Effects of Dietary Vitamin C, E and Fat on CD4 to CD8 T Cell Ratios in Peripheral Blood of Heat-stressed Broiler Chicks

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Summary

The effects of dietary vitamins C (VC), E (VE) and sunflower oil (SFO) on immune responses of heat-stressed male broiler chickens were evaluated. All birds were kept under consistent temperatures from 10:00 to 20:00 and from 20:00 to 10:00 throughout the 1-49 day of age period. Antibodies to Newcastle disease virus (NDV) in serum on the10th day of postimmunization at 18, 35 and 48d of age were determined. At 7wk, CD4/CD8 T lymphocyte ratios in peripheral blood were determined. VC, VE, SFO or their interaction had not significant effects on immune responses indices and total mortality. However, chicks fed the VE-supplemented diet had a higher antibody titer against NDV at 18 and 48d of age and higher CD4/CD8 lymphocyte ratios. In addition, increasing SFO from 0 to 2.5%, enhanced anti-NDV levels at 18 and 35d of age and increased CD4/CD8 ratios at 49d of age. Moreover, total mortality was decreased with increaseing VC concentration from 0 to 255ppm, VE from 0 to 288ppm and SFO from 0 to 2.5 and 5%. These results suggest that immune responses and especially mortality in heat-stressed broiler chickens be ameliorated by use of SFO accompanied by dietary VE or VC supplementation.

Key words: T cell, CD4, CD8, humoral immune response, heat stress, broiler

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Introduction

In many countries, high ambient temperatures induce a large economic losses because of mortality and decreased production. Heat stress leads to generation of free radicals and thereby inducing oxidative degeneration of polyunsaturated fatty acids (PUFA) in cell membranes phospholipids (Laudicina & Marnett 1990). In addition, heat stress stimulates the release of corticosterone (Pardue *et al* 1985) and catecholamins and initiates lipid peroxidation in cell membranes (Freeman & Crap 1982) including membrane of T and B lymphocytes and thereby suppresses antibody production and cell-mediated immunity and also increases heat-stressed dependent mortality (Siegel 1978). Some experiments have shown that these heat-induced adverse effects were all significantly reduced by ascorbic acid supplementation (Schmelings & Nockels 1978, Pardue *et al* 1985). Beneficial effects of additional VE supplementation on immune function in poultry have been demonstrated through several investigations, including studies on innate, cell-mediated and humoral immunity, as well as, on vaccination strategies (Erf & Bottje 1996, Erf *et al* 1998, Swain *et al* 2000). Regarding antioxidant property, there is a positive synergistic effect of VE and VC on the immune response (Putpongsiriporn *et al* 2001). Yin *et al* (1993) reported a mixture of VE and VC delay myoglobin oxidation, whereas VE or VC alone dose not delay metmyoglobin formation. Lipstein and Bornestain (1975) showed that the extra efficiency of fat utilization is more desirable under heat stress condition. The present study was conducted to examine the effects of different levels of dietary vitamins C, E and sunflower oil and their interactions on immune responses and also survival in male broiler chickens subjected to heat stress.

Materials and Methods

Day old male broiler chicks (Ross 208) were placed in a windowless broiler house with floor pens, weighed and divided into 36 groups of 16 chicks. Three pens of chicks were assigned randomly to each of 12 dietary treatments .The experimental

design was a 2×2×3 factorial arrangement of two levels of VE (Rovamix® E-50, 500IU of dl-α-tocopheryl acetate/g. Hoffman-La Roche, Inc.) (0 or 288mg/kg), two levels of VC (97.5% ascorbic acid [coated] Hoffman-La Roche, Inc.) (0 or 250mg/kg) and three levels of sunflower oil (0, 25 or 50g/kg). The basal corn-soybean meal diets (Table1) were supplemented with a vitamin premix free of VE and met all nutrients requirements given in Ross broiler management manual (1999).

The experiment was conducted in summer and temperature controlled to a diurnal cycle from 29°C to 36°C which was 29±1°C from 20:00 to10:00 and 35±1°C from 10:00 to 20:00 throughout the study. For antibody assay chicks were vaccinated against Newcastle disease virus (NDV) (hitchner B1) via drinking water at 8, 25 and 39 days of age. Blood samples from two chicks per pen (6 chicks per treatment) were drawn from wing vein at 10 days after each immunization. Antibodies specific for NDV were detected in serum by the hemagglutination inhibition (HI) procedure of titers. For flow cytometry analysis, on day 49 of feeding dietary treatments, a

Table 1. *Composition of the basal diets (%) in 0 to 28 and 28 to 49 days of age*

Ingredients	0–28 day			28–49 day		
	Oil (%)			Oil (%)		
	0	2.5	5	0	2.5	5
Corn	58.5	51.0	43.0	63.5	55.8	48.0
Soybean meal (43%CP)	37.24	38.8	40.4	32.83	34.3	35
Sunflower Oil	0	2.5	5.0	0	2.5	5.0
Dicalcium Phosphate*	2.1	2.1	2.1	1.8	1.8	1.85
DL-Methionine	0.21	0.21	0.22	0.22	0.22	0.23
L-Lysine	0.18	0.16	0.14	0.18	0.16	0.14
Oyster shell	0.9	0.9	0.9	0.6	0.6	0.6
Salt	0.37	0.37	0.37	0.37	0.37	0.37
Vitamin–Premix**	0.25	0.25	0.25	0.25	0.25	0.25
Mineral-Premix	0.25	0.25	0.25	0.25	0.25	0.25
Sand	0	3.46	7.37	0	3.75	7.5

*Its Ca and P were determined 26% and 13%, respectively.

