EFFECT OF VARYING LEVELS OF BLACK SEED (*Nigella sativa)* POWDER SUPPLEMENTATION ON PERFORMANCE, THERMOREGULATORY PERFORMANCE, BLOOD PROFILE AND HISTOPATHOLOGY OF BROILER CHICKENSDURING HOT SEASON

BY

KAILANI IBRAHIM AL-HABIB
M.Sc./AGRIC/33556/2012-2013

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN ANIMAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE,
FACULTY OF AGRICULTURE
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA

NOVEMBER, 2016

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DECLARATION

I hereby declare that this thesis entitled: '**EFFECT OF VARYING LEVELS OF BLACK SEED (*Nigella sativa)*POWDER SUPPLEMENTATION ON PERFORMANCE, THERMOREGULATORY PERFORMANCE, BLOOD PROFILE AND HISTOPATHOLOGY OF BROILER CHICKENSDURING HOT SEASON'** has been performed by me in the Department of Animal Science under the supervision of Prof. P.P. Barje and Prof. G.T. Iyeghe-Erakpotobor. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at any University.

Kailani Ibrahim Al-habib	________________	________________
Name of student	Signature	Date

CERTIFICATION

This thesis titled '**EFFECT OF VARYING LEVELS OF BLACK SEED (*Nigella sativa)*POWDER SUPPLEMENTATION ON PERFORMANCE, THERMOREGULATORY PERFORMANCE, BLOOD PROFILE AND HISTOPATHOLOGY OF BROILER CHICKENSDURING HOT SEASON"** By Kailani Ibrahim Al-habib meets the regulations governing the award of the degree of Master in Animal Science of Ahmadu Bello University, Zaria and is approved for its' contribution to Scientific knowledge and literary presentation.

Prof. P.P BarjeChairman,Supervisory Committee ______________ Signature ______________ Date

Prof. G.T. Iyeghe-Erakpotobor
Member, Supervisory Committee ______________ Signature ______________ Date

Prof. S. Duru
Head of Department ______________ Signature ______________ Date

Prof. K.Bala
Dean, Postgraduate School ______________ Signature ______________ Date

DEDICATION

This dissertation is dedicated to my dear Mother (Late HajiyaZainab Hassan) whose training, love and care will ever be the reason of my success until I take the last breathe, (Allahummagfirlaha, warahamha, wa’afuanhaa, waakrimnuzulaha, wawasi’imudkhalahawagsilhabilmaai was saljiwal bard yaakramalakrameen) Ameen.

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ABSTRACT

Two experiments were carried out to investigate the effect of varying levels of black seed (*Nigella sativa)* powder supplementation on performance, thermoregulatory performance, blood profile and histopathology of broiler chickens during hot season. The first experiment had a total of 180 one week old broiler chicks which were randomly assigned to four treatments. The birds in each group were further divided into three (3) replicates with 15 chickens in a completely randomized design (CRD). Birds in all the treatment groups were fed same diet for the starter phase and then supplemented with *Nigella sativa* powder at 0, 5, 10 and 15 g/kg of diet to the end of 4weeks, on the last day the growth performance, thermoregulatory parameters and the haematology were investigated. Also at the finisher phase the same diet was given to all the treatments with the same design and supplementations were varied at 0, 10, 20, and 30g/kg for treatment 1 to 4 respectively. Results of the first experiment showed that supplementation of *Nigella sativa* in hot season significantly ($P<0.05$) increased the final body weight, weight gain and daily weight gain with decreased mortality as in the supplemented groups compared to the control. Also both the thermoregulatory parameters and the Haematological parameters were better in the supplemented groups compare to the control. In the second experiment the results of the performance showed better final weight and relative weight of the liver, gizzard, proventriclus, spleen, kidney and the thymus compared to the control. Thermoregulatory parameters measured at the end of 8weeks showed significant ($P<0.05$) decrease in both the body temperature and the respiratory rate on the supplemented groups compared to the control. Haematological parameters also indicated significant ($p<0.05$) for PCV, Hb, WBC and RBC of while Total protein, albumin and globulin showed no significant difference at ($p>0.05$). The histopathological findings clearly depicts no toxic effect as all tissues were not distorted. In conclusion, black seed can be supplemented to broiler chickens during hot season to improve performance, thermoregulation, and blood profile without any detrimental effect to broiler chickens as shown by the Histopathology.

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CHAPTER ONE

INTRODUCTION

The poultry industry has for some time occupied a leading role among Agricultural industries in many parts of the world. Poultry meat production has shown much higher growth than any other type of meat during the past decade. The potential for growth is obvious in view of the value of this kind of meat in modern day human diets. Chicken meat production has been on the increase in all continents with the highest increases in Asia and South America. The rate of increase in chicken meat has averaged 5.7% per year since 1990, (Daghir, 2008). The hot regions of the world (e.g Asia and Africa) have probably the greatest potential for further growth in poultry production since the level of consumption is still very low. Asia now leads the world in poultry meat production, followed by North and Central America which had the lead until 1990. In 2005 Asia and South America contributed 50% to global poultry meat production (Daghir 2008). The rapid expansion of the industry in these regions is very evident in countries like Brazil in South America, Morocco and Nigeria in Africa, and Saudi Arabia in the Middle East. Shane (2006) presented data indicating that there will be an increase of 12.5% in consumption of poultry meat during the present decade. According to (Daghir, 2008) , the highest increase will be in Asia, Africa and South America, the main warm regions of the world.

Poultry production in the tropical and subtropical regions, is characterised by heat stress which is in increase deu to the increase in global warming . Heat stress inflicts heavy economic losses on poultry production as a result of stunted growth (Shahin*et al.*,2001), increased cost of production, high rate of mortality due to depressed immunity, and reproductive failure (Ayo*et al.*, 2010). In Nigeria, the combination of high ambient temperature and high relative humidity, reaching climax at the onset of the rainy season, constitutes extreme heat stress, which depresses the production of broiler and layer birds. (Ayo *et al.*, 2011).

It is also reported by Mills *et al.*,(1999), that high environmental temperatures have deleterious effect on poultry, reducing rate of growth, feed intake, live weight gain, feed efficiency, digestibility of nutrients, egg production, as well as egg weight, mortality and depression of immunity (Naseem*et al.*, 2005). Also the physiological functions of broiler chickens are also affected by heat stress as mentioned by ElHusseiny and Creger (1981) who reported that a high environmental temperature (32^{o}C) decreased concentration of some minerals resulting in abnormal physiological process.

It has however been found recently that natural feed additives like herbs and edible plants have some properties as growth enhancers, instead of using synthetic drugs which may have adverse effects on human health, (Fluck*et al.*,1976). This apparently lead to the increase in the demand for using natural feed additives to overcome the adverse impact of heat stress on broiler performance in northern Nigeria.

Advancesin chemistryand identificationof plant compounds whichareeffective inthetreatmentofcertain diseases have renewedthe interest inherbal medicines. The World Health Organization(WHO) estimated that about 80% oftheworld populationrelies ontraditional medicinesfortheir primary health care. The black seed *(Nigella sativa*L.),belongingtothefamily *Ranunculaceae*, is an arumalspicy herb native to the Mediterranean regionsandisnowcultivatedinotherpartsoftheworld includingMiddle East, NorthAfrica andAsia(Durani*et al.*,2007).TheBlack seedisacropofgreatmedicalimportance,particularlyin Unani(Greek) and Ayurveda system of medicines (Durani*et al.*,2007). Recently theseeds arealso used inpharmaceutical industries.**It**isusedforedibleandmedicinalpurposesin Pakistan, Saudi Arabia, Iran, Sudan and Egyptand manyother countries. Manymedicinal propertieshave been attributedtoits seed and oilincludingAntineoplastic, Antibacterial,Antifungal,Anthelminthicandtreatmentof respiratory organs (Durani*et al.*,*al* 2007).

Nigella sativa as a natural feed additive (medicinal plant) have some properties as growth enhancers to improve birds productive performance under normal or heat stress conditions, the composition and properties of Black seed have been found to be a good source of protein, crude fat, crude fiber and major minerals (Radwan,2003 as cited in Hermes *et al.*,2011).

A lot of trials have been made to investigate the possibility of introducing *Nigella sativa* as a natural feed additive to broiler diets for better performance and there is general agreement that Black seed has no adverse effect on performance of broilers (Herms et al.,2011). Also supplementing broiler diet with *Nigella sativa* seed, meal or oil improves growth performance, biochemical, hematological responses and mortality rate (Nofal*et al.,* 2006). Whereas feeding different levels and forms of Black seed has been reported to show antimicrobial property (Nasir and Grashorn, 2006) and has a stimulatory effect on the immune system (Tolba*et al.*, 2003).

1.1 Justification

The use of Black seed (*Nigella sativa*) is in the increase in humans due to its natural endowmentinterms of nutritive and medicinal potentials and the fact that synthetic drugs and growth enhancers have great residual effect to consumers (Naseem*et al*., 2005). This makes it necessary to explore edible herbs and plants that will enhance proper production of animals specifically broiler chickens that suffers lots of losses in both performance and survival as a result of adverse and sudden fluctuation of weather condition especially during hot season.

1.2 Statement of Hypothesis

Null hypothesis (Ho): there is no significant effect in the supplementation of Black seed (*Nigella sativa*) powder on the performance, thermoregulatory parameters, blood profile and Histopathology of broiler chickens during hot season.

Alternative hypothesis (Ha): there is significant effect in the supplementation of Black seed (*Nigella sativa*) powder on onthe performance, thermoregulatory parameters, blood profile and Histopathology of broiler chickens during hot season.

1.3 Objectives

The objectives of this study are:

1. To determine the nutrient and chemical compositon of black seed (*Nigella sativa*) as a natural additive in ameliorating the effect of heat stress on broiler chickensduring thehot season in the savannah region.

2. To determine the effect of varying levels of black seed(*Nigella sativa*) onthe performance, thermoregulatory parameters, blood profile and Histopathology of broiler chickens at both starter and finisher phases during hot season.

3. To determine the effect of varying levels of black seed(*Nigella sativa*) on haematology of broiler chickens at both starter and finisher phases

4. To evaluate the histopathologic effect of black seed on the liver, kidney and small intestine of broiler chickens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Broiler

Broilers (*Gallus domesticus*) are meat- type chickens that have been specifically bred and can be marketed at an early age. They are usually sold when weight is about 1.9kg in North America but in many south East Asian countries broilers are marketed at smaller size (< 1.4Kg) and at younger age (Payne, 1990).

Also according to (Oluyemi and Roberts, 1979), broilers are specifically bred for fast growth and slaughter when they weigh about two kilograms, usually between seven and nine weeks of age.

2.2 Nutrients Requirement of Broiler

The table below shows the nutrient required by a broiler chicken from day old to table weight for adequate productivity.

Table 2.1 Nutrient requirement of Broiler Chicken

Age (weeks)	Energy kca/kg	Protein (%)	Lysine (%)	Methionine (%)	Calcium (%)	Phosphorus (%)
0-4	2800	23.00	1.25	0.86	1.00	0.70
5-8	3000	20.00	1.10	0.75	1.00	0.70

Aduku (2005)

2. 3 Temperature Effects on Poultry:

High temperature coupled with high humidity usually imposes severe stress on birds and leads to reduced performance. Several methods to alleviate the effects of high environmental temperature on performance of poultry have been recommended. Some of these methods are housing, ventilation, and cooling systems are now issues that are probably applicable on a regional basis (Armstrong*et al.*,1999). Effects of heat stress can also be ameliorated by acclimation (Yahav and Plavnik, 1999; Altan*et al.,* 2000; Yalcin*et al.*,2001). Some of these methods cannot be applied in some regions and farms because of their impracticality and high cost. Instead, because of being practical, nutritional manipulation with its low cost is a common approach in poultry production (Austic, 1985; Leeson, 1986; Shane, 1988).

Charles (2002)reviewed the optimum temperature for performance and concluded that for growing broilers it is between 18 and 22°C. Higher ambient temperature (30°C) was found to depress growth rate and meat yield of commercial broilers as compared with normal ambient temperature (25°C), thus making it a major factor hindering poultry meat production in hot climates (Deeb and Cahaner, 2001)**.** Exposure of broiler chickens to high ambient temperatures (30^{o}c) caused a series of physiological changes such as elevated body temperature, panting and respiratory alkalosis and metabolic status elicited by decreased levels of plasma triiodothyronine(Tao *et al.,* 2006).

According to the findings of Al-Fatah and Abu-Dieyeh (2007), environmental temperature above 25°C has a significant ($P<0.05$) negative effect on the performance of 4-8 week-old broilers reared in open-sided poultry house particularly, during summer season. They explained that the depression in growth rate and body weight gain at high environmental temperatures (30 and 35°C) might be due to many factors which include decreasing feed consumption, inefficient digestion, impaired metabolism, genetic makeup of birds and temperature per se. They added that some of the energyfeed used for muscle contraction associated with panting might be another factor and that, a decrease in growth rate at high environmental temperature is accompanied with a reduction in thyroid size and thyroxin secretion.

Teetre*et al.* (1992)studied the effect of heat stress (24 to 35ºC) and normal temperature (24 ºC) on body temperature of birds. They noted that rectal temperature was increased to 44 ºC under heat stress versus 42.3 ºC in the normal temperature group. In well-fed chickenshowever, that are neither dissipating heat to the environment nor gaining heat from the environment, the upper limit of the circadian rhythm is usually about 41.5ºC while the lower limit is about 40.5ºC. When birds exposed to a hot environment and/ or performing vigorous physical activity, body temperature might rise by 1 or 2ºC as heat is stored (Daghir, 2008).Lu *et al.*(2007)investigated the effect of heat stress (34°C) and optimal ambient temperature (21°C) on growth, proportion of carcass and fat deposition, and meat quality in chickens. Theirresults showed that feed intake as significantly ($P<0.001$) decreased by heat exposure at 8 weeks of age. At 34°C, broilers exhibited great decrease in weight gain and lower breast proportion compared with the broilers at 21°C.

2.4 Heat Stress In Poultry

2.4.1 Definition of stress

Stress, a response to adverse stimuli, is difficult to define and understand because of its nebulous perception (Lucas & Marcos. 2013). According to Selye (1976), “stress is the none-specific response of the body to any demand”, whereas stressor can be defined as “an agent that produces stress at any time”. Therefore, stress represents the reaction of animal or organism (i.e., a biological response) to stimuli that disturb its normal physiological equilibrium or homeostasis.

Heat stress results from a negative balance between the net amount of energy flowing from the animal’s body to its surrounding environment and the amount of heat energy produced by the animal. This imbalance may be caused by variations and combination of environmental factors (e.g., sunlight, thermal irradiation, and air temperature, humidity and air movement), and characteristics of the animal (e.g., species, metabolism rate, and thermoregulatory mechanisms). Environmental stressors, such as heat stress, are particularly detrimental to animal agriculture, (Nienaberand Hahn 2007, Nardone, *et.,al* 2010 and Renaudeau, et., 2012).

