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Effect of safflower oil supplementation in quail (*Coturnix coturnix japonica*) diets on growth performance, blood antioxidant status, caecal short-chain fatty acid content, and biomechanical properties of bones

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Abstract

The aim of this study was to investigate the effect of safflower oil supplementation in quail diets on growth performance, blood antioxidant status, caecal short-chain fatty acid (SCFA) concentrations, and tibia–femur biomechanical properties. A total of 180 one-day-old quail chicks were randomly divided into three groups, each containing 60 chicks. Each group was randomly divided into six subgroups, each containing 10 chicks. All chicks were fed a diet based on corn and soybean meal. The control group was fed the basal ration and experimental groups were fed the basal ration plus 0.5% and 2% safflower oil. The use of safflower oil in quails did not affect the growth performance parameters. Malondialdehyde, glutathione, superoxide dismutase, glutathione peroxidase, and catalase exhibited a linear response to the addition of safflower. Ceruloplasmin, albumin, total protein, and globulin were not affected by the addition of safflower oil. Acetic acid and SCFA were linearly associated with safflower oil content. There were no statistical differences in propionic, butyric, isobutyric, valeric, isovaleric, isocaproic, and caproic acids and BCFA in quails fed different percentages of safflower oil. Feeding a diet containing safflower oil did not affect the biomechanical properties of the tibia and femur in quails. It was concluded that diets containing safflower oil can be used to improve antioxidant status and caecal short-chain fatty acid content in quails.

Keywords: antioxidant status, cecal short-chain fatty acids, growth performance, quail, safflower oil, bone strength
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Introduction

Safflower (*Carthamus tinctorius*) is an oilseed plant from the Compositae family that contains 20–40% oil in its seeds (Coşge *et al*., 2007). Safflower oil, grown mainly for its oil, is also used in the cooking and oil industries. The oil content in the seed varies depending on factors such as variety, climatic conditions, sowing time, and soil structure. The main factor determining the fatty acid composition of the safflower plant is the variety (genotype). Safflower oil consists mainly (96–99%) of oleic, linoleic, stearic, and palmitic fatty acids. Oleic and linoleic acid ratios can vary from 10–32% and 58–81%, respectively, depending on the variety (Coşge *et al*., 2007). The seeds of the safflower plant contain 30–50% oil of two types: linoleic (omega-6) and oleic (omega-9, olive oil quality). It is a high quality, edible oil and can be used in biodiesel production and its pulp is considered suitable for animal feed. It is an annual, long-day, oily plant with a durable, summer character and can grow for an average of 110–140 days (Trakya Agricultural Research Institute, 2023).

It is important to be cognisant of the sustainable use of energy and protein resources in the preparation of poultry feed. Turkey, which is sufficient in terms of high-energy grain resources such as corn and wheat, imports many feed raw materials, especially quality protein resources. Protein-dense feed raw materials for poultry compound feeds that can be produced under Turkey conditions include safflower, camellia, guar, fenugreek, and lupins. It is possible to use oilseed meals. There is a need for research on alternative protein source feeds. Fenugreek, one of the medicinal and aromatic plants, has the potential to be both a feed raw material and a feed additive due to its high antioxidant capacity, antidiabetic, anticholesteromic, hypoglycaemic effects, and many other pharmacognosy properties (Singletray, 2017). The use of these sources in exogenous enzyme supplements in order to increase feed value should also be specifically examined. In closing the vegetable protein gap, the use of oil industry by-products (e.g., hazelnut, sunflower, cottonseed, safflower, and corn gluten meals) in accordance with the patterns in Turkish plant production is evident (Shariatmadari and Forbes, 2005).

In recent years, many studies have been carried out on the use of safflower oil as a feed additive. However, there are very few studies in the literature on the use of safflower oil in Japanese quail diets. Therefore, the aim of this study was to investigate the effect of safflower oil in Japanese quail diets on growth performance, biomechanical properties of femur–tibia bones, blood antioxidant status, and caecal SCFA concentrations.

