Effect of nano-selenium and different stocking densities on performance, carcass yield, meat quality, and feathering score of broilers

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Abstract

Effects of nano-selenium (nano-Se) and stocking density (SD) on growth performance, carcass yield, meat quality, and feathering score of broilers were investigated in this study. One-day-old 480 broiler chickens (45.3±2.4 g body weight) (Ross 308) were randomly divided into 4 treatments each comprising of 8 replicates. In the experiment, treatments consisted of a 2 × 2 factorial arrangement of dietary Se form (inorganic or nano) and SD (low = 12 birds/m^2; LSD, and high = 18 birds/m^2; HSD). No interaction was noted between Se form and SD for any trait. Nano-Se had no effect on growth performance, however, HSD decreased the body weight gain (BWG) ($P<0.05$) and feed intake (FI) ($P<0.001$) while feed conversion ratio (FCR) was unaffected. Neither nano-Se nor HSD had any effect on the relative carcass, breast, and thigh yields. Nano-Se improved the water-holding capacity (WHC) of breast meat 72-hpost-mortem ($P<0.05$). However, pH, colour, and cooking loss of meat remained unaffected by Se form or SD. There were no differences between nano- or inorganic Se and LSD or HSD regarding feathering scores for back and wing. In conclusion, dietary nano-Se improved the WHC and had no significant effect on other parameters. In addition, HSD may negatively affect the growth performance.

Introduction

Selenium (Se) belongs to the class of essential elements required for a wide range of functions such as sustaining of life, growth, meat quality, and feathering. The major factors that determine the effectiveness of Se are its dietary level and form. Today Se is still one of the most discussed elements in poultry nutrition (39). In a living organism, Se is present in the form of selenocysteine as part of selenoproteins (17, 38).

The bioavailability of Se is related to its physical form and is found in diets in two basic forms, inorganic and organic. Mostly inorganic Se sources (e.g. sodium selenite and sodium selenate) used in broiler diets (39). However, there has been an increasing interest in using organic selenium sources (e.g. selenocysteine and selenomethionine) in poultry diets. Se in plants occurs only in organic form and mainly selenomethionine (10). Recently, another form of Se that has been of particular interest is nano-selenium (nano-Se). Nanotechnology is used in animal feeding as well as many other areas and the most important application of nanotechnology in this area are nanominerals. Nanominerals are characterized by a particle size of 1 to 100 nm. (40). Nano minerals have a larger surface area, higher surface activity and are absorbed more easily and effectively than other forms of the same mineral (21).

Optimal housing and nutrition are essential to keep the performance at the highest level in broiler production. One of these environmental conditions is stocking density (SD). High stocking density (HSD) can reduce fixed production costs (such as labor and maintenance) and increase the kilogram of chicken weight produced per unit of area, thus increasing the profitability (19). However, HSD creates stress among the animals, increasing the amount of ammonia in the environment, decreasing the litter quality, and adversely affecting animal health, performance, and product quality (13, 36). Some minerals, such as Se, may be used in broiler diets in order to decrease the adverse effects of HSD (38).

Some studies have reported the use of supplemental nano-Se on growth performance, carcass yield, and meat quality of broiler (10, 29). Moreover, dietary nano-Se has been used to alleviate the negative effect of heat and oxidative stress in broilers (9, 18). However, no study has reported the effect of supplemental nano-Se in broilers under the stressful condition of HSD. Therefore, this study aimed to examine the effect of dietary nano-Se on growth performance, carcass yield, meat quality, and feathering score of broiler chicks under HSD.

Materials and Methods

Experimental design and diets: The experiment comprised of 480 one-day-old Ross 308 male broiler chickens (45.3±2.4 g body weight) randomly allotted to 4 experimental groups with 8 replicates/group as a completely randomized design with 2×2 factorial arrangement of dietary Se form (inorganic or nano) and the SD (low = 12 birds/m^2(LSD) or high = 18 birds/m^2). At the end of experiment, an average 34.3 kg and 50.2 kg body weight/m^2 were determined in groups subjected to LSD and HSD, respectively. Birds were housed in floor pens occupying 1 m^2 floor space (feeders and drinker space excluded) with wood shavings as litter material. A 23L:1D lighting program was implemented up to 7 days and 18L:6D thereafter until day 42. The temperature of 32 °C was maintained during the first week followed by a reduction of 3°C per week until d 21 and a temperature of 24-26°C was maintained afterwards. Free access of birds to feed and water was ensured throughout the experiment. The duration of the experiment was 42 days.

