EFFECT OF DIETARY VITAMIN A AND *NIGELLA SATIVA* ON THE PERFORMANCE OF BROILER CHICKS

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بسم الله الرحمن الرحيم

﴿وقل ربى زدنى علماً﴾

سورة طه الآية 114

In the name of Allah, the Beneficent, the Merciful

(And say: May Lord increase me in knowledge)

(Surah 20: 114, Taha, Holly Quran)

DEDICATION

To the two most important people in my life ; my father , Mohamed , and my mother , Fayza .

To the soul of my grandmother .

To my brother and sisters and my nephews

With love and gratuities

ABSTRACT

A study was conducted to assess the effect of feeding different added levels of vitamin A and *Nigella sativa* seeds on broiler performance, blood chemistry and carcass characteristics.

One-hundred fourty four, one-day old unsexed (Lohman) broiler chicks were divided randomly into eight groups, each represented a treatment (18 birds/treatment), with 2 replicates for each treatment 2x4 factorial arrangement in a completely randomized design was used. The experimental basal rations were formulated to meet requirement for essential nutrients for broiler chicks according to NRC (1984) recommendation. Four graded levels of added vitamin A (0, 3000, 4500 and 9000 IU/kg) and two levels of *Nigella sativa* (0, 0.25%) were used. The experiment lasted for eight weeks.

Body weight, weight gain, feed intake and feed conversion ratio were determined. In addition blood chemistry, absolute weight of internal organs and carcass characteristics were measured.

The added level 3000 IU of vitamin A significantly increased weight gain ($P \leq 0.01$), decreased feed conversion ratio ($P \leq 0.01$) and increased muscle: bone ratio ($P \leq 0.05$). The level 0.25% of *Nigella sativa*

with no added vitamin A significantly ($P \leq 0.01$) increased body weight and weight gain.

High level of added vitamin A decreased absolute weight of liver ($P \leq 0.01$), and increased fat content of muscles ($P \leq 0.01$) and abdominal fat ($P \leq 0.01$). Vitamin A and *Nigella sativa* significantly affect serum Ca, P, Zn and alkaline phosphatase (alk.ase) and cholesterol ($P \leq 0.05$). Addition of 0.25% *Nigella sativa* significantly decreased serum cholesterol ($P \leq 0.01$).

The treatments had no significant effect on serum glucose and ash content of meat. Significant interactions between vitamin A and *Nigella sativa* were observed on body weight gain, feed conversion ration, feed intake ($P \leq 0.01$, $P \leq 0.05$ and $P \leq 0.01$), respectively. Liver weight ($P \leq 0.01$), heart weight ($P \leq 0.01$), serum Ca ($P \leq 0.01$), IP ($P \leq 0.01$), cholesterol ($P \leq 0.01$) and alk.ase ($P \leq 0.01$), moisture content ($P \leq 0.01$) and EE ($P \leq 0.01$) were also affected by the interaction.

However in the present study the added levels of vitamin A and 0.25% level of *Nigella sativa* have no adverse effect on performance, blood chemistry and carcass characteristics of broiler chicks.

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CHAPTER ONE
INTRODUCTION

Poultry is a good source of high quality protein in form of meat and eggs. So it is among the most efficient biological machine for reproduction and production that makes it cheap for the consumer. However, feed accounts about 70% of the cost of poultry production. Recent research tended towards using non-conventional feed resources as additives in order to maximize the production and reduce the cost.

It is well known that appropriate vitamin A supplementation of poultry feeds is critical for growth, maximum egg production and egg hatchability. In addition, vitamin A has been described as an epithelial protector and anti-infective vitamin (Zhuge and Klopfenstein, 1986; Kolb, 1997).

Nigella sativa is a famous plant because of its various uses, especially in the old people medicine. Traditionally, the seeds are mixed with honey and the mixture is taken early in the morning to stimulate the appetite. Seeds are also used as a remedy for many diseases and general anti-poison therapy (Bolous, 1993).

The volatile oil of *Nigella sativa* seeds posses an anti-microbial property against gram-positive microorganisms (El Alfy *et al.*, 1975).

To the best of our knowledge, only one study has been conducted for evaluating the nutritive value of *Nigella sativa* seeds as feed additives for broiler chicks in Sudan (Abdel Majeed, 1999).

However, findings of the above mentioned literature that both vitamin A and *Nigella sativa* have a positive effect on growth and immunity. Therefore, the attempts of the present study is to investigate the response of feeding broiler chicks different added levels of vitamin A, *Nigella sativa* and their interaction on general performance, blood chemistry and carcass characteristics.

CHAPTER TWO
LITERATURE REVIEW

2.1 Chemistry of Vitamin A

Vitamin A, or retinol, is a polyisoprenoid compound containing a cyclohexenyl ring (Fig. 2.1).

Vitamin A is a generic term referring to all compounds other than the carotenoilds that exhibit the biologic activity of retinol. In recent years, the term retinoids has been used to describe both the natural forms and the synthetic analogs of retinol. Vitamin A is necessary in higher animals to support growth and health and is particularly necessary for vision, reproduction, mucus secretion and the maintenance of differentiated epithelia (Martin *et al.*, 1981).

2.2 Vitamin A Occurrence

In animal products, dietary vitamin A exists as long chain fatty acid esters of retinol. In vegetables dietary vitamin A exists as a provitamin in the form of β-carotene, which are yellow pigments (Fig. 2.2).

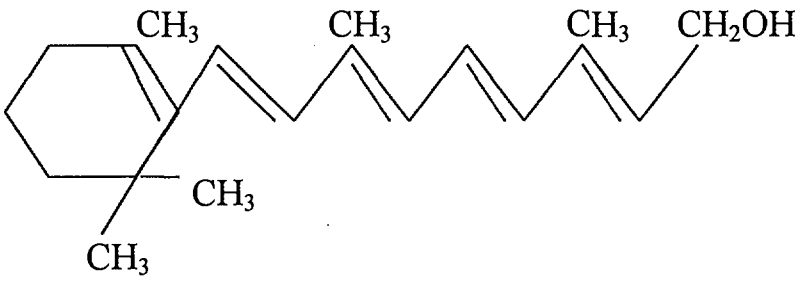


Fig. 2.1. Retinol (vitamin A)

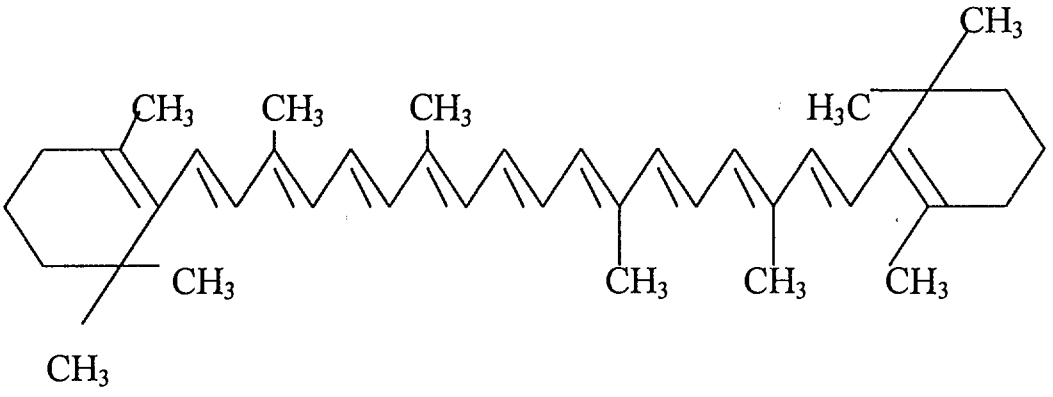


Fig. 2.2 β-carotene

The other naturally-occurring provitamins are α, γ carotenes, myxoxanthin is found in algae, Echinenon in the red fungus, Aphanin and Aphanicin also in certain algae, Torularhodin in red yeasts and leprotin in *Mycobacterium phlei* (Takeda *et al.*, 1941; Drumm *et al.*, 1945; Karrer *et al.*, 1946; Tischen *et al.*, 1936and Grundmann *et al.*, 1937).

2.2.1 Occurrence in Plants

Kutsky (1973) reported that the provitamin carotenoids are generally found in green leafy materials. In fruits (apricots, yellow melons, peaches, prunes). In vegetables (beet greens, broccoli greens, carrots, endive, kale, lettuce, mint, mustard, parsley, pumpkins, spinach, sweet potatoes, turnip greens, cress) also in small quantities in most nuts.

2.2.2 Occurrence in Animals

Vitamin A presents in all vertebrates, and carotenoids in certain invertebrates. Animal organisms and products show an accumulation of vitamin A in liver, heart, lungs, adrenals, retina, kidney, milk (Kutsky, 1973). Milk is relatively rich in vitamin A and so are all fat-containing milk products (Ahmad, 1931).

In blood, about one-fifth of total vitamin content is present as ester, this figure rises with large vitamin A supplies, while the vitamin A alcohol level in the blood remains unchanged (Popper, 1948).

Lazar *et al.* (1986) reported that highest concentrations of β-carotene were found in egg yolks from hens given diets supplemented with ascorbic acid concentrations. Also the same authors showed that increasing the amounts of ascorbic acid, vitamin A and cholecalciferol in diets for laying hens increased the concentrations of Ca and P in egg yolk. In hen's egg, it was reported by Cai-Hy *et al.* (1993) that most of the retinol and other nutrients in the yolk sac were utilized in the first 18 to 48 h after hatching.

Liver stores about 70 to 90% of the body content of vitamin A (Wolf, 1984), because liver storage of vitamin A is a linear function of vitamin A intake (Hennig *et al.*,1985). Fish liver oils, being particularly rich in vitamin A, are only natural source for commercial extraction, the livers of polar bears are the richest source of vitamin A (Rodahl, 1950).

Liver vitamin A is a far better response criterion for assessing vitamin A status than either growth or plasma vitamin A. (Erdman *et al.*, (1986). Therefore, the vitamin content of liver is a useful criterion in determining the stability, availability and utilization of vitamin A from different products (Harms *et al.*1955).

2.3 Vitamin A Metabolism

Vitamin A metabolism in the chicken is basically similar to that in human (Abe *et al.*1975).

2.3.1 Conversion of β-carotene to Vitamin A *In Vivo*

Thampson *et al.* (1950) demonstrated that vitamin A appeared in the wall of small intestine 0.5 hours and in liver 3.5 hours, after dossing chicks with carotene. It was noticed that the conversion of β-carotene to vitamin A was not impaired after ligation of the bile duct, removal of the small intestines, or removal of 60 to 75% of liver. These results suggest that various body tissues are able to convert carotene to vitamin A (Bieri and Pollard, 1954).

