

BLOOD BIOCHEMICAL PARAMETERS IN CHICKENS FOLLOWING DIETARY SUPPLEMENTATION WITH DIFFERENT LEVELS OF VITAMIN C AND E

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SUMMARY

A 25 week-long study was conducted to determine the effect of dietary supplementation with vitamins C and E on blood biochemical parameters in chickens. Day-old layer chicks, Black Nerra, were divided into 7 groups depending on the level and type of vitamin supplementation. Vitamin C was supplemented at 120 and 240 mg/kg feed, whereas vitamin E was supplemented at 140 and 280 mg/kg feed, as low and high levels, respectively. According to vitamin levels supplemented to the basal diet, the groups were referred to as low vitamin C (LC); high vitamin C (HC); low vitamin E (LE); high vitamin E (HE); low vitamin C and E (LCE) and high vitamin C and E (HCE). A control group received only the basal diet. Erythrocyte percentage haemolysis, blood haemoglobin, total plasma protein and plasma ascorbic acid were determined to assess biochemical picture. The data shows that vitamin supplementation was effective in reducing percentage erythrocyte haemolysis ($P < 0.001$). Chickens in LCE and HCE groups had the highest haemoglobin content while total plasma protein and plasma ascorbic acid content were significantly higher ($P < 0.001$) in HCE chickens group. A negative correlation ($r = -0.4$; $P < 0.001$) was observed between haemoglobin content and red cell membrane fragility. Plasma ascorbic was also correlated to total plasma protein. It was therefore established that dietary supplementation of vitamin C and E together especially at high dose of 240 and 280 mg/kg feed respectively, is beneficial in that it improves RBC membrane integrity, blood haemoglobin, total plasma protein and plasma ascorbic acid bioavailability in layer chickens.

INTRODUCTION

Two vitamins, ascorbic acid and alpha-tocopherol are known to be potent biological antioxidants (Newberne and Conner, 1989; Eicher-Pruiet *et al.*, 1992). Ascorbic acid has a critical role in the biosynthesis of collagen. It effects hydroxylation of proline and lysine residues within the nascent peptides in connective tissues (Stryer, 1988; Lehninger, 1987). Its deficiency therefore leads to classical lesions typical of scurvy. Alpha-tocopherol plays a major role as biological membrane stabilizer

through antioxidation of polyunsaturated fatty acids (PUFA). The PUFAs are integral component of biological membranes (Diplock and Lucy, 1973).

Experiments have shown that supplementation with vitamin C and / or E improves resistance to infections and in some instances it improves performance in several animal species (Rajmane, 1992; McIlroy *et al.*, 1992; Bains, 1990)

However, information regarding the biochemical roles of vitamins C and E their mode of action remains to be fully elucidated (Church and Pond, 1988). There

has also been some conflicting reports with regards to whether the two vitamins when combined together elicit synergism. The aim of the present study was to assess the influence of dietary supplementation with vitamin C and E or in combination on some biochemical parameters in chickens and find out if the results can explain the reason for improved immunological and performance parameters.

MATERIALS AND METHODS

A total of 200 day old layer chickens of a commercial strain (Black nerra) were obtained from a commercial hatchery and randomly assigned into 7 groups. The birds were raised under intensive deep litter system at a density of 5 birds per square metre with free access to water and feed. Artificial light was provided day and night throughout the first 40 days of life in order to maintain the temperature at 38 °C. The litter, woodshavings, was changed regularly. The house had concrete floor, corrugated iron sheet roof and was well ventilated by 12 m x 1.5 m wire meshed windows.

Feeds were obtained from the same feed formulation firm (Interchick, Tanzania). The type and proximate analysis of the feed is presented in Table 1. Different types of feed were supplied as follows: chick starter from the 1st to 9th week; growers' mash from 10th to 19th week and layers' mash form 20th to the end of the experiment i.e., at the age of 25 weeks.