Starter (d 1 to 10), grower (d 11 to 24), and finisher (d 25 to 42) diets based on corn-soybean meal were formulated according to Aviagen (5) in mash form (Table 1). Sodium selenite with 99.0% purity (Sigma-Aldrich Co., USA) was used as inorganic Se source. Nano-Se was prepared at the Department of Nanotechnology Engineering, Cumhuriyet University, Sivas, Türkiye. The nano-Se had 99.95% purity, ranging in size between 30 and 60 nm. Sodium selenite and nano-Se were supplemented at 0.66 and 0.30 mg/kg, respectively, in order to provide 0.3 mg/kg Se in diets. First, the feed additives (vitamins and minerals) were mixed among themselves, then this mixture added to the feed mixture and mixed again.

Table 1. Ingredient and nutrient composition of diets.

Ingredients (%)	**Starter (1-10 d)**	**Grower (11-24 d)**	**Finisher (25-42 d)**
Corn	55.52	56.01	58.95
Soybean meal (48% crude protein)	37.51	36.05	33.44
Vegetable oil	2.51	4.16	4.24
Limestone	0.88	0.84	0.81
Dicalcium phosphate	2.31	2.01	1.72
Salt	0.36	0.34	0.36
Methionine	0.36	0.24	0.13
Lysine	0.20	-	-
Vitamin premix*	0.25	0.25	0.25
Mineral premix**	0.10	0.10	0.10
Nutrient composition (calculated)			
Metabolizable energy (ME), kcal/kg	3033	3151	3191
Crude protein (CP)	22.96	22.00	21.00
Calcium	1.00	0.91	0.82
Available phosphorus	0.50	0.45	0.40
Methionine-cystine	1.09	0.95	0.80
Lysine	1.42	1.21	0.15
Selenium, mg/kg	0.35	0.35	0.35
Nutrient composition (analyzed)			
Dry matter	91.2	90.8	90.5
CP	23.16	22.18	21.24
Ether extract	6.32	8.04	8.23
Neutral detergent fiber	9.25	8.74	8.38
Crude ash	5.51	5.45	5.39
Selenium, mg/kg	0.34	0.34	0.34

*Vitamin premix (per kilogram diet): vitamin A 12 000 IU, vitamin D_3 3 000 IU, vitamin E 50 mg, vitamin K_3 5 mg, vitamin B_1 3 mg, vitamin B_2 6 mg, niacin 30 mg, calcium-d-pantothenate 10 mg, vitamin B_6 5 mg, vitamin B_{12} 0.03 mg, d-biotin0.1 mg, folic acid 1 mg, choline chloride 400 mg.
**Mineral premix (per kilogram diet): manganese 100 mg, iron 60 mg, copper 5 mg, cobalt 0.2 mg, iodine 1 mg, zinc 80 mg.

Chemical analysis of feed: The proximate composition of experimental diets was determined according to AOAC (3). Van Soest (42) method was used to measure the neutral detergent fiber (NDF) values of the feeds. The Zn content of feeds was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Feed sample (0.3 g) was subjected to wet digestion with 8 mL 65% nitric acid and 2 mL 37% hydrochloric acid in a sealing vessel at 180 °C in a microwave (MARS 6, CEM Corporation, Matthews, NC) for 20 min. The digested samples filtered in flask and diluted to 25 mL by adding deionized water for analysis in ICP-OES.

Performance parameters and carcass yield: The data for feed intake (FI) and body weight (BW) on per pen basis were recorded. Body weight gain (BWG) and feed conversion ratio (FCR) of broilers were calculated with these data for growth phases and overall growth period. Mortality was recorded daily. At d 42, three chickens from each replicate were slaughtered by decapitation. Carcasses, breasts, and thighs were weighed individually, and relative weights were calculated as a percent of live weight.

