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# The effects of black cumin (*Nigella sativa l.*) seed on carcass characteristics, kidney oxidant antioxidant levels and ileum histomorphology in Japanese quails

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**ABSTRACT:** This study aims to determine the effects of supplementation of different level of black cumin seeds (NS) to quail feeds on carcass characteristics, kidney oxidant antioxidant levels, and ileum histomorphology. A total of 432 mixed-gender three days old Japanese quailswere randomly divided into four groups, including 108 animals each and further into 6 replications consisting of 18 chicks. The groups were fed on either a basal diet alone (Control Group) or a basal diet supplemented with NS at rates of 0.5% (NS-0.5 Group), 1% (NS-1 Group), and 2% (NS-2 Group).It was determined that the back+neck weight in the NS-2 group and head weight and ratio in the NS-1 and NS-2 groups decreased. The thigh rate increased in the NS-0.5 group compared to the control and other NS groups. While the wing weight increased in male quails fed with NS-supplemented feed in NS-1 and NS-2 groups compared to the control group, the abdominal fat weight decreased in NS-supplemented groups.Also, the slaughter weight and hot carcass weight in female quails were significantly reduced in the NS-2 group compared to the control group.Kidney glutathione (GSH), glutathione peroxidase (GPx), and male quails' catalase (CAT) values were increased in NS groups compared to the control group. The kidney vitamin C (Vit C) increased only in the NS-2 group compared to the control. In the NS-2 group, the villus height decreased both on a group basis and in female quails, while the villus width decreased only in female quails. Consequently, it was determined that dietary supplementation of NS at rates of 0.5% increased the thigh rate, dietary supplementation at three different level of NS were effective in maintaining the kidney oxidant-antioxidant balance, and the ileum histomorphology did not change except for dietary supplementation of NS at rates of 2%. Therefore, the result of this study can be stated that NS can be used as a natural antioxidant source in the quail diet.

***Keywords*:** animal nutrition;antioxidant;gender; glutathione;histomorphology; vitamin C

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## INTRODUCTION

Increasing concerns about the use of antibiotics and synthetic antioxidants in the nutrition of farm animals are directing breeders to plants containing these substances(Rahman et al., 2022). Antimicrobial and antioxidant extracts contained in plants improve the health and performance of the animal by positively affecting the digestive system microflora (Cimrin, 2018). Due to its phytogenic compounds, black cumin (*Nigella Sativa L.*) plays a significant role as an endogenous antioxidant, and it can be used as a cytoprotective agent against tissue damage (El-Hack et al., 2018; Guler et al., 2007). Black cumin seeds(NS) consist of polyphenolic compounds including caffeic acid, ellagic acid, epicatechin, ferulic acid, kaempferol, rutin, protocatechuic acid, and quercetin (Gueffai et al., 2022; Toma et al., 2015). Previous studies have shown that NS is a natural antimicrobial, antiviral, antioxidant, immunostimulant in poultry and can provide multisystemic beneficial effects that support poultry health and growth performance, among other pharmacological properties (Azeem et al., 2014; Kumarand Patra, 2017). Black cumin seed oil or compounds obtained by the cold pressing method show strong antimicrobial and antioxidant effects against gram-negative and gram-positive strains. As it is known, black cumin has been widely used in the treatment of various diseases in traditional medicine for centuries. It is also considered safe for health (Erturk,et al. 2020). The slaughter weight of broiler chickens whose feed is supplemented with NS increases and the antioxidant properties of the meat is improved (Guler et al., 2007; Kumar et al., 2018; Kumar and Patra, 2017). The main active ingredient of black cumin, thymoquinone, reduces oxidative stress (Meral et al., 2018) and has a protective effect on the kidney (Guzelsoyet al., 2018). The decrease in malondialdehyde (MDA) level and increase in glutathione (GSH) level, catalase (CAT), and glutathione peroxidase (GPx) activities in the liver of quails with 0.05% and 0.1% black seed oil supplementation to quail feed reveal the antioxidant properties of black cumin (El-Hacket al., 2018). While Hamedi et al. (2014) stated that villus height increased and crypt depth decreased in broiler chickens with NS supplementation, Saeid et al. (2013), on the other hand, stated that both the villus height and the crypt depth increased in quails with 0.5% black seed supplementation. In the literature, there are mostly studies examining the effects of using NS in quail feeds on the performance, food digestibility and economic efficiency of quails (Karadagoglu et al., 2019; Tufan et al., 2015). In addition, in previous studies, serum oxidant and antioxidant levels of NS in broiler feeds were examined (Aydogan et al., 2020; Hassan, 2021). However, in the literature reviews, the number of studies related to the effect of the usage of NS in quail rations on carcass properties, kidney oxidant-antioxidant parameters and ileum histomorphology in quails was extremely insufficient. Moreover, a study similar to the present study was not found in the literature. Hence, this study was carried out to determine the effects of supplementation of different levelsof NS to quail diet on carcass characteristics, kidney oxidant-antioxidant parameters (MDA, GSH, and Vitamin C (Vit C) levels, GPx and CAT activities), and ileum histomorphology.