**The vitamin mix in the basal diets did not contain added vitamin E.

sample of 2ml of blood was taken in a heparinized syring from wing vein of 6 cockerels per treatment (two birds/pen). Blood samples then were immediately placed on ice and transported to laboratory for flow cytometry. To examine subsets population of T lymphocytes, peripheral blood was incubated with a panel of mouse anti-chicken monoclonal antibodies (mAb) specific for CD4 (T helper cells) and CD8 (cytotoxic T cells). The CD8 and CD4 population were detected with phycoerythrin (PE) conjugated with CT8mAb (CT8-PE) and fluorescein isothiocyanate conjugated with CT4 (CT4-FITC) obtained from Southern Biotech. Assoc (Birmingham, Al), respectively. In the direct immuno fluorescence staining of lysed whole blood described by Becton Dickinson (Southern Biotechnology Assoc. Inc., Birmingham, Al) and Li *et al* (2000) that modified by the authors, 30µL of PBL was incubated with 20µl of diluted CT8-PE for 5min and then 20µl of CT4-FITC for 15min at room temperature.Then 1ml of FACS lysing solution was added and incubated again for 10min.After centrifugeation and washing with PBS the fluorescence intensities were measured by two-color flow cytometric analysis. Data from 10,000 live cells were collected on a FACStar flow cytometer. Analysis of flow cytometry data also was performed using the Lysis II software and the percentage of positive cells were determined. All data were subjected to statistically analysis of variance using the General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1996). Significance was declared at $P<0.05$ and, where found, comparisons among multiple means were made by Duncan's Multiple Range Test.

Results

No treatment effects were found on antibody responses to NDV at 18, 35 and 48 days of age (day 10 postimmunizaton). However fed diets containing 2.5% SFO and 250ppm VC or 288ppm VE and/or combination of 250ppm VC and 288ppm VE caused a remarkable increasing in antibody titers. There were no significant effects

associated with the levels of VC, VE, SFO and their interaction on average ratios of CD4:CD8 Lymphocytes. However, the ratios were increased markedly with diets containing 2.5% SFO supplemented by VC or VE or diet contains combination of 250ppm VC, 288ppm VE and 5% SFO. Moreover, VC (as a main effect) did not change CD4:CD8 ratios (2.14 vs. 2.18) but supplemented VE improved it, to some extent (2 vs. 2.33). It is also worth noting that increasing SFO (as a main effect) in the diets from 0 to 2.5% caused an increase in CD4:CD8 ratios from 1.94 to 2.45 but increasing from 2.5 to 5% SFO have accompanied by a decrease in the ratios from 2.45 to 2.1.

Discussion

VC supplementation (as a main effect) failed to enhance antibody responses to NDV at different ages. But it is demonstrated that heat-induced immunosuppression and mortality were reduced in VC supplemented (1000mg/kg) chicks following exposure to high environmental temperature and it has been correlated with a reduction in the glucocorticoid out put and/or protection of cells from the cytotoxic effects of adrenal steroid hormones (Pardue *et al* 1985, Pardue & Thaxton 1984). In comparison to VC, VE supplementation (as a main effect) had a positive but no significant effect on antibody responses to NDV particularly at the ages of 18 and 49 day. In this connection has been reported that the effect of VE on antibody responses to SRBC was stock-dependent, and no consistent differences were found between dietary levels of VE in cockerels (Boa-Amponsem *et al* 2000). Leshchinsky and Klasing (2001) demonstrated in the humoral immune response, the effect of added VE depend on the nature of the antigen.Yang *et al* (2000) also reported that at 6 and 10 days after inoculation with SRBC, differences between VE concentrations (10 and 300ppm) were observed in the White Leghorn line selected for low antibody responses to SRBC but not in the line selected for high antibody responses.

