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Effects of Different Levels of Direct Fed Microbial (*Primalac*) on Growth Performance and Humoral Immune Response in Broiler Chickens

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Abstract: Probiotics are claimed to have beneficial effects on health. The production parameters, including were monitored weekly during a 6-wk trial. Body weight was affected by dietary treatments in 21, 28 and 42 days of age ($p<0.05$). The BW in the 0.1% DFM treatment was significantly ($p<0.05$) higher than 0.15% DFM and control in 28 and 42 days. Body weight gain in probiotic treatments was significantly more than control treatment ($p<0.05$). Moreover, body weight gains in 0.1% DFM were higher than 0.15% DFM treatment ($p<0.05$). Feed intake in control treatment was significantly higher than ($p>0.05$) 0.1 or 0.15% DFM treatments. Feed conversion ratio in 0.1 and 0.15% DFM treatments was significantly ($p<0.05$) better than control treatment. The 0.1% DFM and control treatments have lowest and highest FCR, respectively. The antibody titer against IBDV in 0.1 and 0.15% DFM treatments were significantly ($p<0.01$) more than control treatment but no significant difference among 0.1 and 0.15% treatments. It is concluded that dietary supplementation of DFM probiotic improved body weight, body weight gain and feed conversation ratio and enhanced humoral immune response.

Key words: Probiotic, performance, humoral immune response, broiler chickens

INTRODUCTION

Due to their several negative effects, antibiotics have gradually been replaced by probiotics in controlling intestinal pathogenic bacteria (Fuller, 1992). Using of probiotics as an alternative instead of antibiotics in animal production to be without causing damage to normal intestinal flora and without leaving residues in carcass. Probiotics are food supplements based on live microorganism balance in the intestinal micribiota (Fuller, 1992). Direct-Fed Microbial (DFM) as live microbial feed supplements that improve microbial balance in the animal gastrointestinal tract and, therefore, are beneficial (Fuller, 1989). The Food and Drug Administration considers DFM to be a source of live (viable) naturally occurring microorganisms. Direct fed microbials benefit the host animal by stimulating appetite (Nahashon *et al*., 1992; Nahashon *et al*., 1993), improve intestinal microbial balance (Fuller, 1989), synthesize vitamins (Coates and Fuller, 1977), stimulate the immune system (Toms and Powrie, 2001; Huang *et al*., 2004; Kabir *et al*., 2004), produce the digestive enzyme (Gilliland and Kim, 1984; Saarela *et al*., 2000; Jin *et al*., 2000), utilize undigestible carbohydrate (Prins, 1977), stimulate lactic acid and volatile fatty acids production (Bailey, 1987). Improvement in growth performance by addition of probiotics to the diets of broilers and layers have been reported by several researchers (Nahashon *et al*., 1994; Mohan *et al*., 1996; Jin *et al*., 1998; Zulkifli *et al*., 2000; Kalavthy *et al*., 2003; Angel *et al*., 2005; Khaksefidi and Goorchi, 2006).

Nevertheless, contradictory results have been reported by other researchers (Kabir *et al*., 2004; Gunal *et al*., 2006). Several probiotics are claimed to stimulate the immune system and their modes of action appear to be nonspecific, resulting in increased immune responsiveness to a wide variety of antigens (Roos and Martin, 2000). The immune response after vaccination is an elegant tool with which to study the effects of probiotics (Ross *et al*., 2000). These live organism after residing intestinal tract and their metabolites can act as immunomodulatory agent by activating specific and non-specific host immune responses in chicks, which help in prevention and control of various infectious diseases. (Kostiuk *et al*., 1992; Koenen *et al*., 2004). Probiotics can significantly increase the humoral immune response in chickens (Koenen *et al*., 2004).

The purpose of this study was to determine the effects of Direct-Fed Microbial (*Primalac*) as a dietary probiotic source on growth performance and humoral immune response in broiler chickens.

