05). There was no significant difference on serum HDL cholesterol concentrations of birds fed dietary treatments compared to the control group (P>0.05). Serum LDL cholesterol concentrations were significantly reduced by dietary treatments compared to the control group (P<0.05 or P<0.01) and the treatment containing pro × pre and pre × but showed the lowest LDL among all dietary treatments (P<0.05). Serum VLDL cholesterol concentrations were not significantly reduced by dietary treatments when compared to control group (P>0.05). Similar cholesterol depressing effect due to probiotic supplementation in broiler chicken was observed by Joy and Samuel (1997). In addition, in some study, probiotic supplementation reduced the serum LDL cholesterol (kalavathy et al., 2003) and triglycerides (Santos et al., 1995) in broiler chicken. The finding of our study and previous studies indicated that feeding of probiotic has a cholesterol depressing effect in broiler chicken. Besides, it is reported that some of the microorganisms present in the probiotic preparation could utilize the cholesterol present in the gastro intestinal tract for their own metabolism, thus, reduce to absorption the amount cholesterol (Nelson and Gilland, 1984; mohan et al., 1995). Lactobacillus, which has a high bile salt hydrolytic activity, is responsible for deconjugation of bile salts (Sarono, 2003). Deconjugated bile acid are less soluble at low pH and less absorb in the intestine and is more likely to be excreted in feces (Klaver and van der meer, 1993). In addition, probiotic microorganism inhibits hydroxymthyl- glutaryl- coenzyme A, an enzyme involved in the cholesterol synthesis (Fukashima and Nakon, 1995). The most important mechanism by which prebiotic eliminates cholesterol would likely be through reducing lipid absorption in intestine by binding bile acids, which results in increased cholesterol elimination and hepatic synthesis of new bile acid (Zhang et al., 2003).

On the other hand, Salma et al. (2007) have shown that cholesterol concentration in thigh and breast muscle of the broilers had a positive correlation with the change of the cholesterol contents in serum. Thus, it is expected that with decreasing of serum cholesterol, the amount of meat cholesterol is tending to decrease too. In view of dietary health, food that contains these supplementations can help in reducing the occurrence of cardiovascular heart diseases. Therefore, these results led to the conclusion that there were similar improvements on performance of birds fed diets with single supplementation of probiotic, prebiotic and butyric acid glycerides as well as their combination, and their performance was improved better than birds fed control diet, and choice preference of supplementation should be based on its economic value. In addition to, it is possible that all supplementation diets can help in reducing the occurrence of cardiovascular heart diseases in consumers.

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