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The effect of threonine and vitamin A on immune system in broiler chickens

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ABSTRACT

Two independent experiments were conducted with broilers to determine the effect of threonine and vitamin A on immune system. In first experiment, dietary treatments contained 0.8%, 0.87%, 0.94 % and 1.01 % total threonine. In second experiment, diets were as follows: basal diet without vitamin A; basal diet plus 1500 IU/Kg vitamin A; basal diet plus 6250 IU/Kg vitamin A; basal diet plus 11000 IU/Kg vitamin A. Fourteen days after feeding the treatments, cutaneous basophil hypersensitivity, cell-mediated immune response, was determined as the increase in toe-web skin thickness after an injection with phytohemagglutinin-P and twenty-two days after feeding the diets, heterophils and lymphocytes were enumerated. Humoral immunity was assessed by intravenous injection of 7% sheep red blood cell at 22 days of age. In first experiment, the titers of immunoglobulin for responses were numerically, but not significantly, increased in birds fed diet containing 0.87 % threonine and decreased in 0.94% and 1.01% threonine. Cutaneous basophil hypersensitivity was not affected in chicks, whereas there was a tendency for an increase in birds fed a diet containing 0.87 % threonine at 24 h after injection. In second experiment, the titers of immunoglobulin for responses were significantly increased in birds fed diet containing vitamin A. Cutaneous basophil hypersensitivity was also increased by addition vitamin A in the diet. The results obtained on the present study indicated that threonine and Vitamin A requirements of broiler based on recommendation of national research council are not sufficient to meet the requirement of the new commercial poultry.

Key words: threonine, vitamin A, cutaneous basophil hypersensitivity, hematological profile, broiler

INTRODUCTION

The effect of nutrition on immunology is a new scientific subject and nutrients affect metabolism and function of cells in immune system at molecular, cellular, tissue and whole body levels (Peng et al. 2007). Interactions, Synergisms and antagonisms between nutrition and immunity affect poultry industry (Kidd, 2004). Supplementation of poultry diets with synthetic amino acids may improve the overall amino acid balance (Sepehri et al. 2011) and increase immunoglobulins synthesis (Kogut, 2009). Also, deficiency of protein or amino acids in diet impairs immune function (Wu et al. 1999). Furthermore, broiler nutrient needs for immunity or breast meat may not be similar to growth needs (Kidd, 2004). Threonine (Thr) was discovered over 60 years ago and is considered to be the third limiting amino acid for broilers fed corn-soybean meal (Sepehri et al. 2011). The nutrient Thr must be considered in dietary formulations for commercial broilers because it's excess is costly and its deficiency will decrease the sufficiency of total sulfur amino acids and lysine use (Kidd, 2000). The portal-drained viscera have a high obligatory visceral requirement for Thr and the high rate of intestinal Thr utilization is due to incorporation into mucosal proteins (Schaart et al. 2005). Threonine directly make mucin protein that is required for maintaining intestinal immune function, inhibition of apoptosis, stimulation of lymphocyte proliferation and

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enhancement of antibody production (Kidd, 2004). The NRC (1994) recommendations for broiler chickens are used in research studies but most of these recommendations are from refereed publications of studies. The rapid development commercial broiler strains, genetic selection, management practices and feed formulation changes cause these recommendations are not efficient for giving the best performance (Lesson, 2006). Also, NRC (1994) recommendations are usually based on the needs of optimal conditions, but in commercial conditions, susceptibility of animals to infectious disease increase requirements (Lesson, 2006). Ross requirement for threonine is 14% more than NRC (1994). On the other hand, vitamin A is one of the most widely studied nutrients in relation the immune function. The term vitamin A designates a group of retinoid compounds with the biologic activity of all-trans-retinal. The field of vitamin A immunology has greatly benefited from animal experiments. These studies have provided a vast body of knowledge on the cellular and molecular mechanisms by which vitamin A and its metabolites influence the immune function at various levels (Villamor and Fawzi, 2005).
In this report, NRC requirement for vitamin A and Thr was compared to Ross recommendation for vitamin A and Thr and the more and less levels of it. More specially, our aim was to determine the effect of these levels on immune responses.

