

**SERUM RETINOL LEVELS AND ASSOCIATED BIOCHEMICAL AND
HAEMATOLOGICAL PARAMETERS IN CHICKENS
EXPERIMENTALLY INFECTED WITH *Ascaridia galli***

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ABSTRACT

The levels of serum retinol, total serum proteins, serum albumin, serum globulins, albumin/globulin ratio, haemoglobin concentration, haematocrit, body weights as well as dynamics of *A. galli* (namely eggs per gram of faeces, worm establishment rate, total counts, male and female counts, females to males ratio, total worms weight and fecundity) were investigated in chickens experimentally infected with 350 eggs of *A. galli* and daily supplemented with 1500 I.U retinyl acetate. Forty, ISA brown chickens aged eight weeks were divided into four equal groups. The effect of infection alone was investigated in the infected chickens in group I. The effects of infection and supplementation were investigated in chickens in group II. Chickens in group III were neither infected nor supplemented. The effect of supplementation alone was investigated in chickens in group IV. The dynamics of *A. galli* was investigated in supplemented and non-supplemented chickens. Mean levels of retinol, albumin, albumin/globulin ratio, haematological parameters and body weights were significantly ($p < 0.05$) low in the infected than in non-infected groups. *A. galli* infection had significant effect on globulin levels when birds were supplemented ($p < 0.05$). The above mentioned parameters were significantly higher in the supplemented than in the non-supplemented groups ($p < 0.05$). Mean EPG, fecundity, female: male ratio and female worms counts were significantly ($p < 0.05$) higher in the non-supplemented than in the supplemented groups. Vitamin A supplementation did not significantly ($P > 0.05$) affect total worm counts, male worms counts, establishment and total worms weights.

It is concluded that *A. galli* infestation lowers serum retinol, 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 and lowers egg counts and fecundity and favours development of male worms. Supplementation of commercial feeds with stabilized vitamin A is highly recommended.

DECLARATION

I, Elifuraha Barnabas Mngumi do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and has neither been submitted nor being concurrently submitted for a degree awarded in any other University.

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DEDICATION

This work is dedicated to my wife Sabina and the children Barnabas, Sheppa Jr, and Esther.

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ABBREVIATIONS AND SYMBOLS

%	Percent.
μl	Micro liter.
μmol	Micromole.
A/G	Albumin/globulin ratio.
ALB	Albumin.
ANOVA	Analysis of Variance.
APP	Acute Phase Proteins.
BCG	Bromocresol green.
BHT	Butylhydroxytoluene.
CM	Chylomicron.
Ctn	Carotene.
DASP	Department of Animal Science and Production.
dl	Deciliter.
EDTA	Ethylene diamine tetraacetic acid.
EPG	Eggs per gram.
FAO	Food and Agriculture Organisation.
FVM	Faculty of Veterinary Medicine.

g	Gram.
Hb	Haemoglobin.
HPLC	High Performance Liquid Chromatography.
i.e.	That is.
IU	International units.
kg	Kilogram.
l	Litre.
LRAT	Lecithin retinol acyltransferase.
M	Mole.
mg	Milligram.
min	Minutes.
ml	Milliliter.
nm	Nanometer.
°C	Degree Celcius.
PCV	Packed Cell volume.
RAL	Retinal.
rbp	Retinol binding proteins.

RE	Retinyl esters.
RECM	Retinyl esters-chylomicron.
ROL	Retinol.
rpm	Revolutions per minute.
RRBP	Retinol-rbp.
S.E	Standard error.
SUA	Sokoine University of Agriculture.
US	United States.
USA	United States of America.
UV	Ultraviolet.
Vol	Volume.
α	Alpha.
β	Beta.
χ^2	Chi- square.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Vitamin A refers to all ionone derivatives with a side chain of three isoprenoid units linked at the 6-position of the ionone ring forming a chain of five carbon-carbon double bonds and a functional group at the end of the acyclic portion as shown in Figure 1 (D'Ambrosio *et al.*, 2011; McLaren and Frigg, 2001). These compounds are generally termed retinoids and include retinol, retinal, retinoic acid and retinyl ester, which animals are able to produce (Deming and Erdman, 1999). Retinol, retinal and retinoic acids are the active forms of retinoids present in the circulation while retinyl esters constitute a major storage form, which is stored in the liver (Deming and Erdman, 1999). Retinol is the form of retinoid, which can readily be converted to other forms i.e retinal and retinoic acid. This makes retinol a prototype representative of vitamin A. It is for this reason that vitamin A is synonymously referred to as retinol.

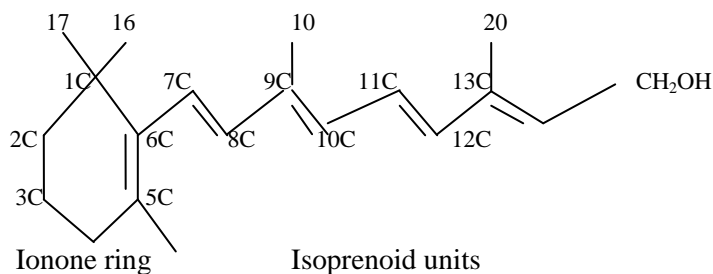


Figure 1: Ionone structure, with three isoprenoid units linked at position 6

Source: D'Ambrosio *et al.* (2011).

Animals are unable to synthesize vitamin A making it an essential nutrient that must be supplied in the diet. There are two main sources of vitamin A, i.e. preformed vitamin A from animal sources (primarily as retinyl esters) and provitamin A in the form of carotenoids from plants (Von Lintig, 2010). Carotenoids are the isoprenoid compounds related to retinoid and are formed by head-to-tail linkages of eight isoprenoid units to provide a 40 carbon skeleton containing up to 15 conjugated double bonds (D'Ambrosio *et al.*, 2011). Carotenoids are divided into two major groups according to their chemical structures i.e. pure hydrocarbons called carotenes such as β , β -carotene, α -carotenes and γ -carotene and the oxygenated derivatives called xanthophylls such as cryptoxanthin (Surai, 2012). There are over 750 carotenoids but only 10% of these can be converted to vitamin A (Surai, 2012). The carotenoids that are convertible to vitamin A are known as provitamin A carotenoids and include β -carotene, α -carotenes, γ -carotene and β -cryptoxanthin (Von Lintig, 2010). It is estimated that 0.6 μ g of β -carotene is equivalent to one I.U of vitamin A and to 0.3 μ g of retinol (D'Ambrosio *et al.*, 2011).

The ability and extent of absorption and metabolism of carotenoids differ between animals. Cattle, horses and carnivores (cats in particular) readily absorb carotenes while pigs and goats can absorb few carotenoids. Birds can absorb xanthophylls only (McLaren and Frigg, 2001). Absorption of carotenoids in most animals except cattle and horses requires that the carotenoids are first converted to retinal and subsequently to retinol in the gut enterocytes (Deming and Erdman, 1999). The

formed retinol is further converted to retinyl esters that are packed with chylomirons that are absorbed from intestinal mucosa into lymphatic system and transported to the liver (Yeum and Russell, 2002; D'Ambrosio *et al.*, 2011). Carnivores are exceptionally incapable of converting carotenoids to vitamin A (McLaren and Frigg, 2001). On the other hand, the preformed retinyl esters are first hydrolysed to retinol within the enterocytes brush border and then absorbed into the enterocytes where they are re-estrified to retinyl esters that are packed with chylomicrons to follow the carotenoid absorption and transport pathways (Yeum and Russell, 2002; D'Ambrosio *et al.*, 2011).

Vitamin A plays essential physiological roles that encompass gene expression (Omori and Chytil, 1982), reproduction (Chew, 1993; Bermudez *et al.*, 1993), vision (E-Siong, 1995), immunity (Wiedermann *et al.*, 1993; Stephensen, 2001), maintenance of the integrity of epithelial tissue (McLaren and Frigg, 2001), growth (McLaren and Frigg, 2001) and cellular differentiations (Ma and Ross, 2009). Vitamin A is also known to protect the body against infections and maintain the optimal functions of body systems; for example stimulation of humoral response (Sijtsma *et al.*, 1989; Koski and Scott, 2003). Thus vitamin A deficiency can be a source of many disorders in animals.

In chickens vitamin A deficiency has been reported to be associated with increased susceptibility and mortality due to helminthosis (West *et al.*, 1992). On the other hand, vitamin A supplementation has been reported to modulate the infection of *A.*

galli by reducing the size, egg counts and fecundity (Idi *et al.*, 2007). Other health conditions associated with vitamin A deficiency in chickens include swollen and exudation of eyes, weight loss, reduced weight gain, and reduced egg production and hatchability (Beynen *et al.*, 1989; West *et al.*, 1992; Bermudez *et al.*, 1993; Idi *et al.*, 2007, Bhuiyan *et al.*, 2004; Schweigert *et al.*, 1991).

Damage of the intestinal mucosa is considered to be the major hindrance to absorption of nutrients including vitamin A. Thus low total serum proteins, albumin, cholesterol and iron have been reported in chickens infected with *A. galli* (Tanwar and Mishra, 2001; Crompton and Nesheim, 2002; Adang *et al.*, 2010a and Ali *et al.*, 2011). Impaired absorption of proteins and fat abets the deficiency of vitamin A, considering that fat is necessary for carotenoids uptake and transport in the intestine (Von Lintig, 2010) and proteins are required for synthesis of albumin and retinol binding proteins, which are both required to carry retinol in the circulation (D'Ambrosio *et al.*, 2011). Indeed, Watts (1991) reported that normal serum levels of retinol can be restored when protein is added to the diet.

Despite the above account, there is scanty information on the association between vitamin A deficiency and gastrointestinal infestation in chicken in Tanzania. Gastrointestinal parasitic infestations are considered to be among the major health problems in chickens in Tanzania and other parts of Africa and Asia, the prevalence of which is estimated to go up to 100% in Tanzania in free ranging chickens (Magwisha *et al.*, 2002). The chickens are usually infected by mixed

species of worm including nematodes, cestodes and trematodes (Magwisha *et al.*, 2002). *A. galli* is the most common infestation in Tanzania with a prevalence of 69% (Permin *et al.*, 1997; Magwisha *et al.*, 2002).

It is evident from the above discussion that *A. galli* is likely to influence the levels of vitamin A in the blood of infected chickens. The present study was, therefore, thought to investigate serum retinol levels and other associated haematological and biochemical parameters and body weights as well as dynamics of *A. galli* in chickens experimentally infected with *A. galli* and supplemented with vitamin A in Tanzania.

1.2 Problem statement and Justification

Vitamin A deficiency is one of the nutritional disorders that has been reported mainly in humans in association with helminthosis. For example low levels of serum retinol have been reported by Curtale *et al.* (1995) in persons infected by *Ascaris lumbricoides*. Stephensen (2001) reported a loss of up to 1µmole/l retinol/day in humans infected by hookworms, while Mwaniki *et al.* (2002) found low serum levels of retinol in children infected by *Schistosoma mansoni*.