2.3.2 Digestion and Absorption of Vitamin A

The digestion of fat-soluble vitamins A, D, E and K take place in the proximal part of small intestine through three steps, emulsification, enzymatic hydrolysis; and solubilization of insoluble lipids in amicellar phase (Carey, 1983). Lipid-soluble vitamins dissolve in small fat globules (Weber, 1981).

Hollander (1981) and Carey (1983) reported that the lipid molecules, including lipid-soluble vitamins and constituents of the mixed micelle except bile salt

Weber (1981) showed that lipid-soluble vitamins are in ester form such as vitamin A. These lipid-soluble vitamins esters are incorporated into mixed micelles and solubilized in the aqueous micellar phase. Pancreatic carboxylic ester hydrolase then hydrolyses the esters of vitamin A, D_3 and E (Lomburdo *et al.*, 1980). The retinol esters are hydrolyzed within the intestinal lumen and absorbed directly in the intestines. The ingested β-carotenes are oxidatively cleaved by β-carotene dioxygenase. This cleavage utilizes molecular O_2 and requires bile salts to generate 2 molecules of retinaldehyde. Also in the intestinal mucosa, the retinaldehyde is reduced by a specific reductase utilizing NADPH to form retinol.

The absorbed retinol is reesterified with long chain saturated fatty acids, incorporated into chylomicrons, to the blood stream through retinal-binding protein (RBP) (Martin *et al.*1981).

2.3.3 Storage and Release of Vitamin A

Stored retinol is mobilized from the liver by hydrolysis of its ester and by binding of retinol to aporetinol-binding protein, which is synthesized in the hepatocyte. The retinol-binding protein complex, called holoretinol-binding protein, then enters the circulation and delivers retinol to the target tissues. When retinol enters its target cell, it is promptly bound to a cellular-retinol-binding protein (CRBP) distinct from the retinol-binding protein present in serum. The (CRBP) transports the retinol within

the cell, where it appears to bind specifically to nuclear proteins, perhaps with a function analogous to that of the intracellular steroid hormone receptor molecules.

It appears that vitamin A toxicity occurs *in vivo* only after the capacity of retinol-binding protein has been exceeded and the cells exposed to unbound retinol. Thus, the nonspecific and unregulated delivery of free vitamin A to tissues may lead to vitamin A toxicity (Martin *et al.*, 1981).

2.4 The Role of Vitamin A

Vitamin A nutrition has been implicated an adjunct in the protection of animals against a variety of stresses such as cold exposure (Odagiri *et al.*1961), X-irradiation (Ershoff, 1952) and infectious diseases (Panda *et al.* 1963).

2.4.1 Growth

Vitamin A markedly influences rate of growth and average body weight at various time is a valid parameter for estimating requirement of animals for this vitamin (Hafez and Dyer, 1969).

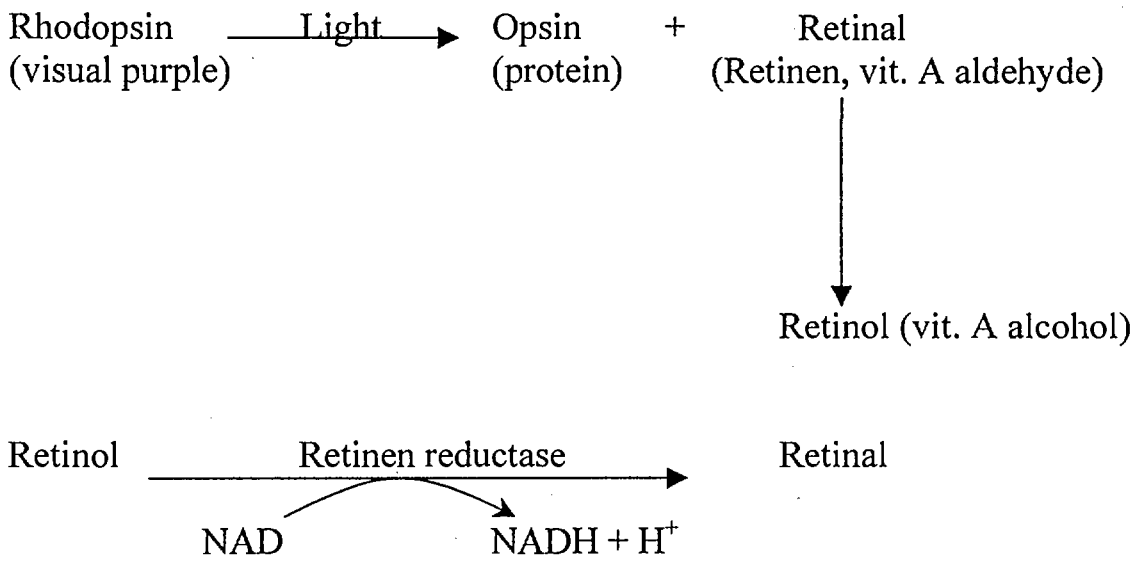
Parrish *et al.* (1963) concluded that, as a result of their study, that for growth of chicks, carotene and vitamin A are utilized to a similar degree on a unit basis, but under certain circumstances, storage of vitamin A by the chick may be somewhat higher if vitamin A fed. Reddy *et al.* (1989) reported that the effect of vitamin A on weight gain was very conspicuous

with low vitamin A diet, growth was poor and mortality was high. Jensen *et al.* (1983) found that chicks fed vitamin A at a level of 1200 IU/kg diet showed a reduction in growth, reduced shank colour and an increased incidence of leg abnormalities.

Huan *et al.* (1992) reported that depression of serum vitamin A concentration occurred at the same time as the decrease in growth rate.

2.4.2 Role in Vision

Harper *et al.* (1979), reported that the retinal pigment (rhodopsin), or visual purple, which has long been recognized in the rod cells of the retina, is a conjugated protein. When light strikes the retina, two reactions happen. Regeneration of rohdopsin takes place in the dark under normal circumstances, equilibrium is maintained in the retina of the eye such that the rate of breakdown of rhodopsin is equaled to the rate of regeneration. If, however, a deficiency of vitamin A exists, the rate of regeneration of rhodopsin is retarded, probably because of storage of substances (Harper *et al.*, 1979).



The same authors reported that the cone cells of the retina are responsible for detection of colour. There are three different retinal containing pigments in the cones; porphyrinopsin, iodopsin and cyanopsin sensitive to red, green and blue, respectively. When light strikes the retina, one or more of these pigments are bleached in accordance with the colour of light and retinal and opsin are released.

Retinol (vitamin A) is required not only for rod but also for cone vision. The only chemical difference between rod and cone visual pigments is that within the opsin component.

2.4.3 Maintenance of Skin and Epithelial Cells

Vitamin A plays an important part in maintaining the function of epithelial and glandular cells, thus protecting them from infection.

Ganguly *et al.* (1980) drew attention to vitamin A importance in cell proliferation, as evidenced by studies in epithelia of the trachea, intestine, cornea, oestrogen-primed chick, oviduct and regenerating liver cells. They suggested that vitamin A may have a common role in the normal proliferation and perhaps differentiation of cells in regenerating tissue.

Studies by Morales *et al.* (1997) demonstrated that level up to 44800 IU/kg of vitamin A in finishing diets for broiler did not have an effect on skin pigmentation or performance.

In vitro retinoic acid increases the number of epidermal growth receptors on surfaces of cultured cells, stimulation of differentiation of embrynoal carcinoma cells, prevention of the expression of the Epstein-Barr virus in virus-infected cells, and the reversible inhibition of growth of human breast cancer cell lines in long-term tissue culture (Martin *et al.*, 1981).

2.4.4 Bone Development

Vitamin A is necessary for normal skeletal development cellular differentiation and maintenance of epithelial and skeletal tissues (Barnicot and Datta, 1972).

Haviv and Wolf (1967) found that vitamin A-deficient chicks bone was longer, thinner and had an increased level of organic matrix,

chronodroitin sulfate and water content than normal bone. Vitamin A-deficient chicks have a higher content of phospholipids in the epiphyseal cartilage than normal chicks (Haviv and Tal, 1974). Veltman *et al.* (1986) found that chicks can tolerate 30 times the requirement of vitamin A without showing any skeletal or performance impairment.

2.4.5 Resistance to Infection

Vitamin A has been described as an anti-infective vitamin. It plays a key role in the health of immune system. Its effects in immune cells that immunocompetent cells (immunocytes) contain the retinoic acid receptor α, which transport all-trans and 9-cis-retinoic acid into the nucleus, where it induces the production of specific types of information carrier (mRNA) for protein synthesis. Animals well supplied with vitamin A break down pathogenic microorganism more rapidly than deficient counterparts. The uptake of retinoic acid increase the ability of monocytes and macrophages to destroy bacteria and viruses (Kolb, 1997).

Lessard *et al.* (1997) demonstrated that both humoral and cellular immune responses were modulated by levels of vitamin A in the diet, and suggest that vitamin A deficient chickens developed a lower immune response, whereas the chickens fed highly enriched vitamin A diet showed a higher immune response.

Friedman and Sklan (1990) showed that vitamin A depletion caused severe impairment of T. lymphocyte activity in both chicks and rats, and this was directly related to vitamin A status in both species. Immune responses impairment preceded other manifestation of vitamin A deficiency.

Taylor *et al.* (1946) found that when the conditions were favorable for the development of cecal coccidiosis, that the severity of the disease was enhanced in chicken receiving less than the minimum requirement of vitamin A. Further more, Richter *et al.* (1986) reported that vitamin A supplementation reduced oocyst sporulation of *Emeria tenela.*

2.4.6 Maintenance of Adrenal Cortex and Steroid Hormone

Some research evidence suggests that vitamin A is active in the synthesis of certain steroid hormones of the adrenal cortex as well as in the maintenance of the integrity of the adrenal cortex. Studies by Johnson and Wolf (1960) demonstrated that in certain animals a vitamin A deficiency will produce a deficiency in adreno-corticoid production.

Glick (1961) reported that the adrenocorticoids, viz. the glucocorticoids of the chicken will significantly increase the circulating number of heterophils. Huble (1955) observed a reduced heterophil count in blood of vitamin A-deficient chicks which may indicate a reduced functioning of the adrenal cortex.

Martin (1981) reported that the reproductive function of the retinoids depends upon retiol acting as a sterol hormone.

2.4.7 Maintenance of Myelin and Membranes

Dingle and Lucy (1965) reported that excess vitamin A appear to have indirect actions on the lipoprotein membrane, furthermore, the single large dose of vitamin A completely restored the ability of the mucosa to bind zinc by 24 h with peak binding at 72 h (Berzin, 1986).

