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Effects of Cecal Cultures and a Commercial Probiotic (PremaLac®) on Performance and Serum Lipids of Broiler Chickens

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Abstract: A study was conducted to evaluate the effects of healthy broiler breeder undefined cecal culture and commercial probiotic (PremaLac®) on broiler performance and the serum lipids of broiler chickens from 1-42 days of age. A total of 288 days old Ross male broiler chickens were used in a 3×2 factorial arrangement with three levels of cecal cultures (0.00, 2.50, 5.00 mL L^{-1} in drinking water) and two levels of PremaLac (0.00, 0.09% in the diet). The cecal cultures and PremaLac were used >7 days of age. Body weight gain was significantly increased in cecal culture-inoculated compared to control broilers at the critical period of 0-21 days of age. A higher body weight gain was also observed in PremaLac-treated compared to control broilers for the whole growing period of 0-42 days. The feed intake and feed conversion ratio decreased in PremaLac-fed compared to control broilers from 0-21 days of age. Cecal cultures and PremaLac did not decrease serum lipid components. More research is needed to clarify the beneficial or adverse effect of cecal cultures as probiotics in poultry.

Key words: Cecal cultures, probiotics, performance, serum lipids, broilers, Iran

INTRODUCTION

For many decades antibiotic growth promoters have been used in farm animals to improve performance and to suppress disease. With increasing concerns about antibiotic resistance, the ban on sub-therapeutic antibiotic usage in Europe and the potential for a ban in the United States, there is increasing interest in finding alternatives to antibiotics for poultry production (Patterson and Burkholder, 2003). At present, a large number of natural growth promoters are commercially available including probiotics, prebiotics and immune-modulators. These products have the potential to influence the intestinal tract in a positive way thus, improving the health, well-being and performance of animals (Fuller, 1989). Probiotics and prebiotics are two of several approaches that have the potential to reduce enteric diseases and subsequently to reduce contamination of poultry products (Patterson and Burkholder, 2003).

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal balance (Fuller, 1989). Probiotics have been administered to farm animals to enhance production performance and immune responses (Huang *et al*., 2004). They are direct-fed viable microbial products and often contain live microbial cultures that are isolated from the Gastrointestinal Tract (GIT) of a healthy adult animal of the same species to which the probiotic product will be administered. Probiotics can be used as an alternative to sub-therapeutic levels of antibiotics to prevent the colonization of the GIT by unfavorable microorganisms (Dea *et al*., 2006). Some data indicate that multispecies probiotics are more effective than mono species probiotics (Timmerman *et al*., 2004).

The knowledge about the microbial ecology of the gastrointestinal tract is still limited (Bjerrum *et al*., 2006). Previous studies have shown that the majority of the culturable bacteria in the cecum belong to the *lactobacilli*, *enterococci*, *bacteroides* and *clostridia* (Salanitro *et al*., 1978; Barnes, 1979; Mead, 1989; Engberg *et al*., 2000). However, it is recognized that many bacteria have not yet been cultured in laboratory conditions because their growth requirements remain unknown. Cecum cultures contain a large number of various microorganisms (Yu *et al*., 1999). This study was conducted to investigate the effects of undefined cecal cultures and a commercial probiotic (PremaLac) on the performance and serum cholesterol levels of broilers.

MATERIALS AND METHODS

A total of 288 days old commercial Ross male broiler chickens were randomly allocated to 6 treatments with 4 replicates and 12 chicks per replicate for the 42 days of the

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experiment. Birds were reared on floor pens (1×1 m). The broiler chickens were allowed access to feed and water *ad libitum*. A completely randomized design with a factorial arrangement was used with three levels of healthy cecal cultures (0.00, 2.50 and 5.00 mL L^{-1} in drinking water) and two levels of PremaLac (0.00, 0.09% in the diet). PremaLac was purchased from Star-Lab Company, 8755 S.W. Hway, Clarksdale, MO, 64430, USA. Cecal cultures and PremaLac were administered up to 7 days of age. Cecal cultures were prepared using the method reported by Revolledo *et al*. (2003) and dissolved at the rate of 0.00, 2.50, 5.00 mL L^{-1} in the drinking water. Diets were formulated as mash to meet the nutrient requirements of broilers for the starter (0-21 days) and grower (22-42 days) periods (Table 1). PremaLac was used in this experiment as a probiotic and included *Lactobacillus acidophilus*, *Lactobacillus casei*, Bifidobacterium and *Enterococcus faecium*.

The body weight gain, feed intake and feed conversion ratio were measured weekly. At the end of the experiment, one bird from each replicate of treatments was randomly selected and blood samples were taken from the brachial vein and collected into EDTA blood collection tubes, centrifuged (10 min at 3000×g) and the serum was separated and then stored at -20°C until assayed for blood lipids using appropriate laboratory kits (Friedewald *et al*., 1972). Data were analyzed as a completely randomized design in a 3×2 factorial arrangement using the General Linear Model (GLM) procedure of SAS (1996). Duncan's multiple range test (Duncan, 1955) was used when treatment means were significantly different ($p<0.05$). The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran.

