

Effects of Dietary Combinations of Vitamin A, E and Methionine on Growth Performance, Meat Quality and Immunity in Commercial Broilers

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ABSTRACT : The experiment was conducted to study the effect of dietary combinations of vitamin A (VA), vitamin E (VE) and methionine (Met) on growth performance, meat quality and immunity in commercial broilers. Ross chicks (n=3,630) were allocated to five experimental treatments with three replicates per diet. The dietary treatments were: VA 8,000 IU, VE 10 IU (diet 1); VA 12,000 IU, VE 10 IU (diet 2); VA 8,000 IU, VE 100 IU (diet 3); VA 12,000 IU, VE 100 IU (diet 4) and; VA 12,000 IU, VE 100 IU/kg diet and 20% Met higher than other groups (diet 5). The Met content in diet 1 to diet 4 were as per the requirement suggested by NRC. Separate vitamin premixes were prepared for each treatment diet as per the requirement of study. The 35 d study revealed significantly ($p < 0.0001$) higher weight gains in broilers fed diet 3 and diet 5, than in the rest of the groups during starter phase (0-3 weeks) only. The feed intake did not vary significantly at all phases of study, but feed efficiency was significantly ($p < 0.05$) lower in diet 1 during starter and overall phase (4-5 weeks). The bone strength and bone composition, except bone calcium, remained unaffected due to experimental diets studied after 35 d of experimental feeding. The thio-barbituric acid reactive substances (TBARS) were significantly ($p = 0.0013$) lower in the breast meat in group 5, followed by group 3, than in the rest of the groups. The immune studies conducted, antibody titers to sheep red blood cells, thickness index to phytohaemagglutinin-P, and heterophil: lymphocyte ratio, did not show any significant difference among treatments. It could be concluded that supplementation of VA, VE and Met at higher levels could be beneficial to broilers only during the starter phase. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 4 : 516-523)

Key Words : Vitamin A, Vitamin E, Methionine, Broiler, Bone, Immunity

INTRODUCTION

There are some interactions among fat-soluble vitamins, although it is far from clear if such effects are meaningful at normal level of inclusion. High dietary vitamin A (VA) levels have been shown to reduce vitamin E (VE) absorption in several species (Abawi and Sullivan, 1989; Blakely et al., 1991; Aburto and Britton, 1998). Dietary VA seems to affect the apparent absorption and retention of VE in the weaned pig confirming that an interrelationship exists between these two vitamins (Ching et al., 2002). They found that as the dietary VA level increased there was a negative impact on both the serum concentration and liver retention of α -tocopherol. There are abundant researches available where supplementation of VA and VE had improved growth and immunity (Friedman and Sklan, 1997; Guo et al., 2001). The vitamin levels as suggested by NRC (1994) are far below that is required for best performances to exploit the full genetic potential of the birds under certain environment (Coelho, 2000). Hence VA and VE fortification levels in commercial starter diets are generally added in excess of NRC standards. Various indigenous and environmental factors present on many commercial farms might cause the animal to have a higher requirement or factors that could cause deterioration of dietary vitamins (Coelho, 2000).

VA levels necessary to maximize immuno-competence have also been shown to be much higher than that needed for optimum growth and feed efficiency (Friedman and Sklan, 1997; Korver and Klasing, 2003). Optimum immune responses in growing chicks and turkeys were obtained with VA intakes that were 3-10 fold higher than NRC recommended levels (Friedman and Sklan, 1997). VA keeps the mucous membrane of eyes, respiratory and digestive tract healthy, which are immune system first line of defense against invading pathogens. Clear evidence exists that VA deficiency is associated with impaired immune functions and reduced resistance to infections (Friedman et al., 1991). Cytokine production, lymphocyte blastogenesis, natural killer cell activity and phagocytosis have been reported to increase after VA treatment (Ross, 1992). However Veltmann et al. (1985) found that supplementing broiler diets with VA increased the morbidity due to malabsorption (stunting) syndrome presumably by interaction with vitamin D or VE. They observed a significant interaction for bone ash in the malabsorption syndrome affected chicks, where bone ash was significantly lower in those chickens fed the higher levels of supplemental VA in the diets with a high level of vitamin D. The lower bone ash values correlated with an increased incidence of rickets, which shifted to tibial dyschondroplasia during 3rd to 4th week. They concluded that a nutritional antagonism between excess VA and VE or vitamin D might account for exacerbative effect of VA on malabsorption syndrome.

Role of VE as a biological antioxidant stabilizing the oxidation of sensitive fatty acids in the cellular metabolism

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is well established. It has to play role in growth, immunity and the protection of biological systems against oxidative damage in live animals as well as in the meat and meat products (Jacobsen et al., 1995; Guo et al., 2001). The levels to achieve these effects are typically in the order of 100-300 mg/kg feed. Meager studies were carried out with respect to dietary combinations of VA and VE and their effect on immunity.

