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The Investigation of Intestinal Microflora and Growth Response of Young Broilers Given Feed Supplemented with Different Levels of Peobiotic and Prebiotic

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Abstract: Effects of a probiotic (PRIMALAC) and a prebiotic (FERMACTO) on performance, ileum and crop microbial flora and carcass characteristics have been examined on 480, 1 day old Ross 308 broiler chicks. The experiment carried out in a factorial design including three levels of FERMACTO (0, 1 and 2 g ton^{-1}) and two level of PRIMALAC (0 and 900 g ton^{-1}). Each of treatment was allocated to 5 replicates of 16 male broilers and reared for 42 days. The microflora population in crop and ileum parts was measured on appropriate bacteriological media. Results of experiment indicated that application of primalc (900 g ton^{-1}) significantly improved body weight gain and feed conversion ratio ($p<0.01$). Feed consumption and feed conversation ratio were not affected with supplementation of different levels of FERMACTO. The percent of carcass, thigh, breast and abdominal fat were not affected by treatments. Coliform counts in the ileum of birds receiving PRIMALAC (900 g ton^{-1}) and FERMACTO (2000 g ton^{-1}) were significantly lower than those of the control birds ($p<0.05$). Different levels of PRIMALAC or FERMACTO had no effect on total bacteria counts in the ileum and crop parts. In 3rd week of rearing periods, the highest significant ($p>0.05$) value of lactobacilli population was recorded for broilers fed diet supplemented with PRIMALAC (900 g ton^{-1}) but different levels of FERMACTO relatively increased lactic acid bacteria population in crop. The results of present study revealed that these feed additive by acting on microbial population of digestive system significantly ($p<0.05$) affects broiler performance.

Key words: PRIMALAC, FERMACTO, microbial population, broilers chicks, digestive system, Iran

INTRODUCTION

Gut microflora has significant effects on host nutrition, health and growth performance (Barrow, 1992) by interacting with nutrient utilization and the development of gut system of the host. When pathogens attach to the mucosa, gut integrity and function are severely affected and immune system threatened (Neish, 2002). In the modern intensive poultry production, newly hatched chicks have little chance to contact with their mother, thereby normal microflora is slow to colonize in the intestine (Fuller, 1989). Animals including poultry are vulnerable to potentially pathogenic microorganisms such as *Escherichia coli*, *Salmonella* ssp., *Clostridium perfringens* and *Campylobacter sputorum*. Pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids (Engberg *et al*., 2000). This leads to depressed growth performance and to increased incidence of disease. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathologies (Truscott and Al-Sheikhly, 1977; Miles *et al*., 1984; Waldroup *et al*., 2003). However, the antibiotic growth promoters have been under scrutiny for many years and have been removed from the market in many countries (Ratcliff, 2000). Probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance has been used for the alternative tools for helping newly-hatched chicks colonize normal microflora as conventionally hatched chicks do (Fuller, 1989). Recent research and understanding of probiotics has lead to the development of prebiotics.

Prebiotics are not digested by the animal's digestive enzymes and affect the host beneficially by selectively stimulating the growth and/or metabolic activity of one or more either naturally present, or orally fed (probiotics) bacterial species in the intestine. The intestinal epithelial layer constitutes a barrier that protects the host against luminal pathogens (Deitch *et al*., 1995). Reduced epithelial cell proliferation and mucosal atrophy

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of the intestine allow various pathogens in the intestinal lumen to invade. Feed additives such as antibiotic, probiotic or prebiotic can help intestinal tissue, since supplementation of their to diets decrease pathogens.

The main objective of the study was to determine the performance and small intestinal microbial flora of broiler chickens fed a probiotic, a prebiotic alone and also the prebiotic in combination with the probiotic to find the most effective synergistic combination of these products.

