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Effect of Dietary Antibiotic, Probiotic and Prebiotic as Growth Promoters, on Growth Performance, Carcass Characteristics and Hematological Indices of Broiler Chickens

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Abstract: This experiment was conducted for comparison the effects of antibiotic (flavomycin), probiotic (primalac), prebiotic (Biolex-MB) and mixture of probiotic and prebiotic (primalac plus Biolex-MB) as dietary growth promoter on growth performance, carcass characteristics and hematological indices of broiler chickens. Three hundred day old Ross 308 broilers were equally distributed into 30 floor pens and reared for 42 day. A basal diet was formulated covering the recommendations of NRC (1994) for starter (0-21 days) and grower (22-42 days) periods and considered as control diet. Four tested diets were formulated by supplemented the basal control diet with antibiotic (flavomycin), probiotic (primalac), prebiotic (Biolex-MB) and mixture of primalac plus Biolex-MB, respectively. Six replicates were used for each treatment. The results of present study showed that all growth promoters used was improved growth indices of Ross 308 broilers. The highest significant ($p<0.05$) values of carcass and thigh were recorded for broilers fed diet supplemented with flavomycin. The highest ($p>0.05$) value of breast was recorded for broilers fed the diet supplemented with primalac, meanwhile the lower value were showed for birds fed either diet or diet supplemented with Biolex-MB. The percent of carcass and cuts followed the same trend. Hematological parameter including cholesterol was recorded the highest ($p>0.05$) values groups fed the diets either control or supplemented with flavomycin, meanwhile the lower value was showed for bird fed diet supplemented primalac plus Biolex-MB. Triglycerides and very low density lipoprotein cholesterol (VLDL) were recorded the highest concentration for bird fed both control and diet supplemented with flavomycin groups while least concentration was found for bird fed diet supplemented with primalac. The results of present study revealed that probiotic and prebiotic as growth promoters can use as alternatives non-antibiotic feed additives to their free harmful side effects on the consumers and to improve broiler chickens growth indices.

Key words: Broiler, flavomycin, primalac, Biolex-MB, hematology

INTRODUCTION

For many years, antimicrobial compounds have been used in the poultry breeding industry for improvement in health status and performance of birds by reduction or correction of the population of the bacteria present in the gestero-intestinal (GI) tract (Fairchild *et al*., 2001). Growth stimulating antibiotics, by the spread of antibiotic-resistant bacteria, are a threat to human health (Wray and Davies, 2000; Turnidge, 2004). Following a severe limitation or a general inhibition of using antibiotics as growth stimulating and therapeutic agents in the poultry industry, probiotics and prebiotics have been suggested as appropriate alternatives (Piray *et al*., 2007). Probiotics are live microbial complements that leave useful effects on the host through an improvement in the intestine's microbial equilibrium (Fuller, 1989). Different microbial species such as bacteria (*Bacilli*, *Bifidobacteria*, *Enterococcus*, *Lactobacillus*, *Streptococcus*), yeasts (*Saccharomyces cerevisiae*), fungi (*Aspergillus oryzae* and *Aspergillus niger*) and indefinite mixed cultures have been used as probiotics (Simon *et al*., 2001). 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 faesium* (Chichlowski *et al*., 2007a, b). Prebiotics such as indigestible carbohydrates that leave a desired effect on the host, by selective growth

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stimulation or activation of one or more bacteria in a large part of the gestero-intestinal tract (Gibson and Roberfroid, 1995). Oligosaccharides such as fructooligosaccharides, galactooligosaccharides and mannan oligosaccharides are among the most important prebiotics that have been studied as alternatives to antibiotics (Spring, 1999). 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*. Several studies have shown that use of probiotics and prebiotics additive in the ration, improves the performance of broiler chickens (Kermanshahi and Rostami, 2006; Piray *et al*., 2007).

The combination of probiotics and prebiotics is called, synbiotic (Gibson and Roberfroid, 1995; Collins and Gibson, 1999; Schrezenmeir and De-Vrese, 2001). This combination can improve the viability of probiotic microorganisms, since they are able to use prebiotics as a substra for fermentation (Bengmark and Bengmark, 2001).

The search for new additives effective on the animal growth and free from harmful side effects on the consumers' health is still continuing. The purpose of this study was comparing the effects of the antibiotic flavomycin and alternatives non-antibiotic feed additives containing primalac and Biolex-MB as alternatives for the growth stimulating antibiotics on the performance and hematological measures parameters of broiler chickens.

MATERIALS AND METHODS

In this study, 300 broiler chickens of the commercial Ross 308 strain were used in a randomized completely design with 5 treatments (6 replicates in each treatment 10 birds/replicates) and reared on the floor pens for 42 days. Before beginning this study, the dry matter, crude protein, ether extract, crude fiber and ash contents of main feed ingredients were determined (AOAC, 1984) in the laboratory to make sure of the presence of sufficient amounts of protein and crude fiber content of the ration.