Table 1: Composition of experimental diets for male broiler chickens

	Age (days)		
Ingredients (%)	0-7	7-21	21-42
Corn	52.12	52.12	54.90
Soybean meal	34.42	34.42	26.89
Wheat	4.00	4.00	10.00
Wheat bran	3.77[1]	3.77	3.95
PremaLac	0.00	0.00	0.00
Dicalcium phosphate	1.40	1.40	1.01
Limestone	1.15	1.15	1.25
Veg. oil	2.00	2.00	1.10
Salt	0.38	0.38	0.30
Vit. and min. premix[2]	0.50	0.50	0.50
DL-methionine	0.21	0.21	0.05
Endofeed W[3]	0.05	0.05	0.05
Calculated analysis			
ME (kcal kg^{-1})	2850.00	2850.00	2900.00
CP (%)	20.50	20.50	18.12
Ca (%)	0.89	0.89	0.82
Na (%)	0.17	0.17	0.14
Arg. (%)	1.31	1.31	1.14
Lys. (%)	1.10	1.10	0.92
Met (%)	0.87	0.87	0.65

[1]0.09% PremaLac replaced with 0.09% wheat bran from 0-7 days of age; [2]Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 9790 IU, vitamin E, 121 IU; B_{12}, 20 μg; riboflavin, 4.4 mg; calcium pantotenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamin, 4 mg; zinc sulfate, 60 mg; manganese oxide, 60 mg; [3]Endofeed W, a multi enzyme from GNC Bioferm Inc., Canada

RESULTS AND DISCUSSION

The effects of cecal cultures and PremaLac on body weight gain, feed intake and the feed conversion ratio are shown in Table 2. Body weight gain was significantly ($p<0.05$) higher in cecal culture-treated compared to control broiler chickens during the starter period (0-21 days). The 5.00 mL L^{-1} cecal cultures significantly ($p<0.05$) increased body weight gain compared to the 2.50 mL L^{-1} cecal cultures. The effects of cecal cultures on body weight gain were not significant during the growing (22-42 days) and experimental (0-42 days) periods ($p>0.05$). Supplementation of PremaLac in the broiler diet did not enhance body weight gain during the starter and growing periods but body weight gain significantly increased during the experimental period.

The feed conversion ratio and feed intake were not improved by inoculation of cecal cultures into drinking water during the experimental period. A significant decrease in the feed conversion ratio and feed intake was observed in PremaLac-treated compared to control broiler chickens from 0-21 days of age. The feed conversion ratio and feed intake did not significantly differ between control and PremaLac-fed broilers from 22-42 and 0-42 days of age. No significant difference was observed in serum total cholesterol, High Density Lipoprotein (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol, Very Low Density Lipoprotein (VLDL) cholesterol or triglycerides between treatments (Table 3). In this experiment, the only significant effect ($p<0.05$) of the interaction between cecal cultures and PremaLac was on body weight gain from 0-21 days of age; the other measured parameters were not significantly affected by this interaction.

Using probiotics in broiler nutrition gives inconsistent results. However, some studies have shown that probiotics have positive effects on the health and performance of broiler chickens (Yu *et al*., 1999; Revolledo *et al*., 2003). In this experiment, cecal cultures improved body weight gain from 0-21 days of age but had no beneficial effects on the feed conversion ratio or feed intake. These results confirm the findings of Yu *et al*. (1999) who reported that body weight gain was increased in cecal culture-treated compared to control broiler chickens during the starter period (0-21 days) but with no

Table 2: Effects of cecal culture[1] and PremaLac on performance of broiler chickens from 0-42 days of age

	Cecal cultures (mL L^{-1} water)				PremaLac (%)			Contrasts		
Items	0.00	2.50	5.00	±SEM	0.00	0.09	±SEM	Cecal cultures	PremaLac	Interaction
FI[3]										
0-21	906.90	932.30	926.80	12.690	961.40^{b}	882.50^{a}	10.36	NS	**	NS
21-42	2743.00	2776.00	3426.00	448.000	3221.00	2742.00	365.80	NS	NS	NS
0-42	3650.00	3708.00	4352.00	449.100	4183.00	3624.00	366.70	NS	NS	NS
BWG										
0-21	507.20^{c}	520.70^{b}	539.70^{a}	6.030	521.10	524.00	4.92	**	NS	**
21-42	1307.00	1268.00	1229.00	25.910	1238.00	1297.00	21.15	NS	NS	NS
0-42	1815.00	1788.00	1768.00	25.420	1760.00^{b}	1821.00^{a}	20.80	NS	*	NS
FCR										
0-21	1.82	1.82	1.74	0.207	1.84^{a}	1.74^{b}	0.022	NS	**	NS
21-42	2.07	2.19	2.77	0.340	2.50	2.09	0.280	NS	NS	NS
0-42	1.99	2.08	2.46	0.240	2.37	1.99	0.200	NS	NS	NS

