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Effect of vitamin C supplementation on haematological and serum biochemical indices of laying hens diagnosed with fowl typhoid under tropical environment

Opoola. E[1]., Atte, P.I[2]., Makinde, O.J.[3] and Aganbi, B.[4]

[1]*Department of Animal Science, Ahmadu Bello University, Zaria*
[2]*Kabba College of Agriculture, Ahmadu Bello University, Zaria*
[3]*Department of Animal Science, Federal University, Gashua.*
[4]*Nigerian Meteorological Agency-NIMET*

Corresponding Author: emmycom123@yahoo.com **Phone no.:** 07032217712

Targeted Audience: Poultry nutritionist and feed mill industry

Abstract

To evaluate the effect of four different levels of vitamin C supplementation on the haematological and serum biochemical parameters of laying hens infected with fowl typhoid, a trial was conducted with 300 Isa Brown layer hens. The experiment was conducted by using a completely randomized design with 5 treatments, 3 replicates, and 20 hens in each replicate. Treatments included basal diet (control) and basal diet plus 100, 150, 200, and 250 mg of vitamin C/kg of diet, respectively. At the end of the 12-week treatment period, samples of blood were collected to determine haematological and serum biochemical values. The results showed that white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), uric acid and cholesterol were significantly ($p<0.05$) higher in layer hens fed vitamin C than those fed the control diet. However, Packed cell volume (PCV), haemoglobin count (HC), red blood cell (RBC), urea, creatinine, glucose, alkaline Phosphatase (ALP), albumin, total protein and serum vitamin C were not significantly ($p>0.05$) affected by dietary treatments. These results indicated that the inclusion of vitamin C at the level of 150mg/kg of diet may have a positive effect on immunity of laying hens.

Keywords: Vitamin C, haematology, serum, layer hens, tropics

Description of Problem

The combination of high ambient temperature (AT), relative humidity (RH) and disease conditions continue to cause major environmental distress in laying birds, impairing their performance, egg quality and increasing the risk of birds' mortality rate (1). These aforementioned conditions are mostly common in the tropics where high ambient temperatures ranging from 30 – 44^{o}C and relative high humidity ranging from 81 – 85% are major causes of mortality, decline in egg production, and egg quality in laying hens (2).Although the environmental approach through modification of housing is the best option to obtain optimal performance, it is nevertheless very expensive, thereby making nutritional strategies more viable alternatives (1).Vitamin C inclusion in poultry diet is one of the cheapest means of alleviating stress, this is due to its antioxidant properties, neutralizing the free radicals generated during heat stress

(2, 3).However, inclusion of Vitamin C in poultry diet is not only because of its anti heat-stress effects but also due to the fact that its biosynthesis is reduced in the birds during heat stress (4).

Research conducted by (5) showed that when chicks were infected with fowl typhoid, their plasma vitamin C concentrations were reduced. In addition to performance, evidence also suggests an association between ascorbic acid and the animal's ability to tolerate or resist certain bacterial infections (4). Haematological changes are used to determine various status of the body and determine stress due to nutrition, health and environment, (6). In earlier work in the temperate region, chickens infected with fowl typhoid had reduced levels of ascorbic acid in the blood and the administration of ascorbic acid at 1,000 mg per kg (454 mg per lb) of feed resulted in reduced early mortality from typhoid infection (7). Chickens fed a diet containing supplemental ascorbic acid showed increased resistance to a combined Newcastle disease virus *Mycoplasma gallissepticum* infection and to a secondary E. coli infection, as well as to a primary *E. coli* challenge (8). The aim of this study, therefore, was to evaluate the effect of dietary vitamin C on the haematological parameters and serum biochemical indices of laying birds diagnosed with fowl typhoid under tropical environment.

