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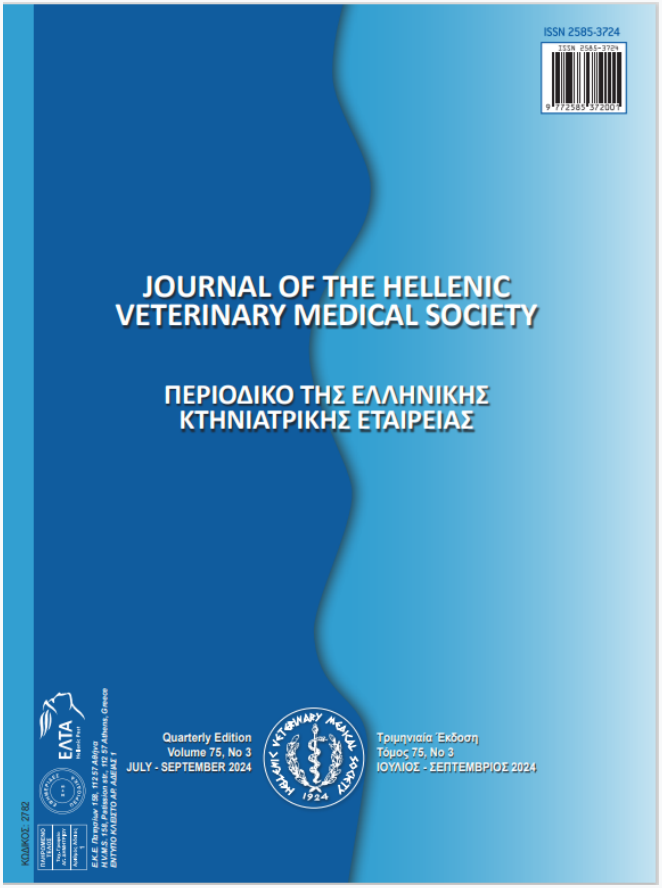

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Growth performance, carcass characteristics, fatty acid composition, blood parameters, and gut morphology of Italian quails fed diets containing chia seed (*Salvia hispanica L.*)

Ö. Karadağoğlu[1*], I. Ahmed[2], T. Şahin[1], M. Ölmez[1], B. Özsoy[3], I. Şerbetçi[4], G. Karadağoğlu[5], E.K. Sarı[6], M. Öğün[7], R. Riaz[1]

[1]*Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkiye*

[2]*Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkiye*

[3]*Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Aydın Adnan Mendres University, Aydın, Turkiye*

[4]*Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, CH-8057, Zurich, Switzerland*

[5]*Department of Food and Food Technology, Kars Vocational High School, Kafkas University, Kars, Turkiye*

[6]*Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkiye*

[7]*Department of Medical Biochemistry, Faculty of Medicine, Kars, Turkiye*

ABSTRACT: The main objective of the study was to determine the growth, hematologicalparameters, and gut-associated attributes in Italian quails fed chia seed (*Salvia hispanica L.*) (CS). Two hundred mixed-sex, 1-day-old Italian quails were randomly distributed into four treatment groups (n=50 birds/group) kept in five replicate pens with 10 birds per pen from day 1 to 35, under a completely randomized design. The control group received a basal diet with no CS, whereas the other groups were fed diets containing 1%, 2%, and 3% CS. Results revealed that for the 0-5 weeks study period, the live weight increased, and the feed conversion ratio (FCR)demonstrated statistically significant ($P = 0.05$) decrease with supplementation of 3% CS. However statistically non-significant ($P > 0.05$) difference was noticed between control groups and groups fed diets containing 1%, 2%, and 3% of CS for slaughter liveweight, hot carcass, cold carcass, dressing percentage, heart and liver weight, and pH of carcass at the time of slaughter and after 24 hours. Similarly, breast meat dry matter, ash percentage, cooking loss, and water holding capacity were also not influenced($P > 0.05$) by supplementation of CS. Regarding gut associated attributes, the ileal villus height (VH) of the control diet is higher in comparison to all inclusion levels of CS (1%, 2%, and 3%) in diets, means supplementation of CS resulted in reduced ($P< 0.05$) VH, whereas crypt depth (CD) was reduced ($P = 0.001$) quadratically. The total protein and cholesterol profile showed statistically significant ($P = 0.001$) increase,and the lipid profilesdecreased at a statistically significant level ($P = 0.001$) with the supplementation of CS. The CS-fed groups had substantially greater values of myristoleic acid, linoleic acid, docosahexaenoic acid, PUFA, USFA, PUFA/SFA, UFA/SFA, and omega 6 (n6), whereas lower values of palmitoleic acid, stearic acid, eicosanoic acid, erucic acid, SFA in the breast meat of quails comparedto the control group. In conclusion, the supplementation of 3% CS resulted in better growth performance, improved blood parameters, and an enhanced nutritional profile of quail breast meat for human consumption.

Keywords: Blood parameters; Carcass characteristics; Chia seed; Fatty acid composition; Gut morphology; Italian quail.

Corresponding Author:
Ö. Karadağoğlu Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkiye
E-mail address: drozlemkaya@hotmail.com

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INTRODUCTION

Global consumption and production of chicken products (eggs and meat) have steadily grown over time and are predicted to continue to rise to fulfil the nutritional needs of the world's constantly rising population (Mahmood et al., 2022, Nardoia, 2016). Quail meat consumption has progressively increased in recent decades, but it is still less popular than the consumption of chicken meat. The increased production of quail is due to its rapid growth rate, illness tolerance, good adaptability, small size, and inexpensive capital and maintenance expenses (Khosravi et al., 2016, Mir et al., 2017). It is also regarded as a viable source of protein for personal food, particularly in underdeveloped nations (Rincon et al., 2020). The application of nutritional amendments to enhance the grade and chemical properties of quail meat used in human diets constitutes an essential relationship among animal farming, food science, and human nutrition. Chia seeds are a novel nutritional additive being used in quail feed (El-Rayes et al., 2023, Şengül, 2022, Yildiz et al., 2022), leading to increased nutrient utilization and ω-3 fatty acid accumulation in the pectoral and leg muscles (Mendonça et al., 2020, Xing and Li, 2023). The consumers PUFA rich food consumption is rising because of non-infectious diseases, obesity, heart, and degenerative diseases (Xing and Galati, 2013; Gul and Alsayeqh, 2022). Such diets also improve eggshell fracture toughness (Yildiz et al., 2022), boosting the humoral immune response, which means a surge in the number of type B lymphocytes, resulting in higher immune cells, and ultimately a higher quantity of globulin in the bloodstream of birds (Habib et al., 2020).