Although helminthosis is well documented in chickens, with chickens being infected by different types of worms including nematodes (i. e *Tetrameres americana*, *Heterakis gallinarum*, *Ascaridia galli*, *Syngamus trachea*, *Trichostrongylus tenuis*, *Capillaria annulata* and *Allodapa suctoria*), cestodes (i.e *Davainea proglottina*, *Raillietina tetragona*, *Raillietina echinobothrida*,

Hymenolepis cantaniana and *Choanotaenia infundibulum*), there is scanty information on the association between vitamin A deficiency and helminthosis in chickens. The limited information about the association of retinol and *A. galli* infestation in chickens formed the justification of the present study, bearing in mind that Vitamin A is a key nutrient in many physiological functions, hence its deficiency is likely to cause a disturbance of many parameters. The study also included investigations on other parameters including biochemical, haematological and dynamics of *A. galli*.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to establish the association between *A. galli* infestation and serum levels of vitamin A and other associated parameters in chickens experimentally infected with *A. galli* and supplemented with Vitamin A.

1.3.2 Specific objectives

- To investigate the effect of *A. galli* infection on serum levels of vitamin A in chickens supplemented with vitamin A and non-supplemented chickens.
- To investigate the effect of *A. galli* infection on biochemical (total serum proteins, albumin, globulin and albumin/globulin ratio) and haematological (haemoglobin concentrations and haematocrit) parameters in chickens supplemented with vitamin A and non-supplemented chickens.

- To investigate the effect of *A. galli* infection on body weights in chickens supplemented with vitamin A and non-supplemented chickens.
- To investigate the dynamics of *A. galli* worms (eggs per gram of faeces, establishment rate, total worm counts, counts of male and female worms, ratio of females to males, total worms weight and fecundity) in chickens supplemented with vitamin A and non-supplemented chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Occurrence of *A. galli* and its effects in chickens

In Tanzania the chickens are usually infected by mixed species of helminths including *A. galli* the prevalence of which has been reported to be 69% (Permin *et al.*, 1997; Magwisha *et al.*, 2002). Elsewhere prevalences of *A. galli* infestations have been reported to be 64% in Denmark (Permin *et al.*, 1999), 55.3% in Ethiopia (Ashenafi and Eshetu, 2004), 9% in Morocco (Hassouni and Belghyti, 2006), 28.8% in Zambia (Phiri *et al.*, 2007), 75.6% in Palestine (Rayyan *et al.*, 2010) and 31.4% in South Africa (Mwale and Masika, 2011). Young chickens of less than three months old and naive chickens are the most susceptible to *A. galli* infestation (Anwar and Rahman, 2002).

Infection of *A. galli* follows ingestion of infective *A. galli* eggs from the environment. The eggs hatch in the intestine and L3 migrate into the intestinal mucosa and spend about eight weeks to reach maturity. During this time the larvae migrate in between the lumen and mucosa of intestine, causing mucosa damage that shows up as haemorrhagic enteritis (Permin and Hansen, 1998). The haemorrhagic enteritis leads to anemia and diarrhea. The damaged mucosa also leads to impaired digestion and absorption of nutrients (Anwar and Rahman, 2002; Crompton and Neishein, 2002). Similarly the migrating larvae and adult worms feed on nutrients present in the intestine resulting into low nutrients available for absorption (Crompton and Neishein, 2002). In chickens, absorption of carotenoids

and retinyl esters starts in the intestine (Deming and Erdman, 1999; D'Ambrosio *et al.*, 2011). It is for this reason that *A. galli* infestation has been documented to be associated with vitamin A deficiency in chickens (Adang *et al.*, 2010b).

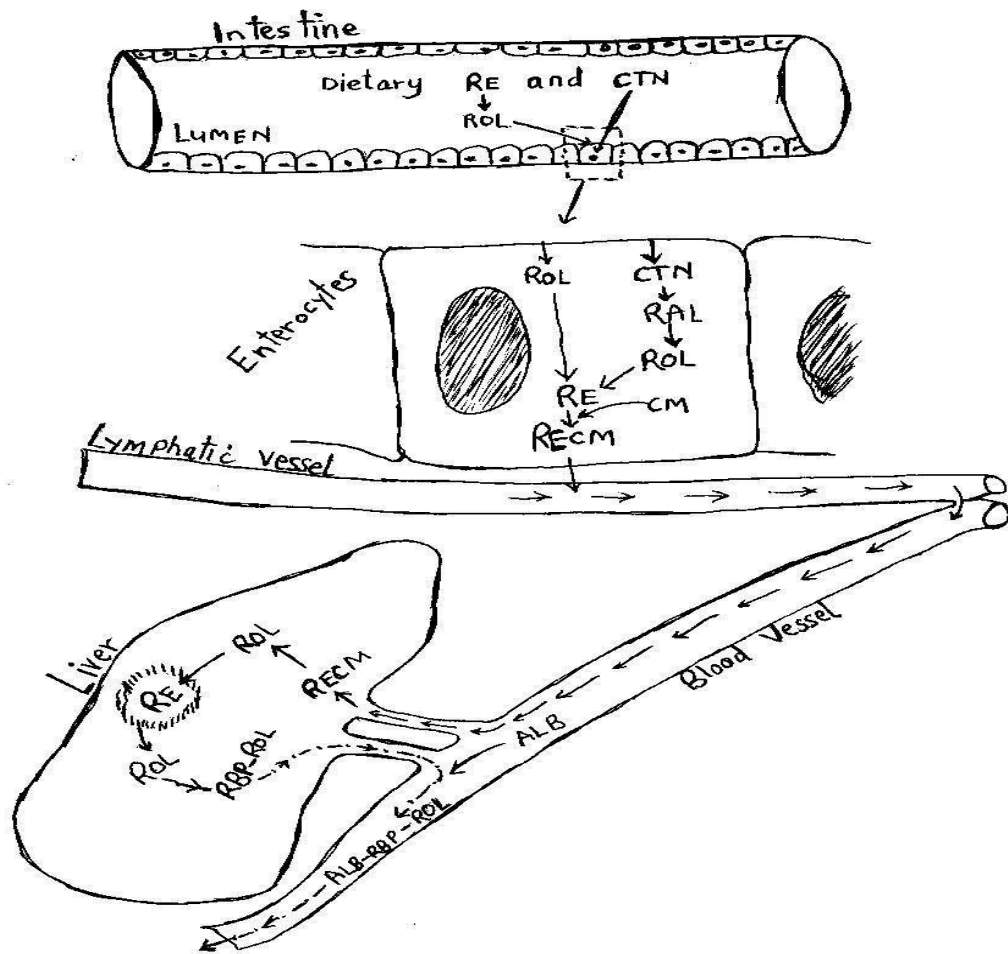
2.2 Metabolism of retinol (Vitamin A)

There are three active forms of Vitamin A in the circulation namely retinol, retinal and retinoic acid while retinyl esters occur in the storage form in the liver (Deming and Erdman, 1999). Retinol is fat soluble, which cannot be synthesized in the body, and has, therefore, to be supplied in the diet either as carotenoids from green plant feeds or as preformed retinyl esters from animal feeds. The carotenoids and retinyl esters consumed are converted to retinol through a number of steps as described by Yeum and Russell (2002) and D'Ambrosio *et al.* (2011). The steps are illustrated in Figure 2.

Intestine and liver are the central organs that play a major role in the metabolism of vitamin A. In this respect the intestine acts as the first organ where metabolism of carotenoids and vitamin A starts while the liver stands for further processing and storage of the vitamin. It should be noted that the steps described in Figure 2 apply for animals that have the ability to convert carotenoids to vitamin A before it is absorbed and for those, which absorb carotenoids directly without being converted to Vitamin A.

Briefly, the metabolism of carotenoids start with β -carotene, which are first absorbed into the enterocytes and converted to retinal through oxidation under the catalytic action of β -carotene monooxygenase enzyme. Retinal is then reversibly

reduced by retinal reductase to retinol. The dietary retinyl esters on the other hand are first hydrolysed in the brush border of the enterocytes to retinol, which is then passively absorbed into the enterocytes.



ALB: Albumin, CM: Chylomicron, CTN: Carotene, RAL: Retinal, RBP: Retinol binding protein, RE: Retinyl esters, RECM: Retinyl esters-chylomicron, ROL: Retinol.

Figure 2: Schematic pathways of carotenoids and retinyl esters metabolism drawn according to steps described by Yeum and Russell (2002) and D'Ambrosio *et al.* (2011).

Under the catalytic activity of lecithin retinol acyltransferase (LRAT), retinol is esterified by combining with long chain fatty acids to form retinyl esters. The retinyl esters combine with chylomicrons to form retinyl esters-chylomicrons (RECM), which are secreted from the enterocytes to the lymphatics and transported to the general circulation through the liver. In the liver, retinyl esters-chylomicrons enter the hepatocytes where according to need are hydrolysed to retinol. Part of the retinol in the hepatocytes is transferred to the hepatic stellate cells where it is re-esterified and the resulting retinyl esters are stored. The stored retinyl esters are reversibly converted to retinol in the hepatocytes. For retinol to be released from the hepatocytes to the circulation, it has first to be bound to retinol binding protein (rbp) to form retinol-rbp (RRBP). The released RRBP in circulation binds to albumin to form albumin-rbp-retinol. The retinol bound to albumin provides a basis for measurements of the status of vitamin A in the body (E-Siong, 1995).

It is evident from the above explanation that the bioavailability of vitamin A depends on the amount of carotenoids from plants, which are able to be converted to retinol and on the amount of retinyl esters from animal source. Other determining factors include activities of enzymes involved in metabolic pathways of carotenoids and presence of rbp and albumin, which are the transporting media of retinol. Any abnormalities in any steps of retinol metabolism may result into deficiency of Vitamin A. The following sections provide an account on the factors that influence levels of retinol in the blood:

2.2.1 Factors affecting availability of dietary carotenoids and retinyl esters

The status of Vitamin A is influenced by season, and deficiencies have been seen during the dry season, when green plants that are rich in carotenoids are minimal (Bates *et al.*, 1994; Mwaniki *et al.*, 2002). Furthermore, naturally occurring precursors of vitamin A tend to be unstable because of oxidation, which occurs in the course of storage. Commercial feeds that lack antioxidants are likely to result into less amount of retinol in the circulation and retinyl esters stored in the liver.

The amount of carotenoids or preformed vitamin A available for absorption also depends on the presence of other competitors for vitamin A. For example, presence of intestinal parasites that consume sources of vitamin A have detrimental effect on serum retinol. Similarly, the same scenario applies for diarrhoeic diseases in which the vitamins and other nutrients are lost, making them unavailable for absorption (Salazar-Lindo *et al.*, 1993; Stephensen, 2001).

2.2.2 Adequate amounts of proteins and fat for binding retinoids

Retinol binding protein, albumin and chylomicrons are critical for retinol availability. Infections and factors that interfere with synthesis of rbp result into less amount of retinol available in the circulation. Conditions associated with release of acute phase proteins (APP) have also been shown to create deficiencies in rbp (Fujita *et al.*, 2009). Similarly, liver diseases and insufficient intake of proteins affect synthesis of albumin, rbp and chylomicrons that are essential in the transport of retinoids (Sommer, 1995). Intestinal diseases and parasitism affects the levels of dietary fat (Crompton and Neisheim, 2002), which is considered to be a

vehicle for the transport of both carotenoids and retinoids (Jalal *et al.*, 1998; Roodenburg *et al.*, 2000).

