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Effect of Chrome Nanoparticle on Growth Performance, Liver Tissue, and Oxidative Stress in Japanese Quails

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Abstract

In this study, the effects of adding different levels of chromium nanoparticles to quail feeds on growth performance and some blood and liver parameters of quails were examined. A total of 160 broiler quails were used, split into four groups (4 × 40 pieces) each with four replications (4 × 10 pieces). The experiment was designed according to a completely random design, including one control and three experimental groups: basal diet (control); basal diet + 0.4 mg kg^{-1} Nano-Cr (40–50 nm) (NCr-04); basal diet + 0.8 mg kg^{-1} Nano-Cr (40–50 nm) (NCr-08); basal diet + 1.2 mg kg^{-1} Nano-Cr (40–50 nm) (NCr-1.2). In order to determine the growth performance of quails, live weight gains, feed conversion rate, and feed consumption were determined. Oxidative stress index, total antioxidant status, total oxidant status,) and liver fat levels were determined using blood and liver samples. Weight gain was significantly higher in the Nano-Cr supplemented groups than in the control group only in the first week. The best performance of final body weight was achieved in group I (NCr-04). Feed consumption during 0–35 days was found to be lower in the Nano-Cr added groups compared to the control group ($p < .05$). Supplementing different levels of Nano-Cr in quails diet significantly improved feed conversion rate ($p < .05$). Fatty liver level was lower in group II (NCr-08) and group III (NCr-1.2) than in the control and group I (NCr-04) ($p < .01$). Cell infiltration was highest in group II. In conclusion, it was determined that the addition of Nano-Cr to the feed decreased feed consumption (average 7.3%), improved feed efficiency (average 8.7%), and decreased the level of liver fat (average 69.4%).

Keywords: Chrome, fatty liver, nanotechnology, poultry, quail

Introduction

Nanominerals, a product of developing technology, are employed extensively in various industries, including agriculture, livestock, and the production of food. Nanominerals have growth-stimulating, immunomodulatory, and antibacterial effects in animal nutrition even at lower levels than conventional mineral sources. It has been stated that using nanominerals in the diet increases the absorption and therefore reduces the excretion of minerals (Swain et al., 2015).

Chromium is one of the most common elements found in soil layers and sea water. It has a significant role in carbohydrate, insulin, and blood lipid metabolism. Chromium (III) stabilizes nucleic acids against structural degradation. It stimulates fatty acid and cholesterol synthesis in the liver. Chromium (III) is included in the glucose tolerance factor structure, which works like a hormone and facilitates the passage of glucose from the blood to various tissues together with insulin, and also supports the intracellular metabolism of nutrients (Apanoğlu, 2008). Chromium (III) aids in controlling hypoglycemia and excess intake of protein and protects against heart diseases and diabetes. At the same time, chromium has the ability to improve the immune system and its development. Chromium supplementation in the diets of humans and healthy animals increases the level of insulin binding and glucose tolerance, so that blood glucose is at normal levels. Symptoms of chromium deficiency are related to insulin, some of which are glucose tolerance, glycosuria, increased insulin concentration, brain diseases, decreased reproduction, and peripheral neuropathy. Cardiovascular risks also increase in relation to the decrease in insulin, glucose, and lipid metabolism due to low chromium concentration (Aydın & Şehu, 2021).

The effects of chromium (III) on immunological factors in poultry have been examined in many studies. Only about 0.5–3% of inorganic chromium is absorbed from the gastrointestinal tract. However, chromium nanoparticles (in the form of $CrCl_3$ and 30–60 nm in size) are absorbed much more efficiently. Desai et al. showed that nanoparticles are absorbed from the gastrointestinal tract 15 to 205 times more than microparticles. The electrical, magnetic, mechanical, and biological properties of nanoparticles change as the size decreases and the surface area increases. In previous studies, it was stated that when inorganic chromium was added to poultry feed, growth, feed conversion rate (FCR), and carcass yield increased,

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while blood total cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and glucose concentrations decreased (Feng et al., 2021; Hossain et al., 2017; Huang et al., 2016; Kheiri & Toghyani et al., 2009; Kroliczewska et al., 2004; Mohammed et al., 2014; Samanta et al., 2008; Toghyani et al., 2012).

