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# Effects of a Probiotic and Other Feed Additives on Performance and Immune Response of Broiler Chicks

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**Abstract:** The effects of dietary supplementation of a probiotic, Toxiban, Formycine and probiotic-Toxiban mixture on performance and immune response of broiler chicks were investigated. In a completely randomized design, one hundred fifty 14-days-old broiler chicks were assigned to 5 treatments with 5 replicates and 6 chicks in experimental unit. The experimental treatments were added to basal (starter and finisher) diets as follow: T (1): control group (C) that received starter and finisher diets, T (2): C plus 0.15 percent probiotic, T (3): C plus 0.1 percent Toxiban, T (4): C plus 0.1 percent Formycine and T (5): C plus mixture of 0.15 percent probiotic with 0.1 percent Toxiban. Additives except Toxiban, significantly ($p<0.05$) increased blood Newcastle antibody titer compared with the control group. Regarding Influenza antibody titer, there was significant differences between treatments except Formycine feeding. Only probiotic caused a significant ($p<0.05$) increased in blood Bronchitis antibody titer. Consumption of Formycine and probiotic+Toxiban mixture resulted in a significant decrease in blood Gamboro antibody titer ($p<0.05$). Chicks fed diets supplemented with Toxiban significantly ($p<0.01$) had higher body weight and better Feed Conversion Ratio (FCR) than other treatments. Results indicated that, consumption of Toxiban had the most positive effect on performance and probiotic alone or combination of probiotic and Toxiban had the best effect on blood antibody titers of broiler chicks.

**Key words:** Probiotic, Toxiban, Formycine, broilers, immune response

## Introduction

Probiotics are as a source of live micro-organisms that includes bacteria, fungi and yeasts (Fox, 1988; Miles and Bootwalla, 1991). Lactic acid bacteria such as *Lactobacilli streptococci* and *Bifidobacteria* are the most common organisms used in probiotics preparations. The mechanism of action of probiotics has not been fully explained although there are several hypothesis (Ahmad, 2006). Its inhibitory action against pathogens may be mediated by competition for receptors on the gut mucosa, competition for nutrients, the production of antibacterial substances and the stimulation of immunity (Piard and Desmazeaud, 1991; Perdigon and Alvares, 1992; Bal *et al*., 2004).

As feed additive, probiotics has a good impact on the poultry performance (Stavric and Kornegay, 1995). These live organisms after residing intestinal tract and their metabolites can act as immunomodulatory agent by activating specific and non-specific host immune responses in chicks, which in turn help in prevention and control of various infectious diseases (Fuller, 1992; Koenen *et al*., 2004).

The most important advantage of probiotic is that doesn't have any residues in animal production and in contrast to antibiotics which could have serous consequences such as drug resistance and harmful alternation of bacterial population in the intestine (Abe *et al*., 1995), probiotics are not made any resistance by consumption. Therefore, some researchers have replaced antibiotics with probiotics as therapeutic and growth promoting agent (Donovan *et al*., 2002; Martins *et al*., 2005).

The dietary supplementation of probiotic benefit the host animal by stimulating appetite (Nahashon *et al*., 1992), stimulate the immune system (Toms and Powie, 2001; Koenen *et al*., 2004), improve microbioal balance (Fuller, 1989), produce the digestive enzymes (Saarela *et al*., 2000), stimulate lactic acid (Bailey, 1987), decrease pH and release bacteriocins (Rolfe, 2000), synthesize vitamins (Coates and Fuller, 1977), improve egg production, egg weight and egg size in layers and turkey (Thayer *et al*., 1978; Nahashon *et al*., 1992; Jin *et al*., 1998), feed consumption in layers and broilers (Nahashon *et al*., 1994; Kim *et al*., 2003), lower serum and egg and yolk cholesterol levels in hens (Mohan *et al*., 1995; Jin *et al*., 1998; Haddadin *et al*., 2001; Kim *et al*., 2003; Kurtoglu *et al*., 2004; Hajjaj *et al*., 2005) improve feed conversion ratio of the host (Raymane, 2000; Cavit, 2003), lower motility rate in broiler (Samanta and Biswas, 1995) and have beneficial effect on the health of the host (Soomro *et al*., 2002). The strain of selected microorganisms in propiotics, method of preparation, the dosage and condition of animals could be partially responsible for such description (Huang *et al*., 2004).

This study was conducted to investigate the effects of dietary supplementation of a probiotic and other additives available in Iran market on performance and immune response of broiler chicks.

