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EVALUATION OF *NIGELLA SATIVA* SEEDS ON BROILER CHICKS HEMATOLOGICAL, BLOOD BIOCHEMICAL PARAMETERS AND ANTIOXIDANT ENZYMES

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ABSTRACT: The present study targets to look deep at the influence of *Nigella sativa* seeds (NSS) supplementation on hematological, blood biochemical measurements, liver and kidney functions and antioxidant enzymes of Arbor Acres broiler chicks. A total of 140 unsexed one day old Arbor Acres broiler chicks were randomly divided into four treatment groups during the period from 1-35 days of age. The chicks were randomly assigned in a straight run experimental design among four treatments, each replicated 7 times with 5 unsexed chicks per replicate. The 1st group was fed a commercial basal diet without any supplementation (control), the 2nd, 3rd and 4th groups were fed control diet supplemented with NSS at 0.5, 1.0 and 1.5% levels. Chicks fed basal diet and basal diet without supplementation or with 1.5% NSS had significantly higher RBCs than the 0.5 and 1% NSS supplemented groups. Chicks fed the NSS at 0.5% showed a higher plasma albumin level than the control group, 1 and 1.5 NSS groups. Feeding diet supplemented with 1% NSS had significantly (P≤0.05) achieved the lowest triglyceride and cholesterol compared with chicks fed diet that contained 0.5 and 1.5% NS seeds groups. Significantly (P≤0.05) higher high-density lipoprotein (HDL) was recorded in chick fed diet that contained 1.5% NSS compared with the control and the other treatments. Chicks fed diet that contained 1% NSS had significantly (P≤0.05) achieved a lower plasma aspartate amino transferase (AST). All of supplementation with different NSS levels had a significantly (P≤0.05) higher plasma creatinine and lower plasma urea to creatinine ratio than the control group. Chicks fed diet contained 0.5% NSS had significantly (P≤0.05) achieved a higher plasma superoxide dismutase and glutathione peroxidase activity compared with the control, 1 and 1.5% NSS supplemented groups. Chicks fed the diet containing 1% NSS showed higher plasma glutathione level than the control group, 0.5 and 1.5% NSS groups. Feeding diet with supplemented 0.5 and 1.5% NSS were significantly (P≤0.05) increased plasma glutathione reductase and catalase compared with the control and 1% NSS groups.

Conclusion: These findings indicated that NSS had improved lipid profile, liver function and antioxidant profile of broiler chicks. Thus, 1% NSS is considered safe due to having no acute toxic side effects as reported through the experimental period.

Key words: Broiler; *Nigella sativa* seeds; liver and kidney functions; antioxidant.

INTRODUCTION

Poultry industry has advanced in several focus points for instance; feeding, genetics, immunity enhancement and improved management for a higher productivity, better growth performance and more meat yield (Gunal *et al*., 2006). Modern poultry farming is under a continuous demand to provide for high quality meat and egg simultaneously with a lower price under the condition of doing without antibiotics and other therapeutic utilization in animals´ diets.

Medicinal plants and their derivatives are known to be safe and efficient substitute for animal production industry because of their positive influence on different physiological responses in poultry as they provide a wide variety of activities for the biological systems in terms of immunity, endocrine gland, digestive tract and a wide range of other body systems El-kashef (2020). The black seed is one of those medicinal plants which is also known by the name ´´black cumin´´ (*Nigella sativa* L.). In the Islamic literature (hadith) the Prophet Mohammed (Peace Be Upon Him (PBUH)) had described the healing capacity of the black seed by saying "Keep on the using of this black seed because it possesses a cure for every disease except for death" (Bukhari, 2018). *Nigella sativa* is a popular herbal plant that has been commonly used to work out various health issues for about 2000 years (Darakhshan *et al*., 2015). NSS also consists of significant levels of minerals such as calcium, iron, zinc, copper, phosphorus, niacin, thiamine, pyridoxine, and folic acid (Takruri and Dameh, 1998; Ramadan, 2007). Moreover, the phytochemical analyses of NSS has demonstrated that they contain over a hundred phytoconstituents, predominantly alkaloids, saponins, sterols and essential oils, nonetheless, the structure of all of them has not been chemically realized nor biologically confirmed (Mamun and Absar, 2018; Ghahramanloo *et al*., 2017). Furthermore, they possessed a number of biologically active constituents, for instance; dithymoquinone, thymol, thymoquinone, which are known to be a pharmaceutically active substances of NSS, the most primarily bioactive component that the black seed possesses is the essential oil in the range of 27.8-57% (Ali and Blunden 2003), alpha-hedrin, carvacrol, nigellidine and nigellicine-N-oxide (Al-Homidan *et al*., 2002; Nasir *et al.*, 2005). The composition of NSS makes it yield to many properties that are demonstrated in figure (1) (Badary *et al*., 2003; Khader *et al*., 2010; Mohideen *et al*., 2003; Srinivasan, 2018).

