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# Effect of a multivitamin complex and probiotic blend in drinking water before and after vaccination on performance traits, blood biochemistry and humoral immune response of broilers

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**ABSTRAK**

Penelitian ini dilakukan untuk menganalisis efek campuran multivitamin kompleks dan probiotik dalam air minum sebelum dan sesudah vaksinasi terhadap kinerja pertumbuhan, sifat karkas, parameter biokimia darah dan respon imun *humoral* pada ayam broiler. Seratus lima puluh anak ayam broiler jantan berumur satu hari (Ross 308) ditempatkan secara acak ke lima kelompok perlakuan, dengan tiga ulangan (10 ekor per ulangan) dalam rancangan acak lengkap. Perlakuan terdiri dari: 1) pakan dasar dan air minum tanpa aditif sebagai kontrol (C); 2) C + air minum + multivitamin kompleks dari 2 hari sebelum vaksinasi hingga 3 hari setelah vaksinasi; 3) C + air minum + multivitamin kompleks selama 3 hari setelah vaksinasi; 4) C + air minum + campuran probiotik dari 2 hari sebelum vaksinasi hingga 3 hari setelah vaksinasi; dan 5) C + air minum + campuran probiotik selama 3 hari setelah vaksinasi. Pada seluruh periode penelitian, asupan pakan, bobot badan dan paha serta lemak perut secara nyata dipengaruhi (P<0,05) oleh perlakuan. Sebaliknya, tidak ada pengaruh nyata yang diamati pada karakteristik karkas, rasio konversi pakan, parameter darah, produksi antibodi terhadap sheep red blood cells (SRBC) dan immunoglobulin G (IgG). Titer IgM lebih tinggi pada perlakuan (2) dibandingkan dengan perlakuan lain pada umur 28 hari (P<0,05). Sebagai kesimpulan, sebuah kompleks multivitamin dimasukkan dalam air minum selama 2 hari sebelum vaksinasi hingga 3 hari setelah vaksinasi memungkinkan untuk meningkatkan kinerja dan kekebalan ayam broiler.

*Kata kunci: pakan, multivitamin kompleks, complex, probiotic blend; imunitas, broiler*

**ABSTRACT**

This study was conducted in order to investigate the effects of a multivitamin complex and probiotic blend in drinking water before and after vaccination on growth performance, carcass traits, blood biochemical parameters and humoral immune response of broiler chickens. A total of 150 one day-old male broiler chicks (Ross 308) were randomly allocated to five treatment groups, with three replicates (10 birds per replicate) in a completely randomized design. Experimental treatments consisted of: 1) a basal diet and drinking water without any additives as control (C); 2) C + drinking water + multivitamin complex from 2 days before vaccination until 3 days after vaccination; 3) C + drinking water + multivitamin complex for 3 days after vaccination; 4) C + drinking water + probiotic blend from 2 days before vaccination until 3 days after vaccination; and 5) C + drinking water + probiotic blend for

3 days after vaccination. In the whole experimental period, feed intake, body and thigh weight and abdominal fat were significantly affected (P<0.05) by dietary treatments. On the contrary, no significant effect was observed on carcass characteristics, feed conversion ratio, blood parameters, antibody production against SRBC and IgG. The titer of IgM was higher in treatment (2) than other treatments at 28 d of age (P<0.05). In conclusion, a multivitamin complex supplied in drinking water for 2 days before vaccination till 3 days after vaccination is enable to improve broiler performance and immunity.

*Keywords: nutrition, multivitamin complex, probiotic blend, immunity, broiler*

## INTRODUCTION

The gastrointestinal tract acts as a physical, chemical and immunological lines of defense to counter potential pathogens found in feed, bedding and environment. Beneficial bacteria (commensal) are an important part of this system colonizing the intestine of animals starting from the first day of life. However, in the modern broiler production systems newly hatched chicks have little chance to contact with their mother, thereby normal microflora is slow to colonize the intestine (Khan *et al*., 2011). Probiotics, or direct fed microbial (DFM), are feed additives defined as "a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance" (Heyman and Ménard, 2002) of good bacteria versus pathogenic bacteria for healthier, more profitable flocks.

