



AMELIORATIVE EFFECT OF VITAMIN C SUPPLEMENTATION ON PARASITOLOGICAL AND HAEMATOLOGICAL INDICES IN EXPERIMENTAL *ASCARIDIA GALLI* INFECTED BROILER CHICKENS

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ABSTRACT

This study evaluates the effects of vitamin C administration on parasitological and haematological indices in experimental *Ascaridia galli*-infected, broiler chickens. Ninety-day-old broiler chicks were divided into three groups of thirty birds each. Group A (*A. galli*), B (*A. galli* + vitamin C 250 mg daily) and C (uninfected control). Each group was subdivided into two replicates with 15 chicks each. The dose of infective *A. galli* eggs given was 1000 orally. Blood sample was taken weekly for determination of haematological parameters. McMaster technique was carried out to determine faecal egg count. On day 47 post infection, three birds per group were humanely sacrificed to determine worm count. Results showed that, the red cell counts in the infected, supplemented group B ( $5.4 \pm 0.8 \times 10^{12}/l$ ) birds, was significantly higher ( $p < 0.05$ ) than those of the infected unsupplemented group A ( $3.5 \pm 0.2 \times 10^{12}/l$ ) birds, but lower than the uninfected group C ( $5.8 \pm 0.9 \times 10^{12}/l$ ) birds. Similar patterns were observed for changes of PCV, TP and Hb in the groups. Group A had significantly higher ( $p < 0.05$ ) white blood cell counts compared to groups B and C. At termination of the experiment on day 54, the supplemented group B had significantly lower faecal egg count ( $380 \pm 0.0$ ) than the unsupplemented group A ( $1200 \pm 0.0$ ). Similarly, the mean worm counts in group B ( $85 \pm 1.2$ ) was significantly lower ( $p < 0.05$ ) than those of group A ( $155 \pm 1.2$ ). In conclusion, Administration of vitamin C ameliorates the effects of *Ascaridia galli* infection on parasitological and haematological parameters in broilers.

**Keywords:** *Ascaridia galli*, broiler, infection, supplementation, vitamin C,

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INTRODUCTION

Infectious and parasitic diseases are problems that limit the expansion and profitability of poultry industries throughout the world [1]. Studies conducted in different parts of the world indicated that the proportion of chicken infection with gastrointestinal helminths is high; therefore, helminths are considered to be an important cause of ill health and reduction in poultry productivity [2, 3]. *Ascaridia galli* is the largest, most prevalent and one of the most pathogenic nematodes of birds. Infection with *A. galli* may cause reduction in growth rate, weight loss, economic losses which are usually associated with treatment cost, reduction in feed efficiency and egg production, and occasionally, mortality [4, 5]. Increased malondialdehyde (MDA) level in chicks and changes in the activity of the enzymes glutathione peroxidase (GPx) and sodium dismutase showed a development of oxidative stress during the infection with *A. galli* [6] has been reported.

Antioxidants, including vitamin C, (ascorbic acid) are required for the sustenance of animals because they protect the cells from adverse effects of reactive oxygen species (ROS). They are present in fruits, vegetables, meat and fish [7]. There has been

interest in a possible nutritional role of ascorbic acid (AA) on the basis of the fact that endogenous synthesis may not be adequate to meet the full needs of animals at all times, and that requirements for ascorbic acid (AA) may be increased under stressful conditions [8, 9]. Haematological parameters have been demonstrated to be important indices of health, production and adaptability to prevailing environmental conditions in livestock [10] and also as an indicator of stress in livestock [11]. There is paucity of information on the ameliorative effects of vitamin C on parasitological and haematological parameters of *Ascaridia galli* infected broiler chickens that are given daily dietary Ascorbic acid supplementation. Therefore, the objective of this study was to determine the ameliorative effects of vitamin C supplementation on haematological and parasitological indices in experimental *Ascaridia galli* infected broiler chickens.