MATERIALS AND METHODS

In this study, 480 broiler chickens of the commercial Ross 308 strain in a randomized block design experiment arranged in a 2*3 factorial schedule with 6 treatments (5 replicates in each treatment 16 birds/replicates) and reared on floor pens for 42 days. 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. The composition of the diets and the content of nutrients are shown in Table 1. Five tested diets were formulated by supplemented the basal control diet with FERMACTO (1000 and 2000 g ton^{-1}), PRIMALAC (900 g ton^{-1}) , mixture of FERMACTO (1000 g ton^{-1}) + PRIMALAC (900 g ton^{-1}) and mixture of FERMACTO (2000 g ton^{-1}) + PRIMALAC (900 g ton^{-1}), respectively.

FERMACTO is a commercial prebiotic of the mannan-oligosaccharides family which is obtained by extraction from the outer cell wall of the yeast *Saccharomyces cerevisiae*. PRIMALAC is a kind of commercial probiotic, consisting of a combined preparation of live microorganisms including *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterim bifidum* (PRIMALAC® Star*labs).

Feed and water were provided ad libitum. Air temperature and humidity were adjusted according to the ROSS technological procedure for broiler fattening. During the experimental period, the growth performance of broiler chickens was evaluated by recording body weight, feed intake and feed conversion ratio. Weighing of the feed and chickens were made on a weekly basis. At the 21st of the experiment, 30 chicken birds of similar body weight to the group average were selected from each treatment group (1 chicken per replicate), weighted and killed by severing of the bronchial vein. After evisceration, hot carcasses were weighted immediately to determine the hot carcass yield. The weights of the Carcass, Breast, Thigh and abdominal fat were recorded individually. The weights of these selected internal parts were expressed as a percentage of preslaughter live weight of the broilers.

Table 1: The ingredient and chemical composition of diets administered to broiler chickens

Ingredients	0-21 days	22-42 days
Corn	56.000	61.60
Soybean meal 44%	29.000	26.00
DCP	0.850	0.85
Corn gluten meal	3.500	0.00
Meat-born meal	3.500	0.00
Fish meal	1.500	7.00
Sunflower oil	3.500	2.60
Limestone	0.500	0.60
DL-Methionine	0.350	0.05
Vitamin premix[1]	0.500	0.50
Mineral premix[2]	0.500	0.50
Salt	0.300	0.30
Calculated composition		
Cp	21.900	20.60
ME, kcal kg^{-1}	3,158.000	3,194.00
Lysine	1.180	0.99
Methionine and cystine	0.960	1.75
Calcium	0.900	0.89
Phosphorus	0.440	0.42

[1]The vitamin premix supplied the following per kilogram of complete feed: vitamin A, 4,500 IU (retinyl acetate); cholecalciferol, 1,000 IU; vitamin E, 25 IU (dl-a-tocopheryl acetate); vitamin B12, 0.02 mg; menadione, 1.5 mg; riboflavin, 3 mg; thiamine, 1.5 mg; pantothenic acid, 5 mg; niacin, 20 mg; choline, 150 mg; folic acid, 0.5 mg; biotin, 0.5 mg; pyridoxine, 2.5 mg. [2]The mineral premix supplied the following per kilogram of complete feed: manganese ($MnSO_4 \cdot H_2O$), 60 g; zinc (ZnO), 40 mg; iron ($FeSO_4 \cdot 7H_2O$), 80 mg; copper ($CuSO_4 \cdot 5H_2O$), 8 mg; selenium (Na_2SeO_3), 0.2 mg; iodine (Iodized NaCl), 0.8 mg; cobalt ($CoCl_2$), 0.4 mg

Bacteriological examinations: On day 21, randomly chosen 30 males (from each treatment) chicks were killed by cervical dislocation. Crop and small intestine was opened immediately after killing and digesta were obtained from them. Ileum was defined as extending from Meckel's diverticulum to a point 4 cm to distal. Approximately 1 g of the small intestinal (ileum contents) and crop contents were mixed with 9 mL of prereduced sterile dilution blank solution (Bryant and Burkey, 1953) and homogenized for 3 min. From the initial 10^{-1} dilution, 10 fold serial dilutions were subsequently made in sterile prereduced dilution blank solution in 0.1% peptone for aerobic bacteria.