Chia (*Salvia hispanica L.*) seeds(CS), also known as the golden seedare considered one of the best botanical sources of alpha-linolenic acid.This plant is indigenous to northern Guatemala and southern Mexico (Azcona et al., 2008). In the twenty-first century, the European Parliament recognized chia seeds (CS) a novel source of nutrition due to their incredible nutritional profile (Orona-Tamayo et al., 2017). It contains between 26-41 % carbohydrates, 30-35 % fat, predominantly polyunsaturated fatty acids (ω-3 and ω-6), 10 % saturated fats, 15-24 % non-gluten protein, and between 18-30 % fiber (Ayşan and Ayaşan, 2020; Ullah et al., 2016). Supplementation of chia seed in the diets of broiler results in incorporation of healthy fatty acids into meat, thereby improving its quality (Mendonça et al., 2020)as CS contain high content of ω-3 fatty acids and antioxidants(da Silva Marineli et al., 2014).

Italian quails are mainly used for meat purpose (Almeida et al., 2002). There has been limited attention paid to the nutritional studies on Italian quails, and no study is available on the effects of chia seeds included in the diet of quails. Hence, the present study was designed to evaluate the effects of dietary CS on growth performance, blood metabolites, gut morphology, and breast meat composition of Italian quails.

MATERIAL AND METHODS

This experiment was in line with the protocols and approved animal experiments' local ethics board of Kafkas University (Approval no: KAÜ-HADYEK 2017-095).

Husbandry, diet, and experimental design

Two hundredmixed-sex, one-day-old Italian quail were obtained from the poultry unit belonging to the Kafkas University Faculty of Veterinary Medicine Prof. Dr. Ali Riza Aksoy research and application farms. The quailswere randomly distributed across four treatmentswith 50 birds per group, with five replicate pens and each pen contains 10 birds from 1 to 35 days of age(Karadağoğlu et al., 2019). The pens were installed in a standard quail shed with a concrete floor, elevated 55 cm from the ground.To provide cushioning for the birds' feet, sawdust was used as bedding, and covered with paper.Each pen was equipped with a bell-shaped-feeder and drinker, providing*ad libitum* feed and fresh water.During the first week, the shed temperature was kept at 35°C and humidity at 67%. The temperature was graduallyreduced by3°C per week.Regular lighting was provided throughout the trial(Karadağoğlu et al., 2019).All the diets were isocaloric (3000 kcal/kg ME) and isonitrogenous (22% CP)(NRC, 1994).The CS used in study (CP 16.11% and crude fat 27.12%) was obtained from the local market, ground in a mill, and added to the feed in appropriate proportions (0%, 1%, 2%, and 3%). Table 1 represents the composition and chemical content of experimental diets.To analyze the nutritional composition of the experimental diets,conventional chemical tests were performed in accordance with-AOAC(1975).Dry matter (DM) was determined by drying materials at 105°C to a constant weight was achieved.Ash content was measured using an ash ovenfor 8 hours for complete combustion at 550°C. The nitrogen concentration was estimated using the Kjeldahl procedure with CuSO4 as a catalyst, and crude protein (CP) was calculated as N×6.25. Ether

Table 1 Ingredients and chemical composition of the experimental diets for quails

Ingredients %	Control	CS 1%	CS 2%	CS 3%
Corn	52.4	52.4	51.75	51
Full Fat Soya 38%	15.85	15.15	15.00	15.30
Soybean Meal 48%	23.60	23.60	23.60	23.15
Corn Gluten Meal 60%	4.4	4.4	4.4	4.4
Vegetable Oil	1	0.7	0.5	0.4
Chia Seed	-	1	2	3
Lysine	0.05	0.05	0.05	0.05
Methionine	0.05	0.05	0.05	0.05
Limestone	1.1	1.1	1.1	1.1
DCP	1	1	1	1
Salt	0.30	0.30	0.30	0.30
KAVIMIX VM*	0.25	0.25	0.25	0.25
Calculated Nutrition Profile (%)				
Dry Matter	88.02	88.22	88.60	88.89
Crude Protein	22	22	22	22
Crude Fat	6.02	5.60	5.47	5.37
ME Kcal/kg	3008	3003	2999	3002
Crude Fiber	4.09	4.08	4.06	4.06
Ca	0.73	0.73	0.73	0.74
P	0.30	0.30	0.31	0.31
Methionine + Cysteine	0.80	0.80	0.81	0.80
Lysine	1.29	1.29	1.30	1.30
Linolenic Acid	3.52	3.45	3.41	3.40
Analyzed Values				
Crude Protein	22.12	21.93	22.04	22.01
Crude Fat	5.70	5.64	5.53	5.61

[1]CS=Chia seed

*KAVİMIX VM 214: Vit A: 12000000 IU; Vit D3: 1500000 IU; Vit E: 30000mg; Vit K3: 5000mg; Vit B1: 3000mg; Vit B2: 6000mg; Vitamin B12: 30mg; Folic Acid: 750mg; Calcium. D.Panth: 10000mg; D Biotin: 75mg; Choline Chloride: 375000mg; Nicotine Amide: 40000mg; Manganese: 80,000 mg; Iron: 40000mg; Zinc: 60000mg; Copper: 5000 mg; Cobalt: 100mg; Iodine: 400mg; Selenium: 150mg; Antioxidant: 10000 mg (per 1 kg).

extract (EE) was evaluated in a continuous extractor with HCl treatment prior to extraction with petroleum ether (AOAC, 1975).

Fatty acid profile of diet and chia seed

The fatty acid content of experimental dietsand chia seeds was determined using the fatty acid methyl esters (FAME) method(Karadağoğlu et al., 2019). Soxhlet equipment was used with diethyl ether as solvent to extract oil from pulverized chia seeds via ether extraction. Boron trifluoride in 35% methanol and sodium hydroxide were used for fatty acid esterification and saponification, respectively. The methyl esters of fatty acids were isolated, filtered, and further examined by refluxing with n-hexane under nitrogen gas. Gas chromatography-mass spectrometry (GC-MS) apparatus (Hewlett Packard 6890/5972 GCMS system, Santa Clara, CA, US) equipped with FID senor employing 100 m×0.25mm×0.2 μm (Agilent J&W HP88 capillary column, Santa Clara, CA, US) column was used to examine the FAME. The initial and final temperatures were 120 °C and 230 °C respectively. The split ratio was 1:50, the injection volume was 1 L, and Helium was utilized as the carrier gas.The results were displayed as a percentage of the FID area(-Karadağoğlu et al., 2019). The fatty acid profile of experimental dietsand chia seed is presented in Table 2.

Growth performance

All birds were weighed weekly usingan electronic weighing balance (range 0.1 to 1000 grams). Weight gain (WG) was measured by subtracting initial weight from final weight. Weekly feed intake (FI) was recorded for all replicatesandcalculated by subtracting the left-over feed from the weekly given feed.The feed conversion ratio (FCR) was measured by dividing FI with WG. Adjustments were made for FI, WG and FCR in case of mortality.