The use of probiotics as feed additives in poultry nutrition emerged not only as one solution to maintain animal welfare without affecting performance parameters but also as substitutes for the antibiotic growth promoters (AGP) used since 1940. The AGP were supposed to promote muscle growth in poultry as a result of improved gut health, resulting in better feed digestion (Visek, 1978). However, the emergence of antibiotic resistance (Imperial and Ibana, 2016) with a potential risk for humans (Adegoke *et al*., 2017), lead to the ban on the use of antibiotics in livestock production in EU.

Probiotic species mainly used in broiler nutrition belong to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces*. Among the advantages claimed as benefits from probiotics in animals, DFM have been proved to create a gastrointestinal environment that enables better nutrient absorption leading to greater weight gain and feed efficiency, as well as to improve immune system and reduce incidence of diseases (Dhama *et al*., 2011; Kamiya *et al*., 2017). Humoral antibodies levels can determine the susceptibility of chickens to disease (Parmentier *et al*. 2004). Chichlowski *et al*. (2007) demonstrated the functionality of probiotics in preventing disease. The study developed by Salim *et al*. (2013) indicated that white blood cells, monocytes and plasma immunoglobulin levels were improved in broilers supplemented with DFM. Further, a trial conducted in broilers reared under heat stress conditions assessed that a multi-strain probiotic supplement could induce favorable effect on performance characteristics, immune responses and cecal microflora (Landy and Kavyani, 2014).

The function of the immune system can also be modulated by certain nutrients through a variety of mechanisms. Vitamin A deficiency decreased the cellular immune response in chickens and several indicators of immune responsiveness are depressed when chicks are vitamin E and/or selenium deficient (Latshaw , 1991). What is more, Gao *et al*. (2004) reported a significantly increased anti-Newcastle disease virus (NDV) titers of chickens with non-starch polysaccharides (NSP)-degrading enzyme supplementation. The immune response after vaccination is a valuable tool to investigate the effect of probiotics (Roos and Katan, 2000). The method of probiotic administration can influence the performance and immune competence of birds, and supplementation via drinking water appears to be superior to the more conventional in-feed supplementation method (Torshizi *et al*., 2010).

The objective of this study was to determine the effect of different levels of a multivitamin supplement and a probiotic blend in drinking water, before and after vaccination, on performance, carcass characteristics, blood biochemistry and humoral immunity of broiler from hatch to 42 days of age.

## MATERIALS AND METHODS

All procedures followed in this study were approved by the Animal Ethics Committee at the Islamic Azad University, Rasht Branch, Iran. Care

was taken to minimize the number of animals used.

**Animals, Mnaging and Diets**

A total of 150 day-old male broiler chickens (Ross 308; Aviagen, Newbridge, Scotland, UK), with similar body weight, were randomly assigned to five treatments with three replicates of 10 birds per replicate. Rearing conditions were similar for all treatment groups. A heater was used and temperature program was according to the instructions for Ross 308 broilers rearing. Air humidity was kept at 55 to 65 % in the early growing period by spraying the floor with water. Twenty watts lamps were installed at a height of 2.2 m above the floor. The lighting program consisted of a period of 23 h light and 1 h darkness.

Dietary treatments were a basal diet (as control); a diet with a multivitamin solution (Tolide Darouhai Dami, Iran) or a diet including a probiotic blend (PrimaLac, Star Labs Inc., Clarksdale, MO), both products were added to the drinking water before and/or after vaccinations. Birds were vaccinated against Bronchitis disease [(at 1 and 23 days of age (DoA)], Newcastle disease (at 23 and 31 DoA), Influenza disease (at 1 DoA) and Gumboro disease (at 16 DoA). Diets were formulated to meet the commercial nutrient recommendations for Ross 308 for starter (1-21 DoA) and finisher (22-42 DoA) periods and were based on corn and soybean meal.

The probiotic blend was added as a lyophilized product containing: $1\times10^8$ CFU/g of *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium*. The multivitamin solution contained per gram of water soluble powder: vitamin A 10000 IU, vitamin $B_1$ 2 mg, vitamin $B_5$ 5 mg, potassium chloride 1 mg, vitamin $D_3$ 3000 IU, vitamin $B_2$ 2 mg, vitamin $B_6$ 1 mg, magnesium chloride 3 mg, vitamin E 5 mg, vitamin $B_3$ 10 mg, and sodium chloride 10 mg.