The samples from the ileum and crop were diluted to 10^{-5}, 10^{-7} and 10^{-9}. For each dilution, 0.1 mL was inoculated on agar plate for aerobes (Jin *et al*., 1996). The plate media used were MRS agar for lactobacilli and MacConkay agar for coliforms.

All the plates were incubated at 39°C. Total numbers of bacterial colonies were counted at the end of each incubation period. MRS agar plates were incubated anaerobically for 2 days in a Gas-Pak container and MacConkay agar plates were incubated aerobically for 1 day. The microbial counts were determined as colony forming units (cfu) per gram of samples.

Statistical analysis: All data were analyzed using the one-way ANOVA procedure of SAS (1998) for analysis of variance. Significant differences among treatments were identified at 5% level by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Results of the analyzed data for body weight, food consumption and feed conversion ratio are shown in Table 2. Levels of FERMACTO had no effect on Body Weight (BW) at 21 days of age but at 42 days of age, the dietary supplemented with FERMACTO (2000 g ton^{-1}) increased the weight of birds to 5.6%, compared to other levels of FERMACTO. The treatment with PRIMALAC (900 g ton^{-1}) of feed produced a significantly greater BW ($p<0.05$) and feed to gain ratio than the control treatment. FERMACTO (1000 g ton^{-1}) had no effect on BW when compared to control (no added FERMACTO). The dietary supplemented with different levels of FERMACTO had no effect on feed intake but there was only a slight decrease in feed to gain ratio on day 21 and 42 of current study.

The interaction between different levels of PRIMALAC and FERMACTO was not significant. On 21st day of experiment, the birds under treatments of PRIMALAC (900 g ton^{-1}), feed conversion ratio improved significantly when compared to control group ($p<0.05$). The FI at 21 and 42 days of age in PRIMALAC treatment was significantly higher than the Control treatment but on 42 days, PRIMALAC had negative effect on feed conversion ratio and made it increased.

Carcass composition: The effect of experimental treatments on the composition of the bird carcasses are shown in Table 3. Carcass weight at 21st day of the experiment was increased numerically by addition of PRIMALAC or FERMACTO. The birds under treatments of PRIMALAC and FERMACTO had no significant effect on carcass weight, percent breast and thigh when PRIMALAC or FERMACTO. The birds under treatments of PRIMALAC and FERMACTO had no significant effect on carcass weight, percent breast and thigh when compared with control group ($p<0.05$). In the present study, probiotic and prebiotic alone or in combination together, increased numerically the carcass percentages of the male birds at 21st day. However, these increasing wres not statically significant. The ratio of fat pad to live body weight in treatments with supplemented by PRIMALAC and FERMACTO slightly decreased.

Crop and intestinal microbial contents: The results on the intestinal microbial population and crop microbial contents are shown in Table 4. There was no significant difference in total aerobe counts in the small intestine of broilers fed on diets with or without PRIMALAC and FERMACTO supplementation on 21st day of experiment. However, supplementing diets with FERMACTO (2000 g ton^{-1}) increased the in total aerobe counts in the crop contents. The number of coliforms was reduced significantly ($p<0.05$) in the ileum of broilers fed diets containing FERMACTO (2000 g ton^{-1}) or PRIMALAC (900 g ton^{-1}). There were no significant differences in the numbers of lactobacilli in the crop of broilers fed with or without FERMACTO at 21st day of experiment and different levels of FERMACTO relatively increased lactic acid bacteria population in crop. However, the Lactobacillus populations in the crop of birds receiving PRIMALAC (900 g ton^{-1}) were significantly ($p<0.05$)

Table 2: Body weight, feed intake and feed conversion ratio of broilers receiving diet supplemented with different levels of probiotic and prebiotic