Table 2 Fatty acid profile of experimental diet and chia seed

Fatty acid	Control	CS 1%	CS 2%	CS 3%	Chia
C8:0	0.02	0.01	0.01	0.01	0.02
C10:0	0.01	0.01	0.01	0.01	-
C12:0	0.02	0.01	0.01	0.02	-
C14:0	0.1	0.08	0.08	0.08	0.05
C15:0	0.04	0.03	0.03	0.03	0.01
C16:0	13.39	11.24	11.94	11.65	8.2
C16:1	0.07	0.06	0.06	0.06	0.02
C17:1	0.07	0.05	0.05	0.06	0.04
C18:0	2.53	2.78	2.75	2.72	4.06
C18:1	25.59	20.13	21.37	22.5	8.26
C18:2n-6	49.45	39.86	42.59	43.19	22.33
C18:3 n-3	7.82	25.08	20.34	18.92	56.44
C18:3 n-6	0.42	0.32	0.38	0.38	0.13
C20:0	0.32	0.23	0.27	0.27	0.12
C20:2	0.03	0.02	0.03	0.02	0.02
C20:5 n-3	0.03	0.03	0.02	0.02	0.05
C22:2	0.05	0.04	0.04	0.04	-
C22:6 n-3	0.01	0.01	0.01	0.01	0.08
C24:1	0.03	0.01	0.01	0.01	0.17
∑SFA*	16.65	14.56	15.26	14.94	12.5
∑MUFA*	25.73	20.24	21.48	22.62	8.31
∑PUFA*	49.9	40.21	42.99	43.59	22.51
∑UFA	75.63	60.45	64.47	66.21	30.82
PUFA/SFA	3	2.76	2.82	2.92	1.8
UFA/SFA	4.54	4.15	4.22	4.43	2.47
n-6	49.87	40.18	42.97	43.57	22.46
n-3	49.49	39.9	42.62	43.19	22.33
Nutritive Value	2.1	2.04	2.02	2.16	1.5
Atherogenic Index	0.04	0.05	0.05	0.05	0.14
Thrombogenic Index	0.08	0.09	0.09	0.08	0.14

[1]CS=Chiaseed

Carcass characteristics

After a 2-hour feed restriction, 40 birds (10 birds from each treatment) were picked randomly and weighed. They were slaughtered using the conventional technique at 35 days of age(Gheisari et al., 2017). The carcass yield characteristics, hot carcass weight, and giblet (liver, heart) were weighedusing-sensitive weighing balance with a range 0.1 to 1000 grams. The hot carcass weights of the quails were determined, and after being kept at +4 ºC for 24 hours, their cold carcass weights were calculated (Cellat et al., 2022).

To determine meat quality,10quails (two birds from each replicate) per group were slaughtered, and their chest meat samples were stored in a Ziplock bag at -18 °C until further processing (Mazizi et al., 2020).

Meat quality

The pH the *pectoralis major* (PM) muscle was measured using apH electrode at two different time:at the time of slaughter and after 24 hours(Ahmad et al., 2023). The PM was stored at +4 ºC prior tothe second pH reading.After slaughtering 5 g of PM muscle was placed on drying paper for 5 minutes, 2250g weight pressure was applied. After the procedure, the meat was again weighted and the percentage ratio of water holding capacity was measured.After the carcass was cut, 10-g pieces of PM were put in Ziplock plastic air-tight bags in a water bath at 80 °C for 1 hour. The bags were stored at +4 ºC for 12 hours, dried, and weighed to measure cooking loss.

Nutrient composition of meat

After cutting a five-g piece of PM, it was kept in the oven at 110 ºC for 12 hours to measure the mois-

ture and DM percentage of the meat. A five-g piece of pectoralis superficialis muscle was incinerated for six hours in an ash oven at 550 ºC. After incineration, the remaining product was weighed, and the ash percentage was measured.

Fatty acid profile

A total of 10 breast meat samples were analyzed from each group, with two samples taken from each replicate. The fatty acid profile betweenC_4 and C_{24} was determined using the method Wołoszyn et al.(2020) with minor modifications.Diethyl ether was used as the solvent in the Soxhlet equipment to extract the fat from each sample.After saponification with sodium hydroxide, FAMEs were created using methylated boron trifluoride (35 % methyl alcohol). The FAMEs were then isolated using n-hexane, vortexed, compressed under nitrogen gas, and separated for GC-MS analysis (Hewlett Packard 6890/ 5972 GCMS system, Santa Clara, CA, US). With an injection volume of 1 µLand a split ratio of 1:50.A capillary column measuring 100 m by 0.25 mm by 0.2 mm (HP88 capillary column, Agilent J&W, Santa Clara, CA, US) was utilized and helium was used as a carrier gas(Ölmez et al., 2023).

Blood metabolites

On the 35th day, 10 blood samples from each group (two from each replicate)were randomly collected after 3 h of fasting from brachial vein. After clotting, the blood samples were centrifuged (NF 200 Bench-Top Centrifuge, Ankara, Turkey) at 4500 rpm for 15 min to separate the serum. Serum total protein, total lipid, and cholesterol were assayed spectrophotometrically using commercial kits reagents (EbraLachema, Brno, Cz) (Aslam et al., 2021).

Ileal morphology

At the end of the experiment (35th day), to examine the morphology of ileum, a 2 cm long sample was dissected from the center of the ileum, washed with cold normal saline (0.9 % saline), and immediately preserved in Bouin's solution.Within 24 hours, they were transferred to 70% ethanol, fixed in paraffin, and sectioned into 5 µm thick slices.Cross-sections of intestinal slide of birds were prepared for histological analysisfollowing the protocol outlined by Nazir et al. (2022). Villus length was measured from the tip of the villus to the intersection of the villus and crypt, and crypt depth (CD) was calculated as the length between the connection and the basal lamina of the endothelial cells at the crypt's bottom. This was doneon 10 complete, well-aligned villi per quail using the Triple staining methodand a compound light microscope equipped with a video camera.

Statistical analysis

The data were evaluated using one-way ANOVA in the statistical software program SPSS (version 26.0, Armonk, NY, US). Each replica was considered an individual experimental unit. The Shapiro-Wilk test was applied to detect non-normally distributed traits, which were then transformed using either a logarithmic or square root method. The confidence interval was set at 95%. Duncan's multiple range test was used as a post-hoc test to distinguish the significantly different means between groups.The results are presented as mean ± SEM. Statements of statistical significance were determined at a level of $P < 0.05$.

RESULTS

Growth performance

Table 3 summarizes the influence of CS on growth performance of Italian quail. Supplementation with 3% CS resulted in astatistically significant improvement in live weight in 1 ($P = 0.001$), 2 ($P = 0.003$), and 5 ($P = 0.017$) weeks.In addition, inclusion at 3% resulted in statistically significant high weekly live weight gain during weeks 1 ($P = 0.001$), and 5 ($P = 0.001$), as well asduring theoverall, 0-5 weeks ($P = 0.017$) period. Overall feed intake (FI) during the 0-5 weeks trial was not statisticallysignificant ($P = 0.210$) whereas a statistically significant difference was observed during weeks 2 ($P = 0.046$) and 3 ($P = 0.001$) with supplementation of CS. The feed conversion ratio (FCR) was statistically significantly ($P = 0.050$) decreased during the 0-5-week trial with supplementation of 3% CS.