Materials and Methods

The study was conducted with the approval of the Kafkas University Animal Experiments Local Ethics Committee (Decision No: KAU-HADYEK/2022-063). A total of 180 unsexed, one-day-old chicks (*Coturnix coturnix japonica*) were included in the study. All chicks were randomly divided into three groups each containing 60 chicks. Each group was then randomly divided into six subgroups each containing 10 chicks. The animals were fed a corn and soybean meal basal ration and trial continued for 35 days (Table 1).

All diets were formulated according to NRC (1994) standards. Nutrient analyses of the feed were performed according to AOAC (2000). Birds were caged in breeding cages. Each subgroup was equipped with manual feeders and automatic nipple drinkers. Water and feed were given *ad libitum*. The house temperature was monitored thermostatically throughout the study. The temperature, which was 32–35 °C on the first day, was gradually lowered and maintained at 22 °C for the last two weeks. An artificial lighting program was implemented in accordance with commercial conditions (23 h of lighting throughout the experiment per day). The experimental diets were: C, basal diet (control; without additional safflower oil); S1, 0.5% safflower oil; and S2; 2% safflower oil. Plant oil was obtained from Botalife (Isparta, Turkey). The fatty acid composition of the safflower oil is given in Table 2.

Table 1. Composition (%) of basal diets used to feed quail to 35 days[1]

Feed materials	**%**
Corn	58.00
Soybean meal	33.00
Corn gluten (CP, 60%)	5.60
Limestone	1.35
Dicalcium 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
Calculated values	
Crude protein, %	22.52
ME (kcal/kg)	2941.00
Ca, %	0.85
Total P, %	0.58
Analysed values	
ME (kcal/kg)	2947,13
Crude protein, %	22,83
Ca, %	1,02
Total P, %	0,60

[1] As-fed basis
[2]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. Safflower oil fatty acid composition used in this study

***Safflower oil**	%
Palmitic acid	6.04
Oleic acid	33.01
Linoleic acid	56.47
Linolenic acid	0.07
Stearic acid	3.19

*Botalife (Isparta, Turkey)

Live weights (LW) were recorded weekly for each subgroup. Live weight gain (LWG) was determined by the difference between these measurements. Each subgroup's feed intake (FI) was recorded weekly and used for the calculation of feed conversion ratio (FCR).

At the end of the experiment, blood samples were taken from the wing veins into anticoagulant (ethylene diamine tetra acetic acid, EDTA) tubes. After a sufficient amount of blood sample was separated as whole blood, plasma of the remaining blood was obtained. The samples were centrifuged at 3000 rpm for 15 min and stored at -20 °C until analysis. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) antioxidant enzyme activities in plasma were determined using an ELISA device (Epoch, Biotek, USA) using commercial kits (Cayman Chemical Company, USA). Reduced

glutathione (GSH) analysis in whole blood was determined colorimetrically (Epoch, Biotek, USA) according to the method of Beutler *et al.* (1963). Malondialdehyde (MDA) in plasma was determined using the method of Yoshioka *et al*. (1979), ceruloplasmin by the method of Colombo & Ricterich (1964), and albumin and total protein levels were determined using a commercial test kit (Biolabo, Maizy, France). The globulin value was determined by subtracting albumin from total protein (Doumas, 1971).

At the end of the study, the cecum contents were obtained and stored at -18 °C. Frozen caecal digesta were thawed at 4 °C and diluted 10-fold with double-distilled water in sterile screw-cap tubes before analysis. The caecal digesta were centrifuged at 4,000 rpm for 15 min at 4 °C for homogenization. The supernatant was placed into a 750-µl Eppendorf tube and mixed with 150 µl ice-cold 25% metaphosphoric acid solution. After that, the tubes were kept ice-cold for 30 min to ensure the denaturation of proteins. Subsequently, tubes were centrifuged for 10 min at 10,000 rpm at 4 °C. Supernatants were analysed using a gas chromatography (Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan) coupled with a 30 m × 0.53 mm internal diameter column (Teknokroma TRB-FFAP, Teknokroma, Barcelona, Spain) and flame ionization detector to determine SCFA concentrations in caecal digesta. The analysis was performed according to the method of Zhang *et al.* (2003). The injector-port and flame ionization detector (FID) temperatures were fixed at 250 °C. In the temperature program, the initial temperature was held at 120 °C for 4 min after injection and then increased at 4 °C /min to 160 °C, where it was held for 4 min. Helium was used as the carrier gas. The injection volume was set at 1 µL and analyses were performed in duplicate.

