Effects of dietary *Nigella sativa* seed supplementation on broiler productive performance, oxidative status and qualitative characteristics of thighs meat

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**ABSTRACT**

The objective of our study was to evaluate the effects of *Nigella sativa* seeds (NS) supplementation on meat quality and antioxidants content of chicken meat. Two hundred 1-d-old male broiler chicks were divided into four diet treatment groups: normal control (baseline feed only), NS supplemented (1 and 2%) groups and a standard (200 IU/kg vitamin-E) VE group. At the end of this period, 10 birds were randomly selected from each group for examination. Feed conversion ratio was significantly lower in the treatment groups than the control one. There were no significant differences in moisture or crude ash percentage in thigh muscle among groups, but dietary NS powder supplementation resulted in a significant increase in crude protein content and decrease in crude fat content relative to the control group \((p < 0.05)\). After 24 h, thigh muscle pH was higher while drip loss, cooking loss and shear force were lower in the NS groups than the control group. Lightness values of thigh muscle colour were decreased and redness and yellowness values were increased by NS supplementation. Muscle lipidperoxidation (malondialdehyde) level, which is correlated with significantly higher levels of glutathione peroxidase and superoxide dismutase, were significantly lower \((p < 0.001)\) in the NS groups and VE group than the normal group. These results suggest that NS supplementation is effective at improving broiler performance and meat quality by enhancing antioxidant activities and suppressing lipidperoxidation in meat.

**Introduction**

Maintaining the meat quality (nutritional status, colour, texture, flavour, etc.) during storage to ensure acceptability to the customer is a major challenge for the meat industry. Nutritional status of meat and meat products depends on the level of oxidative deterioration of muscle components, such as lipids and myoglobin. Oxidative deterioration of meat is responsible for discoloration, development of off flavour, formation of toxic compounds, poor shelf life, drip losses and decreased nutrient value (Falowo et al. 2014). Meat oxidative stability is greatly influenced by animal diet (Aouadi et al. 2014). Retarding the lipid peroxidation process is important to maintain a good meat quality. Antioxidant feeds have the capacity to protect against tissue damage by preventing the formation of radicals, by scavenging radicals or by promoting radical neutralization. However, continuous use of synthetic antioxidants as feed additives has harmful effects on human health, as these antioxidants subsequently enter in the food cycle (Falowo et al. 2014). Plant-derived antioxidants are considered suitable alternatives to synthetic antioxidants to minimize the oxidative instability of lipids and proteins in meat (Aouadi et al. 2014).

*Nigella sativa* seeds (NS) have been used as a folk medicine for more than 2000 years due to its multi-systemic beneficial actions. It is commonly known as black seed and is the seed of an annual herb that belongs to the botanical family Ranunculaceae (Abdel-Fattah et al. 2000; Adams et al. 2016). Many active components of NS have been identified, including thymoquinone, dithymoquinone, thymohydroquinone, nigellone, melanthin, nigilline, nigelianine, damascenone, \(p\)-cymene and pinene. NS contain minerals such as magnesium, calcium, phosphorus, potassium, iron, cobalt, zinc and manganese and vitamins A, B, C, D and E (Guler et al. 2006; Cheikh-Rouhou et al. 2007; Khan et al. 2012). NS are rich in both fixed and essential oils, proteins, alkaloids, saponins, polyphenols and flavonoids. The seeds have analgesic, anti-inflammatory, hypolcholestermic, anthelmintic, digestive and appetite stimulant, antidiarrheal, antiulcer, diuretic, spasmyolytic and bronchodilatory, antimicrobial,
anthihypertensive, antidiabetic, anticancer, hepatoprotective and renal protective activities and possess antioxidant properties, including free radical scavenging (Guler et al. 2006; Adam et al. 2016). Previous work has already demonstrated that NS have a wide spectrum of biological activities in broiler chicken, including growth-promoting, immune stimulating and antimicrobial effects (Khan et al. 2012; Azeem et al. 2014). Our main objective in this paper was to investigate the effects of NS supplementation on the quality of chicken meat with emphasis on meat lipid peroxidation and antioxidant activity.