In particular, the dietary treatments were: (1) basal control (C) diet without supplements, (2) C + 0.5 ml multivitamin/l in drinking water starting from 2 days before each vaccination until 3 days after vaccination; (3) C + 0.5 ml multivitamin/l drinking water for 3 days after each vaccination; (4) C + 0.06 g probiotic/l drinking water starting from 2 days before each vaccination until 3 days after each vaccination; and (5) C + 0.06 g probiotic/l drinking water for 3 days after each vaccination. The ingredient composition and nutritional value of diets are reported in Table 1.

**Performance Parameters, Carcass and Organs Characteristics**

Feed intake (FI) and body weight (BW) were weekly recorded. Feed conversion ratio (FCR) was calculated by dividing FI and BW gain (BWG). At 42 DoA and after 4 h of fasting for complete evacuation of gut, three birds from each replicate were selected and euthanized. These birds were used to assess the final weight of carcass, viscera, breast, thighs, wings, gizzard, liver and bile and abdominal fat.

**Blood Biochemical Parameters**

Before blood collection, feed was removed from all the birds for a period of 4 h in an attempt to allow stabilization of plasma constituents. Further, blood sampling was done in the morning to further reduce the variability of the plasma constituents. At 42 DoA, blood samples were collected from wing vein of three birds per replicate. The whole blood sample was transferred from the syringe into a tube coated with 10 mg of EDTA. After centrifugation at 3,000 *rpm* × 20 min, plasma was harvested and stored at -20°C until assayed.

Blood parameters analyzed in serum were: glucose (GLU), total triglycerides (TG), total cholesterol (TC), very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and uric acid (UA). Unless otherwise stated, the concentrations of these parameters were determined by routine methods using commercial kits (Teif Azmoon Pars Co., Tehran, Iran) according to the manufacturer's instructions. The GLU was measured by a glucose-oxidase photometric assay based on the combined action of glucose oxidase (GOD) and peroxidase (POD) (GOD-POD assay; Barham and Trinder, 1972). Briefly, in the COD-POD assay glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide reacts, in the presence of peroxidase, with phenol and 4-aminoantipyrine to form a quinoneimine dye (Trinder's reaction). The intensity of the pink color formed is proportional to the glucose concentration.

Plasma TG were measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. The glycerol is converted to pyruvate and then to lactate. Decreased absorbance, measured

Table 1. Ingredients and Nutrient Analysis of Diets Fed to Broiler Chickens

| Ingredients | 1-21 DoA | 22-42 DoA |
|---|---|---|
| Corn | 60.16 | 64.12 |
| Soybean meal (43% CP) | 31.78 | 27.00 |
| Corn oil | 4.50 | 5.00 |
| Dicalcium phosphate | 1.68 | 1.85 |
| Oyster shell | 0.79 | 1.00 |
| DL-methionine | 0.22 | 0.18 |
| Vitamin premix* | 0.25 | 0.25 |
| Mineral premix** | 0.25 | 0.25 |
| Salt | 0.37 | 0.35 |
| Chemical composition | | |
| ME (KJ/kg) | 3,200 | 3,220 |
| Crude protein (%) | 21.30 | 19.50 |
| Ca (%) | 0.85 | 1.03 |
| Available P (%) | 0.42 | 0.58 |
| Met (%) | 0.50 | 0.38 |
| Lys (%) | 0.96 | 1.12 |
| Met + Cys (%) | 0.78 | 0.73 |

* Vitamin A: 7.2 mg; vitamin $D_3$: 1.6 mg; vitamin E: 14.4 mg; vitamin $K_3$: 1.6 mg; vitamin $B_1$: 0.72 mg; vitamin $B_2$: 3.13 mg; vitamin $B_3$: 4 mg; vitamin $B_6$: 1.2 mg; vitamin $B_9$: 0.5 mg; vitamin $B_{12}$: 6 mg; vitamin $B_5$: 12 mg; vitamin H: 2 mg; antioxidant 10 mg; ** Mn: 13227 mg; Fe: 100 mg; Zn: 4235 mg; Cu: 16 mg; I: 0.64 mg and Se: 8 mg. DoA: days of age.

spectrophotmetrically, is proportional to the triglyceride concentration in the sample (Schmid and Forstner, 1986).