MATERIAL AND METHODS

This study was conducted in two independent experiments with same methods.

Stocks and husbandry:
In first experiment, 160 one-d-old male Ross 308 chicks were allocated to 16 pens measuring 0.9×1.2 m. Birds received 24 hour/day of light throughout the experiment. Chicks were allowed *ad libitum* access to the feed and water. Experiment was conducted in accordance with the principles of Ross manual (2007). The experiment was divided in a starter (1 to 21 d) and a grower phase (22 to 42 d), however, in order to evaluate immunoglobulin; chickens were maintained until day 48. The basal diet formulated to either meet or exceed the recommended NRC (1994) nutrient requirements for broilers. Dietary treatments contained NRC (1994) requirements (0.8 % threonine), between Ross (2007) and NRC (1994) (0.87 % threonine), Ross (2007) recommendations (0.94 % threonine) and more than Ross (2007) (1.01 % threonine). Treatment additions of L-threonine (98.5 % threonine, Degussa Co., Essen, Germany) was made at the expense of washed builders sand. Corn and soybean meal samples were analyzed for Met, Lys, Thr, and crud protein by Degussa Co., in Essen, Germany. In second experiment, one hundred sixty day-old male broiler chicks (Ross 308) were randomly assigned to 16 pens measuring. All birds fed wheat-soybean meal based diets and water and feed were provided *ad libitum*. The diets were formulated to either meet or exceed the recommended NRC (1994) nutrient requirements for broilers. Experimental diets were as follows: basal diet without vitamin A; basal diet plus 1500 IU/Kg vitamin A (NRC 1994 requirement); basal diet plus 6250 IU/Kg vitamin A (between Ross 2007 and NRC 1994 requirement); basal diet plus 11000 IU/Kg vitamin A (Ross 2007 requirement).

Cell Mediated Responses:
Cutaneous basophil hypersensitivity (CBH) response elicited by an intradermal injection of a T-cell mitogen, provided an in vivo assay for cell-mediated immunity (Boa-Amponsem et al. 2000). Fourteen days after feeding the treatments, 100 μg of phytohemagglutinin-P (PHA-P) in 0.1 mL of physiological salin solution, was injected intradermally between the third and fourth digits of the right foot in 1 bird per pen. The left foot was as control and 0.1 mL of physiological saline solution was injected to it. The right and left toe web thickness was measured in millimeter prior and 20, 24 and 28 h following the injection with PHA-P (Boa-Amponsem et al. 2000). PHA-P response was determined by subtracting preinjection skin thickness, of right foot from post injection skin thickness of right foot (postinjection skin thickness, right foot- preinjection skin thickness, right foot). Salin response was postinjection skin thickness of left food and the cutaneous basophil hypersensitivity (CBH) response was calculated by subtracting saline response of left foot of PHA-P response (Boa-Amponsem et al. 2000).

Humoral Responses:

On day 22 of feeding treatments, two chicks per pen, were immunized intravenously via the wing with 1 mL of 7 % suspension of sheep red blood cell (SRBC) in 0.85 % physiological saline solution. For SRBC preparation, first, blood of a male sheep was collected into tubes with EDTA 1 d before use. Then the samples were centrifuged at 2000 rpm for 15 min. Finally were washed 3 times with 0.85 % physiological salin. Seven and 26 days after injection, chicks were bled via the brachial vein into tubes with EDTA (Bartlett and Smith, 2003), and immediately, plasma was tested for IgG (2-mercaptoethanol-resistant antibodies, IgM (2-mercaptoethanol-sensitive antibodies) and IgA. Serum antibodies were determined by using ELISA kit (Viro-Immune) according to the manufacturer's instruction. Twenty two days after feeding of treatments, blood samples were obtained from 2 birds per pen and were placed in tubes with EDTA as anticoagulant for measuring hematocrit, hemoglobin, lymphocyte and monocyte. The hematocrit was determined by comparing the volume of red cells to the total volume of blood sample. Hematocrit was reported as a percentage (Estridge et al. 2000). Leukocytes were stained by using Wright-Giemsa method (Ritchie et al. 1994). Leukocytes were counted to a total of 60 cells per slide.