Material and Methods

Study location

The experiment was carried out at the Poultry Unit of Kogi State Ministry of Agriculture, Kabba. Kabba is located within the Southern Guinea Savannah Zone on latitude $7^05^{'}$N, longitude $6^0 4^{'}$E and altitude of 640m above sea level. It has an annual rainfall of 1500mm and rain starts between late April and early May to mid October. The dry season begins around the middle of November, with cool weather that ends in February. This is followed by relatively hot-dry weather between March and April just before the rain begins. The minimum daily temperature is from 14^oC - 20^oC during the cool season while the maximum daily temperature is from 19^0C-40^0C during the hot season. The mean relative humidity during dry and wet seasons is 21% and 72%, respectively (9).

Experimental Diets

Five layer mash diets were formulated. Diets 1 was formulated without vitamin C while diets 2, 3, 4and 5 contained graded levels of supplemental vitamin C at: 100, 150, 200 and 250mg/kg (Table1). The diets were formulated to be isocaloric and isonitrogenous (CP=17%, ME=2750 Kcal/Kg). Diets were also formulated to meet the (10) nutrient requirement. The birds were fed the experimental diets for 12 weeks. All the diets were chemically analyzed according to the standard of (11) methods for their proximate composition.

Experimental Design and Management of Birds

A 12 week experiment involving 300 Issa Brown laying hens of 44weeks of age and average live weight of 1.91 ± 0.08 kg was carried at the Poultry Unit of Kabba College of Agriculture; Ahmadu Bello University after a case of fowl typhoid was noticed on the College research farm during the month of February 2018. The outbreak was investigated by combining recognition of clinical signs and post-mortems examination. The clinical signs were monitored for a period of one month; all the birds were treated with a range of antibiotics and isolated when necessary. There were three replicates, each replicate had 20 birds. The experiment was a completely randomized design. The birds were reared in a deep litter system. Routine vaccination and medications was carried out and drugs

administered as needed in the course of the experiment. Feed and water was provided *ad libitum*.

Haematological and Serum Biochemical Analyses

At the end 12th week of the experiment, 2ml of blood samples were collected from each of three birds per replicate via the wing veins into sterile tubes containing an anticoagulant (ethylene diamine tetra acetic acid, EDTA) for the determination of haematological parameters like Packed Cell Volume (PCV) which was determined by the micro-haematocrit method, haemoglobin concentration (HC) which was determined photometrically at the wavelength of 540nm, the erthtocyte (RBC) and leucocytes (WBC) were done using the improved Neubauer haemocytometer, Differential leucocyte counts were determined by the thin slide method. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated from Hb, PCV and RBC (12).

However, 2ml of blood also was allowed to clot and then centrifuged. The serum was separated and stored at -20°C until analyzed for serum parameters (albumin, total protein, glucose, cholesterol, urea, uric acid, Alkaline Phosphatase (ALP) and Creatine Kinase (CK) according to the methods described by (13) at the Haematology Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Each sample was analyzed in triplicates. Mortality record was recorded as it occurred.

Data analysis

All data obtained were statistically analyzed using the General Linear Models (GLM) procedure of (14) for the analysis of variance. Tukey's honestly significant difference multiple range test was performed. Means were considered different at $P<0.05$.

General Linear Model

$Y_{ij} = \mu + K_{i+} e_{ij}$

Y_{ij}= Observation of the i^{th} level of Vitamin C effect on haematological and blood serum indices

μ= Overall mean

$K_i = i^{th}$ effect of Vitamin C

e_{ij} = Random error

Results and Discussion

It is well established that high ambient temperature, high relative humidity and disease condition impairs absorption of vitamin C thereby increasing the dietary requirement of this vitamin (15). Results from the present study showed that graded dietary vitamin C levels significantly ($P<0.05$) affected the white blood cells, MCH, MCV, MCHC, uric acid and cholesterol (Table 2). This present study is a clear indication that the layer chickens were subjected to heat and pathological stresses. However the total RBC counts in this study were not affected. It has been documented that the total RBC counts and HC should not be interpreted clinically, as they vary almost exactly in parallel with the PCV (16). Their function is to allow the calculation of MCV, MCH and MCHC, respectively (17). HC and PCV values (13.63-15.77g/100ml and 31.0-37.0%) obtained in the present study were observed to be higher than the values obtained by (16), who reported 6.0-13g/100ml and 29.0-38.0% respectively. These differences in the values might probably be due to their diseased condition. However, dehydration suffered by the chickens as a result of disequilibrium in acid-base balance, leading to respiratory alkalosis (18) may be responsible for the differences. The MCHC values of 33.0-35.0 pg recorded by (18) are slightly related to the values obtained in this present study (33.31 -34.24pg).