## MATERIALS AND METHODS

### Ethical guidelines and experimental design

This study was accepted with the letter numbered 40960114-604.01.01-2920 dated 14/01/2020, and the decision numbered 2020/10-13 of Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee. Animal materials of the study were fed in Hatay Mustafa Kemal University Experimental Research Application and Research Center for 35 days. A total of 432 quail chicks were divided into four groups (4x108 units), each with six replicates (6x18). Commercial chick grower feed based on corn and soy, prepared in accordance with the nutritional recommendations of the National Research Council (NRC, 1994), was used as a basal diet. The metabolizable energy and crude protein values of basal diet and NS were determined as 2935 kcal/kg - 22.40% and 2991 kcal/kg - 19%, respectively.Therefore, the metabolizable energy and crude protein value corresponding to each kg of NS supplementation were deducted from the feeds of the treatment groups and the diets were prepared as isocaloric and isonitrogenous.The fatty acid composition of the NS was determined 8,11-octadecadienoic acid (44.11%), 9-octadecenoic acid (31.26%), hexadecanoic acid (14.97%), 11,14-eicosadienoic acid (3.13%), and octadecanoic acid (2.78%). The groups were formed as basal diet + 0% NS (Control Group), basal diet + 0.5% NS (NS-0.5 Group), basal diet + 1% NS (NS-1 Group), and basal diet + 2% NS (NS-2 Group). At least twice a day throughout the trial, the feeder, drinker, and litter were checked. Feed and water were given *ad libitum*.The gender of the quails was differentiated by looking at the breast feathers at the 3rd week (spotted ones females, plain brown ones males) (Kazak et al., 2020).

At the end of the fifth week a total of 48 female and 48 male quails from 4 groups (namely 12 females (2 x 6 replications) and 12 males (2 x 6 replications) with a live weight close to the mean live weight of each group were slaughtered by cervical dislocation.

**Determining the proportional values of carcass characteristics in experimental groups**

Slaughter weight, hot carcass weight, eviscerated carcass weight, breast, thigh, wing, and back+neck weights, edible visceral weights (liver, heart, gizzard), abdominal fat and head weights were determined as carcass characteristics of quails with the measurements made before and after slaughter.

Hot carcass yield (%) = (Hot carcass weight / Slaughter weight) x 100

Eviscerated cold carcass yield (%) = (Eviscerated carcass weight / Slaughter weight) x 100

Breast percentage (%) = (Breast weight / Hot carcass weight) x 100

Thighpercentage (%) = (Thigh weight / Hot carcass weight) x 100

Wing percentage (%) = (Wing weight / Hot carcass weight) x 100

Back+neck percentage (%) = (Back+neck weight / Hot carcass weight) x 100

Liver percentage (%) = (Liver weight / Hot carcass weight) x 100

Heart percentage (%) = (Heart weight / Hot carcass weight) x 100

Gizzard percentage (%) = (Gizzard weight / Hot carcass weight) x 100

Abdominal fat percentage (%) = (Abdominal fat weight / Hot carcass weight) x 100

Headpercentage (%) = (Headweight / Slaughter weight) x 100

**Taking tissue samples**

The kidney tissue taken during slaughter was fixed at -80 °C until the biochemical analyzes, and the intestinal tissue taken for histopathological analyzes was fixed in formalin.

**Biochemical analyzes**

For the homogenization of kidney tissue, approximately 1 g of tissue sample was taken and homogenized in 10 ml of phosphate buffer using an ultrasonic homogenizer. Tubes containing homogenate were centrifuged at 5000 rpm, 4°C, for 30 minutes (Kazaket al., 2020).Obtained supernatants were stored at -80°C until spectrophotometric analyzes.MDA levelswas determined by Ohkawa et al. (1979)'s method and presented as nmol/g tissue.GSH levelswas measured byEllman (1959)'s method and presented asμmol/g protein. Vit C levelswas determined byHaag (1985)'smethod and presented as μg/g tissue.GPx activitywas measured byPaglia and Valentine(1967)'s method and presented as U/g protein.CATactivitywasdetermined byAebi (1984)'s method and presented as k/g protein.