Meat quality parameters: After weighing the breast, samples from the left part of breast meat were taken immediately and placed in a refrigerator at 4 °C for storage. Breast muscle pH was measured at a depth of 2 cm using a pH meter (Orion Model 720, Thermo Electron Corporation, Beverly, MA, US) from three different sites and the average value was used. A chromameter (CR-400, Minolta Camera Co., Osaka, Japan) was employed to determine the meat colorfor L* (lightness), a* (redness), and b* (yellowness). Water-holding capacity (WHC) of breast meat samples were determined according to Joo (24). For this purpose, five pieces of breast meat (approximately 5 g) were weighed and placed between the weighed two filter papers with 100 cm^2 (10×10 cm) area. Then the meat samples and filter papers were placed between two 15×15 cm (225 cm^2) glass plates. Loads of 2250 g were applied on the glass plates to create a pressure of 10 g/cm^2 for 5 min followed by weighing the filter paper and calculation of percent WHC as follows:

$$\frac{\text{Final weight of filter paper} - \text{Initial weight of filter paper}}{\text{Initial weight of meat sample}} \times 100$$

The breast meat samples were weighed, put in a polyethylene bag, heated at 75°C internal temperature in a water bath for 45 minutes, and weighed again after cooling and drying between the layers of filter paper to determine the cooking loss (CL). CL was calculated by ascertaining the weight loss after cooking (22).

Feathering score: At d 42 of the experiment, 10 chickens from each pen were subjected to feather scoring according to Lai et al. (25). The back feathering was evaluated by using 5-point scoring method (1 to 5) with 1 indicating minimal coverage and 5 for complete coverage. A 3-point scoring method (0 to 2) was used for wing feathering. Wings without any defect were scored as 0, wings with lesions and torn feathers as 1, and wings with broken feathers and retarded feathering were scored as 2.

Statistical analysis: The effect of dietary Se form and SD was assessed using the GLM procedures of SPSS (version 22.0; IBM Corp., Armonk, NY, US). Feathering scores between groups were tested with Kruskal-Wallis and scores between main effects (Se form and SD) were compared with the help of the Mann-Whitney U test. The Duncan's Multiple Range Test was used for the comparison of means. The difference at 95% confidence interval ($P<0.05$) was assumed as significant.

Results

In the current study, no interaction was found between SD and dietary Se form for performance, carcass yield, meat quality, and feathering scores of broilers (Tables 2 and 5).

Table 2 shows the effect of dietary Se form on BWG, FI, and FCR of broilers under low or high stocking densities. Growth performance of broilers was not different regardless of dietary Se form. However, HSD significantly decreased the BWG ($P<0.05$) and FI ($P<0.001$) on d 25 to 42 and 0 to 42 whereas; there was no significant effect on FCR at any phase.

Neither dietary Se form nor SD had any significant effect on carcass, breast, and thigh yields (Table 3).

Dietary nano-Se significantly decreased the WHC of breast meat 72-h post-mortem ($P<0.05$) but did not affect other meat quality parameters (Table 4). HSD had no effect on pH, meat colour, WHC, and CL of breast meat at 15 min, 24-h and 72-h after slaughter.

Both dietary Se form and SD did not affect the feathering score of back and wing of broilers (Table 5).

Discussion and Conclusion

Owing to the varying reports, growth performance of broilers remains inconclusive in response to dietary Se. The current study showed that growth performance was similar in broiler chickens fed nano- or inorganic Se in conformity with the results of Boostani et al. (9), Liu et al. (27), and El Deep et al. (18). On the contrary, Selim et al. (34) and Mohammadi et al. (29) stated that 0.3 mg/kg dietary nano-Se improved the growth performance of broilers compared with inorganic Se. The incoherent results may depend on the differences in preparation of nano-Se, the strain of birds, the composition of diets, and housing conditions.

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Table 2. Effects of stocking density and dietary selenium form on growth performance of broilers.