The fact that quails are small-bodied creatures and the frequency of settlement in their rearing allows for intensive production in small areas. The short incubation period, early sexual maturity, and low feed consumption per animal have made quail egg production a branch of the commercial livestock industry. However, the production of quail meat is not as widespread as that of eggs, although it contains more protein and less fat than other meats. It is also very rich in minerals such as calcium, phosphorus, iron, copper, zinc, and selenium (Sarıca et al., 2014).

It is thought that chromium nanoparticles added to feed may provide economic benefit by reducing feed consumption and increasing the weight of quails, and may reduce mortality rates by improving some blood and liver parameters. In the study carried out for this purpose, the effects of chromium nanoparticles added to quail feeds at various levels on live performance, weight gain, feed consumption, and some blood and liver parameters were examined.

Materials and Methods

A total of 160, 1-day-old mixed-sex Japanese quail (*Coturnix coturnix japonica*) chicks were used. Each chick was visually checked for quality and weighed, and the chicks were then distributed to the groups so that there was no statistical difference between the initial average weights of the groups. The quail chicks were placed on three floors from top to bottom of five-layer rearing cages, each layer consisting of two cells (47.5 × 45 × h25 cm; VGS brand). In the rearing cages, the chick level temperature was 33–35°C for the first week, and they were raised at room temperature (24°C) from the second week until the end of the study. During the study, the feeder, drinker, and litter materials of the quails were inspected twice a day or more, and any problems encountered were resolved. Sex discrimination was made by examining the breast feathers of the quails starting from the third week. Quail with spotted chest feathers are female, while those with unspotted, smooth brown chest feathers are male. During the study, the quails were fed ad libitum. This study was carried out after the animal experiment was approved by Hatay Mustafa Kemal University Local Ethics Committee (Approval no: 2020/04-25, Date: June 24, 2020).

Experimental Design

One hundred sixty-one-day-old Japanese quails (*Coturnix coturnix japonica*) were distributed randomly to four treatment groups with four replicates of ten chicks. The groups were as follows: basal diet (control); basal diet + 0.4 mg kg^{-1} Nano-Cr (40–50 nm) (NCr-04); basal diet + 0.8 mg kg^{-1} Nano-Cr (40–50 nm) (NCr-08); basal diet + 1.2 mg kg^{-1} Nano-Cr (40–50 nm) (NCr-1.2). The basal diet consisted of commercially produced chick grower feed. The main ingredients of this feed were corn and soy, and it was prepared following the nutritional recommendations of the National Research Council. The basal diet's composition has been shown in Table 1. The study lasted 35 days.

Body weight gain and feed intake of quails were recorded on a weekly basis. Feed consumption of quails was determined by subtracting the quantity of feed left in the feeder at the end of the week from the total quantity of weekly feed given. The average daily feed consumption was calculated by dividing the feed consumption per week by the number of days and animals. Feed efficiency was determined by dividing the feed consumption per week by the live weight gain per week.

Table 1.
Composition of Basal Diet

Ingredient	Amount (g kg^{-1})
Corn	508.0
Wheat	77.0
Wheat berry	45.0
Soybean meal	275.0
Fish meal	55.0
Vegetable oil	15.0
Limestone	10.0
Dicalcium phosphate	7.5
Sodium chloride	2.5
Vitamin–mineral premixes[1]	5.0
Calculated composition	
Crude protein (g kg^{-1})	221.0
Metabolizable energy (kcal kg^{-1})	12.6
Calcium (g kg^{-1})	9.0
Available phosphorus (g kg^{-1})	6.0
Lysine (g kg^{-1})	11.0

Note: [1]The premix provided the following, per kilogram of diet: retinyl acetate, 1.8 mg; cholecalciferol, 0.025.mg; α-tocopherol acetate, 1.25 mg; menadione sodium bisulfite, 1.1 mg; thiamine, 1.1 mg; riboflavin, 4.4 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; pyridoxine, 2.2 mg; folic acid, 0.55 mg; cyanocobalamin, 0.02 mg; manganese, 74 mg; zinc, 45 mg; copper, 4 mg; iron, 12.5 mg; iodine, 0.3 mg; selenium, 0.15 mg.

In the study, 12 female and 12 male quails were separated from each group for slaughter (2020/04-25), based on the average live weight at the fifth week determined for each group at the close of the growth period. As there were four study groups, 4 × 12 = 48 female and 4 × 12 = 48 male quails were included in the slaughter. The quails separated for slaughter were individually weighed, their slaughter weights were determined, and their wing numbers were attached.