In clinical trials, *Nigella sativa* showed to have an effect when included as an alternative for the traditional hypolipidemic and antidiabetic medicines. Inhibiting dietary cholesterol absorption suppressed hepatic cholesterol production and up-regulation of LDL receptors leading to the lipid-reducing effects of *Nigella sativa*. Some studies and researches pointed out that NSS plays a role in the antibacterial activity (El-Kamali *et al*., 1998; Mouhajir *et al*., 1999; Nair *et al*., 2005), where the NSS and their abundant extracts have an effect on gram-positive and gram-negative bacteria and suppress the production of

aflatoxin (Nasir and Grashorn, 2006), and have many biological characteristics such as antiparasitic (Mahmoud *et al.*,2002) The effects of dietary *Nigella sativa* meal (NSM) or oils on poultry performance have been investigated and it has been concluded decisively that the consumption of feed, body weight and efficient performance in broilers are unquestionably influenced. (Halle *et al*., 1999; Tollba and Hassan, 2003; Guler *et al*., 2006; Ziad *et al*., 2008; AL-Beitawi *et al*., 2009; Erener *et al*., 2010; Toghyani *et al*., 2010). Some studies showed that diets which contained 10% of NSM demonstrated no effects on growth performance (Al-Homidan *et al*., 2002). Consequently, the current study targeted the investigation of the effect of the supplementation of NSS on hematological, blood biochemical parameters, liver and renal functions and antioxidant enzymes of broiler chicks.

MATERIALS AND METHODS

The study was conducted at the Poultry Research Unit at El-Bostan Farm, animal and poultry department, Faculty of Agriculture, Damanhour University approved the experiment. This study was carried out to investigate the effectiveness of dietary inclusion of NSS on blood hematological, biochemical parameters, liver and renal functions and antioxidant enzymes of broiler chickens.

Nigella sativa seeds sources

The NSS was purchased commercially from the local commercial market at Damanhour city, Egypt.

Chicks, diets and experimental design

One hundred and forty (140) unsexed one day old Arbor Acres broiler chicks were fed the experimental diets (Table 1) during the period from 1-35 days of age. The chicks were randomly assigned in a straight run experimental design among four treatments, each replicated 7 times with 5 unsexed chicks per replicate. Corn-soybean meal basal diet was formulated to be isocaloric and isonitrogenous to meet the nutritional requirements according to NRC (1994). The basal diet was fed without supplements (control group) or supplemented with NSS levels of 0.5, 1.0 and 1.5% (Table 1). NSS contain crude protein, crude fiber, ether extract, moisture and ash (21.16%, 10.92%, 31.97%, 5.58% and 1.88%), respectively according Mohammed (2016).

Husbandry of chickens:

Chicks were kept in battery brooders (40×45×60 cm) under similar managerial and hygienic conditions in a semi-open house. Water and mash diets were given *ad libitum* throughout the whole experimental period. The brooding temperatures were 33, 31 and 30°C during the 1st, 2nd and 3rd weeks of age, respectively. During 21-35 days of age, the average ambient temperature and relative humidity (RH %) were 30 ± 3°C and 45 ± 4%, respectively. The lighting regimen was 23:1 light-dark cycle.

Data Collection:

Blood samples collection

At 35 days of age, six chicks from each group were randomly taken at 08:00-09:00 am and about 3 ml blood was collected from the wing vein into vacationer tubes with K3-EDTA (1 mg/mL).

Non-coagulated blood was divided into two parts. The first part was used, shortly after collection, for estimating blood picture, whereas, the second part was centrifuged at 4000 rpm for 15 minutes and the clear plasma was separated and stored in a deep freezer at -20^{o}C until biochemical analysis. All blood

biochemical variables were determined calorimetrically using commercials kits.

Hematological parameters

Red blood cells´ count (RBCs 10^6/ml^3) was done according to Feldman *et al*. (2000). Hemoglobin (Hb) concentration (g/dl) and the percentage of packed cells volume (PCV %) were measured according to Drew *et al*. (2004). The average volume (size) of RBC (MCV, µm3) = [hematocrit (%)/RBC] × 10, the average weight of hemoglobin in RBC (MCH, pg) = [hemoglobin concentration (g/dL)/RBC] × 10 and the average concentration of hemoglobin in the RBC (MCHC, %) = [hemoglobin (g/dL)/hematocrit (%)] × 100 counts were calculated. A thin blood film was prepared by using a small drop of blood. The blood film was completely dried before staining using Giemsa stain.

Blood biochemical parameters

Protein profile

To evaluate the changes in the protein profile at 35 days of age in chickens, plasma total protein (g/dl) and albumin (g/dl) were determined using special kits delivered from Diamond diagnostics (23 EL-Montazah St. Heliopolis, Cairo, Egypt, http://www. diamonddiagnostics.com) by means of spectrophotometer (Beckman DU-530, Germany) according to guidelines of Armstrong and Carr (1964) and Doumas *et al*., (1977), respectively. Plasma globulin level (g/dl) was calculated by the difference between total protein and albumin, since the fibrinogen usually comprises a negligible fraction (Sturkie, 1986). Albumin to globulin ratio was also calculated. Albumin to globulin ratio (A/G) was calculated by dividing the total level of albumin of the total level of globulin.

Lipid profile

Plasma total lipid, triglyceride concentrations (mg/dl) were determined in blood plasma using special kits delivered from Diamond diagnostics (23 EL-Montazah St. Heliopolis, Cairo, Egypt, http://www. diamonddiagnostics.com) by means of spectrophotometer according to recommendation of (Fringes *et al*., 1972). Plasma total cholesterol (mg/dl) was determined on individual bases using the specific kits according to recommendation of (Bogin and Keller, 1987). Plasma samples were analyzed for high-density lipoprotein (HDL, mg/dl), low-density lipoprotein (LDL, mg/ dl) and HDL to LDL ratio (HDL/LDL) was calculated by dividing the total level of HDL of the total level of LDL.