The vitamins were obtained from F. Hoffmann La Roche Company, Switzerland with trade marks Rovimix C and Rovimix E-50 SD for vitamins C and E, respectively. Rovimix C is a silicon coated white to yellowish powder which is 96% pure ascorbic acid. Rovimix E-50 SD is a yellowish powder, containing dl-tocopheryl

acetate (dl-TA) finely dispersed in a matrix of fish gelatin and dextrin. It is 50% pure dl--TA. The vitamins were thoroughly mixed with the feed by using a feed mixer to attain levels indicated in Table 2. The levels were attained by adding the following respective amounts of vitamin powders:

Low vitamin C - 156 mg of Rovimix C

High vitamin C - 344 mg of Rovimix C

Low vitamin E - 330 mg of Rovimix E-50 SD

High vitamin E - 660 mg of Rovimix E-50 SD

The final mixtures were fed to the animals within 3 days of preparation.

Treatments and sampling methods

The study was set up as a complete randomised design with 7 groups and seven treatments as shown in Table 2. The treatments (supplementation) commenced from day one to the end of the study (25 weeks). Sampling for biochemical analyses, i.e., haemolysis test, haemoglobin content and total plasma protein was done between day 95 and day 115 of age.

Analytical procedures

The haemolysis test was performed as explained by (Hamada and Matsumoto, 1980). The test measures the resistance of erythrocyte cell membrane against stress which may be imposed on them by the detergent such as hydrogen peroxide and polyethylene sorbitol monolaurete (Tween 20). The percentage haemolysis was calculated after determination of extinction at 540 nm on a spectrophotometer (Spectronic 1001 Roy Company).

The blood haemoglobin content was estimated spectropho-tometrically using

cyanmethaemoglobin method (Baker and Silverton, 1976). The method is based on the oxidation of haemoglobin to methaemoglobin by ferricyanide and subsequent conversion, under controlled pH conditions, to cyanmethaemoglobin (haemoglobincyanide, HICN) by cyanide, utilising Drabkins' reagent. Cyanomethaemoglobin solution is free from turbidity and possess excellent stability which is an advantage in spectrophotometry.

Plasma protein was determined by biuret method (Coles, 1986). The method utilizes the principle that when a solution containing proteins is reacted with tartrate complexed cupric ions and alkali, the copper binds to the peptide bond structure of the proteins, forming a purple chromogen with an absorbance maximum at 540 nm. The absorbance of this colour can be compared with that produced by solutions with a known protein content.

A colorimetric method for determination of plasma ascorbic acid, as explained by McCormic (1986), was employed. Ascorbic acid in plasma can be oxidized by Cu (II) to form dehydroascorbic acid which reacts with acidic 2,4-dinitrophenyl-hydrazine (DNPH) to form a red bis-hydrazone, which is measured at 520 nm.

Statistical analysis

The data were evaluated statistically by the General Linear Models procedure of SAS (SAS Institute, 1987). The means were partitioned by Duncan's multiple range test while correlation between biochemical parameters were analysed by Pearson's partial correlation.

RESULTS

Percentage erythrocyte haemolysis of control and LC groups was significantly ($P < 0.01$) higher than that of other groups. No significant differences were apparent in all other group comparisons. The dosage of vitamin C alone had a significant influence on degree of haemolysis. Increasing the level of vitamin C from 40 (basal diet) to 120 mg/kg (LC) decreased percentage haemolysis by 3%. Also doubling the level of vitamin C from 120 (LC) to 240 mg/kg (HC) halved the percentage haemolysis from 13% to 6.5% (Fig.1). Both levels of vitamin E alone did not lead to significant reduction of percentage haemolysis as compared to high level of vitamin C alone. Combining vitamins C and E significantly reduced percentage haemolysis when compared to control group. However percentage haemolysis of chickens in LCE and HCE groups did not differ significantly.

Table 1: Content of metabolizable energy (ME Kcal/Kg), crude protein (CP %), ether extract (EE %), crude fibre (CF %), calcium (Ca), phosphorus (P), vitamin C (VTC mg/Kg), vitamin E (mg/Kg) in chick starter (CS), growers (GM) and layers marsh (LM) in the basal diet

	CS	GM	LM
ME	2800	3000	3000
CP	18.1	19.8	16.7
EE	4.24	13.7	6.20
CF	9.80	13.7	13.7
Ca	0.86	0.82	0.71
P	0.66	0.66	0.67
VTC	40	40	32
VTE	22	13	7

