www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Vitamin A supplementation moderates the effects of *Ascaridia galli* infestation on haematological parameters and serum proteins in experimentally infected chickens

Muhairwa, A.P[1*]., Barnabas, E[2]., Maselle, R.M[2]., Kassuku, A.A[3]. and Msoffe, P.L.M[1&4]

[1]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Chuo Kikuu, Morogoro Tanzania, [2]Department of Veterinary Pathology, Sokoine University of Agriculture, Morogoro Tanzania, [1&4] Directorate of Undergraduate Studies, University of Dodoma, Dodoma Tanzania

Article History
Received: 13 Sep, 2014
Revised: 29 Nov, 2014
Accepted: 5 Dec, 2014

Abstract

The objective of this work was to investigate the effects of vitamin A supplementation on the levels of total serum proteins, albumin, globulins, albumin/globulin ratio, haemoglobin concentration and haematocrit in chickens experimentally infested with Ascaridia galli. One hundred, ISA brown chickens, aged eight weeks, were divided into four equal groups, and, depending on the groups, they were experimentally infected with 350 embryonated eggs of *A. galli* and daily supplemented with 1500 I.U retinyl acetate. Group I was infested with *A. galli* alone, group II infested and supplemented, group III neither infested nor supplemented and group IV was supplemented with vitamin A alone. The results show that chickens that were infested with *A. galli* alone (Group I) had significantly lower values of the measured parameters than the non infested (group III). However, levels of globulin were not statistically different between the two groups. Similarly, group II (Infestation with supplementation) had significantly lower values of total serum proteins, albumin, globulin), albumin/globulin ratio, haematocrit and haemoglobin than group IV (Non infested and supplemented). On the other hand group I (Infestation alone) had significantly lower values of the above mentioned parameters than group II (Infestation with supplementation) ($P<0.005$). Similarly, group IV (supplemention alone) had significantly higher values than group III (non supplemented). It is concluded that *A. galli* infestation lowers serum total proteins, albumin, albumin/globulin ratio as well as haemoglobin concentrations, haematocrit and body weight, and that, vitamin A supplementation improves the levels of the above mentioned parameters.

Keywords: Poultry; nutrition; vitamins A; helminthosis

To cite this article: Muhairwa AP, E Barnabas, RM Maselle, AA Kassuku and PLM Msoffe, 2015. Vitamin A supplementation moderates the effects of *Ascaridia galli* infestation on haematological parameters and serum proteins in experimentally infected chickens. Res. Opin. Anim. Vet. Sci., 5(1): 3-12.

Introduction

Studies in chickens show that helminthosis affects the concentration of total serum proteins, albumin and globulin (Anwar and Rahman, 2002; Deka and Borah, 2008; Rewat et al., 2010; Ali et al., 2011). Furthermore, migrating helminth larvae cause damage of intestinal mucosa,which leads to leakage of iron that culminates into reduced erythropoiesis due to iron deficiency (Crompton and Nesheim, 2002). Birds with severe

***Corresponding author:** Muhairwa AP, Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Chuo Kikuu, Morogoro Tanzania; E-mail: muhairwa@yahoo.co.uk

worm burdens are, therefore, likely to develop iron deficiency anemia and furthermore, worm burdens have been associated with reduction in serum fat levels (Crompton and Neisheim, 2002) which may result into negative energy balance that ultimately makes the body mobilize and catabolize proteins to release energy. The mechanisms through which such invasive gastrointestinal parasites induce deficiency of micronutrients include loss through diarrhoea, consumption and interference in absorption of nutrients (Marinho et al., 1991; Curtale et al., 1995; Kidala et al., 2000). Thus, worm infestation in chickens can cause severe loss to the farmers by reducing growth rate, egg production and mortalities.

Where management is suboptimal helminthosis is a significant health problem, and where nutrition of birds is inadequate problem is likely to be exaggerated (Ali et al., 2007). Permin et al. (1997) and subsequent studies by Magwisha et al. (2002) showed high prevalence of (69%) in free range chickens. Young chickens of less than three months old and naive chickens are the most susceptible to *A. galli* infestation (Anwar and Rahman, 2002). In free ranging chickens, where the feed quality variations is significant during dry and wet seasons (Mutayoba et al., 2012), worms infestation can exacerbate the clinical signs in chickens. Despite the above account, there is scanty information on the association between vitamin A supplementation, gastrointestinal worm infestation and haematological and biochemical parameters in chicken in Tanzania. The present study was, therefore, thought to investigate the relationship between vitamin A supplementation and haematological and biochemical parameters in chickens experimentally infested with *A. galli* and supplemented with vitamin A.

Materials and Methods

Study design

A controlled experiment involving 100 ISA brown chickens were used for the trial. One hundred, day old chicks, obtained from a commercial supplier were raised under a helminth-free environment at experimental houses, Sokoine University of Agriculture (SUA). On arrival, the chicks were given oxytetracycline (1g/l) in drinking water for five days and then vaccinated against Newcastle disease on day 7 and 21. Similarly, the chicks were vaccinated against Infectious bursal disease on day 14 and 28 post arrival respectively. During the first seven weeks, the birds were given locally available compounded chick starter ration, which was replaced with a finisher ration from 8^{th} to 18^{th} week. The composition and proximate analysis of the feed rations given to the chickens are shown in Table 1. Feed and water were given *ad-libitum*.

