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RESEARCH ARTICLE

THE GROWTH AND HAEMATOLOGICAL EFFECTS OF ASCORBIC ACID SUPPLEMENTATION ON COCCIDIA-CHALLENGED BROILER CHICKENS

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ABSTRACT

A total of one hundred and ninety two day old broiler chicks were used to evaluate the growth and heamatological effects of Ascorbic acid (AA) supplementation on coocidia-challenged broiler chickens. The design was a 4 x 2 factorial in a completely randomized design. The main-factor was the period or length of time of administering ascorbic acid (AA), while the sub-factor was the dosage of AA used for the feed composition.

The birds were challenged with oocysts of *Eimeria tenella* at the end of the third week; and started manifesting signs of coccidiosis four days after challenge. The oocyst count and percentage mortality were significantly ($P<0.05$) different for the main-factors and the interactions, but not for the sub-factors. At the end of eight weeks experimental period, percentage mortality were significantly ($P<0.05$) different for the main-factors and the interactions, but not for the sub-factors; while the oocyst count were significantly ($P<0.05$) different for the main-factors, sub-factors and interactions.

The haematological parameters – Packed Cell Volume (PCV), Haemoglobin (Hb), White Blood Cell (WBC), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were significantly ($P<0.05$) different for the main-factors, sub-factors and the interactions; except for the MCHC which was not significantly ($P<0.05$) different for the sub-factors and the interactions.

The plasma constituents – plasma protein and plasma lipid were significantly ($P<0.05$) different for the main-factors and the sub-factors. Whereas plasma lipid was significantly ($P<0.05$) different for the interactions, it was not so for the plasma protein. It may be concluded that supplementing broiler diets with 150mgAA per Kg of feed has profound effect on growth and haematology which in turn leads to reduction in the severity of coccidiosis in birds.

INTRODUCTION

Ascorbic acid (AA) is a non-essential component of poultry diet (Oluyemi and Roberts, 1979) as it can be synthesized intrinsically by birds. This means that chickens do not require any exogenous supplementation of AA (Tuleun and Njoku, 2000). However, it had been suggested that under certain conditions such as climatic stress, the demand for AA becomes greater than the amount provided by normal tissue synthesis, and dietary supplementation may be beneficial (Mcdonald et.al., 1995). Cyclic high temperature led to increased mortality and reduced growth rate in broilers (Joiner and Houston, 1975).

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In addition to growth, many of the biochemical functions of AA are involved in protection against infections and response to vaccination (Bendich, 1987). In formulating poultry rations, AA is frequently ignored as a result of the belief that it can be synthesized in the body (Oluyemi and Roberts, 1979); this could be counter-productive because any disturbance of the synthetic process could result in a deficiency state leading to general unthriftness and reduced immune response (Arijeniwa, 1995).

Supplementary AA appear necessary to overcome immunosuppression induced by heat as well as maintaining normal performance under pressure from infections in most parts of the tropics (Arijeniwa, 1995). AA also helps in normalizing the reduced chemo-tactic and bactericidal activities of neutrophils from individuals with inherited phagocytosis disorders.

Among layers, supplementing diets with AA serves to enhance absorption of iron from the intestine (Oluyemi, 1979) which aids erythropoiesis by promoting incorporation of iron into tissue ferritin.

Apart from ambient temperature and nutrition, one other problem that could contribute a stress to birds is disease infestations. For instance, coccidiosis (especially caecal coccidiosis) is one of the most prevalent poultry diseases in the tropics. It occurs mostly under stress conditions, and stress is a factor that can hardly be totally eradicated in poultry farms in the tropics. Coccidiosis has been implicated to be a major cause of mortality in poultry farms, and birds that survive the infection are most often debilitated (Reid, 1975).