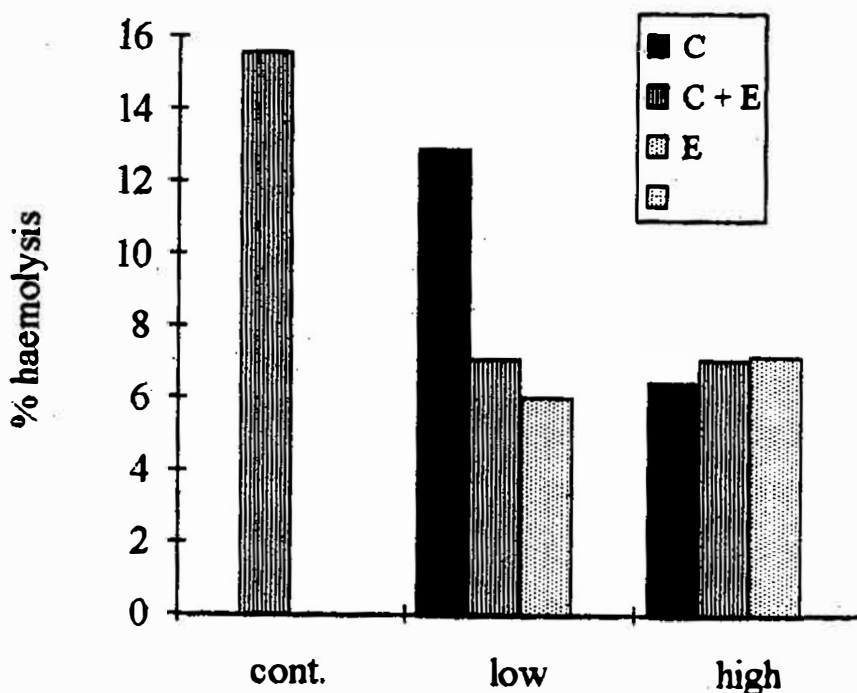


Fig. 1: Percentage erythrocyte haemolysis as affected by supplemental vitamins C and E in chickens.

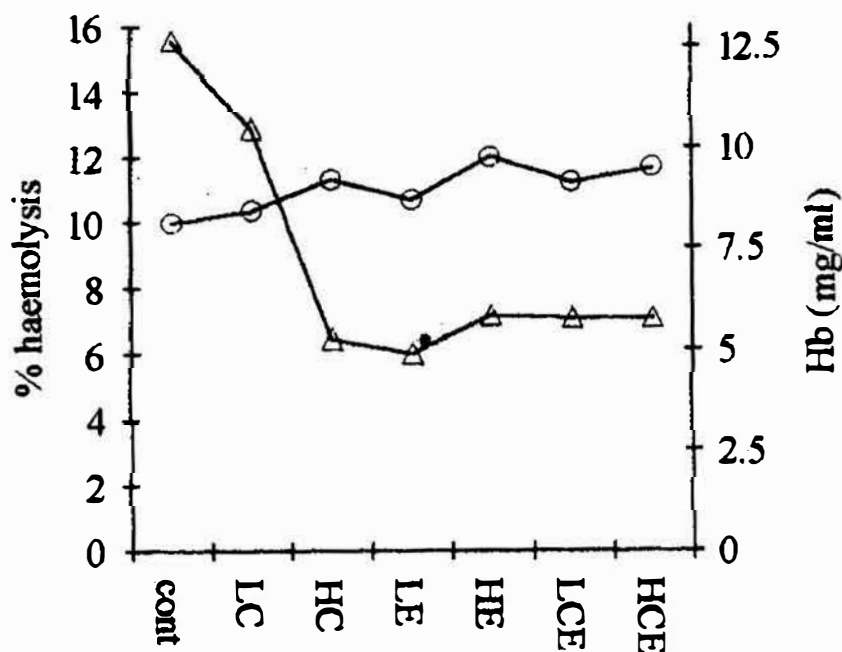


Figure 2: Relationship between percentage erythrocyte haemolysis and blood haemoglobin as affected by supplemental vitamin C and E. cont. = control, LC = low vitamin C, HC = high vitamin C, LE = vitamin E, HE = high vitamin E, LCE = low vitamin C and E, HCE = high vitamin C and E.