Protein is especially important for empowering the immune system, as antibodies and T-cells are made up of proteins and need a constant supply to make sure that the chicken's body is well defended. Tsiabge et al. (1987) suggested that the requirement of methionine (Met) for maximum antibody titers was greater than that for growth. Dietary Met levels slightly in excess of the requirements for maximum growth rate were required in order to maximize the antibody titers. They noted that the immune response of chicks challenged with an antigen changes with supplemental Met. Met was observed to activate certain parts of the chicks immune system. The mechanism was unclear and the therapeutic effectiveness of Met was not studied. It has also been researched that 0.65% Met and 3,300 mg/kg diet choline enhanced the humoral mediated immunity in broilers (Swain and Johri, 2000). But Bhargava et al. (1970) reported increased antibody levels in Met deficiency. Results of studies in which deficiencies in total

sulfur containing amino acids were caused have mixed results. Total sulfur amino acids (TSAA) deficiency may limit the availability of cysteine for production of glutathione, and therefore limit antioxidant defenses against reactive oxygen species produced during an immune response. Discrepancies in these results may be the result of differing experimental designs and antigens used.

Because of the discrepancies and uncertainty about the levels of VA, VE and Met, their dietary interaction/combination effect on immunity, a trial was conducted on a commercial farm to investigate the effects of supplemental levels of VA, VE individually and in combination with Met on the performance, immunity and meat quality of broilers.

MATERIALS AND METHODS

This experiment was conducted on a commercial broiler farm with 3,630 Ross female broiler chicks. A total of 242 chicks were kept in each pen with the pen size as 3.6×3.6 m and three replicates per group. Rice hull was used as litter material. The chicks were reared under temperature-controlled house to 5 weeks of age, and the temperature of the room was 35-33°C in the first 3 days, 32-30°C during the following week, and then it declined 3°C weekly until to 22-24°C. The basal composition of the starter and finisher

Table 1. Formula and chemical composition of experimental diets during feeding trial for different groups

Ingredients (%)	Diets 1 to 4		Diet 5	
	Starter	Finisher	Starter	Finisher
Maize	55.96	62.43	55.96	62.43
SBM (47.5%)	27.00	24.00	26.70	23.76
Corn gluten meal	8.10	6.00	8.10	6.00
DCP	1.80	1.55	1.80	1.55
Calcium carbonate	1.36	1.26	1.36	1.26
Choline chloride (25%)	0.20	0.20	0.20	0.20
DL-methionine (50%)	0.20	0.03	0.40	0.19
L-lysine (78%)	0.09	0.09	0.09	0.09
Salt	0.30	0.30	0.30	0.30
Trace mineral premix ¹	0.14	0.14	0.14	0.14
Vitamin premix ^{2*}	0.10	0.10	0.10	0.10
Tallow	4.75	3.90	4.85	3.98
Total	100.00	100.00	100.00	100.00
Chemical composition calculated (%)				
ME (Kcal/kg)	3,200	3,200	3,200	3,200
CP	22.44	20.31	22.30	20.19
Ca	1.00	0.90	1.00	0.90
Avail. P	0.45	0.40	0.45	0.40
Lysine	1.10	1.00	1.10	1.00
Methionine	0.50	0.38	0.60	0.46
Methionine+cystine	0.89	0.73	0.98	0.81

¹ Supplied per kg diet: 56 mg Fe, 56 mg Cu, 70 mg Zn, 84 mg Mn, 1.4 mg I, 0.07 mg Co, 0.2 mg Se.

² Vitamin premix per kg diet: 1,800 IU vitamin D, 1 mg vitamin K₃, 1 mg vitamin B₁, 10 mg vitamin B₂.

4 mg vitamin B₆, 0.02 mg vitamin B₁₂, 30 mg niacin, 12 mg pantothenic acid, 0.5 mg folic acid, 0.2 mg biotin.

* Vitamin A and vitamin E were added as per the levels shown below for groups 1 to 5 per kg diet in the vitamin premix.

Group 1- 8,000 IU vitamin A and 10 IU vitamin E. Group 2- 12,000 IU vitamin A and 10 IU vitamin E.

Group 3- 8,000 IU vitamin A and 100 IU vitamin E. Group 4- 12,000 IU vitamin A and 100 IU vitamin E.

Group 5- 12,000 IU vitamin A, 100 IU vitamin E.

diet is presented in Table 1, which was fed to groups 1 to 5. Separate vitamin premixes were prepared for each group, which was added at the level of 1 kg per ton of feed. The vitamin premixes contain all the other vitamins levels in common except the levels of VA and VE as per the requirement of the study.

The Met contents in groups 1 to 4 were as per the NRC (1994) levels that are 0.50% for starter and 0.38% for finisher. For group 5, which contains 20% higher Met level than NRC (1994), a separate formulation was made with little adjustments, as shown in Table 1. The diets were prepared in mash form and were fed *ad libitum*. The supplemental VA fed was retinyl acetate and VE was α -tocopherol acetate supplied by Roche Vitamins, Korea and the Met was fed as DL-Met from Degussa amino acid supplier in Korea. The managemental practices remained the same for each group, which is normally followed. In short, the dietary treatments were: VA 8,000 IU, VE 10 IU (diet 1); VA 12,000 IU, VE 10 IU (diet 2); VA 8,000 IU, VE 100 IU (diet 3); VA 12,000 IU, VE 100 IU (diet 4) and; VA 12,000 IU, VE 100 IU/kg and 20% Met higher than other groups (diet 5).