Liver function

The transaminase enzymes activities of plasma aspartate amino transferase (AST) and plasma alanine amino transferase (ALT), as U/L were determined by calorimetric method of (Reitman and Frankel, 1957) and AST to ALT ratio was calculated. Alkaline phosphatase (ALP, U/L) concentration was determined according to the colorimetric method of (Bauer, 1982).

Kidney function

Renal function, urea (mg/ dl) and creatinine (g/ dl) were assessed in the serum based on the suggestions of Bartles *et al*., (1972) and Sampson *et al*., (1980), respectively, and the urea-to-creatinine ratio was calculated.

Oxidative status

superoxide dismutase (SOD, IU), glutathione (GSH, mg/dl), glutathione

peroxidase activity (GPx, U/ml) and glutathione reductase (GRx, U/ml) and were determined according to Koracevic *et al*. (2001), Chiu *et al*. (1976), Beutler *et al*. (1963) and Misra and Fridovich (1972), respectively. Catalase (nmol/min/ml) was determined according to Rindler *et al.*, (2013). Total antioxidant capacity (TAC, nmol/l) was determined according to Koracevic *et al*. (2001). Malondialdehyde (MDA, μmol/L) was determined according to Richard *et al.* (1992).

Statistical analyses:

Analysis of variance was done using a one-way analysis of variance, as described by SAS® (2009), using the following model:

Yij= μ+Fi+eij,

Where: Y=the dependent variable, μ= the overall mean; Fi= the effect of NSS treatments and eij= the random error. The replicate was the experimental unit. The mean difference at P≤0.05 was tested using Tukey's HSD test.

RESULTS AND DISCUSSION

Effect of *Nigella sativa* seeds on blood hematological

The effect of supplementation of NSS on hematological parameters of broiler chicks is presented in table (2). Chicks fed basal diet and basal diet supplemented with 1.5% NSS had significantly higher RBC's than the 0.5 and 1% NSS supplemented groups. No significant differences were observed between the control group and all of levels of NSS treatment groups for Hgb, PCV, MCV, MCH and MCHC.

In accordance with the current results, the positive effect of *Nigella sativa* seeds on hematology could be linked to the highly active components, particularly thymoquinone and thymohydoquinone, as they have a robust antioxidation activity (Arslan *et al*., 2005) and thus, the increased RBC counts in NSS treated quail chicks may be attributed to the decreased lipid peroxide in RBC membrane bringing on a decreased sensitivity of RBC to hemolysis (Toghyani *et al.*, 2010). Shokrollahi and Sharifi (2018) noticed that RBC counts showed a significant improve in the 1% and 1.5% NSS treatments (P≤0.05). There were no big variations in Hb and PCV levels among the treatments (P>0.05). In addition to that, Toghyani *et al.* (2010) discovered that NSS were the cause of the increased RBC, Hb and PCV levels in broilers. Mixed outcomes on the effect of NSS on the hematological profile of healthy broiler are available (Al-Mansour *et al*., 2011; Ghasemi *et al*., 2014). Haqa *et al*., (2020) who quoted that including NSS in the diet showed no major effect on RBC count and Hb concentration in broiler chicks.

Effect of *Nigella sativa* seeds on plasma proteins and lipid profile

The results in table (3) showed the effect of supplementation of NS on plasma proteins and lipid profile of broiler chicks. The results indicated that chicks fed diet that contained 1.5% NSS had significantly (P≤0.05) achieved a lower plasma total protein compared with the control and 0.5% NSS supplemented groups. The highest plasma total protein observed in chicks fed basal diet. Chicks fed the NSS at 0.5% showed higher plasma albumin level than the control, 1 and 1.5% NSS groups. Feeding diet supplemented with 1% NSS significantly (P≤0.05) increased plasma total lipid compared with the control and the other treatment (0.5 and 1.5% NSS) groups. Chicks fed diet containing 1% NSS had significantly (P≤0.05) achieved the lowest triglyceride and cholesterol

compared with chicks fed diet containing 0.5 and 1.5% NSS groups. The highest triglyceride was observed in birds fed diet containing 0.5% NSS. Significantly ($P \leq 0.05$) higher HDL was recorded in chick fed diet containing 1.5% NSS compared with the control and the other treatments. While, the lowest HDL observed was in the control group. Feeding diet with supplementation of 1% NSS significantly ($P \leq 0.05$) decreased LDL compared with the control and 0.5% NSS groups. Chicks fed diet containing 1.5% NSS presented a higher HDL to LDL ratio than the other groups and the lowest level of HDL to LDL ratio was observed with the control group.