Table 1: Composition (%) of basal diets

| Ingredients | Starter (14-28d) | Finisher (28-42d) |
|---|---|---|
| Corn | 55.97 | 61.52 |
| Soybean meal | 35.71 | 31.52 |
| Sunflower oil | 4.49 | 3.46 |
| Dicalcium phosphate | 1.33 | 1.10 |
| Oyster shell | 1.43 | 1.47 |
| DL methionine | 0.17 | 0.08 |
| Salt | 0.40 | 0.35 |
| Vitamin and mineral perimix* | 0.50 | 0.50 |
| Total | 100.00 | 100.00 |
| Nutrient content | | |
| ME (Kcal/Kg) | 3053.00 | 3058.00 |
| Crude protein | 20.47 | 19.10 |
| Methionine +Cysteine | 0.82 | 0.70 |
| Calcium | 0.95 | 0.90 |
| Phosphrous (available) | 0.40 | 0.35 |
| Sodium | 0.17 | 0.15 |

*Vitamin and mineral provided per kilogram of diet: Vitamin A, 3600000 IU; Vitamin $B_1$, 720 mg: Vitamin $B_2$, 2640 mg; Pantothenic acid, 4000 mg; Nicotinic acid, 12000 mg; Vitamin $B_6$, 1200 mg; Folic acid, 400 mg; Vitamin $B_{12}$, 6 mg; Vitamin $D_3$, 800000; Vitamin E, 7200 IU; Vitamin $K_3$, 800 mg; Biotin, 40 mg; Antioxidant, 100000 mg; Choline chloride, 5000 mg; Manganese, 40000 mg; Zinc, 33880 mg; Iron, 20000 mg; Copper, 4000 mg; Iodine, 400 mg; Selenium, 80 mg

## Materials and Methods

A total of one hundred fifty 14-days-old broiler chicks (Lohman strain) were obtained from the local market and were divided into 25 groups of 6 with similar average body weight (255±10 gr). All birds were fed a standard commercial diet based on corn and soybean meal (starter diet) during the first 14 days of life and then each treatment switched to their respective experimental diets. Each experimental treatment was repeated 5 times and fed experimental diets for 28 days. Experiment was conducted as completely randomized design and five dietary treatments were utilized. The chicks were fed diets based on soybean meal and corn (without added antibiotics, coccidiostats or growth promoter). The composition of starter and finisher diets is shown in Table 1. The basal diets (starter and finisher) were formulated to meet or exceed National Research Council (1994) nutrient requirements of broiler chicks and were fed from days 14-42. The treatments were:

T (1): control groups (C) that received starter and finisher diets, T (2): C plus 0.1 percent of a commercial probiotic (Bactocill, Pakgostar Parand, Tehran, Iran), T (3): C plus 0.1 percent Toxiban, T (4): C plus 0.1 percent Formycine and T (5): C plus mixture of 0.15 percent probiotoc with 0.1 percent Toxiban. These levels of supplementations selected based on optimum recommended levels in some researches. Strict sanitation practices were maintained in the house before and during the course of experiment. The probiotic contained *Pediococcus acidilactici* (MA185M) with a minimum of 1_×$10^{10}$ CFU/1 gr of the product. Formycine (a mixture of formaldehyde, propionic acid, sodium bentonite and ammonia) and Toxiban (a mixture of aluminosilicate and ammonium propionate), are two commercial feed additives available in Iran marketed by IQF, Spain.

The chicks were housed in temperature controlled room with continuous lighting and feed and water were provided *ad libitum* throughout the experiment. Feed intake (hen-day) and body weight were determined weekly and their Feed Conversion Ratio (FCR) was calculated. FCR was corrected for mortality. Birds were vaccinated against Gamboro (Biomen+D78), Newcastle (Lasota) according to laboratory recommendations.

At the end of experiment (42 d), five birds from each treatment were bled by wing vein for serum antibody titer analysis.

All serum samples were tested using HI test (Newcastle) according to Xu *et al.* (1997) and indirect antibody enzyme-linked immunosorbent assay (ELISA) kit (Influenza, Bronchitis and Gamboro) according to the manufacturer's (Svanova Biotech, Uppsala, Sweeden) instruction (Looraine and Clarke, 1982).

Analysis of variance was performed on the data using the General Linear Model of SAS software (2002). Means were compared using Duncan's multiple range test. Level of significance used in all results was 0.05.

## Results and Discussion

The main effect of treatments on broiler performance and antibody titers are presented in Table 2. Only treatment 4 (Formycine, commercial product 2) decreased ($p<0.01$) feed consumption by 7.1 percent as compared with the control diet. This product (Formycine) contained formaldehyde acid which coagulate cytoplasmic proteins of pathogenic bacteria through releasing aldehyde and increasing the population of unpathogenic microflora in the gut (Roser, 2006; Kralik *et al.*, 2004). Also this product could maintain acidic condition in the gut and lower the population of saprophytic bacteria. Treatment 5 (probiotic+Toxiban) resulted in non-significantly lower feed consumption, which indicate the beneficial effects of probiotic, formaldehyde acid and zeolites on feed consumption (Bailey *et al.*, 1998). Probiotic alone had no significant on feed consumption which is in agreement with findings of Miazzo *et al.* (2000), Ledoux *et al.* (1999) and Mutus *et al.* (2006) but not in the line of the findings of Jernigan *et al.* (1985) and Yeo and Kim (1997) who reported that the use of probiotic in broiler chicks diets significantly improved the daily body weight gain, feed intake and feed efficiency. The reason of variable effect of biological additives may be confounded by variations in gut flora and environmental conditions (Mahdavi *et al.*, 2005).