Carcass characteristics

Table 4 representsthe carcass characteristics of quails fed chia seed-supplemented diets. The inclusion of CS did not have influence on the live slaughterweight, hot carcass weight, cold carcass weight, dressing percentage, heart and liver weight, and pH of carcass at the time of slaughter (approximately 30 min) and after 24 hours. Similarly, the supplementation of CS did not demonstrated statistically significant influence on DM content, ash percentage, cooking loss, and water holding capacity of breast meat.

Table 3 Growth performance of quails (0-5 weeks) fed diets containing chia seed

Weeks	Control	CS[1] 1%	CS 2%	CS 3%	p-value
Weekly Live Weights (g)					
0	15.32±0.28	15.53±0.24	15.16±0.30	15.52±0.33	0.78
1	29.86±0.77^{b}	29.34±0.82^{b}	30.35±0.79^{b}	34.80±0.90^{a}	0.001
2	61.93±1.50^{b}	61.91±1.36^{b}	63.11±1.55^{b}	68.95±1.61^{a}	0.003
3	101.97±1.70^{ab}	99.04±1.53^{b}	102.37±1.70^{ab}	104.88±1.54^{a}	0.091
4	139.38±2.48	137.33±2.19	142.23±2.22	142.10±2.21	0.367
5	172.45±3.57^{b}	173.11±2.51^{b}	177.48±2.67^{ab}	183.68±2.16^{a}	0.017
Weekly Live Weight Gains (g)					
1	14.54±0.52^{b}	13.81±0.60^{b}	15.19±0.52^{b}	19.28±0.89^{a}	0.001
2	32.07±1.03	32.57±0.91	32.76±0.92	34.15±1.19	0.515
3	40.04±0.97^{a}	37.12±1.83^{ab}	39.26±0.89^{ab}	35.92±1.38^{b}	0.108
4	37.41±1.38	38.29±1.91	39.86±0.93	37.22±1.47	0.572
5	33.06±1.54^{b}	35.77±1.20^{b}	35.24±1.56^{b}	41.57±1.70^{a}	0.001
0-5	157.13±3.34^{b}	157.58±2.39^{b}	162.31±2.47^{ab}	168.17±2.12^{a}	0.010
Feed (consumption) Intake (FI) (gram/day)					
1	34.36±0.39	34.65±0.92	35.60±0.51	35.36±0.82	0.564
2	72.40±1.55^{b}	80.18±4.91^{ab}	80.25±2.49^{ab}	93.68±7.79^{a}	0.046
3	105.05±2.60^{a}	82.01±4.92^{c}	93.64±3.77^{b}	80.11±2.93^{c}	0.001
4	123.92±4.35	115.94±3.54	121.18±5.13	111.38±6.90	0.349
5	138.38±7.52	110.60±15.75	129±5.16	123.16±12.03	0.364
0-5	474.13±13.93	423.39±17.27	459.73±12.37	443.69±21.94	0.210
Feed Conversion Ratio (FCR=Feed consumed/Live body weight)					
1	2.37±0.08^{b}	2.53±0.11^{a}	2.34±0.03^{a}	1.92±0.22^{a}	0.028
2	2.26±0.02	2.46±0.09	2.47±0.10	2.81±0.33	0.230
3	2.63±0.11	2.27±0.20	2.40±0.11	2.26±0.14	0.274
4	3.33±0.12	3.05±0.18	3.04±0.12	3.07±0.30	0.694
5	4.19±0.12^{a}	3.10±0.44^{b}	3.69±0.26^{ab}	2.96±0.27^{b}	0.036
0-5	3.02±0.05^{a}	2.69±0.11^{b}	2.83±0.06^{ab}	2.26±0.12^{b}	0.050

[1]CS=Chia seed

[a, b, c] Means with different superscripts within the same demonstrate statistically significant difference ($p<0.05$)

Table 4 Carcass characteristics and organ yields of quails fed diets containing chia seed

Parameters	Control	CS 1%	CS 2%	CS 3%	p-value
Slaughter LW (g)	186.04±9.79	188.97±4.27	188.57±4.83	185.89±5.76	0.979
Hot Carcass (g)	116.49±4.53	118.48±2.23	119.23±3.44	120.83±2.79	0.834
Cold Carcass (g)	115.02±4.52	117.29±2.19	117.85±3.50	119.32±2.73	0.837
Dressing (%)	63.18±1.25	62.79±0.87	63.30±1.20	65.24±1.28	0.458
Heart weight (%)	1.49±0.07	1.53±0.08	1.38±0.08	1.35±0.07	0.309
Liver (g)	5.40±0.52	4.98±0.39	4.72±0.56	4.70±0.30	0.682
pH (30 min)	5.73±0.16	5.72±0.08	5.73±0.04	5.89±0.04	0.523
pH (24 Hours)	5.44±0.16	5.54±0.05	5.60±0.05	5.59±0.06	0.204
DM %	24.60±0.46	24.21±0.41	25.52±0.42	25.37±0.30	0.090
Ash %	1.46±0.08	1.46±0.10	1.63±0.09	1.60±0.10	0.470
Cooking Loss	27.93±1.42	28.14±1.06	26.89±1.20	27.32±1.84	0.921
WHC[2]	23.15±0.90	25.40±1.19	26.40±0.69	26.05±0.88	0.086

[1]CS = Chia seed

[2]WHC = Water holding capacity

Gut morphology

Table 5 and Figure 1 present the effects of dietary CS on ileal gut morphology including villus height (VH), crypt depth (CD) and VH to CD ratio. The ileal VH of the birds fed control diet was higher than those fed CS. Supplementation of CS resulted in statisticallysignificant (P< 0.01)reduction in VH,with a decrease of 42.18% with the supplementation of 3% CS. Similarly, the crypt depth showed statistically significant reduction quadratically (P< 0.01) with supplementation of CS, with a decrease of 26.11% with a supplementation of 3% CS. Likewise, VH:CD ratio was decreased with supplementation of CS.

Blood metabolites

Table 6 represents the blood metabolite levels of quails fed CS-supplemented diets. The total protein and cholesterol profiles demonstrated statistically significant (P = 0.001) increase, whereas lipids profile reduced with statistically significance (P = 0.001).

