

## Effect of chia oil addition to quail (*Coturnix coturnix japonica*) diets on growth performance, blood antioxidant status, caecal concentrations of short-chain fatty acids, and biomechanical properties of bones

Kadir Önk<sup>1</sup>, Özlem Durna<sup>2</sup>, Gültekin Yıldız<sup>3</sup>, Oğuz Merhan<sup>4</sup>, Fatma Kübra Erbay Elibol<sup>5</sup>, Elif Naz Perdecı<sup>5</sup>, Oktay Kaplan<sup>2</sup>, Hatice Durna<sup>4</sup>

<sup>1</sup>Kafkas University, Veterinary Faculty, Animal Breeding and Husbandry Department, 36040, Kars, Türkiye, ORCID: 0000-0002-5618-2988

<sup>2</sup>Dicle University, Veterinary Faculty, Animal Nutrition and Nutritional Diseases Department, 21200, Diyarbakır, Türkiye, ORCID: 0000-0003-4532-6795, ORCID: 0000-0001-6143-8987

<sup>3</sup>Ankara University, Veterinary Faculty, Animal Nutrition and Nutritional Diseases Department, 06830, Ankara, T Türkiye, ORCID: 0000-0002-1003-9254

<sup>4</sup>Kafkas University, Faculty of Veterinary Medicine, Department of Biochemistry, 36040, Kars, Türkiye, ORCID: 0000-0002-3399-0667, ORCID: 0000-0002-5281-5039

<sup>5</sup>TOBB University of Economics and Technology, Biomedical Engineering Department, 06560 Ankara, Türkiye, ORCID: 0000-0002-4117-1098, ORCID: 0009-0002-2857-448X

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### Abstract

The aim of this study is to investigate the effect of chia oil supplementation in quail diets on growth performance, blood antioxidant status, caecal short-chain fatty acid (SCFA) concentration, and tibia–femur biomechanical properties. A total of 180, one-day-old quail chicks were randomly divided into three groups of 60 chicks each. Each group was randomly divided into six subgroups, each containing 10 chicks. All chicks were fed a diet based on corn and soybean meal. While the control group was fed with the basal ration, the experimental groups were fed with the basal ration and were given 0.2 g/kg and 0.4 g/kg chia oil in addition to the basal ration. The use of chia oil in quails did not affect growth performance parameters. Malondialdehyde and glutathione exhibited a linear response to the increase in dietary chia oil. The superoxide dismutase value exhibited a quadratic response to chia oil use; the glutathione peroxidase value showed a linear and quadratic response to the use of chia oil. Catalase, ceruloplasmin, albumin, total protein, and globulin were not affected by the addition of chia oil. Acetic acid, propionic acid, and total SCFA were linearly affected by the graded level of chia oil. No statistical difference was found in the concentrations of butyric, isobutyric, valeric, isovaleric, isocaproic, and caproic acids and BCFA in quails fed with different levels of chia oil. Feeding a diet containing chia oil did not affect the biomechanical properties of the tibia and femur in quail. It was therefore concluded that diets containing chia oil could be used to improve antioxidant status and caecal short-chain fatty acid values in quails.

**Keywords:** Antioxidant status, chia oil, caecum short-chain fatty acids, growth performance, quails, tibial biomechanical properties

\* **Corresponding author:** [odurna36@gmail.com](mailto:odurna36@gmail.com)

### Introduction

Traditionally, antimicrobials have been widely used to improve health and growth performance in poultry; however, increasing public awareness of the risk of pathogens developing cross-resistance to

antibiotics has resulted in the phasing out of antibiotics for therapeutic and prophylactic purposes in food animals (Ricke *et al.*, 2020). The shift away from antibiotic supplementation has led to tremendous growth in research focused on the implementation of effective alternative control methods, management, and dietary changes aimed at improving animal health, welfare, and productivity (Abdelli *et al.*, 2021). In the search for alternative feed additives to antibiotics, phytogetic feed additives (PFA), also called phytobiotics or botanicals, have become the focus of increasing attention. Herbs and plant extracts used in animal feed, now called phytogetic feed additives (PFA), are defined as plant-derived compounds added to animal feed to improve digestibility, nutrient absorption, and animal productivity by eliminating pathogens living in the animal intestine (Athanasiadou *et al.*, 2007).

