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THE EFFECTS OF VITAMIN E SUPPLEMENTATION ON SERUM LIPID PEROXIDATION LEVEL AND FEED INTAKE IN BIRDS INFECTED WITH INFECTIOUS BURSAL DISEASE OF CHICKENS

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SUMMARY

The effects of dietary supplementation of vitamin E on feed intake and serum lipid peroxidation formation were examined in 200 light breed cockerels infected with infectious bursal disease (IBD) virus. The birds were reared from day-old in deep litter, and were divided into 8 groups of 25 birds each by day 21. Half of each group was fed either basal (10 IU/kg) or high (supplemented) (210 IU/kg) level of vitamin E – containing chick mash. All the birds were vaccinated against Newcastle disease. By day 35, half of each group was vaccinated against IBD using a local IBD vaccine, while the other half was untreated. At day 42, half of each group was challenged with a virulent field strain of IBD virus (experimental groups) while the other half was left unchallenged (control groups). Malondialdehyde level in the serum and feed intake were monitored in all the groups. Serum peroxidation level was significantly lower in birds receiving supplemental vitamin E throughout the experimental period irrespective of vaccination history, while significant differences existed in feed intake among groups only during and after infection periods. Vitamin E supplementation was very efficient in reducing systemic lipid peroxidation, whereas its combination with vaccination led to faster recovery as shown by significant increase in food intake during and after infection periods.

Key words: Infectious bursal disease, vitamin E, lipid peroxidation, Feed intake.

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious disease of young chickens of about two to eight weeks of age (with highly active bursa of Fabricius), and is caused by infectious bursal disease virus which belongs to the family *Birnaviridae* and of the genus *Birnavirus* (Dobos, 1979; OIE, 2000). Its primary target is the lymphoid tissues, particularly the bursa of Fabricius, and when ingested, the virus destroys the lymphoid follicles in the bursa of FabricIUs as well as the circulating B-cells in the secondary lymphoid tissues such as gut-associated lymphoid tissue (GALT), conjuntiva-associated lymphoid tissue (CALT), Bronchial-associated lymphoid tissue(BALT) caecal tonsils, Harderian

gland, etc. (Kahn and Line, 2005). Acute disease and death are caused by the necrotizing effect of the virus on the host tissue in which it causes extensive destruction of immature lymphocytes, leading to severe immunodepression, decreased feed intake and mortality, and also increased susceptibility of the affected flock to other diseases (Bains, 1979; Giambrone, 1996). It has a short incubation period lasting two to four days, and birds which survive may remain immunocompromised and non-responsive to vaccination in the face of outbreak; thus the disease is economically important to the poultry industry worldwide.

In recent times, very virulent strains of IBDV (vvIBDV) which cause severe mortality in chicken have emerged in Europe, Latin America, South-East Asia, Africa and the Middle East (Kahn and Line, 2005). Stoichiometric studies have shown that every disease process is capable of causing oxidative stress to the host, through the initiation of free radical mechanism (Proćtor, 1989). This oxidative stress brings about a misbalance in the antioxidant/pro-oxidant balance, such that the pro-oxidant state is favoured (Niki, 1996).

The above process gives rise to free radical insult termed lipid peroxidation or oxidative degradation of polyunsaturated fatty acids, hence, a quantifiable end-product of lipid peroxidation is malondialdehyde (MDA) which is formed by radical cleavage reactions of polyenoic acids (Goldberg, 1994).

Many studies on antioxidants, lipid peroxidation and disease process, have strongly indicated that antioxidants can be very effective in mopping up free radicals *in-vivo*. Relevant studies have shown that vitamin E, a biologically potent antioxidant, when supplemented in the diet is of immense value in the reduction of lipid peroxidation, osteoarthritis-associated pain, lipid-lowering therapy, coronary heart diseases, Alzheimer's disease and increased resistance to lipoproteins (Murray *et al*., 2000).

This paper reports the effect of dietary supplementation of vitamin E on feed intake, and the progress of IBD virus infection as shown by the level of serum lipid peroxidation formation in chickens experimentally infected with virulent field strain of IBD virus.