**Histopathological examination**

Ileum tissues were fixed with 10% buffered formalin. According to the routine methods, after passing through alcohol and xylol series, 5 μm thick sections were embedded in paraffin and deparaffinized in xylol and stained with Hematoxylin-Eosin (H&E) after passing through 100, 96, 80, and 70 alcohol series. Measurements were made after examination with an optical microscope (Olympus CX31, Japan) (Luna, 1968).

**Statistical analysis**

SPSS (version 23.0, IBM Corp. Armonk, New York, USA) software package was used for statistical analysis of the study data. Differences between group means were determined by analysis of variance (One-Way ANOVA), and differences between groups were determined by Duncan multiple comparison Test. A difference of $P<0.05$ was considered significant. The values were expressed as mean ± standard error means (SEM).

## RESULTS

The carcass characteristics of quails are presented in Table 1. There was no significant difference between the groups in terms of slaughter weight, hot carcass weight, breast weight, thigh weight, wing weight, liver weight, heart weight, abdominal and fat weight ($P>0.05$). While NS supplementation to the diet did not affect the eviscerated carcass weight and gizzard weight compared to the control ($P>0.05$), it was determined that the differences between the NS groups were significant ($P<0.05$).It was determined that the back + neck weight significantly decreased in the NS-2 group compared to the control group ($P<0.05$), and the head weight significantly decreased

**Table 1.Carcass characteristics of quails (g)**

| Characteristics | Gender | Groups | | | | SEM | p-value |
|---|---|---|---|---|---|---|---|
| | | Control | NS-0.5 | NS-1 | NS-2 | | |
| **Slaughter weight** | Mixed | 205.59 | 210.41 | 203.42 | 198.58 | 1.581 | 0.072 |
| | Male | 192.56 | 199.43 | 190.61 | 190.64 | 1.495 | 0.134 |
| | Female | 218.63$^{a}$ | 221.38$^{a}$ | 216.24$^{a}$ | 206.53$^{b}$ | 1.527 | 0.008 |
| **Hot carcass weight** | Mixed | 155.34 | 158.84 | 152.77 | 148.19 | 1.268 | 0.090 |
| | Male | 145.34$^{ab}$ | 149.51$^{a}$ | 141.16$^{b}$ | 142.12$^{b}$ | 1.070 | 0.036 |
| | Female | 165.34$^{a}$ | 165.17$^{a}$ | 164.38$^{a}$ | 154.26$^{b}$ | 1.374 | 0.021 |
| **Eviscerated carcass weight** | Mixed | 126.23$^{ab}$ | 128.82$^{a}$ | 123.10$^{b}$ | 122.98$^{b}$ | 0.811 | 0.036 |
| | Male | 122.67$^{ab}$ | 126.50$^{a}$ | 118.25$^{b}$ | 120.38$^{b}$ | 0.939 | 0.023 |
| | Female | 229.89 | 131.13 | 127.96 | 125.58 | 1.144 | 0.350 |
| **Breast weight** | Mixed | 50.71 | 51.76 | 49.28 | 50.16 | 0.439 | 0.249 |
| | Male | 48.51 | 49.72 | 48.16 | 48.65 | 0.571 | 0.789 |
| | Female | 52.92 | 53.79 | 50.40 | 51.68 | 0.581 | 0.200 |
| **Thigh weight** | Mixed | 28.18 | 29.27 | 28.13 | 27.80 | 0.213 | 0.087 |
| | Male | 27.75$^{ab}$ | 29.45$^{a}$ | 27.87$^{ab}$ | 26.80$^{b}$ | 0.301 | 0.028 |
| | Female | 28.60 | 29.09 | 28.39 | 28.80 | 0.290 | 0.852 |
| **Wing weight** | Mixed | 6.75 | 6.94 | 7.17 | 6.87 | 0.071 | 0.202 |
| | Male | 6.35$^{b}$ | 7.14$^{a}$ | 7.20$^{a}$ | 6.89$^{a}$ | 0.089 | 0.006 |
| | Female | 7.16 | 6.75 | 7.14 | 6.85 | 0.103 | 0.394 |
| **Back+neckweight** | Mixed | 40.12$^{a}$ | 40.38$^{a}$ | 38.38$^{ab}$ | 37.18$^{b}$ | 0.431 | 0.032 |
| | Male | 39.52$^{a}$ | 39.79$^{a}$ | 35.95$^{b}$ | 37.03$^{ab}$ | 0.543 | 0.040 |
| | Female | 40.72 | 40.96 | 40.82 | 37.34 | 0.638 | 0.146 |
| **Liver weight** | Mixed | 4.33 | 4.04 | 4.06 | 4.08 | 0.094 | 0.666 |
| | Male | 3.70 | 3.56 | 3.33 | 3.63 | 0.086 | 0.460 |
| | Female | 4.96 | 4.51 | 4.80 | 4.53 | 0.120 | 0.493 |
| **Heart weight** | Mixed | 1.93 | 1.90 | 1.73 | 2.06 | 0.062 | 0.315 |
| | Male | 1.81 | 1.89 | 1.57 | 2.18 | 0.117 | 0.327 |
| | Female | 2.04 | 1.91 | 1.90 | 1.94 | 0.042 | 0.630 |
| **Gizzard weight** | Mixed | 4.10$^{ab}$ | 4.45$^{a}$ | 3.92$^{b}$ | 3.98$^{b}$ | 0.068 | 0.031 |
| | Male | 3.78 | 4.08 | 3.66 | 3.62 | 0.077 | 0.152 |
| | Female | 4.43 | 4.81 | 4.17 | 4.33 | 0.092 | 0.103 |
| **Abdominal fat** | Mixed | 3.73 | 2.91 | 2.72 | 2.81 | 0.161 | 0.109 |
| | Male | 4.08$^{a}$ | 2.80$^{b}$ | 2.32$^{b}$ | 2.89$^{b}$ | 0.201 | 0.025 |
| | Female | 3.38 | 3.02 | 3.12 | 2.73 | 0.254 | 0.838 |
| **Head weight** | Mixed | 9.31$^{a}$ | 9.29$^{a}$ | 8.49$^{b}$ | 8.45$^{b}$ | 0.051 | 0.001 |
| | Male | 9.32$^{a}$ | 9.32$^{a}$ | 8.43$^{b}$ | 8.72$^{b}$ | 0.070 | 0.001 |
| | Female | 9.29$^{a}$ | 9.26$^{a}$ | 8.55$^{b}$ | 8.17$^{b}$ | 0.071 | 0.001 |