Statistical analysis:

The data were analyzed using General Linear Model's procedure of SAS software (SAS, 1985) in a completely randomized design. Duncan (1995)'s multiple range test was used to determine differences among treatment means. Means were considered different at $p<0.05$.

RESULT AND DISCUSSION

The effects of the incremental Thr levels on primary and secondary antibody responses presented in table 1. There was a tendency for an increase in IgG (596.3mg/dl), IgM (245mg/dl), and IgA (45mg/dl) antibodies as dietary Thr increased from 0.8% to 0.87% , however it was not significant and then decreased in 0.94% and 1.01% Thr. The titers of IgG, IgM and IgA antibodies for secondary response were higher than those for primary response. In our previous research, the best performance was belonged to birds were fed the diet containing 0.87 % Thr (Sepehri et al. 2011) and according to the present study and our previous research, there was no difference between the Thr need in chickens for antibody production to SRBC and performance. This result was not consistent with reports Takahashi et al (1994). They reported a diet deficient in Thr leads to reduced growth but not reduced antibody responses. Kidd et al (1997) found no influence of dietary Thr (0.68 to 0.86 % of diet) on immune organ development in young broilers. Increasing dietary Thr intake increased levels of IgG in sows (Cuaron et al. 1984) and antibody production and serum IgG levels in pig (Wang et al. 2006). In our review, there was a shortage of information about the effect of incremental levels of Thr on immune system specially more than NRC (1994) requirements. Bhargave et al (1971) reported the Thr requirement in broilers for antibody production was found to be more than Thr need for growth. A deficiency of Thr impairs antibody production because Thr is a major component of γ-globulin in animals, also Thr affect on inhibition of apoptosis and stimulation of lymphocyte proliferation (Li et al. 2007). Threonine is a precursor of Glycine and a dietary excess of Thr changes concentrations of Glycine, arginine and methionine, because diets containing surfeit Thr increase glycine and a dietary excess in glycine makes creatine by arginine and methionine. Under these conditions, amino acids imbalance decrease performance and immune functions (Li et al. 2007). Prior to antigenic challenge, serum from birds was analyzed for hematological parameters.

Table 1: Immune system responses of broilers fed different levels of Threonine on the serum level (mg/dl) of Immunoglobulin G (IgG), Immunoglobulin M (IgM) and Immunoglobulin A (IgA) in broiler chickens

	Primary			Secondary		
Treatment	IgG	IgM	IgA	IgG	IgM	IgA
0.80	449.1	139.1	38.7	519.5	151.2	40.0
0.87	484.5	139.7	45.5	596.8	245.0	45.0
0.94	459.5	139.3	39.1	546.3	150.0	44.0
1.01	420.3	138.8	36.7	508.8	144.5	36.2
±SEM	15.01	5.22	1.18	18.62	6.19	2.06
Pvalue	0.75	0.83	0.06	0.12	0.08	0.05

The levels of Thr in the diet did not affect the hematological profile analysis, however, birds fed diet containing 0.87and 0.94 % Thr had heightened HT and HB relative to birds fed the other treatments (Table 2). Hematological parameters determine health status and are important in clinical evaluation of the state of health. There is a dearth of information on the effect of Thr on hematological parameters in broiler; therefore, direct comparisons cannot be made. Amino acids have an important role in immune responses by regulating innate and acquired systems (Li et al. 2007). High hematocrit values indicate dehydration and are as an important signs of stress. In this condition, the corticoids released in the blood decrease the number of lymphocytes .Immune challenges increase the number of heterophils in the first 6 to 12 hours of the immune response, and this cells are the first line of defense in broiler (Da silva et al. 2010). The maximum CBH response occurred in broilers fed 0.87 % Thr at 20, 24 and 28 h after PHA-P injection (Table 3).