MATERIALS AND METHODS

**Isolation, culture and recovery of infective *Ascaridia galli* eggs**

*Ascaridia galli* eggs for this experiment were obtained from live adult females *A. galli* that were collected

from the intestines of local chickens, slaughtered in a poultry slaughter slab in Sabon Gari, Zaria, Kaduna State, Nigeria. The intestines were collected in a beaker, and transported to Helminthology Laboratory, of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. Adult female *Ascaridia galli* worms were obtained from the infected birds according to standard parasitological techniques described by Stephen *et al.* and Skallerup *et al.* [12, 13]. In the laboratory, the intestines were opened up and the ascarid worms, removed and kept in a beaker, containing physiological saline (0.9%). The uteri of the gravid female worms were dissected and the eggs recovered. The recovered eggs were washed with 0.5 M potassium hydroxide (KOH) solution and agitated gently for 30 minutes in order to dissolve the sticky albuminous layer [14]. The preparation was thereafter centrifuged at 1500×g for 3 min, with supernatant decanted and eggs washed with distilled water initially and then with embryonating fluid (0.1 M sulphuric acid) thrice each. The eggs collected were suspended in the embryonating fluid, transferred to Petri dishes and incubated for 21 days in an oven at 25°C. The embryonating fluid was periodically added to the culture to avoid desiccation [15]. The culture in test tubes was centrifuged and the eggs washed twice. The eggs were then pipetted onto the glass slide and examined under the microscope at ×10 for evidence of embryonation.

#### Ethical clearance

Ethical approval for the use of animal for this study was obtained from Ahmadu Bello University, Committee on Animal Use and Care (ABUCAUC/2016/0019), ABU, Zaria.

#### Experimental birds

Ninety (90) day- old broiler chicks of Cobb 500 breed were purchased from a hatchery in Ibadan, Nigeria, for this study. They were housed in separate cages. The birds were randomly divided into three groups, namely A, B and C, each group comprising 30 birds; each group was sub divided into two replicates with 15 chicks in each. They were fed on commercial broiler feed (Vital feed<sup>®</sup>). The feed and water were provided *ad libitum* throughout the experiment. Furthermore, group B birds were given vitamin C in water at 250mg/kg daily throughout the experiment.

#### Infection of the experimental chicks

The embryonated eggs of *A. galli* were suspended in water inside a beaker and thoroughly agitated. 0.1ml of the suspension was dropped on a glass slide and the eggs in it were counted under a light microscope at ×10 objective. This was done three times and average number of eggs in it was taken. The birds in groups A and B were orally infected at three weeks of age with 1000 *A. galli* eggs contained in 0.32ml of water per bird using an 18G needle attached to a 2ml syringe, birds in group C serves as uninfected control. Patency

of the infection was monitored by simple flotation method.

#### Samples collection and processing

**Faeces:** Collection and examination of faeces were done twice in a week until the infection was established using simple flotation protocol [16]. Briefly, a suspension of about 3 g of faeces in flotation fluid was filtered through layers of gauze (in a funnel) into a test tube until a convex meniscus was formed. A cover slip which had been allowed to stay on the meniscus for 3–5mins was carefully removed, placed on a clean glass slide for examination at ×10 objective of a light microscope. Faecal samples were also collected per group after patency of infection (46days post infection) and processed daily using the modified McMaster technique for estimation of egg count per gram of faeces [17].

**Blood:** Two ml of blood was taken weekly from each bird for 9 weeks and placed in a customized sample bottle, containing ethylene diamine tetra acetic acid (EDTA) for determination of haematological parameters viz:

Red blood cell (RBC) counts were determined with the Nan-Herrick solution (1:200 dilutions) and the Improved Neubauer haemocytometer [18]. Packed Cell Volume (PCV) was determined by the microhaematocrit method described by Feldmann *et al.* and Rehman *et al.* [19, 20]. A non-heparinized capillary tube was filled up to about ¾ of its length from one end and the second end was heat-sealed using a Bunsen burner. The blood in the sealed capillary tube was then centrifuged for 5 minutes at 4,383 x g using the Saitexiangyi TG12MX<sup>®</sup> Micro-haematocrit centrifuge machine. Thereafter, the proportion of cells in the total volume of blood was measured and recorded as a percentage (%) using Hawksy<sup>®</sup> Micro-haematocrit Reader.