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 for 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 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 was collected, which were used to form a load–displacement curve (Figure 1).

One-way analysis of variance was used for the statistical calculations of the groups and polynomial contrast tests were used to determine the effect of the safflower oil used at different levels in the groups. The statistical analysis was done with the SPSS software package (SPSS, 2011).

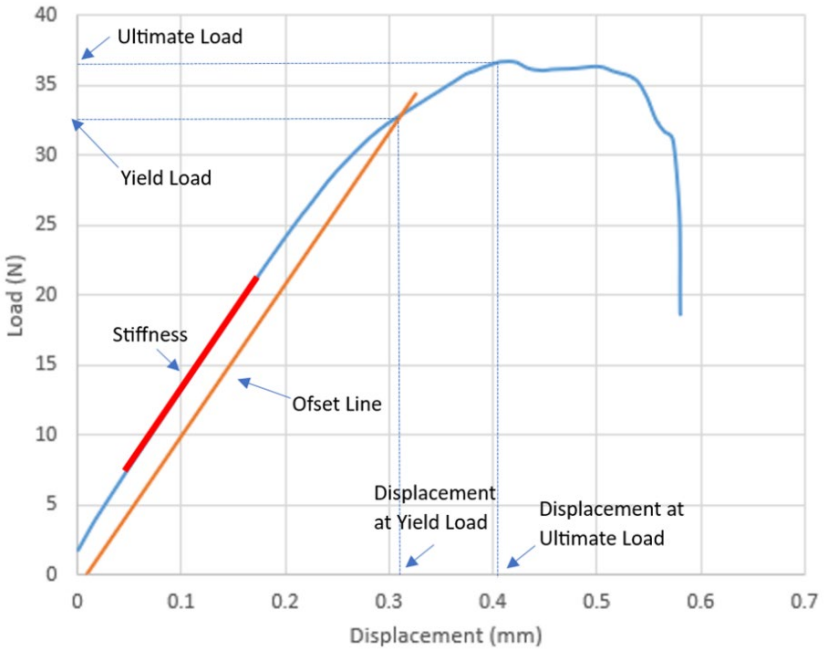

Figure 1. Load–displacement curve produced by the sequential loading of quail femur and tibias

Results

Initial LW, final LW, LWG, FI, and FCR values were not statistically affected by the addition of safflower oil at the end of the experiment (Table 3).

Table 3. Influence of *Carthamus tinctorius* (Safflower) seed oil on growth performance

Groups	Control	S1	S2	SEM	Significance	
Performance Parameters	**x̄**	**x̄**	**x̄**		**L**	**Q**
Initial Live Weight, g	8.136	8.134	8.135	0.00	0.321	0.144
Final Live Weight, g	212.54	203.72	204.45	2.53	0.205	0.380
Live Weight Gain, g	204.41	195.58	196.31	2.53	0.205	0.380
Feed Intake, g	566.16	589.45	574.38	6.51	0.612	0.183
Feed Conversion Ratio	2.77	3.01	2.93	0.45	0.127	0.080

[1]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
[2]C: Control, basal ration, S1: 0,5% *Carthamus tinctorius* (safflower) seed oil added to basal ration; S2: 2% *Carthamus tinctorius* (safflower) seed oil added to basal ration
[3]Polynomial contrasts: L = linear and Q = quadratic effect of supplemental *Carthamus tinctorius* (safflower) seed oil

MDA, SOD, GPx, and CAT showed a linear response (linear, P = 0.000, for all) at the end of the experiment to the addition of safflower oil. SOD and CAT showed a linear and quadratic response (linear, P = 0.000 for all and P = 0.004 and P = 0.022, respectively) to the increase in safflower oil at the end of the experiment. However, ceruloplasmin, albumin, total protein, and globulin were not affected by the addition of safflower oil. The influence of the experimental diets on antioxidant capacity is shown in Table 4.