2.4.8 Gluconeogenesis and Mucopolysaccharide Synthesis

Studies by Nockels and Phillips (1971) demonstrated that vitamin A might be concerned in certain aspects of carbohydrate metabolism. This has been supported by the findings that chicks on a low dietary intake of vitamin A had significantly raised concentration of liver glycogen and a tendency for increased glucogen and ATP in white muscle. Glycogenolysis was not affected by the deficiency of vitamin A and red muscle showed no changes.

Martin (1981) reported that it has been proposed that the retinyl phosphate functions as a carrier of oligosaccharides across the lipids bilayer of the cell by way of an enzymatic trans-cis isomerization. A deficiency of vitamin A can cause an 80% reduction in the amount of mannose bound to liver glycoproteins in experimental animals.

Homer and Philip (1980) demonstrated that vitamin A probably plays a prominent role in mucopolysaccharide formation. Vitamin A may be involved in activation of sulfate prior to its incorporation into mucopolysaccharides.

2.5 Vitamin A Relationship with Minerals

There is a relationship between vitamin A and serum phosphorus and calcium minerals. Tang *et al.* (1982) observed a decrease in serum Ca and P in broiler chicks that received 300 and 660 IU of vitamin A/g/body wt/day.

Chertow (1977) demonstrated that, however, the vitamin A and D_3 interaction seemed to decreased plasma Ca in man at moderate level of vitamin D and low levels of vitamin A; increasing the level of vitamin A, increased plasma Ca level. Thus may be due to the effect of vitamin A on parathyroid hormone secretion.

Talyor *et al.* (1968) reported that excess dietary vitamin A decreased plasma Ca and increased plasma acid phosphatase activity in chicks.

Fisher and Skillern (1974) found that hypervitaminosis A increased plasma Ca.

There is a relationship between Zn and vitamin A reported by many researchers. Harper *et al.* (1979) reported that Zn is an essential component

of a number of enzymes present in animal tissues. The retina contains Zn metalloenzyme, retinene reductase, which is required for the reconstitution of retinen (vitamin A aldehyde) during the rhodopsin cycle. Zinc is necessary to maintain normal concentration of vitamin A in plasma. Zinc may be required for mobilization of vitamin A from the liver. When vitamin A concentrations in the plasma are lower than normal and supplementation and unresponsive to therapy, with vitamin A, zinc supplementation may be effective.

Strozha *et al.* (1987) showed that the deficient diet in vitamin A and Zn inhibited growth, increased the thiobarbituric acid index, and decreased concentrations of vitamin A and E in blood and caused changes in digestion, such as accumulation of hexoses, tryptophan and dipeptidase activity, whereas, Zinc added alone or with antioxidant or with vitamin A normalized digestion and metabolism.

Berzin (1987) reported that there is a stimulatory action of vitamin A on absorption of Zn in the ileum, independent of the presence of copper and calcium salts in equimolar amounts, or 5 times as much. Zinc transport chicks ileum under the influence of vitamin A is based on the special binding with the Zn-binding protein.

Berzin (1986) mentioned that retinyl acetate 20000 IU caused 72 h after administration an increase in secretion of Zn^{65} into all the segments of

the intestine, that increase being greatest at the distal segments. Stimulation of Zn^{65} secretion by vitamin A could be related to the action of the vitamin on differentiation of epithelial cells and their permeability.

There is a relationship between dietary vitamin A and cobalt. Aleksiev *et al.* (1987) reported that vitamin A in the liver increased with increasing vitamin A and with increasing Co up to 2.0 mg/kg feed.

2.6 Factors Influencing Utilization of Vitamin A

Vitamin A is susceptible to destruction in varying degrees by certain physical and chemical agents which may become operative in the course of some of the process to which feed are subjected (Leonard *et al.*1962).

All lipid-soluble vitamins require bile salts for absorption (Lombardo *et al.*, 1980) and are poorly observed in the absence of bile for instance, in case of biliary obstruction (Harper *et al.*, 1979).

Hollander (1981) showed that intestinal pH may have some effect on the utilization of lipid-soluble vitamins. It has shown that under acidic conditions in the intestine better absorption of lipid-soluble vitamins was observed that under alkali conditions. On the other hand, the addition of polyunsaturated fatty acids was found to depress absorption of all-lipid-soluble vitamins.

Weber (1981) reported that short and medium chain fatty acids enhanced the absorption of vitamin K and A. The relative concentration of lipid-soluble vitamin may have some effect on the absorption of other vitamins.

Bar *et al.* (1980) mentioned that the utilization of lipid-soluble vitamins is influenced by factors such as the polarity of vitamin. The more polar the vitamin derivative, the more absorption occurs.

Evidence has been presented by Stoewsand and Scott (1964) showing that the consumption of high protein diets by young chicks is accompanied by marked reduction in liver vitamin A reserves and increases in adrenal size. Deshmukh *et al.* (1964) showed that activities of enzymes of the pancreas and intestinal mucosa hydrolysing and synthesizing vitamin A ester decreased progressively with lowering of dietary protein level.

Further investigations by Nir and Ascarelli (1966) showed that lowering of plasma protein hinders vitamin A mobilization from liver.

Increasing the energy level resulted in better growth and greater storage of the vitamin A in liver. However, this increased storage may not be due to the higher energy level as such, but to the higher fat content of the diets. Thoranton and Whittet (1962) showed that the high energy carbohydrate diet promoted earlier death dates. This diet, caused a more

rapid rate of vitamin A depletion. Males died sooner than females under these conditions.

Further investigations by Huan *et al.* (1992) showed that vitamin A level in both serum and liver tissue were significantly decreased by feeding Tansy ragwart (a dietary source of PA) (Pyrrolizidine alkaloids). Because of the inhibitory effect of PA on hepatic retinol-binding protein (RBP) synthesis and impaired biliary excretion resulting in reduced vitamin A absorption. Also the transport of vitamin A from parenchymal cells to the blood is depressed resulting in low serum vitamin A values.

Further research indicated that vitamin A absorption affected by the age; it was low in younger rats and increased to 37% when the rats were 39 months old (Hollander and Morgan, 1976b).

Wall and Kelly (1951) reported that temperature, concentration, type of carrier and source of vitamin A had a marked effect on the stability of a number of carotene and vitamin concentration. Leonard *et al.*(1962) reported that both carotene and vitamin A are destroyed by oxidation, the process is accelerated at high temperature, but heat without oxygen has a minor effect.

Excessive heat of the feed resulted in destruction of vitamin A, Jones (1986) reported that vitamin A values were reduced 6.5% by pelleting at 80°C.

Harper *et al.* (1979) pointed out that intestinal diseases such as a severe dysentery, celiac disease, cystic fibrosis of the pancreas and sprue al limit the absorption of vitamin A.

Ascarelli *et al.* (1964) reported that at any rate in the chick vitamin A utilization is certainly improved by thyroxine. However, such improvement is strictly dependent on the thyroxine level supplied and to certain extent also on vitamin A level.

2.7 Hypervitaminosis A

Vitamin A, when given in excessive amounts causes severe untoward results usually referred to as toxic. It appears that the high dietary levels of vitamin A may produce a generalized phenomenon that affects the overall metabolism of the body, Ascarelli *et al.* (1964) so high level of vitamin A exerts a direct or indirect effect, involving some physiological or biochemical mechanism.

Susceptibility to hypervitaminosis A in poultry is dependent on breed (Veltmann, 1984), age (Rodahl, 1950), the dose level and frequency of dose used (Veltmann *et al.*, (1984), the form of vitamin used (Veltman *et al.*, 1984), the route of administration (Veltmann *et al.*, 1984), factors involved in the absorption of fat-soluble vitamins (Hollander, 1981).

Veltmann *et al.* (1984) also found that the disease state of bird influences vitamin A toxicosis.

It was demonstrated that excessive dietary concentration of vitamin A can be toxic to chicks and poults and in larger levels over 50000 IU/kg diet caused a decline in egg production besides other deleterious effects (NRC, 1984).

Mehrjnger (1988) pointed out that in broiler chickens a concentration of vitamin A 30000 IU/kg feed resulted in reduced growth and higher mortality, however, feed intake and efficiency were unchanged. Also he claimed that decreased bone weight, and changes in fat content, organic matter and ash were seen in broilers given vitamin A 30000 IU/kg.

Jensen *et al.* (1983) showed that the growth inhibition, poor feathering and high incidence of leg problems in broilers fed excess dietary vitamin A may have been a consequence of a nutrition disease interaction rather than a nutritional interaction between lipid-soluble nutrients.

Taranu *et al.* (1993) stated that feeding higher vitamin A levels with lowered protein: energy ratio was found to reduce broiler performance.

Veltmann *et al.* (1986) reported that excess vitamin A produced an osterodystrophy in long bones. In the faster growing broilers, excess vitamin A was characterized by a rachitic lesion (widened zone of

proliferating cartilage). Jensen *et al.* (1983) observed skeletal disorders and difficulty in walking in broiler fed 12 times NRC vitamin A requirement.

Stevens *et al.* (1986) demonstrated that a 7% dietary supplement of tallow and a combination of 44000 IU/kg vitamin A and 900 IU/kg vitamin D_3 caused hypomineralization of bones and rickets in young poults, whereas vitamin A at this level but with a lower dietary fat level had no significant effect, on the other hand a study using the same level of fat and vitamin A for poults failed to cause hypomineralization of bone (Abdel Ati *et al.*1994). However, Lucy *et al.* (1961) found that excess vitamin A decreased the protein and chondroitin sulfate content of bone matrix.

The leg abnormalities associated with vitamin A could be due to the reduction of collagen and mucopoly-saccharide of bone as influenced by high levels of this vitamin resulting in arachitic-like condition, even though, bone ash was not influenced (Clark and Smith, 1964).

McCuaig *et al.*(1970), reported that high dietary vitamin A markedly depressed the growth of chicks. Also he stated that the livers of birds fed high vitamin A alone were small and clay colored.

Veltmann *et al.* (1986) reported that the parathyroid gland (PTG) from broiler given excess vitamin A was hyperplastic with an increased number of cords of chief cells and numerous mitotic figures. Hyperparathyroidism and parathyroid hormone (PTH) secretion generally

follow a fall in serum Ca in order to maintain calcium homeostasis (Roth and Raiz, 1964).

Mehrjnger (1988) showed that at vitamin A 30000 IU/kg for broilers erythrocyte stability to hypotonic salt solution decreased.

2.8 *Nigella sativa*

Nigella sativa L. is a member of a family Ranunculaceae or Batter-cup family. In England the plant received the popular name of Fennel-flower.