2.4.2 **Effects of Heat Stress on Behavioral and Physiological status of Animals**

Under high temperature conditions, birds alter their behavior and physiological homeostasis seekingthermoregulation, thereby decreasing body temperature. In general, different types of birds react similarly to heat stress, expressing some individual variation in intensity and duration of their responses(Lucas and Marcos 2013). A recent study by Mack *et al*. (2013) showed that birds subjected to heat stress conditions spend less time feeding, more time drinking and panting, as well as more time with their wings elevated, less time moving or walking, and more time resting. Animals utilize multiple ways for maintaining thermoregulation and homeostasis when subjected to high environmental temperatures, including increasing radiant, convective and evaporative heat loss by vasodilatation and perspiration (Mustaf et al.,2009) . Birds have an additional mechanism to promote heat exchange between their body and the environment, which are the air sacs. Air sacs are very useful during panting, as they promote air circulation on surfaces contributing to increase gas exchange with the air, and consequently, the evaporative loss of heat (Fedde, 1998). However, it is worth noting that increased panting under heat stress conditions leads to increased carbon dioxide levels and higher blood pH (*i.e.*,alkalosis), which in turn hampers blood bicarbonate availability for egg shell mineralization and induces increased organic acid availability, also decreasing free calcium levels in the blood. This process is very important in breeders and laying hens, as it affects egg shell quality (Marder, and Arad, 1989).

Heat stress can affect the reproductive function of poultry indifferent ways. In females, heat stress can disrupt the normal status of reproductive hormones at thehypothalamus, and at the ovary, leading to reduced systemic levels and functions (Rozenboim*et al*., 2007). Also,negative effects caused by heat stress in males have been shown in different studies. Semen volume, sperm concentration, number oflive sperm cells and motility decreased when males were subjected to heat stress (Mc Daniel, 2004).

High environmental temperatures alter the activity of the neuroendocrine system of poultry, resulting in activation of the hypothalamic-pituitary-adrenal (HPA) axis, and elevated plasma corticosterone concentrations Star *et al*. 2008 and Quinteiro-Filho*et al*., 2012). Moreover, findings reported by Geraert*et al.*(1996)indicated that endocrinological changes caused by chronic heat stress in broilers stimulate lipid accumulation through increased *de novo* lipogenesis, reduced lipolysis, and enhanced amino acid catabolism.

2.4.3 General effect of heat stress on animals

Heat stress decreased milk yield and reduced reproductive performance in ruminant and some non-ruminant animals (Lucy *et al*., 1992). It altered some haematological and biochemical characteristics such as packed cell volume, haemoglobin concentration and red blood cells (Abdel-salemee*et al*., 1992). It causes haemolysis of erythrocyte (Bezeziska-slebodziiska 2003), decreased plasma protein level (Star *et. al* (2009) and plasma ascorbic acid concentration (Padilla *et al*., 2006). In addition, it distrupts growth performance and increased mortality rate (Ondruska*et al*.,2011).

Chronic heat leads to breakdown of normal physiological and biological mechanisms (El-sobhy, 2000). It significantly reduces feed intake and its efficiency, lowers body weight, fat deposition and impaired growth performance in broilers (Deeb and Chaner, 2002). It causes economic losses due to heat prostration and reduced intake of some essential nutrients which resulted in depressed egg production in layers (Bottje and Harrison, 1985). Chronic heat result in less effective behavioral and physiological thermoregulatory mechanisms. It also leads to higher mortality rate, decreased meat quality and reduced welfare status of broiler birds (Warris*et al*., 2005)

2.4.4 Effect of heat stress on the immune response

Changes in immune response by the central nervous system is mediated by a complex network that operates bi-directionally between the nervous, endocrine and immune systems (Niu*et al*., 2009). Heat stress has immunosuppressant effect on animals (Niu*et al*., 2009). Broilers subjected to heat stress had lower levels of total circulating antibodies, decreased number lymphocytes, reduced bursa weight, reduced systemic humoral immune response and reduced phagocytic ability of macrophages (Prieto and Campo, 2010). Nutritional manipulations, such as the addition of antioxidants, enzyme mixtures and some herbs is reported to be beneficial in alleviating some of the heat stress related physiological responses and improving thermo tolerance Herms *et al*.(2011)

2.5 Black seed (*Nigella sativa* L.,)

This is an annual aromatic plant native toSouthwest Asia and the Mediterranean region. Itscultivation has been traced back more than 3,000 years tothe kingdom of the Assyrians and ancient Egyptians(Khan, 2009). Presently, it is cultivated in various parts ofthe world, including Asia, the Middle East and NorthAfrica. It belongs to family Ranunculaceae. The species isgenerally a short-lived annual, herbaceous plant. Theheight of the plant is approximately 20-60 cm. Itpossesses grayish green linear leaves that are wispy andthread-like. The flowers are delicate, pale blue or white,with a variable number of sepals and 5-10 petals that areabout 2.5 cm wide (Ahmad andGhafoor, 2007). The flowers are distinguished by theoccurrence of nectaries. The gynoecium consists of variednumber of multi-ovulate carpels, which develop intofollicles after pollination. The fruit is large and inflated,with 3-7 integrated follicles, each one with numerousseeds. The seeds are normally small (1-5 mm long), black ordark-grey with a rough grooved surface and an oily white interior (Benkaci-Ali*et al.*, 2007). They are roughly triangular and possess astrongly pungent smell. They contain about 21% protein,35% carbohydrates and 35-38% plant fats and oils (Anon.,2006).Black seedreproduces with itself and forms a fruit capsule which consist of many white trigonal seeds, once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air becoming black in colour (black seeds), seeds are triangular in shape, black in colour, possess a pungent smell and contains considerable amount of oil (Chevallier, 1996).

The seeds have animmense medicinal value and are known to havenumerous medicinal properties, mainly in the Unani-Tibb/Greco-Arab and Ayurveda systems of medicine (Abdulelah andZainal-Abidin, 2007).

2.5.1Scientific Classification of the Plant

Kingdom:	*Plantae*
Sub kingdom:	*Tracheobionata* (i.e vascular plant)
Order:	*Ranunculases*
Family:	*Ranunculasceae*. Butter cup family
Genera:	*Nigella*
Species:	*sativa* (In Ahmad *et al*., 2004)

2.5.2 **Synonym of Black Seed in Various Language**

English:	Black seed or Black cumins
Arabic:	Habatussauda
Hindi:	kalonji. (In Ahmad *et al.*, 2004)

Plate I: showing a dense population of *Nigella sativa* plant on the field
(Thierry Lucas 2008)

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Plate II: showing close image of *Nigella sativa* plant on the field. (shericopans 2008)

Plate III: showing the Nigella sativa seed at maturity.
(Thierry Lucas 2008)

2.2 Chemical Composition Including Active Principles of Black Seed (*Nigella sativa*)

. **Group**	**Sub-group**	**Components**
Fixed oil (32-40 %)	Unsaturated fatty acids	Arachidonic, eicosadienoic linoleic, linolenic, oleic and almitoleic acid. Palmitic, stearic and myristic acid. Beta-sitosterol, cycloeucalenol, cycloartenol, sterol esters and sterol glucosides
Volatile oil (0.4-0.45 %)	Saturated fatty acids	Nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, α & β-pinene, d-limonene, d-citronellol, p-cymene and 2-(2-methoxypropyl)-5-methyl-1,4-benzenediol6,16-18
Proteins (16-19.9 %)	Amino acids	Arginine, glutamic acid, leucine, lysine, methionine, tyrosine, proline and threonine, etc.13
Alkaloids	-	Nigellicine, nigellidine, nigellimine-N-oxide
Coumarins	-	6-methoxy-coumarin, 7-hydroxy-coumarin, 7-oxy-coumarin
Saponins	Triterpenes, Steroidal	Alpha-Hedrin, Steryl-glucosides, acetyl-steryl-glucoside
Minerals (1.79-3.74 %)	-	Calcium, phosphorous, potassium, sodium and iron

Source: Tembhurne*et al.*(2014)

2.6 Utilization of Black seed (*Nigella sativa)* on Broiler health and production.

Black seed have shown positive effect on broiler health and production, it was reported by Al-homidan*et al*.(2002) supported by Osman and El-barody (1999) thatblack seed supplemented diet improvedfeed efficiency in broiler compared to the control. Also reported by Hassan *et al*. (2004), El-ghammary*et al*. (2002) an increase in body weight by incorporating ground black seed in broiler feed. Hermes et al. (2011) reported an increase in live body weight, reduced mortality rate decrease rectal temperature and respiratory rate on a heat stressed broiler fed different levels and forms of black seed during Egyptian summer.

Moreover, feed conversion ratio was reported to have been improved by feeding 4g/kg of feed and 1.5% of black seed fed to four weeks old broilers by Toghyani*et al*. (2010) and Zaid*et al*. (2008). The positive effect of black seed on performance are associated with high nutritive value as well as pharmacological active ingredients found in black seed which include a mixture of essential fatty acids, particularly oleic, linoleic and linolenic acids that cannot be synthesized by the body. Addition of black seed also increases bile flow rate which result in increased emulsification that activates the pancreatic lipases, which is vital in fat digestion and absorption of fat soluble vitamins (Crossland, 1980).

Black seed oil having an active ingredient(thymoquinone) is reported to have hepatoprotective effects (Mahmoud *et al.*,2002), this enable the seed to be used in wide range of gastrointestinal disorders (El-Abhar, 2003). The increase in performance reported might be as a result of the antimicrobial effects of the active ingredient of black seed (Gilani*et al.,* 2004) whereas the anthelmintic activity was reported by Agrawal*et al*.(1978). It is quite intresting to note that black seeds have the potentials to exert on body metabolism in different ways some of which are as sited by Mahfouz and El-dakhakhny(1960) concluded that different component such as thymoquinone and thymohydroquinone present have possessed good antimicrobial properties. It hasalso been observed by (More *et al*. 1980) that black seed can stimulate thyroid gland directly or indirectly through the pituitary gland. Thyroid hormone increases the metabolic rate that can lead to enhanced amino acid utilization.

2.7 Haematology

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease.(Merck Manual, 2012). Haematological values are widely used to determine systemic relationship and physiological/pathological adaptation including the evaluation of general health conditions, diagnosis and prognosis of various types of animal diseases (Shah *et al*.,2007). Blood constituents change in relation to the physiological conditions of health and they are affected by certain factors such as breed, age, sex and management systems (Etim*et al.,* 2014).

Haematological parameters consist of packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin Concentration (MCHC). Each of these parameters signify certain body reaction or interpret certain body reactions.

Red blood cells are produced in the red bone marrow and they contain haemoglobin molecules. They function in transportation of gases, primarily oxygen and some carbon dioxide (OpenStax College, 2013). Thus, a reduced RBC count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Isaac *et al.*, 2013).

The WBC is a major component of the body’s defense against disease. The WBC protects the

Body against invading microorganisms and body cells with mutated DNA, and they clean up debris. White blood cells are divided into two groups: granular and agranular leukocytes **(**OpenStax College, 2013**).** Granular leukocytes include heterophils, eosinophils and basophils while agranular leukocytes include monocytes and lymphocytes. Heterophils are rapid responders to the site of infection and are efficient phagocytes with a preference for bacteria. The eosinophils counteract the activities of inflammatory chemicals produced by basophils and mast cells. Some eosinophils are also capable of phagocytosis and are particularly effective when antibodies bind to the target and form an antigen-antibody complex. High counts of eosinophils are typical of birds experiencing allergies, parasitic worm infestations, and some autoimmune diseases. Low counts may be due to drug toxicity and stress.

Lymphocytes provide generalized, nonspecific immunity, against specific pathogens (disease-causing microorganisms) and are involved in specific immunity. Monocytes release antimicrobial defensins and chemostactic chemicals that attract other leukocytes to the site of an infection (OpenStax College, 2013). Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable ofgenerating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Soetan*et al*., 2013) and enhance adaptability to indigenous environmental and disease prevalent conditions (Kabir*et al*., 2011).

Platelets are critical to haemostasis, through the stoppage of blood flow following damage to a vessel. They also secrete a variety of growth factors essential for growth and repair of tissue, particularly connective tissue. Infusions of concentrated platelets are now being used in some therapies to stimulate healing (OpenStax College, 2013).

Packed cell volume is the volume percentage of erythrocytes present in a sample of centrifuged blood **(**OpenStax College, 2013**).** According to Isaac *et al.* (2013) PCV is involved in the transport of oxygen and absorbed nutrients. Increased PCV shows better transportation and thus results in increased primary and secondary polycythemia. Mean corpuscular volume give an indication as to the type of anaemia and may also suggest the aetiology of the disease process (Coles, 2007). For most birds the MCV lies between 121–200 fl. An increase in this value is most useful for indicating a regenerative anaemia. In a non-regenerative anaemia, the cells are either normal or reduced in size. A reduced value of MCHC usually indicates a deficiency anaemia resulting in an insufficiency of haemopoetic factors. An increase in this value does not occur since RBCs do not become supersaturated with haemoglobin. Mean corpuscular haemoglobin (MCH) is not a useful index as the MCHC (Coles, 2007).

2.8 Biochemical

Biochemical blood parameters include enzymes, metabolites and electrolytes. The enzymes include Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatse (ALP); the metabolites include Cholesterol (CHO), Glucose (GLU) and Creatinine (CRE); while the electrolytes include Sodium (Na), Potassium (K), Calcium (Ca) and Chlorine (Cl),(Coles, 2007).

Normal plasma values for AST in most birds should normally be below 230 IU/l but can be in the range of 52–270 IU/l. As with the biochemistry of many of the blood constituents, the level varies with the age and seasonal activity (Coles, 2007). AST has a wide distribution in body tissues and although it is not liver specific it is possibly the single most useful enzyme for indicating liver disease. However, soft tissue damage, intramuscular injection, particularly with irritant drugs, hepatopathy and toxic chemicals increases the level of AST (Coles, 2007).

Normal levels of ALT in birds vary considerably from 6.5–263 IU/l.ALT is an enzyme found in the cells of many avian tissues. In other animals elevations have been shown to be associated with hepatocellular disruption, however no such association has been consistently demonstrated in birds. Little clinical significance can therefore be applied to ALT values in avian patients (Harris, 2000 and Coles, 2007).

ALP is an enzyme which is found mostly in the duodenum and kidney. According to Coles (2007), ALP is considered to be a good indicator of osteoblastic activity. Its level increases in growing birds, egg laying birds when calcium metabolism is increased in the medullary bone and in some cases in liver disease, such as aflatoxin poisoning. Low levels of ALP may be seen indietary zinc deficiency.

According to Coles (2007), normal values for cholesterol in birds are in the range of 108–330 mg/dl. This substance is the precursor of all steroid hormones and bile acids. The remainder of the cholesterol is produced in the liver and is also gotten from the diet. Plasma levels of cholesterol are increased in cases of fatty liver and kidney syndrome. Very high levels of plasma cholesterol accompanied by a lipaemia indicate fatty degeneration of the liver.

Glucose is required for cellular respiration and is the preferred fuel for all body cells (OpenStax College, 2013). Elevations in blood glucose levels are the principal pathological change noted in avian species (Harris, 2000). Increased glucose level results in hyperglycaemia while decreased glucose level results in hypoglycaemia.

Creatinine levels in avian serum samples typically fall below a measurable range and certain technical factors contribute to a high incidence of artefactual changes. Elevations have been associated with kidney disease, but creatinine is not considered to be a reliable indicator of renal function (Harris, 2000).

Electrolytes play important roles in various physiological processes, such as osmotic pressure maintenance, synthesis of tissue proteins, intra and extracellular homeostasis maintenance, maintenance of the electrical potential of the cell membrane, acid-base homeostasis, as well as the functioning of the enzyme and Nerves (Borges *et al.*, 2003).

Normal calcium levels in most birds are within the range of 8–12 mg/dl (Coles, 2007). Total blood calcium levels tend to be higher during ovulation in birds (corresponding with increased ALP) and coincident with the transport of protein-bound calcium to the shell gland, but the level of calcium ions in the blood remains constant. Immature birds tend to have lower blood calcium levels.