Hematological changes are routinely used to determine various status of the body and to determined stresses due to environmental, nutritional and/or pathological factors. Because of these facts, during the recent decades the avian physiology is found to be of great importance to the scientists, researchers and veterinarians as well as poultry growers (Islam et.al., 2004). Hematological values of chickens are influenced by age, sex, breed, climate, geographical location, season, day length, time of day, nutritional status, life habit of species, present status of individual and such other physiological factors (Dukes, 1955). For proper management, feeding, breeding, prevention and treatment of diseases; it is desirable to know the normal physiological values under local conditions but normal hematological information of the valuable birds is hardly available in the literature as researches on these line have rarely been carried out under local condition (Islam *et al.,*2004).

MATERIALS AND METHODS

A total of one hundred and nine-two day old broiler 'Anak' chicks were used for the study. Eight chicks were randomly selected, weighed and assigned to each of the twenty-four pens. The chicks were reared on deep litter floor. Brooding lasted for three weeks, after which oocysts of Eimeria tenella were introduced into the experiment. The basal ration for both broiler starter and finisher diets are as given below:

Table 1. Composition and calculated analysis of basal diets

Ingredients	Broiler Starter (%)	Broiler Finisher (%)
Maize	49.50	57.00
Soyabean meal	30.00	14.00
PalmKernel cake	4.00	8.00
Bone meal	3.00	2.50
Wheat offal	2.50	4.50
Fish meal	6.00	5.00
Blood meal	1.50	3.50
Palm oil	1.50	2.50
Oyster shell	1.00	2.00
Vitamin-mineral premix	0.30	0.30
Salt	0.30	0.30
Methionine	0.20	0.20
Lysine	0.20	0.20
Total	100	100
Calculated Nutrient Levels		
Crude protein (%)	23.15	19.47
Metabolizable energy (Kcal/Kg)	2896.40	2950.30

**The vitamin-mineral premix supplied per kg diet:- vitamin A-1500w, vitamin D3-3000w, vitamin E-48mg, vitamin K-3mg, vitamin B1-2.4mg, vitamin B2-7.2mg, Nicotinic acid-42mg, calcium D-pantothenate-12mg, vitamin B6-0.2mg, vitamin B12-0.03mg, folic acid-1.2mg, Biotin-0.06mg, choline chloride-360mg, zinc bacitracin-48mg, manganese-120mg, iron-60mg, iodine-1.86mg, cobalt-0.27mg and selenium-0.12mg.*

The experiment was conducted at the poultry unit of the Teaching and Research farm of College of Animal Science and Animal Production (CASAP), Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. Umudike is located at Latitude 5^{o} 29$^{!}$ North and longitude 1.7^{o} 32$^{!}$ East in the rain forest zone of Nigeria. The climate of the region is characterized by a mean daily temperature of between 27^{o}C and 35^{o}C all through the year. Average rainfall of Umudike is about 2000mm per annum with double maxima pattern.

The experiment was a 4x2 factorial design with three replications for each treatment group. The main-factors were (1) No Ascorbic acid (AA) supplementation before and after challenge with coocidia oocysts, this group was termed 'ZeroAA'. (2) No AA supplementation and no challenge with coccidia oocysts. This group was the 'Control'. (3) AA supplementation after challenge with coccidia oocysts, this group was termed 'PostAA). (4) Supplementation of AA before and after challenge with occidian oocysts, this group was termed 'Continuous AA'. The sub-factors were (1) 150mg dose of AA per Kg of feed. (2) 300mg dose of AA per Kg of feed. Thus, the first group of birds was fed unsupplemented diets before and after challenge with coccidian oocysts. The second group of birds was the control. The third group of birds was fed unsupplemented diets before they were challenged with coocidia oocysts, but had their diets supplemented with AA after challenge with coccidia oocysts. The fourth group of birds was fed supplemented diet continuously throughout the experimental period.