The chia plant (*Salvia hispanica* L), from the Lamiaceae family, grown for the seeds and oil, is native to central and southern Mexico and Guatemala. Economic historians have noted that chia as a food crop is no less important than maize (Baginsky *et al.*, 2016). Chia is an annual plant that has been increasingly used in both animal and human nutrition in recent years; it is a plant rich in protein, oils (especially omega-3 fatty acids), polyunsaturated fatty acids, and fibre (Ayaşan and Ayaşan, 2020). Chia is also a rich source of gluten-free protein, vitamins, minerals, and phenolic compounds that are beneficial for the digestive system, as well as fibre-rich sources that control diabetes mellitus (Ullah *et al.*, 2015). Chia appears as an herbaceous plant that has been used for medicinal purposes for thousands of years. Chia seeds are high in nutritional value and are especially rich in cellulose and fat (Sosa-Baldivia *et al.*, 2018). Ayerza and Coates (2011) determined the crude protein levels of chia as 15.95–26.02%; the fat content is 29.98–33.50%. Grancieri *et al.* (2019) found that chia seeds were a good source of vegetable protein and contain 18–24% protein. The amount of saturated fatty acids contained in chia seeds is 8.65–9.99 %, whereas the amount of mono-saturated fatty acids is 7.33–10.95% (Kulczynski *et al.*, 2019). Adding 0.4 g/kg chia oil to quail diets positively affected growth performance, some blood parameters, lipid profile, and immunity (Alagawany *et al.*, 2020). The addition of chia seed oil to the diet affected the omega-3/omega-6 ratio of egg yolk in quails. Increasing the omega-3 level in diets positively affects the polyunsaturated fatty acid (PUFA)/saturated fatty acid (SFA) ratio (Şengül, 2022).

The current study aimed to determine the effect of different levels of chia oil supplementation in mixed feeds on growth performance, blood antioxidant status, caecal base short-chain fatty acid concentrations, and biomechanical properties of Japanese quails (*Coturnix coturnix japonica*).

## Materials and Methods

A total of 180, one-day-old chicks (*Coturnix coturnix japonica*) were included in the study, regardless of gender. All chicks were randomly divided into three groups of 60 chicks each. Each group was then randomly divided into six subgroups of 10 chicks each. The animals were fed a basal diet of corn and soybean meal, and the experiment continued for 35 d (Table 1). All diets were determined according to NRC (1994) standards. Nutrient analyses of the feed were made according to the methods of the AOAC (2000). The birds were kept in breeding cages. Each subgroup was equipped with manual feeders and automatic nipple drinkers. Water and feed were given as desired. House temperature was monitored thermostatically throughout the study. The temperature, which was 32–35 °C on the first day, was gradually decreased and was kept at 22 °C for the last two weeks. An artificial light program was applied in accordance with commercial conditions (23 h of illumination per day throughout the experiment). For each of the replicates, one cage was used with dimensions 22×95×45 cm for height, length, width, respectively. Experimental diets were: C, basal diet (Control; no chia was added); CO1, 0.2 g/kg chia oil addition; and CO2, 0.4 g/kg chia oil addition. Plant oils were obtained from Botalife (Isparta, Türkiye). The fatty acid composition of the chia oil is given in Table 2.

**Table 1.** Composition of basal diets used to feed quail (%)<sup>1</sup>

<b>Feed materials</b>	<b>%</b>
Corn	58.00
Soybean meal (CP,46%)	33.00
Corn gluten (CP, 60%)	5.60
Limestone	1.35
Di calcium phosphate	1.00
DL-Methionine	0.15
L-Lysine Hydrochloride	0.15
L-Threonine	0.15
Vitamin- mineral premix	0.30
Salt	0.20
Phytase	0.10
Total	100.00
<b>Calculated values</b>	
Crude protein, %	22,52
ME (kcal/kg)	2941,00
Ca, %	0.85
Total P, %	0.58
<b>Analysed values</b>	
ME (kcal/kg)	2915.25
Crude protein, %	22.91
Ca, %	1.00
Total P, %	0.61

<sup>1</sup> As-fed basis

<sup>2</sup>Vitamin–mineral premix provided per kg diet: Vit. A 8000 IU, Vit. D3 1000 IU, Vit. E 20 IU, Vit. K 0.5 mg, Vit. B1 3 mg, Vit. B2 9 mg, Vit. B6 7 mg, Vit. B12 0.03 mg, niacin 35 mg, D-pantothenic acid 10 mg, folic acid 0.55 mg, biotin 0.18 mg, Fe 100 mg, Cu 8 mg, Zn 100 mg, Mn 120 mg, I 0.7 mg, and Se 0.3 mg