Potassium is considered the most abundant intracellular cation and is involved in many metabolic processes, including nerve conduction, muscle excitation-contraction and cell volume regulation. Consequently, changes in K+ homeostasis profoundly affect cellular functions (Vieites*et al*., 2005).

Sodium is also essential in the production of hydrochloric acid in the stomach which is vital for digestion. Changes in sodium values usually reflect serious conditions. According to Harris, (2000), elevated levels of sodium occur with salt poisoning, water deprivation and dehydration while decreased levels occur due to sodium loss through renal disease or diarrhoea. Harris, (2000) also reported changes in chloride levels are rarely observed in avian species.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Experiment 1 (one): Effect of Nigella sativa supplementation on weight gain, haematological and thermoregulatory parameters of broiler chi*cks (starter phase)*

3.1.1 Experimental Site

The experiment was conducted at the Poultry Unit of the Teaching and Research farm of the College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road Kaduna located at (10^0 20'N 07^0 45'E) in the Northern Guinea Savannah, characterised by average annual maximum and minimum temperatures of 31.8 ± 3.2°C and 18.0 ± 3.7°C, respectively. The monthly average rainfall during the rainy season (May–October) is 148 ± 68.4 mm (69.2–231.9 mm), while the monthly relative humidity is 71.1 ± 9.7%. The zone is characterized by three seasons: Harmattan (November–February), hot-dry (March–May), and rainy (June–October) seasons (Ayo *et al*., 2011)

3.1.2 Source of Experimental Materials

The experimental birds were obtained from Best line while the Black seed powder was obtained from Kurmi Market Kano State.

3.1.3 Chemical Analysis of Black Seed (*Nigella sativa*) powder

Chemical analysis of the black seed powder was conducted according to A.O.A.C(1990) procedure where the proximate composition were determined at the Biochemistry laboratory of Food Science and Technology Program of the Institute for Agricultural Research, Ahmadu Bello University Zaria.

Also some vitamins (A,B1,C and E) and minerals (Ca,Na,Mg and K) presents in *Nigella sativa* powder were determined at National Research Institute for Chemical Technology(NARICT) Zaria and the Federal Department of Agricultural Land Resources and Climatic Change Management Services km2 Kaduna-Abuja Expressway Gonin-gora, respectively.

3.1.4 Experimental Design and Management of Birds

A total of one hundred and eighty (180) one week old broilers were used for this study. The chicks were randomly allocated into four experimental treatments with three replicates having 15 birds per replicatein a complete randomized design. The birds were reared in a deep litter system and managed with all necessary routine management practice with feed administered using a trough and water using a drinker, both were provided *ad libitum* and mortality was recorded as occurred.

3.1.5 Experimental Diet

A diet was formulated to meet the NRC (1994) requirement for the starter feed and at the end of brooding the chicks were separated according to treatments and the supplement was added to their feed at different levels as thus;

T_1 Control no supplementation

T_2 5g per kg of diet

T_3 10g per kg of diet

T_4 15g per kg of diet

Table 3.1: Composition of Broiler Starter Diet fed to the Chicks (0-4 weeks)

Feed ingredient	Composition (kg)
Maize	50
Maize offal	5
Groundnut cake	23
Soya bean cake	13
Fish meal	4.5
Limestone	0.5
Bone meal	2.8
Salt	0.3
Premix	0.3
Lysine	0.3
Methionine	0.3
Total	100
Calculated analysis	
ME (Kcal/kg DM)	2849
Crude protien (%)	23.20
Ether extract (%)	4.69
Crude fibre (%)	4.14
Calcium (%)	1.22
Phosphorus (%)	0.69
Lysine (%)	1.35
Methionine (%)	0.99

Biomix broiler starter premix include per kg diet: vit A,1,000 I.U; Vit D,2000 IU; Vit E,5.0mg; Vit K,2mg; Vit B1, 1.80mg; Vit B2, 5.5mg; Niacin, 27.5mg; pantothenic acid, 0,5mg Vit B6 0.30mg; Vit B12, 0.15mg; Folic acid, 0.75mg; biotin, 0.6mg; chlorine chloride, 300mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; selenium, 0.2mg; zinc 30mg; Antioxidant, 1.25mg. ME= Metabolizable Energy

3.1.6 Parameters Measured

3.1.6.1 Rectal temperature and respiratory rate

These are indicators of heat stress, Rectal temperature was measured using a digital thermometer inserted 2-3cm into the cloaca at the 28 days of age at (7:30-9:00am) while the Respiratory rate was measured by counting the movement of the body surface of a quite bird for one minute using a stop watch.

3.1.6.2 Growth performance

At the 28th day (i.eend of starter phase) the chickens were weighted in grams (gm) using a weighing scale and the experiment was terminated.

3.1.6.3Haematological analysis.

On the 28th day, two birds per replicate were randomly selected and 2mls of blood was collected via the wing vein into sample bottles containing ethylene Di-amine Tetra Acetic Acid (EDTA) as an anti-coagulant. The samples were taken to the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for the determination of parked cell volume (PVC), Red blood cells (RBC), white blood cells (WBC) and haemoglobin concentration (Hb).

3.1.6.4Estimation of haematological parameters

Packed cell volume (PCV) was estimated from the blood samples collected in bottles containing EDTA by gently mixing and drawing up in a micro haemmatocrit capillary tube to ¾ of its length with one end of the tube sealed with plasticine. The capillary was placed in micro-haematocrit centrifuge, ensuring that the plasticine end is outward and centrifuged at the 12,000 rpm for 4 minutes. The tubes were then read in the haematocrit reader. The reading expressed the red blood cells as a percentage (%) of the total volume of the blood. The white blood cell (WBC) counts and haemoglobin (Hb) concentration were determined following procedures described by Davice and Lewis (1990).

3.2 Experiment Two: Effect of *Nigella sativa* supplementation on weight gain, haematological, thermoregulatory parameters and Histopathology of broiler chickens at (Finisher phase)

3.2.1 Experimental site

It was carried out at the same place with experiment 1, as reported on page 24

3.2.2 Source of experimental materials

Same as experiment 1.as reported on page 24

3.2.3 Experimental design and management of birds

A total of one hundred and eighty (180) four weeks old broilers were used for this study. The chickens were randomly allocated into four experimental treatments with three replicates each having 15 birds in a completely randomized design, the chickens were reared in a deep litter system and managed with all necessary routine management practices with feed and water provided *ad libitum*,Mortality was recorded as it occurred, with zero drug administration.

3.2.4 Experimental diet

A diet was formulated to meet the NRC (1994) requirement of 19% Cp and 2900Kcal ME for finisher phase and the supplement was added as thus;

T_1 Control no supplementation

T_2 10 per kg of diet

T_3 20g per kg of diet

T_4 30g per kg of diet

Table 3.2: Composition of Broiler Finisher Diet Fed to the Birds (5-8 weeks)

Feed ingredients	Composition(Kg)
Maize	57.00
Maize offal	8.00
Ground nut cake	20.00
Soya bean cake	9.00
Fish meal	2.00
Bone meal	2.00
Limestone	1.00
Salt	0.30
Premix	0.25
Lysine	0.20
Methionine	0.25
Total	100

Calculated analysis:

ME(kcal/kg)	2924.00
Crude protein (%)	19.90

Ether Extract (%)	4.44
Crude fibre (%)	4.07
Calcium (%)	1.05
Available phos (%)	0.44
Lysine (%)	1.02
Methionine(%)	0.84

Biomix broiler finisher premix include per kg diet: vit A,10,000 I.U; Vit D3,2000 IU; Vit E,23mg; Vit K,2mg; Vit B1, 1.80mg; Vit B2, 5.5mg; Niacin, 27.5mg; pantothenic acid, 0,5mg Vit B6 0.30mg; Vit B12, 0.15mg; Folic acid, 0.75mg; biotin, 0.6mg; chlorine chloride, 300mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; selenium, 0.2mg; 30mg; Antioxidant, 1.25mg. ME= Metabolizable Energy

3.2.5 Parameters measured

3.2.5.1 Rectal temperature and respiratory rate

On the 56th day the same procedure for the above parameters were employed at the same time as it was carried out during the first experiment and data were recorded.

3.2.5.2 Growth and weight of some internal organs

Initial weight, final weigh, average weight gain, were measured using a table scale in grams and mortality were recorded, also average weight of the liver, empty Gizzard, and Immune organs (spleen and thymus gland), thymus gland found at the neck of the bird while the spleen in the abdomen between the liver and the stomach were also recorded after evisceration of the bird.

3.2.5.3 Haematological analysis

On the 56th day, three birds per replicate were randomly selected, 4mls of blood each bird was collected via the wing vein into two different sets of sample bottles containing ethylene Di-amine Tetra Acetic Acid (EDTA) as an anti-coagulant and the other without the EDTA for the serum biochemical analysis, the samples were taken to the Clinical Pathology Laboratory, Faculty of

Veterinary Medicine, Ahmadu Bello University, Zaria for the determination of parked cell volume (PCV), Red blood cells(RBC), White blood cells(WBC) and haemoglobin concentration(Hb). The above was done as it was performed on Experiment I.

Also the plasma constituents of blood biochemicals taken includes, total protein(TP), albumin(A), globulin (G), total cholesterol and Triglyceride.

3.2.5.4 Histopathology

At the end of the experiment, two chickens were randomly selected from each treatment and slaughtered. Three vital organs (Small intestine, Liver and Kidney) were immediately removed and a section of each was taken and immersed into sample bottle with buffered neutral 10% formalin 48 hours. The fixed tissues were dehydrated by dipping in concentration of alcohol (100%). The tissues were cleared with xylene for about 2 hours, infiltrated with molten paraffin wax at 50^oC to 60^oC for 2 hours, embedded in molten paraffin wax and labeled appropriately. Thick sections of 5µ were cut from the the embedded tissues using a microtome knife attached to a microtome. The sectioned tissues were mounted on grease free, clean glass slides, dried at room temperature and stained with Haematoxylin and Eosine (H and E) stain. The tissue sections on slides were studied using light microscope at ×400 magnification. Photomicrographs of the slides were taken using a digital camera, transferred to a computer and labeled appropriately.

3.3 Statistical analysis

Data collected from the experiment were subjected to analysis of variance (ANOVA), using the General Linear Model procedure of statistical analysis

(SAS, 2001) and means were separated using Duncan's multiple range test (DMRT). The model used for the experiment was:

$Y_{ij} = \mu + t_i + e_{ij}$

Where Y_{ij} =individual observation

μ = Overall mean.

t_i=Fixed effect of treatment at varying level of supplementation.

e_{ij} = Experimental Error

CHAPTER FOUR

RESULTS

4.1 Chemical compositions of Black seed (*Nigella sativa*) powder

The chemical composition of *Nigella sativa* powder is presented (Table 4.1). The proximate composition was determine to have high Dry matter,Crude Protein,Ether Extract with relatively low Moisture, Crude Fiber and Ash, among the minerals investigated, it contained potassium with the highest amount , followed by sodium and magnesium with calcium having the lowest. Also it contained Vitamins with the highest value of vitamin E followed by vitamins B1, C and vitamin A with the lowest value.

Table 4.1: Chemical composition of Black seed (*Nigella sativa)*powder

Parameters	Composition
Dry matter (%)	97.60
Moisture (%)	2.65
Crude protein (%)	19.25
Crude fiber (%)	16.00
Ether extract (%)	35.60
Ash (%)	4.00
Calcium (mg/100g)	2.31
Sodium (mg/100g)	5.65
potassium (mg/100g)	10.12
Magnesium (mg/100g)	3.02
Vitamin A (mg/100g)	4.87
Vitamin C (mg/100g)	6.31

Vitamin B1 (mg/100g)	21.51
Vitamin E (mg/100g)	9.01

4.2 Growth Performance of Broiler Starter Chicks During Hot Season

Table 4.3 shows the effect of Black seed powder on the growth performance of starter broiler chicks. Black seed powder significantly ($P<0.05$) increased final body weight, weight gain and daily weight gain as compaired to the control

Final body weight was signifcantly ($P<0.05$) higher for chicks on 15g/kg black seed supplementation than 10, 5 and 0g/kg black seed. Body weight gain was significantly ($P<0.05$) higher in the group with 15g/kg black seed supplementation than 10, 5 and 0g/kg of diet supplementation. Average daily weight gain is significantly ($P>0.05$) lower in the control than 5, 10 and 15g/kg of diet black seed supplementation.

Also mortality was recorded highest in the control which followed by the group with $5gkg^{-}$ of diet supplementation and none was recorded with the rest of the groups with higher level of supplementation.

4.3 Thermoregulatory Performance of Broiler Starter ChickensDuring Hot Season

Figure1 shows the result of the Rectal temperature of broiler chicks at the end of starter phase during hot season which decreases significantly ($p<0.05$) among the groups with Black seed supplementation in contrast to the control.

Also figure 2 shows the Respiratory rate of broiler chicks at the end of starter phase during hot season decreases significantly ($p<0.05$) among the groups with Black seed supplementation in contrast to the control.

Table 4.2: Effect of varying levels of Black seed (*Nigella sativa*) Supplementation on the Performance of Broiler Starter Chicks

	Levels of Black seed (gkg^{-1})					
Parameters	0	5	10	15	SEM	LOS
Initial weight(g)	166.87	166.84	166.85	166.86	0.18	NS
Final weight (g)	703.10^{d}	807.92^{c}	829.12^{b}	890.74^{a}	3.31	*
Total weight gain (g)	536.23^{d}	640.57^{c}	662.25^{b}	724.06^{a}	3.32	*
Avg. daily weight gain (g)	25.53^{c}	30.50^{b}	31.53^{b}	34.48^{a}	0.74	*

Mortality %	13.33^{a}	4.44^{b}	0.00^{c}	0.00^{c}	0.42	*

$^{abc;}$ Means with different superscripts along same rows shows significant differences (p>0.05)SEM=standard Error of means, LOS=level of significance,NS=Non significant difference at (p>0.05) * = significant difference at (p>0.05)