Allocation of chickens into experimental groups

Chickens were randomly divided into four groups of 25 birds: randomization of chickens was done using random numbers generated by Microsoft excel and was done for each stage of grouping. The groups were Group I (Infected, non supplemented with vitamin A), Group II (Infected, Supplemented), Group III (Non-infected, non supplemented), and Group IV (Non-Infected, supplemented). The selected birds were transferred from the raising house to the poultry experimental unit where they were put and maintained in individual cages for four weeks before the commencement of the infection. On the same day, the allocated chickens were dewormed using piperazine citrate in water and were screened for helminths every week before they were experimentally infested.

Establishment of embryonated *A. galli* eggs

Establishment of *A. galli* infective eggs was done according to procedures described by Adang et al. (2010a). Briefly, *A. galli* eggs for infection were obtained from live adult *A. galli* worms collected from chickens slaughtered at the Morogoro Municipal market. Female worms were crushed using a mortar and pestle in distilled water to release the eggs. The crushed worms were then filtered using a 0.01mm mesh into a beaker and allowed to stand for one hour after which the supernatants were decanted. The sediments were washed with 0.5M sodium hydroxide solution into a beaker and agitated gently for 30 min. The sediments were centrifuged at 1500rpm for 3 min. to recover the eggs. The recovered eggs were washed three times in distilled water and three times in 0.05M sulphuric acid. For embryonation, the collected eggs were cultured by suspending in 0.05M sulphuric acid and placed in plastic specimen and left to stand for 20 d in the laboratory at room temperature.

The embryonated eggs in sulphuric acid were washed in normal saline and diluted in 50 ml normal saline. After thorough mixing, one ml of the egg suspension was transferred into a Rafter chamber where the embryonated eggs were quantified under the light microscope. The volume of egg suspension required to give a dose of 350 eggs was calculated by dilution method using the following formula:

Vol = 350 / Total counted eggs per ml

Infestation and supplementation of experimental birds

Infection of the experimental chickens in groups I and II was done when the birds were 8 weeks old, which was considered as day 0 of the experiment. Each bird was infected orally using a Pasteur pipette with 350 embryonated *A. galli* eggs suspended in 1 ml of normal saline as described by Anwar and Rahman

(2002). On the other hand, the chickens in group II and IV were orally supplemented with vitamin A at rate of 1500 I.U/kg of feed/day using retinyl acetate beginning one day before the infection. All chickens were monitored daily for 10 weeks for signs of disease and samples of blood and body weights were taken from each chicken as described below.

Sampling of blood

The schedule of collecting blood was based on the life cycle of *A. galli* to capture the effects on the study parameters in different stages of the growth of worms (Soulsby, 1982). Sampling was, therefore, done on weeks 0, 2, 3, 5 and 10 post-infection. Five ml syringes, twenty three gauge needles and EDTA containing and plain vacutainers were used to collect the blood from the wing and jugular veins after the areas were sterilized using methylated spirit. Five ml of blood were collected from each chicken and immediately transferred into an EDTA containing vacutainer tube, which was stored at 4^{o}C. Another 3ml of blood was collected from the jugular vein from the same chicken and were gently transferred into a plain vacutainer tube and left to stand in an inclined position in dim light for one hour for serum to separate. Serum from each sample was gently separated and transferred into vials and then stored at -70^{o}C until the time of analysis, which was around 2-3 days.

Measurement of serum proteins

Total serum proteins was determined by the biuret method (Lumeij, 2008) using ERBA analytical kits composed of total protein working reagent (containing copper II sulphate, potassium iodide, potassium sodium tartrate and sodium hydroxide) and total protein standard (4 g/dl). A reaction mixture for each sample consisting 20 µl of each serum sample, 20 µl standard solution of total proteins, 20µl distilled water and 1 ml total proteins working reagent was pipetted into a test tube. The reaction mixture was incubated for 10 min. at 37^{o}C and the absorbencies of standard and serum sample mixture read at 546 nm using a spectrophotometer (Spectronic 21, Milton Roy, USA) against distilled water.

Albumin was determined by the bromocresol green (BCG) method (Ueno et al., 2013) using an albumin analytical kit (AGAPPE diagnostics, India) consisting of albumin working reagent (containing succinate buffer and bromocresol green) and albumin standard (3 g/dl). Ten µl of each sample were pipetted in a test tube followed by 10µl standard total protein solution, 10µl distilled water and 1 ml of working reagent. The serum sample mixture was incubated for 1 min. at 37^{o}C and the absorbencies of standard and serum sample mixture read at 630 nm using a spectrophotometer against distilled water.

The amount of globulins in each sample was determined by subtracting the amount of serum albumin obtained from the amount of total serum proteins (Silva et al., 2007). The ratio of albumin to globulin in each sample was determined by dividing the amount of albumin obtained by the amount of globulin (Lumeij, 2008).

Determination of haematological parameters

The packed cell volume was determined from each sample by the microhaematocrit method (Wakenell, 2010). Briefly, for each sample of blood, duplicate of sodium heparin coated capillary tubes (Paul Marienfeld GmbH, German) were filled up to three quarters of their column and sealed at the filling ends using seal easy (seal easy$^{(R)}$-USA). The tubes were then centrifuged at 3000rpm for 5 minutes in a haematocrit centrifuge (Heraeus Christ GmbH Osteorode). The volume of packed red blood cells was read using the haematocrit reader (Gelman Hawksley, England).

