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Effects of Vitamin E, Vitamin C and Mannanoligosaccharide (Bio-Mos®) Supplements on Performance and Immune System in Broiler Chicks

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Abstract: Maintaining gut health is important for the production of high quality and profitable poultry. The goal of this study was to examine the effects of supplemental mannan oligosaccharide (Bio-MOS®), Vitamin E (VE) and C (VC) on the growth performance and immune response of broilers given a corn based diet over a 6 weeks experimental period. About 1 day old male broilers (n = 300) were randomly distributed to 4 groups (75 birds in each group and 15 birds in each subgroup for repetation 5 times) and reared under similar conditions. Standard husbandry and good management practices were followed that met or exceeded industry guideline. At each feeding, the following treatments were administered: control (no Bio-MOS®, VE and VC), 1.5 g kg^{-1} Bio-MOS®, 500 μg kg^{-1} VE and 500 μg kg^{-1} VC. Body Weights (BW), Feed Intake (FI) and Feed Conversion Ratio (FCR) were measured on day 1, 21 and 42. Blood samples were taken from vena ulnaris every 7 days and were analyzed on IgG concentration. The results showed that there was no significant difference in BW, FI and FCR among the treatment groups. During the 6 weeks of trial period considering plasma IgG levels significant differences were only found as following: compared to control group it was significantly lower in VE group at week 1 and 2, higher in VC and Bio-MOS® groups at week 2 and lower in Bio-MOS® group at week 6. Additionally, at 4 week lowest (0.90±0.06 mg, n = 15) and at 5 week highest (2.85±0.18 mg, n = 15) plasma IgG level was found after applying the dietary treatment in Bio-Mos® group. Consequently, this data suggest that supplementation of Bio-MOS®, VE or VC may not improve either broiler performance or immune response in healthy broilers.

Key words: Broiler chick, immune system, vitamin E, vitamin C, mannanoligosaccharide, performance

INTRODUCTION

Dietary composition has an impact on immune function of the broiler chicken. Nutritional immunology is becoming popular. Clearly, commercial poultry producers do not have the luxury of focusing on specific disciplines when field problems occur. Hence, in practice interaction exists among nutrition, genetics, management and diseases (Kidd, 2004). Nutrition has some effects on both innate and cellular immunities if the feed is severely restricted (Hangalapura *et al*., 2005). Antibody responses, blood lymphocyte proliferative responses and production of Reactive Oxygen Intermediates (ROI) in chickens on different levels of feed restirictions results in lower spleen weight a marked reduction in natural antibodies binding lipoteichoic acid. The recent European Union's ban on the prophylactic use of in-feed antibiotics has escalated the search for alternatives in poultry industry (Janardhana *et al*., 2009). Given the current interest in the use of safe alternatives, natural antioxidants including the mannan oligosaccharides and vitamins E and C are important to animal health. Enhancing the immune response and resistance to pathogens of birds through nutrients is considered to be both practical and efficient in terms of improving performance in modern poultry production. Antimicrobials have traditionally been used as a supplement in the poultry industry to improve health and performance of birds. Nowadays, antibiotics are removed from animal feed because of increasing percentage of antibiotic-resistant bacteria.

Therefore, researchers have focused on alternative feed additives to antibiotics such as pre- and probiotics. One class of prebiotics is represented by oligosaccharides. Oligosaccharides have been shown to increase beneficial bacteria like bifidobacteria or lactobacilli in the intestinal tract of broilers (Patterson *et al*., 1962). Mannan Oligosaccharides (MOS) are derived from mannans on yeast cell surfaces. Based

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on the literature, MOS improve antibody response in broilers and layers (Cotter *et al*., 2000) modulate the immune response in chickens (Cotter *et al*., 2002; Savage *et al*., 1996; Shashidhara and Devegowda, 2003) and increase serum IgG levels in turkeys (Cetin *et al*., 2005) but do not enhance plasma IgG levels in dogs (Swanson *et al*., 2002). In addition, beneficial effects of MOS supplementation on body weight gain in broilers (Rosen, 2007) and feed conversion ratio in birds (Waldroup *et al*., 2003) as well as turkeys (Fritts and Waldroup, 2003) were observed. Conversely, several previous reports did not result in improved performance in terms of these parameters in broilers (Waldroup *et al*., 2003; Stanczuk *et al*., 2005) and Turkeys (Yalcinkayal *et al*., 2008), respectively. However, data regarding effects of MOS on chicken performance are limited. As mentioned above, although some information is available on data regarding effects of MOS, the role of MOS on performance and immune response on chicken is not completely understood.