In most of Arab countries it takes the popular name "El-Habba-el-Sawda". In the old people medicine, it received the name of "Habatt-el-baraka". The center of origin was thought to be the Mediterranean Sea region, Turkey and neighboring parts of Asia. Redgrove (1933). The important producing countries are: India, Pakistan, Iran, Iraq, Syria, Egypt and United States (Abu-Zeid, 1986). In Sudan the crop is produced in the Northern Region and Darfur at Melit and Jebel Marra (Andrews, 1950).

2.8.1 Botany

Nigella sativa L. is an erect annual herb which grows to a height of 40-50 cm (16 inches), easily raised from the seed but does not tolerate transplanting (Kybal, 1980 and Redgrove, 1933). The seeds are small about 2-3 mm long more or less wedge-shaped and are intense dull black colour.

They are markedly angular and their surfaces appear somewhat wrinkled (Redgrove, 1933).

2.8.2 Environmental Requirements

The crop is successfully grown under low temperature and high humidity climates, so it is known as winter crop in Northern Africa and the Mediterranean and cultivated in October and November (Gutb, 1980).

2.8.3 Chemical Composition

Nigella sativa seeds are rich in nutrients, organic compounds and minerals. The seed content of these compounds was investigated by Babayan *et al.* (1978). They reported that the protein content was about 21%, fat 35.5%, ash 3.71% and the rest being total carbohydrates. Al Jassir (1992) showed that the seed component of amino acids were; arginine, methionine, lysine, glycine and leucine. Also he showed the fatty acids in *Nigella sativa* seed were; linoleic acid, oleic acid, palmitic acid and glutamic acid.

Recently, Abdel Majeed (1999) showed the seed content of minerals (ppm): Ca 170, Mg 155, K 125, Fe 24.10, Mn 1.84, Zn 2.00, Na 70, Co 0.17 and Cu 1.63.

Al-Jassir (1992) showed that the active ingredients in *Nigella sativa* seed are: thymoquinone (an alkaloid), nigellone (a carbonyl polymer of thymoquinone and fixed oils).

2.8.4 The Oil Extract

Gad *et al.* (1963) investigated chemical and physical properties of the oil extract from *Nigella sativa* seed cultivated in Egypt. They found that specific gravity was about 0.92, acid value 30.30, saponification value 196.30 and oleic acid represent about 48.76.

Ustan *et al.* (1990) investigated the oil content of three cultivated *Nigella sativa* varieties of Turkish origin. They found that oil contents of seeds collected from the three regions were 29.40, 29.50 and 29.70%, respectively in which the major fatty acids were: linoleic which represents 61.84, 62.53 and 58.38 and oleic acid.

2.8.5 The Uses of *Nigella sativa*

It was used for different purposes in many countries, Gutb (1980) mentioned that *Nigella sativa* seeds used for seasoning and flavouring of food. Moreover, El Khadi and Kandil (1986) discussed the effect of *Nigella sativa* seeds on enhancing the natural immunity.

Ghunaim (1982) reported that the effect of *Nigella sativai* on blood coagulation. In addition, the anti-microbial effect of the seeds was investigated by Hanafy and Hatem (1991) who found the inhibitory effect of Gram-positive bacteria. It was reported that, the seeds are also used as a remedy for cough and chest diseases (Gutb, 1980). Whereas, the alcoholic

extract of seeds after boiling in water relief pain from patients of oral cancer (Salomi *et al.*, 1989).

CHAPTER THREE
MATERIALS AND METHODS

The experiment was carried out in the premises of Animal Production faculty, University of Khartoum in November 1998. The Min. and Max. Temperature were 16.57°C and 33.42°C, respectively. The min. and max. humidity were 21 cm^3 and 35 cm^3 respectively. The experiment lasted for eight weeks.

3.1 Experimental House

The experiment was carried out in the deep litter floor system. In a house ($4x4m^2$) which was constructed from iron pots, short brick walls on the north, south and east sides.

The floor was made of bricks covered with concrete, and the roof was made of galvanized aluminum. The house was separated into sixteen pens ($1x1$ m^2 pen). Each pen was provided with clean, disinfected feeder and drinker. Wood straw was used to create convenient bedding, light was provided continuously throughout the experiment period, using 16 watt bulbs.

3.2 Experimental Birds

One-hundred and fourty four one day-old unsexed commercial broiler chicks (Lohman), were purchased from commercial poultry company, on arrival, chicks were placed in two pens, they were fed the

control diet *ad libitum* for 3 days as adaptation period. They offered water fortified with antibiotic for 3 days. Also vitamin and minerals premix offered for 5 days.

3.3 Experimental Materials

1. Vitamin A; Trade name: Rovimix A-500, Type P with potency 500.000 IU/g. from Roche company.
2. Locally available *Nigella sativa* seeds purchased from Al Goupush company for seeds and medical plants. The seeds were grounded to a fine size to facilitate its inclusion in the ration.

3.4 Experimental Diets

Eight experimental diets were formulated from local ingredients. With one basal diet. The diets were approximately isocaloric and isonitrogenous, with differed levels of added vitamin A (0, 3000, 4500 and 9000 IU/kg feed) and two levels of *Nigella sativa* (0, 0.25%). The chemical analysis of *Nigella sativa* was shown in table (3.1).

The ingredients constituents of the experimental diets are shown in Table (3.2), their calculated and determined chemical compositions are tabulated in table (3.3 and 3.4) respectively.

The experimental diets were formulated and adjusted to meet the nutrient requirements of broiler as outlined by the National Research Council of USA (NRC, 1984).

Table (3.1): Chemical analysis of *Nigella sativa*

Constituents	%
Dry matter	94.314
Crude protein	28.350
Ether extract	35.675
Ash	4.221
Calcium	175 ppm

Table (3.2): *Composition of the experimental diets (starter and finisher diets)*

Ingredients %	Starter	Finisher
Sorghum	63.5	64.5
Groundnut meal	17	9
Sesame meal	13	7
Wheat bran	-	13
Super concentrate*	5	5
Oyster shell	1	1
Salt (NaCl)	0.5	0.5

*Chemical constituent of broiler super concentrate in %: Crude protein 33.0, Lysine 12.1, Methionine 2.74, Meth+Lysine 2.35, Calcium 8.1, phosphorus 5.6 and ME 1720 Kcal/kg.

Table (3.3): Calculated and determined composition of starter diet

Ingredients (%)	Experimental diets							
Nigella sativa (%)	0.0				0.25			
Added Vit. A (IU/kg)	0	3000	4500	9000	0	3000	45000	9000
Calculated composition (as fed)								
CP	22.48	22.48	22.48	22.48	22.48	22.48	22.48	22.48
MEKcal/kg[1]	3104.1	3104.1	3104.1	3104.1	3104.10	3104.10	3104.10	3104.10
Lysine	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19
Methionine	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Phosphorus	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Calcium	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Crude fiber	4.33	4.33	4.33	4.33	4.33	4.33	4.33	4.33
Determined								
DM	93.11	NP*	NP	NP	93.16	93.42	93.13	93.01
CP	29.87	NP	NP	NP	28.00	29.000	25.5	28.68
EE	4	NP	NP	NP	8	8	9	6
C.F	4	NP	NP	NP	7	6	7	4
Ash	8.8	NP	NP	NP	9.7	11.8	9.45	9.9

1. ME was calculated according to proximate analysis reference to Lodhi *et al.* (1976).

Vitamin and mineral premix (water soluble): Per ml: Vit. A 5000 IU (retinyl-palmitate) vit. D_3 500 IU, vit. E 10 mg, vit B 21.6 mg, vit. B5 0.8 mg Nicotinamide 4.0 mg, vit. C 20 mg, vit. K_3 0. 2 mg, Ca 0.8 mg, Mn, 0.02 µg, Co 0.002 µg, Zn 2.0 mg, Fe 5.0 mg, copper 0.01 µg, Mg 0.005 µg.

* Not performed.

Table (3.4): Calculated and determined composition of finisher diet

Ingredients (%)	Experimental diets							
Nigella sativa (%)	0.0				0.25			
Added Vit. A (IU/kg)	0	3000	4500	9000	0	3000	45000	9000
Calculated composition (as fed)								
C.P	19.49	19.49	19.49	19.49	19.49	19.49	19.49	19.49
MEKcal/kg[1]	3012	3012	3012	3012	3012	3012	3012	3012
Lysine	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04
Methionine	0.35	0.35	00.35	00.35	00.35	00.35	00.35	00.35
Phosphorus	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Calcium	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Crude fiber	4.72	4.72	4.72	4.72	4.72	4.72	4.72	4.72
Determined								
DM	98.43	NP*	NP	NP	98.50	98.45	98.75	98.25
CP	23.8	NP	NP	NP	23.8	22.02	19.95	22.05
EE	4.5	NP	NP	NP	3.7	4.4	6.25	6.45
CF	7.9	NP	NP	NP	11.87	8.6	6.8	12.1
Ash	3.2	NP	NP	NP	4.0	6	5.5	5.5

1. ME was calculated according to proximate analysis Reference to Lodhi *et al.* (1976).

* Not performed.

3.5 Experimental Procedure

The eight diets were allotted randomly to 16 experimental units. The chicks were divided randomly evenly among the assigned experimental units in such away that each dietary treatment consisted of two replicates of nine birds each, (18 birds/treatment). Feed and water offered *ad libitum* (*ad lib*). Feed intake, live Body weight were recorded weekly. Feed conversion ratio and body weight gain were calculated.

3.6 Carcass Preparation

At the end of the experimental period, the birds were starved from feed for 12 hours whereas water was available. Six chicks from each treatment (3birds/replicate) were selected randomly then they were leg-tagged then individually weighed and slaughtered, blood was immediately collected for later serum analysis P, Ca, Zn, alkaline phosphates, glucose and cholesterol.

The birds were weighed, manually plucked, eviscerated, washed and allowed to drain. Hot and cold carcass weights were recorded, three birds from each treatment were cut into two equal parts. The right halves of each treatment were minced twicely in a power mince, the resulting mince was mixed thoroughly, placed in polythene bags and immediately stored in deep-freezer (-20°C).

The other remaining left halves of the carcass were dissected, hence average weights of some cuts (thigh, drum stick and breast), their muscle and bone tissue were recorded.

3.7 Chemical Analysis

The experimental diets and meat were subjected to a proximate analysis according to A.O.A.C (1980). Serum Ca was determined according to the calorimetric method (Tinder, 1960). Serum inorganic phosphorus was determined colorimetrically by the method described by Fiske *et al.* (1925).