Table 2: Hematological profile of 22-d-old broilers fed different threonine levels on the serum level (%) of Hematocrit (HT), Hemoglobin (HB), Monocyte (M), Lymphocyte (L), Hetrerophil (H) and Heterophil/Lymphocyte ratio (H/L) in broiler chickens

Treatment	HT	HB	M	L	H	H/L
0.80	28.0	8.8	6.0	58.7	23.4	0.40
0.87	28.5	9.3	7.2	58.9	23.0	0.39
0.94	28.5	9.2	5.7	58.5	22.2	0.38
1.01	27.0	8.8	5.7	57.5	23.5	0.41
±SEM	1.92	0.79	0.54	3.06	2.01	0.03
P value	0.06	0.07	0.08	0.07	0.07	0.06

Table 3: Cutaneous basophil hypersensitivity response of birds fed incremental levels of threonine

	Toe webb swelling in mm		
Treatment	20 h	24 h	28 h
0.80	0.41	0.46	0.41
0.87	0.42	0.49	0.43
0.94	0.41	0.45	0.40
1.01	0.40	0.43	0.39
±SEM	0.03	0.04	0.08
P value	0.07	0.08	0.07

The least response was belonged to the diet containing 1.01 % Thr. In all treatments, at 24 h after PHA-P injection, CBH response increased and at 28 h after PHA-P injection, this response decreased. The CBH response made in broiler chickens by intradermal injection of PHA-P is a thymus-dependent response mediated by T cells (Aslam et al. 1998). Increase of Thr in the diet containing 1.01 % Thr reduced T cell-mediated immunocomptence in chicks. Because of a negative impact of amino acid imbalance and antagonism on nutrient intake and utilization, an excess supply of amino acids in the diet can be deleterious to the immune system (Li et al. 2007). CBH test was a valid test in 12- to 19-d-old broiler chicks. In the current studies, this test was performed on 14-d-old chicks.

In the second experiment, the titers of primary and secondary antibodies in chickens fed accordance to Ross (2007) requirement were the highest. The results of serum immunoglobulins in chickens were showed in table 4. IgA at the primary response and IgG and IgM at the primary and secondary responses significantly increased ($p<0.05$) in chickens fed vitamin A.

Table 4: Immune system responses of broilers fed different levels of Vitamin A on the serum level (mg/dl) of Immunoglobulin G (IgG), Immunoglobulin M (IgM) and Immunoglobulin A (IgA) in broiler chickens

	Primary			Secondary		
Treatment	IgG	IgM	IgA	IgG	IgM	IgA
0	423^{b}	140^{c}	40^{b}	401^{c}	140^{d}	42
1500	683^{a}	190^{a}	56^{a}	472^{c}	191^{c}	41
6250	507^{b}	143^{c}	42^{b}	614^{b}	187^{b}	41
11000	800^{a}	174^{b}	61^{a}	740^{a}	208^{a}	46
±SEM	31.17	2.88	1.47	14.66	16.98	0.94
Pvalue	0.0001	0.0001	0.0001	0.001	0.001	0.12

$^{a-d}$ Means within columns with different superscripts are significantly different ($P<0.05$).