$^{a,b}$Differences between values with different superscripts on the same line are statistically significant (P<0.05, P<0.001).

in the NS-1 and NS-2 groups compared to the control group (P<0.001).While the effect of gender was insignificant in breast weight, liver weight and heart weight (P>0.05), it was significant in other measured carcass characteristics (P<0.05).In male quails, the addition of NS to the feed did not affect the hot carcass weight, eviscerated carcass weight and thigh weight compared to the control group (P>0.05), yet the differences between the NS groups were significant (P<0.05).In addition, the wing weight of male quails increased in the NS-1 and NS-2 groups compared to the control group (P<0.01), while the abdominal fat weight decreased in the NS-supplemented groups (P<0.05).Moreover, the back + neck weight of male quails decreased in the NS-1 group compared to the control group (P<0.05).On the other hand, in female quails, the slaughter weight (P<0.01) and hot carcass weight decreased in the NS-2 group compared to the control group (P<0.05).The head weight decreased in both male and female quails in NS-1 and NS-2 groups compared to the control group(P<0.001).

The carcass piece rate characteristics of quails are presented in Table 2.There was no statistical difference between the groups in terms of hot carcass yield, eviscerated carcass yield, breast percentage, wing percentage, back+neck percentage, liver percentage, heart percentage, gizzard percentageand abdominal fat percentage (P>0.05). However, the thigh percentage increased significantly in the NS-0.5 group compared to the control group (P<0.001). Also, it was determined that the head percentage decreased significantly in the NS-1 and NS-2 groups compared to the control group (P<0.01).

Altough the effect of gender was insignificant in eviscerated cold carcass yield, back+neck percentage, liver percentage, heart percentage, and gizzard percentage (P>0.05), it was significant in other calculated carcass characteristics (P<0.05).In male quails, the thigh percentage increased in the NS-0.5 group compared to the control group (P<0.001), yet the head percentage decreased significantly in the NS-1 group (P<0.05). The wing percentage increased in male quails of NS-1 and NS-2 groups compared to the control group (P<0.01), while the abdominal fat percentage decreased in all three NS groups (P<0.05).In female quails, while the hot carcass yield percentage decreased (P<0.05), the thigh percentage increased (P<0.001) in the NS-0.5 group compared