**Table 2.** Fatty acid composition of the chia oil used in this study

<b>*Chia oil, %</b>	
Myristic acid	0.04
Palmitic acid	6,79
Palmitoleic acid	0.08
Oleic acid	5,89
Linolenic acid	64.65
Linoleic acid	19.00
Arachidonic acid	0.25
Stearic acid	2,72

\*Botalife

Live weights (LW) of each subgroup were recorded weekly. Live weight gain (LWG) was determined according to the difference between these measurements. Feed intake (FI) of animals in each subgroup was recorded weekly and used to calculate the feed conversion ratio (FCR).

In the fifth week of the experiment, blood samples were taken from the wing veins of a total of 18 animals, one from each of the subgroups. Collection of blood has been made into anticoagulant (EDTA) tubes. After taking enough blood, samples were separated into whole blood and the plasma of the remaining blood was obtained. Samples underwent centrifuging at 3000 rpm for 15 min, after which they have been stored at -20 °C until analysis. Activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were investigated using an ELISA device (Epoch, Biotek, USA), using commercial kits (Cayman Chemical Company, USA). Analysis of whole blood reduced glutathione (GSH) was made colorimetrically (Epoch, Biotek, USA) and the method used

was similar to that of Beutler *et al.* (1963). Determination of malondialdehyde (MDA) inside the plasma was determined using the method of Yoshioka *et al.* (1979). Determination of ceruloplasmin was made using the method of Colombo and Ricterich (1964). For the determination of albumin and total protein, a commercial test kit (Biolabo, Maizy, France) was used. Determination of globulin was made by the subtraction of albumin from total protein (Doumas *et al.*, 1971). Serum nitric oxide content was determined according to the method of Miranda *et al.* (2001).

When the study was concluded, cecal contents were stored at -18 °C. Afterwards, frozen caecal digesta were thawed at 4 °C. Thawed samples were diluted 10-fold by mixing with double-distilled water in sterile screw-cap tubes. The caecal digesta were centrifuged at 4,000 rpm for 15 min at 4 °C in order to homogenize the sample. Supernatant was stored in a 750 µl Eppendorf tube and was mixed with 150 µl ice-cold 25% metaphosphoric acid solution. Following that process, the tubes were kept at an ice-cold temperature for 30 min. This was to ensure that the proteins would collapse. Afterwards, tubes were centrifuged again for 10 min at 10,000 rpm at 4 °C. Analysis of supernatants was done using gas chromatography (GC) (Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan). The GC column had a 30 m × 0.53 mm internal diameter (Teknokroma TRB-FFAP, Teknokroma, Barcelona, Spain). A flame ionization detector was used to determine SCFA concentrations in the caecal digesta. The analysis procedure was designed according to that of Zhang *et al.* (2003). Temperatures were fixed at the injector-port; the flame ionization detector (FID) was constant at 250 °C. For the temperature program and the first 4 min after injection, the temperature was constant and following injection, it was increased at a rate of 4 °C/min until it reached 160 °C. This temperature was held constant for 4 min. The carrier gas was helium. The injection volume used was a constant 1 µl and duplicate analyses were performed.

Left femur and tibia samples were thawed at 4 °C and cleared of all tissues. The lengths and widths of the femur and tibia samples were measured using digital callipers. Bone samples were then stored at -20 °C until further analysis. Femurs and tibias were subjected to three-point bending tests to failure with the Instron 5944 testing frame (Instron, Norwood, MA, USA). The loading speed was 5 mm/min. The length of the bones was 70 mm. The load was applied to the midpoint of the shaft. Load versus displacement data were collected for each sample. Stiffness values were determined from the slope of the linear region of the load displacement curves. Ultimate load (UL) and displacement at ultimate load (DUL) were also calculated from the load displacement curves. The load at which permanent deformation of the system begins is the yield load (YL); the displacement at which permanent deformation begins is the displacement at yield load (DYL) (Gürgül *et al.*, 2008). During tests, load and displacement data were collected and were used to form a load–displacement curve (Figure 4).