Materials and methods

Birds and experimental design

A total of 200 1-d-old broiler chickens (40.6 g) were used in this study. Chicks were divided into four diet treatment groups: normal control, 1% NS, 2% NS and 200 IU/kg of VE (vitamin E, Sigma-Aldrich, St. Louis, MO). All birds were housed in wire-floored cages in an environmentally controlled room with continuous light. The lighting regimen and ventilation were continuously monitored from day 1 to day 35. Birds had access to feed and water ad libitum. During the experimental period, relative humidity was 44 ± 6%. The room temperature was maintained at 35 °C for the first 3 d, after which, the temperature was gradually reduced to 32 °C, which was maintained during the 35 d of the experiment to generate a high ambient temperature. Strict biosecurity were maintained for the duration of the experiment, and no vaccinations were provided. Experimental diets were formulated to meet broiler nutrient requirements (NRC 1994) for starter (1–21 d) and finisher (22–35 d) growth periods (Table 1). Level of VE in the diets (200 IU/kg) was determined according to study of Choi et al. (2010). Feed intake was calculated by the difference between supplied feed and feed left in each pen. Weight gain was determined as the difference between the initial weight and the weight at 35 d of age. Feed conversion was calculated as the ratio of total feed intake to weight gain.

Slaughter procedure

At the end of the experimental period (5 weeks), birds were fasted for 6 h, and 40 birds were randomly selected, 10 per each treatment group. Birds were slaughtered with sharpened knives according to the Halal method of slaughtering and excised manually to determine carcass yield. Knives were manually sharpened at the beginning and were washed before each bird was slaughtered and excised. All skin (subcutaneous fat and visible connective tissues) was removed from the thigh muscles before the other assessments. Thigh muscles were packed in sealable plastic bags and stored for 1 d at 4 °C (Kim et al. 2009).

Measurement of meat moisture, crude protein (CP), crude fat, crude ash and pH

Moisture, CP, crude fat and crude ash contents of thigh muscle were analysed according to the AOAC (1998). Approximately 10 g of the thigh meat was removed by bones, homogenized with 90 mL of distilled water and then pH was measured by using a portable pH meter (PB-10, Guangzhou Hongtong Machinery Co., Ltd., Guangzhou Guangdong, China). Before measurement, the pH electrode was calibrated using three buffers with pH values of 4.01, 7.00 and 9.01. The average pH value was obtained from three repeated measurements on the same muscle samples.

Drip loss, water holding capacity (WHC), cooking loss, shear force value and colour

An initial muscle strip weight was determined. At 24 h post-mortem, the muscle strip was blotted dry with a paper towel and then weighed to determine muscle strip weight. Percent drip loss was calculated by subtracting the muscle strip weight from the initial weight, multiplying by 100, and then dividing by the initial weight.

WHC of meat samples was evaluated by using the procedure of Naveena and Mendiratta (2001).
Twenty grams of minced meat samples were stirred with 30 mL of 0.6 M sodium chloride in a centrifuge tube. The tube was then kept at 4 ± 1°C for 15 min, stirred again, and centrifuged at 3000 × g (Model 5500, Kubota Corporation, Tokyo, Japan) for 25 min. The supernatant was measured and WHC was expressed as a percentage.

To determine cooking losses of meat, samples were packed in plastic bags and heated in water till the internal temperature of the meat reached 85°C. Meat samples were weighed after cooling to room temperature for 1 h. Loss in weight by cooking was recorded and percent loss in weight was considered to be cooking loss.

Cubes of 1 cm³ were taken from cooked samples after overnight chilling at 4 ± 1°C and sheared using a Warner Bratzler Shear press (Model no. 81031307, GR Elect. Mfg. Co., Smyrna, GA). The force required to shear the samples was determined (kg/cm²).

After 24 h, the lightness, redness and yellowness of thigh meat were measured perpendicular to the meat surface using the photometer Colour-Guide 45/0 (BYK-Gardner GmbH, Geretsried, Germany).

Measurement of muscle lipid peroxidation and antioxidant activities in thigh muscle

Muscle tissues were homogenized in ice-cold isotonic physiological saline solution to form homogenates at a concentration of 0.1g/mL. Samples were centrifuged and the SOD and GSH-Px activities as well as MDA levels of prepared supernatants and sera were measured by spectrophotometric methods. The reaction products were determined by measuring the absorbance at different nm using a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA). Activity of SOD was measured by the xanthine oxidase (Sigma-Aldrich, St. Louis, MO) method, which was measured in absorbance at 450 nm, monitored the inhibition of reduction of nitro blue tetrazolium by the sample (Winterbourn et al. 1975). Activity of GSH-Px was detected with 5,5'-dithiobis (2-nitrobenzoic acid) (Sigma-Aldrich, St. Louis, MO) and the change in absorbance at 412 nm was monitored (Hafeman et al. 1974). MDA level was analysed with 2-TBA (2-thiobarbituric acid) (Sigma-Aldrich, St. Louis, MO), by monitoring the change in absorbance at 532 nm (Jensen et al. 1997).