Serum cholesterol and serum glucose were determined according to Enzymatic Colorimetric Test (CHOD-PAP) (Richmond, 1973; Flegg, 1973 and Trinaer, 1969).

The King Armstrong method described by White and Frankel (1965) was used for the measurement of serum alkaline phosphatase activity. Serum zinc was determined in atomic absorption according to Division of Analytical Technology Inc. (1991). (Zinc and cholesterol Appendix 1).

3.8 Experimental Design and Statistical Analysis

The experimental design of the trial was 2x4 factorial arrangement in a completely randomized design. Independent variables used were two

levels of *Nigella sativa* (0.00, 0.25%) and four levels of added vitamin A (0, 3000, 4500, 9000 IU/kg) of feed.

Eight dietary treatments were randomly assigned to the experimental pens, each pen contained (9) birds and constituted one of the two replicates per dietary treatment.

The data obtained, were tabulated and subjected to analysis of variance (ANOVA) according to Gomez *et al.* (1984). The least significant difference (L.S.D) test was used for treatments mean separation.

CHAPTER FOUR
RESULT

4.1 Broiler Performance

4.1.1 Body Weight

Fig. (4.1) shows the effect of dietary added levels of vitamin A and *Negilla sativa* on body weight of broiler chicks, the results indicated that all groups of birds showed consistent increase in body weight throughout the eight weeks.

The chicks fed the diet with no added vitamin A showed significantly lower body weight, this difference was not significant compared to chicks fed 3000 IU of vitamin A at the 5^{th} weeks of age.

During the first four weeks of age the birds fed the level 3000 IU of vitamin A with 0.25% *Negilla sativa* recorded significantly higher body weight.

At the 5^{th} weeks of age there was no appreciable difference with the tested groups in body weight, except the group fed no added vitamin A diet and the birds fed dietary level 3000 IU of vitamin A, where they showed significantly lower body weight.

At the 6^{th} weeks of age chicks received the dietary levels 4500 IU of vitamin A with 0.25% *Nigella sativa* and 9000 IU of vitamin A only showed significantly higher body weight.

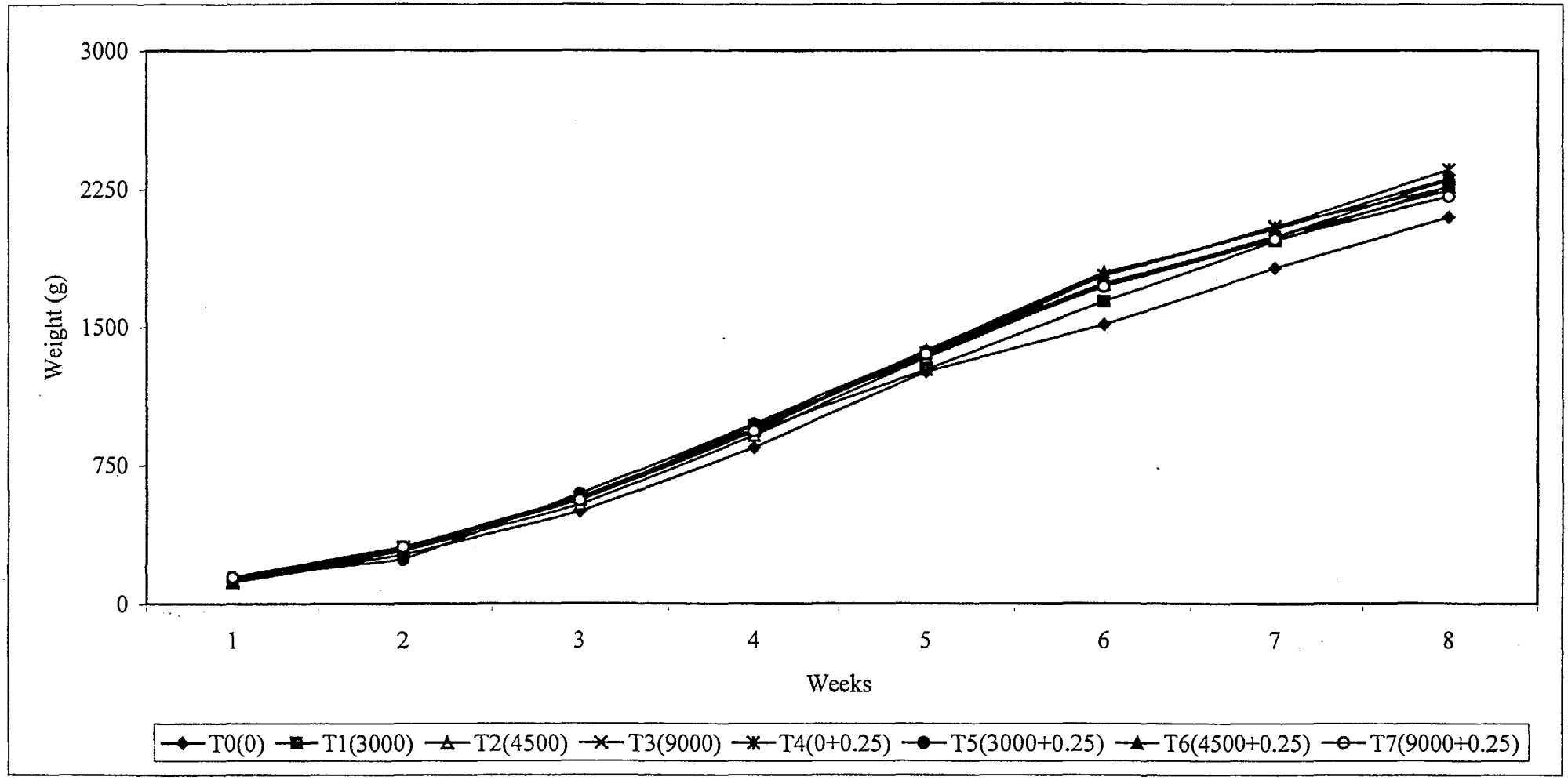


Fig. 4.1: The effect of dietary levels of vitamin A and *Nigella sativa* on body weight of broiler chicks

At the 7th weeks of age chicks fed the dietary levels 4500 IU of vitamin A only, 4500 IU of vitamin A with 0.25% *Nigella sativa* and no added vitamin A diet with 0.25% *Nigella sativa* recorded significantly higher weight.

At the 8th weeks of age the body weight of no added vitamin A with 0.25% *Nigella sativa* supplemented birds showed consistent increase and recorded significantly higher body weight.

4.1.2 Overall Performance

Table (4.1) shows the effect of dietary levels of vitamin A and *Nigella sativa* on performance of broiler chicks. Statistical analysis indicates that the dietary levels of vitamin A had a significant effect on body weight gain ($P \leq 0.01$), the birds received the dietary level 3000 IU and 4500 IU of vitamin A showed significantly higher body weight gain.

In case of *Nigella sativa*, the results indicated that the tested levels had a significant effect ($P \leq 0.01$), the chicks supplemented with 0.25% *Nigella sativa* recorded significantly higher weight gain compared to those unsupplemented.

There was a significant interaction between vitamin A and *Nigella sativa* on body weight gain ($P \leq 0.01$).

Table (4.1): Effect of dietary levels of vitamin A and *Nigella sativa* on performance of broiler chicks (0-8) weeks

Added Vit. A (IU)/Kg (A)	Body W/gain g/bird	Feed intake g/bird	Feed conversion Ratio %
0	2178b	3994c	1.83c
3000	2213a	3993c	1.79d*
4500	2222a*	4179a	1.87b
9000	2163b	4120b	1.90a
Significance	**	**	**
Linear	N.S	N.S	NP
Quadratic	**	*	NP
±SE	±10.87	±16.11	±0.10
Nigella sativa % (B)			
0.00	2158b	4046b	1.87a
0.25	2230a	4099a	1.83b
Significance	**	*	*
±SE	±7.12	±11.40	±0.007
AxB	**	**	*

1. a-d means in the same column with different letters were significantly different.
2. * Significant at 0.05.
3. ** Significant at 0.01 of probability.
4. AxB = interaction between vitamin A and *Nigella sativa.*
5. NP. = Not performed.

Table (4.2) shows the interaction between vitamin A and *Nigella sativa*, although the responses were not consistent chicks fed no added vitamin A with 0.25% *Nigella sativa* recorded significantly higher weight gain.

Statistical analysis shows that the dietary levels of vitamin A had a significant effect on feed intake ($P \leq 0.01$) although the responses were not consistent. Chicks fed the dietary 4500 IU of vitamin A recorded significantly higher value of feed intake.

In case of *Nigella sativa* the effect was significant ($P \leq 0.05$), where the birds supplemented with 0.25 *Nigella sativa* showed significantly higher value of feed intake. Compared to un-supplemented birds.

A significant interaction was observed between vitamin A and *Nigella sativa* on feed intake ($P \leq 0.05$). as shown in Table (4.2). Although the responses were not consistant, birds supplemented with no added vitamin A diet showed significantly lower feed intake.

FCR tended to increase as the dietary levels of vitamin A increase. Where the birds received the dietary level 9000 IU of vitamin A showed significantly higher FCR.

Table (4.2): Interaction of vitamin A with *Nigella sativa* on performance of broiler chicks (0-8) wk

Nigella sativa %	0.00				0.25				
Added Vit. A (IU)/kg	0	3000	4500	9000	0	3000	4500	9000	±SE
Body Wt. Gain	2049^{e}	2212^{bc}	2191^{cd}	2180^{cd}	2308^{a}	2214^{bc}	2253^{b}	2147^{d}	14.25
Feed intake	3849^{d}	3939^{c}	4162^{a}	4235^{a}	4149^{b}	4047^{c}	4195^{ab}	4004^{c}	22.79
Feed conversion ratio	1.88^{b}	1.77^{f}	1.89^{b}	1.94^{a}	1.79^{e}	1.82^{d}	1.86^{c}	1.86^{c}	0.014

a-e means in the same row with different letters were significantly different.

The addition of *Nigella sativa* had a significant effect on FCR ($P \leq 0.05$), where the birds supplemented with 0.25% level recorded significantly lower value of FCR.

There was a significant interaction Table (4.2) between vitamin A and *Nigella sativa* ($P \leq 0.05$)., although the responses were not consistent chicks consumed the dietary level 9000 IU of vitamin A only recorded significantly higher value of FCR.

4.2 Weight of Internal Organs

Table (4.3) shows the effect of dietary levels of vitamin A and *Nigella sativa* on weights of, liver, heart, spleen, bursa and abdominal Fat. Statistical analysis indicates that, the dietary levels of vitamin A significantly affected liver weight ($P \leq 0.01$), although the responses were not even uniformity. Birds fed the dietary level 4500 IU of vitamin A showed significantly higher value of liver weight, whereas those birds supplemented with 9000 IU/ Kg vitamin A exhibited the lowest liver weight.