Table 4. Influence of *Carthamus tinctorius* (safflower) seed oil on antioxidant capacity

Groups	Control	S1	S2	SEM	Significance	
Blood Parameters	**x̄**	**x̄**	**x̄**		**L**	**Q**
MDA (µmol/L)	4.86	4.44	3.87	0.10	**0.000**	0.189
GSH (mg/dL)	8.12	15.76	48.40	4.54	**0.000**	**0.004**
SOD (U/mL)	17.57	27.41	43.92	2.88	**0.000**	0.228
CAT (nmol/min/mL)	2.92	3.36	3.50	0.06	**0.000**	**0.022**
GPx (nmol/min/mL)	29.61	41.70	67.50	4.20	0.000	0.099
Ceruloplasmin (mg/dL)	14.16	13.93	13.90	0.59	0.867	0.943
Albumin (g/dL)	2.23	2.18	2.14	0.03	0.318	0.948
Total protein (g/dL)	3.25	3.24	3.25	0.01	0.855	0.850
Globulin (g/dL)	1.02	1.06	1.10	0.03	0.412	0.977

[1]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
[2]C: Control, basal ration; S1: 0,5% *Carthamus tinctorius* (safflower) seed oil added to basal ration; S2: 2% *Carthamus tinctorius* (safflower) seed oil added to basal ration
[3]Polynomial contrasts: L = linear and Q = quadratic effect of supplemental *Carthamus tinctorius* (safflower) seed oil

Acetic acid (*P* = 0.043) and SCFA (*P* = 0.025) were linearly affected by the addition of safflower oil. There were no significant differences in propionic, butyric, isobutyric, valeric, isovaleric, isocaproic, and caproic acids, and BCFA concentrations in the blood of quails fed different levels of safflower oil. Effect of the experimental diets on caecal short-chain fatty acids (µmol/g) is reported in Table 5.

Table 5. Influence of *Carthamus tinctorius* (safflower) seed oil on some caecal short-chain fatty acid concentrations (µmol/g)

Groups	Control	S1	S2	SEM	Significance	
Blood Parameters	**x̄**	**x̄**	**x̄**		**L**	**Q**
Acetic acid	45.46	60.79	58.36	2.76	**0.043**	0.099
Propionic acid	1.73	2.15	0.87	0.38	0.374	0.314
Isobutyric acid	0.81	0.91	0.81	0.08	1.000	0.589
Butyric acid	3.01	4.36	5.62	0.96	0.299	0.984
Isovaleric acid	0.07	0.31	0.46	0.09	0.105	0.838
Valeric acid	0.19	0.19	0.21	0.02	0.682	0.902
Isocaproic acid	0.10	0.10	0.08	0.01	0.593	0.905
Caproic acid	0.20	0.27	0.37	0.07	0.377	0.925
BCFA	1.08	1.42	1.49	0.13	0.216	0.638
Total SCFA	51.61	69.10	66.81	3.00	**0.025**	0.082

[1]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
[2]C: Control, basal ration; S1: 0,5% *Carthamus tinctorius* (safflower) seed oil added to basal ration; S2: 2% *Carthamus tinctorius* (safflower) seed oil added to basal ration
[3]Polynomial contrasts: L = linear and Q = quadratic effect of supplemental *Carthamus tinctorius* (safflower) seed oil

Feeding a diet containing safflower oil did not affect the biomechanical properties (length, width, UL, DUL, YL, DYL, and stiffness) of the tibia and femur in quails. Effect of the experimental diets on biomechanical properties of femur–tibia strength is given in Table 6.