Slaughter and Carcass Characteristics

After the quails had been slaughtered, their skin and feathers were cleaned. Before the skinless carcass was disintegrated, the solid (not eviscerated) carcass was weighed, and then the eviscerated carcass was weighed after removing the internal organs. Heart, liver, and gizzard weights were determined as edible internal organ weight. Abdominal fat weight and chest weight were also measured. Solid carcass yield, eviscerated carcass yield, breast yield, heart yield, gizzard yield, liver yield, and abdominal fat yield were calculated (Abedpour et al., 2017; Foroutankhah et al., 2019).

Collecting Blood and Liver Samples

In the study, two females (2 × 4 = 8) and two males (2 × 4 = 8) were slaughtered from each subgroup, a total of 16 quails, and their blood samples were taken. The collected blood samples were transferred to

pre-numbered heparinized tubes. After being centrifuged for 1700g × 15 minutes for serum sampling, they were stored at −18ºC until biochemical analysis. Tissue samples were taken from livers whose weights were determined during slaughter, and they were mashed by a mixer. Then, 2 g was weighed and the chloroform–methanol solution prepared in a ratio of 2:1 was added until the sample was covered. The vial was tightly closed, covered with Parafilm to prevent airflow, and stored at −18ºC until analysis.

Histopathological Analysis

All animals were necropsied after euthanasia. 10% buffered formalin was used to fix the collected liver tissues. Thick sections of 5 µm were taken. They were passed through alcohol and xylol series, embedded in paraffin, and then deparaffinized in xylol. They had passed through decreasing alcohol series (100, 96, 80, and 70), and they were subjected to staining with hematoxylin and eosin (H & E). After examination through a light microscope (Olympus CX31), microphotographs (Olympus DP12) were taken. Histopathological evaluations in the liver were made using the criteria defined by Güvenç et al. (2020) and Özkan et al. (2022).

Oxidative Stress Analysis

Total antioxidant status (TAS) and total oxidant status (TOS) were determined by employing ready-made commercial kits and Erel's method (2004). The oxidative stress index (OSI) was determined using the ratio of these two values. Total antioxidant status values are given as mmol Trolox equiv./L and TOS values as µmol H_2O_2 equiv./L.

Determination of Protein Translation Levels

Interleukin 1β and tumor necrosis factor alpha (TNF-α) levels in the serum samples of the blood taken from the experimental groups were determined by following the protocols of the ready-made ELISA kits (BT-lab, Korea). The data obtained are given in ng L^{-1}.

Statistical Analysis

The Statistical Package for Social Sciences version 22.0 software (IBM Corp.; Armonk, NY, USA), was used to statistically analyze the data. For the characteristics determined in the study, one-way analysis of variance (ANOVA) was employed to determine whether the group averages differed from each other, and the Duncan test was used to calculate between-group differences. The Kruskal–Wallis test was used to compare the groups in terms of fatty liver level, and the Mann–Whitney *U*-test was used for different groups.

Results

Initial and weekly live weights of the four groups are shown in Table 2. Adding Nano-Cr to the diet significantly increased the live weight ($p = .004$) in the first week compared to the control. In addition, the live weights in the second, third, and fifth weeks of the growth period were found to be higher in the groups with Nano-Cr added to the feed than in the control group ($p > .05$).

The total amount of feed consumed, live weight gains, and feed conversion ratios of the study groups between days 0 and 35 are presented in Table 3. Nano-Cr supplemented groups differed significantly from the control group in terms of total feed consumption and FCR ($p = .030$ and $p = .017$, respectively).

The slaughter and carcass part characteristics of the groups are given in Table 4. The differences between the groups were found to be insignificant with regard to solid carcass weight, eviscerated carcass weight, breast weight, heart weight, liver weight, heart weight, and abdominal fat weight ($p > .05$). However, in terms of slaughter weight, the NCr-08 group was significantly lower than that of the control, NCr-04, and NCr-1.2 groups ($p = .027$). In terms of liver weight and liver ratio, the difference between the control group and the Nano-Cr supplemented groups was insignificant ($p > .05$), while

Table 2.
Live Weights of the Experimental Groups

Periods	Control	NCr-04	NCr-08	NCr-1.2	SEM	*p*
Beginning	9.01	8.95	9.12	9.03	0.071	.866
First week	31.15[b]	33.53[a]	34.09[a]	34.02[a]	0.325	.004
Second week	77.38	77.89	78.88	78.73	0.681	.847
Third week	133.58	133.82	133.34	134.38	1.102	.989
Fourth week	172.57	170.83	170.10	172.68	1.322	.872
Fifth week	197.14	202.39	197.19	199.23	1.735	.683

Note: [a,b]Different superscripts in the same row indicate the difference between the groups for the feature ($p < .05$).