2.2.3 Impaired absorption and metabolism of retinol and retinyl esters

As explained in section 2.2, the integrity of intestinal mucosa is critical for adequate metabolism and absorption of retinol (Stephensen, 2001) and, is likely to be impaired when the intestine is damaged (Adang *et al.*, 2010b).

2.3 Functions of retinol

Retinol has many essential physiological functions including formation of visual pigments i.e. rhodopsin (E-Siong, 1995), maintenance of the integrity of epithelial tissue (McLaren and Frigg, 2001), promotion of growth (McLaren and Frigg, 2001), reproduction (Chew, 1993; Bermudez *et al.*, 1993), cellular differentiations (Ma and Ross, 2009), gene expression (Omori and Chytil, 1982) and immunity (Wiedermann *et al.*, 1993; Stephensen, 2001). Deficiency of retinol in blood is, therefore, expected to induce many dysfunctions. Some of the dysfunctions reported to be associated with Vitamin A deficiency in chickens include increased susceptibility to diseases, increased mortality, swollen and exudation of eyes, weight loss, reduced weight gain, and reduced egg production and hatchability (Beynen *et al.*, 1989; Schweigert *et al.*, 1991; West *et al.*, 1992; Bermudez *et al.*, 1993; Bhuiyan *et al.*, 2004; Idi *et al.*, 2007).

2.4 The effect of helminthosis on retinol and other micronutrients in birds

Studies in birds show that helminthosis affects the concentration of serum retinol as well as total serum proteins, albumin and globulin (Anwar and Rahman, 2002;

Deka and Borah, 2008; Rewat *et al.*, 2010; Ali *et al.*, 2011). It has also been established that migrating helminth larvae cause damage of intestinal mucosa that leads to leakage of iron that culminates in reduced erythropoiesis due to iron deficiency (Crompton and Nesheim, 2002). Birds with severe worm burdens are, therefore, likely to develop iron deficiency anemia. Moreover, worm burdens have been associated with reduction in serum fat levels (Crompton and Neisheim, 2002). The low levels of fat may result into low negative energy balance that ultimately makes the body to mobilise and catabolise proteins to release energy.

Apart from birds, the association between gastrointestinal parasites and vitamin A has also been described by several investigators in humans. For example low serum retinol levels have been reported in persons infected by *Ascaris lumbricoides* (Curtale *et al.*, 1995), schistosomiasis (Friis *et al.*, 1996) and hookworms (Stephensen, 2001).

The mechanisms through which such invasive gastrointestinal parasites induce deficiency of vitamin A and other micronutrients include loss of retinol through blood diarrhea, interference in bioconversion and absorption of pro vitamin A (Marinho *et al.*, 1991; Curtale *et al.*, 1995; Kidala *et al.*, 2000).

2.5 Assessment of serum vitamin A

Serum vitamin A is routinely assessed by measuring the concentration of retinol in blood (Fujita *et al.*, 2009) using both the spectrophotometric (Rutkowsky and Grzegorzcyk, 2007) and chromatographical methods (Craft *et al.*, 1999; Kane *et al.*, 2008). High performance liquid chromatography (HPLC) is the most recent

standard analytical method involving lipid extraction of retinol from the sample, followed by separation and quantification of retinol (Gunter and Miller, 1986; McLaren and Frigg, 2001).

2.6 Normal levels of vitamin A in birds

The normal levels of serum vitamin A concentrations in White Leghorn birds have been reported as shown in Table 1.

Table 1. Normal levels of serum vitamin A in White Leghorn chickens

Species/Breed of bird	Serum Vitamin A	Reference
White Leghorn Chicken	2.4 to 3 μ mol/l	West <i>et al.</i> (1992)
White Leghorn chickens	0.89 to 0.91 μ mol/l	Anwar and Rahman (2002)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

The study was designed as a controlled experiment involving 100 ISA brown chicks from which a sample of forty was used for the trial as calculated using the Altman normogram as described by Whitley and Ball (2002). Briefly a standard deviation of 1.75 and effect difference of 1.65 was estimated from a related study by Anwar and Rahman (2002) to calculate a standardized difference of 0.94 using a formula: Standardized difference = estimated effect size/estimated standard deviation, the value of which was used to select a required sample size with power of 80% at 5% significant level. The key activities of the study were: purchase and management of experimental chickens, establishment of infective *A. galli* eggs, treatment of experimental chickens, infection of experimental birds, measurement of body weights, determination of serum retinol and other associated biochemical and haematological parameters and assessment of the dynamics of *A. galli*.

3.2 Purchase and management of experimental chickens

One hundred, day old chicks were purchased from Interchick Company in Dar es Salaam and raised under a helminth-free environment at Sokoine University of Agriculture (SUA) in the Department of Animal Science and Production (DASP) before commencement of the experiment. On arrival the chicks were given oxytetracycline (1 g/l) in drinking water for five days and then vaccinated against Newcastle disease and Gumboro on days 7, 21 and 14, 28 post arrival respectively.

During the first seven weeks the birds were given locally compounded chick starter ration, which was replaced with a finisher ration from 8th to 18th weeks. The composition and proximate analysis of the feed rations given to the chickens are shown in Table 2. Both the feed and water were given *adlibitum*.

Table 2: 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
<u>Proximate analysis (percent)</u>		
Dry matter	92.04	88.08
Crude protein	26.23	13.4
Crude fiber	11.91	10.03
Ash	12.64	9.7
Ether extract	9.04	10.11

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

3.3 Randomization selection of chickens into experimental groups

The 100 raised chickens were tagged when they were 4 weeks old. Forty treatment chickens were randomly selected from the raised chickens. The 40 chickens were further randomly divided into four experimental groups, each having 10 birds. The four groups were stratified based on the *A. galli* infection and vitamin A supplementation as presented in Table 3. 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.

Table 3: Experimental groups categorised based on infection and Vitamin A supplementation

Group	Number of chickens	Treatment	
I	10	Infected	Not supplemented
II	10	Infected	Supplemented
III	10	Non-Infected	Not supplemented
IV	10	Non-Infected	Supplemented

3.4 Establishment of embryonated *A. galli* eggs

Establishment of *A. galli* infective eggs was done according to procedures described by Adang *et al.* (2010b). Briefly, *A. galli* eggs for infection were obtained from live adult *A. galli* worms collected from chickens slaughtered at the Morogoro Municipal market. The worms were collected in bottles containing

normal saline and brought to the laboratory in the Department of Microbiology and Parasitology, FVM. In the laboratory, 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 minutes in order to dissolve the sticky albuminous layer of eggs. The sediment were then placed in centrifuge tubes and centrifuged at 1500rpm for 3 minutes to recover the eggs. The recovered eggs were washed three times in distilled water and three times in 0.05M sulfuric acid. The collected eggs were suspended in 0.05M sulfuric acid and placed in plastic troughs and left to stand for 20 days in the laboratory at room temperature, approximately 25-30°C.

The embryonated eggs in sulphuric acid were washed in normal saline and diluted in 50ml normal saline. After thorough mixing, one ml of the egg suspension was transferred into a Rafter chamber where the embryonated eggs were counted and 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:

$$Vol = 350 / \text{Total counted eggs per ml}$$

3.5 Infection 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 week 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 one day before the infection.

The infected and non infected as well as the supplemented and non-supplemented chickens were monitored daily for 10 weeks during when signs of disease were monitored and samples of blood and body weights were taken from each chicken as described below.

3.6 Sampling of blood and measurement of body weights

The schedule of sampling of 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 millilitres syringes, twenty three gauge needles and EDTA containing as well as plain vacutainers were used to collect the blood from the wing and jugular veins after the areas were sterilized using methylated spirit. Five millilitres of blood were collected from each chicken and immediately transferred into an EDTA containing vacutainer tube, which was stored at 4°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 inclined position in dim light for one hour in order for serum to separate. The serum from each sample was gently separated and transferred into two vials. The serum in one vial was intended for retinol analysis and was stored at -70°C while serum in the second vial was

stored at -20°C for subsequent determinations of total serum proteins, albumin and globulins. The body weight of each chicken was measured by placing the chicken on a digital balance (BOECO, Germany).

3.7 Determination of serum retinol

Serum retinol was determined by the high performance liquid chromatography (HPLC) according to Gunter and Miller, (1986). The HPLC calculations and quantifications were based on Catagnani and Bieri (1983). All chemicals were purchased from Sigma Aldrich unless otherwise noted.

3.7.1 Chemicals used in the determination of retinol by HPLC

- I. Sodium chloride-analytical grade.
- II. Butylhydroxytoluene (BHT).
- III. Hexane-HPLC grade.
- IV. Retinol standard (99.5% purity).
- V. Retinyl acetate.
- VI. Methyl acetate.
- VII. Ethanol- HPLC grade.
- VIII. Methanol- HPLC grade
- IX. Acetonitrile- HPLC grade.

3.7.2 Preparation of reagents for determination of retinol by HPLC

- i). 0.9% w/v sodium chloride: Prepared by dissolving 0.9g sodium chloride in distilled water and diluted to 100ml.

- ii). 0.01% w/v BHT: Prepared by dissolving 10mg BHT in hexane and diluted to 100ml.
- iii). 50 mg/dl retinol Stock solution: Prepared by dissolving 50mg of retinol in ethanol and diluted to 100ml.
- iv). 25 mg/dl retinyl acetate stock solution: Prepared by dissolving 25mg retinyl acetate in ethanol and diluted to 100ml.

3.7.3 Preparation of calibration curves for determination of retinol by HPLC

The calibration curve was prepared from four graded concentrations of retinol, which were prepared as follows: Ten millilitre of retinol stock solution was diluted to 100 ml with ethanol. Four different amounts of retinol working solution were diluted with 1 ml retinyl acetate to get four standard solutions of retinol as shown in Table 4.

Table 4: The preparation of standard solutions of retinol.

Retinol working solution	Retinyl acetate	Standard solution of retinol
0.0 ml	1.0 ml	1
0.5 ml	1.0 ml	2
1.0 ml	1.0 ml	3
2.0 ml	1.0 ml	4

The standard solutions of retinol were chromatographed and the peak of retinol and retinyl heights recorded. The calibration curve (Figure 3) was then prepared from

the ratios of the retinol peak height to the retinyl acetate peak height against the weight ratios of retinol and retinyl acetate.

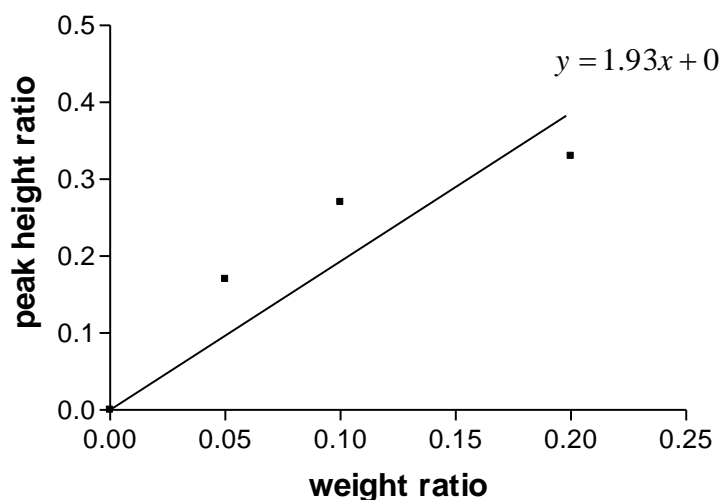


Figure 3: Calibration curve for determination of retinol by HPLC generated by use of linear regression.