Body weight and feed intake were measured at 21 days and 35 days of feeding respectively, and feed efficiency was calculated. The mean body weights by pen were taken at respective intervals. The chicks were not fed for 4 h prior to take the body weight. Accurate records were kept for all feed added to each replicate and the amount of residual feed remaining at the end of feeding periods. Feed conversion efficiency was calculated. Mortality was recorded.

At 35 days of age, randomly 6 birds per group (2 from each replicate), which represents the flock visually, were decapitated to measure the effect of vitamin supplementation on the bone strength and composition. Bone mineralisation was studied in terms of bone breaking strength of tibia, and ash, calcium and phosphorus content. For this, both the tibia was freed of soft tissues. The dried (100°C, 3 h) bone samples were defatted in petroleum ether for 48 h. The right tibia was separated and stored at -20°C and then used for bone breaking strength using Universal testing machine (Model SFM-20, United Calibration Corporation, USA) and the force required was measured as kg/mm². The left tibia was used for determining its chemical composition on dry fat free bones as described by AOAC (1990). The breast meat was stored at 1°C for one week after slaughtering to determine the thio-barbituric acid reactive substances (TBARS) as milligrams of malonaldehyde (MDA/kg) by the method of Sinnhuber and Yu (1977). Chicken meat color of the breast meat was measured with a color difference meter (Yasuda Seiko Co. CR 310, Minolta, Japan) and compared with standard color values.

The immune studies were done at 35th day of age.

Blood smears were prepared from 6 birds per group and measured the heterophil:lymphocyte ratio to study the immune status of the bird. The PHA-P mitogen was injected intradermally in the right wattle to study the cutaneous basophilic hypersensitivity. The concentration of PHA-P was 250 micrograms in 0.1 ml normal saline and was injected in marked six birds per group and in left wattle only 0.1 ml normal saline was injected intradermally and then thickness index (TI) was measured. The thickness of the injected region was measured prior to injection and 24 h after injection with the help of vernier callipers. The TI was expressed as percentage increase in wattle thickness as suggested by Praharaj et al. (2002). The TI was calculated as (right wattle thickness due to PHA-P at 24 h post inoculation/corresponding right wattle thickness before inoculation)-(left wattle thickness due to normal saline at 24 h post inoculation/corresponding left wattle thickness before inoculation). On the same day, 2.5% SRBC in normal saline was injected in marked 6 birds per group and then blood was collected after 5 days of injection. Serum was separated and the haemagglutination titers were measured.

Statistical analysis was done by using the GLM procedure of SAS software (1985) using a completely randomized design. The treatments were the main effect. Data was subjected to one-way analysis of variance using GLM procedure of SAS. When significant interactions were found, comparisons among means were made by LSD's multiple range test and significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Growth performance

The growth performance data revealed a significant ($p < 0.0001$) increase in body weight gain fed higher levels of VE (Group 3) and the least in lower VA and VE levels (Group 1) during starter phase (Table 2). The average day-old weight of chick was 39.99 g, 39.15 g, 39.47 g, 39.39 g, and 39.45 g for groups 1, 2, 3, 4 and 5, respectively. The weight gains in bird fed diets 4 and 5 also showed higher values of weight gains but comparatively little lower than group 3. During finisher phase (4-5 week), supplementation of higher levels of VA, VE or Met did not affect the body weight gain though numerically higher values were noted in broilers fed diet 5. Similar trend was also noticed when overall study was done. The feed intake remained unaffected due to dietary treatment at all phases of measurements. The feed efficiency was significantly ($p = 0.0327$) higher in broilers fed 100 IU VE along with VA 8000 IU during starter phase (Group 3). The supplementation of VE at higher levels was beneficial to reduce the growth and environmental stress, which generally encompasses at early age, which helped to gain

Table 2. Effect of VA, VE and Met on the growth performance of broilers

	A - 1 ¹	A - 2 ²	A - 3 ³	A - 4 ⁴	A - 5 ⁵	SE	P-value
Avg. day-old weight (g)	39.99	39.15	39.47	39.39	39.45		
0-3 weeks							
Weight gain (g)	508.64 ^c	558.93 ^b	588.53 ^a	574.93 ^{ab}	582.56 ^a	8.08	<0.0001
Feed intake (g)	916.67	921.52	919.05	946.56	991.34	11.34	NS
Feed efficiency	0.555 ^c	0.608 ^{ab}	0.640 ^a	0.608 ^{ab}	0.588 ^b	0.01	0.0327
4-5 weeks							
Weight gain (g)	784.18	781.08	798.12	791.51	803.12	9.79	NS
Feed intake (g)	1,556.10	1,521.73	1,561.40	1,549.66	1,549.12	14.48	NS
Feed efficiency	0.504	0.514	0.511	0.511	0.519	0.01	NS
0-5 weeks							
Weight gain (g)	1,292.82	1,340.00	1,386.65	1,366.44	1,385.68	13.46	NS
Feed intake (g)	2,473.07	2,443.25	2,480.46	2,496.22	2,540.46	15.56	NS
Feed efficiency	0.523 ^b	0.548 ^a	0.559 ^a	0.547 ^a	0.545 ^a	0.00	0.0237

¹ vitamin A: 8,000 IU, vitamin E: 10 IU. ² vitamin A: 12,000 IU, vitamin E: 10 IU.