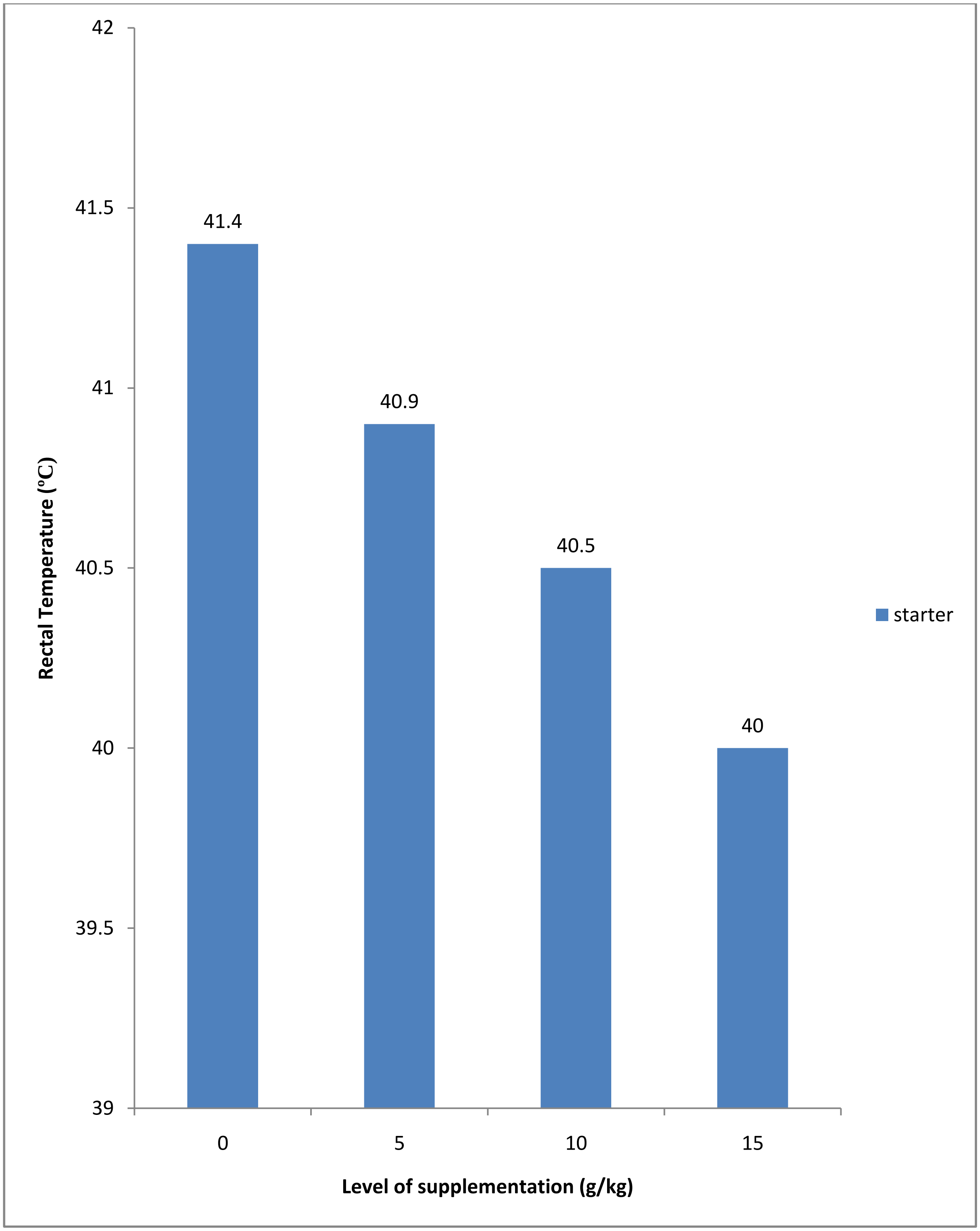

Fig 4.1. Black Seed Supplementation on the Rectal Temperature of Broiler Chicks During the starter phase. SEM = 0.18

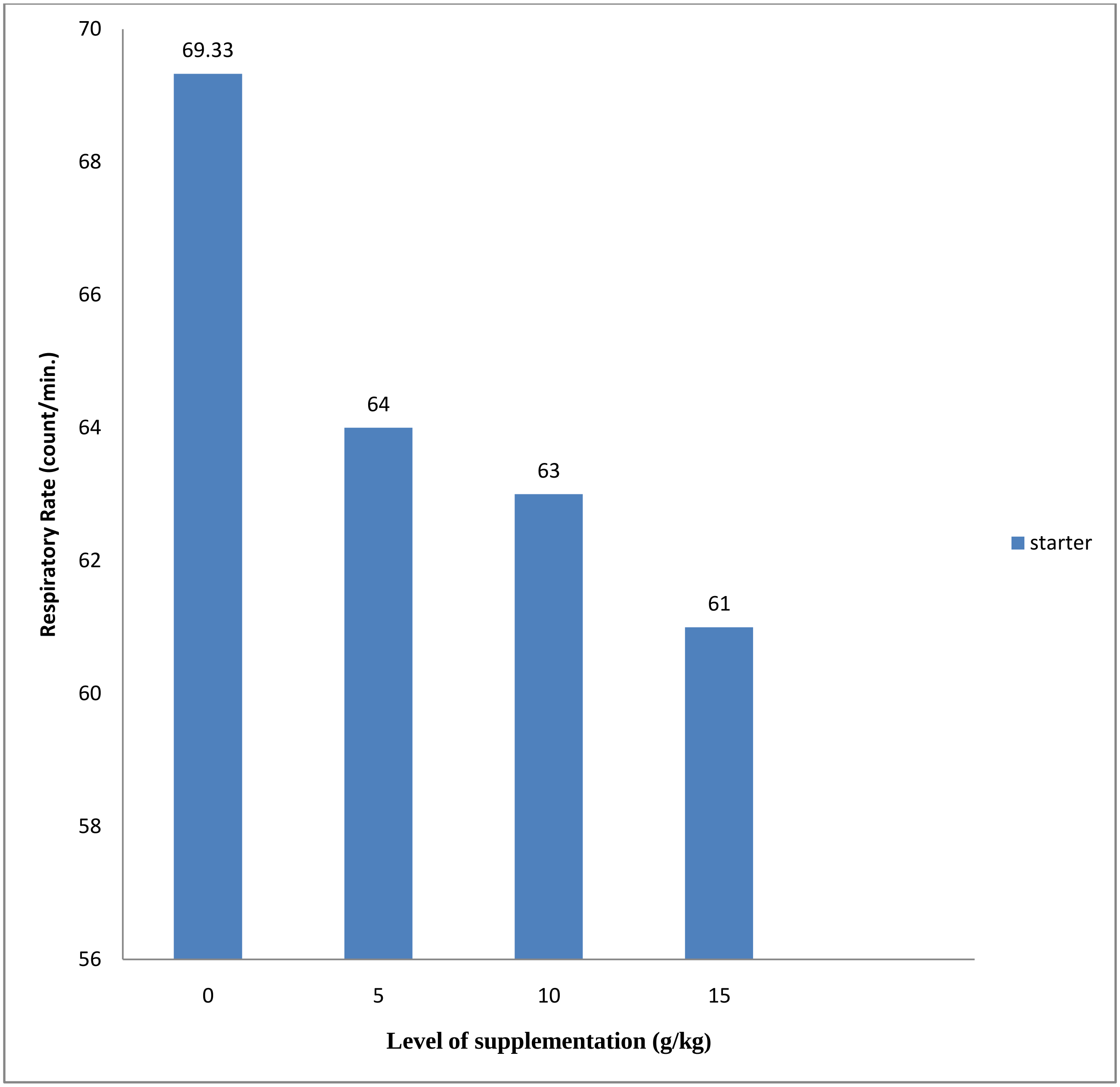

Fig4.2. Black Seed Supplementation on the Respiratory Rate of Broiler Chicks During the starter phase. SEM = 0.54

4.4 Haematological parameters of Broiler ChickensDuring Hot Season

The haematological parameters of broiler chicks at the end of starter phase as presented in Table 4.3. The results showed that only the packed cell volume (PCV) differs significantly at ($P< 0.05$) between the control and the supplemented group while the haemoglobin (Hb), white blood cells (WBC) and the red blood cells (RBC) did not differ statistically at ($P>0.05$).

4.5 Growth Performance and Weight of Some Internal Organs of Broiler Chickens at Finisher Phase During Hot Season

As shown in Table 4.4, the performance of broiler finisher chickens supplemented with different levels ofblack seed powder during the hot season indicating significant differences at ($P<0.05$) in the final weight of the other treatments compaired with the contol with a highest value in the group with 20gkg^{-1}, followed by the group with 30gkg^{-1} and 10gkg^{-1}, respectively. Also Mean liver wieght, gizzard, Proventriclus and kidney differs significantly at ($p<0.05$) in the same pattern with the result of the final body wight compaired to the control.

The result showed that mortality rate decreases as the supplementation ofblack seed increases with the highst in the control (0gkg^{-1}) .

Table 4.3: Effect of Varying Levels of Black seed (*Nigella sativa*)powder on Haematological Parameters of Broiler starter Chicks.

	Levels of Black seed (gkg^{-1})					
Parameter	0	5	10	15	SEM	LOS
PCV (%)	24.11^{b}	29.17^{a}	28.17^{a}	27.17^{a}	1.09	*
Hb (g/dl)	8.00	8.97	9.17	8.00	0.47	NS
WBC(10^9/l)	5.47	5.06	4.57	4.29	0.75	NS
RBC (10^{12}/l)	2.09	2.12	2.06	2.20	0.04	NS

$^{abc;}$ Means with different superscripts along same rows shows significant differences (p>0.05), PCV= pack cell volume, Hb=Haemoglobin, RBC= Red blood cell, WBC= white blood cell SEM: standard Error of means, LOS=level of significance, NS:=Non significant difference at (p>0.05)*: = significant difference at (p>0.05)

Table 4.4: Effect of Varying Levels of Black seed supplementation on the performance andweight of Some Internal Organs of Broilers at the Finisher phase during hot season

	Levels of Black seed (gkg^{-1})					
Parameters	0	10	20	30	SEM	LOS
Initial body weight(g)	702.83	700.53	701.15	700.00	0.69	NS

Final body weight (g)	1935.00^{d}	2005.63^{c}	2080.70^{a}	2016.67^{b}	3.08	*
Total weight gain (g)	1232.17^{d}	1305.10^{c}	1379.55^{a}	1316.67^{b}	0.05	*
Av daily weight gain (g)	44.00^{d}	46.61^{c}	49.27^{a}	47.02^{b}	0.05	*
Liver weight (g)	31.87^{c}	44.00^{ab}	44.64^{a}	42.85^{b}	0.66	*
Gizzard (g)	31.53^{b}	40.09^{a}	39.83^{a}	32.62^{b}	0.82	*
Proventriclus (g)	9.71^{b}	10.57^{ab}	12.40^{a}	8.95^{b}	0.58	*
Spleen (g)	1.48^{c}	2.55^{b}	3.09^{a}	2.51^{b}	0.29	*
Kidney (g)	6.82^{d}	9.72^{c}	11.98^{a}	10.93^{b}	0.24	*
Thymus (g)	1.99^{b}	2.23^{b}	3.01^{a}	2.03^{b}	0.25	*
Mortality (%)	15.55	6.66	2.22	2.22	0.46	*

$^{abc;}$ Means with different superscripts along same rows shows significant differences ($p>0.05$), SEM:standard Error of means, LOS=level of significance,NS=Non significant difference at ($p>0.05$)* = significant difference at ($p>0.05$)

4.6 Thermoregulatory Performance of Broiler FinisherChickens During Hot season

Figure 3, showed the results of Rectaltemperature of broiler chickensduring hot season significantly ($p<0.05$) variability was observed among the groups with black seed supplementationwith the lowest value in the group with ($30gkg^{-1}$) and the highst valuein the control ($0gkg^{-1}$).

Similarly, figure 4, showed the result of the Respiratory rateof broiler finisher chickens during hot season which indicated significant ($p<0.05$) decrease in the groups with black seed supplementation compaired to the control. The lowest value in the group with ($30gkg^{-1}$) and the highst valuein the control ($0gkg^{-1}$).

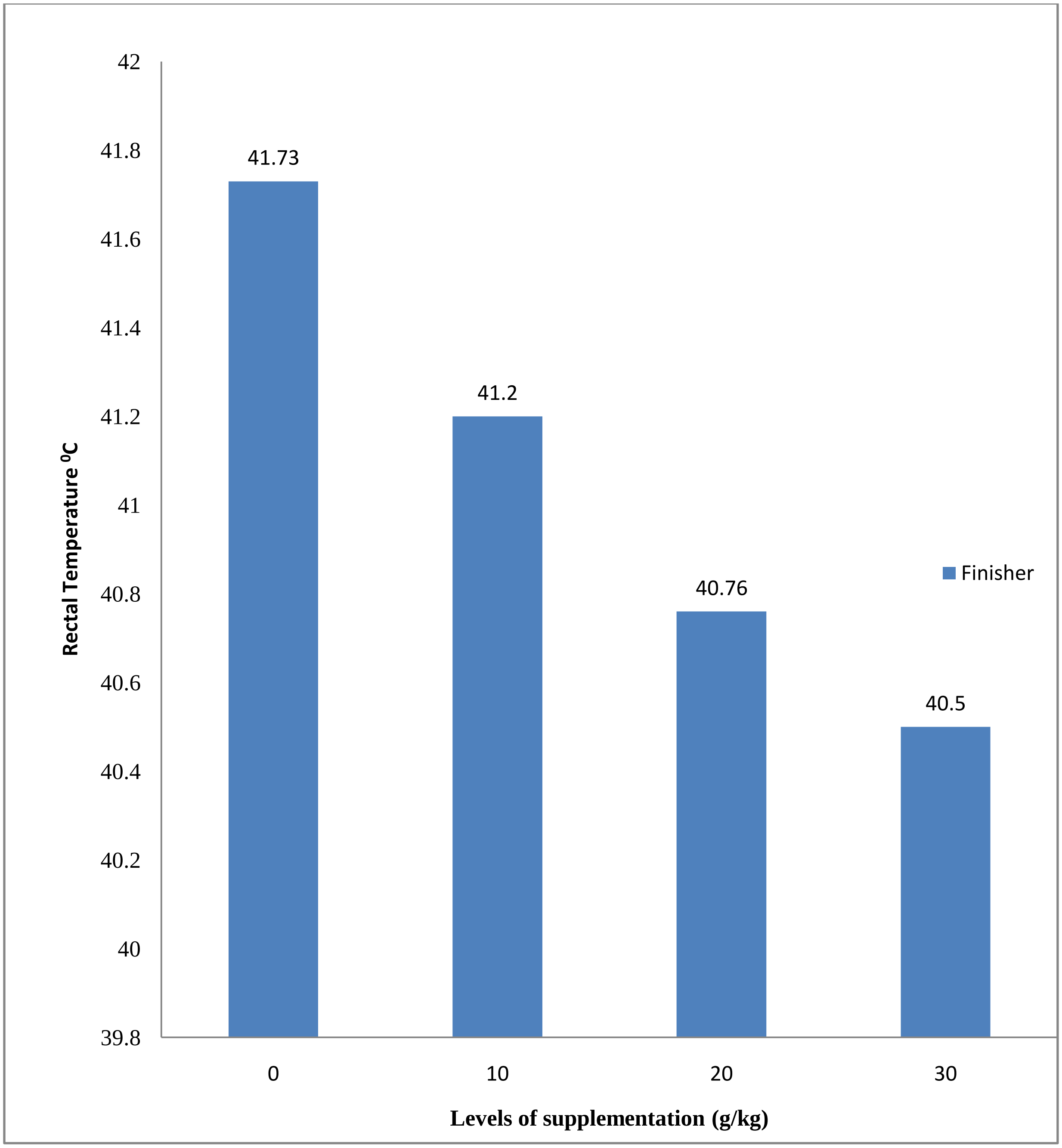

Fig 3: The Effect of Varying Levels of Black seed supplementation on the Rectal Temperature of BroilerFinisher Chickens During Hot Season. SEM = 0.17