The body weight gain was affected by treatments, except treatment 4 (Formycine). As compared with the control diet, probiotic, Toxiban and probiotic+Toxiban increased

Table 2: The main effects of treatments on performance and titer of antibodies of boilers

| | Treatments+ | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | 1 | 2 | 3 | 4 | 5 | SEM | CV |
| Feed consumption (g/hen/d) | 133.48 | 137.68 | 131.79 | 124.02** | 126.91 | 1.01 | 3.92 |
| Body weight gain (g) | 1464.64 | 1680.99** | 1735.39** | 1440.39 | 1586.29* | 3.34 | 3.54 |
| Feed conversion ratio (g/g) | 2.49 | 2.26* | 2.17** | 2.37* | 2.19** | 0.06 | 0.92 |
| Antibody titers | | | | | | | |
| Newcastle | 1.154 | 1.294* | 1.222 | 1.274* | 1.41* | 0.47 | 6.07 |
| Bronchitis | 1.166 | 1.266* | 1.046* | 1.110* | 1.166 | 0.55 | 11.12 |
| Influenza | 1.313 | 1.322 | 1.346* | 1.306* | 1.370* | 0.49 | 7.11 |
| Gambro | 1.166 | 1.266 | 1.138 | 0.918* | 0.918* | 0.45 | 4.97 |

+: 1) control, 2) probiotic, 3) Toxiban; commercial product 1; 4) Formycine; commercial product 2 and 5) probiotic + Toxiban.
SEM = Standard Error of the mean, CV = coefficient of variability, * = Significant at p<0.05, ** = Significant at p<0.01

body weight gain by 12.9, 15.6 and 7.7 percent, respectively. Mohan *et al.* (1995) reported 15.1 percent increase in body weight gain of broilers as compared with the control diet with supplementing diet with probiotic. The higher body weight gain with Toxiban and probiotic+Toxiban might be due to the synergistic effect of probiotic and zeolite+ammonium propionate in Toxiban (Miazzo *et al.*, 2000; Ledoux *et al.*, 1999).

The Feed Conversion Ratio (FCR) was affected (p<0.01) by treatments compared with the control. Probiotic, Toxiban, combination of probiotic and Toxiban and Formycine improved FCR by 9.2, 12.80, 12.0 and 4.8 percent, respectively compared with control diet. The highest (2.49) and the lowest (2.17) FCR was found for control and Toxiban, respectively. The improvement of FCR with feeding probiotic and Toxiban (contained zeolite) is in agreement with the findings of Miazzo *et al.* (2000), Haddadin *et al.* (2001), Pelicia *et al.* (2004) and Papaioannou *et al.* (2005).

The manipulation of gut microbiota via the administration of probiotics influences the development of the immune response (McCracken and Gaskins, 1999). Blood Newcastle antibody titer was affected (p<0.05) by treatments (except feeding Toxiban). The highest value was seen with combination of probiotic and Toxiban followed by probiotic feeding. The positive effect of feeding probiotic on immunity response is in agreement with the findings of Fuller (1992), Zulkifli *et al.* (2000), Dalloul *et al.* (2003) and Koenen *et al.* (2004). Combination of probiotic and Toxiban, probiotic and Formaycine increased the Newcastle antibody titer by 22.18, 12.10 and 10.4 percent compared with the control diet. Only feeding probiotic increased (p<0.05) but Toxiban and Formycine resulted in lower (p<0.05) bronchitis antibody titration. In the case of influenza, feeding probiotic (p>0.05), Toxiban and combination of probiotic and Toxiban increased and formycine decreased (p<0.05) antibody titration. Gamboro antibody titration decreased with feeding Formycine and combination probiotic and Toxiban but increased (p>0.05) with feeding probiotic (Kostiuk *et al.*, 1992; Koenen *et al.*, 2004). The strains of selected microorganisms in probiotics, method of preparation, the dosage and condition of birds could be reasons for any observed discrepancies of the effect of probiotics (Huang *et al.*, 2004).

Based on the results of the current study, feeding Toxiban (a mixture of aluminosilicate and ammonium propionate) had the most positive effects on performance parameters and probiotic alone or probiotic+Toxiban supplementations had the best effect on immunity response of broiler chicks.

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