The concentration of haemoglobin in each sample was determined using the cyanomethemoglobin method (Thrall et al., 2006; Wakenell, 2010). Briefly, for each blood sample, 5ml of Drabkin's solution was mixed with 20µl of EDTA stabilised blood in a centrifuge tube. The sample mixture was mixed and left to stand at room temperature for 10 min. Then the tube was centrifuged at 3000rpm for 5 min. to remove nuclear material that could over-estimate the optic densities. Then 4ml of the supernatant was put into curvettes and the optic densities were read at 540 nm (Spectronic 21, Milton Roy, USA) against distilled water.

Statistical analysis

Initially, data were entered in Microsoft Excel and then imported to GraphPad prism (GraphPad Software Inc., USA) version 3.0 where descriptive statistics (Mean, standard errors of the mean) of the blood analytical variables were determined. One way analysis of variance (ANOVA) was used to compare the four groups. Unpaired Student's t-test was used to compare levels of total protein, albumin, globulin, albumin/ globulin ratio, PCV and haemoglobin between the infected versus non-infected and between supplemented and non-supplemented groups. On the other hand, a paired t-test was used to compare the differences between the initial and final values of the research variables of the individual birds within the group. All statistical analyses were determined using GraphPad prism (GraphPad Software Inc., USA) version 3.0.

Results

Total serum proteins

The results of levels of total serum proteins in the four groups of experimental chickens are presented in Figure 1.

Table 1: Feed composition and proximate analysis of the feeds given to the experimental chickens

Feed ingredient (percent)	Feed ration	
	Starter	Growers/Finisher
Maize	28	35
Maize bran	25	28
Rice bran	20	20
Sunflower seed cake	9	7
Fish meal	10	3
Blood meal	2	1
Limestone	3.45	3.45
Bone meal	2	2
Salt	0.5	0.5
Premix*	0.05	0.05
Proximate analysis (percent)		
Dry matter	92.04	88.08
Crude protein	26.23	13.4
Crude fibre	11.91	10.03
Ash	12.64	9.7
Ether extract	9.04	10.11

*Vitalyte® Biotec. Each kg of feed contained 1600IU vitamin A, 600IU vitamin D, 1.6IU vitamin E, 0.2mg vitamin B1, 0.5mg vitamin B2, 2mg niacin, 1mg pantothenic acid, 0.1mg folic acid, 30mg choline, 4mg iron, 16mg manganese, 1.6mg copper, 10mg zinc, 0.045mg cobalt, 0.4mg iodine, 0.02mg selenium and 1.2mg antioxidant.

Changes of levels of total serum proteins within the experimental groups

The levels of total serum proteins in chickens that were non-infected and non-supplemented (group III) ranged between 3.3 and 3.8 g/dl and formed the base-line values of total serum proteins of chickens in this study. The total serum proteins in chickens in group IV that were the same at the beginning of the experiment as the base-line levels increased significantly ($P<0.05$) from 3.22 to 4.52 g/dl by week 3 of supplementation (Fig. 1). On the other hand, the initial level of total serum protein in the infected and non-supplemented chickens (group I), which was within the normal values of 3.42 g/dl decreased non-significantly ($P>0.05$) to between 3.03 and 3.20 g/dl between weeks 3 and 10 post-infection. Figure 1 further shows that total serum proteins in the infected and supplemented chickens (group II) increased non-significantly ($P>0.05$) from 3.34 to 3.53 g/dl by week 3 post-infection.

Comparative total serum proteins levels between the experimental groups

Figure 1 shows differences on levels of total serum proteins measured at different weeks of infection. The four groups had statistically the same levels of total serum proteins on week 0 ($P=0.945$) and week 2 ($P=0.825$) of the experiment. From week 3 post-infection, group I and IV had significant different levels ($P<0.05$) of total serum proteins compared with other groups, with group I having lower total serum protein than the other three groups and group IV having higher

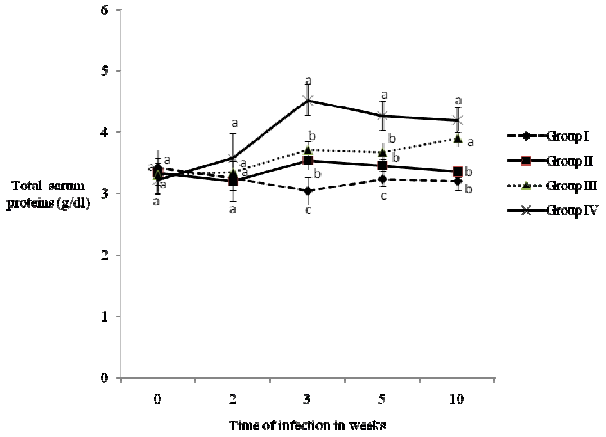

Fig. 1: Total serum proteins levels in the four groups of experimental chickens measured at different weeks of infection. Error bars with different letters between the groups show significant difference ($P<0.05$).