3.7.4 Extraction of retinol from serum sample

0.5 ml serum was used to extract retinol. Samples were first mixed with 0.5ml of 0.9% sodium chloride, 1ml of 25 μ g/dl retinyl acetate and 2ml 0.01% butylhydroxytoluene. The mixture was then vortexed vigorously for 5 minutes and centrifuged at 3000rpm for 3 minutes. One ml of the supernatant was removed and put in the crimp vials ready for HPLC analysis.

3.7.5 HPLC analysis

The top 200 μ l of organic phase was removed and reconstituted into the isocratic mobile phase consisting of 95% acetonitrile, 5% methanol. The mobile phase was

maintained at pH 7.15 and a flow rate of 1.0 ml/min. HPLC analysis was performed by use of a C₁₈ reverse-phase column and a high performance guard column. A UV/visible detector (Waters 484) was set at 325nm, and peaks identification and quantification of retinol were made by comparing samples to a purified all-*trans* retinyl acetate internal standard.

3. 8 Determination of biochemical parameters

3.8.1 Measurement of total serum proteins

Total serum proteins was determined by the biuret method 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 20µl of each serum sample, 20µl standard solution of total proteins, 20µl distilled water and 1ml total proteins working reagent was pipetted into a test tube. The reaction mixture was incubated for 10 minutes at 37°C and the absorbances of standard and serum sample mixture read at 546nm using a spectrophotometer against distilled water.

3.8.2 Determination of albumin

Albumin was determined by the bromocresol green (BCG) method 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 microlitres of each sample were pipetted in a test tube followed by 10µl standard total protein solution, 10µl distilled water and 1ml of working

reagent. The serum sample mixture was incubated for 1 minute at 37°C and the absorbances of standard and serum sample mixture read at 630nm using a spectrophotometer against distilled water.

3.8.3 Determination of globulin

The amount of globulins in each sample was determined by subtracting the amount of serum albumin obtained from the amount of total serum proteins.

3.8.4 Determination of albumin/globulin ratio

The ratio of albumin to globulin in each sample was determined by dividing the amount of albumin obtained by the amount of globulin.

3.9 Determination of haematological parameters

3.9.1 Determination of packed cell volume

The packed cell volume was determined from each sample by the microhaematocrit method. 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-USA). The tubes were then centrifuged at 3000rpm for 5 minutes in a haematocrit centrifuge (Heraeus Christ GmbH Osterode). The volume of packed red blood cells was read using the haematocrit reader (Gelman Hawksley, England).

3.9.2 Determination of haemoglobin concentration

The concentration of haemoglobin in each sample was determined using the cyanmethemoglobin method. 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 minutes. Then the tube was centrifuged at 3000rpm for 5 minutes to remove nuclear material that could over-estimated 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 the distilled water.

3.10 Assessment of worm egg counts and burden

Assessment of egg counts were done from faecal sample collected from each chicken on week 0 and weeks 2, 4, 6, 8 and 10 post-infection. The sample was processed according to procedures described by Permin and Hansen (1998). Briefly, approximately five grams of faecal sample was collected from single chicken and sent to the Parasitology laboratory. Three grams of faecal sample was added to 42ml of saturated salt solution, mixed and filtered. A small volume of the filtrate was charged into McMaster slide and the eggs were counted under the light microscope.

The worm burden from each infected chicken was determined on the 10th week post-infection after the infected chickens were sacrificed. The chickens were sacrificed by neck disarticulation and the intestines were opened and washed into plastic trough to recover the worms. The recovered worms were collected in

specimen bottles in 70% ethyl alcohol and sent to the Parasitology laboratory for description. The description included the sex of the worm, number of worms for each sex, total worm counts and weight of the total worms recovered from each bird. The fecundity was calculated by dividing the egg counts by the number of female worms.

3.11 Data handling and statistical analysis

The raw data were entered into the Microsoft excel workbook according to the experimental groups and research variables. Descriptive statistics of the analytical variables, worm counts and egg counts were determined and tested for normality. One way analysis of variance (ANOVA) was used to test for the significant differences between the four groups. Unpaired Student's t-test was used to test for the significant differences on body weights, retinol, total protein, albumin, globulin, albumin/globulin ratio, PCV and haemoglobin between the infected versus non-infected and between supplemented and non-supplemented groups. Similarly, differences on worm counts, egg counts, worm weight, and worm fecundity recorded from individual chickens from supplemented and non-supplemented groups were tested using unpaired t-test. Chi-square test was used to compare proportions of total worms, sex and eggs recovered from supplemented and non-supplemented infected groups. On the other hand, a paired t-test was used to compare the differences between the initial and final values of the research variable of the individual birds within the group. All statistical analyses were determined using GraphPad prism (GraphPad Software Inc., USA) version 3.0.

CHAPTER FOUR

4.0 RESULTS

4.1 Serum retinol levels in experimental chickens

The results of serum retinol levels in the four groups of experimental chickens are presented in Table 5. A plot of the values against time in weeks after infection is shown in Figure 4.

Table 5: Serum retinol concentrations (mean±se) ($\mu\text{mol/l}$) at different weeks of the experiment.

	Week 0	Week 2	Week 3	Week 5	Week 10
Group I	1.158±0.126	1.007±0.107	0.829±0.075	0.783±0.057	0.731±0.071
Group II	1.048±0.097	1.281±0.056	1.34±0.069	1.389±0.076	1.395±0.072
Group III	1.126±0.177	1.085±0.117	1.267±0.093	1.238±0.111	1.304±0.094
Group IV	1.046±0.079	1.333±0.213	1.855±0.186	2.041±0.172	2.322±0.171

Group I: Infected and non-supplemented; Group II: Infected and supplemented
Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

4.1.1 Changes of serum retinol levels within the experimental groups

Table 5 shows that the levels of serum retinol in chickens that were non-infected and non-supplemented (group III) ranged between 1.0 and 1.3 $\mu\text{mol/l}$ and formed the base-line values of serum retinol of chickens in this study. These values were below the normal levels of 2.4-3.0 $\mu\text{mol/l}$ reported by West *et al.* (1992). However, Figure 4 show that the levels of serum retinol in chickens in group IV that were the same at the beginning of the experiment as the base-line levels increased significantly from 1.04 to 2.3 $\mu\text{mol/l}$ ($p < 0.0001$) clearly indicating a positive

response of supplementation on retinol levels in the absence of infection. On the other hand the values of retinol in the infected and non-supplemented chickens (group I) which were initially 1.1 $\mu\text{mol/l}$ decreased significantly to 0.73 $\mu\text{mol/l}$ ($p=0.0025$) by week 10 post-infection, indicating that absence of supplementation aggravated the decrease of serum retinol of infected chickens. The effect of supplementation in the infected chickens (group II) is seen to prevent a similar decrease in serum retinol concentrations as evidenced by an increased levels from 1.04 to 1.39 $\mu\text{mol/l}$ by week 10 post-infection and supplementation ($p=0.0032$).

4.1.2 Comparative serum retinol levels between the experimental groups

Figure 4 shows differences on levels of serum retinol measured at different weeks of infection. The four groups had statistically the same levels ($p=0.89$) of retinol on week 0 of the experiment. Differences on serum retinol between the groups started to be seen on week 3 post-infection, where infected non-supplemented group (I) had statistically lower retinol levels of 0.829 $\mu\text{mol/l}$ compared to 1.267 $\mu\text{mol/l}$ of the non-infected non-supplemented group (III) ($p=0.0018$). This difference continued to be significant throughout the experiment ($p<0.0001$). From week 3 to 10 post-infection, infected-supplemented group (II) had significant lower retinol levels of 1.395 $\mu\text{mol/l}$ compared to 2.32 $\mu\text{mol/l}$ of the non-infected supplemented group (IV) ($p<0.0001$). This indicates that *A. galli* lowered serum retinol both in supplemented and non supplemented chickens. On the other hand, the infected non-supplemented group (I) had statistically lower serum retinol levels of 0.73 $\mu\text{mol/l}$ compared to 1.395 $\mu\text{mol/l}$ of the infected supplemented group (II) ($p<0.0001$). Similarly, non-infected supplemented group (IV) had 2.32 $\mu\text{mol/l}$ of

serum retinol, which was statistically higher than 1.3 $\mu\text{mol/l}$ obtained from the corresponding non-infected non-supplemented group (III) ($p < 0.0001$). This indicates that vitamin A supplementation improved serum retinol both in infected and non infected chickens. Retinol levels of group II and III did not differ significantly ($p > 0.05$) throughout the experiment, although group II had slight higher retinol values than group III.

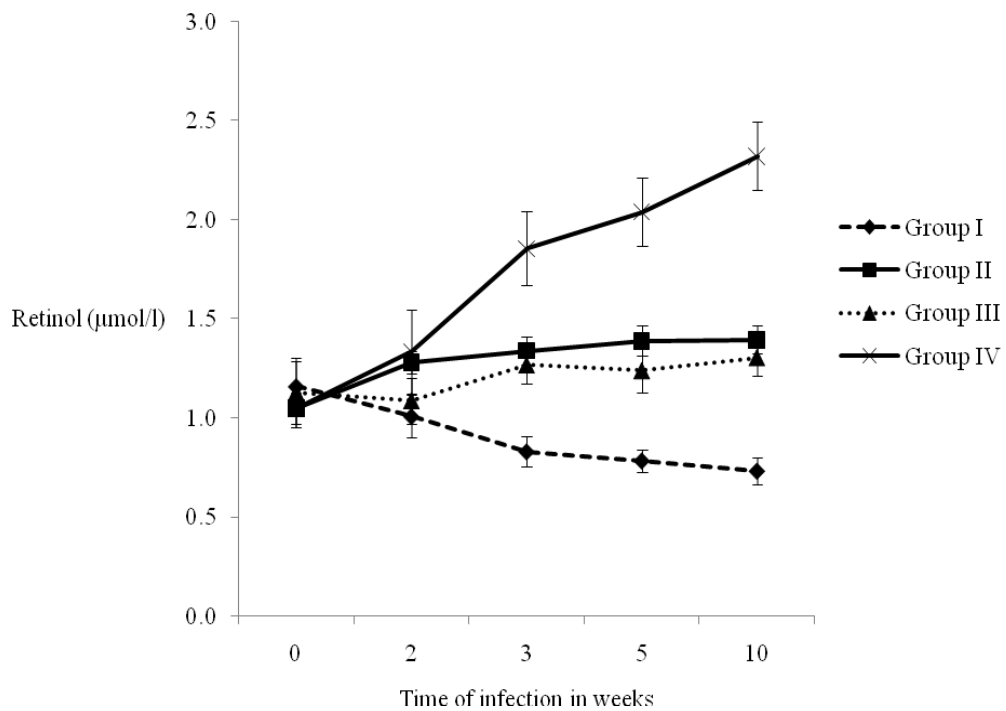


Figure 4: Serum retinol levels in the four groups of experimental chickens measured at different weeks of infection.