The hand refractometric method described by Sirois [21] was used to determine the total plasma protein in g/dl, while Blood haemoglobin concentration was assayed colorimetrically as cyanomethaemoglobin [22]. Total white blood cell (TWBC) counts were also determined with the Nan-Herrick solution (1:200 dilutions) and the Improved Neubauer haemocytometer [18]. Blood smears were prepared for differential leucocyte count, and the blood smears examined using a light microscope (Olympus-XSZ-107BN), and the cells counted using the marble<sup>®</sup> Blood Cell Calculator [23, 24, 18]. The differential WBC count was then expressed as a percentage of the individual cell group.

#### Worm isolation and counting

On day 47 post-infection; three birds per group were randomly selected and humanely sacrificed. Thereafter, the intestines were removed, opened and then examined for the presence of adult *Ascaridia galli*, which were removed, put in a petri dish

containing physiological saline, following which the worms were counted and preserved in 10% buffered formalin [25].

#### Data analysis

All data collected were presented as groups mean  $\pm$  standard error (S.E.) and subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test [26]. Differences between group means were considered significant at  $P < 0.05$ .

## RESULTS

Table 1 shows the faecal egg count of birds supplemented with vitamin C and infected with *Ascaridia galli*, by day 45 of infection, all the infected birds were shedding eggs in their faeces. The egg per gram of the infected groups did not follow a discernable pattern until days 53 and 54 of infection, when the infected, supplemented group had significantly ( $p < 0.05$ ) lower egg per gram. By day 54 of infection the faecal egg count of the infected, unsupplemented birds was  $1200 \pm 0.0$ , while the count in infected, vitamin C-supplemented birds was  $380 \pm 0.0$ .

**Table 1:** Faecal egg count (FEC) of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

Day 41	Day 42	Day 43	Day 44	Day 45	Day 46	Day 47	Day 48	Day 49	Day 50	Day 51	Day 52	Day 53	Day 54
A	—	—	—	+	+	640 $\pm$ 0.1	710 $\pm$ 0.2	320 $\pm$ 0.0	190 $\pm$ 0.0	100 $\pm$ 0.1	360 $\pm$ 0.6	220 $\pm$ 1.4	1200 $\pm$ 0.0
B	—	—	—	—	+	320 $\pm$ 0.0	300 $\pm$ 1.4	140 $\pm$ 0.0	60 $\pm$ 2	400 $\pm$ 0.0	140 $\pm$ 0.0	100 $\pm$ 1.3	380 $\pm$ 0.0
C	—	—	—	—	—	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group, The mean worm count in infected, vitamin C, supplemented group was  $85 \pm 0.0$ , which was significantly lower ( $p < 0.05$ ) compared to the mean counts of ( $155 \pm 0.2$ ) in infected group A birds (Table 2).

**Table 2:** Worm counts of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

GPOUP/WC	A	B	C
1	145	86	00
2	170	80	00
3	150	90	00
TOTAL	465	256	00
MEAN	155 $\pm$ 0.2 <sup>a</sup>	85 $\pm$ 0.0 <sup>a</sup>	00

Means having same superscript alphabets along the same row are significantly different at ( $p \leq 0.05$ ) WC= worm count, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group.