This finding was consistent with previous reports that a diet deficient in vitamin A leads to reduce antibody production to protein antigens (Sklan et al. 1994). Also administering vitamin A supplements to children resulted in a significant increase in the immunoglobulin G response generated against a vaccine dose of tetanus toxoid (Semba et al. 1994). In contrast to this finding, Livingston and Klasing (2011) reported the chickens were allotted to a diet that contained 130% of NRC (1994) recommendation for vitamin A had a lower primary IgM and IgG antibody response ($p<0.02$) and the secondary IgG response was significantly lower ($p=0.004$). There are so many differences between NRC (1994) recommendations for vitamin A in comparison to the requirement of Ross (2007). It is 1500 IU/Kg in NRC (1994) and 11000 IU/Kg in Ross (2007). Maggini et al (2007) reported that vitamin A deficiency is associated with diminish phagocytic and oxidative burst activity of macrophages activated during inflammation and a reduce number and activity of natural killer cells. Vitamin A supplementation has been shown to improve antibody titer response to various vaccines. Villamor and Fawzi (2005) reported that vitamin A does have direct effect on B cells and immunoglobulin synthesis. The hematological values for the 22 d-old broilers (Table 5) were within the normal parameters for this type of bird. The level of HT and HB did not induce an effect in these responses. Similarly, no effects of L, H and H/L were seen for hematological values. In the majority of avian species, healthy animals have more lymphocytes than heterophils in circulation, which influences the H/L ration. An immunological challenge can raise the number of heterophils in the 6 to 12 h of an immune response because these cells participate in the first line of response in birds (Silva et al. 2009). The CBH response elicited in chickens by an intradermal injection of PHA-P is a thymus dependent response mediated by T cells (Carrier and Deloach, 1990). The effect of incremental vitamin A levels on CBH responses presented in table 6. Cutaneous basophil hypersensitivity assay had linear increased with increased vitamin A in all sampling hours ($p<0.05$). Chickens fed no vitamin A significantly depressed CBH responses of 20, 24 and 28 h after PHA-P ($p<0.05$). There was a tendency for an increase in CBH responses at each time period as dietary vitamin A increased. The maximum CBH response occurred at 24 h after PHA-P injection. These results agree with those of Lessard et al (1997) who reported that interdigital skin reactions to phytohemagglutinin (PHA) were significantly reduced in chickens fed the low vitamin A diet.

Table 5: Hematological profile of 22-d-old broilers fed different Vitamin A levels on the serum level (%) of Hematocrit (HT), Hemoglobin (HB), Monocyte (M), Lymphocyte (L), Hetrerophil (H) and Heterophil/Lymphocyte ratio (H/L) in broiler chickens

Treatment	HT	HB	M	L	H	H/L
0	31.9	6.7	4.0	71.4	23.2	0.55
1500	32.4	7.0	3.4	70.4	25.0	0.53
6250	32.4	6.8	3.0	71.2	24.0	0.50
11000	32.9	7.0	4.0	71.0	24.0	0.57
±SEM	1.26	0.89	0.48	0.67	0.57	0.15
P value	0.58	0.58	0.75	0.94	0.60	0.19

Table 6: Cutaneous basophil hypersensitivity response of birds fed incremental levels of Vitamin A

	Toe webb swelling in mm		
Treatment	20 h	24 h	28 h
0	0.38^{b}	0.39^{b}	0.35^{b}
1500	0.46^{a}	0.47^{a}	0.45^{a}
6250	0.48^{a}	0.49^{a}	0.46^{a}
11000	0.47^{a}	0.49^{a}	0.47^{a}
±SEM	0.03	0.04	0.03
P value	0.04	0.04	0.03

^{a,b} Means within columns with different superscripts are significantly different (P<0.05).

CONCLUSION

The present study has revealed the threonine and vitamin A on immune response and compared NRC requirement for Thr and vitamin A to Ross recommendation. Knowledge gained in this study find direct application of Thr and Vitamin A in the management of poultry health, promising to yield better immune response so better disease resistance and health would be possible. Under the conditions of this study, it was concluded that Thr and Vitamin A might have some positive effects on immunity response and may be used directly in the diet as immune system fortification in broiler chickens.

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