Fatty acid profile of breast meat

Table 7 represents the influence of CS on the fatty acids profile of quail breast meat. The experimental groups demonstrated substantially higher values of myristoleic acid (C14:1), linoleic acid (C18:2n6), docosahexaenoic acid (DHA; C22:6n3), PUFA, USFA, PUFA/SFA, UFA/SFA and omega 6 (n6), whereas lower values of palmitoleic acid (C16:1), stearic acid (C18:0), eicosanoic acid (C20:0), erucic acid (C22:1n9), SFA in breast meat of quails than the control group. The total PUFA concentration in the breast meat fatty acid profileincreased by 23% with supplementing of 3% CS. The atherogenic and thrombogenic index decreasedat a statistically significant level with increasing CS supplementation in the diets. The SFA content was reduced with supplementation of CS in the quail diets.

Table 5 Villus height (µm), crypt depth (µm) and villus height and crypt depth ratio (VH:CD) of the Ileum from quail birds fed diet containing chia seed.

Parameter	Control	CS[1] 1%	CS 2%	CS 3%	p-value
Villus Height	227.94±6.08[a]	144.00±4.78[b]	135.32±5.20[b]	131.80±8.72[b]	0.001
Crypt Depth	54.84±1.50[a]	33.94±0.95[d]	45.21±1.42[b]	40.52±1.40[c]	0.001
VH: CD	4.25±0.15[a]	4.38±0.22[a]	3.06±0.13[b]	3.49±0.29[b]	0.001

[1]CS = Chia seed

[a, b, c] Means with different superscripts within the same row demonstrate statistically significant difference (p<0.05)

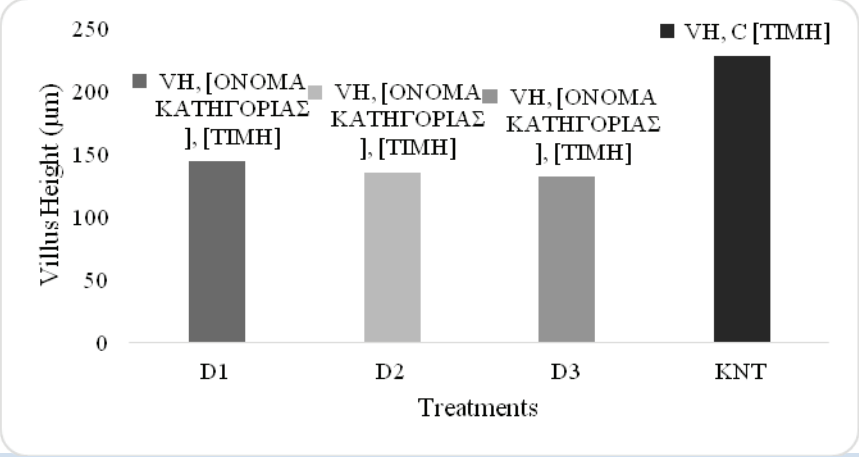

Figure 1. Villus height (µm) in groups with different levels of chia seed supplementation

D1: 1% CS; D2: 2% CS; D3: 3% CS; KNT: control

Table 6 Some blood parameters of quails fed diet containing chia seed

	Control	CS[1] 1%	CS 2%	CS 3%	p-value
Total Protein	43.88±0.46[a]	42.17±0.25[c]	38.52±0.61[b]	36.54±0.52[d]	0.001
Total Cholesterol	279.07±2.03[a]	271.12±0.97[b]	265.77±0.79[c]	259.60±0.89[d]	0.001
Total Lipid	15.58±0.35[a]	14.34±0.19[b]	12.53±0.25[c]	11.89±0.19[c]	0.001

[a, b, c] Means with different superscripts within the same row demonstrate statistically significant difference(p<0.05),

[1]CS = Chia seed

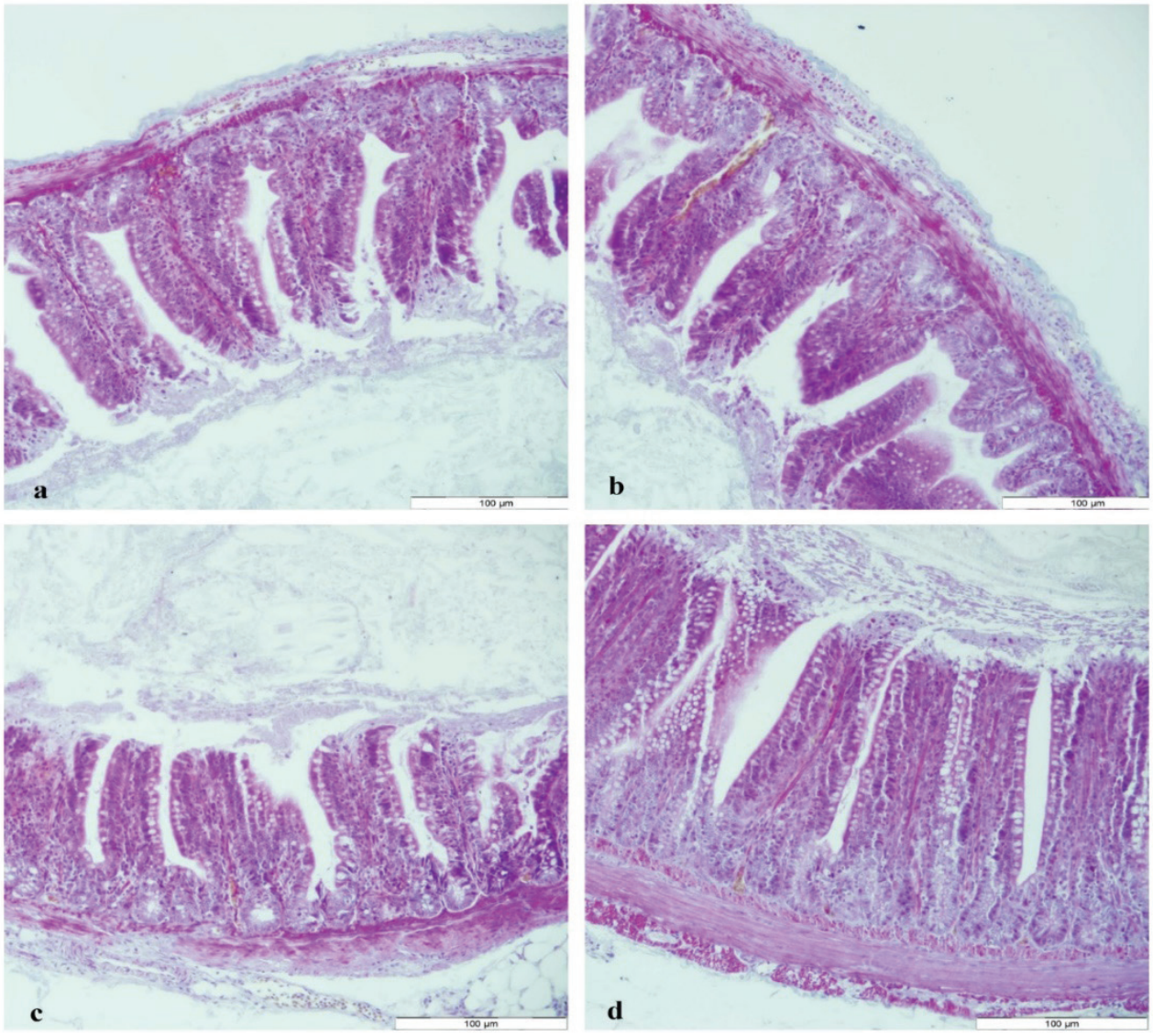

Figure 2. Ileal morphology of quail, a: 1% CS, b: 2% CS, c: 3% CS, d: control group