4.2 Total serum proteins in experimental chickens

The results of levels of total serum proteins in the four groups of experimental chickens are presented in Table 6. A plot of the values against time in weeks after infection is shown in Figure 5.

4.2.1 Changes of levels of total serum proteins within the experimental groups

Table 6 shows that 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. These values are within the normal levels of 2.5-4.5 g/dl reported by Thrall *et al.* (2006). Figure 5 shows that 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. On the other hand the initial levels of total serum proteins in the infected and non-supplemented chickens (group I), which were 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 5 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.

Table 6: Total serum protein concentrations (mean±se) (g/dl) at different weeks of the experiment.

	Week 0	Week 2	Week 3	Week 5	Week 10
Group I	3.423±0.282	3.251±0.200	3.035±0.226	3.237±0.129	3.205±0.154
Group II	3.34±0.1497	3.198±0.324	3.538±0.151	3.456±0.101	3.356±0.097
Group III	3.306±0.266	3.349±0.233	3.708±0.131	3.67±0.151	3.894±0.129
Group IV	3.224±0.237	3.572±0.406	4.519±0.256	4.263±0.239	4.191±0.198

Group I: Infected and non-supplemented; Group II: Infected and supplemented
Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

4.2.2 Comparative total serum proteins levels between the experimental groups

Figure 5 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 levels than the other three groups and group IV having higher levels than the other groups. There were no 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 confirm that vitamin A supplementation improved the levels of total serum proteins, both in the infected and non-infected chickens.

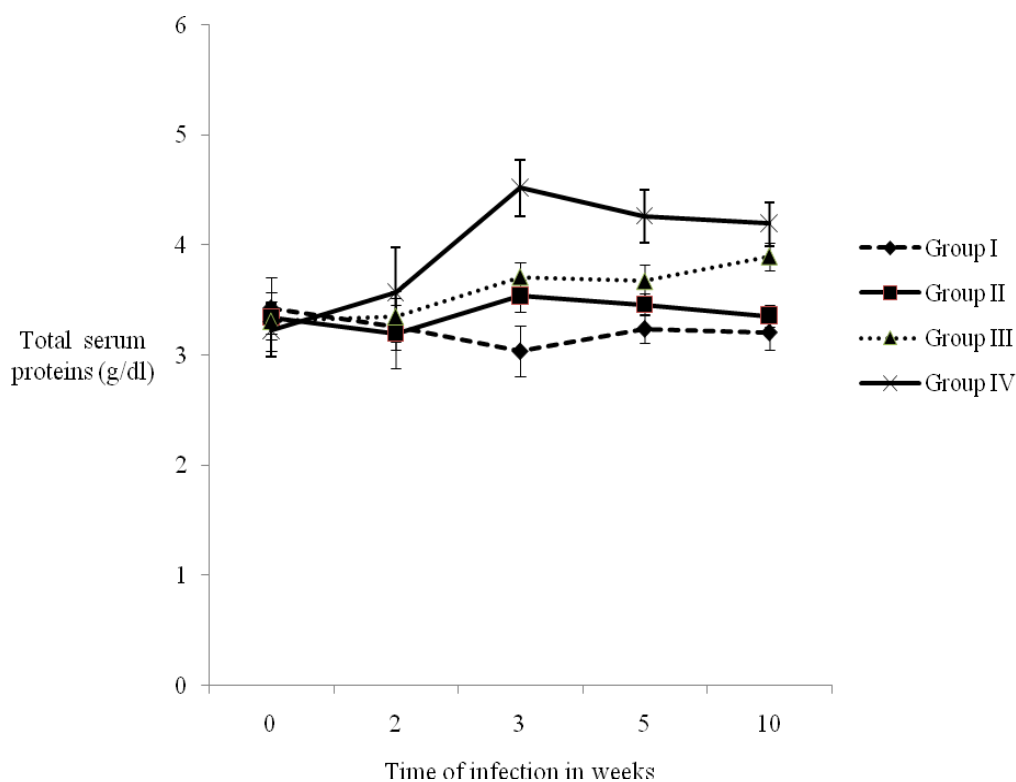


Figure 5: Total serum proteins levels in the four groups of experimental chickens measured at different weeks of infection.

4.3 Albumin levels in experimental chickens

The results of serum albumin levels in the four groups of experimental chickens are presented in Table 7. A plot of the values against time in weeks after infection is shown Figure 6.

4.3.1 Changes of serum albumin levels within the experimental groups

Table 7 shows that 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 lower margin

of these values was below the normal levels of 1.5-3 g/dl reported by Thrall *et al.* (2006). Figure 6 shows that the levels of albumin in chickens in group IV that were the same at the beginning of the experiment as the base-line values increased significantly ($p<0.05$) from 1.40 to 2.26 g/dl by week 5 of supplementation. 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 1.16 g/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.

Table 7: Serum albumin concentrations (mean \pm se) (g/dl) at different weeks of the experiment.

	Week 0	Week 2	Week 3	Week 5	Week 10
Group I	1.459 \pm 0.133	1.428 \pm 0.098	1.235 \pm 0.095	1.261 \pm 0.042	1.164 \pm 0.047
Group II	1.4 \pm 0.084	1.366 \pm 0.135	1.454 \pm 0.084	1.394 \pm 0.043	1.293 \pm 0.089
Group III	1.415 \pm 0.130	1.378 \pm 0.119	1.737 \pm 0.094	1.619 \pm 0.076	1.725 \pm 0.083
Group IV	1.403 \pm 0.102	1.627 \pm 0.167	2.112 \pm 0.141	2.261 \pm 0.187	2.227 \pm 0.147

Group I: Infected and non-supplemented; Group II: Infected and supplemented
Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

4.3.2 Comparative serum albumin levels between the experimental groups

The significant differences on the levels of serum albumin between the groups were seen from week 3 through the end of 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-infected 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) ($p = 0.0002$). This indicates that vitamin A supplementation improved serum albumin in both infected and non-infected chickens.

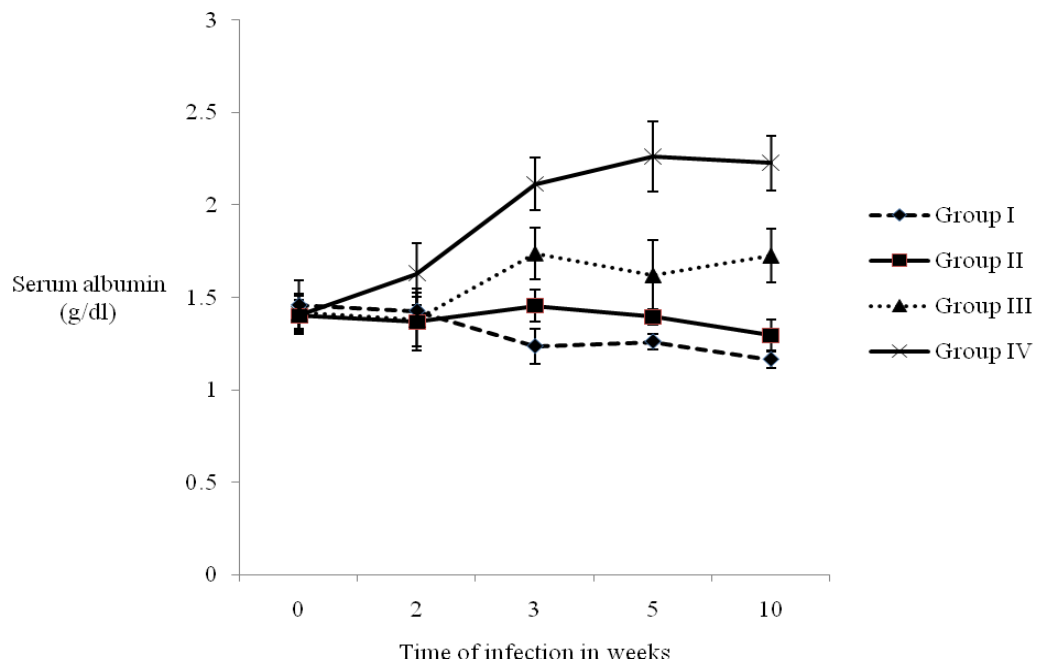


Figure 6: Serum albumin in the four groups of experimental chickens measured at different weeks of infection.

4.4 Globulin levels in experimental chickens

The results of serum globulin levels in the four groups of experimental chickens are presented in Table 8. A plot of the values against time in weeks after infection is shown in Figure 7.

4.4.1 Changes of serum globulin levels within the experimental groups

Table 8 shows that 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 0.5-1.8 g/dl reported by Thrall *et al.* (2006). Figure 7 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), which were 1.96 g/dl decreased non-significantly ($p > 0.05$) to within the normal range of 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.

Table 8: Serum globulin concentrations (Mean±se), (g/dl) at different weeks of the experiment.

	Week 0	Week 2	Week 3	Week 5	Week 10
Group I	1.964±0.172	1.823±0.121	1.8±0.144	1.976±0.117	2.041±0.135
Group II	1.94±0.141	1.832±0.199	2.084±0.096	2.062±0.094	2.063±0.069
Group III	1.891±0.161	1.968±0.158	1.970±0.073	2.051±0.105	2.169±0.123
Group IV	1.821±0.156	1.945±0.254	2.407±0.148	2.002±0.19	1.964±0.22

Group I: Infected and non-supplemented; Group II: Infected and supplemented
 Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

4.4.2 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 supplemented 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.

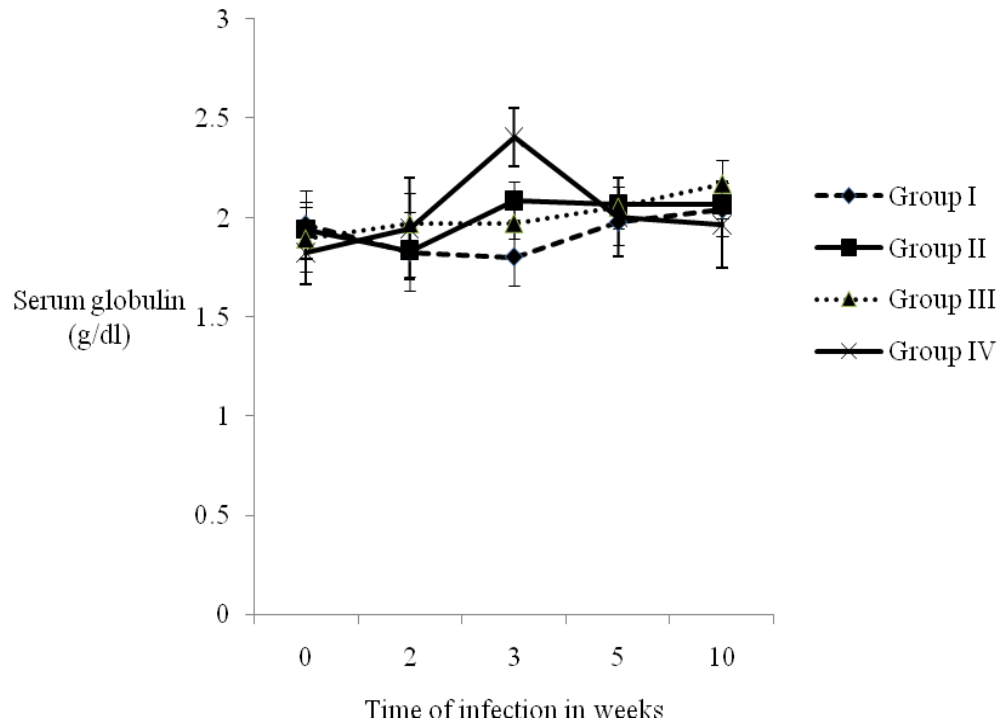


Figure 7: Serum globulin in the four groups of experimental chickens measured at different weeks of infection.