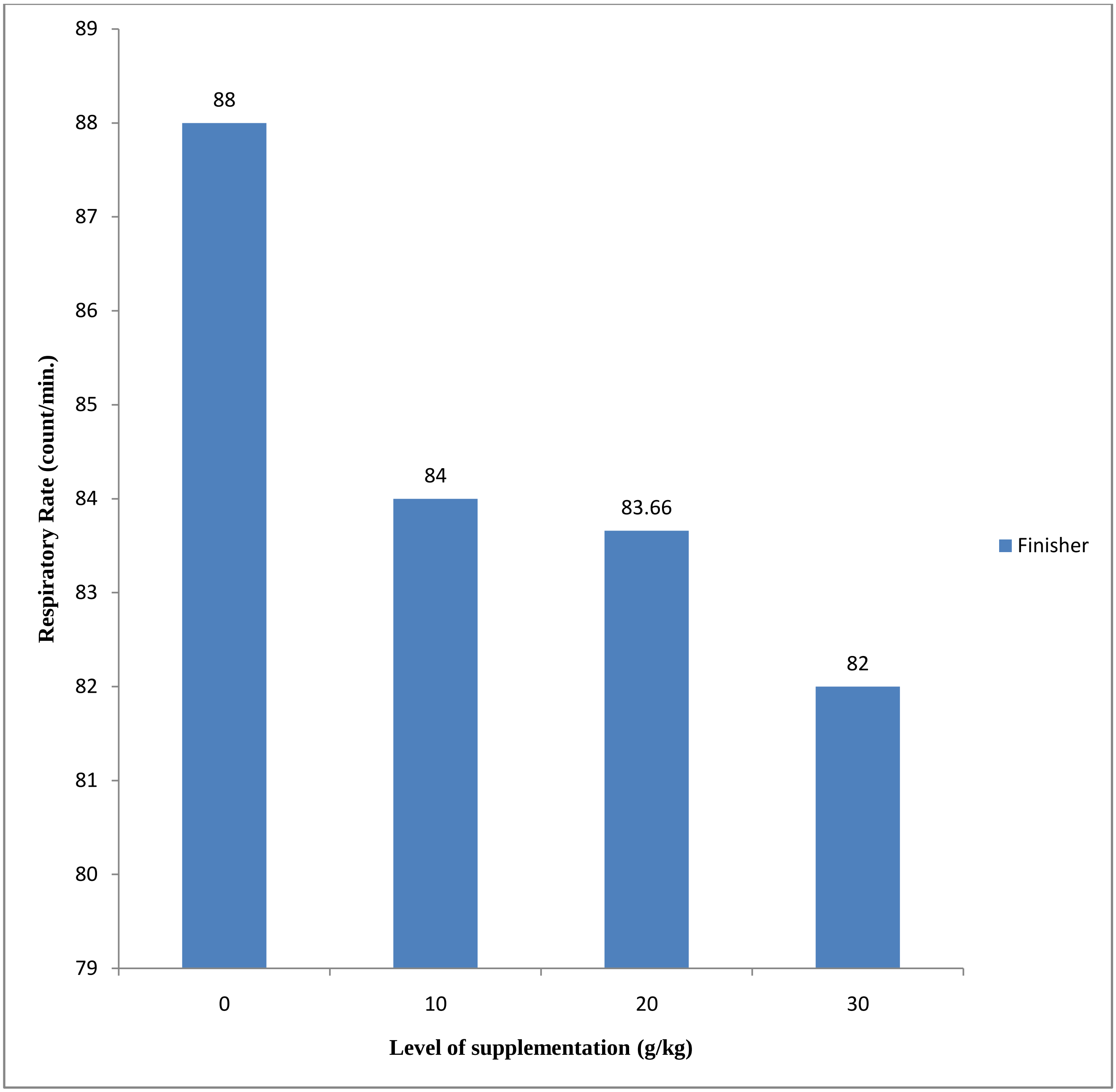

Fig 4: The Effect of Varying Levels of Supplementation of Black seed powder on Respiratory Rate of Broiler Finisher ChickensDuring Hot Season. SEM = 0.23

4.7 Haematological and Blood Biochemical Parameters of Broiler Finisher Chickens During Hot Season

The haematological indices shown in Table 4.5. shows that Supplementation of black seedhad significant($p<0.05$) increase on PCV, Hb, WBC and RBC, over the control with the highest and lowest values of (28.67-24.11 %),(9.68-7.35g/dl), (18.47-12.08 10^9/l) and (4.64-3.93$10^{12}$/l) respectively. The result of serum biochemichemistry. shows no significant difference ($P>0.05$) in both Albumin and Globulin. The Total cholesterol and Triglycerides differs significantly with the highest values of both in the control and the lowest in the group with 30gkg^{-1} of suplementation.

4.8 Histopathology of Broiler Finisher Chickens During Hot Season

The photomicrograph of sections of the, liver, kidney and the small intestine of both the control and the supplemented groups at 10, 20, 30 g/kg presented in plates 1 to 12 which indicated no histopathological findings.

Plates I to IV, showed the liver with no degeneration, changes or inflammation of the hepatocytes.

Plates V to VIII, showed the intestinal mucusa and the villi clearly no alterations or degenerations of the small intestine.

Plates IX to XII, showed no change, no degeneration and no inflammation in the glomeruli, renal corpuscles, glomerular basement membrane and the proximal convulated tubule of the kidney.

Table 4.5: Effect of Varying Levels of Black seed (*Nigella sativa*)Powder on Haematological parameters and serum biochemichemistry of Broiler Finisher Chickens

	Levels of supplementation (gkg^{-1})					
Parameter	0	10	20	30	SEM	LOS
PCV (%)	24.11^{b}	28.22^{a}	28.67^{a}	24.22^{b}	0.76	*
Hb (g/dl)	7.35^{c}	9.68^{a}	9.35^{ab}	8.18^{bc}	0.67	*
WBC(10^{9}/l)	18.47^{a}	17.58^{a}	14.07^{b}	12.08^{b}	1.15	*
RBC (10^{12}/l)	3.93^{b}	4.64^{a}	4.60^{a}	4.01^{a}	0.16	*
Biochemicals						
Total protein(g/dl)	2.08	2.88	2.58	2.29	0.36	NS
Albumin (g/dl)	2.23	2.65	2.71	2.61	0.28	NS
Globulin (g/dl)	1.42	1.55	1.56	1.64	0.19	NS
TCH (mg/dl)	162.64^{a}	137.56^{b}	129.56^{c}	128.22^{c}	1.54	*
TRG (mg/dl)	102.56^{a}	85.22^{b}	74.44^{c}	73.78^{c}	1.00	*

$^{abc;}$ Means with different superscripts along same rows shows significant differences ($p<0.05$). PCV= pack cell volume, Hb= Haemoglobin, RBC= Red blood cell, WBC= white blood cell, TCH= Total cholesterol, TRG= Triglycerides, SEM: standard Error of means, LOS= level of significant.

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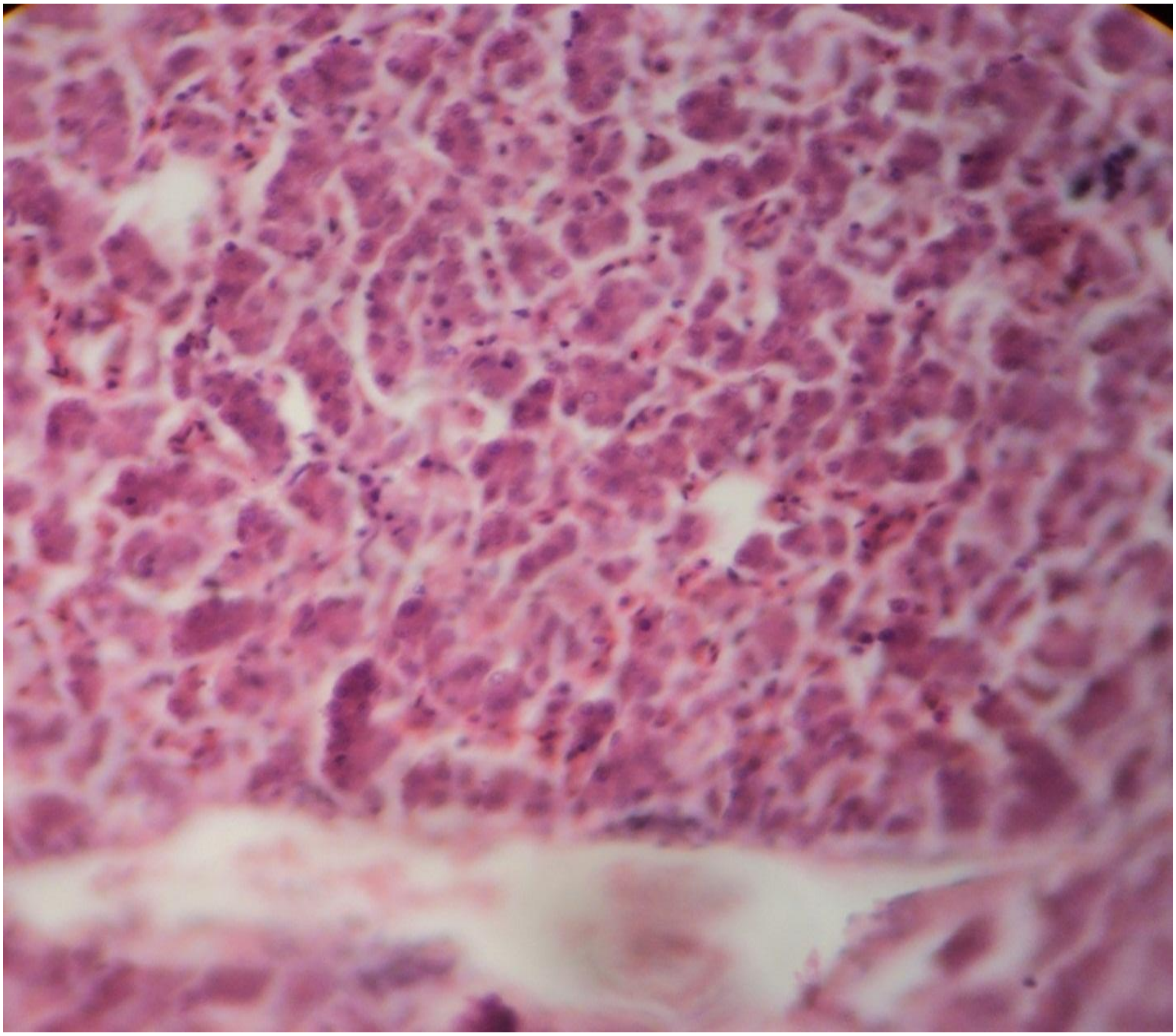

Plate I: photomicrograph of the liver of broiler Finisher chicken on 0g supplementation of Black seed, Showing no Histopathological Changes (H and E X 200)

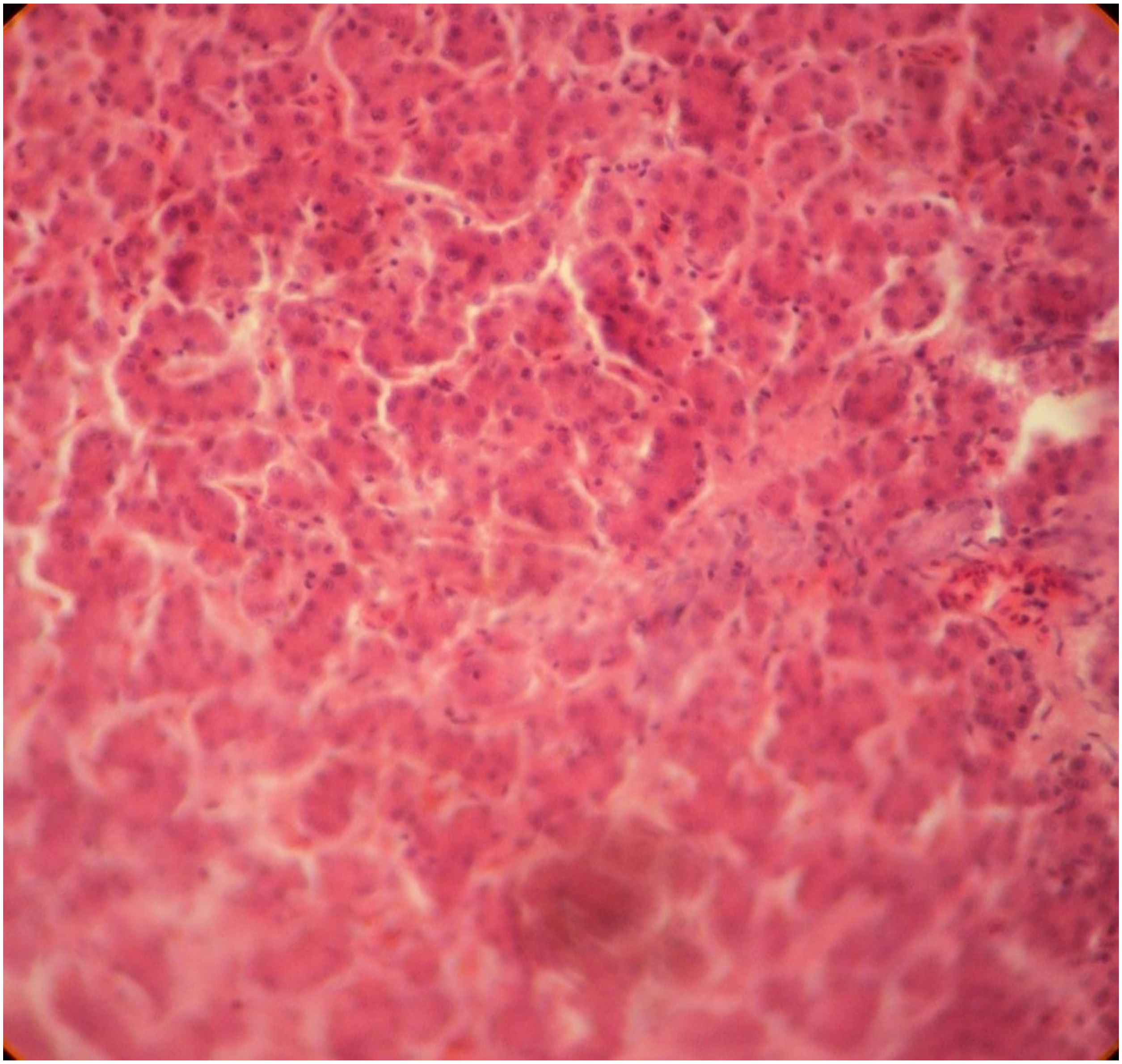

Plate II: photomicrograph of the liver of broiler chicken with 10g supplementation of Black seed, showing no histopathological changes (H and E X 200)