Table 3.
Body Weight Gain, Feed Intake, and Feed Conversion Ratio of the Experimental Groups

Properties	Control	NCr-04	NCr-08	NCr-1.2	*p*
Body weight gain between days 0 and 35 (g)	188.13	193.44	188.07	190.20	.792
Total feed intake between days 0 and 35 (g)	714.12[a]	660.34[b]	660.20[b]	665.83[b]	.030
Feed conversion ratio between days 0 and 35	3.80[a]	3.41[b]	3.51[b]	3.50[b]	.017

Note: [a,b]Different superscripts in the same row indicate the difference between groups for the feature ($p < .05$).

Table 4.
The Slaughter and Carcass Traits

Characteristics	Control	NCr-04	NCr-08	NCr-1.2	*p*
Slaughter weight	196.84 ± 3.87[ab]	204.27 ± 5.17[a]	181.24 ± 8.43[b]	200.88 ± 3.99[a]	.027
Solid carcass weight	145.18 ± 2.90	151.54 ± 3.98	139.44 ± 4.04	146.45 ± 3.41	.136
Hollow carcass weight	122.48 ± 2.19	127.00 ± 3.08	118.86 ± 3.35	125.19 ± 3.01	.233
Breast weight	48.58 ± 1.05	49.73 ± 1.23	47.70 ± 1.44	49.72 ± 1.37	.626
Heart weight	1.86 ± 0.06	1.94 ± 0.07	1.79 ± 0.14	1.89 ± 0.07	.696
Gizzard weight	4.74 ± 0.14	4.73 ± 0.21	4.62 ± 0.14	4.52 ± 0.18	.780
Liver weight	4.72 ± 0.28[ab]	5.31 ± 0.28[a]	4.21 ± 0.22[b]	4.45 ± 0.17[b]	.014
Abdominal fat weight	1.75 ± 0.23	1.89 ± 0.23	1.32 ± 0.16	1.51 ± 0.15	.177
Proportional Values (%)					
Solid carcass yield	73.74 ± 0.25[a]	74.16 ± 0.22[a]	73.45 ± 0.32[ab]	72.80 ± 0.42[b]	.022
Hollow carcass yield	62.28 ± 0.28	62.26 ± 0.47	62.65 ± 0.30	62.22 ± 0.48	.857
Breast ratio	33.46 ± 0.21	32.91 ± 0.42	34.24 ± 0.39	33.96 ± 0.54	.108
Heart weight %	1.28 ± 0.04	1.28 ± 0.03	1.29 ± 0.10	1.29 ± 0.04	.998
Gizzard ratio	3.27 ± 0.08	3.13 ± 0.12	3.33 ± 0.07	3.08 ± 0.09	.202
Liver ratio	3.22 ± 0.14[ab]	3.49 ± 0.14[a]	2.99 ± 0.10[b]	3.03 ± 0.08[b]	.013
Abdominal fat ratio	1.18 ± 0.14	1.22 ± 0.14	0.93 ± 0.10	1.03 ± 0.10	.298

Note: [a,b]Different superscripts in the same row indicate the difference between groups for the feature ($p < .05$).

the difference between the Nano-Cr groups was statistically significant ($p < .05$). In addition, the mean of the NCr-04 and NCr-08 groups was similar to that of the control group ($p > .05$) in terms of solid carcass yield, but the mean of the NCr-1.2 was significantly different from that of the control group ($p < .05$).

Values related to liver fat level and mononuclear cell infiltration are given in Table 5. While the mean of NCr-04 group was similar to the control group in terms of liver fat levels, the mean of NCr-08 and NCr-1.2 groups demonstrated a significant ($p < .05$) decrease compared to the control group (Figure 1A and 1B). Liver fat level was lower in the Nano-Cr supplemented NCr-08 and NCr-1.2 groups. Mononuclear cell infiltration was higher in the NCr-08 group than in the other groups (Figure 1A and 1B). Microscopic images of liver tissue are shown in Figure 1.