A basal diet was formulated and considered as control according to recommendation of NRC (1994) for starter (0-21 days) and grower (22-42 days) diets. Four tested diets were formulated by supplemented the basal control diet with antibiotic flavomycin (650 g t^{-1}), probiotic (Primalac, 900 g t^{-1}), prebiotic (Biolex-MB, 2000 g t^{-1}) and mixture of Primalac (900 g t^{-1}), + Biolex-MB (2000 g t^{-1}), respectively. Six replicates were used for each treatment (Table 1).

During the experiment, water and feed were given to the birds ad libitum. Weighing of the feed and chickens were made on a weekly basis. At 42nd day of the experimental period, 5 mL of blood was collected from wing vein from 6 birds in each treatment. Blood samples were centrifuged (at 2, 000×rpm for 10 min) and serum was separated and then stored at -20°C until assayed for measuring blood parameters (glucose, total protein, albumin, cholesterol, triglyceride and high density lipoprotein cholesterol (HDL)) using appropriate laboratory kits (Friedewald *et al*., 1972; Gordon *et al*., 1977; Gowenlock *et al*., 1988). The serum globulin was calculated by subtracting serum albumin from serum total protein levels. Very low density lipoprotein cholesterol (VLDL) was calculated from triglycerides by dividing the factor 5. The low density lipoprotein cholesterol (LDL) was calculated by using the formula:

Table 1: The experiment basal diets composition and calculated proximate analysis (on dry matter basis)

Ingredients	Starter (0-21 days)	Grower (22-42 days)
Corn	58.07	63.25
Soybean meal	27.12	21.52
Cotton meal	10.00	10.00
Soybean oil	1.16	1.75
Ground limestone	1.17	1.34
DCP	1.34	1.13
Salt	0.40	0.32
Vitamin and mineral premix	0.50	0.50
Coccidiostat	-	0.04
Vitamin E	0.03	0.03
DL-methionine	0.11	0.04
L-lysine	0.10	0.08
Nutrient content		
ME (kcal kg^{-1})	2850.00	2950.00
Crude protein (%)	20.48	18.44
Nitrogen free extract (%)	4.37	4.10

Nitrogen free extract (%) mixing 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, 200000 mg; Manganese, 40000 mg; Iron, 20000 mg; Zinc, 40000 mg; Copper, 4000 mg; Iodine, 400 mg; Selenium, 80 mg

LDL cholesterol = Total cholesterol–HDL cholesterol–VLDL cholesterol

The birds were slaughtered for separation of carcasses (Perreault and Leeson, 1992).

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 effect of experimental treatments on the performance of broiler chickens is given in Table 2.

The dietary supplemented with flavomycin, primalac, Biolex-MB and the primalac-Biolex-MB mixture, were increased the weight of the birds to 130.8, 73.5, 81.3 and 148.8 g, respectively compared to control diet (Table 2).

The weight gain was increased for birds fed primalac and Biolex-MB by 7.4% compared to control diet.

The above mentioned growth stimulating had no effect on the feed consumption of the birds. As similar trend mentioned for weight gain, in the birds under treatments of flavomycin, Biolex-MB and the primalac and Biolex-MB mix, the feed conversion ratio improved significantly when compared to control group ($p<0.05$). In general the positive effects of experimental additives tested on performance is in agreement with the results reported by several researchers. Fairchild *et al.* (2001), who showed that flavomycin as antibiotics has favorable effects on the weight gain of broiler chickens. Kermanshahi and Rostami (2006), Thitaram *et al.* (2005) and Nayebpor *et al.* (2007), reported that probiotics and prebiotics can improve the weight of birds. Esteve-garcia *et al.* (1997) observed that addition of flavomycin to a wheat-based ration could improve significantly the chickens' feed conversion ratio in all breeding periods (0-21 and 21-42 days). Moreover, adding probiotic and synbiotic to the ration has been effective in improving the feed conversion ratio (Zulkifli *et al.*, 2000; Cavit, 2004). In contrast, Ignacio, (1995) reported that the use of prebiotics in the ration of broiler chickens reduces the feed conversion ratio. Yalcinkayal *et al.* (2008), Gunal *et al.* (2006), Willis *et al.* (2007), Jamroz *et al.* (2004) and Denli *et al.* (2003) reported that using this additive shed in the broiler ration had no significant effects on body weight gain and feed conversion ratio after experimental period of 42 days 42. In agreement with the results of this study, there are numerous reports showing that the use of such additives has no effect on the feed consumption (Yalcinkayal *et al.*, 2008; Willis *et al.*, 2007; Pelicia *et al.*, 2004). Adverse results were reported by Piray *et al.* (2007) and Nayebpor *et al.* (2007).