4.5 Albumin/globulin ratios in experimental chickens

Table 9 shows the results of serum albumin/globulin ratio in the four groups of experimental chickens.

4.5.1 Changes of serum albumin/globulin ratios within the experimental groups

Figure 8 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.

Table 9: Serum albumin/globulin ratio (Mean \pm se) at different weeks of the experiment.

	Week 0	Week 2	Week 3	Week 5	Week 10
Group I	0.743 \pm 0.049	0.796 \pm 0.043	0.695 \pm 0.035	0.659 \pm 0.044	0.592 \pm 0.043
Group II	0.754 \pm 0.061	0.765 \pm 0.037	0.703 \pm 0.037	0.688 \pm 0.04	0.636 \pm 0.052
Group III	0.762 \pm 0.048	0.712 \pm 0.063	0.889 \pm 0.054	0.799 \pm 0.041	0.826 \pm 0.074
Group IV	0.787 \pm 0.047	0.863 \pm 0.049	0.891 \pm 0.059	1.255 \pm 0.081	1.295 \pm 0.024

Group I: Infected and non-supplemented; Group II: Infected and supplemented
Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

4.5.2 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-infected 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-supplemented 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) ($p<0.05$). This indicates that vitamin A supplementation did influence the levels of albumin/globulin ratio in the non-infected chickens and not in the infected chickens.

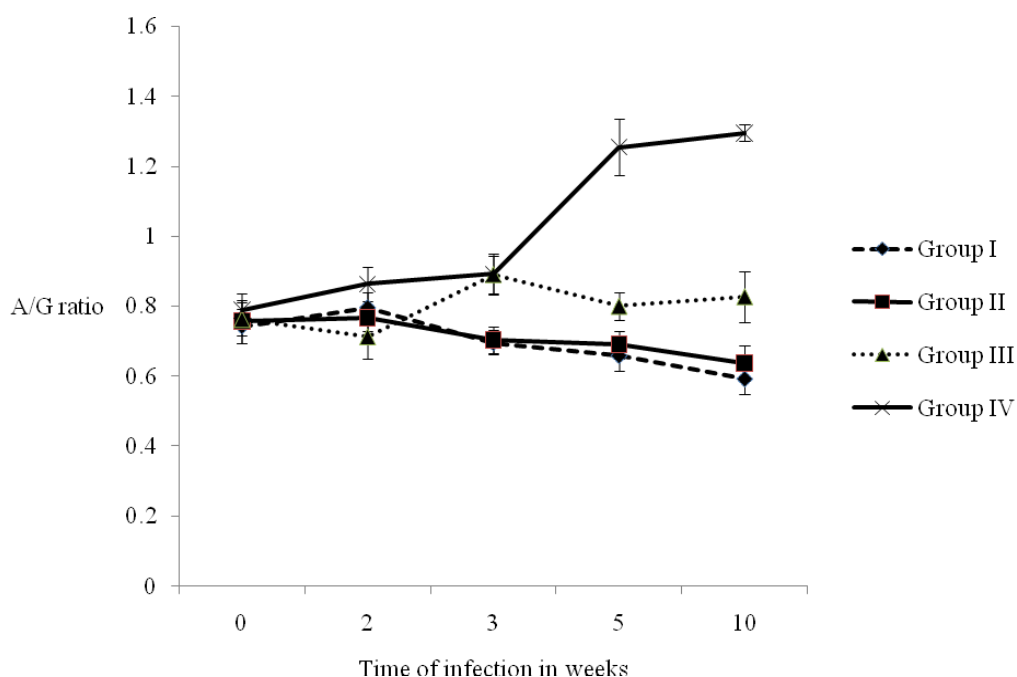


Figure 8: Serum albumin/globulin ratio in the four groups of experimental chickens measured at different weeks of infection.

4.6 Haematocrit levels in experimental chickens

The results of haematocrit in the four groups of experimental chickens are presented in Table 10. A plot of the values against time in weeks after infection is shown in Figure 9.

4.6.1 Changes of haematocrit levels within the experimental groups

Table 10 shows that 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. 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 (Figure 9). 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.

Table 10: Packed cell volumes (Mean \pm se) (%) at different weeks of the experiment.

	Week 0	Week 2	Week 3	Week 5	Week 10
Group I	31.05 \pm 1.01	32.1 \pm 1.005	31.6 \pm 1.072	27.2 \pm 0.638	25.65 \pm 0.597
Group II	33.35 \pm 1.057	35 \pm 0.711	33.65 \pm 0.972	33.1 \pm 0.802	32.95 \pm 0.560
Group III	32.1 \pm 0.649	34.1 \pm 0.640	32.95 \pm 0.932	34 \pm 0.596	35.65 \pm 0.863
Group IV	30.95 \pm 0.801	31.4 \pm 1.077	36.15 \pm 1.455	35.8 \pm 1.119	36.99 \pm 1.048

Group I: Infected and non-supplemented; Group II: Infected and supplemented
Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

4.6.2 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-supplemented 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. This indicates that vitamin A supplementation did influence the levels of haematocrit values both in infected and non-infected chickens.

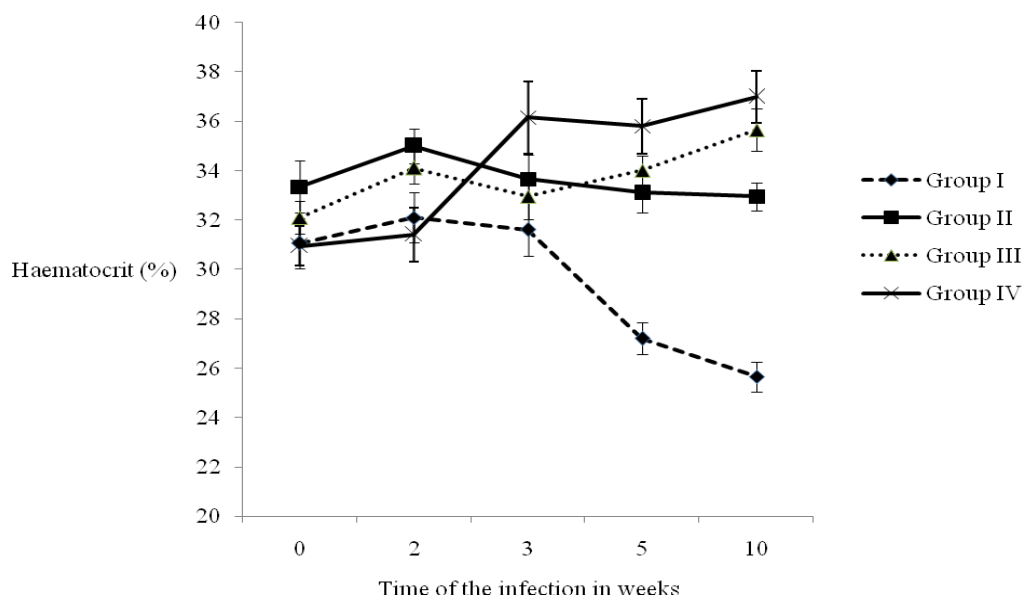


Figure 9: Haematocrit levels in the four groups of experimental chickens measured at different weeks of infection.

4.7 Haemoglobin levels in experimental chickens

The results of haemoglobin concentrations in the four groups of experimental chickens are presented in Table 11. A plot of the values against time in weeks after infection is shown in Figure 10.

4.7.1 Changes of haemoglobin levels within the experimental groups

Table 11 shows that 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 10 shows that the base-line values increased significantly from 10.8 to 12.7 g/dl by 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.

Table 11: Haemoglobin concentrations (Mean \pm se) (g/dl) at different weeks of the experiment.

	Week 0	Week 2	Week 3	Week 5	Week 10
Group I	10.7 \pm 0.398	10.22 \pm 0.348	9.94 \pm 0.297	9.11 \pm 0.285	8.58 \pm 0.263
Group II	10.31 \pm 0.262	10.96 \pm 0.212	10.84 \pm 0.269	10.74 \pm 0.261	10.86 \pm 0.158
Group III	10.77 \pm 0.278	11.39 \pm 0.245	11.16 \pm 0.295	11.44 \pm 0.197	11.6 \pm 0.249
Group IV	10.89 \pm 0.262	11.23 \pm 0.462	12.29 \pm 0.398	12.48 \pm 0.343	12.76 \pm 0.263

Group I: Infected and non-supplemented; Group II: Infected and supplemented
 Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

4.7.2 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 (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-supplemented 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. This indicates that vitamin A supplementation did influence the levels of haemoglobin values both in infected and non-infected chickens.

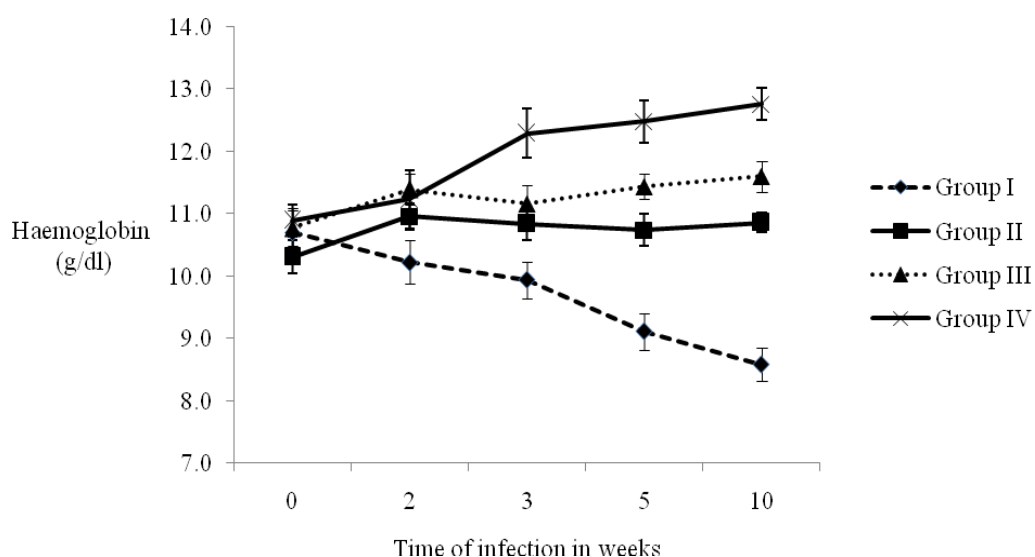


Figure 10: Haemoglobin levels in the four groups of experimental chickens measured at different weeks of infection.

4.8 Live body weights in experimental chickens

The results on live body weights in the four groups of experimental chickens are presented in Table 12. A plot of the values against time in weeks after infection is shown in Figure 11.