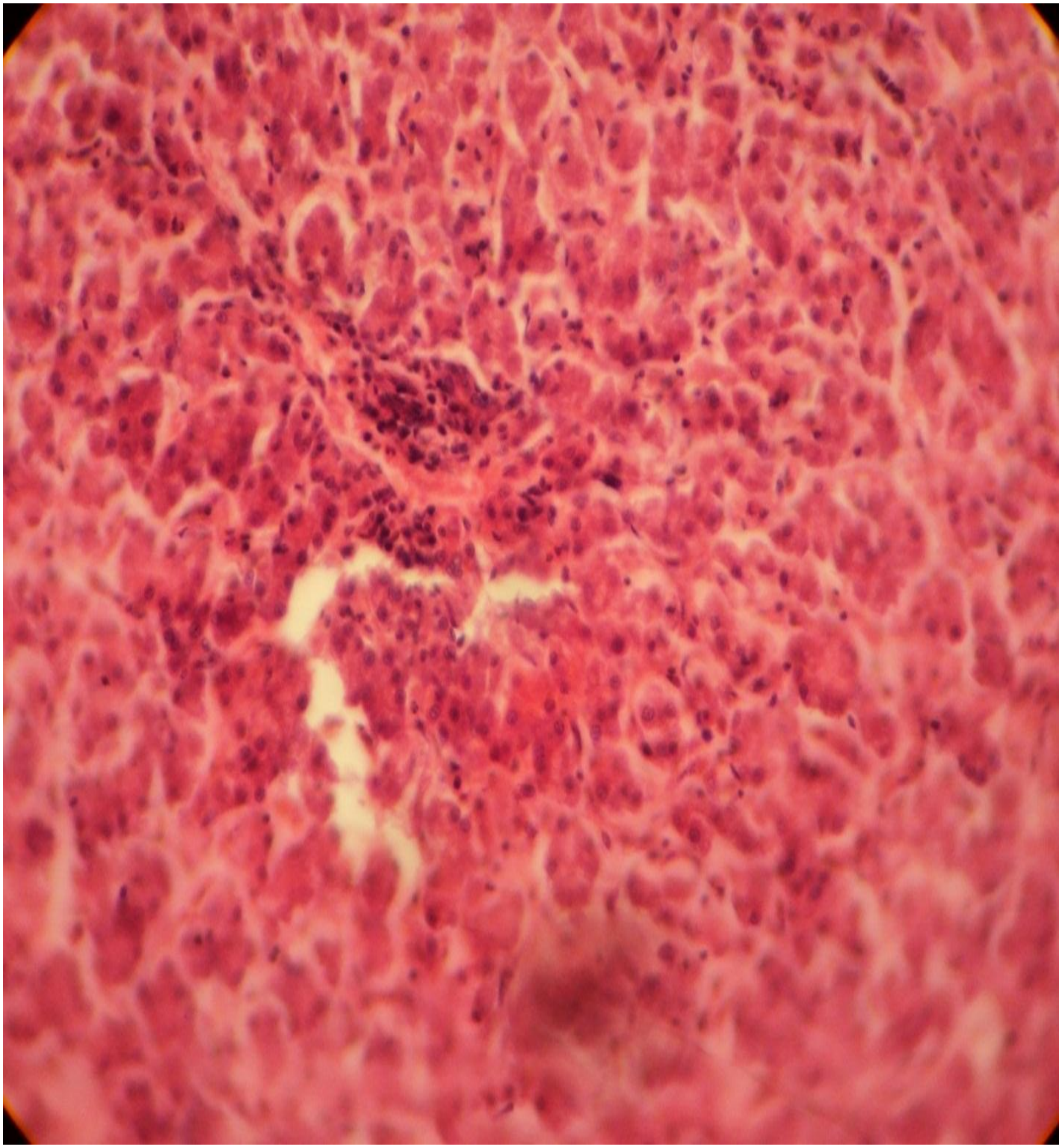

Plate III: photomicrograph of the liver of broiler chicken on 20g supplementation of Black seed, showing no histopathological changes (H and E X 200)

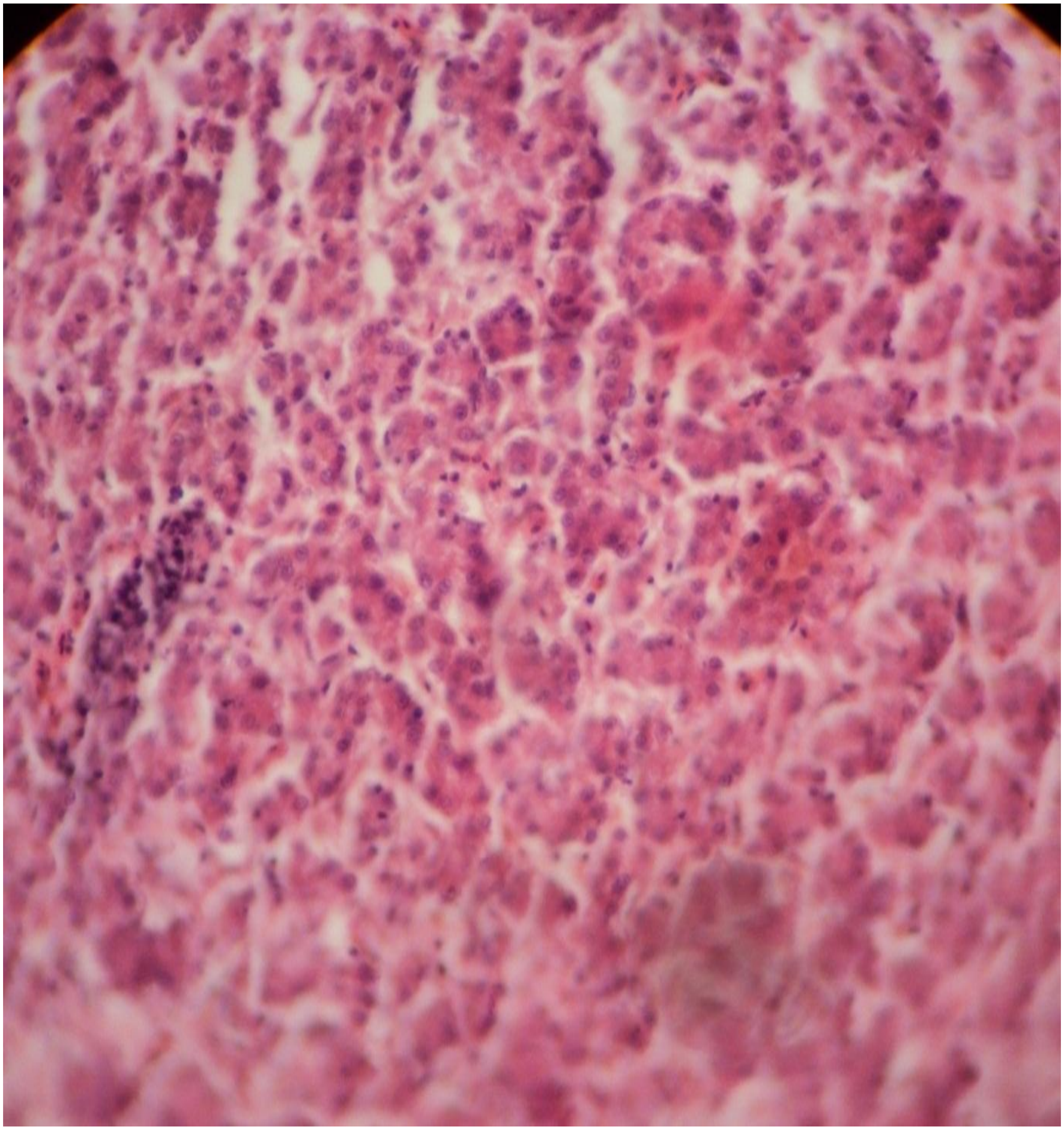

Plate IV: photomicrograph of the liver of broiler chicken on 30g supplementation of Black seed, showing no histopathological changes (H and E X 200)

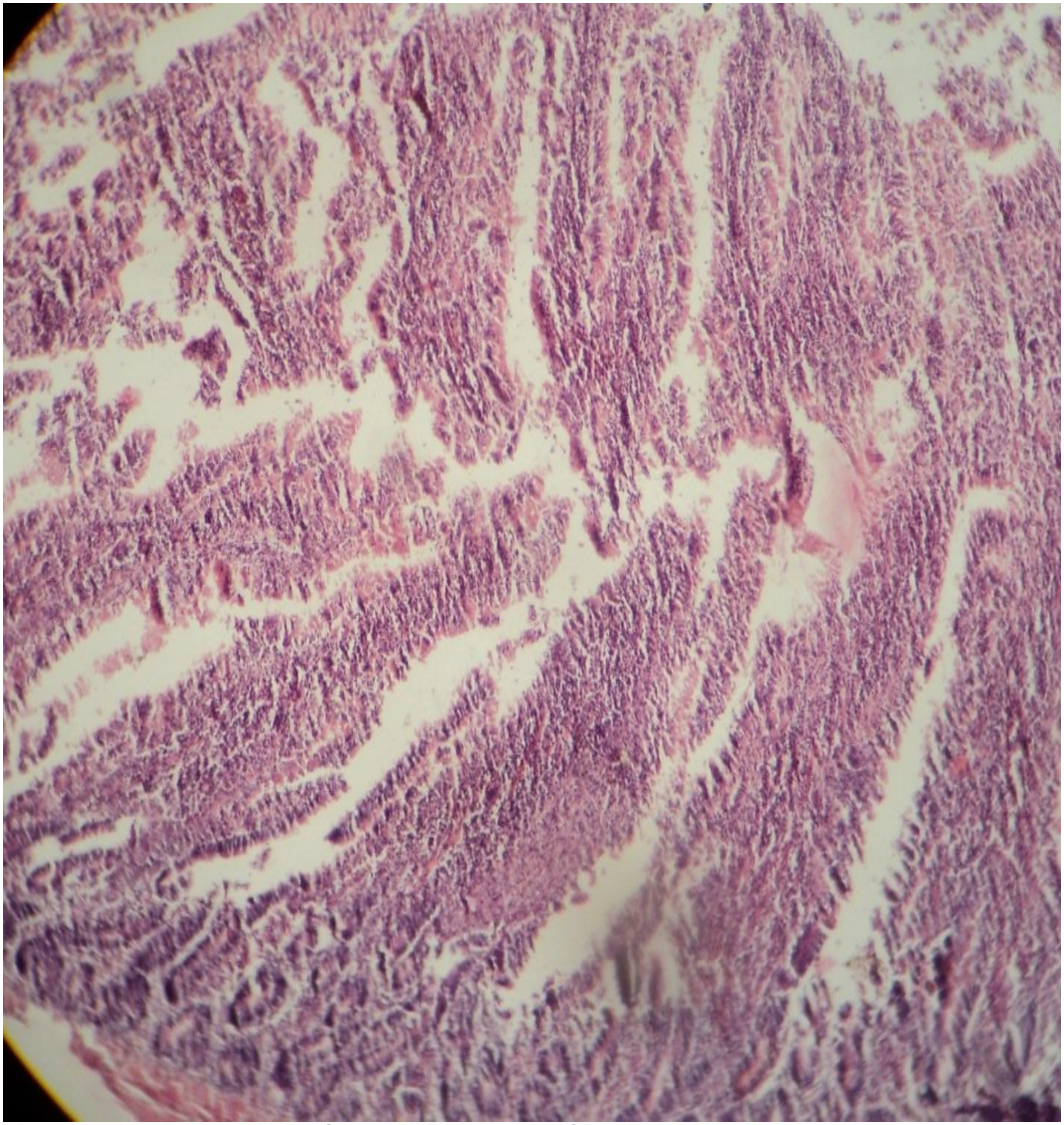

Plate V: photomicrograph of the small intestine of broiler chicken on 0g supplementation of Black seed, showing no histopathological changes (H and E X 200)

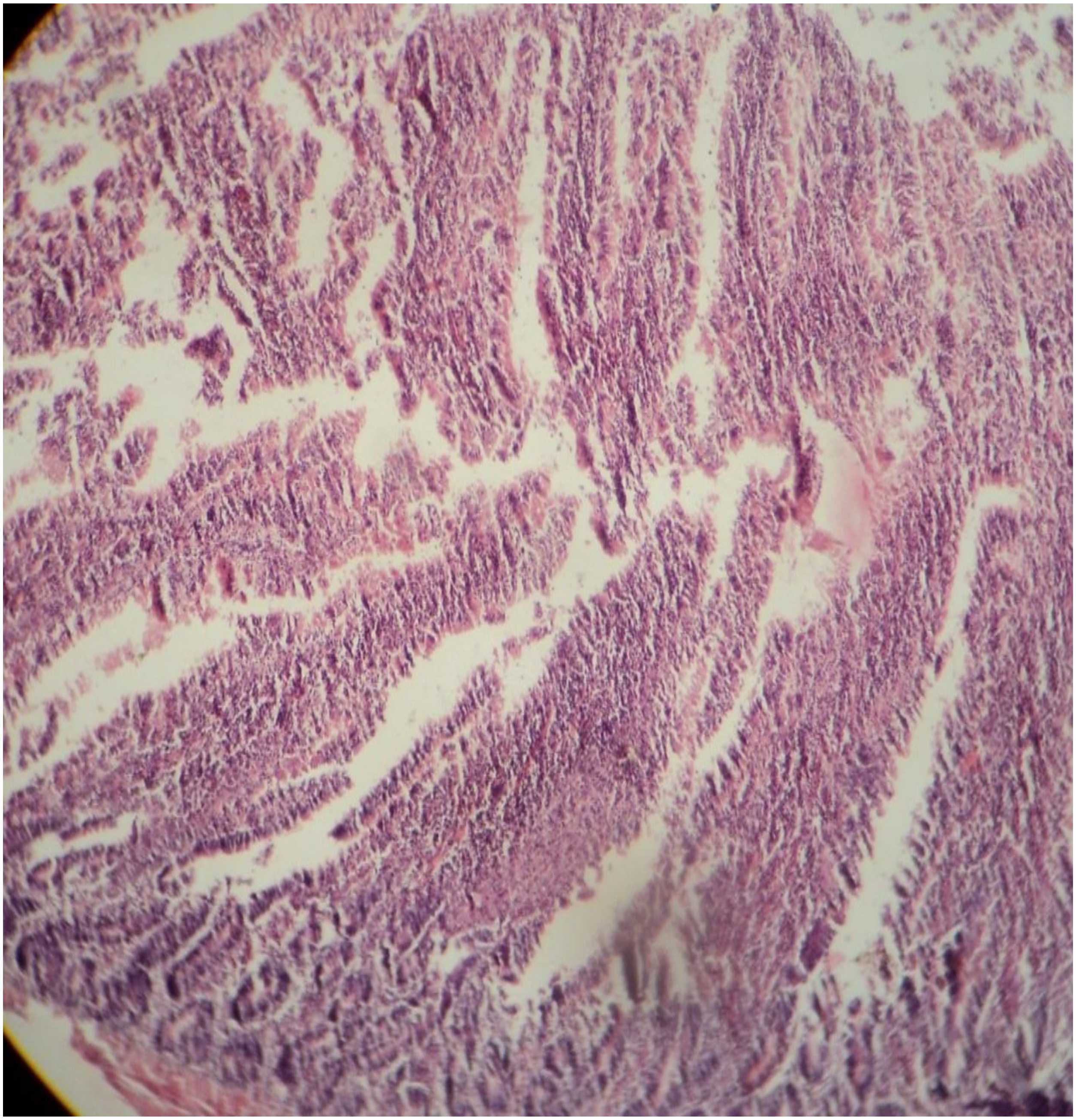

Plate VI: photomicrograph of the small intestine of broiler chicken on 10g supplementation of Black seed, showing no histopathological changes (H and E X 200)