White blood cells are transported to areas of serious infection and inflammation, thereby providing a rapid and potent defence against

infectious agents (19). Laying hens fed 250mg/kg dietary vitamin C had the highest value for white blood cell (WBC) followed by laying hens fed 100, 150 and 200 mg/kg dietary vitamin C. It was observed that the laying hens fed the control diet had the least value for white blood cell. Although no clear pattern was observed for the values obtained across the treatment groups for WBC, these values however fell within the normal range (4.93-8.90 x 10^3/l) for healthy birds as suggested by (20). Increased levels of vitamin C in the diets of laying hens may be responsible for the high WBC production.

The mean values (170.60-237.00mg/dl) of uric acid in this experiment does not agree with the report of (21) who reported 245±49 - 639 ±175, however the lower mean values of uric acid in this experiment showed that there was no incidence of kidney damage as the high mean values reported by (22) was attributed to kidney damage. Dietary treatments had no (P>0.05) significant effects of albumin, total protein and ALP of laying hens. However, the observed numerical increase in the serum albumin concentration as the levels of dietary vitamin C increased could be partially explained, by the reduction of synthesis and secretion of corticoids in birds fed vitamin C. Decreased cholesterol concentrations observed as the dietary levels of Vitamin C increased in the present study were in agreement with previous report of (23). This might be due to the function of vitamin C in transforming cholesterol to bile acids by controlling the microsomal 7α-hydroxylation. As this reaction is the rate-limiting step of cholesterol catabolism in liver, ascorbic acid deficiency induces a marked slowing down of this reaction, leading to cholesterol accumulation in liver and in blood (24). Mortality rate across the treatment groups were not significantly (p>0.05) affected by dietary treatments (Table 4). However, it was observed that treatment with vitamin C supplementations had lower mortality. This might be due to the strict bio security measures like the effective disinfecting of the poultry pens and its surrounding.

Conclusion and Applications

1. Dietary vitamin C supplementation significantly protected haematological and blood serum indices in laying hens infected with fowl typhoid and reared under high ambient temperature.
2. Laying hens supplemented with 150 mg/kg vitamin C had better haematological and serum biochemical profiles across the treatment groups.

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Table 1: Composition of Layer Mash Diets Containing Graded Levels of Vitamin C (44-56Weeks)

	Vitamin C mg/kg				
Ingredient	**1**	**2**	**3**	**4**	**5**
	0.00	**100**	**150**	**200**	**250**
Maize	30.00	30.00	30.00	30.00	30.00
Maize offal	35.95	35.95	35.95	35.95	35.95
Groundnut cake	15.00	15.00	15.00	15.00	15.00
Soya cake	6.00	6.00	6.00	6.00	6.00
Limestone	9.00	9.00	9.00	9.00	9.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Common salt	0.30	0.30	0.30	0.30	0.30
Premix **	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30	0.30
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcal/kg)	2750	2500	2500	2500	2500
Crude protein (%)	17.00	17.00	17.00	17.00	17.00
Ether extract (%)	3.88	3.88	3.88	3.88	3.88
Crude fibre (%)	5.83	5.83	5.83	5.83	5.83
Calcium (%)	4.09	4.09	4.09	4.09	4.09
Lysine (%)	0.80	0.80	0.80	0.80	0.80
Methionine (%)	0.54	0.54	0.54	0.54	0.54
Available P (%)	0.52	0.52	0.52	0.52	0.52
TSAA (%)	0.78	0.78	0.78	0.78	0.78

Total sulfur amino acid =TSSA, P=Phosphorus; ME=Metabolizable energy;**Biomix Premix Supplied per kg of diet: Vit. A, 10,000iu; Vit.D_3, 2000iu; Vit E, 23mg;Vit. K, 2mg: Vit.B1,1.8; Vit. B_2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 7.5mg; Vit. B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

Table 2. Effect of Graded Dietary Levels of Vitamin C on the Haematological Parameters and Serum Biochemical Indices of Laying Hens. (44 - 56Weeks).