MATERIALS AND METHODS

A total of 200 Harco® cockerels of the same parent stock were used for the experiment. They were reared from day-old in deep litter, and after twenty-one days, they were divided into 8 groups of 25 birds each. Half of each group received either basal (10 IU/kg) or high (supplemented) (210 IU/kg) level of vitamin E-containing chick ration. Vaccinations against Newcastle disease were done, on days 11 and 21, using Newcastle disease intraocular and LaSota vaccines, respectively, obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. By day 35, half of each group was vaccinated against IBD using a local intermediate IBD vaccine, while the other half was untreated. At day 42, half of each group was challenged with a virulent field strain of IBD virus (experimental groups), while the other half was left unchallenged (control groups). The treatment groups were sub-divided as follows based on schedules of treatment:

(i) Basal vitamin E unvaccinated/unchallenged (BUU), (ii) Basal vitamin E unvaccinated/challenged (BUC), (iii) Basal vitamin E vaccinated/unchallenged (BVU), (iv) Basal vitamin E vaccinated/challenged (BVC), (v) High vitamin E unvaccinated/challenged (HUC), (vi) High vitamin E unvaccinated/unchallenged (HUU), (vii)

High vitamin E vaccinated unchallenged (HVU) and (viii) High vitamin E vaccinated/challenged (HVC). The study lasted for 63 days.

Collection of Blood and Serum Samples

Blood samples were collected in all the groups from the external jugular vein before infection (day 42), during infection (day 49), and after infection (day 63) periods. The blood was allowed to clot, after which it was centrifuged at 3000g for 15minutes to extract serum **(**Coles, 1986).

Extraction of virus/preparation of viral inoculum

The virus extraction and inoculum were prepared using the method of Okoye and Uzoukwu (1981), whereby bursae from confirmed infected dead birds were used. The bursae which were removed from the birds soon after death weighed 4 g. They were ground with sterile sand using pestle and motar, and suspended in 16 ml of phosphate buffered saline (PBS), thus making up 20% PBS bursa homogenate. Penicillin (1000 IU) and Streptomycin (1000 μg) were added to the homogenate which was then centrifuged at 3000g for 15 minutes.

The supernatant which contained the virus was transferred to a bijou bottle, stored in a deep freezer at –20^{o}C and was used soon after preparation. Test IBDV antigen was prepared using the above method but with 50% bursal suspension. The experimental birds were infected by administering 0.2 ml of the bursal suspension which contained the virulent IBD virus intraocularly (N.V.R.I., 2005).

Lipid Peroxidation (Malondialdialdehyde) Assay

The experimental basis for this test is that thiobarbituric acid reacting substances (TBARS) such as malondialdehyde in the serum or blood will react with thiobarbituric acid to give a pinkish colour which absorbs maximally at 530 nm.

To 1ml of serum sample, 0.1ml of triton x-100 was added, and the content of the tube mixed thoroughly. 3 ml of trichloroacetic acid and 0.6% thiobarbituric acid were added. The tube was shaken vigorously and filtered. 2 ml of 0.6% thiobarbituric acid was then added to 3 ml of the clear filtrate and the reaction mixture was incubated in a boiling water bath for 15 minutes. The mixture was allowed to cool, after which 2 ml of chloroform was added and centrifuged at 7,000 rpm for 5 minutes and the absorbance of the clear supernatant read at 532 nm. Thiobarbituric acid reacting substances were quantified as lipid peroxidation products by referring to a standard curve of malondialdehyde equivalent generated by the hydrolysis of 1, 1, 3, 3-tetra ethoxy propane (TEP) (Walling *et al.*, 1993).

Statistical Analysis

Data were analysed using analysis of variance (ANOVA) with computer package SPSS 10.0, in a 2x2x2 factorial arrangement in complete randomized design (CRD).

RESULTS

Serum lipid peroxidation level of various groups before infection showed a significantly higher MDA level in the basal compared to the supplemented groups (Table I), while the factorial analysis showed significant difference ($p<0.05$) only with respect to vitamin E supplementation. There were no interactions among the independent variables (Table II).