Regarding nutritional supplementation vitamins are of great interest because vitamins like vitamin E and vitamin C, possessing potent antioxidant activities are able to decrease the effects of stress and infection on feed intake and body weight gain in chicken (Colnago *et al*., 1984; McKee and Harrison, 1995). Vitamin E (VE) is necessary as an antioxidant as a regulator of the transcription and of the activity of enzymes. Body weight gain and viability (Colnago *et al*., 1984) of chicks may improve with VE supplementation. However, numerous studies have reported no beneficial effects of VE either on mortality (Richter *et al*., 1985), body weight or feed intake (Nameghi *et al*., 2007; Niu *et al*., 2009). Further, many parameters of the immune system including resistance to infection, specific antibody production and number of antibody producing cells are altered by supplementing diets that are deficient or marginal in VE (Meydani and Blumberg, 1993). Vitamin E is an essential constituent of all the cell membranes including mitochondrial and nuclear membranes (Machlin, 1984). Colnago *et al*. (1984) found out that immunisation of chicken against coccidiosis was enhanced by vitamin E supplementation. An injection of vitamin E into the amnion three days prior to hatch enhanced antibody and macrophage response in Turkey and chicken (Gore and Qureshi, 1997). Abdukalykova *et al*. (2008) examined the effets of Arginin (Arg) and VE on the subpopulations of T Lymphocytes in peripheral blood in broiler chickens after an infectious bursal disease virus vaccination. Broiler chickens were fed diets with both normal and hight levels of Arg and VE. The results suggested that Arg and VE have complementary effects on cellular and humoral immune function and enhance the resistance of broilers to infectious diseases. A Meta-Analysis procedures were made on the effects of VE supplementation in feedlot cattle by Cusack *et al*. (2009). The health outcome morbidity, the production outcomes of Average Daily Gain (ADG) and Gain to Feed ratio (G:F) were analysed. It was concluded that supplemental dietary VE should be fed as an injection within Recommendation which is 15-60 IU kg^{-1} of diet DM. The effects of VE were evaluated on the fatty acids and MDA level in broiler chicken meat quality. It was concluded that the fatty acid concentration in broiler meat changes with respect to the nutrition and dietary composition of liver oil together with VE and VC increases n-3 fatty acid levels that is good for human health in broiler meat.

Several researchers have reported beneficial effects of Vitamin C (VC) supplements given either in diets and/or in drinking water. Vitamin C may also play a role in enhancing the immune system. In general, immunoglobulins and complement factors protect against systemic infections. Studies demonstrate that Vitamin C is required for the systemic availability of substances such as immunoglobulins and interferons (Bendich, 1990). Furthermore, VC increased in chicken the number of CD 8 (+) and IgM (+) cells (Wu *et al*., 2000) increased bacterial killing by heterophils and lowered plasma corticosterone (McKee and Harrison, 1995). Furthermore, beneficial effects of VC supplementation on body weight gain (Blaha and Kreosna, 1997; Jaffar and Blaha, 1996), feed intake (Sahin and Kucuk, 2001) and feed conversion ratio (McKee and Harrison, 1995; Blaha and Kreosna, 1997) were observed in poultry. In contrast, several studies (Blaha and Kreosna, 1997; Jaffar and Blaha, 1996) reported that supplementation of VC did not influence feed intake. VC has been demonstrated to improve immune responsiveness (Nameghi *et al*., 2007). Pulmonary Hypertension Syndrome (PHS, ascites) is a common metabolic disorder of modern, fast-growing strains of broilers. Concurrent supplementation of Arg, VC and VE was conducted to evaluate the effects on cardiopulmonary performance in broiler chickens under cold environmental conditions after an acute Epi challenge with increased no concentrations and lower hematocrit concentrations (Ruiz-Feria, 2009).

In recent decades, deficiencies in feed formulation and management practices have been masked by the routine Use of Antibiotic Growth Promoters (AGP). However, the ban of AGP in Europe has driven the implementation of safe alternatives such as antioxidants for optimizing health and performance of broilers (Awad *et al*., 2008). Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. However, published reports on some natural antioxidants in broiler diets are rare and contradictory. Therefore, there is vast interest in understanding the mechanisms of immunosuppression and developing strategies to

enhance immune responsiveness in commercial poultry. Thus, the aim of this study was to determine the effects of supplemental dietary MOS, VE and VC on parameters of performance and immune responses on healthy broilers. Several micro and macro-nutrients are needed for normal maintenance of the immune system (Bhaskaram, 2001). This includes amino acids, essential fatty acids and several vitamins and minerals. Numerous investigations since the early 1900s have shown that marginal and profound nutrient deficiencies impair the immune response in animal models as well as in humans, leading to an increased morbidity and mortality (Grimble, 2001).