The birds except the control were individually inoculated per os with 1ml of coccidian oocyst suspension at the end of the third week. 1ml of the suspension contains 10^5 oocysts of Eimeria tenella. Coccidiosis was established four days after inoculation through observation of loose droppings and/or droppings mixed with blood (Arijeniwa, 1995). The chicks were treated three days after the establishment of coccidiosis with 'Amprol 20% soluble powder'.

The basal, non-AA diet (Tuleun *et al.,*2000) as shown in table1 consisted of a practical maize-soyabean meal, containing 23.15% crude protein and 2896.40Kcal of Metabolizable energy/Kg; 19.47% crude protein and 2950.30Kcal of Metabolizable energy/Kg for the broiler starter and finisher diets respectively.

Feed and water were provided ad-libitum. Before treatment against coccidiosis was initiated, feed intake, weight gain, feed to gain ratio, oocyst count and percentage mortality were recorded. At the end of the eighth week of the experiment, a bird was selected from each of the twenty-four pens, fasted overnight and weighed. Blood samples were collected from each of the selected birds through the wing vein. The blood samples were collected into labeled sterile universal bottle containing a pinch of dried ethyl diamine tetraacetic acid (EDTA) powder (Awoniyi *et al.,*1999) as the anti-coagulant. The blood samples were used to determine the erythrocytic indices, white blood cell (WBC) count, and the plasma constituents. The erythrocytic indices are packed cell volume (PCV), haemoglobin concentration (Hbg), total erythrocyte count (RBC) and absolute haematological value. The plasma constituents are plasma protein and plasma lipid.

Table 2.1. Values of Growth parameters, Oocyst counts, Percentage mortality, length and width of Caecum and weight of Adrenal glands for the main-factors and sub-factors before treatment against coccidiosis

PARAMETERS	MAIN-FACTORS					SUB-FACTORS		
	Zero AA	Control	Post AA	Cont.AA	SEM	150mgAA	300mgAA	SEM
Av. Final wt. (g)	168.20^{b}	214.20^{a}	209.00^{a}	210.20^{a}	9.38*	205.50	196.00	6.63^{ns}
Av. Initial wt. (g)	38.00	39.00	41.00	38.00	3.49^{ns}	40.50	38.00	2.47^{ns}
Av. Wt gain (g)	130.20^{b}	175.20^{a}	168.00^{a}	172.20^{a}	15.57*	165.00	158.00	11.01^{ns}
Feed intake (g/d/bird)	33.70^{b}	36.55^{a}	35.36^{ab}	35.38^{ab}	0.85*	35.61	34.89	0.60^{ns}
Wt. gain (g/d/bird)	8.65	10.26	10.00	10.15	0.64^{ns}	9.89	9.64	0.45^{ns}
Feed : gain ratio	3.90^{a}	3.56^{b}	3.53^{b}	3.49^{b}	0.35*	3.60	3.62	0.24^{ns}
Oocyst count	10^{5a}	0^{d}	$4x10^{4b}$	$12.5x10^{3c}$	4568.92*	$38.75x10^{3}$	$37.5x10^{3}$	3230.71^{ns}
Mortality (%)	12.5^{a}	0d	6.25^{b}	2.08^{c}	0.46*	5.21	5.21	0.32^{ns}

*a-d on the same row with different superscripts are significantly different(P < 0.05); * * = significant; *ns = not significant; *SEM = standard error of mean; *Zero AA = No supplementation of diet with ascorbic acid; *Post AA = Post challenge supplementation of diet with ascorbic acid; *Cont.AA = Continuous supplementation of diet with ascorbic acid

Table 2.2. Values of Growth parameters, Oocyst counts, Percentage mortality, length and width of Caecum and weight of Adrenal glands for the interactions between the main-factors and sub-factors before treatment against coccidiosis.