However, during infection, significant differences existed in the means between the challenged and unchallenged groups. Viral challenge caused increased serum lipid peroxidation levels among group.

The factorial analysis showed that vitamin, viral challenge and their interactions were all significant ($p < 0.05$).

Lipid peroxidation level between the challenged and and unchallenged groups was significant ($p<0.05$) after infection, particularly in the basal groups, while in the supplemented groups, it was statistically non-significant ($p>0.05$). The factorial analysis showed all the independent variables and their interactions to be significant (Table 2).

Feed intake among groups was not significant ($p>0.05$) in all the groups before infection period (Table 3). Hence, it was significantly higher ($p<0.05$) in the unchallenged group compared to the challenged throughout the period. During infection, feed intake was not significant ($p>0.05$) among the challenged groups irrespective of treatment, while after infection, it became significantly higher in the vitamin E supplemented/vaccinated HVC group compared to the other challenged groups (BUC, BVC, and HUV).

Table I. Serum lipid peroxidation levels in the various groups.

Vaccination	Vitamin E level	Virus	Mean MDA level (µg/ml)		
			Day 42	Day 49	Day 63
Vaccinated	(BVC)	Challenged	10.40^{a}	80.40^{a}	50.40^{d}
	(BVU)	Unchallenged	10.30^{a}	15.95^{b}	34.66^{c}
	(HVC)	Challenged	4.22^{b}	50.25^{c}	24.56^{a}
	(HVU)	Unchallenged	4.60^{b}	7.35^{d}	20.46^{a}
Unvaccinated	(BUC)	Challenged	10.17^{a}	81.78^{a}	56.46^{e}
	(BUU)	Unchallenged	10.18^{a}	16.02^{b}	35.09^{c}
	(HUC)	Challenged	4.61^{b}	48.47^{c}	23.64^{a}
	(HUU)	Unchallenged	4.53^{b}	7.58^{d}	20.66^{a}

Means with different superscripts in a column are statistically significant ($P < 0.05$). MDA= Malonyldialdehde. (i) Basal vitamin E unvaccinated/unchallenged (BUU), (ii) Basal vitamin E unvaccinated/challenged (BUC), (iii) Basal vitamin E vaccinated/unchallenged (BVU), (iv) Basal vitamin E vaccinated/challenged (BVC), (v) High vitamin E unvaccinated/challenged (HUC), (vi) High vitamin E unvaccinated/unchallenged (HUU), (vii) High vitamin E vaccinated unchallenged (HVU) and (viii) High vitamin E vaccinated/challenged (HVC).

Table II. Factorial analysis of serum lipid peroxidation before, during and after infection in the various groups

Source	Day 42	Day 49	Day 63
Vaccination	0.209	0.444	0.000
Vitamin	0.000	*0.000	*0.000
Viral challenge	0.000	*0.000	**0.000
Vaccination x Vitamin	0.283	0.283	**0.000
Vaccination x Challenge.	0.406	0.406	**0.000
Vitamin x Challenge.	0.000	**0.000	**0.000
Vaccination x Vitamin x Challenge	0.104	0.267	**0.000

*Significant; **Significant interaction of independent variables ($p<0.05$).

Table III. Feed intake before, during and after infection periods.

Treatment	Mean feed intake (g)		
	(Day 42)	(Day 49)	(Day 63)
BUU	290.22± 0.04[a]	310.55± 0.02[a]	504.02 ± 0.03[a]
BUC	290.63 ± 0.07[a]	221.63± 0.03[b]	372.05 ± 0.28[b]
BVU	288.44 ± 0.22[a]	316.56± 0.04[a]	502.00 ± 0.10[a]
BVC	288.05 ± 0.08[a]	220.44± 0.02[b]	370.62 ± 0.36[b]
HUU	290.33 ± 0.44[a]	315.12 ± 0.00[a]	506.45 ± 0.01[a]
HUC	290.58 ± 0.04[a]	217.23 ± 0.11[b]	383.22 ± 0.33[b]
HVU	288.44 ± 0.20[a]	318.81± 0.10[a]	506.03 ± 0.05[a]
HVC	286.07 ± 0. 01[a]	222.04 ± 0.14[b]	395.55 ± 0.09[c]

Means with different superscripts down the row are statistically significant ($P < 0.05$).