Table 2: Vitamin C and E content of the final feed mixture as indicated by suppliers

Vitamin (mg/Kg)	Control	LC	HC	LE	HE	LCE	HCE
C	40*	120	240	40*	40*	120	240
E	22*	22*	22*	140	280	140	280

+control= no additional vitamin C or E., LC, HC =low and high levels of vitamin C, respectively. LE, HE = low and high levels of vitamin E, respectively. LCE, HCE = low and high levels of vitamins C and E combined. * basic concentration of vitamins from commercial suppliers.

Table 3: Percentage haemolysis (HLS), blood haemoglobin (Hb, mg/dl), total plasma protein (TPP, mg/ml) and plasma ascorbic acid (AA, mg/m) content of chickens supplemented with vitamin C and E supplementation or non supplemented (Non) (mean s.e.).

	HLS	Hb	TPP	AA
Non	15.56±2.99 ^a	9.96±0.80	4.11±0.44 ^b	1.58±0.23 ^b
LC	12.89±3.25 ^b	10.34±0.86	4.28±0.54 ^b	1.60±0.43 ^b
HC	6.44±1.34 ^c	11.31±0.70 ^{ab}	4.05±0.29 ^b	1.61±0.29 ^b
LE	6.00±1.06 ^c	10.68±0.96 ^c	4.15±0.41 ^b	1.33±0.33 ^b
HE	7.17±1.85 ^c	12.03±0.72 ^a	4.49±0.25 ^b	1.36±0.35 ^b
LCE	7.07±1.75 ^c	11.23±1.02 ^b	4.36±0.51 ^b	2.03±0.24 ^a
HCE	7.09±1.91 ^c	11.66±1.24 ^a	5.13±0.63 ^a	2.21±0.58 ^a
R ² (%)	74.25	37.89	38.34	44.95
cv	24.21	8.32	10.43	21.37
Mean	8.89	11.03	4.37	1.67

^{a, b, c} Means within each column with the same superscript are not significantly different (P < 0.05).

Haemoglobin content in blood of chickens in different groups correlated well with dietary levels of vitamins C and E, (Table 3). Groups receiving high levels of the two vitamins either alone, (LE and HE groups),

or in combination, (HCE group) had significantly (P < 0.05) higher blood haemoglobin content than the remaining groups. While the control and LC groups had the lowest haemoglobin content, HE and

HCE groups had the highest values. There was a negative correlation ($r = -0.4$, $P < 0.001$) between blood haemoglobin and erythrocyte percentage haemolysis (Fig. 2).

The data on total plasma protein indicate that chickens in HCE group had significantly higher ($P < 0.001$) values than the remaining groups (Table 3). Increasing dosage of vitamin E alone resulted into slight increase (not statistically significant) in total plasma protein, this was not true for vitamin C. It was also observed that a combination of vitamins had a measurable effect toward the increase of total plasma protein.

Groups under supplementation with both vitamins (LCE and HCE) had significantly ($P < 0.01$) higher plasma ascorbic acid content than other groups. Plasma ascorbic concentration reflected dietary vitamin C although at a level which was not statistically significant. It is also apparent from this data that vitamin C was more bioavailable in plasma when supplemented in combination with vitamin E than when supplied alone. There was a positive correlation ($r = 0.34$, $P < 0.01$) between plasma ascorbic acid and total plasma protein.

DISCUSSION

The erythrocyte haemolysis bioassay was employed in the present study as an indirect measure of vitamin E status and an intermediary test between those based on biological functions and those involving measurements of tocopherol levels in plasma and tissues (Azaz *et al.*, 1981). The data obtained from this assay shows that vitamin supplementation, especially vitamin E, was significantly effective ($P < 0.001$) in

reducing percentage erythrocyte haemolysis. Since LC group had higher percentage haemolysis than other supplemented groups, it seems that vitamin C could only reduce percentage haemolysis at high doses. Hoppe *et al.* (1989) observed a highly significant protective effect of vitamin C on the cell membrane integrity of stressed pigs when the latter was supplemented at 500 mg/kg feed. Although vitamin C is not directly associated with cell membranes, the mechanism suggested by Cadenas *et al.* (1983) seems to be relevant in explaining the involvement of this vitamin in membrane integrity. Vitamin C in excess at membrane site tends to regenerate vitamin E. Thus by supplementing high dose of vitamin C, the regeneration of vitamin E was improved hence leading to the observed results.