Statistical analysis

Data are expressed as means ± standard errors of the mean (SEMs). Differences between groups were evaluated by analysis of variance (ANOVA) with the Bonferroni post hoc test or by calculation of Spearman’s rank correlation coefficient, as appropriate, using Prism 5.03 (GraphPad Software Inc., San Diego, CA). Statistical significance was set at p < 0.05.

Results and discussion

Effect of NS on broiler performance

Final body weight and weight gain were significantly higher (p < 0.05) in the treatment groups than the control group, but feed intake and FCR were markedly decreased (p < 0.05) (Table 2). Broiler performances of all treatment groups were superior to those of the controlone, consistent with previous studies (Guler et al. 2006; Khan et al. 2012; Saeid et al. 2013). Because NS have a number of active ingredients and pharmacologically active substances, they are attractive for maintaining the health and improving the performance of poultry. NS have been reported to stimulate secretion of digestive enzymes (lipase and amylase) and intestinal mucous in broilers, to stimulate feed digestion, to impair adhesion of pathogens and to stabilize microbial balance in the gut, leading to better feed utilization and assimilation (Khan et al. 2012; Azeem et al. 2014).

Effect of NS on pH, WHC and shear force in chicken meat

There were significant differences in pH values among all treatment groups (p < 0.05). The pH values increased with an increase in dietary NS and VE supplementation. There were significant differences in WHC and shear force in thigh muscle between all

|          | NC         | 1% NS      | 2% NS      | VE         |
|----------|------------|------------|------------|------------|
| Initial BW | 40.6 ± 2.5 | 40.5 ± 2.9 | 40.9 ± 5.5 | 40.8 ± 5.5 |
| Final BW  | 1774.8 ± 164.6 | 1798.8 ± 202.5* | 1833.6 ± 173.7* | 1819.5 ± 152.1** |
| Weight gain | 1734.2 ± 164.2 | 1758.3 ± 202.1* | 1792.8 ± 175.5** | 1778.7 ± 153.3** |
| Feed intake | 3081.0 ± 308.1 | 3059.0 ± 275.5* | 3018.0 ± 171.8* | 3025.2 ± 252.4* |
| Feed conversion | 1.78 ± 0.25 | 1.74 ± 0.31* | 1.68 ± 0.18** | 1.70 ± 0.20** |

NC: normal control; 1% NS: 1% Nigella sativa-treated group; 2% NS: 2% Nigella sativa-treated group; VE: vitamin E-treated group; BW: body weight. Data are reported as means ± SEMs (n = 10). *p < 0.05 and **p < 0.01, Bonferroni post hoc test following one-way ANOVA versus the NC group.
treatment groups and the normal group (Table 3). Muscle pH does not merely indicate muscle acidity, but is also strongly correlated with meat tenderness, drip loss, and meat colour. High pH values were observed in the VE group and 2% NS powder group, potentially because of the antioxidant effects of NS powder and VE. Similar findings were reported in a previous study (Zhang et al. 2012); antioxidant α-tocopherol succinate increased meat pH compared with the control. One of the most important physiological factors influencing drip loss is the extent of the post-mortem decrease in pH. A lower muscle pH after death results in higher drip loss due to the lower ability of muscle proteins to bind water and greater myofibrillar shrinkage brought about by reduced electrostatic repulsion between filaments (den Hertog-Meischke et al. 1997). In particular, muscle WHC, which is assessed in terms of drip loss or water loss, directly influences meat taste, succulence, colour, nutrients and flavour. Meat products can vary in colour, firmness or texture according to WHC and shear force values (Kim et al. 2009).