Therefore, dependency of the immune system on adequate nutritional status is unquestionable. More recent studies, however have looked beyond deficiency of nutrients and focused on the level of nutrients needed for optimal immune response. Therefore, the aim of this study was to investigate the effects of different feed additives (immunomodulators) such as vitamin E, vitamin C, Bio-Mos® on broiler performance and immune system.

The effects of $-mannanase (Hemicell) on growth performance and immunity in broiler chickc fed the same basal diet based on corn-soybean meal were examined and Hemicell was added to the basal diet at 0, 0.025, 0.05 and 0.075%, respectively. The results indicated that hemicell may improve growth performance and immunity of broilers. Hemicell supplementation increased most of the relative immune organ weights and significantly increased the concentration of serum IgM. Hemicell supplementation at 0.05% significantly increased T-lymphocyte proliferation (Zou *et al*., 2006). Broiler chickens were fed with dietary treatments of MOS at 50 ppm in the 1st week, 30 ppm in the week 3 and 2 g of MOS kgG^{1} of diet (Yang *et al*., 2007). MOS supplementation did not improve significantly the growth performance of the broilers. On the other hand, MOS supplement numerically icreased the villus height and specific activity of maltase in the jejunum. The inclusion of MOS did not show a clear positive effect on the intestinal digestibility of nutrients, morphology or the mucosal enzyme activities.

MATERIALS AND METHODS

Birds, housing and diets: About 300 of 1 day old male broiler chicks (Ross) were obtained from a local hatchery (Pak Pilic, Istanbul, Turkey). The birds were weighed at the beginning of the experiment, randomly divided into four groups (75 male birds/group) which are control (no Bio-MOS®, VE and VC), 1.5 g kgG^{1} Bio-MOS®, 500 µg kgG^{1} VE and 500 µg kgG^{1} VC and housed in pens of identical size (1.75×6 m) in a deep litter system. The study was repeated 5 times for each of 4 groups. So, there were 15 birds in each subgroup actually. Wood shavings were used as the litter material. The climatic conditions and lighting program were computer-operated and followed the commercial recommendations. The chicks were raised using standard temperature regimes that gradually decreased from 35-21°C and under a 23L:1D cycle throughout the studies. The control group was fed with starter and grower diets based on corn, soya, vegetable oil and a premix with vitamins, minerals, amino acids (lysine, methionine) and salt (Table 1).

Table 1: Composition of the starter and grower diets

Ingredients and analysis	Starter (%)	Grower (%)
Maize	47.55	55.55
Soya meal	45.00	37.00
Vegetable oil	4.00	4.00
Limestone	1.90	1.90
DCP	0.83	0.83
Vitamin-mineral premix*	0.30	0.30
Salt	0.20	0.20
Lysine	0.08	0.08
Methionine	0.15	0.15
Dry matter	88.56	88.32
Crude protein	23.00	20.00
Calcium	1.08	1.04
Total phosphorus	0.75	0.77
ME kcal kgG^{1}	3100.00	3200.00

The broiler premix provided the following per kilogram of diet: vitamin A, 1204 µg; cholecalciferol, 25 µg; vitamin E, 4.5 mg; riboflavin, 2.25 mg; niacin, 15.0 mg; d-pantothenic acid, 4.0 mg; folic acid, 0.25 mg; vitamin B_{12}, 5 µg; choline cloride, 200 mg; thiamine, 0.5 mg; biotin, 25 µg; ethoxiquin, 12.5 mg; menadione sodium bisulfite, 1.25 mg; pridoxine, 0.50 mg; manganese, 24.9 mg; zinc, 22 mg; iodine, 0.2 µg; iron, 13.6 mg and copper, 1.6 mg. The treatment groups were fed with the basal diet with additional 1.5 g kgG^{1} Bio-MOS® (Alltech, Izmir, Turkey), 500 µg kgG^{1} VE and 500 µg kgG^{1} VC (Roche Vitamins, Inc. Istanbul, Turkey). The chicks were fed with the starter diets from days 1-21 and grower feed from day 21-42 (Table 1). The birds had free access to water and feed. The experimental procedures were approved by the institutional animal care committee

Performance of broiler chicks: Chicks were weighed individually at the beginning of the experiment (initial body weight) as well as at week 3 and at the end of feeding period (at week 6). The feed consumption was measured weekly during the 6 weeks experiment. Cumulative weights gain (BW); Feed Intake (FI) and food conversion ratio (food intake/weight gain, FCR) were calculated. The mortality rate and liveability rate at the end of the feeding period were determined. The European Production Efficiency Factor (EPEF) was calculated according to the following equation:

$$EPEF = \left[\frac{(\text{live weight, kg}\times\text{liveability\%})}{(\text{feed conversion ratio}\times\text{age, days})}\right]\times 100$$