PARAMETERS	150mgAA			300mgAA					
	Zero AA	Control	Post AA	Cont. AA	Zero AA	Control	Post AA	Cont. AA	SEM
Av. Final wt (g)	168.20	221.00	211.80	214.00	168.20	209.00	205.00	207.00	13.26^{ns}
Av. Initial wt (g)	38.00	38.00	41.00	39.00	38.00	39.00	40.00	41.00	4.94^{ns}
Av. Wt gain (g)	130.20	183.00	170.80	175.00	130.20	170.00	165.00	166.00	22.02^{ns}
Feed intake (g/d/bird)	33.70	36.80	35.92	36.00	33.70	36.30	34.80	34.75	1.20^{ns}
Wt. gain (g/d/bird)	8.65	10.52	10.10	10.30	8.65	10.00	9.90	9.80	0.90^{ns}
Feed : gain ratio	3.90	3.50	3.56	3.50	3.90	3.63	3.52	3.55	0.49^{ns}
Oocyst count	10^{5a}	0^{d}	$5x10^{4b}$	$5x10^{3d}$	10^{5a}	0^{d}	$3x10^{4c}$	$2x10^{4c}$	6461.4*
Mortality (%)	12.50^{a}	0^{d}	8.33^{b}	0^{d}	12.50^{a}	0^{d}	4.17^{c}	4.17^{c}	0.65*

*a-d on the same row with different superscripts are significantly different (P < 0.05); * * = significant; *ns = not significant; *SEM = standard error of mean; *Zero AA = No supplementation of diet with ascorbic acid; *Post AA = Post challenge supplementation of diet with ascorbic acid; *Cont.AA = Continuous supplementation of diet with ascorbic acid

Table 2.3. Values of Growth parameters, Oocyst counts, and Percentage mortality for the main-factors and sub-factors at the end of 8 weeks.

PARAMETERS	MAIN-FACTORS				SUB-FACTORS			
	Zero AA	Control	Post AA	Cont.AA	SEM	150mgAA	300mgAA	SEM
Av. Final wt. (g)	1050.00^{c}	1269.00^{b}	1297.00^{ab}	1336.00^{a}	20.90*	1246.50	1230.00	14.81^{ns}
Av. Initial wt. (g)	38.00	39.00	41.00	38.00	3.49^{ns}	40.50	38.00	2.47^{ns}
Av. Wt gain (g)	1012.00^{c}	1230.00^{b}	1256.00^{b}	1298.00^{a}	14.79*	1206.00	1192.00	10.46^{ns}
Feed intake (g/d/bird)	83.13^{c}	93.25^{b}	95.10^{a}	93.23^{b}	0.51*	90.18^{b}	91.62^{b}	0.48*
Wt. gain (g/d/bird)	18.07^{c}	21.97^{b}	22.43^{ab}	23.18^{a}	0.52*	21.54	21.28	0.44^{ns}
Feed : gain ratio	4.60	4.20	4.24	4.02	0.58^{ns}	4.19	4.31	0.41^{ns}
Oocyst count	10^{3a}	0^{c}	375^{b}	0^{c}	30.89*	312.50^{b}	375^{a}	21.84*
Mortality (%)	12.50^{a}	0^{d}	6.25^{b}	2.08^{c}	0.46*	5.21	5.21	0.32^{ns}

*a-d on the same row with different superscripts are significantly different(P < 0.05); * * = significant; *ns = not significant; *SEM = standard error of mean; *Zero AA = No supplementation of diet with ascorbic acid; *Post AA = Post challenge supplementation of diet with ascorbic acid; *Cont.AA = Continuous supplementation of diet with ascorbic acid

All the collected data were subjected to statistical analysis using Analysis of Variance (ANOVA). Differences between the treatment means for the main-factors and sub-factors were separated using least significance difference (LSD), while the interactions between the main-factors and sub-factors were separated by Duncan's multiple range test.