	21 days			42 days		
Treatments	BW (g)	FI (g)	FC	BW (g)	FI (g)	FC
Prebiotic						
P0	612.2700^{a}	541.9000^{a}	1.4920^{a}	2240.8500^{b}	1309.5600^{a}	1.815^{a}
P1	610.2900^{a}	525.6800^{a}	1.4520^{a}	2238.5800^{b}	1321.0000^{a}	1.723^{a}
P2	643.5300^{a}	546.8100^{a}	1.4190^{a}	2365.4100^{a}	1309.8600^{a}	1.654^{a}
SEM	11.3700	11.1100	0.0450	26.9000	23.8000	0.063
p-value	0.0940	0.3880	0.5390	0.0047	0.9270	0.228
Probiotic						
1	565.3800^{b}	518.2100^{b}	1.5240^{a}	2172.9100^{b}	1299.7500^{a}	1.630^{b}
2	678.6800^{a}	558.0600^{a}	1.3850^{b}	2390.3100^{a}	1327.2000^{a}	1.831^{a}
SEM	9.2700	9.0800	0.0360	21.9300	19.4000	0.051
p-value	0.0001	0.0060	0.0163	0.0001	0.3297	0.014
Pro*pre						
1						
P0	570.6300^{c}	522.6400^{b}	1.4940^{ab}	2212.7100^{dc}	1327.6600^{a}	1.648^{a}
P1	542.5900^{c}	520.1700^{b}	1.6100^{a}	2099.9500^{d}	1304.3900^{a}	1.650^{b}
P2	582.9200^{c}	511.8100^{b}	1.4690^{ab}	2306.0800^{dc}	1267.1900^{a}	1.596^{b}
2						
P0	649.9200^{b}	561.1700^{a}	1.4900^{ab}	2268.9900^{bc}	1291.4600^{a}	1.982^{a}
P1	677.9800^{ab}	531.2000^{b}	1.2950^{b}	2377.2100^{b}	1337.6200^{a}	1.797^{ab}
P2	704.1500^{a}	581.8200^{a}	1.3690^{b}	2524.70004^{a}	1352.5300^{a}	1.713^{ab}
SEM	16.0400	15.6700	0.0640	37.94000	33.5600	0.063
p-value	0.2698	0.1981	0.0717	0.00590	0.2198	0.439

Table 3: Effect of different levels of probiotic and prebiotic on carcass characteristics of broiler chickens at 21 days of age

Treatments	Slaughter weight (g)	Carcass weight (g)	Carcass yield (%)	Breast (%)	Thigh (%)	Abdominal fat (%)
Prebiotic						
P0	671.6900	425.220	62.750	26.330	25.070	2.230
P1	698.1800	436.870	64.410	27.150	25.820	2.220
P2	703.2200	451.970	64.770	28.120	26.100	2.150
SEM	11.5000	15.640	2.850	0.930	0.470	0.290
p-value	0.0933	0.072	0.360	0.450	0.160	0.810
probiotic						
1	684.1700	430.970	63.380	26.940	26.140	2.220
2	704.5600	448.390	63.640	27.120	27.450	2.120
SEM	9.3900	12.770	2.320	0.760	0.380	0.230
p-value	0.2390	0.255	0.880	0.653	0.176	0.245
Pro*pre						
1						
P0	664.6900	414.850	62.400	26.460	24.980	2.320
P1	664.5800	433.180	62.810	26.990	25.170	2.290
P2	683.2300	442.900	63.750	27.380	26.450	2.240
2						
P0	678.6900	424.570	62.085	26.200	25.680	2.140
P1	679.7700	449.040	63.034	27.300	26.750	2.120
P2	688.2000	450.450	64.870	27.870	27.520	2.140
SEM	26.2600	22.120	4.030	1.330	0.660	0.410
p-value	0.5690	0.061	0.100	0.805	0.787	0.120