Likewise, Attia and Al-Harthi (2015) revealed that the inclusion of *Nigella sativa* oil at 0.5 and 1.5g/kg in the diet significantly lessened the overall plasma total protein and albumin. While, Hassan *et al.*, (2007) noticed an improvement of serum albumin with the supplementation of NSS. Blood serum proteins are a sign for the state of the body and the changes that occur under the effect of internal and external factors (Toghyani *et al.*, 2010). However, Miraghaee *et al.* (2011) concluded that NSS had no obvious impact on serum protein, yet increased the levels of serum albumin in broiler chicks. The average Plasma protein, albumin and globulin of Hubbard broiler chicks fed on 4 g/kg NSS were approximately alike those of the control group (EL-Ghammry *et al.*, 2002). Shokrollahi and Sharifi (2018) noticed no major contrast in the levels of albumin and total protein in the respective treated groups ($P>0.05$). El-kashef (2020) demonstrated that the total protein was significantly improved in groups 3% and 6% NSM in comparison with those treated with 9% NSM or to the control group and higher albumin levels were observed in the groups receiving 3% and 6% NSM. AboSaleh *et al.*, (2019) and Kumar *et al.* (2017b) discovered that the concentration of total protein tended to be higher in the NSS than in the control group, which had a positive correlation with higher NSS doses (Kumar *et al.*, 2017a). Elevated serum total protein with NSS supplementation was noted by Al-Beitawi and El-Ghousein (2008). Yatoo *et al.* (2012) discovered a significantly higher serum total protein and albumin with 1% NSS supplementation as compared to the control group, and Khan *et al.* (2012) noticed enhanced total protein value compared with the 1.25% antibiotic or non-supplemented diet groups. Tollba and Hassan (2003) and Hassan *et al.* (2007) discovered increased serum total protein, albumin and globulin in birds fed diets with high levels of NSS. Saleh (2014) noted that feeding NS oil at the level of (1 ml/kg) elevated serum total protein and albumin concentrations, but not globulin concentration, and albumin to globulin ratio compared to the control group (Kumar *et al.*, 2017a). The improvement in albumin level may be ascribed to that NSS possess an immune-stimulating effect. El-Ghammry *et al.* (2002) discovered an opposite finding, in which supplementing with 4 g/kg NSS in the diet showed a little effect on plasma total protein, albumin or globulin.

As stated by Aziza *et al.*, (2019), serum total protein and globulin levels were considerably higher in the group supplemented with *Nigella sativa* oil compared to the positive control group. Meanwhile, the albumin content and

albumin/globulin ratio did not indicate any substantial difference among all experimental categories (in quail). Soliman *et al.*, (2017) demonstrated a highly significant improvement ($p<0.01$) in serum total protein and albumin and a highly significant decline ($p<0.01$) in globulin level in broiler chicks fed 5.6% *N. sativa* Linn.

Current results demonstrated that cholesterol and triglycerides` levels were reduced in layer chicks supplemented with 1% NSS. In accordance with our results Akhtar *et al.* (2003) noted that the serum total cholesterol and triglycerides were less in birds fed on a diet containing 1% NSS in than a diet NSS-free (Hermes *et al.*, 2011; El-Bagir *et al.*, 2006; Hassan *et al.*, (2007); Al-Beitawi and El-Ghousein (2008); AL-Beitawi *et al.*, 2009; Ghasemi *et al.*, 2014; Yatoo *et al.* (2012). Shokrollahi and Sharifi (2018) noticed that the levels of cholesterol and triglycerides declined significantly ($P<0.05$) in the groups treated with 1 and 1.5% NSS compared to control one, respectively. LDL concentration declined significantly ($P<0.05$) in NSS treated broiler chicks compared to the control ones. The levels id HDL showed no significant differences among the treatments ($P>0.05$); however, HDL concentration was higher in the treated broiler chicks. Concentrations of cholesterol are likely to be lower for NSS compared to control group, which had a negative linear correlation with increasing doses (5, 10, and 20 g/kg) of NSS (Kumar *et al.*, 2017a). To the contrary, Khalaji *et al.* (2011) demonstrated that 1% NSS in broiler diets had no effect on the serum plasma cholesterol (Khalaji *et al.*, 2011). The decrease in serum cholesterol levels might be ascribed elevated bile secretion (El-Dakhakhny *et al.*, 2000). Brunton (1999) proposed that the decrease in serum cholesterol may be linked to the lowering influence of thymoquinone and unsaturated fatty acids on synthesizing cholesterol by hepatocytes or the fractional reabsorption from the small intestine. Furthermore, NSS constitute considerable amount of sterols, particularly β-sitosterol that could inhibit the absorption of dietary cholesterol (Khan *et al.*, 2012). Those constituents might stimulate the excretion of cholesterol into the intestine being oxidized by bile acids (Tollba and Hassan, 2003). This reduction in serum cholesterol level of broiler chicks fed NSS diets probably proposes a general decline in lipid mobilization.

Ali *et al.*, (2014) found out that 0.50% NSS in diet resulted in a reduced level of blood LDL. Although, they also noticed that raising NSS diet up to 0.75%, resulted in an increase in the levels of HDL. El-kashef (2020) demonstrated that diets containing various levels of NSM showed a reduction in ($P\leq0.05$) serum total cholesterol. Except for the group fed 9% NSM, all treatments showed an increase in the HDL fraction, at the same time decreasing the LDL fraction.