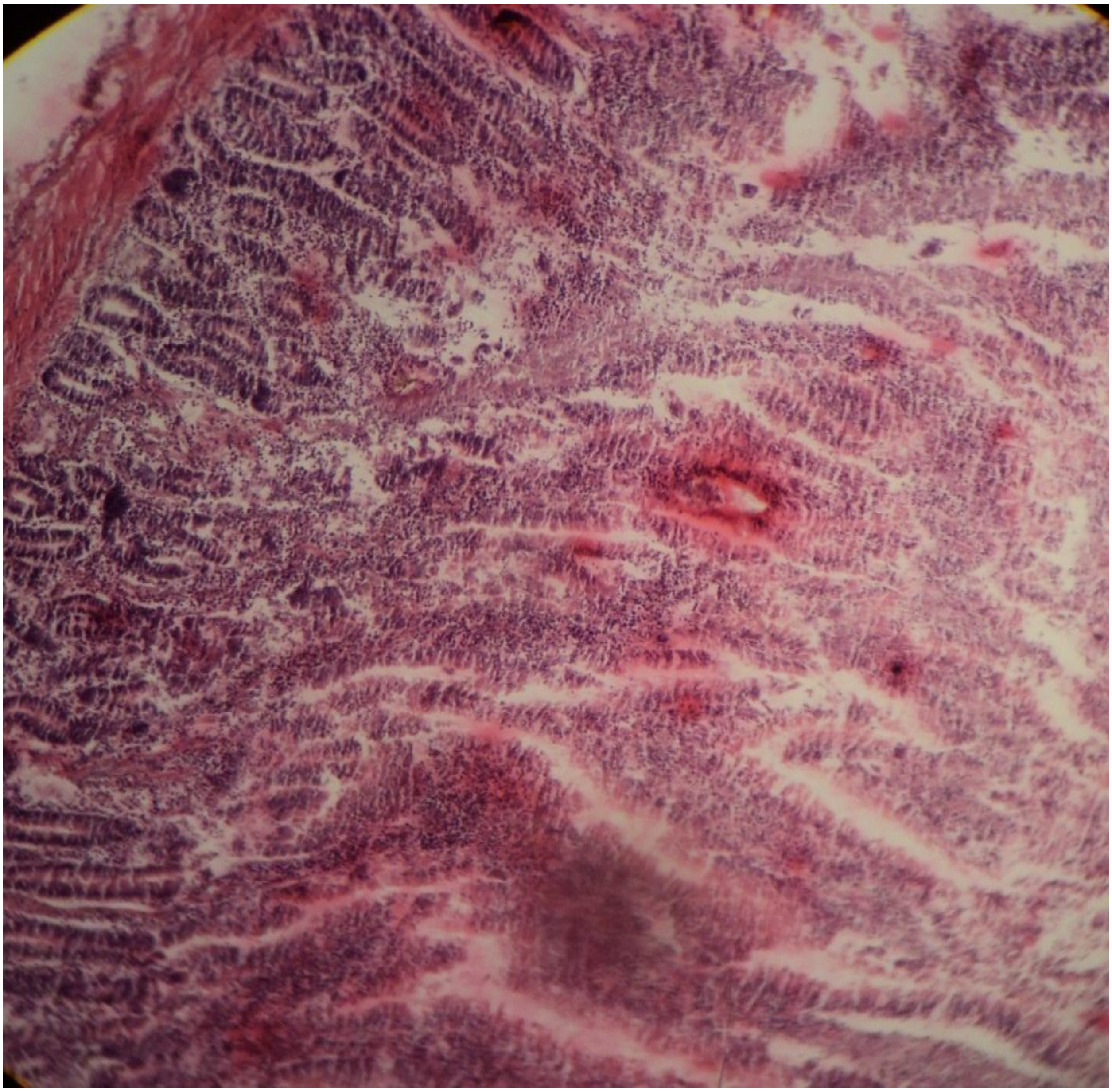

Plate VII: photomicrograph of the small intestine of broiler chicken on 20g supplementation of Black seed, showing no histopathological changes (H and E X 200)

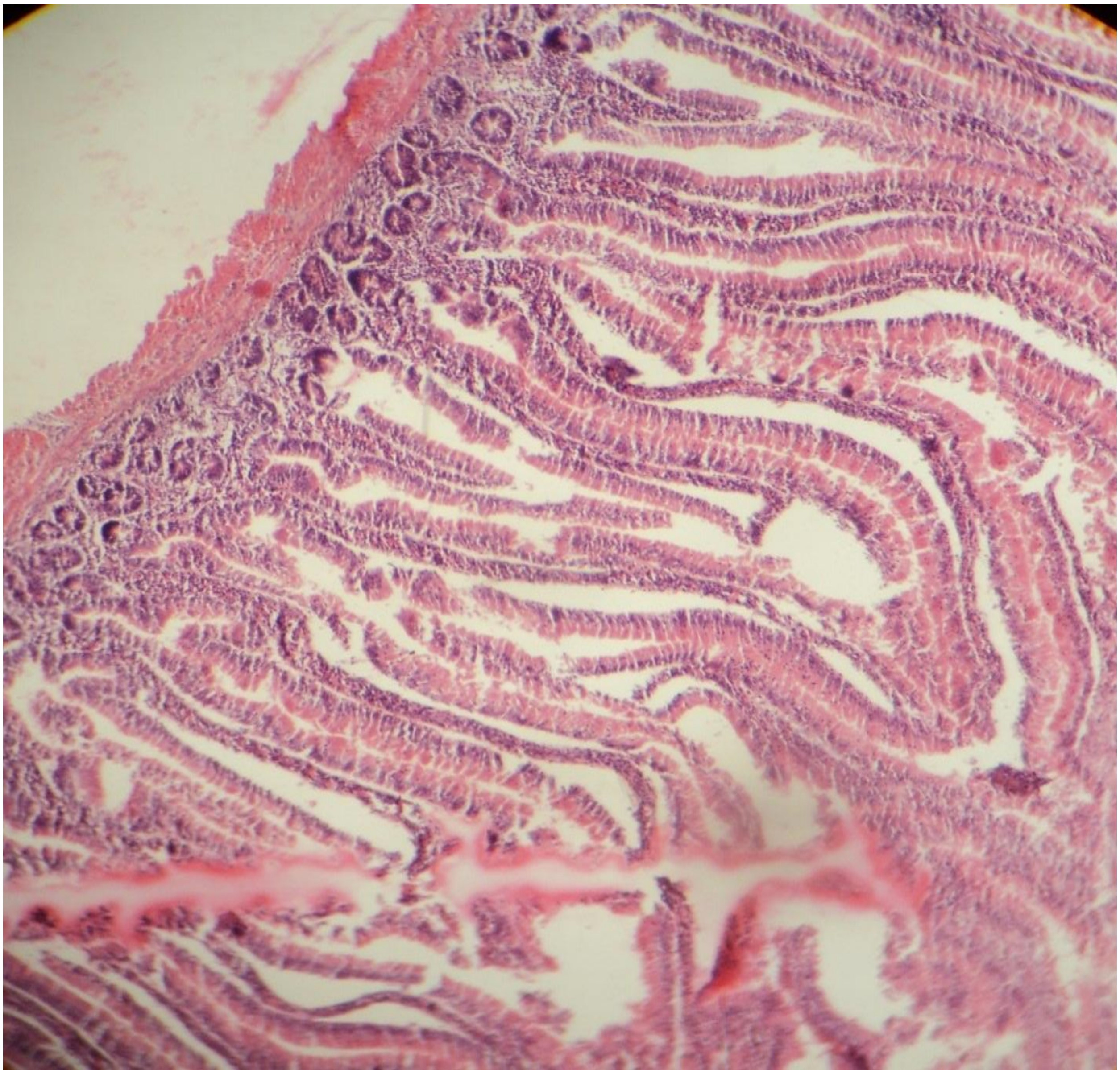

Plate VIII: photomicrograph of the small intestine of broiler chicken on 30g supplementation of Black seed, showing no histopathological changes (H and E X 200)

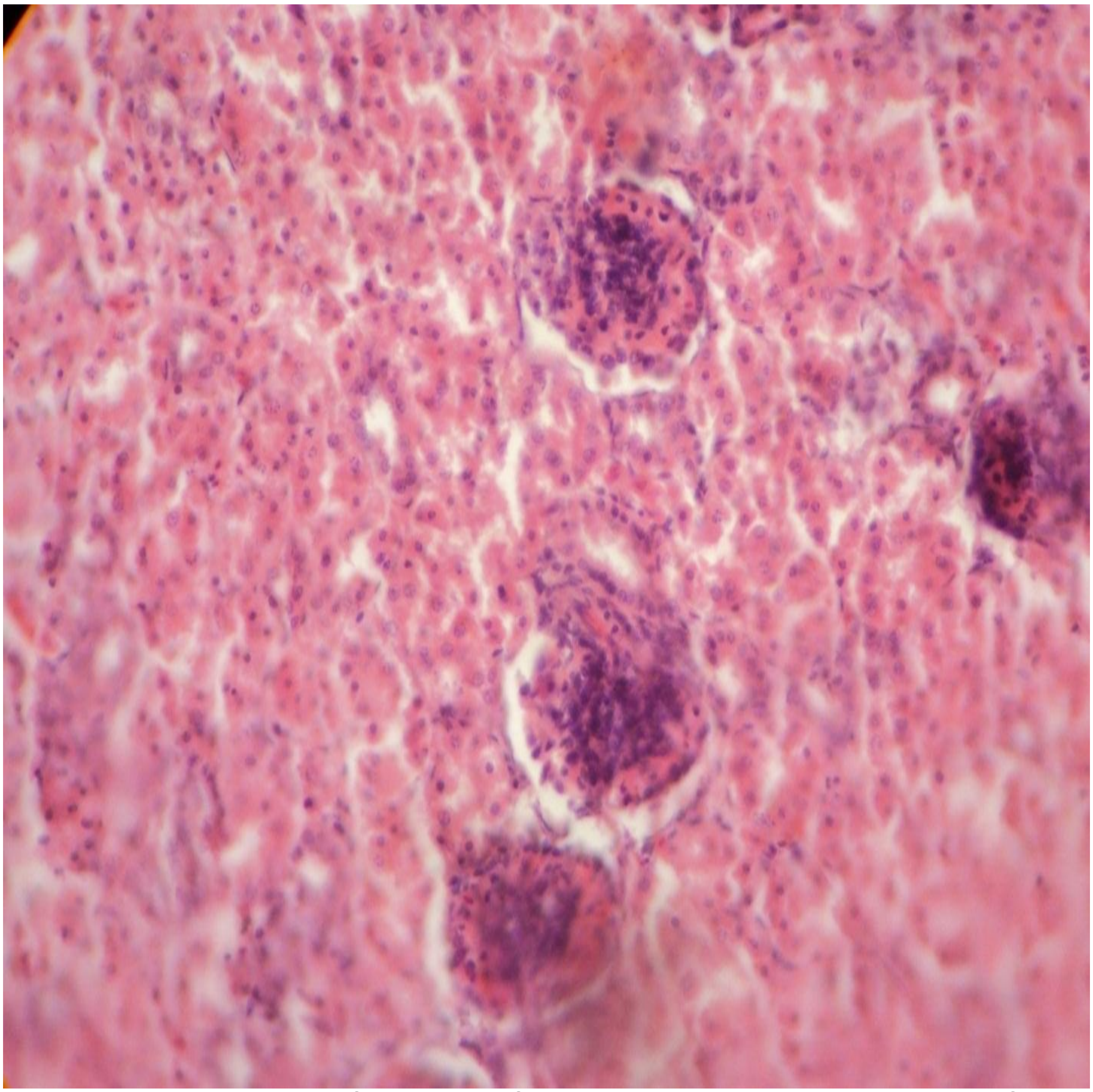

Plate IX: Photomicrograph of the kidney of broiler chicken on 0g supplementation of Black seed, showing no histopathological changes (H and E X 200)

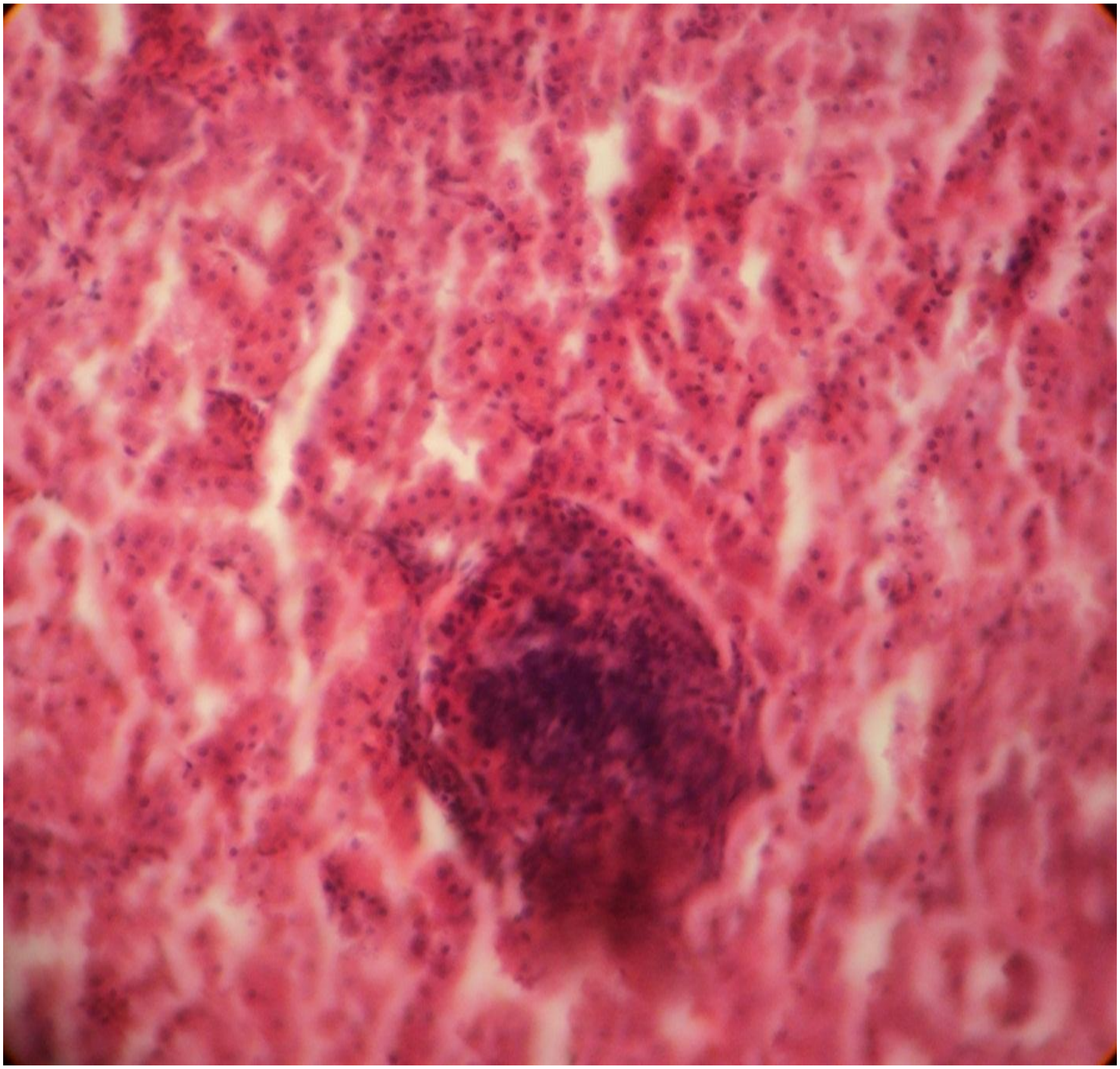

Plate X: Photomicrograph of the kidney of broiler chicken on 10g supplementation of Black seed, showing no histopathological changes (H and E X 200)