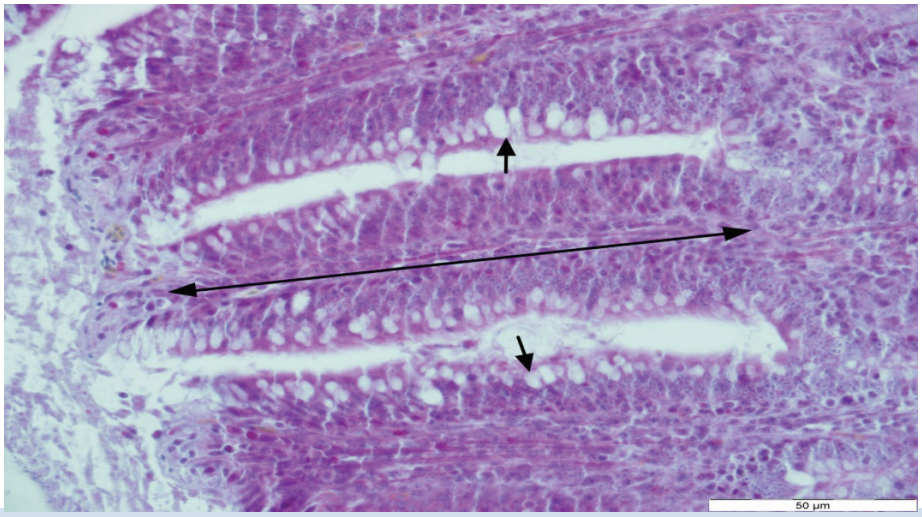

Figure 3. Ileal morphology of quail fed diet containing chia seed

Table 7 Breast meat fatty acid composition (%) of quails fed diet containing chia seed

Fatty acid	Control	CS 1%	CS 2%	CS 3%	p-value
C8:0	0.04±0.02	0.01±0.07	0.02±0.01	0.07±0.07	0.104
C10:0	0.06±0.03	0.01±0.01	0.03±0.01	0.06±0.02	0.172
C12:0	0.07±0.01	0.04±0.001	0.05±0.003	0.09±0.04	0.361
C14:0	0.71±0.03	0.85±0.03	0.92±0.09	0.73±0.10	0.121
C14:1	0.28±0.03^{b}	0.47±0.07^{a}	0.34±0.03^{ab}	0.31±0.05^{b}	0.055
C15:0	0.12±0.03	0.08±0.006	0.09±0.007	0.30±0.01	0.082
C16:0	21.54±0.48	21.59±0.35	22.13±0.54	20.47±0.66	0.173
C16:1	8.47±0.92^{a}	6.22±0.42^{b}	6.50±0.37^{b}	6.98±0.24^{ab}	0.037
C17:0	0.14±0.01	0.16±0.01	0.16±0.008	0.17±0.08	0.312
C17:1	0.14±0.03	0.08±0.008	0.10±0.10	0.16±0.62	0.420
C18:0	8.10±0.29^{a}	5.36±0.43^{b}	6.27±0.25^{b}	5.92±0.41^{b}	0.001
C18:1	28.95±0.51	30.08±0.79	29.82±0.76	27.73±1.05	0.179
C18:2n-6	16.34±0.58^{c}	20.57±0.78^{b}	20.43±0.40^{b}	23.24±0.97^{a}	0.001
C18:3 n-6	1.08±0.14	1.16±0.17	1.36±0.18	1.57±0.16	0.184
C18:3 n-3	0.31±0.13	0.22±0.14	0.45±0.18	0.73±0.24	0.223
C20:0	0.24±0.05^{a}	0.14±0.02^{b}	0.11±0.006^{b}	0.15±0.02^{b}	0.016
C20:1	0.33±0.05	0.28±0.03	0.22±0.02	0.023±0.04	0.114
C20:2	0.09±0.01	0.07±0.006	0.05±0.006	0.076±0.03	0.381
C20:2 n-6	0.54±0.05	0.46±0.04	0.48±0.03	0.42±0.04	0.177
C21:0	0.12±0.02	0.15±0.005	0.14±0.01	0.10±0.02	0.321
C22:2	1.44±0.38	0.95±0.79	0.83±0.11	1.35±0.39	0.376
C22:1 n-9	5.00±0.31^{a}	4.49±0.18^{ab}	3.55±0.33^{bc}	3.06±0.45^{c}	0.001
C20:3 n-3	4.13±0.24	4.36±0.16	3.43±0.33	3.35±0.46	0.082
C20:4 n-6	0.23±0.14	0.04±0.009	0.04±0.005	0.31±0.17	0.217
C20:5 n-3	0.58±0.21	0.39±0.08	0.49±0.11	0.67±0.17	0.607
C22:6 n-3	0.62±0.10^{b}	1.56±0.24^{a}	1.80±0.22^{a}	1.39±0.25^{a}	0.003
C24:1	0.27±0.11	0.20±0.03	0.11±0.01	0.21±0.07	0.449
∑SFA	30.94±0.64^{a}	28.23±0.29^{bc}	29.74±0.58^{ab}	27.83±0.59^{c}	0.001
∑MUFA	38.18±1.09	37.13±0.63	37.03±0.67	35.40±1.13	0.213
∑PUFA	23.22±0.58^{c}	27.21±0.69^{b}	26.69±0.41^{b}	30.45±1.06^{a}	0.001
∑UFA	61.40±0.65^{b}	64.33±0.44^{a}	63.73±0.62^{ab}	65.85±1.28^{a}	0.006
PUFA/SFA	0.75±0.020^{bc}	0.96±0.03^{b}	0.90±0.03^{b}	1.10±0.06^{a}	0.001
UFA/SFA	1.99±0.064^{c}	2.28±0.03^{ab}	2.15±0.06^{cb}	2.38±0.09^{a}	0.002
n-6	18.20±0.52^{c}	22.24±0.78^{b}	22.32±0.37^{b}	25.69±1.15^{a}	0.001
n-3	5.64±0.33	6.53±0.47	6.18±0.50	6.15±0.51	0.603
Atherogenic Index	0.18±0.06^{a}	0.14±0.006^{c}	0.16±0.003^{b}	0.14±0.01^{c}	0.001
Thrombogenic Index	0.51±0.02^{a}	0.43±0.009^{b}	0.46±0.01^{b}	0.43±0.01^{b}	0.001

[1]CS = Chia seed

[a, b, c] Means with different superscripts within the same row demonstrate statistically significant difference($p<0.05$).