³ vitamin A: 8,000 IU, vitamin E: 100 IU. ⁴ vitamin A: 12,000 IU, vitamin E: 100 IU.

⁵ vitamin A: 12,000 IU, vitamin E: 100 IU, 20% Methionine higher than other group.

NS: not significant. SE: Pooled standard error.

more body weight in these groups. Earlier studies in our lab also reported beneficial role of VE in gaining weight at 100 and 200 IU/kg diets in broilers during starter phase (our unpublished observations). Supplementation of VA at higher level (group 2) alone was also beneficial to improve growth than lower level (group 1) only during starter phase. VA has a role as antioxidant. VA at higher levels (12,000 IU) might have prevented the birds from oxidative stress which can also be observed after seeing the TBARS values (Table 4) and thus helped in gaining more body weight.

Met along with higher levels of both vitamins (group 5) did not improve the weight gain and it proved similar to groups 3 and 4. Feed efficiency tends to be significantly lower ($p=0.0237$) at lower levels of both vitamins addition (diet 1). It is a general trend to supplement the vitamins at higher levels than NRC (1994) requirements as indigenous and environmental factors at commercial situation causes deterioration of dietary vitamins (Coelho, 2000). Neither beneficial nor detrimental effects was also recorded by Ching et al. (2002) on post-weaning pig performances when two VE sources were fed at 15 and 90 IU/kg diet along with VA at two levels (2,200 IU/kg and 13,200 IU/kg). But they reported that dietary VA did seem to affect the apparent absorption and retention of VE in the weaned pig confirming that an interrelationship exists between these two vitamins. High dietary levels of VA reduced the absorption of α -tocopherol in chicks (Sklan and Donoghue, 1982). Abawi and Sullivan (1989) also noted decreased plasma tocopherol concentration when chicks received a high (100,000 IU/kg) level of dietary VA. There was no significant difference in body weight of chicks when VA was fed at 12,000 IU level in our study (diet 4) as compared with diet 3 at all levels. High levels of VA did not show any detrimental effect on young chicks VE status and ultimately the antioxidant status, which was contradictory to the study by Ching et al. (2002) in pigs.

Abawi and Sullivan (1989) reported that high levels of VE (100 IU/kg or more) could be toxic in the presence of low VA (1,000 IU/kg) as measured by increased mortality rate. We could not notice such impact in our studies. In vitro hydrolysis of tocopheryl acetate inhibited by retinyl acetate was shown by Lauridsen et al. (2001), which suggest that at high VA levels there may be less hydrolysis of tocopherol acetate and hence it may obstruct the absorption of VE in chickens. The non-significant reduction in body weight in group 4 could be explained on this possibility.

Significant changes in body weight during starter phase may be due to insufficient immune status of the bird during early age and the role of VE in improving immunity. The benefits in performance from high dietary concentration of VE would only be observed in the presence of free radical attack on immune system (Franchini et al., 1988; Rice and Kennedy, 1988). Feeding 6,000-20,000 IU VA/kg has no effect on VE status, while feeding as much as 1,000 IU VE/kg seems to have no influence on VA status was reported by Leeson and Summers (2001) that contradicts to our study. The body weight was reduced if the broiler chickens fed VA levels above 20,000 IU/kg of diet was studied by Aburto and Britton (1998) when they fed 5,000, 10,000, 20,000, 40,000, 80,000 and 160,000 IU/kg VA.

Recent researches have shown that Met in excess of NRC (1984) recommendations have resulted in enhanced performance as studied by Hickling et al. (1990), Schutte and Pack (1995) and Wallis (1999) in broilers. Hickling et al. (1990) fed broilers two Met levels (100 or 116% of NRC, 1984) and found that increasing Met significantly improved body weight and feed conversion ratio (FCR) at 6 wk but not at 3 wk. Schutte and Pack (1995) after feeding a range of Met or TSAA levels for 14 to 38 d, estimated a TSAA requirement of 0.84% for BW gain and 0.88% for FCR. But Jianlin et al. (2004) did not find any effect on body weight of broilers fed four levels of Met and TSAA at 0, 0.05%,

Table 3. Effect on bone strength and bone composition

	A-1 ¹	A-2 ²	A-3 ³	A-4 ⁴	A-5 ⁵	SE	P-value
Bone strength (kg/mm ²)	9.70	11.84	8.40	10.24	8.47	0.47	NS
Bone composition (%)							
DM	53.19	54.18	55.13	51.31	51.82	0.50	NS
Ash	58.49	58.10	59.61	58.81	56.29	0.56	NS
Ca	20.34 ^a	18.25 ^c	20.22 ^a	18.42 ^c	19.36 ^b	0.22	<0.0001
P	9.92	9.58	10.17	9.94	9.93	0.11	NS

¹ vitamin A: 8,000 IU, vitamin E: 10 IU. ² vitamin A: 12,000 IU, vitamin E: 10 IU.