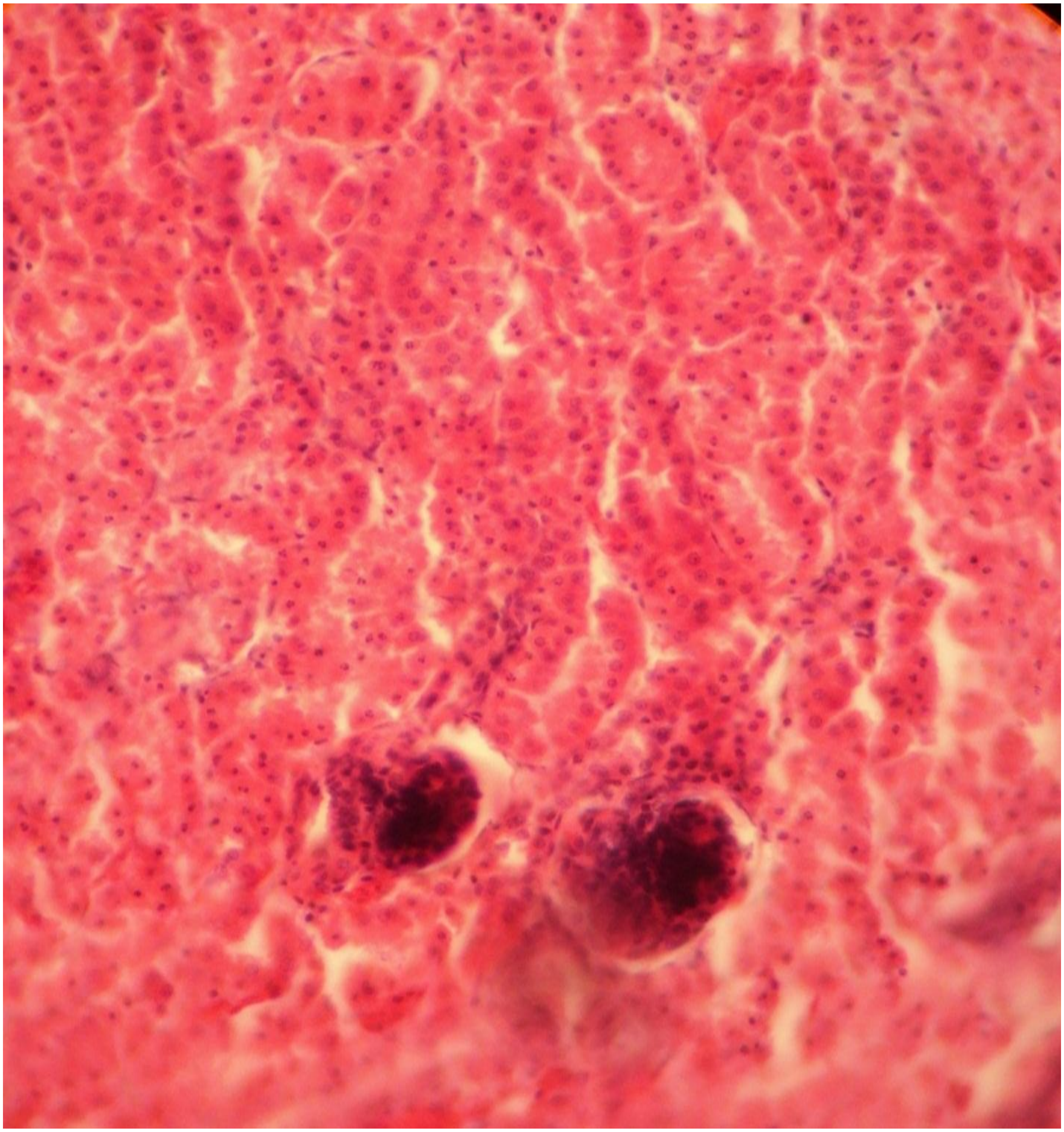

Plate XI: Photomicrograph of the kidney of broiler chicken on 20g supplementation of Black seed, showing no histopathological changes (H and E X 200)

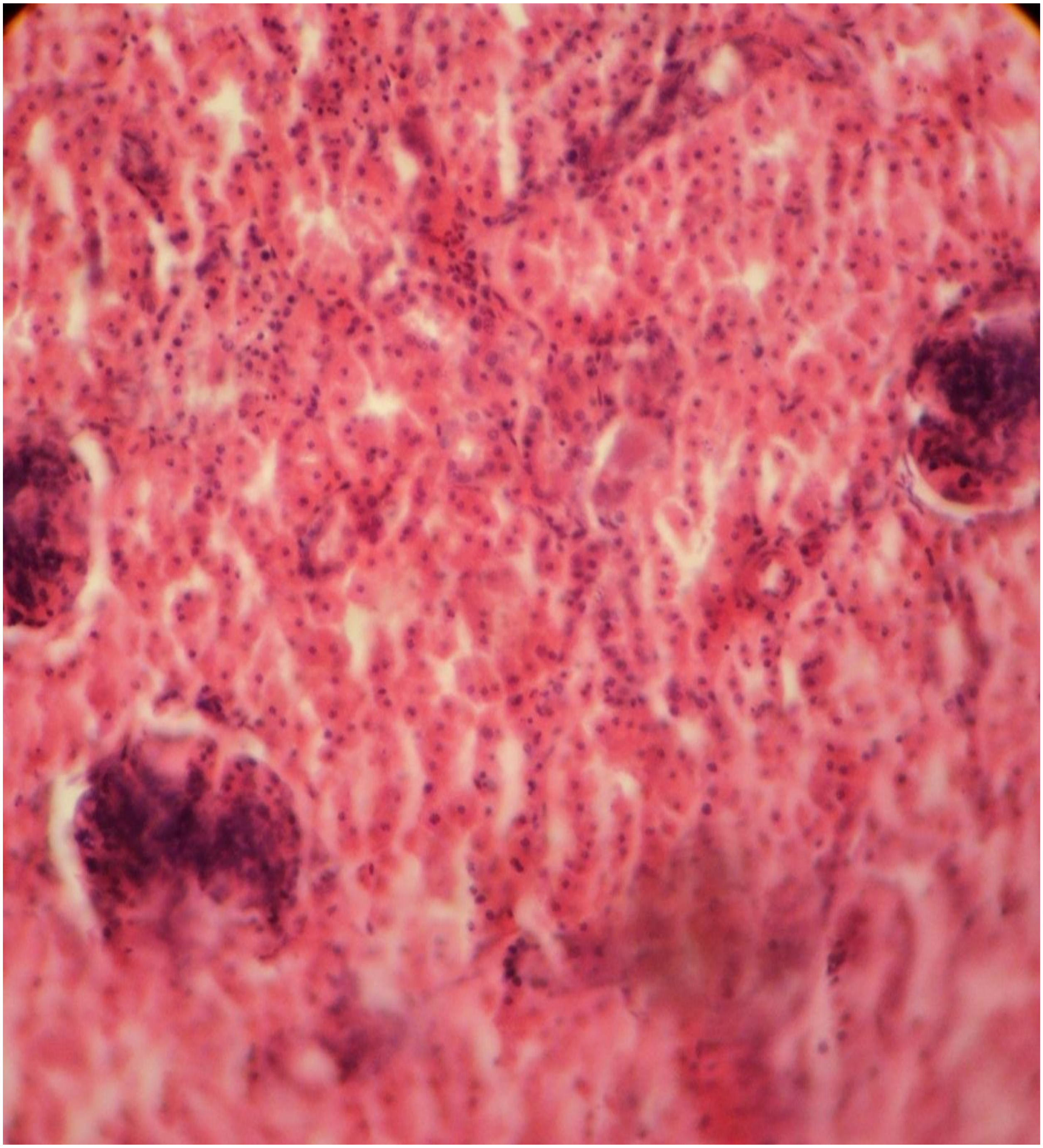

Plate XII: Photomicrograph of the kidney of broiler chicken on 30g supplementation of Black seed, showing no histopathological changes (H and E X 200)

CHAPTER FIVE

DISSCUSION

5.1 Chemical compositions of Black seed (*Nigella sativa*) powder

The result of chemical composition of Black seed powder presented is in close conformity with that reported by Ashraf *et al*.(2006), (Anon.,2006), Cheikh-Rouhou*et al*. (2007) and Muhammad *et al.*(2009), although (Anon 2006) reported the result of crude protein to be 21% which is slightly higher than the findings in this research, the presence of the constituents found may be the basis of increased growth performance and reduced effect of heat stress in the birds that were given the supplementation as it possesses lots of antioxidants such as Vit A, vit. B, Vit. C and Vit E with Minerals which include Na, Ca, Mg and K. . Although slightdifferences may occur due to environmental and soil variability that exist between different agronomical locations.

5.2 Weight Gain of Broiler Chickens at 4weeeks during hot season

The result of weight gain reported agrees with the findings of El-ghammary*et al*. (2002), Hassan *et al*. (2004) and Siddiqui and Abu Sayed (2015),who reported an increaese in growth performance of broiler chickens supplemented with Black seed,it has been established that weight gain is driven by feed conversion efficiency therefore animals with better weight gain has higher feed efficiency, this indicates that the animals in the supplemented group would have higher weight gain. Although in this study feed intake was not taken into cognizance but it was reported that Black seed improvedfeed efficiency in the broiler rations compared to the control, (Osman and El-Barody 1999), Al-homidan*et al*.,(2002). Similarly, the result of the mortality showing higher percentage in the control group compared to the group supplemented with Black seed is in agreement with the report given by Siddiqui and Abu Sayed (2015) that survivability percentage increased as Black seed suplementaion increases, this may be due to the presence of pharmacologically active constituents in Black seed such as thymoquinone, thymohydroquinone and nigellone which may have improved the immunity of the chicks as reported by Osman and El-Barody (1999) and confirmed by Mahmoud et al. (2002) that the active ingredient (thymoquinone) in Black seed have hepatoprotective effects which may result in a sound healthy chickens with stong immune system that feed adequately with no or minimal heat stress that leads to beter weight gain and low mortality.

5.3 Thermoregulatory performance of Broiler Chickens during Hot Season

As observed in this study, rectal temperature and respiratory rate of heat stressed broiler at the end of 4^{th} and 8^{th} weeks of age were significantly ($P<0.05$) decreased by feeding experimental treatmentscompared with the control.Belland Freeman (1971) reported that young chickensswitch from poikilothermic to homeothermic status at the age of four weeks,when they are able to maintain their body temperature by means of their ownmetabolic processes. At this developmental stage, chickens begin to accumulateenormous amounts of muscle. This was why their records were taken at the age of the 4^{th} and 8^{th} weeks which is the end of the fattening period. It is wellknown, that high temperature conditions resulted in physiological changes and reactions of the bird such as the increase of body temperature, respiration rate and heart rate, as the birds consume more energy and accordingly the remaining netenergy for growth is decreasedHermes *et al*. (2011). It is well known that birds in general adjust their feeding as the ambient temperature rises which leads to the rise in the internal body temperature, the reduction in the rectal temperature with 3.5% in the starter phase and 3% in the finisher phase , and respiratory rate with 13.65% in the starter phase and 7.37% in the finisher phase of the chickens compared to the control may be the reason for better weight gain in both phases as the chickens were able to feed better.

Naqvi*etal*. (1995) reported that environmental temperatures above 28ºC causes heat- induced physiological stress, which leads to increase in respiration rate and rectal temperature. The reductions in thermoregulatory parameters corroborated with the findings of Herms et al. (2010) who reported a decrease in both respiratory rate and rectal temperature of broiler chickens fed with different forms and levels of black seed, Tollba and Hassan (2003) that adding 1% Black seed asnatural feed additives to diets of heat stressed broiler significantly decreased theirRespiration rate and body temperature compared with the control.

5.4. Weight Gain and Weight of Some Internal Organs of Broiler Chickens at 8weeks during hot season

The results total weight gain and average daily weight gain increases compaired with the control,this is in agreement with the findings reported by Tolba *et al*. (2005) who found a significant increase in final body weight of broiler chickens fed diet supplemented with different levels of Black seed.This may be due to the enhancement of metabolism as the supplement might have increased the thyroid hormone concentrations which could lead to an increase in the metabolic rate of the chickens which can lead to enhanced amino acid utilization as reported by More et al. (1980) and Mandour *et al.* (1998).

The increase in the weight of the gizzard, proventriculus and kidney disagrees with the result of Hermes*et al.*(2010) who found no increase in the relative weight of internal organs, the study shows an increase in the weight of the lymphoid organs(thymus, spleen and liver) Osman and El-barody (1999), Elkhaiaty *et al.*(2002) and Radwan (2003) reported that the increase in the weight of thymus, spleen and liver may be as a result of the effective substance Nigellon which activates lymphoid organs for better immune response.

5.5 Haematological Parameters of Broiler Chickens at 8week during Hot Season.

The result of the haematolgy at the end of both the 4^{th} weeks and 8^{th} weeks for PCV and Hbwere all within the normal range of 24-45% for PCV and 7-13(g/dl) for Hb reported byMistruka and Rawnsley (1977), the RBC values are also within the normal range of 2-4.0(10^{12}/l) reported by Mistruka and Rawnsley (1977). The WBC are slightlylower at 4^{th} week and higher at the 8^{th} week although differences were only significant at 8^{th} weeks, Aguihe *et al.* (2014) reported that higher WBC values are good indications of no pathological effect. These results are in contrast with the report of Al-homidan *et al.*(2002) who reportedno difference in WBC with the control and it agrees with the result obtained byHerms *et al.* (2010) who reported increase in the values with no statistical difference. This can be supported by the fact thatBlack seed can be used as an immune and growth stimulant as reported by El-Sayed andHashem (2000). Haematology is an index and it reflects the effect of dietery treatments on the animals interms of type and quantity ingested and available for the animal to meet its physiological, biochemical and metabolic requirements (Ewuola *et al.*,2004).

The result of the serum biochemistry revealed no significant difference in the total protein, albumin and globulin but shows slight increase as the levels of supplementation increases. The increase in the total protein suggest good quality protein in the supplement (Eggum 1980), the highest value of globulin recorded in the treatment supplemented with 30 gkg^{-1} while that of albumin was recorded in the treatment supplemented with 20 gkg^{-1} supplementation is in agreement with the report of Babatunde and Oluyemi (2000) that the higher the value of serum globulin the better the ability to fight against diseases and the increase in globulin may be due to the immuno-stimulant effect of Black seed (Aqel, 1993).The results of the albumin and globulin agrees with the findings of Herms *et al.*(2011) The values of total cholesterol found are within the range of 129-279 (Jain, 1993) and its reductions as the level of supplementation increases may be due to high content of unsaturated fatty acids particularly linolic acid (Abdel-Aal and Attia, 1993). Tolba and Hassan (2003) suggested that the compound may stimulate cholesterol excreation into the intestine, being oxidised to bile acids. The reslults of total cholesterol and triglycerides agrees with the findings of El-Dakhakhny*et al.*(2000), Islam *et al.*(2011) and Siddiqui*et al.*(2015) that Black seed fixed oil could have favorable impact on serum lipid profile by decreasing total cholesterol and triglycerides Akhtar*et al.,* (2003) who supplemented commercial layer-ration with black cumin seeds observed that serum triglycerides and total cholesterol contents were reduced. The reducing effect is particularly helpful to humans that are very conscious of cholesterol intake.

CHAPTER SIX

SUMMARY, CONCLUTION AND RECOMMENDATIONS

6.1 SUMMARY

A total of 360 broiler chickens were divided into two groups with 180 per group. Each group were used to conduct a separate experiment in the starter and finisher phases with different levels of black seed (*Nigella sativa*) powder supplementation, The studies were conducted on the effect ofvariiying levels of black seed (*Nigella sativa*) powder supplementation on the performance, thermoregulatory parameters, blood profile and histopathology of broiler chickens during hot season.

It was observed that the supplement has a lot of antioxidant comprising of some minerals, vitamins including some essentials amino acids and some pharmacologically active ingredients that positively fight against harmful micro-organisms.

In both the two experiments it was clearly shown that the supplements have potentials of reducing heat stress and improve on the performance of broiler chickens during hot season.

6.2 Conclusion

It is concluded that the supplementation of black seed during hot season can reduce the effect of heat stress and improve both the performance, physiological responses and the immunity of broiler chickens with no toxic effect on the vital organs of the Animal.

6.3 Recommendations

From this study it can therefore, be recommended that:

- Black seed can be supplemented during hot season in broiler chickens for better performance best at 15 gkg^{-1} at the starter phase and 20gkg^{-1} at the finisher phase.
- Black seed can also be used as broad spectrum antibiotic and feed additive to reduce the effect of synthetic drug's residual effect to the consumers.
- The Agronomical production of Black seed should be encouraged to our farmers so as to eliminate its importation into the country as it has a wide range of utilization by both Humans and Animals.

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