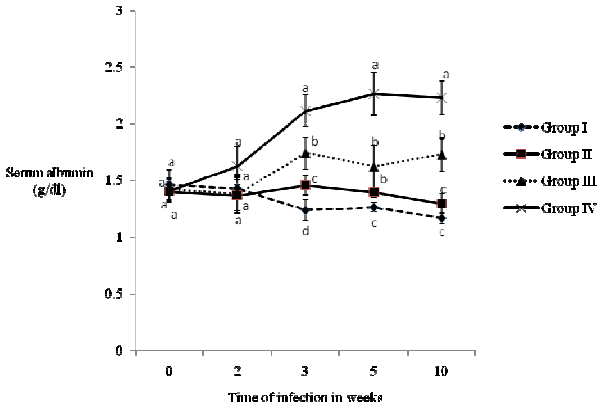

Fig. 2: Serum albumin in the four groups of experimental chickens measured at different weeks of infection. Error bars with different letters between the groups show significant difference ($P<0.05$).

levels than the other groups. There were no statistically significant differences on the levels of total serum proteins between group II and III throughout the experiment ($P>0.05$) except on week 10 when group III had statistically higher total serum protein concentrations ($P=0.0037$). Overall at the end of the experiment, infected non supplemented group (I) had total serum proteins of 3.20 g/dl, which was significantly lower than 3.89 g/dl of non-infected non-supplemented group (III) ($P=0.003$). Similarly, infected supplemented group (II) had 3.35 g/dl total serum proteins, which was significantly lower than 4.19 g/dl obtained from non-infected supplemented group (IV) ($P<0.0001$). The above results show that *A. galli* infection reduced total serum proteins, both in the supplemented and non supplemented chickens. On the other hand, the levels of total serum proteins of 3.20

g/dl from the infected non-supplemented group (I) was statistically lower than 3.35 g/dl of the infected, supplemented group (II) (P=0.04). Similarly, non-infected supplemented group (IV) had 4.19 g/dl of total serum proteins, which was significantly higher than the corresponding 3.89 g/dl of the non-infected non-supplemented group (III) (P=0.0002). The results on supplementation, thus confirmed that vitamin A supplementation improved the levels of total serum proteins, both in the infected and non-infected chickens.

Albumin levels in experimental chickens

The results of serum albumin levels in the four groups of experimental chickens are presented in Fig. 2.

Changes of serum albumin levels within the experimental groups

The levels of serum albumin in chickens that were non-infected and non-supplemented (group III) ranged between 1.38 and 1.74 g/dl and formed the base-line values of serum albumin of chickens in this study. The levels of albumin in chickens in group IV were the same at the beginning of the experiment. The base-line values increased significantly (P<0.05) from 1.40 to 2.26 g/dl by week 5 of supplementation (Fig. 2). On the other hand, the initial levels of albumin (1.45 g/dl) in the infected and non-supplemented chickens (group I) decreased significantly (P<0.05) to 1.16g/dl by week 10 post-infection. The decrease in the serum albumin values was prevented in chickens in group II, which were infected and supplemented as evidenced by a non-significant decrease (P>0.05) from 1.4 to 1.29 g/dl from week 5 post-infection.

Comparative serum albumin levels between the experimental groups

The significant differences in the levels of serum albumin between the groups were seen from week 3 through the experiment. The albumin concentration of 1.16 g/dl in the infected non-supplemented group (I) was statistically lower than 1.72 g/dl from the non-infected non-supplemented group (III) (P<0.0001). Similarly, 1.29 g/dl of albumin in the infected-supplemented group (II) was significantly lower than 2.22 g/dl in the non-infetcted supplemented group (IV) (P<0.0001). This indicates that *A. galli* lowered albumin levels both in supplemented and non supplemented chickens. On the other hand, 1.16 g/dl of albumin in the infected non-supplemented group (I) was statistically lower than 1.29 g/dl from the infected supplemented group (II) (P=0.041). Similarly, non-infected supplemented group (IV) had 2.22 g/dl of albumin, which was statistically higher than the corresponding 1.72 g/dl in the non-infected non-supplemented group (III) (P=0.0002).

Globulin levels in experimental chickens

The results of serum globulin levels in the four groups of experimental chickens are presented in Figure 3.

Changes of serum globulin levels within the experimental groups

The levels of serum globulin in chickens that were non-infected and non-supplemented (group III) ranged between 1.89 and 2.16 g/dl and formed the base-line values of serum albumin of chickens in this study. These values were above the normal levels of 0.5-1.8g/dl reported by Thrall et al. (2006). Figure 3 shows that the base-line levels in chickens in group IV increased significantly from 1.82 to 2.40 g/dl (P<0.05) by week 3 of supplementation and then fell to 1.96 g/dl by week 10 of supplementation. On the other hand, the base-line values of serum globulins in the infected and non-supplemented chickens (group I), decreased non-significantly (P>0.05) to 1.82 g/dl by week 2 post-infection before increasing slightly (P>0.05) to 2.04 g/dl by week 10 post-infection. The effect of supplementation in infected chickens in group II on the values of serum globulins was not statistically significant (P>0.05) as evidenced by a small increase from 1.94 to 2.08 g/dl by week 3 post-infection and supplementation.

Comparative serum globulin levels between the experimental groups

The significant differences on the levels of serum globulin between the groups were seen from week 3 through the end of the experiment (P=0.008-0.002). The level of globulin (2.04 g/dl) in infected non-supplemented group (I) was not statistically different from 2.16 g/dl of non-infected non-supplemented group (III) (P=0.49). However, infected-supplemented group (II) had 2.08 g/dl globulins, which was significantly lower than 2.4 g/dl from the non-infected supplemented group (IV) (P<0.0001) on week 3 post-infection. This indicates that *A. galli* had no significant effect on globulin levels in non supplemeemented chickens, but the effect was on chickens that were supplemented. On the other hand, there was no significant difference on serum globulin levels between the infected non-supplemented group (I) and infected supplemented group (II) (P=0.88). However, the non-infected supplemented group (IV) had 2.4 g/dl globulins levels, which was significantly higher than the corresponding 1.97 g/dl of non-infected non-supplemented group (III) (P=0.016). This further indicates that vitamin A supplementation did not influence serum globulin levels in the infected chickens but the effect was on non-infected chickens.