In case of *Nigella sativa*, a significant effect was observed ($P \leq 0.01$), where the birds supplemented with 0.25% *Nigella sativa* recorded significantly lower value of liver weight. Compared to non-supplemented group.

Table (4.3): Effect of dietary vitamin A and *Nigella sativa* on absolute weights of internal organs and abdominal fat

Added Vit. A (IU)/Kg (A)	Liver	Spleen	Bursa	Heart	Abdominal fat
	◄		(g)		►
0	56.42b	3.85c	3.39c	7.99b	25.35bc
3000	51.02c	4.30b	3.87b	8.72a	27.84b
4500	62.79a	3.33d	4.03a	8.65a	24.86c
9000	44.62d	4.49a	4.20a	8.44a	32.69a
Significant	**	**	**	**	**
±SE	±0.38	±0.01	±0.07	±0.10	±1.24
Nigella sativa (B)					
0	55.76a	3.71b	4.00a	8.54b	31.05a
0.25	51.60b	4.27a	3.74b	8.90a	24.32b
Significant	**	**	**	**	*
±ES	±0.27	±0.01	±0.05	±0.07	±0.62
AxB	**	**	*	**	*

1. a-d means in the same column with different letters were significantly different.

Table (4.4): Interaction of dietary levels of vitamin A and *Nigella sativa* on absolute weights of internal organs and abdominal fat

Nigella sativa %	0.00				0.25				
Added Vit. A (IU)/kg	0	3000	4500	9000	0	3000	4500	9000	SE
Organs weights									
Liver	61.58b	52.37c	64.51a	44.60e	51.26cd	49.67d	61.07b	44.64e	±0.53
Spleen	3.53f	4.14d	2.96g	4.23c	4.18d	4.46b	3.7e	4.75a	±0.01
Bursa	3.42de	3.74cd	4.31ab	4.55a	3.37e	4.00	3.76c	3.86c	±0.05
Heart	7.19d	8.98b	8.28c	8.54c	8.79b	8.47c	9.03ab	9.34a	±0.14
Abdominal	31.07b	29.24bc	28.74bc	35.16a	19.63d	26.45c	20.98d	30.23bc	±1.24

a-g means in the same row with different letters were significantly different.

A significant interaction was observed between vitamin A *Nigella sativa* on liver weight (P≤0.01) although Table (4.4) shows that the responses were not consonant. The dietary level 4500 IU vitamin A supplemented birds showed significantly higher value of liver weight.

The dietary levels of vitamin A had significant effect on spleen weight although the responses were not consistent. The birds received the dietary level 9000 IU of vitamin A showed significantly higher weight of spleen.

Birds supplemented with dietary 0.25% *Nigella sativa* recorded significantly higher value of spleen weight than those unsupplemented.

There was significant interaction between vitamin A and *Nigella sativa* on spleen weight (P≤0.01), even though the responses were not consonant. Birds fed the dietary 4500 IU of vitamin A showed significantly lower value of spleen weight.

The dietary vitamin A levels had a significant effect on bursa of Fabricious weight (P≤0.01). The responses were consistent, values of bursa weight increase, as the dietary vitamin A levels increase. Where chicks supplemented with dietary 9000 IU of vitamin A recorded higher value of the character.

In case of *Nigella sativa*. It had also significant effect on Bursa weight (P≤0.01), where 0.25% level supplemented birds showed

significantly lower value of Bursa weight than unsupplemented birds. A significant interaction was observed between vitamin A and *Nigella sativa* on Bursa of Fabricious weight ($P \leq 0.05$). Table (4.4) shows that the responses were consistent in case of birds fed the dietary levels of vitamin A only without *Nigella sativa*, where the values of bursa weight tend to increase as the vitamin A levels increased. The birds fed the dietary 9000 IU of vitamin A showed significantly higher value of bursa weight, on the other hand the birds fed no added vitamin A with 0.25% *Nigella sativa* recorded significantly lower value of the character.

The dietary vitamin A level had significant effect on heart weight ($P \leq 0.01$). The groups showed some consistent increase in heart weight. As the dietary levels of vitamin A increased where the birds fed no added vitamin A diet showed significantly lower value of heart weight.

The addition of dietary *Nigella sativa* had significant effect on heart weight ($P \leq 0.01$). Birds supplemented with 0.25% level showed significantly higher heart weight compared to unsupplemented birds.

A significant interaction was observed between vitamin A and *Nigella sativa* on heart weight ($P \leq 0.01$), although the responses were not consistent. Table (4.4) shows that chicks received the dietary 9000 IU of vitamin A with 0.25% *Nigella sativa* recorded significantly higher value of heart weight compared to the other groups, except those received the

dietary 4500 IU of vitamin A with 0.25% *Nigella sativa* where the difference was not significant. The dietary vitamin A levels had significant effect on abdominal fat weight, even though the responses were not consistent ($P \leq 0.01$).

Abdominal fat weight of those birds fed the dietary levels 9000 IU of vitamin A was significantly higher than the other groups.

Addition of *Nigella sativa* significantly affect abdominal fat weight ($P \leq 0.05$) chicks received the dietary 0.25% *Nigella sativa* showed significantly lower value of abdominal fat weight.

There was a significant interaction between vitamin A and *Nigella sativa* on abdominal fat weight ($P \leq 0.05$), although the responses were not consonant. Birds fed the dietary 9000 IU of vitamin A only, recorded significantly higher value of abdominal fat weight.

4.3 Serum Biochemistry

The effect of dietary levels of vitamin A and *Nigella sativa* on serum Ca, P, Zn, glucose, cholesterol and alkaline phosphates levels of broiler chicks are shown in Table (4.5). The results indicate that the dietary levels of vitamin A had a significant effect on serum Ca ($P \leq 0.0\ 5$). The responses were quite consistent. Where the Ca levels tend to increase as vitamin A levels increase, but at vitamin A level 9000 IU Ca show a

Table (4.5): Effect of dietary levels of vitamin A and *Nigella sativa* on blood chemistry of broiler chicks

Added Vit. A (IU)/kg (A)	Ca	IP	Zn	Glucose	Cholesterol	Alk. Ase*
			(mg/100 ml)			K.A unit/100 ml
0	12.95c	5.02c	0.14c	151.46a	151.11ab	23.01a
3000	14.91b	4.4d	0.20a	146.98a	142.02c	19.44b
4500	15.45a	5.98a	0.16b	146.35a	154.18a	16.61c
9000	15.02b	5.51b	0.19a	148.17a	145.61bc	22.06a
Significance	**	**	**	N.S	*	**
Linear	NP	**	**	N.S	N.S	N.S
Quadratic	NP	N.S	**	N.S	N.S	**
±SE	±0.09	±0.09	±0.005	±3.49	±2.13	±0.76
Nigella sativa (B)						
0.0	14.42b	5.21b	0.15b	150.00a	157.91a	19.28b
0.25	14.73a	5.24a	0.19a	146.48a	138.55b	21.28a
Significant	**	**	**	N.s	**	*
±SE	±0.12	±0.06	±0.003	±2.46	±1.51	±0.54
Interaction AXB	**	*	**	N.S	**	**

a-d means in the same column with different letters were significantly different.

* Alkaline phosphatase.

NP = Not performed.

decrease. Birds fed the dietary 4500 IU of vitamin A showed significantly higher level of serum Ca.

The addition of *Nigella sativa* had a significant effect on serum Ca ($P \leq 0.0$) where the birds supplemented with 0.25% level revealed significantly higher level of serum Ca.

A significant interaction was observed between vitamin A and *Nigella sativa* on Ca level ($P \leq 0.01$), although the responses were not consistent (Table 4.6). Birds supplemented with 4500 IU of vitamin A and 0.25% *Nigella sativa* recorded significantly higher level of serum Ca.

The dietary level of vitamin A significantly affect serum inorganic P ($P \leq 0.01$), even though the responses were not uniform . The dietary level 4500 IU of vitamin A supplemented birds showed significantly higher level of serum P.

The addition of *Nigella sativa* had no significant effect on P level. There was a significant interaction between vitamin A and *Nigella sativa* on serum P level ($P \leq 0.05$), whereas the responses were not consonant. Birds received the dietary 4500 IU of vitamin A recorded significantly higher level of serum P.

Serum Zn level was significantly affected by dietary levels of vitamin A ($P \leq 0.01$), the responses were consistent. Zn tend to increase as vitamin A levels increased. Although the birds fed Vit.A at levels (3000

Table (4.6): Interaction of vitamin A level and *Nigella sativa* on blood chemistry of broiler chicks

Nigella sativa (B)%	0.00				0.25				SE
Added Vit. A (A)(IU)/kg	0	3000	4500	9000	0	3000	4500	9000	
Ca	12.29e	15.38b	14.82bc	15.20b	13.6d	14.43c	16.07a	14.83bc	±0.17
P	4.70e	5.10de	5.75b	5.32cd	5.35cd	3.71f	6.22a	5.7bc	± 0.13
Zn	0.10d	0.18b	0.15c	0.17b	0.17b	0.21a	0.17b	0.20a	±0.007
Glucose	153.23a	147.18a	150.61a	148.9a	149.69a	146.78a	142.1a	147.36a	±4.93
Cholesterol	154.49bc	161.70ab	170.71a	144.76cd	147.74cd	122.35e	137.65d	146.46cd	±3.01
Alk. ase*	25.3a	14.85cd	20.7b	16.27c	20.72b	24.03ab	12.52d	27.85a	±1.08

a-e means in the same row with different letters were significantly different.

* Alkaline phosphatase.

and 9000) showed similar response, birds fed no added vitamin A diet showed significantly lower level of serum Zn.

The addition of *Nigella sativa* had significant effect on serum Zn ($P \leq 0.01$). The birds supplemented with 0.25% *Nigella sativa* revealed significantly higher level of Zn than unsupplemented birds.

A significant interaction was observed between vitamin A and *Nigella sativa* on Zn level. ($P \leq 0.01$), even though the responses were not uniform. Chicks received the dietary levels 3000 and 9000 IU of vitamin A with 0.25% *Nigella sativa* showed significantly higher level of serum Zinc.

The dietary levels of vitamin A, *Nigella sativa* Table (4.5) and their interaction Table (4.6) had no significant effect on serum glucose level (NS).

The dietary vitamin A had significant effect on serum cholesterol level ($P \leq 0.05$) the responses were not consistant. Birds fed the dietary 4500 IU of vitamin A showed significantly the highest serum cholesterol level compared to the other groups except those fed no added vitamin A diet where the difference was not significant.