The colorimetric determination of TC in blood plasma samples involved the use of the cholesterol oxidase procedure (Allain *et al*., 1974), which is based on the conversion of free cholesterol to cholest-4-en-3-one and hydrogen peroxide in the reaction catalyzed by cholesterol oxidase. Finally, hydrogen peroxide in reaction with phenol and 4-aminoantipyrine in the presence of peroxidase forms a red colored chinoimine derivative. Colour intensity is directly proportional to the amount of cholesterol having a maximum absorption at a wavelength of 500 nm. The HDL and LDL cholesterol were measured directly with diagnostic kits, where serum HDL and LDL/VLDL are firstly separated and then the cholesterol concentration of each is determined by a coupled enzyme assay, which results in a colorimetric (570 nm) product, proportional to the cholesterol present. The UA was determined by enzymatic colorimetric method based on the use of the enzyme uricase (Trinder, 1969; Barham and Trinder, 1972; Fossati and Principe 1980). The UA is oxidized by uricase, and by the action of POD, in the presence of a phenol-derivative and 4-aminoantipyrine, the generated $H_2O_2$ gives a colored indicator reaction which can be measured at 520 nm. The increasing in absorbance is proportional to the UA concentration of sample (Thomas, 1998).

**Immunization Schedule and Immunity Evaluation**

The sheep red blood cells (SRBC) immunization was performed twice, on days 21 and 35, through subcutaneous injection of 0.5 mL of SRBC in PBS (10:1). Blood samples were collected at 28 and 42 DoA to assess the humoral immune response to Newcastle disease vaccine and SRBC immunization. To quantify serum

antibody to Newcastle disease, and to measure the antibody-mediated immune response (IgM and IgG) to SRBC immunization the haemagglutination inhibition (HI) method (Cunningham, 1971) was used. In brief, in U-bottom microtiter plates, two-fold serial dilutions of heat-inactivated (at 56°C) serum were made with PBS (0.01 mol/L; pH 7.4) for total antibody, or PBS with 1.4% 2-mercaptoethanol for immunoglobulin G (IgG) antibody. All antibody titers were recorded as $\log_2$ of the highest dilution of serum that agglutinated an equal volume of a 0.5% SRBC suspension in PBS. The IgM titer was determined by the difference between total Ig and IgG titer.

**Statistical Analysis**

Data were subjected to statistical analysis using the General Linear Model procedure of SPSS (1997). Differences among main effect means were assessed using Duncan's multiple range test. Statement of significance was based on $P \leq 0.05$.

## RESULTS AND DISCUSSION

The FI, BW and FCR during the starter period (1-21 DoA) were not affected (P>0.05) by the administration of multivitamins or probiotic blend in drinking water. Conversely, during the finisher period (22-42 DoA), the FI and the BW were affected by the dietary treatments, which in turn govern the variations observed when the entire experimental period is considered (Tables 2-4). The feed intake (FI) was not significantly affected by multivitamins and probiotic treatments compared with the control diet (treatment 1). However, supplementation of multivitamins for 2 days before vaccination till 3 days after vaccination (Treatment 2) significantly increased (P<0.05) the FI during finisher period (22-42 days of age) compared with the other diets (Table 2). The highest numerical BW value (1457.4 g) was observed in birds supplemented with multivitamins for 2 days before vaccination till 3 days after vaccination (Treatment 2). This value significantly differed (P<0.05) from that observed in birds supplemented with probiotic blend (treatments 4 and 5) (Table 3). The FI, BW and FCR of birds within probiotic treatments were similar (Table 4). In a study, Torshizi *et al*. (2010) compared two ways (drinking water and feed) of probiotic administration in broilers, and concluded that the method of probiotic administration can influence the performance of birds, and the administration via drinking water appears to be appropriate compared to the conventional in-feed supplementation method.