Albumin/globulin ratios in experimental chickens

Figure 4 shows the results of serum albumin/ globulin ratio in the four groups of experimental chickens.

Changes of serum albumin/globulin ratios within the experimental groups

Figure 4 shows that the levels of serum albumin/ globulin ratios in chickens that were non-infected and non-supplemented (group III) ranged between 0.71 and 0.88 and formed the base-line values of serum albumin/globulin ratios of chickens in this study. These values were below the normal ratios of 1.5-3.5 reported by Thrall et al. (2006). The albumin/globulin ratios increased significantly ($P<0.05$) from 0.78 to 1.29 by week 10 in chickens in group IV that were being supplemented. On the other hand, the base-line values of serum albumin/globulin ratio in the infected and non-supplemented chickens (group I) decreased significantly ($P<0.05$) from 0.74 to 0.59 by week 10 post-infection. The effect of supplementation in infected chickens in group II on the values of serum albumin/globulin ratio was not significant ($P>0.05$) as evidenced by a small change from 0.75 to 0.63 by week 5 post-infection.

Comparative serum albumin/globulin ratios between the experimental groups

The significant differences in the levels of albumin/globulin ratio between the groups were seen from week 2 up to the end of the experiment. The albumin/globulin ratio in infected non-supplemented group (I) was 0.592. This was statistically lower than 0.826 of the ratio in non-infected non-supplemented group (III) ($P=0.013$). Similarly, the ratio of 0.636 in the infected-supplemented group (II) was significantly lower than 1.295 from the ratio in non-infetcted supplemented group (IV) ($P<0.05$). This indicates that *A. galli* had significant effect on the albumin/globulin ratio in both the supplemented and non-supplememented chickens. On the other hand, there was no significant difference in the albumin/globulin ratio of 0.592 in the infected non-supplemented group (I) and 0.636 in the infected supplemented group (II) ($P=0.52$). However, the non-infected supplemented group (IV) had a ratio of 1.295, which was statistically higher than 0.826 in the non-infected non-supplemented group (III) ($P<0.05$).

Haematocrit levels in experimental chickens

The results of haematocrit in the four groups of experimental chickens are presented in Figure 5.

Changes of haematocrit levels within the experimental groups

The levels of haematocrit in chickens that were non-infected and non-supplemented (group III) ranged between 32.1 and 34.65% and formed the base-line values of haematocrit of chickens in this study (Fig. 5). These values were within the normal levels of 22-35% reported by Wakenell (2010). Haematocrit values of chickens in group IV increased significantly ($P<0.05$) from 30.95 to 36.99% by week 10 post infection (Fig. 5). On the other hand, the base-line values of haematocrit in the infected and non-supplemented chickens (group I) decreased significantly ($P<0.05$) from 31.0 to 25.6% by week 10 post-infection. The effect of supplementation in the infected chickens in group II on the values of haematocrit is demonstrated by a non significant decrease ($P>0.05$) from 33.35 to 32.95% by week 10 post-infection. The significant effects of supplementation on haematocrit values in the infected chickens were seen as early as week 5 post-infection.

Comparative haematocrit levels between the experimental groups

Differences on the levels of haematocrit values between the groups were seen from week 2 through the end of the experiment ($P<0.0001$). For example, the haematocrit values in infected non-supplemented group (I) was 25.65% and was statistically lower than 35.65% of non-infected non-supplemented group (III) ($P<0.0001$) on weeks 2, 5 and 10 post-infection. Similarly, the infected-supplemented group (II), which on week 2 showed significant higher haematocrit values of 35% compared to 31.4% of the non-infected supplemented group (IV) ($P=0.012$) had its PCV decreased significantly to 32.9% compared to the the PCV of 36.9% in group IV on week 10 post-infection ($P=0.0032$). This indicates that *A. galli* infection had a significant effect on haematocrit values both in supplemented and non-supplememented chickens. On the other hand, the haematocrit value of 25.65% in the infected non-supplemented group (I) was significantly lower than 32.95% in the infected supplemented group (II) ($P<0.0001$). Also, the non-infected supplemented group (IV) had statistically significant higher haematocrit value (36.1%) than the corresponding 32.9% in the non-infected non-supplemented group (III) ($P=0.045$) on week 3 post-infection.

Haemoglobin levels in experimental chickens

The results of haemoglobin concentrations in the four groups of experimental chickens are shown in Figure 6.

Changes of haemoglobin levels within the experimental groups

The levels of haemoglobin concentrations in chickens that were non-infected and non-supplemented (group III) ranged between 10.77 and 11.6 g/dl and formed the base-line values of haemoglobin concentrations in chickens of this study. These values were within the normal levels of 7-13 g/dl reported by Wakenell (2010). Figure 6 shows that the base-line values increased significantly from 10.8 to 12.7 g/dl by

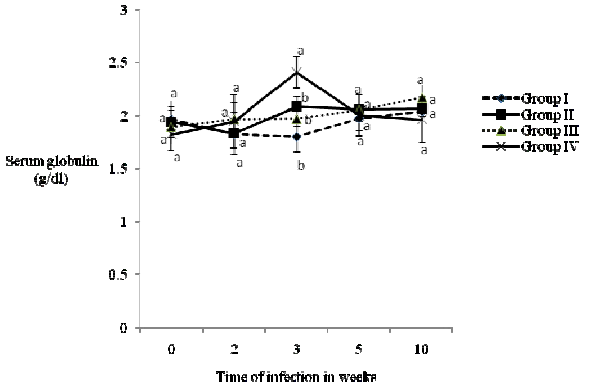

Fig. 3: Serum globulin in the four groups of experimental chickens measured at different weeks of infection. Error bars with different letters between the groups show significant difference (P<0.05)