Item	Body weight gain, g				Feed intake, g				Feed conversion ratio			
	0-10 d	11-24 d	25-42 d	0-42 d	0-10 d	11-24 d	25-42 d	0-42 d	0-10 d	11-24 d	25-42 d	0-42 d
LSD												
Inorganic Se	209.29	690.17	1964.08	2818.21	204.37	1008.55	3595.91	4808.82	0.98	1.46	1.83	1.71
Nano-Se	203.92	686.18	1969.28	2814.05	204.88	987.77	3573.92	4766.57	1.01	1.44	1.82	1.70
HSD												
Inorganic Se	203.07	688.74	1892.14	2738.60	200.22	1000.62	3440.26	4641.10	0.99	1.45	1.82	1.70
Nano-Se	205.72	688.94	1900.13	2749.45	195.33	1003.94	3415.17	4614.44	0.95	1.46	1.80	1.68
SEM	4.36	15.32	26.74	27.95	7.07	25.13	38.36	39.34	0.03	0.01	0.02	0.02
SD												
Low	206.60	688.17	1966.68^{a}	2816.13^{a}	204.62	998.16	3584.91^{a}	4787.70^{a}	0.99	1.45	1.83	1.70
High	204.40	688.83	1896.14^{b}	2744.02^{b}	197.77	1002.28	3427.72^{b}	4627.77^{b}	0.97	1.45	1.81	1.69
Se form												
Inorganic	206.18	689.45	1928.11	2778.40	202.29	1004.58	3518.08	4724.96	0.98	1.46	1.83	1.70
Nano	204.82	687.56	1934.71	2781.75	200.11	995.85	3494.54	4690.51	0.97	1.45	1.81	1.69
SEM	3.08	10.83	18.91	19.76	5.00	17.77	27.12	27.81	0.02	0.01	0.02	0.01
P-values												
SD	0.616	0.966	0.013	0.015	0.340	0.871	0.000	0.000	0.467	0.754	0.444	0.354
Se form	0.757	0.902	0.807	0.906	0.759	0.731	0.544	0.389	0.884	0.531	0.412	0.354
SD × Se form	0.365	0.892	0.959	0.790	0.705	0.635	0.968	0.844	0.328	0.255	0.977	0.839

^{a,b}: Means bearing different superscript within the same column are significantly different (P<0.05), SD: Stocking density, Se: Selenium, LSD: Low stocking density, HSD: High stocking density, SEM: Standard error of the mean.

Table 3. Effects of stocking density and dietary selenium form on relative carcass, breast, and thigh yields of broiler, % of body weight.

Item	Carcass	Breast	Thigh
LSD			
Inorganic Se	73.56	21.94	15.30
Nano-Se	73.34	21.28	15.34
HSD			
Inorganic Se	73.85	21.59	15.41
Nano-Se	73.51	21.63	15.28
SEM	0.324	0.221	0.078
SD			
Low	73.46	21.61	15.32
High	73.68	21.61	15.34
Se form			
Inorganic	73.71	21.76	15.35
Nano	73.43	21.45	15.31
SEM	0.214	0.312	0.111
P-values			
SD	0.466	0.998	0.843
Se form	0.371	0.323	0.680
SD × Se form	0.849	0.261	0.447

SD: Stocking density, Se: Selenium, LSD: Low stocking density, HSD: High stocking density, SEM: Standard error of the mean.

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Table 4. Effects of stocking density and dietary selenium form on pH, meat colour, water holding capacity (%), and cooking loss (%) of broiler breast meat at different times after slaughtering.