DISCUSSIONS

Growth performance

In the present study, quails fed CS showed significantly higher BW during 1st, 2nd, and 5th weeks, whereas weight remained the same in the 3rd and 4th weeks. The improved growth performance of quails in the present study can be attributed to the enhanced immunity of birds fed CS. Uribe et al. (2011) stated that CS contains antioxidants thatenhance the health of the birds. Similarly, Kamranazad et al. (2009) reported that compared with ALA, PUFA n-3 has a more significant impact on the immune response of birds, and CS contain an ample amount of these fatty acids(Vuksan et al., 2007). The CS has a therapeutic effect that reduces cholesterol in the serum of birds which lowers stress and ultimately enhance the immunity and growth (Fernández, 2006). In the present investigation, the explanation for the greater body weight gain in the group given food supplemented with 3% CS than the control group might be the extra

energy supplied by CS (Yildiz et al., 2022). Similarly, Koh et al. (2015) stated that the linolenic acid concentration of CS is the cause of body weight gain. Furthermore, phenolic chemicals in CS improved quail feed nutrient utilization, which might be seen in body weight gain (Hamzah and Mohmad, 2021). Reduced FI with CS supplementation was also observed by Rasul et al. (2019). The improved BW observed in the present experiment is in contrary to Rasul et al. (2019)and Mendonça et al. (2020). This discrepancy could be attributed to the specific condition of the later authors' trial, as they conducted their trial under heat stress conditions whereas in the present study, birds were reared in a normal, healthy environment. Another possible reason of increased BW increase in present trial can be linked to CS physical form (grinded powdered form) which leads to maximize CS nutrient absorption.

Similarly, FCR is also different to Rasul et al. (2019), which can be correlate with the previous trial's heat stress condition. The outcomes of the present study are different from those reported in previous studies of broiler chickens fed different levels of PUFAs from vegetable sources (Ghasemi et al., 2016; Kanakri et al., 2018). Furthermore, as compared to controls, CS feeding induces a considerable reduction in BWG in broilers (Ayerza and Coates, 2002). Likewise, no significant variations in broiler BWG were seen when CS dosage was low or high (Ayerza and Coates, 2002).

The differences in the findings could be related to differences in management approaches, species, dietary composition, and levels of seed incorporation. The diets provided to all experimental groups in this trial were balanced in protein and energy. Furthermore, the diets comprised more digestible feedstuffs than the CS inclusion levels. As a result, there is a possibility that those significant feed additives concealed growth performance-related features compared to the lesser inclusion amounts of CS. Moreover, it is a believed that healthier birds in hygienic housing and environments seem to have little space for increase in growth performance, even in response to growth-stimulating ingredients (Lee et al., 2003), as shown in the present study. The bioactive compounds in these seeds aid in feed digestion and nutrient uptake in the digestive system, resulting in improved growth performance in chickens fed dietary CS. When whole seeds are utilized rather than isolates, the benefits of these bioactive compounds are diminished.

Carcass characteristics

The reduced pH at 24 hours in quails fed CS,as reported by , can be attributed to the linkage between anaerobic glycogen conversion into lactic acid and storage time during postmortem (Mendonça et al., 2020). A lower breast meat pH at 24h can be due to the predominance of white muscle fibers, which exhibit a faster drop in pH after postmortem (Xing et al., 2020). Similarly, Rasul et al. (2019) reported no effect of dietary CS supplementation on breast meat DM, ash percentage, cooking loss, and water holding capacity were not affected by supplementation of CS in the diets. Carcass characteristics are frequently linked to the overall birds' performance, resulting in heavy body parts due to increased protein accretion caused by bioactive compounds found in CS. The trials that reported higher carcass characteristics also showed an increase in the overall performance of poultry birds fed CS diets. Although dietary CS had some effects on quail growth performance in this investigation, carcass characteristics remained unchanged across the groups. Meat pH is crucial factor regarding meat quality as it impacts further quality attributes like water retention, moisture loss, heat loss, storage stability, softness, juiciness, and color (Mir et al., 2017). At the time of the death, hypoxia causes glycogen to convert to lactic acid, decreasing the pH, and causing protein denaturation, protein mobility, and water-bonding reactive groups on muscle fibers. Under the impact of reduced pH, the ionic values (positive and negative) of the intermediates of muscle fibers appeal to each other rather than water binding, achieving an isoelectric point. This decreases the gap between protein molecules for water holding capacity, which is more reduced after sarcoplasmic cations lower the anions on nearby protein sequences, reducing electrostatic interactions between polypeptide chains (Mir et al., 2017). It implies that lowering the pH of meat reduces its capacity to retain water while increasing its loss during heat, or vice versa.

Gut morphology

Improved growth of Japanese quail,rather than reduced VH, can be attributed to the high digestibility of CS,which is enriched with bioactive ingredients (Mohammed et al., 2019). The improved growth performance, instead of lower villus height can be due to improved immunity of quailfed chia seed (Asad et al., 2019). The present study reported that shorter villi can save energy for tissue repair. This energy can be utilized for better growth and nutrients uptake leading

to improved growth performance (Ravindran, 2016). Similarly, Draper and Lowe (1958) stated that nutrient utilization is enhanced with reduced intestinal weight,which can be due to reduced villus height. Another possible reason could be the lack of examination of microvilli on the surface of villi with the help of an electron microscope in the present study, which could have helped to understand the surface area of villi better. Moreover, increased villus height requires faster renewal and, ultimately more energy, leaving fewer resources for the synthesis of other body cells. A larger tissue mass in the GIT to protect cells from the entrance of infection would need more protein or energy to maintain.

Contrarily, da Silva Marineli et al. (2014) stated that supplementation of CS in the amniotic sac of *gallus gallus* resulted in enhanced villus width, area, and length. This difference in the results can be attributed to variations in species and trial execution procedures. The present study uses CS in a quail experiment for 5 weeks but da Silva Marineli et al. (2014) experimented on *Gallus gallus* during the embryonic stage.

Blood parameters

The results obtained in the present study are consistent with thoseAlkenany et al. (2021) and El-Rayes et al. (2023), which stated that supplementation of water extract of chia resulted in reduced cholesterol. This reduction can be attributed to omega richness of chia, which has antioxidative and hypoglycemic activity that leads to enhanced glucose uptake and interfering with gluconeogenesis in the liver (Ayaz et al., 2017). Similarly, Rasul et al. (2019)reported reduced cholesterol in the blood serum of Japanese quails by supplementing CS. Fonte-Faria et al. (2019) conducted a trial by supplanting chia oil in obese mice, andfound reduced obesity, which indicated reduced protein, cholesterol, and lipid concentration in blood. The CS has medicinal qualities such as lowering blood cholesterol levels, which helps to reduce stress and strengthen the immune system (Fernández, 2006). Similarly, Poudyal et al. (2012) conducted a trial on rats and concluded that supplementation of CS resulted in reduced serum cholesterol and lipids content and de Abreu Silva et al. (2021) also described the same trend in systematic review of CS and its impact on lipid.