Animal studies have showed that NSS possess hypocholesterolemic and hypolipidemic properties (Toghyani *et al.*, 2010). In accordance with our results, cholesterol and triglycerides were reduced in chicks given NSS with the levels of 10 g NSS/kg diet (Miraghaee *et al.*, 2011; Shewita and Taha, 2011), 10 and 15 g NSS/kg diet (AL-Beitawi and EL-Ghousein, 2008; Yalçın *et al.*, 2012) led to decline in the levels of cholesterol and triglycerides. Likely, Bölükbasi *et al.* (2009) proposed that NSS was the reason for the reduction of the levels of triglycerides. The decline in serum

triglycerides could be linked to NSS volatile oils (Thymoquinone and dithymoquinone) (Swamy and Tan, 2000). The choleretic activity of NSS has the capacity to lower the levels of serum cholesterol and triglycerides (El-Dhakhny *et al.*, 2000) through either reducing the synthesis of triglycerides and cholesterol by hepatocytes or lessening its fractional reabsorption from the small intestine (Brunton, 1996). Akhtar *et al.* (2003) demonstrated that NSS has the capacity to lower concentration the serum LDL in layer hens. The amount of serum HDL changed a little among the treatments, though HDL showed no significant increase in NSS-treated chicks. 1.5% NSS showed a significant enhancement in HDL concentration (Akhtar *et al.*, 2003; AL-Beitawi and EL-Ghousein, 2008). Contrarily Tufan *et al.* (2015) declared that NSS and their oil showed no effect on the concentrations of HDL and LDL in quail chicks.

Effect of *Nigella sativa* seeds on liver and renal function indices

Table (4) illustrated the effect of supplementation of NSS on liver and renal function indices of broiler chicks. The results indicated that chicks fed diet containing 1% NSS had significantly ($P \leq 0.05$) achieved a lower plasma AST compared with chicks fed diet supplemented with 0.5 and 1.5% NSS groups. The highest plasma AST protein observed in chicks fed diet containing 0.5% NSS group. No significant ($P \leq 0.05$) differences were found among the control group and treatment groups for plasma ALT, AST to ALT ratio and alkaline phosphatase. Chicks fed the NSS 1.5% showed a higher plasma urea level than the control and chicks fed diet containing 0.5 and 1% NSS groups. All of supplementation with different NSS levels had significantly ($P \leq 0.05$) higher plasma creatinine and lower plasma urea to creatinine ratio than the control group.

The scarcity of significant differences in plasma ALT among treatment diets indicates normal liver function in birds fed NSS-containing diets. Although, the decline in the activity of AST noticed in birds fed a NSS diet of 1% may propose that it has properties that may enhance liver health. Consequently, Attia and Al-Harthi (2015) demonstrated that plasma ALT in the control, *Nigella sativa* oil at 0.5 and 1g/kg of diet groups was lower than that in the 1.5g *Nigella sativa* oil/kg of diet group. The levels of AST were comparable in the monitor, 0.5, and 1.5g *Nigella sativa* oil/kg diet categories. The level of alkaline phosphatase showed a significant decrease in 1.5g *Nigella sativa* oil/kg of diet group than in the other groups. The level of plasma urea showed a significant increase in the groups fed 1.5g *Nigella sativa* oil /kg of diet than the other groups. Plasma creatinine had a different pattern, and the plasma urea/creatinine ratio was more in the control and 1.5g *Nigella sativa* oil/kg diet groups than in the 0.5 and 1g *Nigella sativa* oil/kg diet groups. Except for 9% of NSM, plasma AST and ALT declined. As the liver is shown to contain enzymes such as ALT and AST, when it is enfeebled, it secretes those enzymes into the blood stream (Kaplan *et al.*, 2003). Moreover, Soliman *et al.*, (2017) discovered that the enzymes of the liver (ALT, AST) and creatinine levels exerted a highly significant enhancement ($p<0.01$) in broiler chicks fed on a diet containing 5.6% NSS, while urea

demonstrated a significant elevation in all treated groups compared to the normal control group with highly significant differences ($p<0.01$). Liver indices (AST, ALT) and renal functions indices (urea and creatinine) may be useful in decide the health and physiological status of an animal (Toghyani *et al.*, 2010; Attia *et al.*, 2015).

Effect of *Nigella sativa* seeds on antioxidant indices

Effects of experimental treatments on antioxidant indices of broiler chicks presented in table (5). The results indicated that chicks fed diet contained 0.5% NSS had significantly ($P\leq0.05$) achieved a higher plasma superoxide dismutase and glutathione peroxidase activity compared with the control, 1 and 1.5% NSS supplemented groups. Chicks fed the diet contained 1% NSS showed higher plasma glutathione level than the control group, 0.5 and 1.5% NSS groups. Feeding diet with supplemented 0.5 and 1.5% NSS significantly ($P\leq0.05$) increased plasma glutathione reductase compared with the control and 1% NSS groups, the lowest glutathione reductase was observed in chicks fed diet containing 1% NSS group. Chicks fed diet containing 0.5 and 1.5% NSS had significantly ($P\leq0.05$) increased plasma catalase compared with the control and chicks fed diet supplemented with 1% NSS groups. The lowest plasma catalase level was observed in the control group. Significantly ($P\leq0.05$) higher plasma total antioxidant capacity was observed in chicks fed diet containing 0.5 and 1% NSS compared with the control and the chicks fed diet containing 1.5% NSS group. While, the lowest plasma total antioxidant capacity was found in the control group. There were no significant ($P\leq0.05$) differences in plasma malondialdehyde among the control and different levels of diet supplemented NSS groups.