**Table 3:** Total red blood cell counts ( $\times 10^{12}/l$ ) of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

TRBC	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
C									
$\times 10^{12}/l$									
GP A	4.08 $\pm$ 0.20	4.5 $\pm$ 0.13 <sup>a</sup>	3.6 $\pm$ 0.2 <sup>a</sup>	3.5 $\pm$ 0.4 <sup>a</sup>	3.6 $\pm$ 0.3 <sup>a</sup>	3.5 $\pm$ 0.4 <sup>b</sup>	3.6 $\pm$ 0.3 <sup>a</sup>	3.4 $\pm$ 0.1 <sup>a</sup>	3.5 $\pm$ 0.2 <sup>a</sup>
GP B	4.33 $\pm$ 0.25	4.8 $\pm$ 0.14 <sup>b</sup>	4.7 $\pm$ 0.6 <sup>a</sup> <sub>b</sub>	5.1 $\pm$ 0.4 <sup>a</sup> <sub>b</sub>	5.0 $\pm$ 0.3 <sup>a</sup> <sub>b</sub>	5.2 $\pm$ 0.3 <sup>a</sup> <sub>b</sub>	5.4 $\pm$ 0.8 <sup>a</sup> <sub>b</sub>	5.4 $\pm$ 0.4 <sup>a</sup> <sub>b</sub>	5.4 $\pm$ 0.3 <sup>a</sup> <sub>b</sub>
GP C	4.65 $\pm$ 0.38	4.7 $\pm$ 0.19 <sup>a</sup> <sub>b</sub>	5.8 $\pm$ 0.1 <sup>a</sup> <sub>b</sub>	5.8 $\pm$ 0.5 <sup>a</sup> <sub>b</sub>	5.7 $\pm$ 0.9 <sup>a</sup> <sub>b</sub>	5.7 $\pm$ 0.4 <sup>a</sup> <sub>b</sub>	5.7 $\pm$ 0.7 <sup>a</sup> <sub>b</sub>	5.8 $\pm$ 0.2 <sup>a</sup> <sub>b</sub>	5.8 $\pm$ 0.2 <sup>a</sup> <sub>b</sub>

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ). TRBC= Total red blood cell count, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

**Table 4:** Packed Cell Volume values (%) of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*.

PCV	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
	24.86±1	27.80±0	30.5±1.	22.30±2	26.10±1.	28.00±2	29.50±1	27.80±0.	26.6±1.5
<b>A</b>	.33	.6 <sup>a</sup>	5 <sup>a</sup>	.5 <sup>a</sup>	6 <sup>a</sup>	.2 <sup>a</sup>	.4 <sup>a</sup>	8 <sup>a</sup>	<sup>a</sup>
	26.88±1	30.20±0	35.97±3	33.60±2	38.76±1.	39.60±1	38.61±4	37.20±2.	38.40±1.
<b>B</b>	.26	.9	.0 <sup>a</sup>	.7 <sup>a</sup>	9 <sup>ab</sup>	.6 <sup>a</sup>	.7 <sup>a</sup>	3 <sup>ab</sup>	2 <sup>ab</sup>
	26.88±2	27.00±1	36.85±0	38.40±3	42.90±5.	41.9±2.	39.5±4.	38.52±1.	40.8±1.4
<b>C</b>	.10	.0 <sup>a</sup>	.9 <sup>a</sup>	.3 <sup>a</sup>	5 <sup>ab</sup>	4 <sup>a</sup>	5 <sup>a</sup>	4 <sup>ab</sup>	<sup>ab</sup>

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ) PCV= Packed cell volume, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

**Table 5:** Total plasma protein (g/dl) of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

TPg/dl	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<b>GRP</b>	3.12±0.	3.4±0.	3.2±0.3 <sup>a</sup>	3.6±0.3 <sup>a</sup>	3.3±0.2 <sup>a</sup>	3.5±0.3 <sup>a</sup>	4.3±0.3 <sup>a</sup>	4.4±0.4	4.3±0.3
<b>A</b>	32	1 <sup>a</sup>						<sup>a</sup>	<sup>a</sup>
<b>GRP</b>	3.35±0.	5.4±0.	6.15±0.2	6.60±0.4	6.60±0.2	6.75±0.4	8.70±0.5	6.8±0.3	8.3±0.2
<b>B</b>	42	3 <sup>a</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>
<b>GRP</b>	3.10±0.	3.6±0.	6.45±0.3	7.20±0.2	8.25±0.1	7.35±0.4	8.85±0.3	8.0±0.2	9.8±0.2
<b>C</b>	25	2 <sup>a</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ) TP= Total plasma protein, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

Table 3 shows the Total red blood cell count ( $\times 10^{12}$ ) of *Ascaridia galli* infected experimental birds. The infected, supplemented group (B) had significantly higher ( $p < 0.01$ ) counts of RBC than the infected non-supplemented group (A). At week 9 of infection, the value ( $\times 10^{12}$ ) for the infected vitamin C supplemented group was  $5.4 \pm 0.3$  compared to  $3.5 \pm 0.2$  for the infected non-supplemented group A. Throughout the experiment, the uninfected positive control group (group C) had significantly higher counts than the infected supplemented ( $p < 0.05$ ) and infected non-supplemented ( $p < 0.01$ ) group of birds.