Blood sampling and analysis: All the chicks were bled by puncture of the ulnar vein by using a 0.5-heparinized insulin syringe with a 28-gauge needle (Becton Dickinson and Co., Istanbul,Turkey). At the beginning of the study all male chicks in the control group were bled and 1-heparinized syringe with a 25-gauge needle

(Becton, Dickinson and Co) on day 14, 21, 28, 35 and 42. The heparinized blood samples were centrifuged at 700×g for 15 min at 4°C. Subsequently, plasma samples were stored at -20°C until analysis for IgG. In each of the four groups, the concentrations of specific chicken IgG in plasma was measured with Enzyme-Linked Immunosorbent Assay (ELISA) as described by Erhard *et al*. (1992).

Statistical analysis: The Statistical Package for the Social Sciences (SPSS version 14.0) was employed to perform statistical analysis. The experimental design was completely randomized with dietary treatment as the main effect.

Statistically significant differences between group means were determined by Analysis of Variance (ANOVA). When the differences were significant, Duncan's multiple range test was performed. Probability values $p<0.05$ were considered significant. Data are expressed as mean values±SEM.

RESULTS AND DISCUSSION

Feed intake and body weight: The supplementation of VE, VC or Bio-MOS® to the diets did not improve the general BW of broilers (Table 2). The cumulative feed intake of broilers for control group and VE, VC and Bio-MOS® groups at week 3 were 1100, 1150, 1160, 1080 g at week 6 they were 3810, 3800, 3850, 3750 g, respectively.

The food conversion ratio at week 3 was lower for control group (1.56) than VC group (1.65) but it was higher for control group than VE (1.50) and Bio-MOS® group (1.47) at week 6 FCR was higher for control birds (1.74) than Bio-MOS® group (1.68), VC group (1.67) and VE group (1.63).

The liveability was 100% in all groups during the 6 week trial period. Additionally, the European Production Efficiency Factor (EPEF) was higher for VE, VC or Bio-MOS® supplemented groups (VE = 335,96, VC = 313,65 and BM = 316,04, respectively) than for the control group (Control Group = 299,67).

Total igG levels in the chicks plasma: The levels of IgG in the chicks plasma were examined on day 1 and then at 6 time points (week 1-6). Three of male chicks in each group were randomly selected from each group and this selection was repeated 5 times for every subgroup. So, number of 15 was obtained in this way. Plasma IgG value of control group (n = 15) was highest on day 1 (3.41±0.36 mg) and lowest on week 2 (1.53±0.11 mg) during the 6 weeks study period. Considering plasma IgG level, at week 1 after the dietary treatment was applied, no statistically significant differences were observed between the control group and VC group and between the control and Bio-Mos® group while at 2 week the control group was significantly lower than the VC and Bio-Mos® group ($p<0.05$). Both at the 1st and 2nd week of the study plasma IgG levels in VE group was significantly lower than those in the other three groups ($p<0.05$). At 3rd week of the dietary treatment, no statistically significant differences were observed between the groups.

The lowest IgG level after applying the dietary treatment was found in Bio-MOS® group (0.90±0.06 mg) at 4th week and it was significantly lower than that in the other three groups ($p<0.01$). The highest IgG level after applying the dietary treatment was also observed in Bio-Mos® group (2.85±0.18 mg) at week 5 ($p<0.05$). At the 6th week of the dietary treatment, no significant differences were observed between the control and VE group and also between the control and VC group while plasma IgG values of these three groups were statistically higher than that of Bio-MOS® group ($p<0.05$) (Table 3).

Table 2: Effects of vitamin E, vitamin C and Bio-Mos® on growth performance of broilers

Parameters	Control	Vitamin E	Vitamin C	Bio-Mos
Body weight (g)				
Day 0	41±0.43	40±0.42	41±0.49	42±0.47
Day 0-21	705±14.5	720±15.6	700±14.6	730±15.3
Day 0-42	2190±28.5	2300±29.5	2200±30.2	2230±29.6
Feed intake (g)				
Day 0	-	-	-	-
Day 0-21	1100	1150	1160	1080
Day 0-42	3810	3800	3850	3750
FCR				
Day 0	-	-	-	-
Day 0-21	1.56	1.50	1.65	1.47
Day 0-42	1.74	1.63	1.67	1.68
Mortality (%)				
Day 0	-	-	-	-
Day 0-21	0	0	0	0
Day 0-42	0	0	0	0
EPEF	299,67	335,96	313,65	316,04

Table 3: Mean serum IgG concentrations in broilers of vitamin E, vitamin C and Bio-Mos® and control group