NSS possess a highly antioxidative activity and has the capacity to reduce hepatic lipid peroxidation as well as enhancing the activity of many enzymes such as SOD, GSH, catalase, and adenosine deaminase, which they play an important role in reducing the oxidative stress in broilers' livers (Sogut *et al.* 2008; Azeem *et al.*, 2014). Liver indices (AST, ALT) and renal functions (urea and creatinine) could be useful in deciding on the health and physiological status of an animal (Tuluce *et al.*, 2009). The marked antioxidant activity of NSS and thymoquinone could be a realizable modern antioxidant agent to be used as nutrients for the health promotion and disease prevention (Yimer *et al.*, 2019). Recent findings showed an improvement in health status of broilers fed on diets containing various additives, as it was indicated that they have no effects on liver and renal functions and increased antioxidant enzymes.

In accordance with the current outcomes, Attia and Al-Harthi (2015) found out that the groups fed on Nigella sativa oil in the diets exerted a significant increase in the levels of SOD, GSH, GPx, and GRx than the control group. Treating with NSS was linked to the increased levels of GSH activity, which may be ascribed to thymoquinone, which possesses potent anti-oxidative and anti-inflammatory properties (Nili-Ahmadabadi *et al.*, 2011). Aziza *et al*., (2019) discovered that the hepatic GSH level was significantly enhanced just in the groups supplemented with NS oil when compared to both control groups. Accordingly, in a rat model, the fixed and essential oil of NSS had a clear increase

in GR and GPx versus the oxidative stress resulted from potassium bromate (Sultan *et al.*, 2015).

Linked to the key bioactive components of black cumin essential oil, NSS could work as an antioxidant which inhibits the development of free radical and raises the antioxidant enzyme activity (Tülüce *et al.*, 2009). This result is in accordance with El-Hack *et al.*, (2018). Polyphenol-containing substances could suppress nuclear factor kappa signaling; thereby elevating the level of nuclear factor erythroid 2-related factor 2 activation which preserves the cells from oxidative sabotage and promotes antioxidant activity (Tangney and Rasmussen, 2013). As a result, the now time usage of NSS in thirty postmenopausal women demonstrated an un questionable reduction in the levels of plasma MDA with an elevated activity in erythrocyte GPx and SOD after two months of consumption (Mostafa *et al.*, 2015).

CONCLUSION

These findings indicated that NSS had improved lipid profile (decreased triglyceride and cholesterol), liver function (lower AST) and antioxidant indices of broiler chicks. As a result, NSS is marked safe as a result that they have no acute toxic side effects like what was reported throughout the experimental period.

Table (1): Ingredients and compositions of the experimental diets

Ingredients	Starter				Grower diet			
	0	0.5	1	1.5	0	0.5	1	1.5
Corn	54.02	53.52	53.02	52.52	61.02	60.52	60.02	59.52
Soybean meal (44%)	33.0	32.5	32.5	32.5	26.0	26.0	26.0	26.0
Corn gluten meal (60%)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Mixed oils (%)	4.5	5.0	5.0	5.0	4.5	4.5	4.5	4.5
Nigella seeds (%)	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Dl- methionine	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
L-lysine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dicalcium phosphate	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Limestone	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Vit. and min. mixture	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Antioxidant	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
ME kcal/ kg diet[2]	3200	3200	3200	3200	3200	3200	3200	3200
Dry matter[3]	90.15	89.8	89.31	88.87	90.06	89.61	89.17	88.73
Crude protein	23.0	23.0	23.0	23.0	20.0	20.0	20.0	20.0
Calcium	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Non-phytate phosphorus[2]	0.48	0.5	0.48	0.47	0.45	0.45	0.45	0.45
Methionine+cystine[2]	0.76	0.7	0.75	0.74	0.68	0.68	0.68	0.68
Lysine[2]	1.36	1.3	1.34	1.34	1.17	1.17	1.17	1.17
Crude fibre[3]	4.25	4.2	4.18	4.1644	3.91	3.90	3.88	3.8674
Crude fat[3]	6.66	7.14	7.12	7.11	6.81	6.80	6.78	6.77

[1]Vit+Min mix. Provides per kilogram of the diet: Vit. A, 12000 IU, vit. E (dl-α-tocopheryl acetate) 20 mg, menadione 2.3 mg, Vit. D3, 2200 ICU, riboflavin 5.5 mg, calcium pantothenate 12 mg, nicotinic acid 50 mg, Choline 250 mg, vit. B_{12} 10 μg, vit. B_6 3 mg, thiamine 3 mg, folic acid 1 mg, d-biotin 0.05 mg. Trace mineral (mg/ kg of diet): Mn 80 Zn 60, Fe 35, Cu 8, and Selenium 0.1 mg.
[2]Calculated values, [3]Analyzed values.

Table (2): Effect of *Nigella sativa* seeds on blood hematological of broiler chicks at 35 days of age

Item	Control	*Nigella sativa* seeds			SEM	P value
		0.5%	1%	1.5%		
RBC's (10^6/mm^3)	10.54[a]	10.42[b]	10.39[b]	10.55[a]	0.059	0.0002
Hgb (g/dl)	11.3	10.9	11.5	11.5	0.044	0.438
PCV (%)	32.8	32.3	33.3	34.1	0.104	0.296
MCV (fl/cell)	31.11	31.00	32.05	32.32	1.661	0.764
MCH (Ug)	10.72	10.46	11.06	10.90	0.626	0.135
MCHC (%)	34.4	33.7	34.5	33.7	0.155	0.188

[a,b,c] Means within the same row with different superscript letters are significantly different at $p \leq 0.05$