Table 4 shows that, from week 3 of infection to the end of the experiment, the mean (%) PCV of the infected supplemented birds (Group B) was significantly higher ( $p < 0.001$ ) when compared to the corresponding values for the infected non-supplemented birds (groups A). At week 6 of infection, the mean PCV (%) of group B birds were  $39.60 \pm 1.6$  compared to  $28.0 \pm 2.2$  for the infected non-supplemented (group A) birds. By week 9 of infection, when the experiment was terminated, the value for the infected supplemented group B birds was  $38.4 \pm 1.2$  compared to  $26.6 \pm 1.5$  for the infected non-supplemented group (A) birds. From week 4 of infection, the mean PCV values of the uninfected control (group C) were higher compared to those of the infected supplemented group, but the differences were only significant ( $p < 0.01$ ) at weeks 5, 8 and 9 of infection.

In Table 5, the infected, supplemented birds had significantly higher ( $p < 0.05$ ) total protein compared to the infected, non-supplemented group. At week 4 of infection, the mean TP values for the infected, vitamin C, supplemented (group B) birds was  $6.60 \pm 0.4$ , compared to  $3.6 \pm 0.3$  for the infected, unsupplemented group (A) birds. The uninfected control (group C), had significantly ( $p < 0.01$ ) higher TP than the infected supplemented and infected unsupplemented groups.

Table 6 shows Haemoglobin concentration (g/dl) of *Ascaridia galli* infected experimental birds. From week 3, to the end of the experiment, the mean Hb concentration (g/dl) of the infected, supplemented birds (Group B) was significantly higher ( $p < 0.001$ ) when compared to the corresponding values for the infected, non-supplemented birds (groups A). At week 5, the mean Hb (g/dl) of group B birds, was  $12.90 \pm 0.6$  compared to  $8.70 \pm 0.5$  for the infected, non-supplemented group A birds. By week 9, when the experiment was terminated, the values for the infected, supplemented group B birds were  $12.80 \pm 0.4$  compared to  $8.86 \pm 0.5$  for the infected non-supplemented group (A) birds. From week 4, the mean Hb concentrations of the uninfected control group C birds were higher compared to those of the infected supplemented group, but the differences were only significant at weeks 5, 8 and 9.

**Table 6:** Haemoglobin concentration (g/dl) of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

Hb g/dl	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<b>GR</b>	8.28±0.		10.16±0.	7.40±0.8		9.33±0.7	9.83±0.5	9.26±0.	
<b>P A</b>	44	9.3±0.2 <sup>a</sup>	5 <sup>a</sup>	<sup>a</sup>	8.70±0.5 <sup>a</sup>	<sup>a</sup>	<sup>a</sup>	3 <sup>a</sup>	8.86±0.5 <sup>a</sup>
<b>GR</b>	8.96±0.	10.07±0	11.99±1.	11.20±0.	12.90±0.	13.20±0.	12.87±1.	12.4±0.	12.80±0.
<b>P B</b>	41	.3	3 <sup>a</sup>	8 <sup>a</sup>	6 <sup>ab</sup>	6 <sup>a</sup>	6 <sup>a</sup>	8 <sup>ab</sup>	4 <sup>ab</sup>
<b>GR</b>	8.96±0.	9.00±0.	12.29±0.	12.80±1.	14.30±1.	13.67±0.	13.16±1.	12.8±0.	13.60±0.
<b>P C</b>	70	3 <sup>a</sup>	3 <sup>a</sup>	1 <sup>a</sup>	8 <sup>ab</sup>	8 <sup>a</sup>	5 <sup>a</sup>	5 <sup>ab</sup>	5 <sup>ab</sup>

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ) Hb= Haemoglobin, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