Table 4: Total colony count, Lactobacillus and Coliforms populations in broilers fed diets different levels of probiotic and prebiotic

	Crop		Ileum	
Treatments	Total colony count	Lactobacillus	Total colony count	Coliforms
Prebiotic				
P0	7.2150^{b}	7.0950^{a}	8.380^{a}	7.9200^{a}
P1	7.5600^{a}	7.3800^{a}	8.270^{a}	7.8600^{a}
P2	7.7600^{a}	7.4500^{a}	8.360^{a}	7.7000^{b}
SEM	0.2100	0.1800	0.110	0.0800
p-value	0.3480	0.0921	0.169	0.0212
probiotic				
1	7.4200^{a}	7.0930^{b}	8.330^{a}	7.8700^{a}
2	7.6200^{a}	7.4000^{a}	8.340^{a}	7.7700^{b}
SEM	0.1700	0.1300	0.090	0.0400
P value	0.2452	0.0250	0.880	0.0123
Pro*pre				
1				
P0	7.1300^{b}	7.0000^{b}	8.400^{a}	7.9600^{a}
P1	7.5200^{ab}	7.0200^{b}	8.290^{a}	7.9300^{a}
P2	7.6200^{ab}	7.2600^{ab}	8.320^{a}	7.7300^{ab}
2				
P0	7.3000^{ab}	7.1900^{ab}	8.370^{a}	7.8900^{ab}
P1	7.6100^{ab}	7.3800^{ab}	8.250^{a}	7.6800^{ab}
P2	7.9100^{b}	7.6400^{a}	8.410^{a}	7.4900^{b}
SEM	0.3000	0.2300	0.160	0.1000
p-value	0.1600	0.0660	0.121	0.4690

higher than those of the control birds. The interaction between different levels of PRIMALAC and FERMACTO was not significant.

Addition of probiotics to feed is one of the alternatives to used as a replacement for antibiotics. There is sufficient evidence to show that probiotics are effective in enhancing the immune system, increasing body weight gain, reducing diarrhea and improving feed conversion efficiency (Reid and Friendship, 2002; Patterson and Burkholder, 2003).

A significant ($p<0.05$) increase in BW was observed in broilers fed the diets containing PRIMALAC or FERMACTO. Similar improvements in BW have been reported in poultry fed with probiotic or prebiotic supplemented diets (Kermanshahi and Rostami, 2006; Nayebpor *et al*., 2007). But this result is contrary to the findings of Mohan *et al*. (1996) who observed that the beneficial effect of probiotic on chicken occurred only after the 4th week of growth and that of Yeo and Kim (1997) who reported that average daily weight gain of chickens fed probiotics was significantly increased during the first 3 week of growth but not during the 4th to 6th week of growth. Variance among reports of researchers could be related to differences in management and environmental conditions that be exist in various experiments.

It is suggested that under benefit management and/or environmental conditions, the effect of such feed additives may be worthless. Feed to gain ratios were improved significantly ($p<0.01$) for the broilers fed diets with PRIMALAC on 21 and 42 days of current experiment. The present results agree with Mohan *et al*. (1996) who found that feed to gain ratio of broilers fed on diets supplemented with 75 or 100 mg kg^{-1}. Lactobacillus was better than those of the control and those fed on 125 mg kg^{-1} Lactobacillus. Similar improvements in feed efficiency have been reported for poultry receiving probiotics (Cavit, 2004; Yalcinkayal *et al*., 2008).

The improvements in BW and feed to gain ratio of broilers fed PRIMALAC supplement were probably due to the *Lactobacillus* sp., used in the supplement. It has been suggested that to obtain the best effects from Lactobacillus as a growth promotion, the bacteria used must be able to survive and later colonize the gastrointestinal tract so that their beneficial functions could be performed. In agreement with the result of this study, there are numerous reports showing that the use of such additives has no effect on the feed consumption (Cavit, 2004; Yalcinkayal *et al*., 2008). The optimal dose for prebiotics to exert growth-promoting effects is not easy to define; however feeding a higher level (0.8%) of inulin and short-chain fructo-oligosaccharide depressed growth performance, digestibility of amino acids as well as metabolisable energy of birds (Biggs *et al*., 2007). The results of present study showed that FERMACTO treated chickens had higher weight gain and feed conversion ratio. But it seems that the dose of prebiotic was not enough to exert their stark effect.