**Table 2.** Carcass piece rate characteristics of quails (%)

| Characteristics | Gender | Groups | | | | SEM | p-value |
|---|---|---|---|---|---|---|---|
| | | Control | NS-0.5 | NS-1 | NS-2 | | |
| **Hot carcass yield** | Mixed | 75.59 | 74.55 | 75.10 | 74.60 | 0.226 | 0.329 |
| | Male | 75.56 | 74.97 | 74.20 | 74.54 | 0.396 | 0.653 |
| | Female | 75.62$^{ab}$ | 74.13$^{c}$ | 76.00$^{a}$ | 74.66$^{bc}$ | 0.214 | 0.013 |
| **Eviscerated carcass yield** | Mixed | 61.59 | 61.38 | 60.69 | 61.98 | 0.356 | 0.629 |
| | Male | 63.72 | 63.44 | 62.18 | 63.13 | 0.384 | 0.526 |
| | Female | 59.47 | 59.32 | 59.19 | 60.83 | 0.492 | 0.620 |
| **Breast percentage** | Mixed | 32.76 | 33.04 | 32.38 | 33.89 | 0.252 | 0.194 |
| | Male | 33.45 | 33.28 | 34.05 | 34.24 | 0.325 | 0.681 |
| | Female | 32.06$^{ab}$ | 32.80$^{ab}$ | 30.72$^{b}$ | 33.53$^{a}$ | 0.349 | 0.042 |
| **Thigh percentage** | Mixed | 18.22$^{b}$ | 22.74$^{a}$ | 18.51$^{b}$ | 18.79$^{b}$ | 0.149 | 0.001 |
| | Male | 19.11$^{b}$ | 23.28$^{a}$ | 19.71$^{b}$ | 18.87$^{b}$ | 0.154 | 0.001 |
| | Female | 17.34$^{c}$ | 22.19$^{a}$ | 17.30$^{c}$ | 18.71$^{b}$ | 0.201 | 0.001 |
| **Wing percentage** | Mixed | 4.36 | 4.44 | 4.74 | 4.65 | 0.056 | 0.062 |
| | Male | 4.38$^{b}$ | 4.77$^{ab}$ | 5.13$^{a}$ | 4.85$^{a}$ | 0.070 | 0.005 |
| | Female | 4.34 | 4.11 | 4.36 | 4.45 | 0.070 | 0.381 |
| **Back+neck percentage** | Mixed | 25.93 | 25.83 | 25.17 | 25.12 | 0.266 | 0.590 |
| | Male | 27.18 | 26.61 | 25.52 | 26.02 | 0.323 | 0.306 |
| | Female | 24.68 | 25.05 | 24.82 | 24.23 | 0.394 | 0.900 |
| **Liver percentage** | Mixed | 2.77 | 2.57 | 2.64 | 2.74 | 0.051 | 0.478 |
| | Male | 2.55 | 2.38 | 2.37 | 2.54 | 0.059 | 0.547 |
| | Female | 3.00 | 2.76 | 2.92 | 2.94 | 0.071 | 0.689 |
| **Heart percentage** | Mixed | 1.24 | 1.22 | 1.12 | 1.39 | 0.040 | 0.167 |
| | Male | 1.25 | 1.26 | 1.11 | 1.52 | 0.076 | 0.308 |
| | Female | 1.24 | 1.18 | 1.16 | 1.26 | 0.028 | 0.506 |
| **Gizzard percentage** | Mixed | 2.64 | 2.83 | 2.57 | 2.68 | 0.037 | 0.093 |
| | Male | 2.59 | 2.73 | 2.60 | 2.56 | 0.052 | 0.660 |
| | Female | 2.69 | 2.93 | 2.54 | 2.80 | 0.052 | 0.067 |
| **Abdominal fat percentage** | Mixed | 2.41 | 1.87 | 1.77 | 1.89 | 0.101 | 0.112 |
| | Male | 2.77$^{a}$ | 1.87$^{b}$ | 1.65$^{b}$ | 2.01$^{b}$ | 0.130 | 0.024 |
| | Female | 2.06 | 1.87 | 1.89 | 1.76 | 0.155 | 0.925 |
| **Head percentage** | Mixed | 4.55$^{a}$ | 4.43$^{ab}$ | 4.20$^{b}$ | 4.27$^{b}$ | 0.040 | 0.010 |
| | Male | 4.85$^{a}$ | 4.68$^{ab}$ | 4.44$^{b}$ | 4.58$^{ab}$ | 0.047 | 0.025 |
| | Female | 4.25$^{a}$ | 4.19$^{a}$ | 3.96$^{b}$ | 3.95$^{b}$ | 0.033 | 0.003 |

$^{a,b,c}$Differences between values with different superscripts on the same line are statistically significant (P<0.05, P<0.01, P<0.001).

to the control group. Additionally, the breast percentage of female quails ($P<0.05$) in the NS-1 group and the head percentage of female quails in the NS-1 and NS-2 groups decreased compared to the control group ($P<0.01$).