RESULTS

The mean values of growth parameters, oocyst counts, percentage mortality, caecum length and weight of adrenal glands for the main-factors and sub-factors, and the interactions between the main-factors and the sub-factors respectively before treatment against coccidiosis was instituted (Tables 2.1 and 2.2). From table 2.1, there were significant ($P<0.05$) differences in the feed intake and feed to gain ratio for the main-factors, while the weight gain

Table 2.4. Values of Growth parameters, Oocyst counts and Percentage mortality for the interactions between the main-factors and sub-factors at the end of 8 weeks.

PARAMETERS	150mgAA				300mgAA				
	Zero AA	Control	Post AA	Cont. AA	Zero AA	Control	Post AA	Cont. AA	SEM
Av. Final wt (g)	1050.0^{d}	1313.5^{ab}	1269.5^{bc}	1347.5^{a}	1050.0^{d}	1224.0^{c}	1323.0^{ab}	1328.5^{ab}	29.63*
Av. Initial wt (g)	38.0	38.0	41.0	39.0	38.0	39.0	40.0	41.0	4.94^{ns}
Av. Wt gain (g)	1012.0^{c}	1275.5^{a}	1228.5^{b}	1308.5^{a}	1012.0^{c}	1185.0^{b}	1283.0^{a}	1287.5^{a}	20.92*
Feed intake (g/d/bird)	83.13	90.68	93.99	92.91	83.13	93.59	96.21	93.54	0.87^{ns}
Wt. gain (g/d/bird)	18.07	22.78	21.94	23.37	18.07	21.16	22.91	22.99	0.82^{ns}
Feed : gain ratio	4.60	3.98	4.28	3.98	4.60	4.42	4.20	4.07	0.80^{ns}
Oocyst count	10^{3a}	0^{d}	250^{c}	0^{d}	10^{3a}	0^{d}	500^{b}	0^{d}	43.68*
Mortality (%)	12.50^{a}	0^{d}	8.33^{b}	0^{d}	12.50^{a}	0^{d}	4.17^{c}	4.17^{c}	0.65*

*a-d on the same row with different superscripts are significantly different (P < 0.05); * * = significant; *ns = not significant; *SEM = standard error of mean; *Zero AA = No supplementation of diet with ascorbic acid; *Post AA = Post challenge supplementation of diet with ascorbic acid; *Cont.AA = Continuous supplementation of diet with ascorbic acid

Table 2.5. Values of Haematological parameters for the main-factors and sub-factors at the end of 8 weeks.

PARAMETERS	MAIN-FACTORS			SUB-FACTORS				
	Zero AA	Control	Post AA	Cont.AA	SEM	150mgAA	300mgAA	SEM
PCV (%)	25.65^{c}	25.75^{c}	29.20^{b}	32.25^{a}	0.527*	28.89^{a}	27.54^{b}	0.373*
Hb (g/dl)	8.00^{c}	8.10^{c}	9.27^{b}	10.40^{a}	0.186*	9.21^{a}	8.68^{b}	0.132*
WBC (x10^{6}/mm^{3})	38.00^{a}	31.65^{b}	27.70^{c}	25.50^{d}	0.436*	30.03^{b}	31.40^{a}	0.309*
RBC (x10^{6}/mm^{3})	2.71^{c}	2.82^{b}	2.90^{ab}	2.98^{a}	0.045*	2.89^{a}	2.82^{b}	0.032*
MCV (fl)	94.65^{c}	94.67^{c}	100.69^{b}	108.35^{a}	0.559*	101.48^{a}	97.70^{b}	0.395*
MCHC (%)	31.19^{b}	31.46^{b}	31.73^{ab}	32.21^{a}	0.306*	31.80	31.49	0.217^{ns}
Plasma protein (mg/ml)	23.90^{c}	25.90^{b}	27.05^{a}	27.90^{a}	0.402*	26.55^{a}	25.83^{b}	0.284*
Plasma lipid (mg/100ml)	298.00^{c}	305.40^{b}	328.65^{a}	330.00^{a}	1.234*	320.70^{a}	310.33^{b}	0.873*

*a-d on the same row with different superscripts are significantly different(P < 0.05); * * = significant; *ns = not significant; *SEM = standard error of mean; *Zero AA = No supplementation of diet with ascorbic acid; *Post AA = Post challenge supplementation of diet with ascorbic acid; *Cont.AA = Continuous supplementation of diet with ascorbic acid

Table 2.6. Values of Haematological parameters for the interactions between the main-factors and sub-factors at the end of 8 weeks.