One-way analysis of variance method was used for the statistical calculations of the groups and polynomial contrasts were used to determine the effect of the chia oil at different levels. The statistical analysis was done using SPSS software (SPSS, 2011).

## Results

The fatty acid composition and content of chia oil used in the experiment are shown in Table 2. Table 3 shows the effect of chia oil on performance in the study; the effect on antioxidant status is in Table 4; the effect on caecal-based short-chain fatty acid concentrations is given in Table 5; its effect on tibial biomechanical criteria is shown in Table 6.

**Table 3.** Influence of Chia oil on growth performance of quail

Groups Performance Parameters	Control	CO1	CO2	Significance	
	$\bar{x} - S \bar{x}$	$\bar{x} - S \bar{x}$	$\bar{x} - S \bar{x}$	L	Q
Initial Live Weight, g	8,137 ± 0,008	8,140 ± 0,00	8,143 ± 0,00	0,341	1,000
Final Live Weight, g	231,88 ± 2,21	227,56 ± 4,86	227,62 ± 4,72	0,476	0,670
Live Weight Gain, g	223,75 ± 2,22	219,42 ± 4,86	219,48 ± 4,72	0,475	0,670
Feed Intake, g	722,69 ± 28,38	707,71 ± 16,05	678,76 ± 21,51	0,189	0,804
Feed Conversion Ratio	3,22 ± 0,10	3,23 ± 0,09	3,09 ± 0,06	0,310	0,525

<sup>1</sup>Statistically not significant ( $P > 0.05$ ). All values are given as mean ± standard error of mean (SEM). Data represent mean values of six replicates per treatment <sup>2</sup>C: Control basal ration, CO1: 0,2 g/kg chia oil added to basal ration, and CO2: 0,4 g/kg chia oil added to basal ration <sup>3</sup>Polynomial contrasts: L = linear and Q = quadratic effect of supplementary chia oil

**Table 4.** Influence of chia oil supplementation on antioxidant status in quail

Groups	Control	S1	S2	Significance	
Blood Parameters	$\bar{x}$ - S $\bar{x}$	$\bar{x}$ - S $\bar{x}$	$\bar{x}$ - S $\bar{x}$	L	Q
MDA ( $\mu\text{mol/L}$ )	3.01 $\pm$ 0.07	2.90 $\pm$ 0.07	2.64 $\pm$ 0.08	<b>0.005</b>	0.430
GSH (mg/dL)	14.28 $\pm$ 1.79	21.37 $\pm$ 1.12	31.82 $\pm$ 1.59	<b>0.000</b>	0.383
SOD (U/mL)	31.47 $\pm$ 0.98	38.48 $\pm$ 1.74	36.01 $\pm$ 2.11	0.076	<b>0.036</b>
CAT (nmol/min/mL)	3.12 $\pm$ 0.09	3.26 $\pm$ 0.07	3.31 $\pm$ 0.08	0.147	0.632
GPx (nmol/min/mL)	19.45 $\pm$ 0.71	51.28 $\pm$ 0.98	72.93 $\pm$ 1.78	<b>0.000</b>	<b>0.005</b>
Ceruloplasmin (mg/dL)	17.23 $\pm$ 0.32	17.15 $\pm$ 0.57	17.04 $\pm$ 0.44	0.773	0.978
Albumin (g/dL)	1.56 $\pm$ 0.04	1.51 $\pm$ 0.02	1.48 $\pm$ 0.02	0.144	0.781
Total protein (g/dL)	3.67 $\pm$ 0.03	3.61 $\pm$ 0.03	3.58 $\pm$ 0.04	0.135	0.809
Globulin (g/dL)	2.11 $\pm$ 0.06	2.10 $\pm$ 0.02	2.09 $\pm$ 0.05	0.857	1.000

<sup>1</sup>Statistically not significant ( $P > 0.05$ ). The mean ( $\bar{x}$ ) and standard error (S $\bar{x}$ ) values of six replicates in each group.