	Control		Vitamin E		Vitamin C		Bio-Mos®	
Weeks	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	3.41	0.36	-	-	-	-	-	-
1	2.08^{a}	0.16	1.50^{b}	0.23	2.23^{a}	0.07	2.33^{a}	0.08
2	1.53^{b}	0.11	1.01^{c}	0.13	2.29^{a}	0.13	2.31^{a}	0.13
3	2.24	0.15	2.05	0.09	2.00	0.15	2.31	0.21
4	1.88^{a}	0.24	2.13^{a}	0.08	2.13^{a}	0.21	0.90^{b}	0.06
5	1.56^{b}	0.18	2.72^{a}	0.31	1.07^{b}	0.10	2.85^{a}	0.18
6	2.84^{a}	0.20	2.46^{a}	0.07	2.72^{a}	0.15	1.87^{b}	0.16

This study was conducted to evaluate the efficiency of natural antioxidants as Bio-Mos®, VE and VC on performance and immune responses of broiler chickens.

Performance: The results of using MOS as natural growth promoters in poultry diets are inconsistent. The current study identified no significant effect on performance in chicks fed with supplemental 1.5 g kg^{-1} Bio-MOS® on 21 or day 42. In agreement with the observations, addition of 1 or 3 g kg^{-1} of MOS did not influence BW and FCR (Eren *et al*., 1999) or FI (Shafey *et al*., 2001) whereas 5 g kg^{-1} of MOS led to minor improvements in FCR (Iji *et al*., 2001). These results were contradictory to recent studies reported that the inclusion of MOS in broiler diets resulted in significant improvement in BW (Blake *et al*., 2006; Rosen, 2007) and FCR (Waldroup *et al*., 2003). Published report on the effects of MOS on broiler performance is rare and contradictory. This may be due in part as the levels of MOS used in this study were not sufficient to elicit a pronounced response. This warrants further study with levels of inclusion in the diet.

In the present study, either inclusion of 500 µg kg^{-1} VE or VC supplementation to the diet of chicks did not result in a significant influence on performance of broilers that are compatible with previous researches who reported that inclusion of one of these antioxidants into the broiler diet did not resulted in a significant improvement on BW (Coetzee and Hoffman, 2001; Nameghi *et al*., 2007), FI (Niu *et al*., 2009; Blaha and Kreosna, 1997; Jaffar and Blaha, 1996) or FCR (Coetzee and Hoffman, 2001). However, these results disagreed with previous reports indicating that chickens benefited from a dietary supplementation of VC increased BW (Blaha and Kreosna, 1997; Jaffar and Blaha, 1996), improved their FI (Sahin and Kucuk, 2001) or FCR (McKee and Harrison, 1995; Blaha and Kreosna, 1997). The reason for this finding is unclear. However, kidneys which are the principal organs for chickens to synthesize ascorbic acid can not synthesize adequate amounts of ascorbic acid until after 15 days of age (Puls, 1994). Considering VE, Raza *et al*. (1997) showed that supplementary VE had beneficial effects on performance related parameters. An explanation for performance results may be attributable to the fact that there was no increase in protein or energy utilization between dietary VE treatments, since all dietary treatments were balanced energy to protein ration.

Immune response: Birds respond to antigenic stimulation by generating antibodies as well as cellular immunity. Very young chicks are susceptible to many pathogens during the first few weeks of age because their immune system is not fully developed hence, maternal antibodies are the primary means of antigen-specific protection (Hamal *et al*., 2006). In the present study, immune characteristic measured in blood at 1-6 weeks of age and IgG levels did not indicate positive changes in systemic immune capacity as a result of Bio-MOS®, VE or VC supplementation in broiler chicks at 3 and 6 weeks of age.

Mannan and glucan of the yeast cell wall may bind to pattern-recognition receptors on a variety of defence cells of the gut-associated lymphoid tissue and in turn activate immune defences such as phagocytes, the alternative complement pathway and the lectin pathway (Shashidhara and Devegowda, 2003). However, in the present study, supplementation of Bio-MOS® in the broilers diet increased the IgG levels at week 2 and 5 decreased at week 4 and 6 and not influenced at 1 and week 3 compared to control. In particular agreement with the observations, Swanson *et al*. (2002) reported that the addition of MOS did not significantly influence IgG concentrations after 2 weeks trial period in dogs. Cetin *et al*. (2005) pointed out that the addition of MOS significantly increased serum IgG levels at the end of the 15 weeks trial period in Turkeys. Savage *et al*. (1996) reported that feeding Turkeys with diet with 0.11% MOS led to significant increases in plasma IgG level. In the present study the initial decrease in the plasma IgG levels at week 2 may be due to the catabolism of maternal IgG in chicks as the half-life of IgG in the plasma (Patterson *et al*., 1962). The role of Bio-MOS® in modulation of the IgG levels during the initial 6 weeks of broiler chickens is not completely understood.