Carcass characteristics and organ weight of broilers were not markedly affected by dietary

Table 2. Effect of Treatments on Feed Intake (g) of Broilers

| Treatment* | Starter period (1-21 DoA) | Finisher period (22-42 DoA) | Total period (1-42 DoA) |
|---|---|---|---|
| 1 | 1213.78 | 3384.2$^{ab}$ | 4598.0$^{ab}$ |
| 2 | 1240.93 | 3716.7$^{a}$ | 4957.6$^{a}$ |
| 3 | 1204.67 | 3650.2$^{b}$ | 4445.9$^{b}$ |
| 4 | 1254.24 | 3194.2$^{b}$ | 4448.4$^{b}$ |
| 5 | 1205.78 | 3084.8$^{b}$ | 4290.6$^{b}$ |
| SEM | 29.47 | 111.67 | 119.85 |
| P-value | 0.684 | 0.009 | 0.014 |

*(1) Basal control (C) diet without multivitamin or probiotic, (2) C + 0.5 ml multivitamin/l in drinking water from 2 days before each vaccination until 3 days after vaccination, (3) C + 0.5 ml multivitamin/l in drinking water for 3 days after each vaccination, (4) C + 0.06 g probiotic/l in drinking water from 2 days before each vaccination until 3 days after vaccination, and (5) C + 0.06 g probiotic/l in drinking water for 3 days after each vaccination.

Within each column, means with different subscripts indicate significant differences (P<0.05). SEM: Standard error of means. DoA: days of age.

Table 3. Effect of Treatments on Body Weight (g) of Broilers

| Treatment* | Starter period (1-21 DoA) | Finisher period (22-42 DoA) | Total period (1-42 DoA) |
|---|---|---|---|
| 1 | 739.9 | 1321.1$^{ab}$ | 2061.0$^{bc}$ |
| 2 | 779.8 | 1457.4$^{a}$ | 2237.2$^{a}$ |
| 3 | 771.1 | 1402$^{a}$ | 2173.1$^{ab}$ |
| 4 | 762.3 | 1162.9$^{c}$ | 1925.2$^{c}$ |
| 5 | 739.7 | 1234.2$^{bc}$ | 1973.9$^{c}$ |
| SEM | 20.27 | 42.02 | 48.67 |
| P-value | 0.548 | 0.003 | 0.005 |

*See Table 2

Table 4. Effect of Treatments on Feed Conversion Ratio of Broilers

| Treatment* | Starter period (1-21 DoA) | Finisher period (22-42 DoA) | Total period (1-42 DoA) |
|---|---|---|---|
| 1 | 1.64 | 2.56 | 2.23 |
| 2 | 1.59 | 2.55 | 2.22 |
| 3 | 1.56 | 2.60 | 2.23 |
| 4 | 1.65 | 2.64 | 2.31 |
| 5 | 1.63 | 2.50 | 2.20 |
| SEM | 0.056 | 0.077 | 0.043 |
| P-value | 0.789 | 0.280 | 0.336 |

*See Table 2

treatments, although thigh weight was significantly higher ($P<0.05$) in broilers supplemented with probiotic 3 days after vaccination. Wing weight increased with probiotic added in drinking water 3 days after vaccination, despite significant ($P<0.05$). Abdominal fat decreased with multivitamin and PrimaLac supplementation compared with control (Table 5). Khan *et al*. (2011) showed that probiotics supplementation did not change the broilers meat composition or organs weigh; however, abdominal fat content was significantly reduced. Our findings agree the results of Santoso *et al*. (2001) reporting that certain microflora present in gastrointestinal tract of birds impaired the absorption of cholesterol and bile acid. So, there is a possibility that microorganisms in probiotic blend may cause lower absorption and deposition of fat content around the abdomen. Moreover, they suggested that probiotics could significantly decrease the activity of acetyl-CoA carboxylase which catalyses the rate-limiting step in fatty acid biosynthesis. However, it seems not be the case in the study presented herein as we can see further down when analyzing serum biochemistry results.

Several studies have stressed the beneficial microbes used in animal husbandry and having growth promotion effects (Bernardeaua and Vernoux, 2013). These effects may be a result of the active metabolites synthesized by probiotic

Table 5. Effect of Treatments on Body Weight (g) of Broilers

| Treatment* | Carcass | Breast | Thighs | Wings | Gizzard | Liver | Abdominal Fat |
|---|---|---|---|---|---|---|---|
| 1 | 2000 | 733 | 693[b] | 145 | 92.0 | 65.0 | 57[a] |
| 2 | 1890 | 711 | 705[b] | 145 | 91.7 | 63.3 | 30[b] |
| 3 | 2140 | 760 | 701[b] | 135 | 90.1 | 61.6 | 33[b] |
| 4 | 2055 | 751 | 713[b] | 156 | 90.5 | 61.7 | 38[b] |
| 5 | 2110 | 753 | 753[a] | 163 | 90.0 | 64.3 | 41[b] |
| SEM | 0.08 | 0.03 | 0.04 | 0.01 | 1.97 | 1.97 | 0.41 |
| P-value | 0.306 | 0.882 | 0.018 | 0.464 | 0.71 | 0.736 | 0.025 |