<sup>2</sup>C: Control basal ration, CO1: 0,2 g/kg chia oil added to basal ration, and CO2: 0,4 g/kg chia oil added to basal ration <sup>3</sup>Polynomial contrasts: L = linear and Q = quadratic effect of supplemental chia oil

## Discussion

Chia seeds contain high amounts of essential oils, which act as digestive enhancers, balance the intestinal microbial ecosystem, and stimulate the secretion of endogenous digestive enzymes, thereby improving growth performance in poultry (Cross *et al.*, 2007). Dietary essential oils can improve digestion and improve poultry performance. They were shown to stimulate bile salt secretion and digestive enzyme activities of the intestinal mucosa and pancreas (Hernandez *et al.*, 2004). In general, phytochemical substances can be natural and safe growth promoters in animal and poultry feeds (Mohamed and Hassan, 2023). In our study, initial LW, final LW, LWG, FI, and FCR values were not statistically affected by chia oil supplementation at the end of the experiment. While chia seeds or their essential oils did not negatively affect BW, WG, and FCR (Rasul *et al.*, 2019), others did not find any significant findings (Urrutia *et al.*, 2015; Şengül, 2022). There are many current studies that investigate the effect of chia seeds or their essential oils as feed additives on the performance characteristics of poultry; chia seeds or their essential oils improve BW, WG, and FCR (Alagawany *et al.*, 2020; Mendonça *et al.*, 2020; Yıldız *et al.*, 2022). Differences between these studies may be explained by plant-related factors (such as the dose and quality of chia used) and animal and environmental factors (such as differences in care and feeding conditions).

Many of the bioactive compounds in chia seeds are associated with antioxidant activities. Antioxidants are substances that protect cells against oxidative damage caused by excess reactive oxygen species. Oxidative stress, which causes the release of free oxygen radicals in the body, has been associated with various disorders such as cardiovascular diseases, cataracts, diabetes, Alzheimer's disease, cancer, and rheumatism (Noratto and Murphy, 2019). Chia and quinoa seeds and their by-products, like other plant materials, offer good alternatives due to their content of bioactive compounds (mainly phenolic compounds) containing various biological activities and antioxidant properties. Chia products are reported to be a good source of phenolic acids (mainly rosmarinic, ferulic, and caffeic acids) and flavonoids (mainly rutin, myricetin, and quercetin)

**Table 5.** Effect of two concentrations of chia oil on caecal short-chain fatty acid concentrations ( $\mu\text{mol/g}$ ) in quail

SCFA	Groups			Significance	
	Control	CO1	CO2	L	Q
	$\bar{x} - S \bar{x}$	$\bar{x} - S \bar{x}$	$\bar{x} - S \bar{x}$		
Acetic acid	46.22 $\pm$ 6.42	60.74 $\pm$ 3.33	65.55 $\pm$ 4.11	<b>0.012</b>	0.423
Propionic acid	0.89 $\pm$ 0.14	1.98 $\pm$ 0.45	2.40 $\pm$ 0.46	<b>0.014</b>	0.487
Isobutyric acid	0.74 $\pm$ 0.26	1.03 $\pm$ 0.16	1.05 $\pm$ 0.15	0.289	0.582
Butyric acid	5.97 $\pm$ 1.99	8.22 $\pm$ 3.01	6.91 $\pm$ 2.37	0.794	0.570
Isovaleric acid	0.04 $\pm$ 0.01	0.39 $\pm$ 0.22	0.42 $\pm$ 0.22	0.155	0.491
Valeric acid	0.17 $\pm$ 0.04	0.24 $\pm$ 0.035	0.17 $\pm$ 0.05	0.971	0.238
Isocaproic acid	0.09 $\pm$ 0.16	0.16 $\pm$ 0.030	0.17 $\pm$ 0.028	<b>0.035</b>	0.390
Caproic acid	0.06 $\pm$ 0.005	0.23 $\pm$ 0.064	0.19 $\pm$ 0.074	0.111	0.164
BCFA	0.95 $\pm$ 0.25	1.66 $\pm$ 0.25	1.65 $\pm$ 0.23	0.067	0.251
Total SCFA	54.20 $\pm$ 7.29	73.00 $\pm$ 4.04	76.89 $\pm$ 5.90	<b>0.016</b>	0.318

<sup>1</sup>Statistically not significant ( $P > 0.05$ ). The mean ( $\bar{x}$ ) and standard error ( $S\bar{x}$ ) values of six replicates in each group. <sup>2</sup>C: Control basal ration, CO1: 0,2 g/kg chia oil added to basal ration, and CO2: 0,4 g/kg chia oil added to basal ration <sup>3</sup>Polynomial contrasts: L = linear and Q = quadratic effect of supplemental chia oil