³ vitamin A: 8,000 IU, vitamin E: 100 IU. ⁴ vitamin A: 12,000 IU, vitamin E: 100 IU.

⁵ vitamin A: 12,000 IU, vitamin E: 100 IU, 20% Methionine higher than other group.

NS: not significant. SE: Pooled standard error.

Table 4: Effect on TBARS and meat color

	A-1 ¹	A-2 ²	A-3 ³	A-4 ⁴	A-5 ⁵	SE	P-value
TBARS							
Breast meat (mg/kg)	0.62 ^a	0.34 ^b	0.26 ^{bc}	0.34 ^b	0.15 ^c	0.04	0.0013
Meat color							
L	56.83 ^a	56.09 ^a	53.48 ^b	56.17 ^a	56.41 ^a	0.40	0.0381
a	11.07	11.04	11.93	9.96	10.78	0.22	NS
b	16.58 ^a	16.08 ^{ab}	14.87 ^b	17.06 ^a	16.95 ^a	0.26	0.0340

¹ vitamin A: 8,000 IU, vitamin E: 10 IU. ² vitamin A: 12,000 IU, vitamin E: 10 IU.

³ vitamin A: 8,000 IU, vitamin E: 100 IU. ⁴ vitamin A: 12,000 IU, vitamin E: 100 IU.

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0.1% and 0.15% more of NRC (1994) levels. They noted that increasing Met above NRC improved FCR at 42 and 56 d, but not at other periods. They found best FCR at 0.10% Met level than NRC and other levels tested. Ramarao et al. (2003) fed 4 concentrations of Met (3.91, 4.46, 5.00 and 5.54 g/kg) to broilers and found that variation in Met concentration did not influence body weight gain or weight gain/food intake at 1 to 14, or 42 d of age, which contradicts to our study. But at 28 d of age, chicks fed on the 3.91 g Met/kg diet weighed significantly less than those on the other Met concentrations. Supplemental levels of Met (0.0, 1.5, 3.0 and 4.5 g/kg) to a basal diet containing crude protein, 221 g/kg, ME, 12.25 MJ/kg, Met, 3.6 g/kg and choline, 1,300 mg/kg fed to broilers were found to be ineffective in improving the growth, food consumption and food conversion efficiency of broilers (Swain and Johri, 2000).

The various reports mentioned above though suggest mixed results with respect to VA, VE and Met supplementation to broilers, but we found improved growth when VA, VE and Met were fed to broilers (group 5) than those supplemented at lower levels (group 1). Even VA levels studied in this experiment did not pinpoint any negative effect on growth. The study conducted by us took into consideration the practical levels of vitamins supplementation observed globally, and we conclude that these levels do not have any negative effect on growth of broilers.

Bone strength and bone composition

The bone strength was not affected due to experimental

treatments (Table 3). The dry matter, ash and phosphorus content of right tibia bone did not differ significantly because of vitamin levels but the calcium percent was significantly ($p < 0.0001$) higher in group 1 and group 3 than other groups. Fewer studies were conducted with respect to supplementation of VA, VE or Met on bone strength and composition. Though VA has a role in bone growth as VA deficiency in ducks caused a marked retardation and suppression of endochondral bone growth while excess VA results in an acceleration of this bone development (Wolbach and Hegsted, 1952). And the studies by Xu et al. (1994) that supplemental VE enhanced bone formation of chicks showed the possibility of these vitamins on bone metabolism but we could not find any such effect in our studies, but the calcium content increased in group 1 and group 3 remained obscure to us. The calcium content in left femur bone of rat was increased by supplementing VE at 90 IU/kg body weight/day was studied by Norazlina et al. (1999), and they postulated that VE supplementation induced calcium deposition in bones but it inhibited deposition of other minerals that also contribute to bone mineral density such as magnesium, phosphate and zinc.

Murphy et al. (1981) reported a decrease in bone calcium content in chicks after treatment with 10,000 IU/kg body weight/day of VE. They reported reduced calcium and phosphorus in plasma and bone ash and a significant VA×vitamin D interaction when chicks were given a high dose of VE. A significant three-way interaction among vitamins A, D₃ and E was also noted for plasma VE concentrations in broiler chickens (Abawi and Sullivan, 1989). Frankel (1995) conducted experiments in broiler

Table 5. Effect on immune parameters

	A-1 ¹	A-2 ²	A-3 ³	A-4 ⁴	A-5 ⁵	SE	P-value
Heterophil:lymphocyte	0.41	0.31	0.23	0.33	0.34	0.02	NS
Anti-SRBC titers	20.00	18.00	10.00	18.00	20.00	1.96	NS
Thickness index	48.85	7.00	32.11	21.36	45.95	7.28	NS

¹ vitamin A: 8,000 IU, vitamin E: 10 IU. ² vitamin A: 12,000 IU, vitamin E: 10 IU.

³ vitamin A: 8,000 IU, vitamin E: 100 IU. ⁴ vitamin A: 12,000 IU, vitamin E: 100 IU.

⁵ vitamin A: 12,000 IU, vitamin E: 100 IU, 20% Methionine higher than other group.