PARAMETERS	150mgAA				300mgAA				
	Zero AA	Control	Post AA	Cont. AA	Zero AA	Control	Post AA	Cont. AA	SEM
PCV (%)	25.65^{c}	26.00^{c}	29.40^{b}	34.50^{a}	25.65^{c}	25.50^{c}	29.00^{b}	30.00^{b}	0.746*
Hb (g/dl)	8.00^{c}	8.20^{c}	9.33^{b}	11.30^{a}	8.00^{c}	8.00^{c}	9.20^{b}	9.50^{b}	0.263*
WBC (x10^{3}/mm^{3})	38.00^{a}	30.70^{c}	27.40^{d}	24.00^{e}	38.00^{a}	32.60^{b}	28.00^{d}	27.00^{d}	0.617*
RBC (x10^{6}/mm^{3})	2.71^{c}	2.94^{a}	2.90^{a}	3.00^{a}	2.71^{c}	2.70^{b}	2.90^{a}	2.95^{a}	0.063*
MCV (fl)	94.65^{c}	94.89^{d}	101.38b^{c}	115.00^{a}	94.65^{c}	94.44^{d}	100.00^{c}	101.69^{b}	0.791*
MCHC (%)	31.19^{b}	31.54	31.73	32.75	31.19^{b}	31.37	31.72	31.67	0.433^{ns}
Plasma protein (mg/ml)	23.90^{c}	26.80	27.00	28.50	23.90^{c}	25.00	27.10	27.30	0.578^{ns}
Plasma lipid (mg/100ml)	298.00^{c}	308.00^{c}	336.80^{a}	340.00^{a}	298.00^{c}	302.80^{d}	320.50^{b}	320.00^{b}	1.745*

*a-d on the same row with different superscripts are significantly different (P < 0.05); * * = significant ;
*ns = not significant; *SEM = standard error of mean *ZeroAA = No supplementation of diet with ascorbic acid; *Post AA = Post challenge supplementation of diet with ascorbic acid; *Cont.AA = Continuous supplementation of diet with ascorbic acid

was not significantly (P<0.05) different. Table 2.1 also shows significant (P<0.05) differences in the oocyst count and percentage mortality for the main-factors, while there were no significant (P>0.05) differences for the sub-factors. Table 2.2 show significant (P<0.05) differences in the oocyst count and percentage mortality for the interactions between the two factors. Tables 2.3 and 2.4 show the mean values of growth parameters, oocyst counts, and percentage mortality for the main-factors and sub-factors, and the interactions between the two factors at the end of eight weeks experimental period. From table 2.3, there were significant (P<0.05) differences in the feed intake and weight gain for the main-factors, while for the sub-factors there was a significant (P<0.05) difference in the feed intake. From table 2.4, there were no significant (P>0.05) differences in the growth parameters of the interactions between the two factors. But numerically, 'ContinuousAA x 150mgAA' and 'Control x150mgAA' had the best feed conversion ratio of 3.98, while 'ZeroAA x 150mgAA' and 'ZeroAA x 300mgAA' had the poorest feed conversion ratio of 4.60. From table 2.3, there were significant (P<0.05) differences in the oocyst count for the main-factors and sub-factors, while table 2.4 show significant (P<0.05) differences in the oocyst count and percentage mortality for the interactions between the two factors. Percentage mortality was also significantly (P<0.05) different for the main-factors while it was not different for the sub-factors. Tables 2.5 and 2.6 show the mean values of haematological parameters and the plasma constituents for the main-factors and sub-factors, and the interactions between the two factors respectively. From table 2.5, the PCV, Hb, WBC, RBC, MCV, MCHC, plasma protein and plasma lipid were significantly (P<0.05) different for the main-factors. For the sub-factors, PCV, Hb, WBC, RBC, MCV, plasma protein and plasma lipid were significantly (P<0.05) different. For the interactions, table 2.6 shows that PCV, Hb, WBC, RBC, MCV and plasma lipid were significantly (P<0.05) different.