Regarding nutritional supplementation vitamins are of great interest because vitamins like VE and VC, possessing potent antioxidant activities have recently been shown to influence immune system. Previous reports on beneficial effects of VE are as follows: Erf *et al*. (1998) reported that beneficial effects on the development and function of adaptive immunity in 5-7 weeks old broilers. Konjufca *et al*. (2004) suggested that the addition of VE may have a substantial positive impact with respect to disease resistance and improved broiler health. In the case of humoral immune response, the effect of added VE depended on the nature of the antigen and quantity. Typically, addition of VE in amounts between 25 and 50 IU to the basal diet containing the National Research Council suggested requirement of 10 IU was the most immunomodulatory. This emphasize that higher levels of VE were often less effective (Leshchinsky and Klasing, 2001). This may explain the result obtained in this study that the supplementation of VE did not indicate enhanced IgG levels in plasma compared to control during 6 weeks

of study period. VC addition only significantly increased the plasma IgG concentration at 2 weeks of age during 6 weeks dietary trial period compared to control in the present study. In agreement with the observations Hesta *et al*. (2009) found that addition of VC did not show any significant effect on the serum IgG concentration at 5 weeks of age in healthy dogs. This observation differs from those of other researchers. Zhao *et al*. (2002) reported that the plasma levels of the IgG showed a linear increase in pigs with increasing levels of VC supplementation. Wu *et al*. (2000) pointed out that IgG antibody secreting cells in spleen at 3 weeks of age were significantly higher in ascorbic acid supplemented group which was vaccinated against infectious bursal disease in chickens. These results indicated that dietary supplementation of ascorbic acid may improve humoral and cellular immune responses in chickens. Whether or not VC plays a role in stimulating humoral immune function in broilers requires further study since the results of the experiments do not completely rule out the possibility that such a role exists. Although considerable research have been performed on the effects of supplementation of antioxidant such as VE and VC, its effects on performance and immune function have yet to be concluded on healthy broilers.

CONCLUSION

In conclusion, although Bio-Mos®, vitamin E or C addition to broiler diets seemed to alter IgG concentrations in plasma in various times, they did not improve the immune system in broilers. With regard to performance, BW, FI and FCR were not affected by dietary treatment. Therefore, 1.5 g kg^{-1} Bio-Mos®, 500 μg kg^{-1} vitamin E or C may not be used in broiler diets to improve performance and enhancing immune capacity. A wider MOS, vitamin E or C supplementation range and more biochemical data in future research will help to clarify the understanding of the optimal and marginal levels for broilers with respect to performance and immune system.

REFERENCES

Abdukalykova, S.T., X. Zhao and C.A. Ruiz-Feria, 2008. Arginin and vitamin E modulate subpopulations of T lymphocytes in broiler chickens. Poult. Sci., 87: 50-55.

Awad, W., K. Ghareeb and J. Bohm, 2008. Intestinal structure and function of broiler chickens on diets supplemented with a *Synbiotic* containing *Enterococcus faecium* and *Oligosaccharides*. Int. J. Mol. Sci., 9: 2205-2216.

Bendich, A., 1990. Antioxidant vitamins and their functions in immune response. Adv. Exp. Med. Biol., 262: 35-55.

Bhaskaram, P., 2001. Immunobiology of mild micronutrient deficiencies. Br. J. Nutr., 85: S75-S80.

Blaha, J. and K. Kreosna, 1997. Effect of vitamin and electrolytes supplements on broilers performance, slaughter value and chemical composition of meat during the heat stres. Univ. Agric. Praga Press, 30: 103-113.

Blake, J.P., J.B. Hess, K.S. Maklin, S.F. Bilgili, A.E. Sefton and A. Kocher, 2006. Mannan oligosaccharide supplementation of wheatbased diets for broilers. Proc. 12th Eur. Poult. Conf. Suppl. World Poult. Sci., 62: 342-342.

Cetin, N., B.K. Guclu and E. Cetin, 2005. The effects of probiotic and mannan-oligosaccharide on some haematological and immunological parameters in Turkeys. J. Vet. Med., 52: 263-267.

Coetzee, G.J.M. and L.C. Hoffman, 2001. Effect of dietary vitamin E on the performance of broilers and quality of broiler meat during refrigerated and frozen storage. South Afr. J. Anim. Sci., 31: 158-173.