NS: not significant. SE: pooled standard error.

chicks to determine whether dietary imbalances of sulfur amino acids (SAA), VA, or interactions between two nutrients could influence organic bone matrix metabolism measured with L-³⁵S-methionine. They found in vivo incorporation of L-³⁵S-methionine into the tibio-tarsal bone matrix of 2 week old birds was not affected by VA treatment of 10 and 100 times the requirement, when compared with that of birds receiving recommended amounts of VA. Its incorporation was significantly reduced by increasing the sulfur amino acids concentration of the diet to 1.5 times the requirement, relative to lysine. In his second experiment, in vitro incorporation of ³⁵S, derived from L-³⁵S-methionine into bone matrix was reduced in birds, consuming a diet containing 1.5 times the methionine requirement relative to lysine, when compared with those receiving 0.75, 1.0 and 1.25 times the requirement. He pointed out that excess SAA could affect organic bone matrix metabolism and suggest that SAA may play a role in the etiology of tibial dyschondroplasia. The non-significant decrease in ash content and significantly ($p < 0.0001$) reduced calcium content in diet 5 than diet 1 and diet 3 in our study also supported the above results and gives the clue that excess Met has a role to play in bone matrix metabolism.

Effect on TBARS and meat color

The TBARS values in the breast meat were higher in diets fed low levels of both the vitamins (Group 1). The higher the TBARS value the more oxidation of lipids has taken place. The values were measured after one week of storage at 1°C hence higher value of TBARS was an indication of oxidation was somewhat progressed (Table 4). The TBARS value, expressed as malonaldehyde is a good index reflecting the degree of oxidation (Lohakare et al., 2004). The TBARS values were lower in the breast meat of group 5 birds, where cumulatively both vitamins and Met played role. The higher levels of VA (Group 2) and higher levels of VE (Group 3, 4 and 5) have lower TBARS than Group 1 diet, which showed that both vitamins at higher levels have anti-oxidative role. VE improved the lipid stability of meat during storage and was in agreement with that reported by Guo et al. (2001). The antioxidant function of VE persists after slaughter and delays the onset of oxidation reactions in meat and meat products (DeVore et al., 1983). The higher levels of VE and VA (Group 4) could not prevent the breast meat getting deteriorated, possibly

VA may have suppressed the absorption of VE which could have resulted the lower levels in meat as mentioned before. The 'L' and 'b' values of the breast meat measured after week storage at 1°C also showed lower values in broilers fed diet 3. It is established fact that supplementation of diet with higher levels of VE has beneficial role in meat and meat products by preventing it from getting deteriorated. The present findings were in accordance with that recorded by Mitsumoto et al. (1998) and Lauridsen et al. (1999), where VE supplementation of diets of meat producing animals effectively elevates muscle VE levels and lowers the susceptibility of muscle and ultimately products to lipid oxidation and the onset of flavor defects. Here though we have not measured the muscle VE content, but our earlier studies reported that supplementation of VE at higher levels 100 or 200 IU/kg diet positively elevates the muscle VE levels and thus prevents the muscle getting affected during 10 days storage (our unpublished observations).

Effect on immune parameters

The heterophil to lymphocyte ratio, antibody titers to sheep red blood cells and thickness index due to Phytohaemagglutinin-P (PHA-P) remained unaffected due to different experimental treatments (Table 5). The number of birds taken to conduct such studies and the difference in the individual bird response guarded to draw any conclusions out of such immune studies.

Plenty of researches available where immune response increased due to increase in vitamin levels. But there are some contradictory reports, where removal of vitamin and trace mineral premixes during grower phase did not impact immunological competence when antibody titers to SRBC were tested. These results suggested that vitamin and trace mineral contents of corn and soybean meal diet were sufficiently high to maintain a humoral immune response when the grower diet was not fortified with vitamin and trace mineral premix during 28 to 49 days post-hatching (Deyhim and Teeter, 1994). A lot of bird-to-bird variation was noted with respect to antibody titers in all groups measured, which have not ultimately shown any significant difference in our study. Gross and Siegel (1983) mentioned that H:L ratio measured a physiological change and it was a better measure of long-term changes in the environment, but it remained unaffected in our study.

VA levels necessary to maximize immuno-competence

have been shown to be much higher than that needed for optimum growth and feed efficiency (Friedman and Sklan, 1997; Korver and Klasing, 2003). The levels of VA in the present study could not show any additional benefit on the immune parameters studied.

Leshchinsky and Klasing (2001) found that the effect of VE supplementation in the humoral immune response depended on the nature of antigen. A dose-dependent increase in antibody production in response to attenuated infectious bronchitis vaccine between 0 and 25 IU/kg of supplemented VE was recognized. Antibody levels to sheep red blood cells were higher in 50 IU VE supplemented birds than 0 or 200 IU/kg of supplemented VE, while antibody production in response to *Brucella abortus* antigens and live IBV vaccine were not influenced by VE supplementation.

A number of reports cite a benefit of Met supplementation on reduced immunologic stress (Tsiagbe et al., 1987; Klasing and Barnes, 1988). Tsiagbe et al. (1987) noted that the immune response of chicks challenged with an antigen changed with supplemental Met. Met was observed to activate certain parts of chicks immune system. The mechanism was unclear and the therapeutic effectiveness of Met supplementation was not studied.