Table 6. Influence of *Carthamus tinctorius* (Safflower) seed oil on femur and tibia parameters

	Groups	Control	S1	S2	SEM	Significance	
		x̄	**x̄**	**x̄**		**L**	**Q**
Femur	Length, mm	42.53	43.17	42.42	0.40	0.450	0.450
	Width, mm	3.37	3.31	3.15	0.69	0.732	0.732
	UL, N	38.69	32.75	34.19	1.55	0.259	0.269
	DUL, mm	0.70	0.51	0.64	0.03	0.534	0.054
	YL, N	32.94	27.55	29.75	1.17	0.270	0.126
	DYL, mm	0.44	0.32	0.42	0.03	0.773	0.118
	Stiffness, N/mm	84.38	86.76	77.94	4.54	0.596	0.583
Tibia	Length, mm	54.81	55.35	54.41	0.49	0.754	0.510
	Width, mm	2.92	2.99	3.05	0.05	0.395	0.970
	UL, N	38.23	31.39	37.59	1.36	0.834	0.024
	DUL, mm	0.60	0.58	0.61	0.02	0.839	0.560
	YL, N	37.56	28.94	36.53	1.41	0.718	0.004
	DYL, mm	0.57	0.46	0.57	0.02	0.913	0.052
	Stiffness, N/mm	67.01	63.93	67.53	2.58	0.939	0.571

[1]Statistically not significant (P <0.05). The values show the mean (x̄) and standard error (Sx̄) of the six subgroups in each group
[2]C: Control, basal ration; S1: 0,5% *Carthamus tinctorius* (safflower) seed oil added to basal ration; S2: 2% *Carthamus tinctorius* (safflower) seed oil added to basal ration
[3]Polynomial contrasts: L = linear and Q = quadratic effect of supplementary safflower seed oil
UL: ultimate load, DUL: displacement at ultimate load, YL: yield load, DYL: displacement at yield load

Discussion

Antibiotic growth promoters have long been used in poultry feed to improve intestinal health and growth performance. Increasing concerns about the adverse effects of antibiotic growth promoters have led to research on the use of natural feed additives in poultry feed to ensure better performance and safety in the food chain. Herbs and substances of plant origin (garlic, thyme, thyme, anise, rosemary, and cinnamon) are defined as phytogenic, phytobiotic, or botanical natural feed additives. Numerous

studies on such substances in poultry production show their properties as antimicrobial, antiviral, antioxidant, and improving intestinal function. These substances have several advantages over those commonly used as growth promoters and they leave no residue and are safe. Phytogenic substances have attracted great attention as ideal growth promoters in poultry nutrition. However, methods to evaluate their effects and interactions with other medical treatments need to be developed. In general, phytogenic substances can be natural and safe growth promoters in animal and poultry feeds (Mohamed & Hassan, 2023). In the current study, with the increase in dietary safflower oil, initial LW, final LW, LWG, FI, and FCR values were not statistically affected by the addition of safflower oil by the end of the experiment. In a study where more than one aromatic plant oil was used, it was reported that the addition of soy, sunflower, safflower, and olive oil to quail rations did not affect live weight, live weight gain, feed consumption, and feed conversion ratio values (Kara & Bülbül, 2021). In another study with similar results, it was observed that the use of 0%, 7.5%, and 15% safflower seeds in lamb rations did not affect performance parameters (Ferreira *et al*., 2019). Amer *et al*. (2021) found that the use of safflower oil at different doses (0%, 1% and 5%) increased final live weight, total live weight gain, total feed consumption, and relative growth rate. Saminathan *et al*. (2022) reported improved feed and energy consumption, BW, and FCR of broiler chickens fed high-energy diets. In a different study, safflower supplementation substantially increased growth performance (Meng *et al*., 2022). Differences between these studies can be explained by the dose and quality of safflower used and differences in care and feeding conditions.

The physiological effects of flavonoids and lignans in safflower oil are similar to phytoestrogens. They exhibit potent antioxidant activities and anticarcinogenic and cholesterol-regulating properties (Draper *et al*., 1997). In the current study, MDA, GSH, SOD, GPx, and CAT responded linearly to the increase in safflower oil by the end of the experiment. However, ceruloplasmin, albumin, total protein, and globulin were not affected by the addition of safflower oil. The use of safflower oil in quails was reported to decrease serum MAD content in the blood and increased the serum antioxidant activity level (Kara & Bülbül, 2021). Safflower oil added to broiler diets had no marked effect on SOD, GSH, and CAT parameters. With the addition of safflower oil, the MDA level in the chest muscle decreased substantially (Amer *et al*., 2021). Similarly, safflower use in aquatic animals increased CAT but reduced MDA content in the midgut (Meng *et al*., 2022). It is assumed that the strong antioxidant effect of safflower oil may be due to the high levels of vitamin E contained in this oil and the polyphenolic compounds in its seeds.