4.8.1 Changes of body weights within the experimental groups

Table 12 shows that the live mean body weight in chickens that were non-infected and non-supplemented (group III) ranged between 655.8 and 1376.7g and formed the base-line values of body weights of chickens in this study. Figure 11 shows that the mean base-line weight of supplemented chickens in group IV increased significantly from 668.2 to 1465.7g by week 10 of the experiment ($p < 0.05$). Similarly the mean base-line values of body weight in the infected and non-supplemented chickens (group I) also increased significantly from 665.5 to 1156.3g by week 10 post-infection ($p < 0.05$) but the increase was less by 309.4g as compared to the supplemented chickens in group II in which the live body weights increased significantly ($p < 0.05$) from 638.3 to 1334.8g.

4.8.2 Comparative body weight values between the experimental groups

Significant differences in mean body weights between the groups were seen from week 5 ($p = 0.0357$) when group I (infected and non supplemented) differed significantly ($p < 0.0034$) from group IV (non infected and non supplemented). Similarly, on week 10 post-infection the mean body weight of the infected non-supplemented group (I) was 1156.3g being statistically lower than 1376.7g in the non-infected non-supplemented group (III) ($p < 0.0001$). Similarly, the infected-

supplemented group (II) had a mean weight of 1334.8g, which was significantly lower than 1465.7g from the non-infected supplemented group (IV) ($p=0.0001$). This indicates that *A. galli* infection had significant effect on body weights in both the supplemented and non-supplemented chickens. On the other hand, the mean body weight of 1156.3g from the infected non-supplemented group (I) was significantly lower than 1334.8g of the infected supplemented group (II) ($p<0.0001$). Likewise, a mean weight of 1465.7g of the non-infected supplemented group (IV) was statistically higher than the corresponding 1376.7g from the non-infected non-supplemented group (III) ($p=0.012$). This indicates that vitamin A supplementation had the influence on body weights, both in infected and non-infected chickens.

Table 12: Mean live body weights, (Mean±se) (g) at different weeks of the experiment.

Group	Week 0	Week 2	Week 3	Week 5	Week 9	Week 10
I	665.5±16.1	872.1±17.48	949.7±17.73	1035.7±18.46	1136.5±22.2	1156.3±22.14
II	638.3±14.48	839.3±17.37	934.3±19.26	1074.7±18.5	1289.8±16.95	1334.8±18.72
III	655.8±27.45	871.8±29.04	957.3±27.73	1100±29.77	1316.9±26.25	1376.7±25.06
IV	668.2±21.32	886.8±15.64	982.4±16.66	1130±21	1403.1±18.49	1465.7±19.42

Group I: Infected and non-supplemented; Group II: Infected and supplemented
 Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

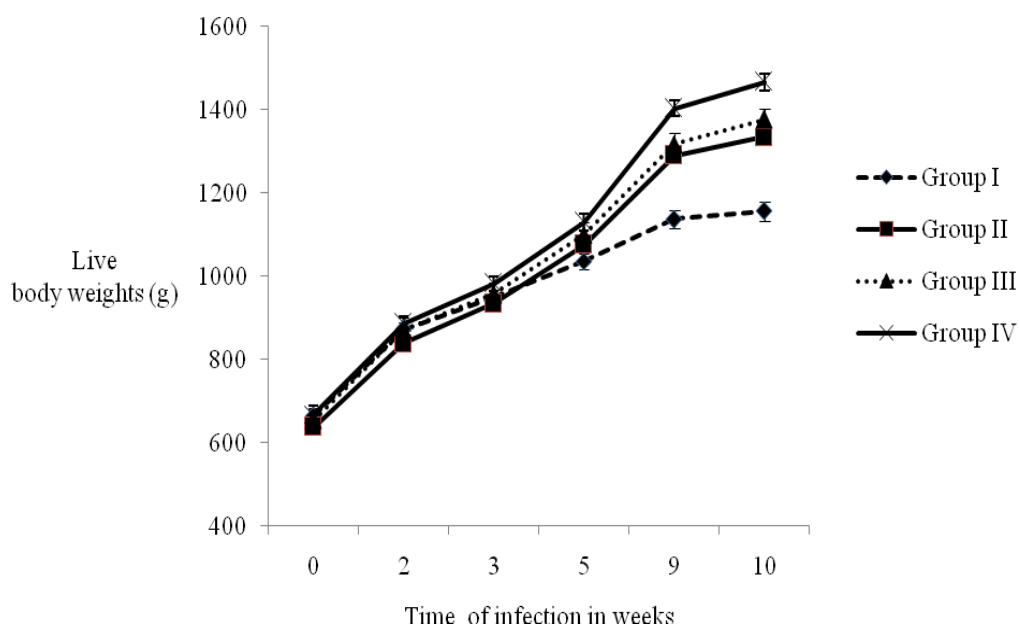


Figure 11: Live body weights in the four groups of experimental chickens measured at different weeks of infection.

4.9 Recovery of *A. galli* eggs from the infected chickens

Table 13 shows the number of eggs per gram of faeces (EPG) recovered from the infected chickens at different weeks after infection. A plot of the EPG against time in weeks is shown in Figure 12.

Table 13: Number of eggs per gram of faeces (Mean±se) recovered at different time in weeks from the infected chickens.

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10
Group I	0	0	0	25±15.37	670±88.25	890±82.26
Group II	0	0	0	15±10.67	285±49.47	480±57.83
Group III	0	0	0	0	0	0
Group IV	0	0	0	0	0	0

Group I: Infected and non-supplemented; Group II: Infected and supplemented
 Group III: Non-infected and non-supplemented; Group IV: Non-infected and supplemented.

Figure 12 shows that eggs started being recovered from the infected chickens by week 6 of the infection with chickens in group I, which were infected and not supplemented showing a significantly higher ($p<0.05$) mean egg recovery than chickens in group II, which were supplemented. The EPG in chickens in group I continued to increase and by week 10 of the experiment it reached a mean of 890 ± 82.26 . The increase in the EPG in chickens in group II on the other hand was only about half the increase in group I at 480 ± 57.83 by week 10 of the experiment, the difference which was statistically significant ($p=0.0007$). There were no eggs

recovered from groups III and IV, hence excluding any possibility of water and feed being contaminated.

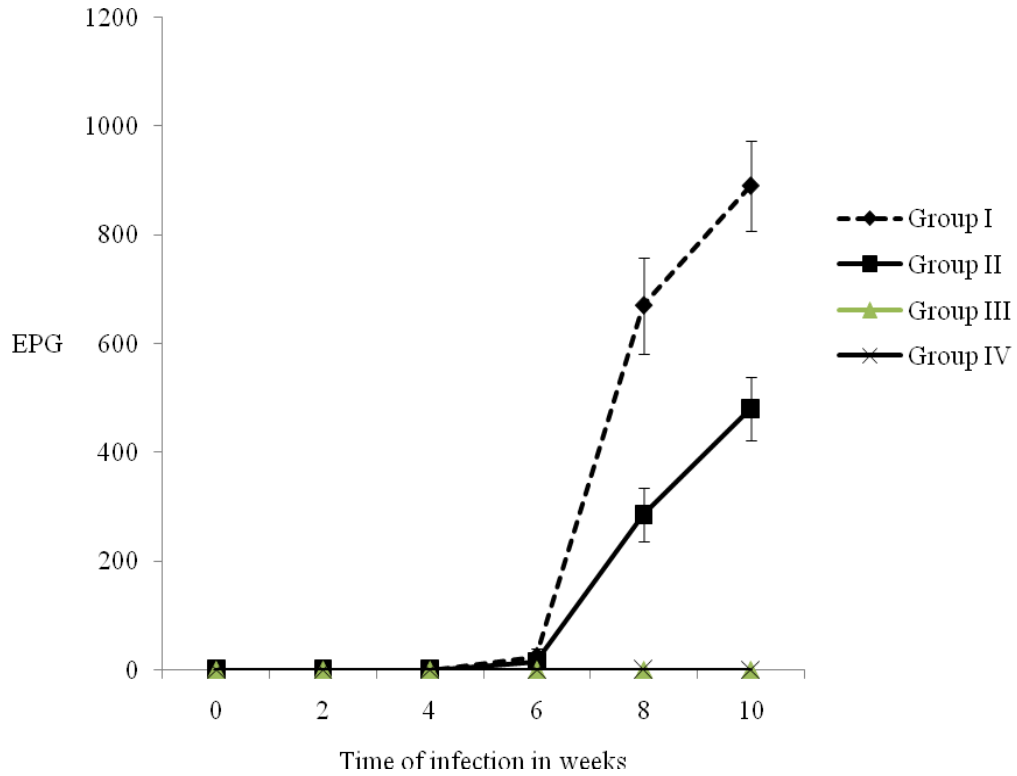


Figure 12: EPG in the two groups of *A. galli* infected experimental chickens measured at different weeks of infection.

4.10 Number of worms recovered from infected chickens at the end of the experiment

Table 14 shows that the mean total number of worms recovered from chickens in group I, which were infected and not supplemented was 21. The number of worms recovered at the same time in chickens in group II, which were infected and supplemented was 18.5, a decrease of 11.9%, which was not significant ($p=0.43$) (Figure 13). Likewise, the mean establishment rate of 5.9% in chickens in group I

was not significantly different ($p=0.44$) from 5.2% observed in group II. Analysis of the recovered worms in each group (Figure 13) shows that of the 21 ± 2.206 mean worms in group I, 9.6 were males while 11.4 were females. A paired t-test showed that group I were significantly higher than males ($p=0.047$). On the other hand, of the 18.5 worms in supplemented chickens in group II, 10.6 were males while 7.9 were females. This difference was statistically significant ($p=0.016$). Chi-square analysis of the proportions of total worms recovered from each sex shows strong association between sex of the recovered worms and vitamin A supplementation (OR= 1.6, $\chi^2= 1.56$, $p=0.02$), with the recovery of male worms positively associated with vitamin A supplementation.

Table 14: The number of *A. galli* worms (Mean \pm se) recovered and the related variables from the infected chickens.

Background variable	Infected chickens	
	Group I	Group II
Total worms counts	21 \pm 2.206	18.5 \pm 2.112
Establishment, %	5.99 \pm 0.635	5.29 \pm 0.701
Male worms counts	9.6 \pm 1.267 ^a	10.6 \pm 1.284 ^a
Female worms counts	11.4 \pm 1.067 ^b	7.9 \pm 1.1 ^{bb}
Male/Female ratio	0.834 \pm 0.071 ¹	1.458 \pm 0.144 ²
Worms weight, g	0.902 \pm 0.055	0.7842 \pm 0.067
Fecundity	78.7 \pm 3.38 ¹	64.95 \pm 5.27 ²

Group I: Infected, non-supplemented; Group II: Infected. Supplemented
The mean values with different **letter** superscripts in the same column or different **number** superscripts in the same row differ significantly ($p<0.05$).

In the same Table, the ratio of male to female worms increased significantly ($p=0.0011$) from 0.8 in the infected non-supplemented group (group I) to 1.4 following supplementation, the results that indicate male dominance with supplementation.