Ramarao et al. (2003) fed 4 concentrations of Met (3.91, 4.46, 5.00 and 5.54 g/kg) to broilers and found that chicks given higher concentration of Met had higher antibody titers to SRBC and E.coli inoculations and greater cutaneous basophilic hypersensitivity response to PHA-P than those given low levels of Met, indicating a higher Met requirement for immunity than for weight gain for the various genotypes in their study. Supplemental levels of Met (0.0, 1.5, 3.0 and 4.5 g/kg) and choline (0, 1,000 and 2,000 mg/kg) to a basal diet containing crude protein, 221 g/kg, ME, 12.25 MJ/kg, Met, 3.6 g/kg and choline, 1,300 mg/kg fed to broilers, the HI test and ELISA indicated a significantly improved cellular immune response receiving 3.0 g/kg Met and 3,300 mg/kg choline showing a significantly better humoral immune response reported by Swain and Johri (2000).

The present research failed to show any additive response of supplementation of VA, VE and Met on the immune parameters studied as compared with low levels (group 1). Overall we conclude that supplementation of VA, VE and Met at higher levels could be beneficial to broilers in starter phase and there was no effect on immune parameters studied during finisher phase.

REFERENCES

- Abawi, F. G. and T. W. Sullivan. 1989. Interactions of vitamins A, D₃, E and K in the diet of broiler chicks. *Poult. Sci.* 68:1490-1498.
- Aburto, A. and W. M. Britton. 1998. Effects of different levels of vitamins A and E on the utilization of cholecalciferol by broiler chickens. *Poult. Sci.* 77:570-577.
- AOAC. 1990. Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists. Arlington, Virginia.
- Bhargava, K. K., R. P. Hanson and M. L. Sunde. 1970. Effects of methionine and valine on antibody production in chicks infected with Newcastle disease virus. *J. Nutr.* 100:241.
- Blakely, S. R., G. V. Mitchell, M. Y. Jenkins, E. Grundel and P. Whittaker. 1991. Canthaxanthin and excess vitamin A alter alpha-tocopherol, carotenoid and iron status in adult rats. *J. Nutr.* 121:1649.
- Ching, S., D. C. Mahan, T. G. Wiseman and N. D. Fastinger. 2002. Evaluating the antioxidant status of weanling pigs fed vitamin A and E. *J. Anim. Sci.* 80:2396-2401.
- Coehlo, M. 2000. Poultry, swine, dairy vitamin supplementation updated. *Feedstuffs*. July 3 p. 12.
- Deyhim, F. and R. G. Teeter. 1993. Dietary vitamin and/or trace mineral premix effects on performance, humoral mediated immunity, and carcass composition of broilers during thermo neutral and high ambient temperature distress. *J. Appl. Poult. Res.* 2:347-355.
- De Vore, V. R., G. L. Colnago, L. S. Jensen and B. E. Greene. 1983. Thiobarbituric acid values and glutathione peroxidase activity in meat from chickens fed a Se supplemented diet. *J. Food Sci.* 48:300-301.
- Franchini, A., A. Meluzzi, S. Bertuzzi and G. Giordani. 1988. High doses of vitamin E in the broiler diets. *Archiv fur Geflugetkunde.* 52:12-16.
- Frankel, T. L. 1995. Sulfate incorporation into organic bone matrix of the tibiotarsus of broiler chicks is reduced by excess dietary methionine. *Poult. Sci.* 74(3):510-516.
- Friedman, A., A. Meidovsky, G. Leitner and D. Sklan. 1991. Decreased resistance and immune response to *Escherichia coli* infection in chicks with low or high intakes of vitamin A. *J. Nutr.* 121:395-400.
- Friedman, A. and D. Sklan. 1997. Effects of retinoids on immune response in birds. *World Poult. Sci. J.* 53:186-195.
- Gross, W. B. and H. S. Siegel. 1983. Evaluation of the heterophil:lymphocyte ratio as a measure of stress in chickens. *Avian Dis.* 27:972-979.
- Guo, Y., Q. Tang, J. Yuan and Z. Jiang. 2001. Effects of supplementation with vitamin E on the performance and the tissue peroxidation of broiler chicks and the stability of thigh meat against oxidative deterioration. *Anim. Feed. Sci. Tech.* 89:165-17.
- Hickling, D., W. Guenter and M. Jackson. 1990. The effect of dietary lysine and methionine on broiler chicken performance and breast meat yield. *Can. J. Anim. Sci.* 70:673-678.
- Jacobsen, K., R. M. Engberg, J. O. Andersen, S. K. Jensen, C. Lauridsen, P. Sorensen, P. Henckel, G. Bertelsen, L. H. Skibsted and C. Jensen. 1995. Supplementation of broiler diets with all-rac-alpha- or a mixture of natural source RRR-alpha, gamma-delta-tocopheryl acetate. 1. Effect on vitamin E status of broilers in vivo and at slaughter. *Poult. Sci.* 74:1984-1994.
- Jianlin, S., J. H. Kersey, C. A. Fritts and P. W. Waldroup. 2004. An evaluation of the interaction of lysine and methionine in diets for growing broilers. *Int. J. Poult. Sci.* 3(1):51-60.
- Klasing, K. C. and D. M. Barnes. 1988. Decreased amino acid