As shown in Table 3, VE and NS dietary inclusion significantly affected the shear force of thigh muscle, which could be due to MDA in thigh muscle. It is well known that MDA is an indicator of endogenous lipid peroxidation, and that muscle oxidation can decrease meat tenderness by inhibiting the activities of protein catabolic enzyme and other proteolytic enzymes in muscle (Rowe et al. 2004). The NS or VE administration appears to have reduced muscle MDA production, in our study. Reduction of the shear force due to inclusion of NS had a tenderizing effect. Similar effects on shear force values were previously observed for broiler chickens fed garlic bulbs and garlic husks (Kim et al. 2009; Choi et al. 2010).

### Effect of NS on meat moisture, CP, crude fat and crude ash of chicken meat

Proximate composition of chicken thigh muscle is shown in Table 4. There were no significant differences in moisture or crude ash content among treatment groups. Crude protein content was significantly increased, and crude fat content was decreased ($p < 0.05$) in all treatment groups compared with the control group (Table 4). CP and crude fat content of meat were inversely proportional (Hossain et al. 2012). Moreover, Choi et al. (2010) found that dietary α-tocopherol and garlic powder supplementation resulted in significantly higher CP and lower crude fat content in comparison with the control, consistent with our study findings.

### Effect of NS supplementation on the colour status of thigh muscles of broilers

The meat colour of the thigh muscle of broilers, as expressed in terms of lightness, redness and yellowness, was significantly influenced by dietary supplementation. The highest lightness values and the lowest redness and yellowness values were obtained in the control group, but treatment with 1 and 2% NS and VE resulted in a decrease in lightness values and higher redness and yellowness values (Table 5). Myoglobin is a haem protein that is an important determinant of meat quality. It is responsible for the colour of meat (haem groups, i.e. prosthetic groups of iron-containing porphyrins that are able to bind oxygen and that give haem proteins their typical colour) and can cause undesirable discoloration when exuded from muscle tissue or extravasated from the circulatory system. Bruises, haemorrhages and post-mortem muscle effluences are considered to be major quality defects.

### Table 3. Effects of dietary NS supplementation on pH, water holding capacity (WHC) and shear force in chicken meat after 35 d.

|                | NC          | 1% NS       | 2% NS       | VE          |
|----------------|-------------|-------------|-------------|-------------|
| pH             | 6.08 ± 0.03 | 6.09 ± 0.02*| 6.20 ± 0.02**| 6.12 ± 0.02**|
| WHC, %         | 55.30 ± 0.23| 56.38 ± 0.38*| 57.21 ± 0.26**| 56.79 ± 0.20*|
| Shear force, kg/cm² | 3.65 ± 0.09 | 3.44 ± 0.07**| 3.35 ± 0.07**| 3.42 ± 0.04* |
| Drip loss, %   | 3.22 ± 0.04 | 2.41 ± 0.06  | 2.50 ± 0.05  | 2.67 ± 0.04  |

NC: normal control; 1% NS: 1% Nigella sativa-treated group; 2% NS: 2% Nigella sativa-treated group; VE: vitamin E treated group. Data are reported as means ± SEMs ($n = 10$). *$p < 0.05$ and **$p < 0.01$; Bonferroni post hoc test following one-way ANOVA versus the NC group.

### Table 4. Effect of NS on proximate composition of broiler meat.

|                | NC          | 1% NS       | 2% NS       | VE          |
|----------------|-------------|-------------|-------------|-------------|
| Moisture, %    | 72.82 ± 0.47| 72.85 ± 0.37| 71.76 ± 0.36| 72.27 ± 0.46|
| Crude protein, %| 21.55 ± 0.34| 22.31 ± 0.12*| 22.68 ± 0.21**| 21.98 ± 0.23**|
| Crude fat, %   | 3.72 ± 0.05 | 2.76 ± 0.05*| 2.38 ± 0.04**| 2.40 ± 0.03**|
| Crude ash, %   | 1.08 ± 0.01 | 1.07 ± 0.01  | 1.06 ± 0.01  | 1.05 ± 0.01  |