The addition of *Nigella sativa* had significant effect on serum cholesterol ($P \leq 0.05$). The birds supplemented with 0.25% *Nigella sativa* recorded significantly lower serum cholesterol level than those non-supplemented.

A significant interaction was observed between vitamin A and *Nigella sativa* on serum cholesterol level ($P \leq 0.01$), although the responses were not consistent. The dietary level 4500 IU of vitamin A supplemented birds showed significantly the highest serum cholesterol level. However, the birds fed 3000 IU/kg of vitamin A showed the lowest.

Serum alkaline phosphates level was affected by dietary vitamin A ($P \leq 0.01$). Increases level of vitamin A in diet resulted in a decrease in serum alkaline phosphates but at the level 9000 IU of vitamin A the alkaline phosphatase level tend to increase. Where the birds fed no added vitamin A diet and dietary 9000 IU of vitamin A showed significantly higher level of the character.

Addition of *Nigella sativa* had significant effect on alkaline phosphates level ($P \leq 0.05$). The birds supplemented with 0.25% level revealed significantly the highest serum alkaline phosphatase level compared to unsupplemented birds.

Significant interaction was observed between vitamin A and *Nigella sativa* on serum alkaline phosphates level ($P \leq 0.01$), although the responses were not consistent. Birds received no added vitamin A diet and 9000 IU of vitamin A with 0.25% *Nigella sativa* showed significantly higher level of serum alkaline phosphates, but the difference was not significant with 3000 IU of vitamin A and 0.25% *Nigella sativa* supplemented birds.

4.4 Carcass Yield and Cuts

Table (4.7) shows the effect of dietary levels of vitamin A and *Nigella sativa* on average weight of total muscle, bone tissue as percentages of selected cuts weight (breast, thigh, drum stick). And dressing percentage of carcass. The dietary levels of vitamin A had a significant effect ($P \leq 0.01$) on these characters. The responses were consistent. The birds fed the dietary level 9000 IU of vitamin A showed significantly higher value for dressing percentage of carcass.

The addition of *Nigella sativa* had no significant effect on dressing percentage values.

The dietary vitamin A level had significant effect on total weight of selected cuts ($P \leq 0.01$) although the responses were not consistent. Birds supplemented with no added vitamin A diet showed significantly lower value of total Wt of selected cuts.

The addition of *Nigella sativa* had a significant effect ($P \leq 0.05$). on total weight of selected cuts. Birds supplemented with 0.25% *Nigella sativa* showed significantly higher value of total Wt of selected cuts compared to unsupplemented group.

A significant interaction was observed between vitamin A and *Nigella sativa* on values of total Wt of selected cuts ($P \leq 0.01$).

Table (4.7): Effect of dietary of vitamin and *Nigella sativa* on average weights of total muscle, bone tissue as percentage of total selected cuts weight, dressing carcass percentage and muscle bone ratio

Added Vit. A (IU)/Kg (A)	Dressing percentage	Total WT of selected cuts	Total muscle %	Total bone %	Muscle bone ratio
0	70.84b	507.25c	78.31d	16.29b	4.80b
3000	71.85b	542.75a	81.72b	16.38b	5.12a
4500	70.64b	527.38b	84.22a	18.04a	4.70b
9000	73.29a	547.13a	79.46c	17.15b	4.69b
±SE	±0.41	±3.46	±0.03	± 0.03	± 0.02
Significance	**	**	**	**	*
Nigella sativa (B)					
0.00	71.60a	526.63b	80.86a	16.86a	4.83a
0.25	71.71a	535.63a	80.99a	17.07a	4.82a
Significance	N.S	*	N.S	N.S	N.S
±SE	± 0.29	±2.80	±0.02	± 0.02	± 0.01
AXB	N.S	**	**	**	**

1. a-c means in the same column with different letters were significantly different.

Table (4.8): Interaction of dietary levels of vitamin A and *Nigella sativa* on average weight of total muscle bone tissue expressed as a percentage from total weight of selected cuts, dressing carcass percentage and muscle: bone ratio

Nigella sativa %	0.00				0.25				
Added Vit. A (IU)/kg	0	3000	4500	9000	0	3000	4500	9000	±SE
Dressing percentage	70.45b	71.98ab	70.55b	73.43a	71.23b	71.73ab	70.73b	73.16ab	±0.58
Total Wt of selected cuts	480.00d	556.75a	515.50c	554.25a	534.50b	528.75bc	539.25ab	450.0ab	±5.59
Total muscle %	74.94f	78.34de	88.33a	81.85c	81.69c	85.10b	80.12cd	77.08e	±0.04
Total Bone %	14.99c	18.58a	18.54a	15.33c	17.59b	14.18c	17.55b	18.97a	±0.05
Muscle Bone ratio	4.95c	4.21e	4.83c	5.33b	4.65d	6.03a	4.57c	4.06e	±0.03

a-f means in the same row with different letters were significantly different.

shows that chicks received no added vitamin A diet showed significantly lower value of total Wt of selected cuts.

Total muscle % was affected by the dietary levels of vitamin A ($P \leq 0.01$). Birds fed the dietary level 4500 IU of vitamin A showed the highest value of the character.

Addition of *Nigella sativa* had no significant effect on values of total muscle %.

A significant interaction was observed between vitamin A and *Nigella sativa* on total muscle % ($P \leq 0.01$), although the responses were not consistant. Chicks received the dietary level 4500 IU of vitamin A only. Recorded significantly higher value of total muscle %.

Table (4.8) the dietary levels of vitamin A had a significant effect on total bone % value ($P \leq 0.01$). the groups showed a consistent increase in total bone %. As the dietary levels of vitamin A increase except the 9000 IU of vitamin A supplemented birds. The birds fed the level 4500 IU of vitamin A recorded the highest value of total muscle %.

In case of *Nigella sativa* the effect was not significant. There was a significant interaction between vitamin A and *Nigella sativa* on total bone % value ($P \leq 0.01$), although the responses were not consistent (Table 4.8). Chicks received the dietary levels (0,4500) of vitamin A showed significantly higher value of total bone %.

Muscle: bone ratio was affected by the dietary levels of vitamin A ($P \leq 0.05$). The birds supplemented with the dietary level 3000 IU of vitamin A. recorded the highest value of muscle: bone ratio, whereas the different between the other groups was not significant.

Addition of *Nigella sativa* had no significant effect on muscle: bone ratio.

A significant interaction was observed between vitamin A and *Nigella sativa* on muscle: bone ratio ($P \leq 0.01$), even though the responses were not uniform birds fed the dietary level 3000 IU of vitamin A with 0.25% *Nigella sativa* showed the highest value of the character (Table 4.8).

Table (4.9) shows the effect of dietary levels of vitamin A and *Nigella sativa* on meat proximate analysis. Crude protein value was significantly affected by dietary levels of vitamin A ($P \leq 0.01$). However, the difference was not quite significant. The level 9000 IU of vitamin A supplemented birds recorded the lowest value.

Addition of *Nigella sativa* had significant effect on crude protein of the muscle value ($P \leq 0.01$). The birds supplemented with 0.25% *Nigella sativa* recorded significantly lower value of crude protein of the muscle compared to non-supplemented group.

A significant interaction was observed between vitamin A and *Nigella sativa* on muscle crude protein value ($P \leq 0.05$).

Table (4.9): Effect of dietary levels of vitamin A and *Nigella sativa* on meat proximate analysis

Added Vit.A (IU)/Kg (A)	Crude protein	Moisture	Ether extract	Ash
	◄	%		►
0	22.26a	73.53b	2.49c	1.11a
3000	22.15a	73.47b	2.51c	1.10a
4500	21.96ab	73.84a	2.7b	1.07a
9000	21.68b	73.56b	2.77a	1.08a
±SE	±0.13	±0.07	±0.02	±0.01
Significant	**	*	**	N.s
Nigella sativa (B)				
0.00	22.49a	73.35b	2.48b	1.11a
0.25	21.53b	73.85a	2.75a	1.08a
±SE	±0.09	±0.05	±0.02	±0.01
Significant	**	**	**	N.S
AXB	*	**	**	Ns

a-c means in the same column with different letters were significantly different.

* Significant at 0.05 level (P≤0.05).

** Highly significant (P≤0.01).

N.S: Not significant.

Table (4.10) shows the responses were consistant. The crude protein values tend to decreased as increasing the dietary levels of vitamin A with 0.25% level of *Nigella sativa* in diet.

The dietary levels of vitamin A had significant effect on Moisture content of the muscle value ($P \leq 0.05$), although the responses were not consonant. The dietary level 4500 IU of vitamin A supplemented birds showed significantly higher value of moisture.

Addition of *Nigella sativa* had significant effect on moisture value ($P \leq 0.01$). The birds supplemented with 0.25% level showed significantly higher value of moisture compared to non-supplemented group.

A significant interaction was observed between vitamin A and *Nigella sativa* on moisture value ($P \leq 0.01$). The responses were not consistent. Table (4.10) shows that the chicks fed the dietary 3000 IU and 9000 IU of vitamin A had lower moisture value than the other groups, but this difference was not quite significant.

Ether extract of the muscle value was significantly affected by dietary vitamin A ($P \leq 0.01$). Increasing levels of vitamin A in diet resulted in an increase in ether extract values.

Addition of *Nigella sativa* had significant effect on ether extract value ($P \leq 0.01$).

Table (4.10): Interaction of dietary levels of vitamin A and *Nigella sativa* on meat proximate analysis

Nigella sativa %	0.00				0.25				
Added Vit. A (IU)/kg	0	3000	4500	9000	0	3000	4500	9000	±SE
Crude protein	21.99b	22.64a	22.70a	22.64a	22.53a	21.66bc	21.22cd	20.72d	±0.19
Moisture	73.50ab	73.18b	73.66ab	73.06b	73.57ab	73.76ab	74.02a	74.07a	±0.10
Ether extract	2.46d	2.31e	2.65c	2.52d	2.53d	2.71bc	2.75b	3.03a	±0.03
ASH	1.11a	1.11a	1.10a	1.10a	1.11a	1.09a	1.05a	1.06[a]	±0.02

a-e means in the same row with different letters were significantly different.

Birds supplemented with 0.25% level recorded significantly the highest value of ether extract compared to non-supplemented birds. A significant interaction was observed between vitamin A and *Nigella sativa* on ether extract values ($P \leq 0.01$). The responses were not consistent in case of dietary level of vitamin A only, but they were consistant in case of dietary levels of vitamin A with *Nigella sativa.*

Increases levels of vitamin A with 0.25% *Nigella sativa* in diet resulted in an increase in ether extract value (Table 4.10).