**Table 7:** Total white blood cell count ( $\times 10^9/l$ ) of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

TWB C	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<b>GRP</b>	7.9±0.	12.83±1.	14.96±1.	16.1±1.	20.78±1.	16.2±1.3	17.3±1.1	16.9±0.	14.9±0.
<b>A</b>	9	2 <sup>a</sup>	8 <sup>a</sup>	4 <sup>a</sup>	6 <sup>a</sup>	<sup>a</sup>	<sup>a</sup>	7 <sup>a</sup>	9 <sup>a</sup>
<b>GRP</b>	7.6±0.	13.21±0.	11.20±1.	9.5±0.9 <sup>a</sup>	11.3±0.7	10.5±0.6		9.0±1.1 <sup>a</sup>	9.9±0.7 <sup>a</sup>
<b>B</b>	9	8	0 <sup>a</sup>	<sup>b</sup>	<sup>ab</sup>	<sup>ab</sup>	9.3±0.5 <sup>ab</sup>	<sup>b</sup>	<sup>b</sup>
<b>GRP</b>	7.9±1.	12.49±0.	10.20±1.	8.1±0.7 <sup>a</sup>	10.2±1.9		10.2±1.5	8.8±0.9 <sup>a</sup>	8.8±1.4 <sup>a</sup>
<b>C</b>	6	6 <sup>a</sup>	2 <sup>a</sup>	<sup>b</sup>	<sup>ab</sup>	9.2±1.7 <sup>ab</sup>	<sup>ab</sup>	<sup>b</sup>	<sup>b</sup>

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ) TWBC=Total white blood cell, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

**Table 8:** Eosinophil count (%) of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

EOSIN %	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<b>GROUP</b>	3.81±0.2	4.7±0.1	5.2±0.2	5.4±0.4	5.4±0.7		5.8±0.7	6.3±0.4	
<b>A</b>	6	7	<sup>a</sup>	<sup>a</sup>	<sup>a</sup>	5.6±0.3 <sup>a</sup>	<sup>a</sup>	<sup>a</sup>	7±0.3 <sup>a</sup>
<b>GROUP</b>	4.08±0.2	4.5±0.1	3.8±0.3	3.8±0.3	4.1±0.3		4.6±0.3	4.8±0.1	4.6±0.2
<b>B</b>	1	1	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	4.2±0.1 <sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>
<b>GROUP</b>	4.02±0.2		3.3±0.2	3.1±0.3	3.3±0.2	3.3±0.11	3.4±0.2	3.5±0.1	3.7±0.1
<b>C</b>	1	4±0.32	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>	<sup>ab</sup>

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ) EOSIN= Eosinophil, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

**Table 9:** Heterophil count of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

HET %	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<b>GRP</b>	16.13±1	7.3±1.	19.65±1.	16.40±1.	17.6±2.	20.4±1.	26.05±2.	21.5±0.	21.3±0.
<b>A</b>	.2	8	1 <sup>a</sup>	4 <sup>a</sup>	3 <sup>a</sup>	5 <sup>a</sup>	3 <sup>a</sup>	7 <sup>bc</sup>	9 <sup>a</sup>
<b>GRP</b>	18.38±1	20.2±1	13.20±1.	10.20±1.	10±2.1 <sup>a</sup>	11.3±1.	13.1±2.4	7.9±1.7 <sup>a</sup>	9.8±1.7
<b>B</b>	.5	.8	2 <sup>ab</sup>	4 <sup>ab</sup>	<sup>b</sup>	3 <sup>ab</sup>	<sup>ab</sup>	<sup>b</sup>	<sup>ab</sup>
<b>GRP</b>	16.25±1	16.2±1	8.30±0.9 <sup>a</sup>		8.1±1.9	9.5±1.3 <sup>a</sup>		7.1±1.4 <sup>a</sup>	8.1±1.7
<b>C</b>	.4	.4	<sup>b</sup>	8.3±1.4 <sup>ab</sup>	<sup>ab</sup>	<sup>b</sup>	9.1±2.1 <sup>ab</sup>	<sup>b</sup>	<sup>ab</sup>

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ) HET= Heterophil, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

**Table 10:** Lymphocyte response of birds supplemented with vitamin C and experimentally infected with *Ascaridia galli*