The effect of NS supplementation to diet on renal MDA and antioxidant levels of quails are presented in Table 3. Compared to the control group, GSH, GPx, and CAT values were found to be increased in all NS groups except NS-1 group females, and Vit C level increased only in the NS-2 group without any gender difference, and the differences between the groups were significant ($P<0.05$;$P<0.001$). The differences between the MDA values of the groups and the CAT values of female quails were found to be not significant.

Intestinal histomorphology of the groups are presented in Table 4. It was determined that the villus height in mixed-gender and female quails of the NS-2 group, and the villus width in the female quails of the NS-2 group were lower than the control and other NS groups, and the differences between the groups were significant ($P<0.001$; $P<0.01$). In terms of crypt depth, the differences between groups were found to be not significant ($P>0.05$).

## DISCUSSION

Supplementation of NS to the diet did not affect other carcass characteristics,including slaughter weight, hot carcass weight, eviscerated carcass weight, breast weight, thigh weight, wing weight, liver weight, heart weight, abdominal fat weight compared to the control group. Back+neck weight in the

**Table 3.** Effect of black cumin seed supplementation to diet on renal malondialdehyde and antioxidant levels of quails

| Parameters | Gender | Groups | | | | SEM | p-value |
|---|---|---|---|---|---|---|---|
| | | Control | NS-0.5 | NS-1 | NS-2 | | |
| **Malondialdehyde (nmol/g tissue)** | Mixed | 71.08 | 66.56 | 63.16 | 64.24 | 1.103 | 0.052 |
| | Male | 70.48 | 69.88 | 64.43 | 67.90 | 1.435 | 0.450 |
| | Female | 71.68 | 63.23 | 61.88 | 60.57 | 1.644 | 0.068 |
| **Glutathione (µmol/g protein)** | Mixed | $9.02^{c}$ | $11.83^{b}$ | $12.74^{b}$ | $16.07^{a}$ | 0.373 | 0.000 |
| | Male | $6.39^{c}$ | $10.40^{b}$ | $12.45^{a}$ | $14.14^{a}$ | 0.519 | 0.000 |
| | Female | $11.65^{c}$ | $13.25^{b}$ | $13.04^{bc}$ | $18.00^{a}$ | 0.435 | 0.000 |
| **Vitamin C (µg/g tissue)** | Mixed | $64.29^{b}$ | $79.40^{b}$ | $77.87^{b}$ | $135.84^{a}$ | 4.442 | 0.000 |
| | Male | $60.84^{b}$ | $83.04^{b}$ | $80.74^{b}$ | $177.17^{a}$ | 8.266 | 0.000 |
| | Female | $67.73^{b}$ | $75.77^{b}$ | $75.00^{b}$ | $94.52^{a}$ | 2.493 | 0.000 |
| **Glutathione peroxidase (U/g protein)** | Mixed | $195.72^{b}$ | $229.05^{a}$ | $229.40^{a}$ | $245.57^{a}$ | 4.018 | 0.000 |
| | Male | $184.18^{c}$ | $211.80^{b}$ | $240.62^{a}$ | $210.31^{b}$ | 4.635 | 0.000 |
| | Female | $207.26^{c}$ | $246.31^{b}$ | $218.17^{c}$ | $280.83^{a}$ | 6.030 | 0.000 |
| **Catalase (k/g protein)** | Mixed | $0.21^{c}$ | $0.24^{ab}$ | $0.24^{ab}$ | $0.26^{a}$ | 0.006 | 0.030 |
| | Male | $0.17^{b}$ | $0.25^{a}$ | $0.24^{a}$ | $0.25^{a}$ | 0.009 | 0.001 |
| | Female | 0.26 | 0.24 | 0.24 | 0.28 | 0.007 | 0.262 |

$^{a,b,c}$Differences between values with different superscripts on the same line are statistically significant ($P<0.05$, $P<0.001$).