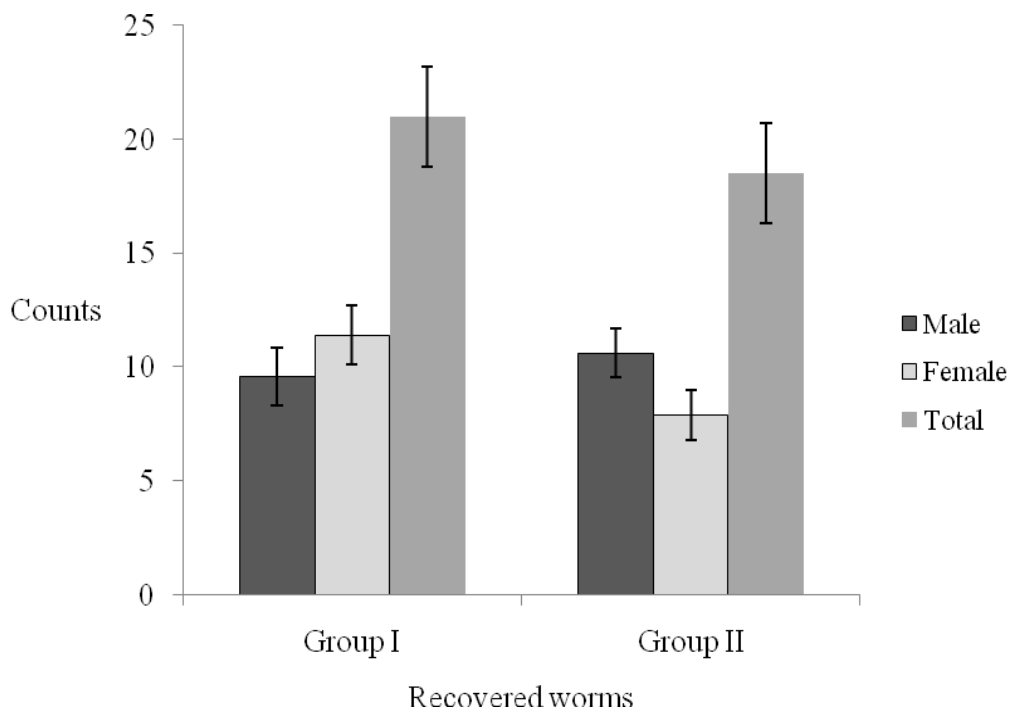


Figure 13: Mean values of worm counts in the non-supplemented (group I) and supplemented (group II) infected chickens.

Table 14 also shows that the total weights of the worms in the infected non-supplemented chickens in group I was 0.9g while the weight in the infected and supplemented chickens was 0.78g. The difference between the two values were not statistically significant ($p=0.19$). Fecundity of worms in the infected and non-supplemented chickens in group I is shown in Table 14 as 78.7. Figure 14 shows that this value was significantly ($p=0.04$) higher than the fecundity (64.9) in the

supplemented group, indicating the role of vitamin A in suppression of egg excretion by worms.

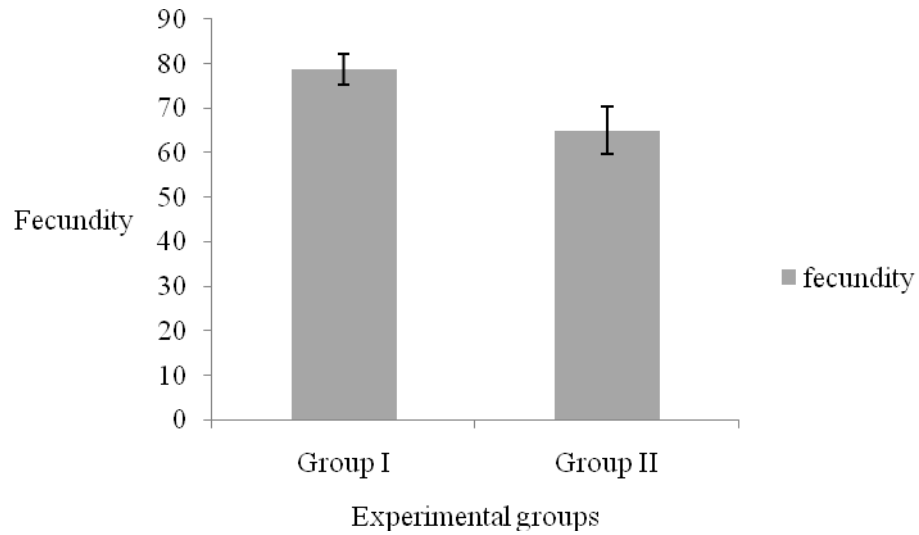


Figure 14: Fecundity in the non-supplemented (group I) and supplemented (group II) infected chickens.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Levels of serum retinol

The results of the present study indicate that *A. galli* infestation caused a significant depression of the concentrations of serum retinol in both non-supplemented and supplemented chickens. These findings are in agreement with the findings made by Anwar and Rahman (2002) in which *A. galli* infestations in chickens were found to decrease both the serum and liver concentrations of vitamin A. Similar observations have also been reported by Mwaniki *et al.* (2002) in human beings infested by *Schistosoma mansoni*. The mechanisms through which *A. galli* infestations reduce retinol concentration are thought to involve interferences in the absorption of precursors of vitamin A or the pre-formed vitamin A during the histotrophic stages of the *A. galli* life cycle in which there is extensive mucosal damage due to migration of the larvae (Crompton and Nesheim, 2002).

The mucosa damage leads to two important events. Firstly there is reduction in the absorptive surface of the intestine (Permin and Hansen, 1998; Kidala *et al.*, 2000; Anwar and Rahma, 2002). Secondly, damage of the mucosa leads to release of APPs, which impair synthesis of retinol binding protein responsible for the transport of retinol in the blood circulation (Fujita *et al.*, 2009, Koski and Scott, 2003). Reduction in the absorption could also be through feeding on and bioconversions of vitamin A in the intestine. It has also been established that absorption of lipids is highly impaired during helminth infestations (Crompton and Neisheim, 2002). Since lipids are a dietary vehicle for vitamin A interferences in

its absorption will affect the availability of Vitamin A (Jalal *et al.*, 1998; Roodenburg *et al.*, 2000).

According to Watts (1991), vitamin A deficiency is also associated with deficiency of protein in the diet, and serum levels of vitamin A can be restored when protein is added to the diet. Deficiency of protein in the diet affects level of retinol binding proteins hence the level of vitamin A (Sommer, 1995). This is supported by the findings of the present study as the infected groups had also low levels of protein.

5.2 Levels of albumin, globulin and albumin/globulin ratio

The findings of the present study that *A. galli* infestations lowered serum albumin, globulin and albumin/globulin ratio are in agreement with observations of a 93% decrease of serum albumin in chickens infected by *A. galli* reported by Ali *et al.* (2011). Furthermore, Kuklina and Kuklin (2006) observed low albumin in Gulls as early as day 4 post-infection. Deka and Borah (2008) did not observe significant differences on the levels of globulin ratio in chickens and Japanese quails infected with *A. galli*. These findings support the results of the present study, in which the effect of *A. galli* on globulin was seen when chickens were supplemented.

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 mobilising 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 thus ensuring the absorption of protein (Tanwar and Mishra, 2001). Vitamin A supplementation is therefore, considered to minimize the pathologic effects of *A. galli* in chickens.

5.3 Levels of haematocrit and haemoglobin

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). Both haematocrit and haemoglobin indirectly measure the status of erythrocytes the integrity of which depends on iron. 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 (Thurnham *et al.*, 2000). Vitamin A increases utilization of iron in the liver, spleen (Roodenburg, 2000) and enhances the uptake of iron in the bone marrow (Sijtsma *et al.*, 1989). It is known that during vitamin A deficiency much of the iron is retained in the liver and spleen and this reduces the incorporation of iron into erythrocyte by 40 to 50% (Semba and Bloem, 2002). The association between haemoglobin and serum retinol has well been described in several studies in human (Molla *et al.*, 1993, cited by Semba and Bloem, 2002; Fujita *et al.*, 2011).

5.4 Live body weights

The results of the present study indicate that chickens that were experimentally infected with *A. galli* had lower body weights than birds which were not infected and that supplementation with vitamin A in the infected chickens improved the live body weights. These observations are similar to those reported by Idi *et al.* (2007) and Phiri *et al.* (2007) in chickens infected with *A. galli* and by Adang *et al.* (2010a) in pigeons infected with *A. galli*. It is thought that *A. galli* affects body weights through interference with feed intake, digestion and absorption of nutrients following damage of the intestinal mucosa. *A. galli* also consumes some of the nutrients in the intestine thus reducing the amount of nutrients required by the host (Crompton and Nesheim, 2002). Improvement of the body weights in birds supplemented with vitamin A is directly related to the influence of vitamin A on

the gut integrity (Tanwar and Mishra, 2001). Vitamin A improves gut integrity and thus improves the digestion and absorption of multi-nutrients necessary for growth and maintenance (Tanwar and Mishra, 2001). Vitamin A also improves the immunity and general health, hence influencing the growth (Stephensen, 2001).

5.5 Recovery of eggs and worms

Recovery of *A. galli* eggs and worms were done in both the supplemented and non-supplemented birds which were infected. A significant reduction in the EPG was recorded in the supplemented chickens. The reduction favoured the development of male worms. These findings are in agreement with the work by Idi *et al.* (2007). The low EPG in the supplemented group in the present study is related to both the low number of female worms recovered in the supplemented birds and the low fecundity. Although statistically not significant, the low worm burden in the supplemented group can partly be related to the immune status of the supplemented birds (Villamor and Fawz, 2005). There are lines of evidence that vitamin A improves immunity, thus when birds are supplemented they develop strong immunity that interferes with establishment of the worms and also lowers fecundity of the fertile female *Ascaridia* (Idi *et al.*, 2007). In addition, vitamin A improves the integrity of the intestinal mucosa and hence interfering with both the migration phases of the *A. galli* larvae and the attachments of the adult worms to the mucosa (Crompton and Nesheim, 2002). These perhaps interfere with the overall establishment of the worms.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

The results of the present study provide three conclusive observations. Firstly, *A. galli* infections in chickens induce low levels of serum retinol, albumin and globulin as well as haematocrit, haemoglobin concentration, and body weights. Secondly, the effects of *A. galli* infection on the above-mentioned parameters can be reversed by supplementing the chickens with vitamin A and lastly Vitamin A moderates *A. galli* infection in chickens particularly by reducing egg counts and fecundity of fertile female worms. Vitamin A has, therefore, beneficial effect on moderating the effect of *A. galli* infestation. The evidence on the effects of *A. galli* on the above-described parameters necessitates strong measures to control helminths infestations in chickens. Similarly, considering the benefits of vitamin A described above supplementation of commercial feeds with vitamin A is recommended to be a routine practice for farmers raising chickens for various purposes. The above-mentioned recommendations are particularly important for free range local chickens, which are usually highly infested by worms and depend on natural sources of vitamin A, which vary significantly with seasons. While the present study reports association of vitamin A deficiency with *Ascaridia galli* infestation under experimental condition, it could be worth to have similar study conducted in the field in order to provide an insight of what happens in a typical field condition, where helminthosis is a problem.

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