*See Table 2

Table 6. Effect of Treatments on Serum Biochemical Parameters of Broilers at 42 Days of Age

| Treatment* | Glucose (mg/dl) | Cholesterol (mg/dl) | Triglycerides (mg/dl) | HDL | LDL | VLDL | Uric Acid (mg/dl) |
|---|---|---|---|---|---|---|---|
| 1 | 140.0 | 164.9 | 78.95 | 71.0 | 78.1 | 15.7 | 5.38 |
| 2 | 129.4 | 168.4 | 71.05 | 79.6 | 74.5 | 14.2 | 6.26 |
| 3 | 129.0 | 182.5 | 69.30 | 77.0 | 81.6 | 13.8 | 5.74 |
| 4 | 144.6 | 164.2 | 90.35 | 82.3 | 63.8 | 18.1 | 5.74 |
| 5 | 126.4 | 189.5 | 91.23 | 74.0 | 97.2 | 18.2 | 6.97 |
| SEM | 9.38 | 8.25 | 13.62 | 6.40 | 10.92 | 2.72 | 0.77 |
| P-value | 0.423 | 0.185 | 0.685 | 0.743 | 0.271 | 0.685 | 0.650 |

*See Table 2

microorganisms, such as organic acids, hydrogen peroxide, bacteriocins or bacteriocin-like substances. Furthermore, probiotics maintain a better microbial environment in the digestive tract of birds by reducing the number of pathogenic microbes (Tufarelli *et al*., 2017). This enhanced digestion, absorption and efficiency of utilization of feed. Our obtained performance characteristics disagree from previous studies where probiotic treatments had a beneficial effect (Talebi *et al*., 2008; Torshizi *et al*., 2010; Khan *et al*., 2011; Giannenas *et al*., 2014). However, growth enhancement effects are more likely in situations involving a stress situation, as found on real farms rather than in university-based trials, assuming that health and zootechnical effects are closely related (Bernardeaua and Vernoux, 2013).

Table 6 summarizes the effect of treatments on broilers serum constituents at 42 DoA. Experimental treatments did not induce any significant effect on the serum concentration of glucose (GLU), cholesterol (CT), triglyceride (TG), HDL, VLDL, LDL and uric acid (UAc). Broilers supplemented with probiotic blend 3 days after vaccination had higher numerical values for CT (189.5 mg/dL), TG (91.1 mg/dL), LDL (97.2), VLDL (18.2) and UAc (6.9 mg/dL). On the contrary, GLU (126.4 mg/dL) was lower. These results disagree from previous studies were probiotics lowered the serum lipid of broilers; in fact, the hypolipidemic effect of probiotics in broilers can be the result of a reduction of

cholesterol synthesis or an increase in degradation and excretion of cholesterol (Fukushima and Nakano, 1995). Probiotic blend rich in *Lactobacillus* strains is able to reduce serum cholesterol through deconjugation of bile salts (Klaver FA and van der Meer, 1993).

Antibody plays a role in the maintenance of immune homeostasis (Bayry *et al*. 2005). Newcastle Disease (ND) ranked as the fourth most important disease in terms of the number of livestock units lost for poultry species. The humoral immunity from vaccination is critical to ND control in chickens (Kapczynski *et al*., 2013,). In our trial, no significant differences ($p>0.05$) were found for antibody titers against ND virus at days 28 and 42 of age (Table 7). Likewise, the effect of treatments on total antibody (IgT), IgG and IgM titers against SRBS at d 28 and at day 42 was not significant ($p>0.05$), except for IgM at d 28 (Table 8). Indeed, multivitamin supplementation 2 days before vaccination and 3 days after, improved IgM at a significant different level when compared with control diet (C) and treatment 5 (C+ probiotic blend 3 days after vaccination).