	Treatments					
	Vitamin C Levels mg/kg					
Parameters	0.00	100	150	200	250	SEM
PCV (%)	31.00±7.54	35.00±4.00	36.33±3.06	36.33±9.07	37.00±5.20	3.58
HC (g/dl)	13.63±2.52	15.00±1.30	15.43±1.03	15.77±2.44	15.57±1.88	1.12
RBC (x 10^6/l)	4.37±0.60	4.73±0.23	4.57±0.40	4.73±0.55	4.73±0.38	0.26
WBC (x 10^3/l)	4.93^{b}±2.52	8.17^{a}±1.70	8.67^{a}±3.71	7.23^{a}±3.62	8.90^{a}±1.56	1.61
MCH (pg)	31.09^{b}±1.44	31.66^{b}±1.63	33.85^{a}±1.09	33.21^{a}±1.41	32.81^{a}±1.45	0.82
MCV (fl)	93.50^{c}±4.28	94.99^{bc}±5.07	97.24^{a}±8.41	101.63^{a}±3.50	99.13^{a}±3.43	3.05
MCHC (g/dl)	33.31^{b}±0.08	33.34^{b}±0.07	33.25^{b}±0.02	34.24^{a}±1.62	33.09^{b}±0.37	0.43

a, b,c = Means with different superscript on the same row differ significantly ($p<0.05$)
SEM = Standard Error of Means
PCV= Packed cell volume
HC=Haemoglobin count
RBC=Red blood cells
WBC= White blood cells
MCH=Mean corpuscularhaemoglobin
MCV=Mean corpuscular volume
MCHC=Mean corpuscular haemoglobin count

Table 3. Effect of Graded Dietary Levels of Vitamin C on the and Serum Biochemical Indices of Laying Hens. (44 - 56Weeks).

	Treatments					
	Vitamin C Levels mg/kg					
Parameters	0.00	100	150	200	250	SEM
Creatine (mg/dl)	53.33±16.26	58.67±3.79	61.00±6.25	80.33±33.81	107.00±46.71	19.60
Glucose (mg/dl)	7.03±1.86	8.00±0.75	6.90±0.10	8.43±0.42	8.00±0.85	0.98
ALP (i.u/l)	41.00±1.73	39.67±3.22	42.00±1.00	40.33±1.15	41.33±17.58	1.03
Cholesterol (mg/dl)	7.40^{a}±1.06	6.03^{b}±0.31	6.10^{b}±0.10	5.10^{c}±4.45	5.27^{c}±0.31	0.32
Albumin (g/dl)	3.97±0.32	4.10±0.17	4.20±0.10	4.03±0.12	4.13±0.06	0.10
Total protein (g/dl)	7.27±2.21	7.26±0.76	7.17±0.38	7.43±0.84	8.03±0.80	0.68
Blood Vitamin C	3.73±2.32	2.93±0.58	4.73±1.21	5.20±1.73	3.27±0.58	0.98

a, b,c = Means with different superscript on the same row differ significantly ($p<0.05$)
SEM = Standard Error of Means
ALP=Alkaline Phosphatase

Table 4. Effect of Graded Dietary Levels of Vitamin C on the Mortality rate of Laying Hens. (44 - 56Weeks).

	Treatments					
	Vitamin C Levels mg/kg					
Parameters	0.00	100	150	200	250	SEM
Mortality	4.32±1.87	3.33±1.11	3.33±1.11	1.67±1.06	3.33±1.11	1.42