The dietary levels of vitamin A had no significant effect on ash values. All the groups showed similar responses.

CHAPTER FIVE
DISCUSSION

In the present study the birds looked healthy and no mortality were recorded throughout the experimental period.

Increasing the level of dietary vitamin A resulted in an increase in body weight of broiler chicks. These results are in agreement with findings of Hafez and Dyer (1969). Who demonstrated that vitamin A markedly influence rate of growth and average body weight. However, the birds fed diet with no added vitamin A showed the lowest body weight. Similar results were reported by Reddy *et al.*(1989).

The present study showed that added level of vitamin A 3000 IU/kg of feed appeared adequate to maintain maximum body weight gain with low feed intake and feed conversion ratio throughout the experimental period.

Addition of *Nigella sativa* 0.25% to broiler ration with unsupplemented vitamin A increased weight and body weight gain. This result confirmed the findings of Abdel Majeed (1999) who demonstrated that, the birds received 0.25% *Nigella sativa* recorded the highest body weight. This may be due to the nutritive value of *Nigella sativa* for instant, fat content, minerals and amino acids composition (Al-Jassir, 1992 and Abdel Majeed, 1999).

The interaction between *Nigella sativa* and vitamin A had positive effect on body weight gain. This may be due to the effect of unsaturated fatty acids in *Nigella sativa* seed which may enhance the absorption of vitamin A this result confirmed the findings of Weber (1981) who showed short and medium fatty acids enhanced the absorption of vitamin A furthermore, Leonardo and John (1962) reported that dietary fats promote the absorption of both vitamin A and Carotene.

Addition of *Nigella sativa* to broiler ration increased feed intake and this may be attributed to its effect on the appetite. This result is agreed with (Bolous, 1983), also dietary *Nigella sativa* 0.25% showed best feed conversion ratio this may be due to the nutritive value of *Nigella sativa* or unknown factor in it which could be more effectively for growth.

Absolute weight of livers was affected by dietary levels of vitamin A the birds fed 9000 IU of vitamin A showed a decreased in liver weight. This level may be slightly toxic, although there was no clinical symptoms of toxicity was observed . Similar results were reported by McCuaig and Motzok (1970). However those birds supplemented with Vit.A 4500 IU/kg showed the highest liver weight and this effect coincided with their highest body weight. Supplementation of *Nigella sativa* to the diet reduced the absolute liver weight of broiler ($P \leq 0.01$). This may be due to its effect on fat mobilization from the liver. It was also noticed that there was an

increase of bursa weight as dietary level of vitamin A increased. This may be due to the effect of vitamin A on the Immunocompetent B-cells which are produced by bursa of fabricius and control humoral Immunity in birds. This results may be explained by the findings of (Kolb, 1997). Who pointed out to the role of vitamin A in health of Immune system. The same author showed similar results that spleen and bursa in 10-week old chickens fed nutritionally complete vitamin A were greater than of those fed vitamin A deficient diet. However, in the present study the responses of spleen weight were not consistent. These observations could be related to vitamin A and retinoic acid concentrations and their effect on proliferation in chicken Immunocompetent cells. These results are in agreement with (Kolb, 1997).

Dietary 0.25% *Nigella sativa* increased spleen and decreased bursa of Fabricious weight of the broiler chicks. However the effect of the interaction showed added 0.25% *Nigella sativa* resulted in an increase of spleen weight in birds fed Vit.A levels and *Nigella sativa.*

Heart weights tended to increase as dietary levels of vitamin A increase, addition of *Nigella sativa* to the diet increase heart weights ($P \leq 0.01$).

The dietary 9000 IU of vitamin A was found to increase the abdominal fat of broiler chicks. This could be due to the direct effect of

vitamin A on adrenal cortex function Huble, (1955). The present results suggested that excess vitamin A may have effect on adrenal cortex specially on its role in metabolism of lipids. This result is in agreement with Martin *et al.* (1981) who reported that many of the nutritional metabolic and hormonal factors regulate the metabolism of adipose tissue act either upon the process of esterification or on lipolysis, the resultant of the two processes determines the magnitude of the free fatty acids pool in adipose tissue, which in turn is the source and determinant of the level of free fatty acids circulating in the plasma. The same authors concluded that adrenal cortex had a role in enhancing fat mobilization.

Supplementation with *Nigella sativa* at 0.25% level resulted in low abdominal fat of broiler chicks, whereas this level of *Nigella sativa* resulted in high fat content of the muscle. Similar results obtained by (Abdel Majeed, 1999). This may indicate that *Nigella sativa* has a role in lipid metabolism.

Added levels of vitamin A significantly affected serum calcium level ($P \leq 0.05$) of broilers. It increased as dietary level of vitamin A increased and this agreed with the findings of (Chertow, 1977). These responses may be due to effect of vitamin A on parathyroid hormone which lead to an increase in serum calcium (Martin *et al.*, 1981). Dietary 0.25% *Nigella sativa* increased serum Ca of chicks. This effect may be related to

Ca content of *Nigella sativa* (Abdel Majeed, 1999), which may contribute to this elevation.

The dietary vitamin A significantly affected serum inorganic P ($P \leq 0.01$), although the responses were not consistent. No explanation can be offered for the unexpected observation.

Added levels of vitamin A resulted in an increase of serum Zn. This result agreed with findings of (Ber Zin *et al.*, 1986, 1987), who showed the stimulatory action of vitamin A on Zn absorption and the influence of vitamin A on Zn transport which based on the special binding with Zn-binding protein.

The addition of 0.25% *Nigella sativa* in broiler ration elevated serum Zn, and this may be related to Zn content of *Nigella sativa* seed which was found to be 2.00 ppm (Abdel Majeed, 1999).

Serum Glucose was not influenced by dietary levels of vitamin A or *Nigella sativa.* However, serum cholesterol of birds was affected by dietary levels of vitamin A chicks received the added level of vitamin A 3000 and 9000 IU/kg in the diet showed low serum cholesterol. Furthermore, dietary supplementation of 0.25% *Nigella sativa* reduced serum cholesterol level of broiler, this maybe attributed to oleic and Linolic acids content of *Nigella sativa* seeds (Al Jassir, 1992). Serum alkaline phosphatase of chicks was influenced by dietary vitamin A level . The added level of 9000 IU/kg of

vitamin A increased serum enzyme. Similar results were obtained by Veltmann *et al.* (1986) who reported an increase of plasma alkalinephosphatase was observed in chicks fed toxic levels of Vit.A.

The addition of 0.25% *Nigella sativa* in broiler diet increased serum alkalinephosphatase, this may be related to Zn content of *Nigella sativa* seed (Abdel Majeed, 1999). Mathies (1958), Trubowitz *et al.* (1961) and Li and Vallee (1980) reported that Zinc is essential for the catalytic and sub unit structure of the enzyme.

Carcass yield was influenced by dietary levels of vitamin A. The added vitamin A level of 9000 IU/kg of feed resulted in maximum dressing percentage. While the added level of vitamin A 3000 IU/kg feed achieved the maximum weight of selected cuts (breast, thigh and drum stick). This may be due to the availability of the vitamin that may enhance the carcass yield and quality grade (bone muscle and fat).

The broiler received 0.25% *Nigella sativa* showed an increase of weight of selected cuts, this effect may be attributed to the nutritive value of *Nigella sativa* (AlJassir, 1992 and Abdel Majeed, 1999).

The added level of vitamin A 4500 IU resulted in highest total muscle % and total bone % these responses coincided with high body weight gain of chicks fed this level. However, the added level 3000 IU of vitamin A increased muscle: bone ration of the broiler chicks.

Increasing dietary levels of vitamin A at 9000 resulted in slightly decrease in crude protein of meat. This may be due to the effect of vitamin A on glycoprotein synthesis (Martin *et al.*, 1981).

The birds supplemented with 9000 IU of vitamin A exhibited highest ether extract (fat) content of the muscle. This may be explained by the indirect effect of vitamin A on fat mobilization and fatty acid pool in adipose tissues (Martin *et al.*, 1981).

Addition of 0.25% *Nigella sativa* in broiler ration improved the ether extract content in the muscle of the broiler meat. Similar results were reported by (Abdel Majeed, 1999).

CONCLUSION

The result of this study showed that:

1. Dietary level of vitamin A had no adverse effect on growth or health of broiler chicks.
2. Dietary level 3000 IU of vitamin A had a positive effect on body weight gain, muscle:bone ratio, feed conversion ratio, moisture and Ether extract content of muscle. Therefore, this level is recommended to be used for broiler ration.
3. A high level of dietary vitamin A increased weights of bursa of Fabricious and abdominal fat.
4. Dietary levels of vitamin A resulted in elevation of serum Ca and fat content in the muscle of meat.
5. Supplementation with 0.25% *Nigella sativa* increased weight and weight gain, decreased abdominal fat of broiler chicks.
6. The dietary level 0.25% of *Nigella sativa* had a lowering effect of serum cholesterol. This may indicate that *Nigella sativa* has a role in lipids metabolism.

Further study is suggested to determine the effect of dietary vitamin A and *Nigella sativa* on the immune response of broiler chicks.

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APPENDIX 1

Chemical Analysis of Serum Zinc and Serum Cholesterol

1. Zinc

Stock solution: 1000 mg liter^{-1} Zn

Dissolve 1000 mg Zn in 30 cm^3 of 5 M HCl. Dilute to 1 liter in volumetric flask with deionized water. Store in a polythene bottle. Or dissolve 1.2450 g of zinc oxide (ZnO) in 5 cm^3 of deionized water followed by 25 cm^3 of 5 M HCl. Dilute to 1 liter in a volumetric flask with dionized water, store in a polythene bottle.

Organo Metallic Standard

Zinc cyclohexane butyrate.

2. Cholesterol

Enzymatic Colorimetric Test (CHOD-PAP)

Principle

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolize the esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol-oxidase according to the same reactions.

Reagents

R_1 (buffer) pipes buffer	90 mmol/L phenol
Phenol	25 mmol/L
R_2 (enzyme) cholesterol oxidase	200 U/L
Cholesterol esterase	300 U/L
Peroxidase	1250 U/L
4-aminoantipyrine	0.4 mmol/L
Reagent 4 (standard): cholesterol	200 mg/dl

Preparation and stability

Dissolve contents of enzyme reagent R_2 with the corresponding volume of buffer /R_1. This working solution is stable:

2 wks at 20-25°C, 8 wks at 2-8°C.

Calculation (by standard)

$$\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{standard conc.} = \text{cholesterol in mg/dl}$$