LY M %	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<b>GR</b>	83.86±2.	82.8±1.	86.1±1.1	90.6±1 <sup>a</sup>	86.5±1.1	89.2±2.3	88.3±1.6	87.3±1.1	90.3±1.4
<b>PA</b>	3	9	ab		a	a	a	a	a
<b>GR</b>	79.63±1.	77.4±1.	79.3±1.4	79.0±1.6	75.0±1.9	74.8±1.5	74.4±2.4	72.2±1.8	78.3±1.6
<b>PB</b>	9	9	ab	ab	ab	ab	ab	ab	ab
<b>GR</b>	82.5±1.9	82.6±3.	78.8±1.2	78.9±1.6	73.7±2.6	69.3±0.7	68.0±2.2	68.3±1.8	76.6±1.7
<b>PC</b>		0	ab	ab	ab	ab	ab	ab	ab

Means having same superscript alphabets along the same column are significantly different at ( $p \leq 0.05$ ) LYM= Lymphocyte, A=A. *galli* infected group, B=A. *galli* infected + vitamin C group, C= uninfected control group

Table 7 shows the Total white blood cell count ( $\times 10^9/l$ ) of A. *galli* infected experimental birds. From week 3, the infected, unsupplemented group (A) had significantly higher ( $p < 0.05$ ) TWBC count than the infected, supplemented group (B). By weeks 5 and 9, the infected unsupplemented group had TWBC of  $18.0 \pm 1.2$ , and  $14.85 \pm 0.9$  respectively, while the infected vitamin C, supplemented group had corresponding values of  $11.3 \pm 0.7$  and  $11.7 \pm 1.4$ . The TWBC for the uninfected control group was significantly lower than those of infected supplemented ( $p < 0.05$ ) and infected unsupplemented ( $p < 0.01$ ) groups.

In Table 8, the Eosinophil Count (%) of A. *galli* infected experimental birds and control shows that, from week 3, the infected, unsupplemented group (A) had significantly higher ( $p < 0.05$ ) eosinophil count than the infected, supplemented group (B). By weeks 5 and 9, the infected unsupplemented group had eosinophil count of  $7.2 \pm 0.3$ , and  $7.0 \pm 0.3$  respectively, while the infected vitamin C-supplemented group had corresponding values of  $4.1 \pm 0.3$  and  $4.6 \pm 0.3$ . The eosinophil count for the uninfected control group was significantly lower than those of infected supplemented ( $p < 0.05$ ) and infected unsupplemented ( $p < 0.05$ ) groups from week 3 post infection.

In Table 9, All the infected birds showed significant increase in heterophil count by week 3, from which time, (to the end of the experiment) the infected unsupplemented group had significantly higher heterophil count than the infected supplemented group. By weeks 6 and 9, the former group had values of  $27.2 \pm 1.1$  and  $21.3 \pm 0.9$ , while the infected supplemented group had corresponding values of  $11.3 \pm 1.3$  and  $12.9 \pm 1.4$  respectively. The uninfected control group had significantly ( $p < 0.05$ ) lower heterophil count than the infected groups throughout the period of the experiment.

In Table 10, the Lymphocyte response of all *Ascaridia galli* infected experimental birds, showed significant increase in percentage lymphocyte by week 3, from which time, (to the end of the experiment) the infected, unsupplemented group had

significantly higher percent lymphocyte than the infected, supplemented group. By weeks 6 and 9, the former group had values of  $89.2 \pm 2.3$  and  $90.3 \pm 1.4$  respectively, while the infected, supplemented group had corresponding values of  $74.8 \pm 1.5$  and  $78.3 \pm 1.6$  respectively. The uninfected control group had significantly ( $p < 0.05$ ) lower lymphocyte values than the infected groups throughout the period of experiment.