**Table 4.** Intestinal histomorphology of the groups

| Parameters | Gender | Groups | | | | SEM | p-value |
|---|---|---|---|---|---|---|---|
| | | Control | NS-0.5 | NS-1 | NS-2 | | |
| **Villus height (µm)** | Mixed | $386.96^{a}$ | $351.41^{a}$ | $368.21^{a}$ | $305.54^{b}$ | 7.568 | 0.001 |
| | Male | 360.87 | 331.28 | 368.62 | 312.95 | 9.763 | 0.151 |
| | Female | $413.04^{a}$ | $371.54^{a}$ | $367.80^{a}$ | $298.13^{b}$ | 11.503 | 0.003 |
| **Villus width (µm)** | Mixed | 87.32 | 84.99 | 88.89 | 73.62 | 2.436 | 0.108 |
| | Male | 79.02 | 79.26 | 99.58 | 83.43 | 3.309 | 0.086 |
| | Female | $95.61^{a}$ | $90.72^{a}$ | $78.20^{ab}$ | $63.81^{b}$ | 3.596 | 0.005 |
| **Crypt depth (µm)** | Mixed | 64.84 | 60.28 | 68.85 | 70.66 | 2.009 | 0.269 |
| | Male | 63.66 | 57.67 | 76.91 | 77.01 | 3.247 | 0.076 |
| | Female | 66.01 | 62.88 | 60.79 | 64.30 | 2.338 | 0.887 |

$^{a,b}$Differences between values with different superscripts on the same line are statistically significant ($P<0.05$ $P<0.01$, $P<0.001$).

NS-2 group and head weight in the NS-1 and NS-2 groups were significantly decreased compared to the control and NS-0.5 groups. Although there was an increase in slaughter weight, hot carcass weight, breast weight, and thigh weight in the NS-0.5 group compared to the control and other NS groups, this increase was not statistically significant. The supplementation of NS to the diet did not affect the eviscerated carcass weight and gizzard weight compared to the control group. However, it was determined that the eviscerated carcass weight and gizzard weight of the NS-0.5 group were found to be significantly heavier than the other NS groups.The supplementation of NS to the diet increased the wing weight of male quails compared to the control group; But decreased the abdominal fat weight, which can be considered a positive development regarding animal health and reproductive performance.However, in this study, the slaughter weight and hot carcass weight of female quails of the NS-2 group containing the highest NS decreased compared to both control and NS groups containing lower NS.Hence, it can be concluded that adding less than 2% black cumin to quail feed contributes more positively in terms of slaughter weight.In terms of carcass characteristics, the increase in the thigh percentage in the NS-0.5 group and the decrease in the head percentage in the NS-1 and NS-2 groups compared to the control group were significant. Likewise, in both male and female quails, the thigh percentage increased in the NS-0.5 group compared to the control and other NS-supplemented groups; Also, the abdominal fat ratio decreased with the supplementation of NS.Therefore, considering that the quails closest to the group mean were slaughtered, it can be stated that in terms of carcass characteristics,compared to other NS-added groups, the best results were achieved in the NS-0.5 group and the rate of NS supplement was significant in this respect. Consistent with the results of this study, Majeed et al. (2010) determined that the supplementation of 0.5% NS to broilers diet increased the thigh percentage but did not affect the breast weight.In different studies conducted with quails; Karadagogluet al. (2019) found that supplementing the diet with 1% and 2% NS did not affect the slaughter weight, hot carcass weight, heart, and liver weight; Arif et al. (2018) found that the supplementation of 0.5% black cumin powder to the diet did not affect liver, gizzard, heart and thigh weights; Tufan et al. (2015) found that the supplementation of 1% NS did not affect carcass yield, breast, thigh and wing ratios. These results are consistent with the result of the present study that supplementation of 0.5%, 1%, and 2% NS to the diet did not affect slaughter, hot carcass, wing, liver and heart weights, compared to the control group.In some studieswith broilers; Amadand Mohammed(2013) found that the addition of 1.5%, 2.5%, and 3.5% NS did not affect slaughter weight, carcass yield, and edible visceral rates; Kumar et al. (2018) stated that slaughter weight, carcass yield, breast, thigh, wing, neck, liver, and abdominal fat weight, and total edible visceral weight increased or tended to increase with the addition of 0.5%, 1% and 2% black cumin to the diet.Contrary to the results of the present study; Tufanet al. (2015) determined that the 1% NS supplementation to the diet increased the slaughter weight and cold carcass weight of quails; Arifet al. (2018) determined that 0.5% black cumin powder supplementation increased carcass weight, breast weight, intestine weight, and intestine length; Erener et al. (2010) determined that 1% NS supplementation increased the carcass weight of broiler chickens.Similarly, El-Hacket al. (2018) stated that 0.05% black cumin oil diet improves carcass characteristics.