MATERIALS AND METHODS

During the first week the chicks fed standard diet (NRC, 1994) then switched to experimental diets. This experiment was carried out at the Application Research Farm of the Agricultural Faculty, Uremia University. The basal diet was formulated to meet nutrient requirements (NRC, 1994) for starter (7-21 day) and grower (22-42 day) periods. The composition of basal diet is shown in Table 1. The dietary treatments were: Basal diet (control), basal diet +0.1% DFM and basal diet +0.15% DFM. Direct-Fed Microbial (*Primalac*) included *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum* and *Enterococcus faecium* with a minimum of 1.0×10^8 cfu g^{-1} of the product. Chemical composition of the feeds was analyzed according to the methods of the AOAC. Feed and water were provided ad-libitum and illumination was 24 h florescent lighting. Body weight, body weight gain, feed intake and feed conversion ratio were monitored weekly. An immunization test was carried out to evaluate the effect of DFM probiotic on the immune system of broiler chickens. A live and inactivated IBDV vaccine was applied to the birds in their drinking water on 24day of the experiment. Seven day after immunization, 3 chickens of each pen were randomly selected and killed by neck cut. Blood samples from 36 chickens were collected and the serum was separated. The tiers of IBDV antibody in serum were measured by ELIZA test as described by Marquardt. Briefly, the IBDV antigen coated plates obtained from IDEXX were allowed to react with the known positive, negative and test serum samples from various treatment groups. After adding the labeled (Horse Radish Peroxidase) goat anti-chicken (H and L chains) against the IBDV antibody and incubating the plates at room temperature for 30 min, the reaction was then stopped by adding Nitric acid (HNO^3). With the help of ELISA reader the optical densities of the well of the plates were determined and used to calculate S/P ratio by using following formula.

The data were subjected to statistical analysis, using a General Linear Model procedure of SAS (1999) for the completely randomized experimental design, Differences between means were determined by Duncan's multiple range test at significance level of $p<0.05$ (Table 1).

The broiler premix provided the following per kilogram of diet: Vitamin A,10000 IU; cholecalciferol, 82.5 μg; vitamin E,25 IU; riboflavin 8mg; niacin, 50 mg; d-pantothenic acid,15 mg; folic acid, 1 mg; vitamin B12,15 μg; choline chloride, 1000 mg; thiamine, 2.5 mg; biotin, 0.1 mg; ethoxyquin, 100 mg; menadione sodium bisulfite, 3.3 mg; pyridoxine 1 mg; manganese, 15 mg; zinc, 50 mg; iodine, 1.5 mg; iron, 30 mg; copper, 6 mg. Selenium, 0.2 mg.

Table 1: Ingredient (%) and nutrient content of the basal diets

Ingredient	Starter (1- 21 day)	Grower (22-42 day)
Corn	57.6	61.43
Soybean meal	36.3	27.47
Wheat bran	-	5.54
Soybean oil	2.00	2.00
Limestone	1.33	1.57
Dicalcium phosphate	1.55	0.99
Vitamin-mineral premix?	0.5	0.5
DL- methionine	0.27	0.18
Salt (NaCl)	0.45	0.32
Calculated composition		
Crude protein, %	21.00	18.28
Crude fiber	3.81	3.88
Lysine, %	1.13	0.933
Met + Cys, %	0.94	0.78
Metabolizable energy, k cal kg^{-1}	2.925	2.925
Ca, %	0.96	0.92
Nonphytin P, %	0.434	0.32

RESULTS AND DISCUSSION

Performance: This study was carried out to evaluate the effect of different level of Direct-Fed Microbial (DFM) on BW, BWG, FI, FCR and humoral immune response. The results presented in Table 2-6. There was significant difference in the body weight between dietary treatments in 21, 28 and 42 days of age. Body weight of birds fed diet supplemented with DFM were significantly ($p<0.05$) higher than control group. The treatment with 0.1% DFM of feed produced a significantly greater BW ($p<0.05$) than the control or 0.15% DFM treatments. Body weight gain was affected by dietary treatment except (14-21) days and BWG in 0.1% DFM treatment was significantly ($p<0.05$) more than 0.15% DFM and control treatments.

Improvement in BW and BWG have been reported by adding DFM in poultry diets (Mohan *et al*., 1996; Yeo and Kim, 1997; Jin *et al*., 1998; Zulkifli *et al*., 2000; Kalavthy *et al*., 2003; Khaksefidi *et al*., 2006; Panda *et al*., 2006). In the present experiment, the improvement of BW and BWG were consistent in both the growing period (0-3 week) and the finishing period (4-6 week). Kalavthy *et al*. (2003) reported that supplementation of 0.1% LC improved the body weight and BW gain from 22-42 day of age ($p< 0.05$) but not during the (0-3) wk of growth. Jin *et al*. (1998) observed that supplementation of broiler diets with DFM increased the BW and BWG in both the growing period (0-3 week) and the finishing period (4-6 week). Khaksefidi *et al*. (2006) reported the inclusion of bacillus subtilis in broiler diets improved BW and BWG during 1-21 and 22-42 days. Where Mohan *et al*. (1996) reported that improvement in body weight gain was observed in broilers only after 4 week of feed probiotic. Yeo and Kim (1997) reported that average body weight gain of chickens fed probiotics was significantly increased during the first 3 week of growth.