Low and high levels of vitamin E were equally capable of significantly reducing the percentage haemolysis when compared with control. From these findings it can be concluded that only a moderate amount of vitamin E is required for optimal cell membrane integrity and that is why combination of both vitamins did not further reduce the percentage haemolysis. Balthazary (1991) observed haemolysis of 18% when the broiler chickens were supplemented with 22 mg/kg DL-TA. This level is similar to that used in the present study which gave 15.6% haemolysis in the control group.

Erythrocyte haemoglobin content is a good measure of the ability of the cells to carry oxygen and therefore the proper functioning of the entire process of oxygen transport throughout the body. Since vitamin E is reported to be involved in the synthesis of haeme molecule, (Caasi *et al.*, (1972) data from haemoglobin determination

would give clues on the status of the vitamin in the animals and perhaps to explain the reason for improved metabolism and performance. The data obtained from this test fairly agree to the fact that vitamin E may influence haemoglobin content of RBC, since HE, LCE and HCE groups had slightly higher haemoglobin content than other groups. The tendency of vitamin C at high level to give effects similar to those of vitamin E was also observed in this test. This can be explained by the previously discussed vitamin E regeneration theory (Cadenas *et al.*, 1983).

The negative correlation ($r = -0.4$, $p < 0.01$) observed between percentage haemolysis and haemoglobin content is another interesting biochemical feature. It is possible that high percentage haemolysis is a result of low vitamin E status and hence decreased synthesis of haemoglobin. This observation can lead to a possibility of using haemoglobin content as an indirect measure of vitamin E status in animals. The synergistic relationship of vitamin C and E was not pronounced at this juncture as there was no significant difference between HE, LCE and HCE groups.

Total plasma levels were within a range of 4.0 - 5.2 mg/dl what agrees with those reported by Swenson (1984) as a normal range in chickens. Literature on plasma protein following supplementation with the two antioxidant vitamins (C and E) indicate that many investigators have concentrated on a particular component of plasma protein such as immunoglobulins (antibodies) (Quereshi *et al.*, 1993; Maurice *et al.*, 1993; Rajmane, 1992; Lewin, 1974). Results obtained in the present study have shown that supplementation of chickens with high dose of vitamins C and E increase total

plasma protein significantly ($P < 0.05$). Franchini *et al.* (1994) found that increasing intake of ascorbic acid during early growth in chicks increases percentage of DNA synthesizing cells and that of cells affecting bases incorporation into molecular DNA chain. This finding is an indication of increased protein synthesis.

Bioavailability of ascorbic acid in experimental animals was determined by directly measuring its plasma concentration. The plasma ascorbic acid concentration of the control group (unsupplemented) ranged from 1.2 - 1.9 mg/dl with a mean value of 1.5 ± 0.2 mg/dl. This value is similar to that obtained by Pardue and Thaxton (1984) i.e., 1.25 - 1.35 mg/dl who also employed a spectrophotometric method but slightly higher than that obtained by Pardue *et al.* (1993), i.e., 0.7 - 1.0 mg/dl when a high performance liquid chromatography (HPLC) method was used. These differences are a result of analytical procedures and probably the level of the vitamin in the basal diet. The finding that increasing level of vitamin C alone in the diet did not significantly increase plasma ascorbic acid while that of the two vitamins in combination did, is a further evidence of synergism in terms of regeneration mechanism. The positive correlation ($r = 0.34$, $p < 0.01$) between total plasma protein and plasma ascorbic acid substantiates the fact that ascorbic acid is involved in protein synthesis.

In conclusion, dietary supplemental vitamins C and E, especially when supplied together at high dose of 240 and 280 mg/kg feed respectively, considerably improve their bioavailability, erythrocyte membrane stability, haemoglobin content and total plasma protein. The most significant observations occur when the two vitamins

are supplied together, an indication of a degree of synergism. Improved immunocompetence and performance which is frequently observed following dietary supplementation with the two vitamins seems to be a result of interrelationship between several biochemical changes which take place simultaneously in the body of an individual. The relationship between vitamin E status and blood haemoglobin content which was observed in this study can be taken to advantage so that the haemoglobin content can be used as an indirect measure of vitamin E status in animals.

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