DISCUSSION

Though feed to gain ratio is not significant, the superior feed conversion ratio of 3.49 for the 'ContinuousAA' showed that the birds exhibited a positive response to a sustained ascorbic acid (AA) supplementation; this is in agreement with several reports of improvement in feed conversion ratio following AA supplementation (Alishekov, 1980; Njoku and Nwazota, 1989; Tuleun and Njoku, 2000). Challenged birds fed on unsupplemented diet (ZeroAA) had the poorest feed intake and feed to gain ratio of because of heavy burden of coccidia oocysts. Furthermore, 'ContinuousAA' had the best growth parameters because the continuous supplementation of their diet with ascorbic acid enhanced their resistance to coccidia infection; this is in agreement with the findings of Arijeniwa (1995).

The results obtained in this work agree with the findings of Kassim *et al.,*(1995) who reported an improved body weight gain and feed conversion following AA supplementation in broilers. Vitamins are needed for the continuation of the metabolic processes even in the absence of feeding. Vitamin needs are also influenced by stress whether induced by disease or environment (Whitehead, 1993). Vitamin C counter metabolic changes including immuno-suppression, this must have contributed to the excellent performance of birds whose diets were continuously supplemented with AA.

The excellent percentage mortality and oocyst count of 'Continuous AA' was due to the effect of AA on the immune system. According to Arijeniwa (1995), AA is necessary in most parts of the tropics to overcome immuno-suppression as well as maintain optimal performance under pressure from infections.

Diets supplemented with vitamin C at 330mg/kg reduced mortality and pericarditis in chicken infected with *Escherichia coli* (Gross *et al.,*1988); the amount of vitamin C needed for the protective effect increased with higher environmental stress. Also Tuleun *et al.* (2000) reported that AA supplementation had considerable influence on mortality of chicks; they recorded on the average a 90% reduction in mortality of chicks by supplementing the feed with 250mg/kg diet compared to the unsupplemented group. In a study carried out by Li and Lovell (1985), they showed that AA deficient-catfish had lower antibody response and depressed phagocytic activity to a septicemic bacterium called *Edwardsiella ictalum* leading to heavy mortality. In this study, mortality was reduced to almost zero percent in 'ContinuousAA' x 150mgAA' and 'Control' probably because of the enhanced antibody and phagocytic activities of the birds in the two treatments.

According to Conway et al. (1992), total plasma lipids are depressed with increasing dose of *Eimeria tenella*; thus the high oocyst count of 'ZeroAA x 150mgAA' and 'ZeroAA x 300mgAA' contributed to the depressed plasma lipid. According to Guyton (1986), the WBC together with the macrophage system and lymphoid tissue function in two different ways to prevent diseases:- (1) by actually destroying invading agents by the process of phagocytosis, and (2) by forming antibodies and sensitized lymphocytes, one or both of which may destroy the invader; the elevated WBC count of 'ZeroAA x 150mgAA' and 'ZeroAA x 300mgAA' was due to increased oocyst count.

In conclusion, Ascorbic acid (AA) from this study is not prophylactic against caecal coccidiosis, but serves to reduce the coccidial load of infected birds because of its anti-stress activity. The present study also shows that the interaction between the dosage of AA supplementation (sub-factor) and the period or length of time of supplementation (main-factor) is very important for optimal performance of birds. It was observed that continuous supplementation of diets with 150mgAA produced the best performance.

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