**Table 6.** Effect of two concentrations of chia oil on tibia biomechanical properties in quail

Bone	Item	Dietary Treatment			Significance	
		Control	CO1	CO2	L	Q
		$\bar{x} - S \bar{x}$	$\bar{x} - S \bar{x}$	$\bar{x} - S \bar{x}$		
Femur	Length, mm	42,57 $\pm$ 0,42	41,96 $\pm$ 0,41	42,62 $\pm$ 0,81	0,948	0,415
	Width, mm	3,40 $\pm$ 0,10	3,46 $\pm$ 0,19	3,17 $\pm$ 0,09	0,222	0,328
	UL, N	49,58 $\pm$ 2,12	41,79 $\pm$ 3,08	42,09 $\pm$ 2,91	0,063	0,257
	DUL, mm	0,61 $\pm$ 0,04	0,54 $\pm$ 0,07	0,46 $\pm$ 0,02	0,041	0,933
	YL, N	47,34 $\pm$ 2,88	40,36 $\pm$ 3,42	41,02 $\pm$ 2,77	0,148	0,332
	DYL, mm	0,54 $\pm$ 0,02	0,47 $\pm$ 0,08	0,43 $\pm$ 0,02	0,126	0,842
	Stiffness, N/mm	93,65 $\pm$ 6,02	97,56 $\pm$ 3,77	99,37 $\pm$ 8,11	0,533	0,900
Tibia	Length, mm	54,40 $\pm$ 0,62	54,19 $\pm$ 0,79	55,02 $\pm$ 0,86	0,578	0,585
	Width, mm	3,05 $\pm$ 0,07	3,01 $\pm$ 0,05	3,24 $\pm$ 0,13	0,173	0,250
	UL, N	42,23 $\pm$ 2,27	48,01 $\pm$ 4,21	42,23 $\pm$ 2,49	1,000	0,151
	DUL, mm	0,70 $\pm$ 0,01	0,68 $\pm$ 0,04	0,71 $\pm$ 0,05	0,892	0,608
	YL, N	39,69 $\pm$ 2,66	46,79 $\pm$ 4,64	38,05 $\pm$ 1,47	0,723	0,062
	DYL, mm	0,60 $\pm$ 0,02	0,63 $\pm$ 0,05	0,58 $\pm$ 0,04	0,786	0,505
	Stiffness, N/mm	69,94 $\pm$ 2,62	76,53 $\pm$ 1,95	74,55 $\pm$ 4,40	0,319	0,287

Statistically insignificant ( $P > 0.05$ ). The values show the mean ( $\bar{x}$ ) and standard error ( $S\bar{x}$ ) of the six subgroups in each group. <sup>2</sup>C: Control basal ration, CO1: 0,2 g/kg chia oil added to basal ration, and CO2: 0,4 g/kg chia oil added to basal ration <sup>3</sup>Polynomial contrasts: L = linear and Q = quadratic effect of supplemental chia oil; *UL*: Ultimate Load, *DUL*: Displacement at Ultimate Load, *YL*: Yield Load, *DYL*: Displacement at Yield Load

Isoflavones (mainly daidzin, genistin, and genistein) and tocopherols (predominantly  $\gamma$ -tocopherol) have also been identified in chia products. All this richness in antioxidant compounds makes them demonstrate antioxidant properties for the control of lipid oxidation (Fernández-López *et al.*, 2020). Most of the phenolic compounds found in chia are not found in other oilseeds (Tuberoso *et al.*, 2007). In our study, the increase in the amount of chia oil in the diet, MDA and GSH showed a linear response and the SOD value showed a quadratic response at the end of the experiment. GPx value showed both a linear and quadratic response. However, CAT, ceruloplasmin, albumin, total protein, and globulin were not affected by chia oil supplementation. In a study, the addition of chia oil to quail diets at doses of 0.4 g/kg and 0.8 g/kg did not affect antioxidant status (Alagawany *et al.*, 2020). Another study showed that chia seeds can be used against stress because they are a good source of antioxidants, which can improve health (Uribe *et al.*, 2012). In a study where chia seed extract was added to broiler drinking water at doses of 2 ml/L, 4 ml/L, and 6 ml/L, albumin, globulin, and total protein values in blood plasma were not affected (Hamzah and Mohmad, 2021). It is thought that the strong antioxidant effect of chia oil may be due to the fact that most of the phenolic compounds contained in this oil are not found in other oilseeds.