DISCUSSION

Lipid peroxidation levels prior to infection were observed to be about twice higher among groups on basal vitamin E diet, compared to the supplemented groups. This indicated the importance of vitamin E in the control of lipid peroxidation even without the establishment of any disease process *in vivo;* hence, the source of stress in this case may be environmental heat. This finding supports the reports of other workers (Burton *et al*., 1993; Murray *et al*., 2000; BÖlűbaşi *et al.,* 2007) who reported on the antioxidative function of vitamin E in the biological system. BÖlűbaşi *et al.,* (2007) also reported that dietary supplementation of vitamin E is capable of decreasing the systemic levels of thiobarbituric acid reacting substances (TBARS); it could in addition reduce the susceptibility of meat to iron-induced lipid peroxidation (Frigg *et al*., 1993).

During and after infection periods, the serum lipid peroxidation levels were higher in all the infected groups irrespective of vaccination, vitamin E supplementation or their combination. This most likely occurred as a result of the effect of viral challenge which may have introduced oxidative stress in the birds. Hence during infection, the factorial analysis was highly significant ($p<0.05$) for vitamin, viral challenge and their interaction only, while the factorial analysis after infection was highly significant for all the independent factors and their various interactions. Feed intake among groups was not statistically significant before infection, however during infection period, particularly by day 49, it decreased significantly ($p<0.05$) as a result of IBD infection in all the challenged groups compared to the unchallenged, whereas intake among the unchallenged groups did not differ significantly ($p>0.05$) irrespective of treatments (vaccination or vitamin E supplementation) (Table II). This agrees with the reports of Maziar *et al.* (2007) who equally noted in their trial (although with non-infected birds) that vitamin E supplementation had no effect both on feed intake and feed conversion efficiency. Consequently, Jerry *et al*., (1997) also reported that supplementation of vitamin E did not change significantly ($p>0.05$) either the feed conversion or feed intake in male

turkey groups, which were either exposed to heat stress or not. However, on the contrary, supplementation of dietary vitamin E at 500 mg/kg was reported to cause a significant increase ($p<0.01$) in feed intake of hens (Bollengier-lee *et al.*,1998) and in Japanese quails exposed to heat stress (Sahin *et al*., 2002).

Moreso, it was observed that IBD infection caused low feed intake in all the infected groups, which became more severe during infection. This is in agreement with the findings of Bains (1979), who reported that affected birds will go off feed and water. Goldberg (1994) also suggested that systemic depletion of vitamin E can occur due to low dietary intake, consumption of large amounts of unsaturated dietary lipids and disease processes, while Leońhardt *et al.* (1997) suggested that the systemic levels of vitamin E is directly related to dietary intake. However, after infection, feed intake increased significantly in the vitamin E supplemented/vaccinated groups, compared to the basal/vaccinated groups. This occurred possibly as a result of immunostimulatory role of vitamin E (Tengerdy, 1989), or in synergy with vaccination considering their roles in antibody production in the system (Kahn and Line, 2005).

There are also other supportive studies which have shown that vitamin E supplementation increases humoral and cell-mediated immune responses to a number of diseases in man and animals (Gibbs, 1982). Consequently, the above evidence is further supported strongly by the presence of a significant interaction ($p<0.05$) among the three independent variables (vitamin E, viral challenge and vaccination), as shown in Table II.

In conclusion, it is suggested that the administration of supplemental vitamin E diets to birds could be beneficial in lowering their lipid peroxidation levels during IBD infection, and that its combination with vaccination may enhance the feed intake in birds during and after IBD infection, and quicken their recovery through improved immunity. This is particularly important in tropical environments where diseases and harsh environmental conditions are most likely to bring about increased oxidative stress in animals.

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