[1]0-7 days of age in the drinking water; [2] 0-7 days of age in the starter diet; [3]FI, Feed Intake (g); BWG, Body Weight Gain (g); FCR, Feed Conversion Ratio (g g^{-1}); [a, b, c]Means in each row with different superscripts are significantly different (NS: Not Significant, *: $p<0.05$, **: $p<0.01$)

Table 3: Effects of experimental treatments on serum lipids (mg dL^{-1}) of broiler chickens at 42 days of age

	Cecal cultures (mL L^{-1} water)				PremaLac (%)			Contrasts		
Serum lipids	0.00	2.50	5.00	±SEM	0.0	0.9	±SEM	Cecal cultures	PremaLac	Interaction
CH	141.6	143.4	152.7	5.90	140.4	151.4	4.80	NS	NS	NS
TG	47.7	52.1	51.3	3.50	50.1	50.6	2.90	NS	NS	NS
HDL	226.6	194.4	194.8	12.80	203.5	207.0	10.50	NS	NS	NS
LDL	94.5	65.6	182.7	71.10	160.1	68.5	58.10	NS	NS	NS
VLDL	9.5	10.4	10.2	0.70	10.0	10.1	0.50	NS	NS	NS

CH: Cholesterol, TG: Triglyceride, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein; NS: Not Significant, *$p<0.05$, **$p<0.01$

significant effects of cecal cultures on the feed conversion ratio and feed intake. Piray *et al.* (2007) reported that administering cecal cultures in the drinking water did not affect performance parameters in broiler chickens. It has also been shown that cecal cultures can increase resistance against potentially pathogenic microorganisms and therefore decrease infections in broiler chickens (Yu *et al*., 1999; Revolledo *et al*., 2003).

Some effects of probiotics on host animals include a decrease in GIT pH and production of H_2O_2, a reduction in the pathogenic microorganism population, the production of organic acid components and enhancement of animal immunity. Cecal cultures may improve the intestinal environment by the mechanisms mentioned above and thus increase body weight gain in the starter period. We used cecal cultures only during the first week after hatching. If we were to use cecal cultures for a longer period or at higher doses, the positive effects of cecal cultures may continue during the grower and overall experimental periods. In this study, the commercial probiotic increased body weight gain over the whole experimental period (0-42 days).

This result is consistent with that of Safalaoh (2006) who showed that body weight gain significantly increased in broilers fed diets supplemented with probiotics compared to control broilers. The findings are also consistent with those of Angel *et al.* (2005) who showed that adding a commercial probiotic to diets significantly increased the weight gain of the broilers. However, other researchers found that supplementing commercial probiotics (Dea *et al*., 2006) and *Lactobacillus bulgaricus* (Cavit, 2004) had no beneficial effects on broiler chickens or Rock partridge performance, respectively. The results show that the feed conversion ratio only improved during the starter period by supplementation with PremaLac. Safalaoh (2006) found that probiotic-supplemented broilers had a better feed conversion ratio than control broilers from 0-42 days of age. Angel *et al.* (2005) also reported that supplementation of a commercial probiotic in broiler diets significantly improved the feed conversion ratio.

In contrast, Rock partridges fed with *Lactobacillus bulgaricus* showed no significant improvement in the feed conversion ratio compared to control Rock partridges (Cavit, 2004). The results of this experiment show that feed intake significantly decreased in the starter period in PremaLac fed chickens. Safalaoh (2006) reported that feed intake was significantly reduced in broilers supplemented with probiotics. Cecal cultures and PremaLac did not have a cholesterol lowering effect in broiler chickens.

These results are consistent with the findings of Kalavathy *et al.* (2003) who showed that cholesterol content was not significantly reduced by probiotic supplementation. However, Kalavathy *et al.* (2003) reported that serum total cholesterol, Low Density

Lipoprotein (LDL) and triglycerides were significantly decreased in lactobacillus culture-fed compared to control broilers.

The results demonstrate that supplementation of a commercial probiotic (PremaLac) in broiler chickens from 1-7 days of age has a beneficial effect on growth performance throughout the whole growth period and the feed conversion ratio during the critical growth period (0-21 days). Use of cecal cultures in broiler chickens for a week increased their growth performance during the critical growth period (0-21 days).

CONCLUSION

These results suggest that the levels of cecal cultures used in this experiment might not have been high enough or that the duration of usage might have been too short for prolonged effects and therefore by changing the duration and dosage of cecal cultures and PremaLac, it might be possible to provide beneficial effects during the overall growing period. More research is needed to verify this hypothesis.

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