- requirement of growing chicks due to immunological stress. *J. Nutr.* 118:1158-1164.
- Korver, D. and K. Klasing. 2003. Influence of nutrition on immune status of the bird. In: *Proceedings of the 24th Technical Turkey Conference* p. 43.
- Lauridsen, C., S. Hojsgaard and M. T. Sorensen. 1999. Influence of dietary rapeseed oil, vitamin E and copper on the performance and the oxidative status of pigs. *J. Anim. Sci.* 77:906-916.
- Lauridsen, C., M. S. Hedemann and S. K. Jensen. 2001. Hydrolysis of tocopheryl and retinyl esters by porcine carboxyl ester hydrolase is affected by either carboxylate moiety and bile acids. *J. Nutr. Biochem.* 12:219-224.
- Leeson, S. and J. D. Summers. 2001. *Nutrition of the Chicken*. (4th Edition). University Books Publication, Guelph, Canada.
- Leshchinsky, T. V. and K. C. Klasing. 2001. Relationship between the levels of dietary vitamin E and the immune response of broiler chickens. *Poult. Sci.* 80:1590-1599.
- Lohakare, J. D., T. W. Hahn, Y. H. Shim, J. Y. Choi and B. J. Chae. 2004. Effects of feeding methods (feed vs. water) of vitamin E on growth performance and meat quality of broilers. *Asian-Aust. J. Anim. Sci.* 17:1260-1265.
- Mitsumoto, M., S. Ozawa, T. Mitsunashi and K. Koide. 1998. Effect of dietary vitamin supplementation for one week before slaughter on drip, color and lipid stability during display in Japanese black steer beef. *Meat Sci.* 49:165-174.
- Murphy, T. P., K. E. Wright and W. J. Pudlakiewicz. 1981. An apparent rachitogenic effect of excessive vitamin E intake in the chick. *Poult. Sci.* 60:1873-1878.
- National Research Council. 1994. *Nutrient Requirements of Poultry*, 9th Edition. National Academy Press, Washington, DC.
- Norazlina, M., S. Ima-Nirwana and B. A. K. Khalid. 1999. Effects of palm vitamin E, vitamin D and calcium supplementation on bone metabolism in vitamin E deficient rats. *Med. J. Islam. Acad. Sci.* 12(4).
- Praharaj, N. K., M. R. Reddy, A. K. Panda, S. V. Ramarao and R. P. Sharma. 2002. Genotype by dietary lysine interaction for growth and response to sheep red blood cells and *Escherichia coli* inoculation in commercial broiler chicks. *Asian-Aust. J. Anim. Sci.* 15(8):1170-1177.
- Ramarao, S. V., N. K. Praharaj, R. V. Reddy and A. K. Panda. 2003. Interaction between genotype and dietary concentrations of methionine for immune function in commercial broilers. *Br. Poult. Sci.* 44(1):104-112.
- Rice, D. A. and S. Kennedy. 1988. Vitamin E and free radical formation. In: (Ed. W. Haresign and D. J. A. Cole) *Recent Advances in Animal Nutrition*, pp. 39-57 (London, Butterworths).
- Ross, A. C. 1992. Vitamin A status: relationship to immunity and the antibody response. *Proc. Soc. Exp. Bio. Med.* 200:303-320.
- SAS Institute. 1985. *SAS User's Guide: Basics*. SAS Institute Inc, Cary NC.
- Schutte, J. B. and M. Pack. 1995. Sulfur amino acid requirement of broiler chicks from fourteen to thirty-eight days of age. 1. Performance and carcass yield. *Poult. Sci.* 74(3):480-487.
- Sinnhuber, R. O. and T. C. Yu. 1977. The 2-thiobarbituric acid reaction, an objective measure of the oxidative deterioration occurring in fats and oils. *J. Jan. Soc. Fish Sci.* 26:259-267.
- Sklan, D. and S. Donoghue. 1982. Vitamin E response to high dietary vitamin A in the chick. *J. Nutr.* 112:759-765.
- Swain, B. K. and T. S. Johri. 2000. Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. *Br. Poult. Sci.* 41(1):83-88.
- Tsiagbe, V. K., M. E. Cook, A.E. Harper and M. L. Sunde. 1987. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. *Poultry Sci.* 66(7):1147-1154.
- Veltmann, J. R., Jr. L. S. Jensen and G. N. Rowland. 1985. Exacerbative effect of vitamin A on malabsorption syndrome in chicks. *Avian Diseases.* 29: 446-456.
- Wallis, I. R. 1999. Dietary supplements of methionine increase breast meat yield and decrease abdominal fat in growing broiler chickens. *Aust. J. Exp. Agric.* 39:131-141.
- Wolbach, S. B. and D. M. Hegsted. 1952. Vitamin A deficiency in the duck. Skeletal growth and central nervous system. *Arch. Pathol.* 54:548.
- Xu, H., B. A. Watkins and M. F. Seifert. 1994. Vitamin E status can alter bone histo-morphometry. *J. Bone Min. Res.* 9(1):S249.