NC: normal control; 1% NS: 1% Nigella sativa-treated group; 2% NS: 2% Nigella sativa-treated group; VE: vitamin E treated group. Data are reported as means ± SEMs ($n = 10$). *$p < 0.05$ and **$p < 0.01$; Bonferroni post hoc test following one-way ANOVA versus the NC group.
(Kranen et al. 1999). Increasing the lightness of meat and decreasing redness might be associated with an increase in metmyoglobin formation due to oxidation of myoglobin (Fernandez-Lopez et al. 2005). Furthermore, the presence of antioxidant compounds in natural extracts could retard metmyoglobin formation in meatballs and decrease lightness values (Fernandez-Lopez et al. 2005). Aksu and Kaya (2005) also found that in the absence of antioxidants, meat had a lower redness value than in their presence of antioxidants. Antioxidant supplements protect against tissue damage by preventing the formation of radicals, by scavenging radicals, or by promoting their decomposition, resulting in better meat colour (Choi et al. 2010; Falowo et al. 2014). Our finding that NS, which contains antioxidant compounds, could retard metmyoglobin formation and oxidation in the thigh muscle of chicks, is, therefore, not surprising.

**Effect of NS supplementation on lipid peroxidation (MDA) and antioxidant status in the thigh muscle of broilers after 24 h**

In the NS-treated group (1 and 2% NS), lipid peroxidation as measured by MDA (malondialdehyde) was 24% and 28% of levels in the control group, while in the VE-treated group, there was a 30% decrease in MDA. Antioxidant activities (GSH-PX and SOD) were also up-regulated in the NS groups (Figure 1). Lipids (in the form of triacylglycerides, phospholipids and sterols) are widely distributed in both the intra- and extra cellular spaces of meat. Lipids are chemically unstable and prone to oxidation, especially during post-mortem handling and storage. Lipid peroxidation is an autocatalytic mechanism that results in the oxidative destruction of cellular membranes. This destruction can lead to the production of toxic and reactive aldehyde metabolites, known as free radicals. Among these free radicals, malondialdehyde (MDA) is the most important and main secondary product of lipid peroxidation, and is, therefore, frequently used to determine oxidative damage (Jensen et al. 1997). In this study, we found lower MDA and higher antioxidant activities (SOD and GSH-PX) in the NS and VE-treated groups than the normal control one. NS have the ability to inhibit lipid peroxidation and act as superoxide anion scavengers. NS have strong antioxidant activity and can decrease hepatic lipid peroxidation and increase the activities of several enzymes such as superoxide dismutase, glutathione-S-transferase, catalase and adenosine deaminase, all of which decrease oxidative stress in the livers of broilers (Sogut et al. 2008; Azeem et al. 2014). A dietary supply of NS to rats had a beneficial effect on the antioxidative enzymes superoxide dismutase (SOD)

![Figure 1](image.png)

**Table 5.** Effect of NS on colour status of thigh muscle of broilers after 35 d.

|       | NC    | 1% NS | 2% NS | VE    |
|-------|-------|-------|-------|-------|
| Lightness, % | 57.18 ± 25 | 55.92 ± 40* | 55.01 ± 0.47** | 56.01 ± 28* |
| Redness, %   | 5.37 ± 0.06 | 5.53 ± 0.06* | 5.83 ± 0.07** | 5.76 ± 0.05** |
| Yellowness, % | 3.48 ± 0.05 | 3.63 ± 0.03* | 3.80 ± 0.04** | 3.85 ± 0.03** |

NC: normal control; 1% NS: 1% *Nigella sativa*-treated group; 2% NS: 2% *Nigella sativa*-treated group; VE: vitamin E-treated group. Data are reported as means ± SEMs (n = 10). *p<0.05 and **p<0.01, Bonferroni post hoc test following one-way ANOVA versus the NC group.
and glutathione peroxidase (GSH-PX) in various tissues. Rodent treated with NS or its constituents had greater levels of antioxidants than the untreated controls (Mahmoud et al. 2002; Adam et al. 2016). Inclusion of NS seeds in the diet resulted in a significant decrease in erythrocyte malondialdehyde (MDA) concentration, production of lipid peroxides and increased glutathione (GSH) concentration in chickens. NS protect against oxidative stress by inhibiting free radical production and by regulating glutathione (Tuluce et al. 2009). Hashemipour et al. (2013) reported that the intake of herbs in chickens resulted in an increase in serum antioxidant enzyme activities and a decrease in MDA levels. It has been extensively demonstrated that meat oxidative stability is greatly influenced by the diet of animals (Aouadi et al. 2014).

Conclusions

Therefore, in view of the above arguments and the new data presented herein, we propose that NS supplementation has a two-fold benefit: it decreases the feed conversion ratio and increases meat quality. It should, therefore, be considered a good supplement in the broiler industry.

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