Although kidney MDA values showed a tendency to decrease in the NS groups, this decrease was not significant compared to the control group. Similar to this study, Hassan (2021) reported that 0.5%, 1%, and 1.5% black cumin supplementation did not affect the plasmaMDA level in broiler chickens. Likewise, Aydoganet al. (2020) reported that 0.5% NS supplementation did not affect serum total antioxidant and oxidant levels. Tuluce et al. (2009) reported that 0.5% and 1% NS supplemented diet decreased erythrocyte MDA levels, while 1.5% NS supplementation did not. Another study reported that 0.5%, 1%, 2%, and 3% NS supplementation to diet tended to decrease MDA levels measured in blood and different tissues (Guler et al., 2007).

Renal GSH levels, GPx activities, and CAT activities were significantly increased in all NS groups. In previous studies, it has been reported that thymoquinone, the main active ingredient of black cumin, reduces oxidative damage (Meralet al.,2018) and increases intracellular GSH production (Daba and Abdel-Rahman, 1998). Tuluce et al. (2009) determined that erythrocyte GSH levels increased in broiler chickens fed diets containing 0.5% and 1% NS, but no change was observed in erythrocyte GSH levels in chickens fed a diet containing 1.5% NS. In studies similar to this study, it has been reported that thymoquinone, the main active ingredient of black cumin, increases CAT and GPx activities (El-Hack et al., 2018; Harzallah et al., 2012; Hassan, 2021; Sogut et al., 2008). It has also been reported to have lipid peroxidation inhibitory effects and antioxidant activity (Harzallah et al., 2012). The hydrogen peroxide causing cell lipid peroxidation is prevented by CAT or GPx (Lee and Kang, 2022). This study shows that black cumin can be effective for detoxification of hydrogen peroxide, which occurs with the increase in renal CAT activities, and the contribution of black seed to nutritional performance. Hassan (2021) stated that there is an improvement in the health status of broiler chickens due to the fact that black cumin increases antioxidant enzymes. The present study suggests that renal GPx activity increases with black cumin supplementation and that GPx activity is used to maintain the oxidant-antioxidant balance. Tuluce et al. (2009) attributed the effect of reducing lipid peroxidation and increasing GSH levels of black cumin to the effect of reducing oxidants such as hydrogen peroxide and hydroxyl radical. This study reveals that NS have an antioxidant effect as a result of the increase in CAT and GPx causes a decrease in hydrogen peroxide, and also the use of NS tends to decrease kidney MDA.

Vitamin C level tended to increase in all NS groups, but the increase was significant only in the NS-2 group. It is known that black cumin contains Vit C (Tekeli, 2014), and some antioxidant compounds such as Vit C reduce lipid peroxidation. In the present study, considering that the levels of Vit C in kidney tissue tend to increase with black seed supplemented diet, especially in the NS-2 group, but MDA values do not change, it is predicted that MDA values may also decrease significantly with the supplementation of black seed to the diet at levels higher than 2%.

It was determined that the ileum villus height and width decreased significantly in female quails of the NS-2 group but remained unchanged in males. Therefore, it can be stated that while black cumin does not adversely affect ileum histomorphology in males, 2% level has a negative effect in females. As it is known, villus height, villus width, and crypt depth in ileum histomorphology are accepted as indicators of the healthy intestine in poultry. Accordingly, short villus reduces nutrient absorption and thus reduces growth performance (Fan et al.,1997; Kumar and Patra, 2017; Samanya et al., 2002). As there may be other reasons, the reduction in slaughter weight of the NS-2 group may be associated with the decrease in villus height of the same group. In parallel with the intestinal histomorphology results of the NS-0.5 and NS-1 groups of the study, Kumar and Patra (2017) stated that the use of black cumin in diets did not affect the intestinal histomorphology.

## CONCLUSION

As a result, the supplementation of NS at a rate of 0.5% to the feed, compared to other rates, had a better effect on carcass characteristics. NS supplemented (0.5%,1%, and 2%) diet positively affected kidney GSH level, Vit C level, GPx activity, and CAT activity. However, 2% NS supplementation negatively affected intestinal histomorphology in female quails. Therefore, it is considered that NS can be used as a protective agent against tissue damage as a good antioxidant; however, more detailed studies are needed in terms of the antioxidant capacity of quail meet and its effects on the intestine. In addition, further studies are recommended to determine the levels of phenolic compounds of NS in quail meat and the effects of NS on the shelf life of quail meat.Consequently, this study suggests that the supplementation of NS at a rate of 0.5% to the feed is beneficial for quails.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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