Use of PRIMALAC or FERMACTO in diet improved slightly body weight at slaughter, thigh and breast yield. The growth-promoting effects of probiotics are dependent on the specific probiotics, the application level of probiotics, the age of birds as well as the delivery method (i.e., via water and/or feed). 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. In the Ammerman *et al*. (1989) study, adding 0.375% oligofructose to the birds ration, on day 47, decreased the percent of abdominal fat. However, present findings on carcass composition were in contrast to those of Cavit (2004) and Yalcinkayal *et al*. (2008). These differences between reported results could be related to management and environmental conditions.

The microbial populations in the gastrointestinal tracts of poultry play a key role in normal digestive processes and in maintaining animal health. Disease and stress induced changes in the physicochemical environment in the gastrointestinal tract or simple changes in feed management practices can significantly influence the microbial populations and their effects on animal performance and health. Various pathogenic microbes, such as Escherichia coli have been implicated to reduce the growth of poultry. Possible mechanisms for this reduction of growth are: toxin production, utilization of nutrients essential to the host and suppression of microbes that synthesize vitamins or other host growth factors. The supplemented diet with different levels of PRIMALAC or FERMACTO slightly increased the lactobacillus population and total count of microorganisms in the ileum of chickens in early ages. This result was also confirmed by Corrier *et al*. (1991). In ileum the populations of useful bacteria like lactobacillus and bifidobacteria (Ziggers, 2000) increases and the pH of the GIT due to increasing production of Volatile Fatty Acids (VFAs), decreases. Therefore, the environment of GIT becomes unsuitable for the activity and proliferation of pathogens like Salmonella. Based on Nurmi concept of competitive exclusion (Nurmi and Rantala, 1973), pathogens will be expelled out of the gut by useful bacteria if it already occupied the gut sites. Colonization of useful micro flora in the gut of young birds that is related to gut conditions can inhibit further colonization of pathogens (Nurmi and Rantala, 1973). Lactobacillus and coliforms can use FERMACTO as a source of fermentable carbohydrate and produce lactic acid and volatile fatty acids like acetic, propionic and butyric acid (Corrier *et al*., 1991; Hume *et al*., 1992).

Increasing iodized VFAs in more acidic conditions of ileum part have bacteriostatic and bactericidal effect on pathogenic bacteria like salmonella (Chambers *et al*., 1997; Hinton *et al*., 1990). Higher susceptibility of the chickens to salmonella colonization is mostly related to the lower concentration of VFAs and higher pH of GIT in early ages (Nurmi and Rantala, 1973). Therefore, use of FERMACTO or PRIMALAC at early ages decreases pH of GIT and inhibits salmonella colonization. In this experiment, the number of coliforms decreased with supplemented diet with different levels of FERMACTO or PRIMALAC at week three. Under the conditions of this study, use of PRIMALAC (900 g ton^{-1}) or FERMACTO (200 g ton^{-1}) had beneficial effects on bird performance at rearing period and ileum and crop microflora at starter period.

CONCLUSION

Competitive exclusion is a popular strategy for preventing poultry from intestinal infectious disease due to the effective inhibition of pathogenic bacteria in the gut. Probiotic with defined bacteria and prebiotic with ability to aid growth of beneficial bacteria had significant effect on growth performance. From the present results, it seems interesting to focus on using these feed additives (PRIMALAC or FERMACTO) in animal diet to reduce the microflara content, particularly pathogens and in this case improve the performance and immunity of the host instead of using antibiotics.

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