Item	15 minutes				24 hours						72 hours				
	pH	L	a	b	pH	L	a	b	WHC	CL	pH	L	a	b	WHC
LSD															
Inorganic Se	6.45	52.13	2.67	5.20	5.87	59.31	3.17	7.69	12.63	32.22	5.87	59.00	3.36	6.83	12.99
Nano-Se	6.49	52.04	2.31	4.59	5.86	58.67	3.05	7.51	12.26	32.13	5.86	58.76	3.10	6.81	12.43
HSD															
Inorganic Se	6.45	52.33	2.56	4.81	5.90	59.21	3.10	7.70	13.03	32.01	5.88	58.52	3.32	6.87	13.13
Nano-Se	6.52	52.12	2.24	4.44	5.84	59.70	2.61	7.74	12.21	32.27	5.90	59.63	2.88	6.54	12.26
SEM	0.04	0.57	0.18	0.36	0.02	0.56	0.22	0.44	0.40	0.53	0.02	0.59	0.22	0.33	0.33
SD															
Low	6.47	52.09	2.49	4.89	5.87	58.99	3.11	7.60	12.44	32.18	5.86	58.88	3.23	6.82	12.71
High	6.49	52.23	2.40	4.63	5.87	59.46	2.86	7.74	12.62	32.21	5.89	59.08	3.10	6.70	12.72
Se form															
Inorganic	6.45	52.23	2.61	5.00	5.89	59.26	3.14	7.18	12.83	32.12	5.87	58.76	3.34	6.85	13.06^{a}
Nano	6.51	52.08	2.28	4.52	5.85	59.19	2.83	7.63	12.23	32.27	5.88	59.20	2.99	6.68	12.35^{b}
SEM	0.03	0.41	0.12	0.26	0.02	0.40	0.16	0.31	0.28	0.38	0.01	0.42	0.15	0.23	0.23
P-values															
SD	0.725	0.807	0.598	0.463	0.853	0.409	0.258	0.747	0.659	0.947	0.161	0.736	0.541	0.721	0.964
Se form	0.144	0.798	0.058	0.185	0.125	0.893	0.171	0.837	0.137	0.770	0.710	0.461	0.108	0.594	0.033
SD × Se form	0.742	0.918	0.910	0.740	0.287	0.316	0.411	0.856	0.579	0.650	0.521	0.254	0.670	0.625	0.635

[a,b]: Means bearing different superscript within the same column are significantly different ($P<0.05$), L: Lightness, a: Redness, b: Yellowness, WHC: Water holding capacity, CL: Cooking loss, SD: Stocking density, Se: Selenium, LSD: Low stocking density, HSD: High stocking density.

Table 5. Effects of stocking density and dietary selenium form on back and wing feathering score of broiler (x±Sx).

Item	Back	Wing
LSD and inorganic Se	4.45±0.08	0.43±0.06
HSD and inorganic Se	4.50±0.08	0.39±0.06
LSD and nano-Se	4.48±0.09	0.34±0.06
HSD and nano-Se	4.56±0.08	0.29±0.05
P-values	0.708	0.348
SD		
Low	4.46±0.06	0.38±0.04
High	4.53±0.06	0.34±0.04
P-values	0.564	0.463
Se form		
Inorganic	4.48±0.06	0.41±0.04
Nano	4.52±0.06	0.31±0.04
P-values	0.305	0.097

SD: Stocking density, Se: Selenium, LSD: Low stocking density, HSD: High stocking density.

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The SD is generally recommended as 13-15 birds/m^2 or 30-35 kg BW/m^2 in environmentally controlled broiler houses (15, 16). Many researchers reported that increasing SD beyond these ranges exerts adverse effects on the growth performance of broilers (4, 6, 12). In our study, 34.3 and 50.2 kg/m^2 were found at d 42 in LSD and HSD groups, respectively. In line with these results, BWG and FI were significantly higher in LSD groups than HSD groups during 25 to 42 and 0 to 42 days. Particularly during the finishing phase, birds might have been difficulty accessing feed and water under HSD. This might have caused a decrease in FI and BWG. Additionally, Feddes et al. (20) stated that low FI and BWG may be due to a decrease in gaseous and heat exchange within the microclimate of the birds. However, disturbance in the digestive microbiota which affects the digestion and absorption of nutrients may decrease the performance of broilers at HSD (12). Increased dust and airborne pathogens may also affect performance negatively at HSD (33). Therefore, the FI might have decreased at HSD resulting in lowered BWG. However, FCR remained unaffected in response to SD in this study. Similar results were reported by Feddes et al. (20) and Madilindi et al. (28). In contrast, Astaneh et al. (4) found that HSD affected FCR negatively. In most of the studies involving SD revealed that HSD decreases both BWG and FI. However, in some studies including the present study, the decrease of FI and BWG might be so similar. In such a case, the FCR may not be affected.