Oxidative stress parameters of serum and liver are given in Table 6. At different concentrations of added chromium (0.4, 0.8, and 1.2 mg kg^{-1}), levels of serum TOS increased (41% ($p = .015$), 34% ($p = .037$), and 19% ($p = .023$), respectively) when compared to the control group. However, this value decreased linearly (about 7%) as the concentration given to quails increased. Chromium added at different concentrations (0.4, 0.8, and 1.2 mg kg^{-1}) caused a decrease in serum TAS levels (41% ($p = .022$), 43% ($p = .018$), and 20% ($p = .024$), respectively) when compared to the control group. However, when the chromium concentration applied to the experimental animals was increased, it was found that the total antioxidant capacity values increased with similar rates. Serum OSI increased by 143%, 137%, and 49%, respectively, in the chromium-treated groups (0.4, 0.8, and 1.2 mg kg^{-1}) compared to the control. It was determined that

Table 5.
Liver Histopathological Values

Properties	Control	NCr-04	NCr-08	NCr-1.2	*p*
Fatty liver	1.08 (0–2)[a]	0.42 (0–2)[ab]	0.33 (0–1)[b]	0.25 (0–1)[b]	.008
Mononuclear cell infiltration	0.00 (0–0)	0.00 (0–0)	0.25 (0-1)	0.00 (0–0)	.024

Note: [a,b]Different superscripts in the same row indicate the difference between groups for the feature ($p < .05$).

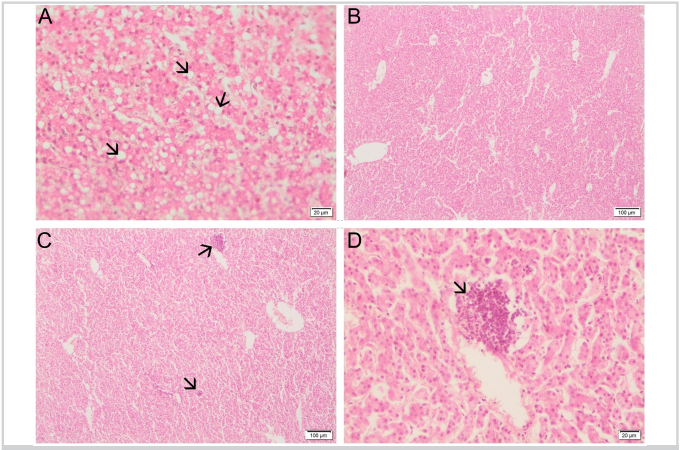

Figure 1.
Microscopic images of liver tissue. (A) Control group female; moderate fatty vacuoles in liver (arrows), hematoxylin and eosin (H&E). (B) NCr-1.2 female; hepatocytes are normal, H&E. (C) NCr-08 female; focal mononuclear cell infiltration, H&E. (D) NCr-04 female; focal mononuclear cell infiltration, H&E.

Table 6.
Serum and Liver Oxidative Stress Parameters

Group	TAS mmol Trolox (equiv./L)	TOS µmol H_2O_2 (equiv./L)	OSI
Serum Oxidative Stress Parameters			
Control	1.8 ± 0.62	6.69 ± 1.10	3.72
NCr-04	1.05 ± 0.66*	9.49 ± 2.92*	9.04
NCr-08	1.02 ± 0.29*	8.98 ± 1.36*	8.80
NCr-1.2	1.44 ± 0.39*	7.97 ± 0.98*	5.53
Liver Oxidative Stress Parameters			
Control	1.3 ± 0.29	19.15 ± 4.67	14.73
NCr-04	0.7 ± 0.07	14.76 ± 7.65*	21.09
NCr-08	1.13 ± 0.24	17.74 ± 5.9	15.70
NCr-1.2	1.2 ± 0.23	23.75 ± 11.74*	19.79

Note: OSI = Oxidative stress index; TAS = Total antioxidant status; TOS = Total oxidant status.
*$p < .05$ relative to control.