Table 2. *Effects of dietary VC (mg/kg), VE (mg/kg) and SFO (%) supplementation on anti-NDV titer, CD4 to CD8 ratios and total mortality percentage in heat-stressed chicks*

Group	Dietary treatments			Anti-NDV level (log value)			CD4/CD8 49d	Mortality% 0 to 49d
	VC	VE	SFO	18d	35d	49d		
1	0	0	**0**	0.67	2.00	2.67	1.57	**10.67**
2	0	0	**2.5**	**1.50**	**3.00**	**2.33**	**1.96**	**12.67**
3	0	0	**5**	**2.66**	**4.67**	**3.50**	**1.96**	**8.33**
4	0	288	**0**	**4.33**	**3.00**	**4.00**	**2.35**	**6.33**
5	0	288	**2.5**	**4.00**	**3.00**	**4.67**	**2.54**	**8.33**
6	0	288	**5**	**2.66**	**1.67**	**2.50**	**1.96**	**4.00**
7	250	0	**0**	**1.67**	**1.00**	**3.00**	**2.16**	**6.33**
8	250	0	2.5	3.00	4.67	2.33	2.44	4.00
9	250	0	5	1.00	2.00	2.67	1.42	2.00
10	250	288	0	2.00	1.00	1.33	1.69	10.67
11	250	288	2.5	2.33	2.67	4.50	2.34	2.00
12	250	288	5	1.67	2.00	3.00	3.08	4.33
Pooled SEM				1.325	1.103	0.9958	0.5032	4.232

In each column, values that don't have common superscripts are not significantly different ($P<0.05$)

The results of current study show that addition of 2.5% SFO to the corn-soybean diets accompanied by VC (255ppm) or especially VE (288ppm) affect developing lymphocytes and thereby cell mediated immunity, as shown by increased proportion of CD4:CD8 lymphocytes in the peripheral blood. It has been suggested that VE protects cells involved in immune responses such as lymphocytes, macrophages and plasma cells against oxidative damage and enhance the metabolic function and proliferation of these cells (Bartow & Friggm 1992, Yu 1994). Erf *et al* (1998) also reported, at levels three to five times higher than that typically used in commercial formulations, VE specifically increased the percentage of mature $CD4^{+}CD8^{-}$ T cells present in the thymus and spleen by the time the broilers were 7wk of age. However, it has been reported that the additional level of VE supplementation to optimize immune function may be dependent on various factors such as genotype (Yang *et al*

2000, Boa-Amponsem *et al* 2000), concentration of vitamin added (Leshchinsky & Klasing 2001), age (Erf *et al* 1998), dietary composition, type and severity of stress(s). However, in present study it seems that inclusion of SFO in the diets ameliorated adverse effects of heat stress by advantages of extra caloric effect (Dale & Fuller 1980) and also improved cell-mediated and humoral immune responses in chicks. Therefor, it seems that above-mentioned notices caused to were found no significant differences between dietary levels of VC, VE, SFO on immune response indices in present work. As shown in Table2, high level of SFO (5%) caused a reduction of CD4:CD8 ratios, to some extent. In this connection, it have been reported that both concentration and type of dietary fats impact on lymphocyte functions in animal models (Keley & Daudu 1993). Some workers have reported that with some exception, high levels of n-6 PUFA in the diet inhibit T and B Cell responses in several animal model studied (Fritsche *et al* 1991, Deckeri *et al* 1988). It seems that inhibiting of immune responses by high level of SFO is through its oxidative properties (Gross & Siegel 1983) because supplementing both VE and VC in the diets (group 12) overcome the inhibition caused by 5% SFO. Hamilton *et al* (2000) also reported in human subjects that the mean relative contribution of α-tochopherol (73.5 mg/day) plus ascorbic acid (500 mg/day) overall 'total' antioxidant power following ascorbic acid or α-tocopherol supplementation increased from 16.1% to 22.1% and from 16.2% to 18.7%, respectively.

About total mortality percentage, vitamins C, E and SFO and also their interaction had no significant effects on this parameter. However, a remarkable reduction in total mortality were noted in birds fed diets containing 2.5% and 5% SFO that supplemented with 250ppm or 288ppm VC or VE, respectively. Also VC and SFO (as main effect) had an important role in reducing mortality up to 42 and 45%, respectively. Numerous researchers have reported that addition of dietary VC to chicks experiencing stressful situation, particularly acute high temperature, appears to be beneficial in reducing deleterious stress responses and enhancing survivability.

In this connection, Pardue *et al* (1985) and Brake (1989) reported that heat and infectious bursal disease-associated mortality was markedly reduced in supplemented chicks with1000ppm ascorbic acid. However in present experiment VE supplementation reduced total mortality by 19%. Moreover in a field trial on the summer with two broiler commercial stock, with the same conditions, that fed diets containing 50 and 150ppm VE we observed that total mortality were 13 and 9.5%, respectively (unpublished). It seems that substantial improvement in livability by adding VC and VE or even SFO may be not associated with their notable and significant effects on the immune response indices. However,in the present experiment probably, due to chicks' age, lack of severe immunological challenge and special disease, chicks' genetic background, or experiment's duration time we failed to observe any significant effects for the treatments. But the results showed that under heat stress conditions use of SFO (esp. 2.5%) accompanied by additional dietary supplementation of VC (250ppm), VE (288ppm) or their combination can support humoral and cell-mediated immunity and particularly their survivability. Moreover, it seems that administration of SFO or probably other vegetable oils are the most efficient route to reduce adverse effects of heat stress on immune response and especially performance of broiler chicks during the heat stress. But in this situation we must supplement diets with sufficient antioxidants such as vitamins C and E to prevent from possibility occurrence of lipid peroxidation and thereby depletion of body reserves antioxidants.

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