Colnago, G.L., L.S. Jensen and P.L. Long, 1984. Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. Poult. Sci., 63: 1136-1143.

Cotter, P.F., A. Malzone, B. Paluch, M.S. Lilburn and A.E. Sefton, 2000. Modulation of humoral immunity in commercial laying hens by a dietary prebiotic. Poult. Sci., 79: 38-38.

Cotter, P.F., A.E. Sefton and M.S. Lilburn, 2002. Manipulating the Immune System of Layers and Breeders: Novel Applications of Mannan Oligosaccharides. In: Nutritional Biotechnology in the Feed and Food Industries, Lyons, T.P. and K.A. Jacques (Eds.). Nottingham University Press, Nottingham, England, pp: 21-27.

Cusack, P., N. McMeniman, A. Rabiee and I. Lean, 2009. Assessment of the effects of supplementation with vitamin E on health and production of feedlot cattle using meta-analysis. Prev. Vet. Med., 88: 229-246.

Eren, M., G. Deniz, H. Biricik, S.S. Gezen, I.I. Turkmen and H.M. Yavuz, 1999. Effects of supplementation of zinc bacitracin, mannanoligosaccharide and Probiotic in broiler feeds on fattening performance. Uludag Univ. Vet. Fak. Derg., 18: 73-84.

Erf, G.F., W.G. Bottje, M.D. Bersi, M.D. Headrick and C.A. Fritts, 1998. Effects of dietary vitamin E on the immune system in broilers: Altered proportions of CD4 T cells in the thymus and spleen. Poult. Sci., 77: 529-537.

Erhard, M.H., I.V. Quistorp, I. Schranner, A. Jungling, B. Kaspers, P. Schmidt and R. Kuhlmann, 1992. Development of specific enzyme-linked immunosorbent antibody assay systems for the detection of chicken immunoglobulins G, M and A using monoclonal antibodies. Poult. Sci., 71: 302-310.

Fritts, C.A. and P.W. Waldroup, 2003. Evaluation of bio-mos mannan oligosaccharide as a replacement for growth promoting antibiotics in diets for Turkeys. Int. J. Poult. Sci., 2: 19-22.

Gore, A.B. and M.A. Qureshi, 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. Poult. Sci., 76: 984-991.

Grimble, R.F., 2001. Nutritional modulation of immune function. Proc. Nutr. Soc., 60: 389-397.

Hamal, K.R., S.C. Burgess, I.Y. Pevzner and G.F. Erf, 2006. Maternal antibody transfer from dams to their egg yolks, egg whites and chicks in meat lines of chickens. Poult. Sci., 85: 1364-1372.

Hangalapura, B.N., M.G. Nieuwland, G. de Vries-Reilingh, J. Buyse, H. van den Brand, B. Kemp and H.K. Parmentier, 2005. Severe feed restriction enhances innate immunity but suppresses cellular immunity in chicken lines divergently selected for antibody responses. Poult. Sci., 84: 1520-1529.

Hesta, M., C. Ottermans, S. Krammer-Lukas, J. Zentek, P. Hellweg, J. Buyse and G.P. Janssens, 2009. The effect of vitamin C supplementation in healthy dogs on antioxidative capacity and immune parameters. J. Anim. Physiol. Anim. Nutr. (Berl.), 93: 26-34.

Iji, P.A., A.A. Saki and D.R. Tivey, 2001. Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide. J. Sci. Food Agric., 81: 1186-1192.

Jaffar, G.H. and J. Blaha, 1996. Effect of ascorbic acid supplementation in drinking water on growth rate, feed consumption and feed efficiency of broiler chickens maintained under acute heat stress conditions. Zivocisna Vgroba, 41: 485-490.

Janardhana, V., M.M. Broadway, M.P. Bruce, J.W. Lowenthal, M.S. Geier, R.J. Hughes and A.G.D. Bean, 2009. Prebiotics modulate immune responses in the gut-associated lymphoid tissue of chickens. J. Nutr., 139: 1404-1409.

Kidd, M.T., 2004. Nutritional modulation of immune function in broilers. Poult. Sci., 83: 650-657.

Konjufca, V.K., W.G. Bottje, T.K. Bersi and G.F. Erf, 2004. Influence of dietary vitamin E on phagocytic functions of macrophages in broilers. Poult. Sci., 83: 1530-1534.

Leshchinsky, T.V. and K.C. Klasing, 2001. Relationship between the level of dietary vitamin E and the immune response of broiler chickens. Poult. Sci., 80: 1590-1599.

Machlin, L.J., 1984. Vitamin E. In: Handbook of Vitamins, Machlin, L.J. (Ed.). Marcel Dekker Inc., New York.