RBC= Red blood cells; Hgb= Hemoglobin; PCV= packed cell volume; MCV= Mean Corpuscular volume; MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin concentration

Table (3): Effect of *Nigella sativa* seeds on plasma proteins and lipid profile of broiler chicks at 35 days of age

Item	Control	*Nigella sativa* seeds			SEM	P value
		0.5%	1%	1.5%		
T.pro (g/dl)	5.49[a]	5.40[ab]	5.27[bc]	5.18[c]	0.018	0.010
Alb. (g/dl)	2.51[b]	2.90[a]	2.53[b]	2.56[b]	0.016	0.0030
Glb. (g/dl)	2.98	2.50	2.73	2.62	0.029	0.1060
A/G ratio	0.866	1.196	0.943	0.986	0.017	0.0590
T. lipid (mg/dl)	106.0[b]	106.0[b]	111.0[a]	104.0[b]	0.407	0.0400
Trig. (mg/dl)	185.0[bc]	191[a]	183[c]	187[b]	0.258	0.0001
Cho. (mg/dl)	209.0[b]	213[a]	206[c]	212[a]	0.32	0.0020
HDL (mg/dl)	36.2[c]	39.0[b]	38.1[b]	40.8[a]	0.177	0.0001
LDL (mg/dl)	135.8[a]	135.8[a]	131.3[b]	133.8[ab]	0.149	0.0020
HDL/LDL ratio	0.267[c]	0.287[b]	0.290[b]	0.305[a]	0.016	0.0030

[a,b,c] Means within the same row with different superscript letters are significantly different at $p \leq 0.05$

T.pro= Total protein; Alb= Albumin; Glb= Globulin; A/G ratio= Albumin to Globulin ratio; T.lip= Total lipids; Trig= Triglycerides; Cho= Cholesterol; HDL= High density lipo-protein; LDL= Low density lipo-protein

Table (4): Effect of *Nigella sativa* seeds on liver and renal function indices of broiler chicks at 35 days of age

Item	Control	*Nigella sativa* seeds			SEM	P value
		0.5%	1%	1.5%		
Liver functions						
AST (U/L)	61.9^{bc}	64.1^{a}	60.9^{c}	63.4^{ab}	0.196	0.0001
ALT (U/L)	56.4	57.7	56.2	57.1	0.249	0.6687
AST/ALT ratio	1.10	1.11	1.08	1.11	0.005	0.0628
Alkaline phosphatase (U/L)	12.4	10.3	11.9	11.0	0.153	0.1057
Renal functions						
Urea (mg/dl)	26.6^{b}	28.0^{b}	28.3^{b}	31.7^{a}	0.232	0.0003
Creatinine (mg/dl)	0.77^{b}	1.29^{a}	1.31^{a}	1.31^{a}	0.02	0.0001
U/C ratio	35.4^{a}	21.9^{b}	22.6^{b}	24.4^{b}	0.522	0.0001

^{a,b,c} Means within the same row with different superscript letters are significantly different at $p \leq 0.05$

AST= Aspartate aminotransferase; ALT= Alanine aminotransferase; AST/ALT= Aspartate aminotransferase to Alanine aminotransferase ratio; U/C ratio= Urea to Creatinine ratio

Table (5): Effect of *Nigella sativa* seeds on antioxidant indices of broiler chicks at 35 days of age

Item	Control	*Nigella sativa* seeds			SEM	P value
		0.5%	1%	1.5%		
SOD (IU)	254^{b}	268^{a}	252^{b}	254^{b}	0.483	0.0001
GSH (mg/dl)	1003^{b}	1012^{b}	1055^{a}	1006^{b}	1.567	0.0001
GPX (U/ml)	0.407^{b}	0.471^{a}	0.411^{b}	0.411^{b}	0.003	0.0001
GR (U/ml)	29.0^{b}	30.2^{a}	27.2^{c}	30.6^{a}	0.11	0.0001
Cat (nmol/min/ml)	32.8^{c}	39.8^{a}	34.7^{b}	38.7^{a}	0.151	0.0001
TAC (nmol/l)	418^{c}	453^{a}	455^{a}	444^{b}	0.754	0.0001
MAD (µmol/l)	1.4	1.43	1.39	1.59	0.023	0.1179

^{a,b,c} Means within the same row with different superscript letters are significantly different at $p \leq 0.05$

SOD = superoxide dismutase; GSH = glutathione; GPx = glutathione peroxidase activity; GR = glutathione reductase; Cat= Catalase; TAC= total antioxidant capacity; MDA= Malondialdehyde.