Table 2: Effect of adding a Direct-Fed Microbial (DFM) to broiler feed on body weight (g)

Dietary treatment	7 day	14 day	21 day	28 day	35 day	42 day
Control	146	348	712^{b}	1177^{b}	1736	2238^{c}
0.1% DFM	145	359	727^{a}	1237^{a}	1814	2370^{a}
0.15% DFMSEM	150	354	717^{ab}	1201^{b}	1754	2314^{b}
Significance	183	1.93	2.02	4.54	14	4.9
	Ns	Ns	*	**	Ns	**

**: (p<0.01); *: (p<0.05); Ns: Non significant. b: Means within a column with no common superscripts differ significantly (p<0.05)

Table 3: Effect of adding a Direct-Fed Microbial (DFM) to broiler feed on body weight gain (g)

Dietary treatment	(8-14 day)	(15-21 day)	(22-28 day)	(29-35 day)	(36-42 day)	(7-42 day)
Control	203.4^{b}	365	464.5^{b}	528^{c}	528^{b}	2093^{c}
0.1%DFM	214^{a}	367	499^{a}	576^{a}	569^{a}	2225^{a}
0.15%DFM	207^{b}	363	488^{ab}	549^{b}	560^{a}	2165^{b}
SEM	1.28	1.3	0.6	2.7	1.9	4.72
Significance	*	Ns	*	**	**	**

**: (p<0.01); *: (p<0.05); Ns: Non significant. b: Means within a column with no common superscripts differ significantly (p<0.05)

Table 4: Effect of adding a Direct-Fed Microbial (DFM) to broiler feed on feed intake (g)

Dietary treatment	(8-14 day)	(15-21 day)	(22-28 day)	(29-35 day)	(36-42 day)	(7-42 day)
Control	312	566.2	857	1014	1135	3896^{a}
0.1% DFM	309	551	850	1011	117.5	3833^{b}
0.15% DFM	308	556	863	1011	1122	3860^{ab}
SEM	1.83	3.85	6.2	3.01	3.9	6.8
Significance	Ns	Ns	Ns	Ns	Ns	*

**: (p<0.01); *: (p<0.05); Ns: Non significant. b: Means within a column with no common superscripts differ significantly (p<0.05)

Table 5: Effect of adding a Direct-Fed Microbial (DFM) to broiler feed on feed conversion ratio

Dietary treatment	(8-14 day)	(15-21 day)	(22-28 day)	(29-35 day)	(36-42 day)	(7-42 day)
control	1.51	1.56^{a}	1.84^{a}	1.89^{a}	2.15^{a}	1.86^{a}
0.1% DFM	1.45	1.48^{b}	1.68^{b}	1.75^{b}	1.97^{b}	1.73^{c}
0.15% DFM	1.51	1.53^{ab}	1.76^{a}	1.84^{a}	2^{c}	1.79^{b}
SEM	0.02	0.009	0.0132	0.0135	0.004	0.0036
Significance	Ns	*	**	*	**	**

**: (p<0.01); *: (p<0.05); Ns: Non significant. b: Means within a column with no common superscripts differ significantly (p<0.05)

No significant difference was observed among treatments on feed intake. But the control group consumed more than DFM treatments (p<0.05) from (7-42) days. Feed conversion ratio in 0.1 and 0.15% DFM treatments was significantly better than control group and the feed conversation ratio in 0.1% DFM treatment higher than 0.15% treatment (p<0.05).