Table 7.
Protein Translation Levels

Group	TNF-α (ng/L)	IL-1β (ng/L)
Control	59.26 ± 0.17	11.37 ± 0.07
NCr-04	100.68 ± 0.04*	10.69 ± 0.05
NCr-08	107.52 ± 0.12*	9.01 ± 0.10
NCr-1.2	104.01 ± 0.11*	10.25 ± 0.14

*$p < .05$ relative to control.

the total oxidant capacity levels in the liver tissue were decreased by 22% ($p = .035$) and 7% ($p = .763$), with 0.4 and 0.8 mg kg^{-1} added chromium, respectively, and 1.2 mg kg^{-1} added chromium increased by 23% ($p = .032$) compared to the control group. Chromium added at various concentrations (0.4, 0.8, and 1.2 mg kg^{-1}) decreased TAS levels in liver tissue compared to the control group (46% ($p = .064$), 13% ($p = .091$), and 7% ($p = .218$), respectively). Various concentrations of chromium (0.4, 0.8, and 1.2 mg kg^{-1}) in liver tissue increased OSI levels by 43%, 6%, and 34%, respectively, compared to the control group.

Protein translation levels are shown in Table 7. It was found that, compared to the control group, chromium (0.4, 0.8, and 1.2 mg kg^{-1}) applied at different concentrations in serum samples decreased IL-1β levels by 6% ($p = .573$), 18% ($p = .065$), and 8% ($p = .362$), respectively. Chromium added at various concentrations (0.4, 0.8, and 1.2 mg kg^{-1}) increased TNF-α translation levels in serum samples compared to the control group (57% ($p = 0 = .034$), 65% ($p = .011$), and 61% ($p = .024$) respectively).

Discussion

Although no significant difference was found between the groups in terms of initial body weight, only the first week body weight was significantly higher in the groups whose feed had been supplemented with Nano-Cr than the control group (Table 2). In addition, weekly body weight values during the study were numerically higher in the groups with Nano-Cr supplementation than in the control group. Body weight values were numerically higher than the other groups in the NCr-08 group quails at the beginning, first and second weeks, in the NCr-1.2 group quails at the third and fourth weeks, and in the NCr-04 group at the fifth week. When Nano-Cr supplemented groups were compared among themselves, although the lowest initial body weight was NCr-04, they reached the highest body weight average in the third, fourth, and fifth weeks. This may be due to the fact that chromium plays a role as a cofactor for insulin activation, preventing glucose from accumulating in fatty tissues and thus increasing muscle development in quails. In studies conducted on broiler chickens and quails, it has been stated that Nano-Cr particles added to the diet increase the average body weight and can reduce the body fat ratio (Vincent, 2000) and that the addition of chromium to the feed significantly improved growth performance by increasing average daily weight gain in broilers (Feng et al., 2021). Yarmohammadi et al. (2020) and Kumari et al. (2021) reported that adding Nano-Cr to the feed of Japanese quails and broilers significantly increased the body weight values on the 21st and 42nd days compared to the control group.

Adding Nano-Cr to the feed decreased the total feed consumption between 0 and 35 days compared to the control group, increased the live weight gain value, and improved the rate of feed conversion (Table 3). In addition, it was determined that the feed conversion efficiency was numerically the best in the NCr-04 group using the lowest amount of Nano-Cr compared to the NCr-08 and NCr-1.2 groups. Ognik et al. (2020) found that the addition of Cr-NP (3 mg kg^{-1} Cr-NP and 6 mg kg^{-1} Cr-NP) to broiler feed improved FCR compared to chickens in the control and chromium picolinate groups. In addition, it was found that supplementing broiler chicken feed with Nano-Cr improved the feed efficiency compared to the control group in the studies conducted by Sirirat et al. (2012) and Kumari et al. (2021). In another study, in which Nano-Cr was added to quail feed, feed consumption decreased and the FCR in quails improved because small particle sizes are important for easy absorption from the intestinal mucosa, and nano-sized feed additives have a stronger absorption ability and have an increasing effect on the bioavailability of feed (Budak, 2018).