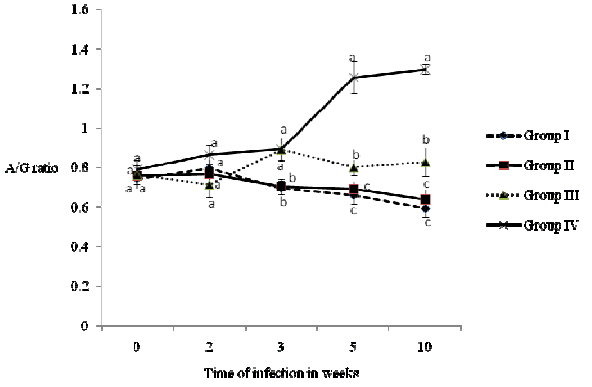

Fig. 4: Serum albumin/globulin ratio in the four groups of experimental chickens measured at different weeks of infection. Error Bars with different letters between the groups show significant difference (P<0.05)

week 10 of the experiment in chickens in group IV that were being supplemented (P<0.05). On the other hand, the base-line values in the infected and non-supplemented chickens (group I) decreased significantly from 10.7 to 8.58 g/dl by week 10 post-infection (P<0.05). The effect of supplementation in the infected chickens in group II on haemoglobin levels is evidenced by a significant increase of haemoglobin from 10.31 to 10.86 by week 10 post-infection (P<0.05). The significant effects of supplementation on haemoglobin values in the infected chickens were seen as early as week 2 post-infection.

Comparative haemoglobin levels between the experimental groups

Significant differences in the levels of haemoglobin concentration between the groups were seen from week 3 (P=0.0001) through the end of the experiment (P<0.0001). For example, the mean value of haemoglobin in the infected non-supplemented group

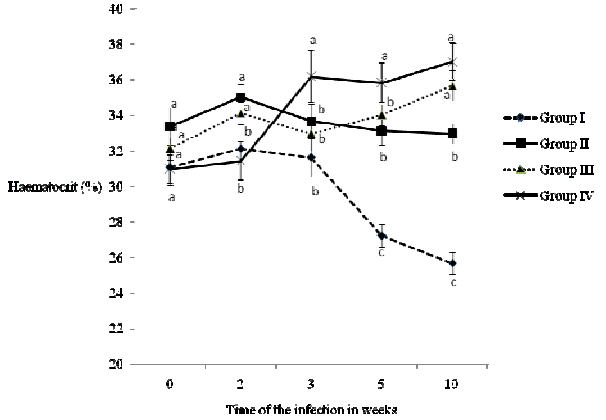

Fig. 5: Haematocrit levels in the four groups of experimental chickens measured at different weeks of infection. Error bars with different letters between the groups show significant difference (P<0.05)

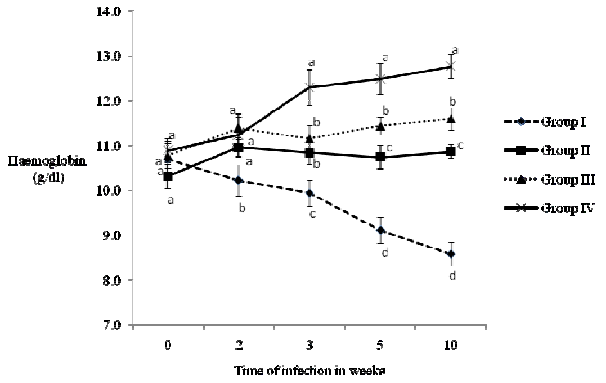

Fig. 6: Haemoglobin levels in the four groups of experimental chickens measured at different weeks of infection. Error bars with different letters between the groups show significant difference (P<0.05)

(I), was 8.58 g/dl and statistically lower than 11.6 g/dl in the non-infected non-supplemented group (III) (P<0.0001) from week 3 to 10 post-infection. Similarly, infected-supplemented group (II) had haemoglobin value of 10.86 g/dl, which was significantly lower than 12.76 g/dl of the non-infected supplemented group (IV) (P<0.0001). This indicates that *A. galli* infection had significant effect on haemoglobin values both in supplemented and non-supplemememented chickens. On the other hand, haemoglobin values of 8.58 g/dl from the infected non-supplemented group (I) were significantly lower than 10.86 g/dl haemoglobin from the infected supplemented group (II) (P<0.0001). Also, haemoglobin concentration of 12.76 g/dl from the non-infected supplemented group (IV) was significantly higher than the corresponding 11.6 g/dl obtained from the non-infected non-supplemented group (III) (P=0.005) on week 10 post-infection.