Response to vaccination can provide information on the immunomodulation properties of dietary components, including probiotics. The use of probiotics increases the amount of immunoglobulins (IgA, IgM and IgG) found in gut and serum of broilers. An increased antigen-specific antibody response following probiotic treatment and ND vaccination has been well documented by Dhama *et al*. (2011). Conflicting results occur since the efficacy of probiotics in poultry industry is multifactorial (Patterson and Burkholder, 2003). Differences in the antigen used to test the immune modulation response of probiotics could affect the significance of the results. Moreover, the same strain of bacteria had different effects on gut ad immune system of poultry species. Many studies have been carry out to study the effect of probiotics on the production efficiency of broiler chickens (Dhama *et al*., 2011, for review). In the present study, FI and BW was improved by addition of multivitamins in drinking water before and after vaccination of broilers; on the other hand, supplementation of probiotic in drinking water after vaccination induced

Table 7. Effect of Treatments on Antibody Titer against Newcastle Virus

| Treatment* | 28- DoA | 42- DoA |
|---|---|---|
| 1 | 2.33 | $2.33^{ab}$ |
| 2 | 2.66 | $1.66^{ab}$ |
| 3 | 3.33 | $1.00^{b}$ |
| 4 | 2.33 | $2.66^{ab}$ |
| 5 | 2.33 | $3.00^{a}$ |
| SEM | 0.490 | 0.611 |
| P-value | 0.621 | 0.984 |

*See Table 2

Table 8. Effect of Treatments on Total Antibody (IgT), IgG and IgM Titer against SRBS at 28 and 42 Days of Age

| Treatment* | Antibody response to SRBC at 28 DoA | | | Antibody response to SRBC at 42 DoA | | |
|---|---|---|---|---|---|---|
| | IgT | IgG | IgM | IgT | IgG | IgM |
| 1 | 2.00 | 1.00 | $1.00^{b}$ | 5.00 | $2.66^{a}$ | 2.33 |
| 2 | 3.00 | 1.00 | $2.00^{a}$ | 3.33 | $1.33^{b}$ | 2.00 |
| 3 | 2.33 | 1.33 | $1.00^{a}$ | 4.00 | $2.00^{a}$ | 2.00 |
| 4 | 2.33 | 1.33 | $1.00^{a}$ | 4.00 | $2.00^{a}$ | 2.00 |
| 5 | 2.66 | 1.33 | $1.33^{b}$ | 4.00 | $1.66^{a}$ | 2.33 |
| SEM | 0.36 | 0.25 | 0.14 | 0.64 | 0.44 | 0.33 |
| P-value | 0.415 | 0.736 | 0.002 | 0.529 | 0.361 | 0.871 |

*See Table 2

beneficial effect (P<0.05) on thigh weight, abdominal fat deposition and IgM level of broilers.

## CONCLUSION

In conclusion, form the present findings it was assessed that a multivitamin complex administrated in drinking water for two days before vaccination till three days after vaccination is enable to improve broiler performance and immunity.

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## REFERENCES

Adegoke, AA, A.C. Faleye, G. Singh and T.A> G, Stenström. 2017. Antibiotic Resistant Superbugs: Assessment of the Interrelationship of Occurrence in Clinical Settings and Environmental Niches. Molecules. 22(1):29

Allain, C.C., L.S. Poon, C.S. Chan, W. Richmond and P.C. Fu. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20: 470-475.

Barham, D. and D. Trinder. 1972. An improved color reagent for the determination of blood glucose by the oxidase system analyst. The Analyst. 97:142-145.

Bayry, J., S. Lacroix-Desmazes, M.D. S.V. Kazatchkine, O. Hermine, D.F. Tough and Kaveri 2005. Modulation of dendritic cell maturation and function by B lymphocytes. J Immunol. 175:15–20.

Chichlowski, M, W.J. Croom, F.W. Edens, B.W. McBride, R. Qiu, C.C. Chiang, L.R. Daniel, G.B. Havenstein and M.D. Koci. 2007. Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac, and salinomycin. Poult Sci. 86(6):1121-32.

Dhama, K., V. Verma, P.M. Sawant, R.K. Vaid and R.S. Chauhan. 2011. Applications of probiotics in poultry: Enhancing immunity and beneficial effects on production performances and health - A Review. J. immunol. Immunopathol. 13(1): 1-19.

Fukushima, M. and M. Nakano. 1995. The effect of a probiotic on faecal and liver lipid classes in rats. Br. J. Nutr. 73(5):701-10.