Short-chain fatty acids are formed as a result of bacterial fermentation in the caecum. In addition to improving intestinal integrity by stimulating cell growth and differentiation in the intestine, they also prevent the growth of pathogenic microorganisms by lowering the pH of the digestive tract (Knudsen *et al*., 2012). Researchers have reported that there is a close relationship between the composition of the caecal microflora and the SCFA concentration (Meimandipour *et al*., 2010). Increased SCFA concentrations have been shown to have beneficial effects on energy, metabolism, microflora, and immune responses (Tan *et al*., 2012). This indicates that an increase in short-chain fatty acids in the caecum has a positive effect on intestinal health. In the current study, acetic acid and SCFAs were linearly affected by increases in safflower oil. There were no statistical differences in propionic, butyric, isobutyric, valeric, isovaleric, isocaproic, and caproic acids and BCFA concentrations in quails fed different levels of safflower oil.

No study was found that investigated the effect of safflower oil on caecal short-chain fatty acids in quails. Therefore, the discussion will be based on different animal species or different plant extracts. Safflower can regulate the contents of SCFAs, especially acetic, butyric, and total acids in the intestinal contents of the common carp (Meng *et al*., 2022). Similarly, acetic, butyric, and isocaproic acids and SCFAs were linearly affected by the increasing content of an essential oils mixture (peppermint, juniper, rosemary, and thyme vulgare oils) in quail diets. No statistical differences were found in the concentrations of propionic, isobutyric, valeric, isovaleric, and caproic acids and BCFAs (Aydin and Yildiz, 2020a). The addition of thyme and black cumin oil to broiler diets did not affect the acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, and caproic acids, and total SCFA and BCFA values in the cecum (Aydin and Yildiz, 2020b). Differences between these studies can be explained by the dose and quality of safflower used, the use of different plant extracts with different animal species, and differences in care and feeding conditions. Seed oil content of safflower seeds varies between 20% and 45%, depending on their shell type.

Leg abnormalities are one of the most important problems in the poultry industry, causing economic losses and welfare problems in poultry breeding (Shim *et al*., 2012). Many nutrients, from micronutrients to macronutrients, have a potential role in skeletal health, especially dietary fat intake (Holl & Allen, 1987). Monounsaturated fatty acids are thought to have a potential role in prostaglandin activity (French, 1943), affecting both bone formation and resorption (Calverley & Kennedy, 1949). Seed oil content of safflower seeds varies between 20% and 45%, depending on their shell type. The oil

contains high levels of linoleic acid, an unsaturated fatty acid that helps lower blood cholesterol content (Fernandez-Martinez, 2002). In the current study, it was determined that feeding safflower oil did not affect the biomechanical properties (length, width, UL, DUL, YL, DYL, and stiffness) of the tibia and femur in quails. No negative effects on bone biomechanics were observed with the addition of safflower oil to the diet. 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). More studies are needed to discuss the effect of safflower oil on bone biomechanical properties.

Conclusion

It was determined that the use of safflower 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, GPx, and CAT values in the blood. Additionally, the marked increase in acetic acid and SCFA values indicated that diets containing safflower oil could improve intestinal health in quail. Safflower oil can therefore be used safely as a feed additive to protect against oxidative stress and improve intestinal health in quails.

Authors' contributions

ÖD, GY, OK, and KO executed the experiment; ÖD statistically analysed the collected data; ÖD completed the manuscript; all authors reviewed the final compilation of manuscript; TA helped in preparing the manuscript. ÖD and GY contributed to the design and execution of the study. GY, ÖD, OM, FKEE, ENP, and HD were in charge of laboratory analyses. ÖD was responsible for supervision and writing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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