Discussion

The present study shows that vitamin A supplementation can reduce effect of A. *galli* infestation in chickens by moderating the levels of serum proteins, albumin and globulin, as well as levels of haematocrit and haemoglobin concentrations. The findings attempts to explain the occurrence of overt nutritional deficiencies reported in free ranging chickens and underlines importance of deworming chickens and supplementation of vitamin A or its precursors in the diet (Bhuiyan et al., 2004). Although the study did not address the role of other nutrients on the infestations it sheds light on the importance of animal feeding in the productivity and health of chickens. The relationship between helminth infestations and nutrients in animals and humans has been documented (Mwaniki et al., 2002; Koski and Scott, 2003; Osazuwa et al., 2011). The present study further provides the same observation in our settings. It shows that *A. galli* infestations lowered serum albumin, globulin and albumin/globulin ratio, the observation which is in agreement with a 93% decrease of serum albumin in chickens infected by *A. galli* reported by Ali et al. (2011) and Kuklina and Kuklin (2006). Similarly, it is apparent that globulin is not affected by helminth infestation, an observation that agrees with Deka and Borah (2008) in a study involving Japanese quails infected with *A. galli*.

Although the mechanism of reduction in the albumin concentration in serum is not well known it is suggested that intestinal parasitism increases albumin catabolism (Tanwar and Mishra, 2001). This is probably due to reduced energy intake during parasitism thus mobilizing the proteins to supply the energy (Crompton and Nesheim, 2002). Adang et al. (2010b) reported degenerative and necrotic lesions in the liver, kidney and the lungs of pigeons infected by *A. galli*. It is, therefore, possible that the low levels of albumin and total proteins observed in the *A. galli* infected chickens in the present study are partly due to hepatic lesions that compromised protein metabolism. More evidence is however needed to justify this claim. The results of the present study also provide evidence that vitamin A supplementation improves both serum albumin and total protein concentrations. This is partly due to the role of vitamin A on maintaining the integrity of intestinal mucosa and general immunity thus ensuring the absorption of protein (Tanwar and Mishra, 2001; Villamor and Fawzi, 2005). Vitamin A supplementation is therefore, considered to minimize the pathologic effects of *A. galli* in chickens.

Both haematocrit and haemoglobin levels were significantly reduced by *A. galli* infections in the present study. Low levels of haematocrit and haemoglobin concentrations in both chickens and quails infected with *A. galli* have also been reported by Deka and Borah (2008). Likewise, decreased levels of haematocrit and haemoglobin concentrations have been reported in children infected with hookworms and *Ascaris lumbricoides* (Osazuwa et al., 2011). Haematocrit and haemoglobin indirectly measure the status of erythrocytes, the integrity of which depends on iron (Crompton and Nesheim, 2002). Hence the intestinal leakage of iron that follows damage caused by migrating larvae is linked to the observed low values of haematocrit and haemoglobin through reduced erythropoiesis (Crompton and Nesheim, 2002). It is also known that helminthosis cause inappetance that ultimately affect dietary intake including iron-containing feeds and other nutrients essential for erythropoiesis (Crompton and Nesheim, 2002).

Improvement in the levels of haematocrit and haemoglobin in chickens that were infected and supplemented with vitamin A is based on the influence of vitamin A on the gut integrity that enhances absorption of nutrients required for the production of red blood cells (Koski and Scott, 2003). Vitamin A increases utilization of iron in the liver and spleen (Roodenburg, 2000) and enhances the uptake of iron in the bone marrow (Sijtsma et al., 1989). Evidence shows that during vitamin A deficiency much of the iron is retained in the liver and spleen and this reduces the incorporation of iron into erythocyte by 40 to 50% (Semba and Bloem, 2002).

We conclude that vitamin A supplemenation moderates the effects of helminthosis on blood parameters in chickens. Moderation of the effect is through among the other mechanisms, reducing worm burden as previously reported (Idi et al., 2007). While the present study reports the effect of vitamin A supplementation on moderating the health effect of helminthosis in chickens under experimental conditions, it provides an insight of the benefits of extrapolating the results to the field condition. Scavenging chickens are usually infected with helminths (with a prevalance of up to 100%) (Magwisha et al., 2002). These chickens are therefore at great risk of helminthosis during dry season, during when dietary vitamin A from plant sources is scarce (Mutayoba et al., 2012). Supplementation of scavenging chickens with vitamin A may provide a relief. This intervention is however lilely to yield more benefit if a study targeting village or scavenging chickens is conducted. In fact it remains a next important step to undertake.

References

Adang KL, Abdu PA, Ajanusi JO, Oniye SJ, Ezealor AU (2010a) Effects of *Ascarida galli* infection on body weight, packed cell volume, hemoglobin

level and total plasma protein of experimentally infected domestic pigeons (*C.l. domestica*) in Zaria, Nigeria. Global J Med Res 10: 6-10.

Adang KL, Abdu PA, Ajanusi JO, Oniye SJ, Ezealor AU (2010b) Histopathology of *Ascaridia galli* infection on the liver, lungs, intestines, heart, and kidneys of experimentally infected domestic pigeons (*C.l. domestica*) in Zaria, Nigeria. Pacific J Sci Technol 11: 511-515.

Ali MA, Devi LI, Lyngdoh WM, Das G, Prasad H, Chanu KV, Prava M, Tolenkhomba TC, Singh YD, Lallinchhunga MC (2011) Comparative biochemical profile of *Ascaridia galli* infected broiler chickens on admiministration of pineapple and neem leaves and piperazine. Int J Poult Sci 10: 542-546.

Anwar H, Rahman Z (2002) Effect of *Ascaridia galli* infestation on electrolyte and vitamins in chicken. Online J Biol Sci 2: 650-651.

Bhuiyan AR, Lauridsen C, Howlider AR, Jakobsen K (2004) Importance of vitamin A supplementation for performance of Sonali chickens under smallholder farm conditions in a tropical climate. Livest Res Rural Dev 16: 1-9.