Table 2: The main effects of treatments on performance of broiler chickens at 42 days (Mean±SE)

Treatments	Body weight gain (g)	Feed consumption (g)	Feed conversion ratio (g g^{-1})
Control	1996.6±30.44^{b}	4457.5±58.52^{a}	2.23±0.027^{a}
Flavomycin	2127.4±15.42^{a}	4462.2±66.70^{a}	2.10±0.030^{b}
Primalac	2070.1±33.40^{ab}	4429.6±110.98^{a}	2.15±0.045
Biolex-MB	2077.9±37.97^{ab}	4459.0±167.58^{a}	2.12±0.041
Synbiotic	2145.4±29.41^{a}	4462.9±82.30^{a}	2.08±0.020^{b}
p-values	0.017	0.999	0.038

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

Variance among reports of researchers could be related to differences in management and environmental conditions that be exist in various experiments. It's suggested that under benefit management and/or environmental conditions, the effect of such feed additives may be worthless. The results of some studies shown that growth stimulating antibiotics, increase the growth of broiler chickens by an increase in the uptake of nutrients (especially fatty acids and glucose), fixation of nitrogen and reduction in excretion of fat in the feces and microbial urea (Anderson *et al.*, 1999). The antibiotics reduce the number of bacteria, toxins and their secondary products in the GI tract (Gunal *et al.*, 2006). Present findings shown that consumption of primalac-biolex mixture, like that of antibiotics, had a positive effect on the body weight gain. The reason may be ascribed to the synergism of primalac and Biolex-MB. Following the concurrent action of prebiotics' fermentation by lactic acid bacteria in the GI tract and production of some acids by this group of bacteria, the pH of the GI tract is further reduced (Fuller, 1989). Reduction in pH is effective in controlling the population of pathogenic bacteria. During the infections due to pathogenic bacteria, lymphocytes crowd up to kill them and after inflammation, the thickness of the muscular layer increases (Gunal *et al.*, 2006). It seems that in our study, due to the synergism between primalac and Biolex-MB, followed by absorption of nutrients by the GI system, the birds under synbiotic treatment had a better feed conversion ratio.

Carcass composition: The effect of experimental treatments on the composition of the bird carcasses (in grams) and the carcass efficiency (in %) are given in Table 3.

Table 3: The effect of feed additives on carcass composition of broiler chickens

	Treatments					
Variables	Control	Flavomycin	Primalac	Biolex-MB	Synbiotic	p-value
As gram						
Carcass	1434.10±19^{b}	1523.30±23.33^{a}	1485.00±24.22^{ab}	1421.60±26.85^{b}	1465.80±41.58^{ab}	0.110
Thigh	396.60±8.02^{b}	438.30±7.92^{a}	419.10±7.12^{ab}	401.60±9.27^{b}	430.00±11.18^{a}	0.010
Breast	389.17±3b^{c}	425.00±14.31^{ab}	428.30±11.66^{a}	385.80±9.69^{c}	417.50±17.83^{abc}	0.051
Abdominal fat	40.80±1.86^{a}	40.30±0.94^{a}	36.60±2.33^{ab}	33.10±1.59	39.00±2.77^{b}	0.071
As %						
Carcass	69.63±0.62^{b}	72.46±0.90^{a}	70.85±0.57^{ab}	69.34±0.60^{b}	70.03±0.53^{b}	0.019
Thigh	27.67±0.61^{b}	28.80±0.62^{ab}	28.22±0.09^{ab}	28.24±0.26^{ab}	29.35±0.28^{a}	0.100
Breast	27.15±0.29^{b}	27.86±0.75^{ab}	28.82±0.44^{a}	27.14±0.47^{b}	28.45±0.68^{ab}	0.095
Abdominal fat	2.84±0.11^{a}	2.64±0.06^{ab}	2.46±0.14^{ab}	2.33±0.11^{b}	2.66±0.17^{ab}	0.085

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

Table 4: The effect of feed additives on serum total protein, albumin, globulin and glucose of broiler chickens

Treatments	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Glucose (mg dL^{-1})
Control	3.89±0.05[a]	1.78±0.08[a]	2.10±0.13[a]	203.50±17.55[a]
Flavomycin	3.76±0.16[a]	1.74±0.13[a]	2.02±0.12[a]	214.00±5.89[a]
Primalac	3.80±0.07[a]	1.80±0.04[a]	1.99±0.10[a]	217.83±13.24[a]
Biolex-MB	3.84±0.11[a]	1.74±0.12[a]	2.09±0.12[a]	208.16±2.51[a]
Synbiotic	3.87±0.09[a]	1.92±0.15[a]	1.95±0.17[a]	213.33±14.73[a]
p-value	0.987	0.884	0.944	0.302