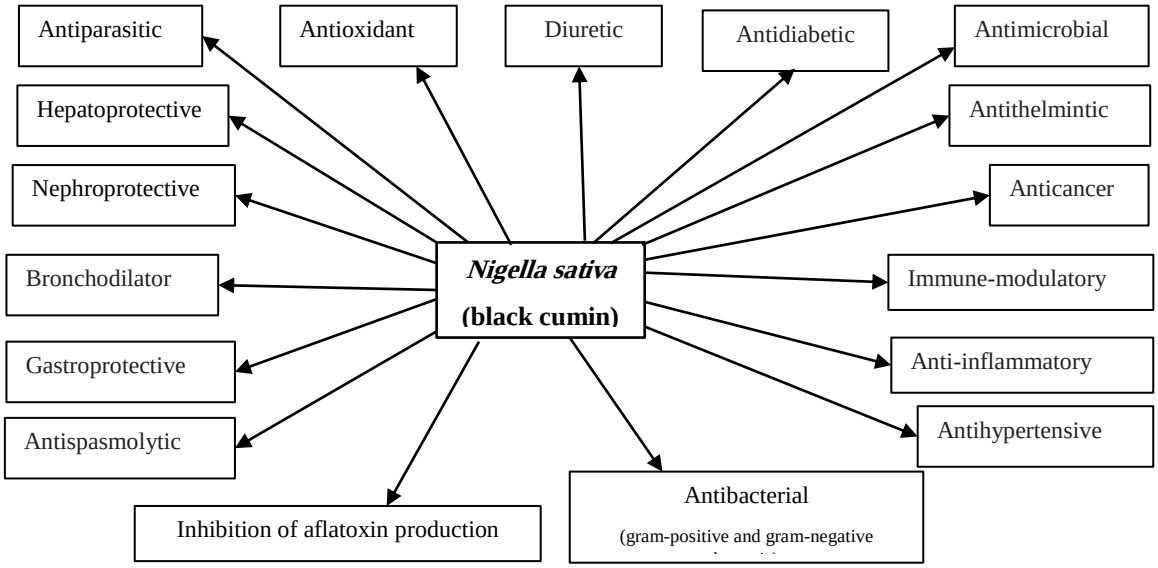

Figure (1): distinguish properties of *Nigella sativa* (black cumin**)**

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Broiler; *Nigella sativa* seeds; liver and kidney functions; antioxidant.

الملخص العربى

تقييم بذور حبة البركة على صفات كتاكيت اللحم الهيماتولوجية والبيوكيميائية في الدم والإنزيمات المضادة للأكسدة

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استهدف البحث دراسة تأثير إضافة بذور حبة البركة على صفات الدم الكيميائية والهيماتولوجية ووظائف الكبد والكلى والأنزيمات المضادة للأكسدة لكتاكيت التسمين. تم استخدام ١٤٠ كتكوتاً تسمين غير مجنسة عمر يوم حتى عمر ٣٥ يوماً. قسمت الكتاكيت عشوائياً في تصميم عشوائي بسيط إلى أربعة معاملات، بكل معاملة ٧ مكررات وبكل مكررة ٥ كتاكيت كالتالي: مجموعة الكنترول أو إضافة بذور حبة البركة بمعدل ٠.٥ و١ و١.٥٪ على الترتيب. زاد عدد كرات الدم الحمراء للكتاكيت التي تم تغذيتها على العليقة الكنترول أو العليقة المضافة إليها ١.٥٪ بذور حبة عن العليقة المضاف إليها بذور حبة البركة بمستويات ٠.٥ و١٪، ارتفع مستوى ألبيومين البلازما للكتاكيت التي تم تغذيتها على ٠.٥٪ بذور حبة البركة مقارنة بالكنترول و١ و١.٥٪ بذور حبة البركة. وكان أقل مستوى للدهون الثلاثية والكوليسترول للكتاكيت المغذاة على عليقة تحتوي على ١٪ بذور حبة البركة مقارنة بالكتاكيت التي تغذت على عليقة تحتوي على ٠.٥ و١.٥٪ بذور حبة البركة، وأدت إضافة بذور حبة البركة بمستوى ١.٥٪ إلى زيادة الليبوبروتين عالي الكثافة مقارنة بالكنترول والمعاملات الأخرى. وانخفض مستوى الأسبارتات أمينو ترانسفيراز في بلازما الكتاكيت التي تغذت على عليقة تحتوي على ١٪ بذور حبة البركة مقارنة بالكتاكيت التي تغذت على عليقة تحتوي على ٠.٥ أو ١.٥٪ بذور حبة البركة. كل المستويات المختلفة من بذور حبة البركة أدت إلى إرتفاع كرياتينين البلازما وانخفاض نسبة اليوريا إلى الكرياتينين مقارنة بالكنترول. إضافة بذور حبة البركة بمستوى ٠.٥٪ أدت إلى زيادة أكسيد ديسموتاز البلازما ونشاط الجلوتاثيون بيروكسيديز مقارنة بالكنترول والمجموعات الأخرى. بينما زاد مستوى جلوتاثيون البلازما بإضافة بذور حبة البركة بمستوى ١٪ مقارنة بالكنترول والمستويات الأخرى. وزاد مستوى الجلوتاثيون باضافة بذور حبة البركة بمستوى ١% مقارنة بالكنترول وإضافة حبة البركة بمعدل ٠.٥ و١.٥%. وزاد مستوى الجلوتاثيون والكاتلاز بإضافة بذور حبة البركة بمستوى ٠.٥ و١.٥٪ مقارنة بالكنترول ومستوى ١٪ من بذور حبة البركة.
الخلاصة: أشارت النتائج إلى أن استخدام بذور حبة البركة أدت إلى تحسن تمثيل الدهون (انخفاض الدهون الثلاثية والكوليسترول)، وتحسن وظائف الكبد (انخفاض AST) وزادت مضادات الأكسدة لكتاكيت التسمين. وبالتالي تعتبر بذور حبة البركة بمعدل ١% آمنة لعدم وجود أي آثار جانبية سامة لها خلال فترة التجربة.

الكلمات الدالة: بدارى اللحم، بذور حبة البركة، وظائف الكبد والكلى، مضاد الأكسدة