Crompton DWT, Neisheim MC (2002) Nutritional impact of intestinal helminthiasis during the human life cycle. Annu Rev Nutr 22: 35-59.

Curtale F, Vaidya Y, Tilden RL (1995) Ascariasis, hookworm infection and serum retinol amongst children in Nepal. Panminerva Med 36: 19-21.

Deka K, Borah J (2008) Haematological and biochemical changes in Japanese Quails *Coturnix coturnix japonica* and chickens due to *Ascaridia galli* infection. Int J Poult Sci 7: 704-710.

Idi A, Permin A, Jensen SK, Murrell KD (2007) Effect of minor vitamin A deficiency on the course of infection with *Ascaridia galli* (Schrank, 1788) and the resistance of chickens. Helminthologia 44: 3-9.

Kidala D, Greiner T, Gebre-Medhin M (2000) Five-year follow-up of a food based vitamin A intervention in Tanzania. Public Health Nutr 4: 425-31.

Koski KG, Scott ME (2003) Gastrointestinal nematodes, trace elements, and immunity. J Trace Elem Exp Med 16: 237–251.

Kuklina MM, Kuklin VV (2006) Effect of helminth infestation on the biochemical parameters of Gulls of the genus Larus in the Barents Sea Region. Doklady Biol Sci 411: 471–474.

Lumeij JT (2008) Avian clinical biochemistry. In: Kaneko JJ, Harvey JW, Bruss ML (editors), Clinical biochemistry of domestic animals, 6th edition. Elsevier, USA, pp: 839-840.

Magwisha HB, Kassuku AA, Kyvsgaard NC, Permin, A (2002) A comparison of the prevalence and burdens of helminthes infections in growers and adult free-range chickens. Trop Anim Health Prod 34: 205-214.

Marinho HA, Shrimpton R, Giugliani R, Burini RC (1991) Influence of enteral parasites on the blood vitamin A levels in preschool children orally supplemented with retinol and/or zinc. Eur J Clin Nutr 45: 539-44.

Mutayoba SK, Katule AK, Minga U, Mtambo MM, Olsen JE (2012) The effect of supplementation on the performance of free range local chickens in Tanzania. Lives Res Rural Dev 24. Retrieved December 31, 2013, from http://www.lrrd.org/lrrd24/5/muta 24093.htm

Mwaniki D, Omondi B, Muniu E, Thiong'o F, Ouma J, Magnussen P, Geissler PW, Michaelsen KF, Friis H (2002) Effects on serum retinol of multi-micronutrient supplementation and multi-helminth chemotherapy: a randomised, controlled trial in Kenyan school children. Eur J Clin Nutr 56: 666-673.

Osazuwa F, Ayo OM, Imade P (2011) A significant association between intestinal helminth infection and anaemia burden in children in rural communities of Edo state, Nigeria. North Am J Med Sci 3: 30-34.

Permin A, Bojesen M, Hansen P, Bisgaard M, Frandsen F, Pearman M (1997) *Ascaridia galli* populations in chickens following single infections with different dose levels. Parasitol Res 83: 614-617.

Rewat N, Shakya S, Singh F (2010) Biochemical and mineral profile of broiler chickens experimentally infected with hydropericardium syndrome virus. Int J Poult Sci 45: 59-62.

Roodenburg A, Leenen R, Van Het Hof K, Weststrate J, Tijburg L (2000) Amount of fat in the diet affects bioavailability of lutein esters but not of alphacarotene, beta-carotene, and vitamin A in humans. Am J Clin Nutr 71: 1187-1193.

Semba RD, Bloem MW (2002) The anemia of vitamin A deficiency: epidemiologyand pathogenesis. Eur J Clin Nutr 56: 271-281.

Sijtsma SR, West CE, Rombout JHWM, Van der Zijpp, AJ (1989) The interaction between vitamin A status and Newcastle disease virus infection in chickens. J Nutr 119: 932-939.

Silva PRL, Freitas Neto OC, Laurentiz AC, Junqueira OM, Fagliari JJ (2007) Blood serum components and serum protein test of hybro-pg broilers of different ages. Braz J Poult Sci 9: 229-232.

Soulsby EJL (1982) Helminths, arthropods and protozoa of domesticated animals. Baillière Tindall, London: p: 809.

Tanwar RK, Mishra S (2001) Clinico-haemato-biochemical studies on intestinal helminthiasis in poultry. Vet Pract 2: 137-140.

Thrall MA, Baker DC, Campbell TW, DeNicola D, Fettman MJ, Lassen ED, Rebar A, Weiser G (2006) Veterinary haematology and clinical chemistry. Blackwell, USA: pp: 225-258, 479-491.

Ueno T, Hirayama S, Ito M, Nishioka E, Fukushima Y, Satoh T, Idei M, Horiuchi Y, Shoji H, Ohmura H, Shimizu T, Miida T (2013) Albumin concentration determined by the modified bromocresol purple method is superior to that by the bromocresol green method for assessing nutritional status in malnourished patients with inflammation. Ann Clin Biochem 50: 576-584.

Villamor E, Fawzi WW (2005) Effects of vitamin A supplementation on immune responses and correlation with clinical outcomes. Clin Microbiol Rev, 18: 446-464.

Wakenell PS (2010) Haematology of chickens and turkeys, In: Weiss, D.J. and Wardrop, K.J. (editors), Schalms Veterinary Haematology, 6th edition. Wiley-Blackwell, USA. p: 965.