Fatty acid profile of breast meat

The dietary fatty acid profile significantly impacts the fatty acid profile of the broilers meat(Long et al., 2020). The CS contains a significant percentage of oil (25-40%) (Timilsena et al., 2016), indicating its importance in changing the fatty profile of birds fed a diet containing CS. The increase in USFA and linoleic acid is due to the enriched profile of CS (Ciftci et al., 2012; Muhammad et al., 2016). With supplementation of 3% CS, the omega-6 fatty acids increased due to the improved nutritional profile of CS (Rodríguez Lara et al., 2021). In our study, α-linolenic acid (C18:3n-3) was not significantly increased with the supplementation of CS;on the other hand, Mendonça et al.(2020) reported an improved α-linolenic acid on supplementing CS in diets. The difference in our result may be due to differences of species because Mendonça et al. (2020) conducted the trial on broilers, whereas in our study, Italian quail were used, which usually gain lower weight and smaller in size compared with Japanese quail. Similarly, the number of days for the experiment also differed from 1-35 in the present study vs. 29-42 days in the study by Mendonça et al. (2020). The significantly increased amount of docosahexaenoic acid in breast meat can be related to the conversion of α-linolenic acids into docosahexaenoic acid and eicosapentaenoic acid in the animal body which are biochemically more active molecules (Jing et al., 2013). Similarly, Ayerza and Coates (2002) and Azcona et al. (2008) also found that CS in broiler diets increase the concentration of DHA in broiler meat. Supplementation of 3% CS reduced SFA by 10% in the current study is good for human health because less consumption of SFA can lead to a reduction in the production of LDL, a hazard cause for cardiovascular disease (CVD) in humans (Liput et al., 2021). Similarly, MUFA concentration was reduced by 7% in the present study which is beneficial for humans because it reduces Ischemic heart disease risk in humans leading to CVD (Shramko et al., 2020). The total PUFA concentration was enhanced by 23% in breast meat fatty acid profile by supplementing 3% CS in present study which leads to positive influence on human health which can lead to reduced chances of diabetes type 2 and CVD (Lenighan et al., 2019).

The degree of lipid production and oxidation reactions determines variations in muscle lipids content (Crespo and Esteve-Garcia, 2001). The richness of distinct total PUFA and PUFAs in the quail breast muscle fed a diet containing CS appears to be due to the fatty acid composition of CS. Furthermore, the susceptibility of PUFAs to oxidative destruction in breast muscle are high (González-Ortiz et al., 2013) which may influence the preservation outcomes of

breast muscle. The CS contains bioactive chemicals with antioxidant properties, which may have slowed the degradation of PUFAs in quail breast muscle. Consequently, PUFA contents in breast tissue were increased in quails fed CS diets.

The atherogenic and thrombogenic indices were significantly reduced with increasing supplementation of CS. It can be attributed to the lower atherogenic and thrombogenic index of CS (Rodríguez Lara et al., 2021). Similarly, Mendonça et al. (2020) stated a reduced atherogenic index compared with the control diet with the supplementation of CS. A lower concentration of atherogenic and thrombogenic index indicates the healthfulness of food for humans due to less danger of CVD and atherosclerosis due to dietary lipid composition (Ulbricht and Southgate, 1991).

The SFA content was reduced with supplementation of CS in the quail diet in the present study, consistent with Cufadar et al. (2021). The level of SFA in avian tissues is proportional to its dietary content, degradation rate, and liver generation (Nir et al., 1988). The decline of fatty acid production in the liver is more remarkable when unsaturated fats are digested than when saturated fats are digested. Therefore, the more significantdecrease in SFA reported with CS diets compared to controls might be due to varying degrees of lipogenesis reduction caused by lesser PUFA absorption from sunflower oil compared to CS oil content. Therefore, the larger drop in SFA reported with CS diets compared with controls might be due to varying degrees of lipogenesis inhibition caused by lower PUFA uptake from sunflower oil relative to CS oil content. MUFA was 7% reduced in present study which is in consistence with Ayerza et al. (2002). The inhibitory impact of PUFA on $^{\Delta}_{9}$-desaturase ability, the primary enzyme utilized to desaturate and extend stearic to oleic and palmitic to palmitoleic fatty acid, might explain the reduction in MUFA concentration in quail. Ratnayake et al. (1989) found that the MUFA concentration of broiler meat depends more reliant on MUFA concentration of diet than on endogenous fatty acid production in the body. The increase in DHA by 55% is consistent with Azcona et al. (2008), which is due high ALA content of CS.

Several factors may be responsible for the discrepancies between the results of this study and those of previous research.These discrepancies were most likely caused by the fact that the samples utilized in this study were skinless, as opposed to those used by Ayerza et al. (2002). Another aspects to consider aregenetic factors related to the birds strain. Cherian et al. (1995) discovered substantial variations in total fat accumulation between bird lines for both kinds of light and dark meat, indicating that genetic could possibly play a role. Because specific types of broilers were utilized, this could potentially explain the discrepancy between the CS treatments in these two experiments.

In conclusion, the results of the present study revealed that 3% chia seed supplementation improved growth performance and feed conversion. Interestingly, chia seed supplementation significantly reduced ileal villus height and crypt depth while enhancing the blood total protein and cholesterol profile and decreasing the lipid profile of quails. Moreover, chia seed-fed groups had significantly greater values of fatty acids in breast meat of quails compared to the control groups, suggesting chia seeds use for the enhanced nutritional profile of breast meat for human consumption. This study provides the basis to investigate the potential of replacing traditional feedstuffs with chia seed as well as rigorous cost assessment. In addition, the benefits of chia seed may be hidden by other dietary factors, mainly feed ingredients, if utilized in low quantities. As a result, further studies on the seed and oil of chia seeds are proposed, as these show potential for beneficial outcomes in this field.

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AUTHORS CONTRIBUTION

This study was conceived and designed by Özlem Karadağoğlu. Özlem Karadağoğlu, and Gencehan Karadağoğlu executed the experiment and collected the samples. Ebru Karadağ Sarı and Metin Öğün carried out the histopathological evaluations. Bülent Özsoy and İdil Şerbetçistatistically analyzed the data. Ibrar Ahmed and Roshan Riaz drafted the original manuscript. Tarkan Şahin, Mükremin Ölmez and Özlem Karadagoglu, Ibrar Ahmed and Roshan Riazreviewed and revised the manuscript. All authors approved the manuscripts submitted version.

CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

DATA AVAILABILITY

Data will be made available on demand.

ETHICAL APPROVAL

This experiment was in line with the protocols and approved animal experiments' local ethics board of Kafkas University (Approval no: KAÜ-HADYEK 2017-095).

CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLISH

Not applicable.

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