The present results agree with (Jin *et al* 1998; Zulkifli *et al*., 2000; Kalavthy *et al*., 2003; Khaksefidi *et al*., 2006; Panda *et al*., 2006). Zulkifli *et al*. (2000) observed that broilers fed a diet containing LC consumed less feed and had better feed efficiency ratios during the growing period (1-21 day), but found that the superior food efficiency did not extend to the finishing period (22-42 day) during which the chicks were subjected to 3-h episodes of heat stress (36_1_C) heat stress (36_1_C) each day. In contrast, others have not found difference in FCR between probiotic-treated birds and untreated (control) birds (Watkins and Kratzer, 1983, 1984; Estrada *et al*., 2001; O'Dea *et al*., 2006). The results of this experiment demonstrated that birds given feeds containing probiotic (*primalac*) as an additive substance to diet had a higher performance than those of the control. The probiotics consumption enters a large amount of lactic acid bacteria in the animal's gastrointestinal tract. These microorganisms by producing acids (such as acetic acid and lactic acid) and other compounds cause to inhibit the pathogenic bacteria growth and help to adhesion or colonization to the intestinal mucosa and rapid proliferation of beneficial bacteria in animal's intestine (Fuller, 1989). So that, probiotics by improving the microflora intestinal microbial balances have beneficially effects on growth performance. In addition, the probiotics increased available energy by increasing the digestibility of carbohydrates, improved the organic matters digestibility, increased the amylase enzyme activity and decreased the bacterial β-glucuronidase, β-glucosidase and urea's enzymes activities (Jin *et al*., 1997; Jin *et al*., 2000). However, probiotics with making a better microbial environment in intestine and by activating the internals and external enzymes of animals help to digestion and absorption of nutrients, efficiently of utilization of feed, at least cause to improve the growth performance of birds.

Humoral immune response: A humoral immune test was carried out to evaluate the effect of DFM on immune system of broiler chickens. The result of DFM on immune system in response to Infections Bursal Disease Virus (IBDV) is presented in Table 6. The concentration of IBDV antibody titer in the serum, were significantly higher in 0.1 and 0.15% DFM treatments than control treatment. But, there was no significant difference among 0.1 and 0.15% DFM treatments.

Munner *et al.* (2002) reported that using of Protexin in broiler diets had better antibody response against IBDV than non treated chicks. Balevi *et al.* (2001) reported that Probiotic supplementation did not affect specific antibody synthesis to ND vaccine antigen administered via drinking water, but antibody titers production in probiotic group was higher than control group. Khaksefidi *et al.* (2006) observed that Antibody production against Newcastle disease virus in 50 mg kg^{-1} probiotic supplemented group was significantly higher at 10 days of post immunization compared to control. Panda *et al.* (2000) and Cross *et al.* (2002) indicated that some probiotics could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens.

Probiotic bacteria are normal inhabitants of microflora and may confer health benefits to the host. The activation of the systemic and secretory immune response by lactic acid bacteria requires many complex interactions among the different constituents of the intestinal ecosystem (microflora, epithelial cells and immune cells). One of the immune modulations by probiotics is antibody production (Perdigon *et al.*, 1999). Several *in vitro* and animal studies have shown that certain strains of probiotics, such as *Lactobacillus rhamnosus*, *L. acidophilus* and Bifidobacterium lactis, were able to stimulate macrophage and neutrophil populations and to enhance natural killer cells activity (Gill *et al.*, 2000; Matsuzaki and Chin, 2000). *L. casei* was found to increase the size of T helper lymphocyte population in the GALT of mice (Perdigon *et al.*, 1999). In addition, an enhanced IgA secretion was observed in mice challenged with cholera toxin and orally treated with *L. acidophilus*, *B. infantis* or *B. bifidum* (Tejada-Simon *et al.*, 1999) and in serum of volunteers challenged with *Salmonella typhimurium* and fed with yogurt containing *L. acidophilus* La1 and bifidobacteria (Link-Amster *et al.*, 1994). With measurement of lysosmic macrophage enzymes (β-glucuronidase, β-glucosidase) and un-lysosomic enzymes activities (Lactate dehydrogenas) as an index of macrophage phagocytosis activity in animal showed that the enteral or interaperitoneal route consumed lactic acid bacterial cause to active the macrophage. It seems that the intestinal bacteria such as lactobacillus may be migrating from lumen and in habits in the omental lymphatic nodes (Fuller, 1989). This migration may be increased with extra growth bacterial in intestine or with addition of probiotic to diets. Produced lymphocine in response to antigenic stimulation (migrating bacteria) by lymphocytes, cases to active the non specific immune mechanisms (macrophage). Stimulation and activation of non specific immune mechanisms activate the involved cells in specific response and consequently, it case to active the cell-mediated and humeral immunity) by stimulating of both responses.

Table 6: Anti-IBDV Eliza titers of the broiler chickens fed on diet with different DFM supplementation

Dietary treatments	24 day
Control	1138[b]
0.1% DFM	1457[a]
0.15% DFM	1420[a]
SEM	17.31

**Significance

CONCLUSION

Results from this study suggests that supplementation of diet with DFM probiotic improved body weight, body weight gain and feed conversion ratio in broiler chickens. But no effect was seen on feed intake. Serological data from the present study showed the effectiveness of probiotic supplementation on systemic immunity.

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