## DISCUSSION

The lower faecal egg count and worm count recorded in infected, supplemented group compared to infected unsupplemented group agrees with the reports of earlier studies [27, 28]. Celi *et al.* [27] observed lower egg count and worm count in selenium supplemented weaned merino sheep, Sweeny *et al.* [28] also observed resistance to gastrointestinal nematode with supplemented nutrition. The lower faecal egg count and worm count in this study, might be due to the supplement status of the birds, which may have influenced the rate of acquisition of resistance to parasite infection. Since the supplements play such an important role in antioxidant defence and immune function, therefore, the supplemented group would have significantly lower faecal egg count. According to Sweeny *et al.* [28], nutritional supplementation can have synergistic impacts on the control of helminthes, because limiting infection in a population requires; lower worm burden/reduce faecal egg count, reduce onward transmission and preventing reinfection. In their study population, they found out that supplementation achieved all the above three requirements, anthelmintic treatment in conjunction with higher quality diet reduced *Heligmosomoides polygyrus* adult worm burdens and egg shedding to almost zero on supplemented grids. This was replicated in the laboratory, where supplemented individuals shed no eggs during secondary challenge despite harbouring adult worms. Finally, the increased resistance to reinfection in supplemented individuals in the laboratory highlights an additional benefit of nutrition in managing nematodes in natural

populations where post-treatment reinfection is common [28].

The statistically significant red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb) and plasma protein values recorded in the control group than the *Ascaridia galli*-infected groups in this study, agrees with the reports of earlier studies [29, 30, 31, 32]. The higher RBC, PCV, Hb and plasma protein values observed in the uninfected control group could be attributed to lowered erythropoiesis in the infected birds. *Ascaridia galli* infection is usually associated with mild/acute enteritis which hampers the absorption of essential nutrients for blood cell formation as reported by Kumar *et al.* [33], who also reported that the fall in blood content might be due to metabolic disturbance caused by the worms rather than direct blood loss. This can also be attributed to the effects of larval migration in the process of penetration with resultant destruction of mucosa of small intestine and rupture of small blood vessels in the tissue phase of the life cycle of the parasite, which involves some blood loss. Adang *et al.* and Christiana & Celia, [34, 35], also reported that chickens infected with a large number of ascarids suffer from loss of blood, reduced blood sugar content, increased urates, causing anaemia in the birds. The higher PCV, RBC plasma protein and haemoglobin concentration observed in the *Ascaridia galli*-infected, supplemented group compared to the infected, unsupplemented group in this study agrees with previous findings [36, 37, 38, 39] in which vitamin C treated group have higher values than other treated groups, and this could be attributed to the effect of vitamin C in protecting the membrane integrity of erythrocyte which directly affects the haemoglobin concentration and the total erythrocyte count, as demonstrated by Adenkola *et al.* and Candan *et al.* [37, 40].

The higher total leucocyte count, eosinophil and heterophil counts observed in the infected birds than the control in this study, agrees with the reports of previous works [41, 33, 29, 30]. Furthermore, leukocytosis and heterophilia have been reported in parasitic diseases in birds [42, 43]. The increase in the total leucocyte count might be due to the increase in heterophils and eosinophils. Leukocytosis by AL-Saffar & Al-Mawla [42] as “an absolute increase in total number of white blood cells in circulation caused by inflammation resulting from parasitic infections”, agrees with the increased leucocyte count observed in infected birds in this study. These authors opined that heterophils are actively amoeboid and phagocytic in nature, and that their phagocytic action may correlate with increased number as a first line of host defence. The increased number of eosinophils in the blood is an indication of parasitic infection as a first line defence of the host [41]. In the present study, the lymphocyte percentage were significantly increased ( $p < 0.01$ ) in all the infected groups than the control group of chickens. This finding was in accordance with the findings of

previous researchers [41, 29, 30]. Birds in the infected, supplemented group had lower total leucocyte counts, heterophil, eosinophil and lymphocytes count as compared to the infected unsupplemented group. This is similar to the findings of Ajakaiye *et al.* [44] in which vitamins-supplemented groups had lower leucocyte counts than the control group. Adenkola *et al.* [37] also observed lower leucocyte count in vitamin C supplemented group than the control group. The lower value of total white blood cell count recorded in the infected, supplemented group could be due to the effect of Ascorbic acid in preventing the release of the white blood cells from their body pool due to its effect in inhibiting corticosteroids which are known to increase in animals under stress [9].

## CONCLUSION

This study shows that, daily administration of vitamin C ameliorates the effects of *Ascaridia galli* infection on parasitological and haematological parameters in broiler birds. It is therefore, recommended that, administration of supplements such as vitamin C in feed, should be part of regimen for broiler production to ameliorate the effects of parasitism like *A. galli*.

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