McKee, J.S. and P.C. Harrison, 1995. Effect of supplemental ascorbic acid on the performance of broilers chickens exposed to multiple concurrent stress ors. Poult. Sci., 74: 1772-1785.

Meydani, S.N. and J.B. Blumberg, 1993. Vitamin E and the Immune Response. In: Nutrient Modulation of the Immune Response, Unnigham-Rundles, S. (Ed.). Marcel Dekker, New York, pp: 233-238.

Nameghi, A.H., H.N. Moghaddam, J.T. Afshari and H. Kermanshahi, 2007. Effects of vitamin E and C supplementation on performance and immune response of broiler chicks. J. Anim. Vet. Adv., 6: 1060-1069.

Niu, Z.Y., F.Z. Liu, Q.L. Yan and W.C. Li, 2009. Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. Poult. Sci., 88: 2101-2107.

Patterson, R., J.S. Younger, W.O. Weigle and F.J. Dixon, 1962. The metabolism of serum proteins in the hen and chick and secretion of serum proteins by the ovary of the hen. J. Gen. Physiol., 45: 501-513.

Puls, R., 1994. Vitamin Levels in Animal Health: Diagnostic Data and Bibliographies. 1st Edn., Sherpa International, Clearbrook, BC, Canada.

Raza, F.K., S.A. Khan, A. Raza, M.A. Saeedand and I.N. Bashir, 1997. Effect of vitamin E deficiency and excess on immune system of broilers. Int. J. Anim. Sci., 12: 39-41.

Richter G., I. Rodel, E, Wunderlich and E. Marckwardt, 1985. Evaluation of laying-hen feed with varied vitamin E and antioxidant supplementation. Arch. Tierernahr., 35: 707-714.

Rosen, G.D., 2007. Holo-analysis of the efficacy of bio-mos in broiler nutrition. Br. Poult. Sci., 48: 21-26.

Ruiz-Feria, C.A., 2009. Concurrent supplementation of arginine, vitamin E and vitamin C improve cardiopulmonary performance in broilers chickens. Poult. Sci., 88: 526-535.

Sahin, K. and O. Kucuk, 2001. Effects of vitamin C and vitamin E on performance, digestion of nutrients and carcass characteristics of Japanese quails reared under chronic heat stress (34EC). J. Anim. Physiol. Anim. Nutr., 85: 335-342.

Savage, T.F., P.F. Cotter and E.I. Zakrzewska, 1996. The effect of feeding a mannan oligosaccharide on Immunoglobulins, plasma IgG and bile IgA of Wrolstad MW male turkeys. Poult. Sci., 75: 143-143.

Shashidhara, R.G. and G. Devegowda, 2003. Effect of dietary mannan oligosaccharide on broiler breeder production traits and immunity. J. Poult. Sci., 82: 1319-1325.

Stanczuk, J., Z. Zdunczyk, J. Juskiewicz and J. Jankowski, 2005. Indices of response of young turkeys to diets containing mannanoligosaccharide or inulin. Vet. Zootech., 31: 98-101.

Swanson, K.S., C.M. Grieshop, E.A. Flickinger, L.L. Bauer and H.P. Healy *et al*., 2002. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. J. Nutr., 132: 980-989.

Waldroup, P.W., E.O. Oviedo-Rondon and C.A. Fritts, 2003. Comparison of Bio-Mos and antibiotic feeding programs in broiler diets containing copper sulfate. Int. J. Poult. Sci., 2: 28-31.

Wu, C.C., T. Dorairajan and T.L. Lin, 2000. Effect of ascorbic acid supplementation on the immune response of chickens vaccinated and challenged with infectious bursal disease virus. Vet. Immunol. Immunopathol., 74: 145-152.

Yalcinkayal, H., T. Gungori, M. Bafialani and E. Erdem, 2008. Mannan oligosaccharides (MOS) from *Saccharomyces cerevisiae* in broilers: Effects on performance and blood biochemistry. Turk. J. Vet. Anim. Sci., 32: 43-48.

Yang, Y., P.A. Iji, A. Kocher, L.L. Mikkelsen and M. Choct, 2007. Effects of mannanoligosaccharide on growth performance, the development of gut microflora and gut function of broiler chickens raised on new litter. J. Applied Poult. Res., 16: 280-288.

Zhao J., D. Li, X. Piao, W. Yang and F. Wang, 2002. Effects of vitamin C supplementation on performance, iron status and immune function of weaned piglets. Arch. Tierernahr., 56: 33-40.

Zou, X.T., X.J. Qiao and Z.R. Xu, 2006. Effect of B-Mannanase (*Hemicell*) on growth performance and ýmmunity of broilers. Poult. Sci., 85: 1276-1279.