Dietary nano-Se was unable to affect the carcass, breast, and thigh yields of broilers. Similarly, Bakhshalinejad et al. (7) reported that dietary Se source (inorganic, organic, and nano) or level (0.1 and 0.3 mg/kg) did not influence the carcass, breast, and thigh yields in broiler. And also Ahmadi et al. (2) stated that different dietary nano-Se levels (between 0.1 to 0.5 mg/kg) had no significant effect on breast and thigh yields of broiler. Se deficiency causes muscular dystrophy as well as exudative diathesis in chicks (39). However, sufficient or excess (up to 1 mg/kg) dietary Se provides proper muscle growth but does not increase muscle growth (2, 11). In this study, since the Se level in the diet (0.34 mg/kg) was sufficient, either inorganic or nano Se could not affect the carcass, breast, and thigh yields.

Carcass, breast, and thigh yields of broilers were similar irrespective of the SD. Likewise, other studies have suggested that SD does not affect carcass, breast, and thigh yields of broiler chickens (1, 41). However, Cengiz et al. (12) reported that HSD decreased the breast yield, increased the thigh yield, and had no effect on the carcass yield in broilers. The contrasting results may be associated with the differences in housing conditions and stocking densities.

In this study, dietary nano-Se did not affect pH, colour, and CL of breast meat of broiler chickens but decreased WHC 72-h post-mortem. Most of the researchers have revealed that nano-Se improved drip loss (DL) or WHC and had no effect on pH. Cai et al. (10) reported that dietary nano-Se reduced DL but did not affect the meat colour of broiler. Mohammadi et al. (28) reported that nano-Se had no significant effect on pH and WHC of broiler breast meat. However, Bakhshalinejad et al. (7) found that dietary nano-Se had no significant effect on pH but increased redness and yellowness, decreased DL and CL of breast meat. It is well documented that Se is essential for the antioxidant systems of the organism (38, 39). According to Huff-Lonergan and Lonergan (23), oxidation of meat could decrease the sensitivity to hydrolysis, increase protein degradation, and reduce the WHC of myofibrils, which would increase the water loss of the meat. Therefore, the decrease of WHC can be explained by an augmented bioavailability of nano-Se in comparison with its inorganic counterpart.

HSD did not affect the meat quality parameters of broilers in the present study. Similar results were reported by Tong et al. (41) and Patria et al. (31). In fact, it may be thought that the decrease in litter quality due to HSD would negatively affect breast meat quality. However, the broiler's breast skin and feathers probably prevented the meat quality from being adversely affected by the HSD. In the current study, it was determined that HSD has no significant effect on feathering.

Dietary nano-Se had no significant effect on feathering score of broiler chickens. These results are in agreement with those of Ravindran and Elliot (32) who noted that Se supplementation (0.4 mg/kg organic Se) was ineffective on the feathering score of broiler chickens. In contrast, Choct et al. (14) stated that organic Se supplementation (0.25 mg/kg) improved the feathering score in broilers as compared to inorganic Se. The inconsistencies in the results may depend on the differences in the feather scoring method, housing conditions of birds, dietary Se level, and form.

According to the results of the current study, HSD had no significant effect on the rate of feathering in broiler. Similarly, Skrbic et al. (35, 37) revealed that HSD (16 birds/m^2) did not affect the feathering score of broilers. In addition, Moreire et al. (30) described that feathering remained unaffected by SD of 16 birds/m^2. However, Beaulac and Schwean-Lardner (8) reported that HSD (50 kg/m^2) has negative effect on feathering in turkeys. According to these studies, it may be concluded that the number of birds in HSD groups was not enough to impose a negative effect on the feathering score. Moreover, it is well known that the process of feather forming is not only determined by environmental conditions but also by

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genetics, the hormonal status of the organism, and nutrition (26). Differences between the results of the studies can be explained by these reasons.

The present study demonstrated that dietary nano-Se had no effect on growth performance, carcass yield, and feathering but may affect meat colour in broiler. However, growth performance is negatively affected by HSD.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

ÖS designed the experiment and took the lead in writing the manuscript. OT, EK, EKK, ÖSÖ, AKA, and ART carried out the experiment and contributed to sample preparation. UA, BHK, ÖC, and AGÖ contributed to the interpretation of the results and edited the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

The study was ethically approved by the committee for ethical use of animals of Aydın Adnan Menderes University, Türkiye (No: 64583101/2017/042).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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