Short-chain fatty acids are a result of bacterial fermentation in the cecum. They not only improve intestinal integrity by stimulating cell growth and differentiation in the intestine, but also prevent the growth of pathogenic microorganisms by lowering the pH of the digestive system. (Knudsen *et al.*, 2012). There is a close relationship between the composition of the cecum microflora and the SCFA concentration (Meimandipour *et al.*, 2010). Increased SCFA concentrations have beneficial effects on energy, metabolism, microflora, and immune responses (Tan *et al.*, 2012). This information leads us to conclude that increasing short-chain fatty acids in the cecum has a positive effect on intestinal health. In our study, acetic acid, propionic acid, isocaproic acid and total SCFA were linearly affected with the graduated increase in chia oil. There were no marked differences in butyric, isobutyric, valeric, isovaleric and caproic acids or BCFA concentrations for quails fed different supplementations of chia oil. In a study supporting our study results, feeding chia flour in a high-fat diet increased intestinal acetic acid, propionic acid, and SCFA production in female Wistar rats (Mishima *et al.*, 2023). In another study, when an *in vitro* fermentation experiment was conducted using hazelnuts, the composition of the chia nut mucilage from ground chia nuts fermented well and produced SCFA (Ang *et al.*, 2023). In a similar study, acetic, butyric, and isocaproic acids and SCFA were linearly affected by an increasing essential oil mixture (peppermint, juniper, rosemary, and thyme vulgare oils) in quail diets (Aydin and Yıldız, 2020a). Adding thyme and black cumin oil to broiler chicken diets did not affect cecum acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, and caproic acids; total SCFA; and BCFA values. (Aydin and Yıldız, 2020b). The differences in SCFA results between studies can be explained by the dose and quality of chia used, the use of different plant extracts in different animal species, and differences in care and feeding conditions.

Leg abnormalities are one of the most important problems in the poultry industry and are known to cause economic losses and welfare problems in poultry farming (Shim *et al.*, 2012). Many nutrients, from micronutrients to macronutrients, have a potential role in skeletal health, especially dietary fat intake (Holl and Allen, 1987). Monounsaturated fatty acids (PUFAs) may have a potential role in prostaglandin activity (French, 1943), affecting both bone formation and resorption (Calverley and Kennedy, 1949). Chia (*Salvia hispanica*) seeds are a plant source known to be the richest in omega-3 ( $\omega$ -3) fatty acids (Nitrayová *et al.*, 2014). Unlike other plant sources of  $\omega$ -3 such as flaxseed, chia has no known anti-nutritional factors for poultry (Azcona *et al.*, 2008). Chia seeds are known to be a good source of calcium (Shafey *et al.*, 1990). In the current study, it was determined that feeding chia oil did not affect biomechanical properties (length, width, UL, DUL, YL, DYL, and stiffness) of the tibia and femur in quails. No studies were found that investigated the effect of chia oil on the biomechanical properties of poultry bones. Therefore, the discussion is based on different animal species or different plant extracts. The addition of oilseeds to the diet of Japanese quails did not affect bone morphology (Ebeid *et al.*, 2011). As a herbal supplement, flaxseed is known to contribute to the maintenance of bone strength and density while preventing bone loss (Batool *et al.*, 2024). Many studies are needed in this field to discuss the effect of chia oil on bone biomechanical properties.

## Conclusions

It was determined that the use of chia oil in quail diets did not affect performance parameters and bone biomechanical properties and had a protective effect against oxidative damage by increasing GSH, SOD, and GPx values in the blood. The substantial increase in acetic, propionic, and isocaproic acids and SCFA values showed that diets containing chia oil can improve intestinal health in quails. In

light of the data obtained, chia oil can be safely used as a feed additive to protect against oxidative stress and improve intestinal health in quails.

### Authors' contribution

KÖ, ÖDA, GY, OK, and KO executed the experiment; ÖDA statistically analysed the collected data; ÖDA completed the manuscript. ÖDA and KÖ contributed to the design and execution of the study. GY, ÖDA, OM, FKEE, ENP, and HD were in charge of laboratory analyses. ÖDA was responsible for supervision and writing of the manuscript. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

### Compliance with ethical standards

Approval to this study has been given by Kafkas University Animal Experiments Local Ethics Committee (Decision No: KAU-HADYEK /2022-064) report.

### Conflict of interest

The authors declare that there is no conflict of interest for this study.

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