Gao, F., Y. Jiang, G.H. Zhou and Z.K. Han. 2004. Effects of non-starch polysaccharide enzyme supplements on the growth, immune function and gastrointestinal microflora of chickens. Chin. J. Vet. Sci. 24:501–503.

Giannenas, I. E. Tsalie, E. Triantafillou, S. Hessenberger, K. Teichmann, M. Mohnl and Tontis. 2014. Assessment of probiotics supplementation via feed or water on the growth performance, intestinal morphology and microflora of chickens after experimental infection with Eimeria acervulina, Eimeria maxima and Eimeria tenella. Avian Pathol. 43(3):209-216.

Heyman, M. and S. Ménard. 2002. Probiotic microorganisms: how they affect intestinal pathophysiology. Cell Mol. Life Sci. 59(7):1151-65.

Imperial, I.C. and J.A. Ibana. 2016. Addressing the antibiotic resistance problem with probiotics: reducing the risk of its double-edged sword effect. Front Microbiol. 7:1983.

Kamiya, T, Y. Watanabe, S. Makino, H. Kano and N.M. Tsuji. 2017. Improvement of intestinal immune cell function by lactic acid bacteria for dairy products. Microorganisms. 5:1-10.

Kapczynski DR, Afonso CL, Miller PJ. 2013. Immune responses of poultry to Newcastle disease virus. Dev. Comp Immunol. 41(3): 447-453.

Khan, S.H, B. Yousaf, A.A. Mian, A. Rehman and M.S. Farooq. 2011. Assessing the effect of administering different probiotics in drinking water supplement on broiler performance, blood biochemistry and immune response. J. App. Anim. Res. 39(4):418-428.

Klaver, F.A. and R. van der Meer. 1993. The assumed assimilation of cholesterol by *Lactobacilli* and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. Appl. Environ. Microbiol. 59(4):1120-1124.

Latshaw. JD. 1991. Nutrition--mechanisms of immunosuppression. Vet. Immunol Immunopathol. 30(1):111-120.

Parmentier, H.K., R. Baelmans, H.F.J. Savelkoul, P. Dorny, F. Demey and D. Berkvens. 2004. Serum haemolytic complement activities in 11 different MHC (B) typed chicken lines. Vet. Immunol. Immunopathol. 100:25–32.

Patterson, J.A. And K.M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production (Review). Poult. Sci. 82: 627–631.

Salim, H.M., H.K. Kang, N. Akter, D.W. Kim, J.H. Kim, M.J. Kim, J.C. Na, H.B. Jong, H.C. Choi, O.S. Suh and W.K. Kim. 2013. Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. Poult Sci. 92(8):2084-90.

Santoso, O.U, K. Tanake, S. Ohtani and M. SakaidaM. 2001. Effect of fermented product from Bacillus subtilis on feed conversion efficiency, lipid accumulation and ammonia production in broiler chicks. Asian-Aust. J. Anim.Sci. 14:333-337.

Schmid, M. ad D. D. ForstnerD. 1986. Laboratory Testing in Veterinary Medicine Diagnosis and Clinical Monitoring, pp. 253 (Mannheim, Germany, Boehringer Mannheim GmbH).

SPSS, 1997. SPSS Base 7.5 for Windows. SPSS, Chicago, IL.

Talebi, A., B. Amirzadeh, B. Mokhtari, H. Gahri . 2008. Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. Avian Pathol. 37(5):509-512.

Thomas, L. 1998. Clinical Laboratory Diagnostics: Use and Assessment of Clinical Laboratory Results. pp. 192-202 (Frankfurt/Main, Germany, TH-Books Verlagsgeselschaft).

Toghyani, M., S.K. Mosavi, M. Modaresi and N. Landy. 2015. Evaluation of kefir as a potential probiotic on growth performance, serum biochemistry and immune responses in broiler chicks. Anim. Nutr. 1(4):305-309.

Torshizi, M.A.K., A.R. Moghaddam, Sh. Rahimi, and N. Mojgani. 2010. Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. Br. Poult. Sci. 51(2):178-84.

Trinder, P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromosome. J. Clin. Pathol. 22:158-161.

Tufarelli, V., A.M. Crovace, G. Rossi and V. Laudadio. 2017. Effect of a dietary probiotic blend on performance, blood characteristics, meat quality and faecal microbial shedding in growing-finishing pigs. South Afr. J. Anim. Sci. 47(6):875-882