[a, b, c]Means in each column with different superscripts are significant different ($p<0.05$)

Table 5: The effect of feed additives on serum lipid concentrations of broiler chickens

Treatments	Cholesterol (g dL^{-1})	Triglycerides (g dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
Control	160.80±1.33[ab]	86.17±6.69[a]	78.33±1.76[a]	65.23±2[a]	17.23±1.33[a]
Flavomycin	167.40±8.14[a]	81.33±3.17[a]	83.67±9.29[a]	67.47±17.15[a]	16.26±0.63[a]
Primalac	143.55±4.64[bc]	55.17±5.84[b]	64.67±2.84[a]	67.85±6.53[a]	11.03±1.16[b]
Biolex-MB	146.10±4.33[abc]	68.00±11.09[ab]	70.33±8.81[a]	62.17±10.80[a]	13.60±2.21[ab]
Synbiotic	138.45±0.88[c]	64.67±10.96[ab]	74.67±0.66[a]	50.85±0.90[a]	12.93±2.19[ab]
p-value	0.036	0.044	0.484	0.731	0.044

[a,b,c]Means in each column with different superscripts are significant different ($p<0.05$)

The weight and the efficiency of the dressing carcass in birds under flavomycin treatment was significantly higher as compared with the control and Biolex-MB treatments ($p<0.05$). The weight and the efficiency of the thigh of birds obtaining a ration containing synbiotic showed a higher mean as compared with the control treatment ($p<0.05$).

Also, the birds under primalac treatment had the highest percent breast and weight and birds under Biolex-MB treatment had the least abdominal fat percent and weight among the experimental treatments. In spite of the highest amount and percentage of abdominal fat in control group, the carcass, thigh and breast of this group were lowest than other groups except Biolex-MB fed group. The positive effect of the antibiotic use on the weight of broiler carcasses the confirm results of this experiment (Woodward *et al*., 1988; Elwinger *et al*., 1998).

Kabir *et al*. (2004) have reported that adding 2 g probiotic per each liter of water consumed by broiler chickens, would increase the efficiency in their thigh and breast as compared with the control treatment that confirm the positive effect of primalac on breast yield in this study. In the Ammerman *et al*. (1989) study, adding 0.375% oligofructose to the birds' ration, on day 47, decreased the percent of abdominal fat. This result was similar to the effect of Biolex-MB on abdominal fat in birds in present experiment. The positive effect of prebiotic on carcass quality were also reported by Piray *et al*. (2007). However, present findings on carcasse composition were in contrast to those of Pelicia *et al*. (2004), Pelicano *et al*. (2003), Willis *et al*. (2007) and Kannan *et al*. (2005). As pointed out before, these differences between reported results could be related to management and environmental conditions.

In this study, it seems that the use of flavomycin, primalac and primalac-Biolex-MB mix, by improving the uptake of nutrients and increase in nitrogen stability (Nahashon *et al*., 1996), can improve the carcass quality. Also, by observing a reduction in the fat level of birds fed by prebiotic, it is suggested that this product can interfere in the accessibility to fat for formation of fat tissue in the birds.

Biochemical parameters: As Table 4 shown all blood parameters not affected by treatments, with exception of cholesterol, triglycerides and VLDL. All non-antibiotic additives had significantly lowered ($p<0.05$) cholesterol level than control and flavomycin treatments. In the birds under primalac treatment, the serum triglyceride and VLDL were lower than these under the control and flavomycin treatments ($p<0.05$) (Table 5).

In agreement with the result of cholesterol in this experiment, it is reported that the use of 100 mg kg^{-1} of the probiotic supplement (*Lactobacillus acidophilus*, *Bifidobacterium* and *Aspergillus oryzae*) significantly reduces the serum cholesterol level of the chickens (Panda *et al*., 2001). Kannan *et al*. (2005) have reported that the use of 0.5 g kg^{-1} mannan oligosaccharide obtained from yeast in the ration of broiler chickens, significantly reduced the serum cholesterol level on day 35 as compared with the control ($p<0.05$). 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, by the activity of lactic acid bacteria, production of enzymes disintegrating bile salts and de-conjugating them, as well as reduction of the pH in the intestinal tract, can be effective in reducing the cholesterol connect ration. 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 feces (Klaver and Van der Meer, 1993). Consequently, the liver, for re-establishment of the hepatic cycle of bile 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 triglyceride carrier.

A reduction in the serum triglyceride level can be due to an increase in the population of lactic acid bacteria in the GI 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 triglyceride 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

It is concluded that by using non-antibiotic additives particularly mixing of both probiotic and prebiotic could obtained the advantages of antibiotic (performance) without their disadvantages (high cholesterol, triglycerides, etc).

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