Addition of Nano-Cr to the diet did not have an effect on slaughter weight, liver weight, solid carcass yield, and other slaughter characteristics except liver yield compared to the control group (Table 4). The NCr-04 group had significantly higher mean values than the other treatment groups for slaughter weight, liver weight, solid carcass yield, and liver yield ($p < .05$). This may be due to the fact that chromium added to the diet affects carbohydrate, lipid, and protein metabolism. El-Kholy et al. (2017) determined that the addition of chromium to the feed of heat-stressed quail chicks can improve carcass characteristics. The animals fed with 3 mg kg^{-1} Cr-NP and 6 mg kg^{-1} Cr-NP added to the broiler's feed were found to have higher values to the control group in terms of slaughter weight and liver weight (Ognik et al., 2020). Moreover, Ahmadi et al. (2013) noted that the slaughter weight of chickens was higher in the 40 mg kg^{-1} nano-zinc oxide group than in the control group in their study on chickens.

In this study, it was determined that adding Cr-Nano in quails at doses of 0.8 and 1.2 mg kg^{-1} reduced ($p = .008$) fatty liver levels compared to the control group (Table 5). Concurrently, mononuclear

cell infiltration was observed only in the group to which 0.8 mg kg^{-1} Cr-Nano was added ($p = .024$). Brody (1999) determined that fatty liver can be formed as a result of free fatty acids increasing and impaired oxidation of hepatic fatty acids. Ognik et al. (2020) determined that the addition of Cr-Picolinate or Cr-Nano 3.0 mg kg^{-1} or 6.0 mg kg^{-1} to the diet of broiler chickens led to hyperemia, mononuclear cell infiltration, and fatty degeneration in the liver compared to the control groups. Liu et al. (2015) stated that excessive chromium consumption causes oxidative stress and histopathological changes in tissues. This study determined that adding Nano-Cr to quail feed did not give rise to morphological changes in the liver.

According to the data obtained from our study, the serum total oxidant level increased in all chromium levels (Table 6). However, serum total antioxidant levels decreased compared to the control group. This could have occurred as a result of the depletion of antioxidant enzymes due to decreased production. In liver tissue, it was observed that low concentrations (0.4 mg kg^{-1}) of chromium decreased the TOS level, while high concentrations (0.8 and 1.2 mg kg^{-1}) led to an increase in the level. Total antioxidant status levels in liver tissue decreased insignificantly at all concentrations, and there was an increase in the OSI at all concentrations. Other data obtained from our study showed that chromium insignificantly affected serum translation levels of IL-1β, one of the inflammatory cytokines, while all concentrations of chromium increased TNF-α translation levels (Table 7). Wang et al. (2010) found that hexavalent chromium exposure increased the release of inflammatory cytokines, including TNF-α, by activating Akt, NF-κB, and MAPK, while also increasing reactive oxygen species. Guéniche et al. (1994) demonstrated that exposure to potassium dichromate can induce TNF-α release for up to 48 hours in normal human keratinocytes. In their study, Sahu et al. (2014) determined that potassium dichromate significantly increased TNF-α levels in an in vivo nephrotoxicity model compared to the control group. Dworzański et al. (2021) showed how serum samples in the groups with added chromium picolinate, chromium methionine, and nanoparticle trivalent chromium significantly increased TNF-α levels compared to the control group in a high-fat diet exposure model. In addition, they reported that oxidative stress triggered inflammation and stimulated NFκB-mediated IL-1B and TNF-α expression. Jain et al. (2007) investigating monocytes of the U937 cell line reported that among the trivalent form of chromium, namely chloride, picolinate, and niacinate, it was chromium niacinate that was most effective in reducing the secretion of IL-6 and IL-8, as well as reducing the malonyldialdehyde concentration. In one study, it was found that, while Cr-Pic caused a significant increase in levels of IL-2 and TNF-a, it was ineffective in the production of IL-6. Compared with picolinate, the main effect of Cr-Met was observed to be a significant increase in IL-6 and TNF-α, while other forms of chromium were more potent in inducing the production of proinflammatory cytokines.

Conclusion and Recommendations

As a result, it was determined that the performance and weight of the quails fed with 0.4 mg kg^{-1} nanochrome diet increased and the mortality rate decreased. However, the least hepatic steatosis and improvement in blood parameters were determined in quails fed 0.8 and 1.2 mg kg^{-1} nanochrome-supplemented diet. In order to collect all the positive effects in a single dose, it is necessary to study different concentrations of nanochrome added to diets.

Ethics Committee Approval: This study was carried out after the animal experiment was approved by Hatay Mustafa Kemal University Local Ethics Committee (Approval no: 2020/04-25, Date: June 24, 2020).

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