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Changes in serum biochemical and lipid profile, and fatty acid composition of breast meat of broiler chickens fed supplemental grape seed extract

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Abstract: A study was conducted to evaluate the changes in serum biochemical and lipid profile, and fatty acid composition of breast meat of broiler chickens fed supplemental grape seed extract (GSE). A total of 240 1-day-old male broiler chickens were randomly allocated to 4 groups, each consisting of 4 replicate pens having 15 birds/replicate pen. One group served as control group fed basal diets and other groups received 100, 200, and 400 ppm GSE supplemented to the basal diets. Liver weight was greater in broilers fed 200 ppm GSE compared to the other groups ($P = 0.004$, $L = 0.024$, $C = 0.010$). Serum AST and ALT levels decreased ($P < 0.001$) whereas ALP levels increased with increasing dietary GSE levels ($P < 0.001$). Serum albumin and total protein levels were lower in control in comparison with other groups ($P < 0.001$). Serum cholesterol, triglyceride, and LDL levels declined ($P < 0.001$) while HDL levels increased with increasing supplemental GSE levels ($P < 0.001$). MUFA levels were greater in breast meat of broiler chickens fed diets supplemented with 100 ppm GSE. On the other hand, PUFA (including n-3 and n-6) were greater in broiler chickens fed control diets or 400 ppm GSE supplemented diets. In general, essential fatty acids such as α-linolenic acid (n-3 C18:3), arachidonic acid (n-6 C20:4), eicosapentaenoic acid (n-3 C20:5), and docosahexaenoic acid (n-3 C22:6) were greater in breast meat of broiler chickens fed supplemental GSE (100, 200, or 400 ppm) while overall UFA concentration in breast meat remained unaffected. In conclusion, findings of this study suggest that dietary supplementation of up to 400 ppm GSE in broilers may improve serum biochemical and lipid profile. It may also improve the fatty acid composition of breast meat to varying extent despite no effect on overall UFA concentration.

Key words: Broiler chickens, grape seed extract, serum biochemical profile, serum lipid profile, breast meat, fatty acid composition

1. Introduction

Supplementation of antioxidants in commercial diets historically stems from the prevention of lipid peroxidation and rancidity, and improvement in the oxidative stability of diets during feed manufacturing, processing, storage, and later stages thereafter. More recently, an augmented antioxidant supplementation in animal diets is in place due to current inclination towards formulation of diets rich in polyunsaturated fatty acids (PUFAs) because such diets are increasingly vulnerable to lipid peroxidation. Exogenous antioxidants act to prevent the denaturation of vital nutrients like vitamins and pigments thus preserving the sensory quality of diets [1]. Generally, antioxidants have been characterized as synthetic and natural depending on their sources. Although the use of synthetic antioxidants in animal diets continues so far, growing concerns are being expressed regarding the toxicity and carcinogenic activities of their pure and oxidized forms, reaction products in animal diets, and metabolites, and residues [2,3]. These concerns are alarmingly important not only from the view point of animal health but from the perspective of toxicity to humans consuming animal-origin foods that contain residues of synthetic antioxidants. In contrast, natural antioxidants largely exist in certain parts of plants, plant-based ingredients, phytochemicals, or phytogenic products. A majority of natural antioxidants are authorized a generally recognized as safe (GRAS) status in addition to more lenient safety assessment for other natural antioxidants in comparison with their synthetic accompaniments. Therefore, an upward trend is seen in consumer acceptances and preferences towards natural antioxidants that represents the prospective image of these antioxidants in near future [4].

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Polyphenols, once known as antinutritional factors in animal production, emerged as antioxidants due to their antioxidative activities [5]. Copious amounts of polyphenols are available in grape seed (by-products of wine and grape juice industry). These seeds may be separated during processing and flavonoid compounds including many polyphenols can be extracted and purified into the grape seed extract (GSE) [6]. Previous studies have reported the effect of dietary GSE or grape derived polyphenols on growth performance, amino acid digestibility, antioxidant status in breast and thigh muscles, plasma biochemical and lipid profile, and gut microbial populations of broilers [6–9]. Contrasting results have been reported for plasma lipid profile whereas no report is available entailing the possible effect of dietary GSE on fatty acid composition of breast meat of broilers. Keeping in view, we hypothesized that supplemental GSE may help to improve the breast meat fatty acid composition, and serum biochemical and lipid profile of broilers. Therefore, the present study was conducted to evaluate the changes in serum biochemical and lipid profile, and fatty acid composition of breast meat of broilers fed supplemental GSE.

2. Materials and methods

The present study was conducted at research and experimental farm located at Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey with prior approval from the animal care and use committee of the university via letter no. KAÜ-HADYEK 2016/047.

2.1. Preparation and characterization of GSE

Syrah red grapes (*Vitis vinifera*) were collected from vineyards located in Elmalı district, Antalya, Turkey. Grapes were separated from peduncles, skin and flesh were removed, and seeds were detached. Subsequently, seeds were washed and air dried at room temperature in the absence of sunlight. Seeds were coarsely grounded (crushed) and defatted by overnight soaking in hexane. Aqueous and methanolic extraction of seeds was carried out by soaking in distilled water and by Soxhlet extraction apparatus, respectively, according to the procedure previously described by Habibian et al. [10]. For the preparation of aqueous extract, fine powder was prepared by crushing and sieving through muslin cloth. Later, the finely powdered seeds were soaked in the distilled water for half an hour and boiled subsequently for half an hour. Afterwards, it was sieved through 2 layers of muslin cloth that was dried at 50 °C–60 °C. For methanolic extract, crushed seeds were finely grounded, extracted using methanol as solvent by refluxing on hot water bath in a Soxhlet apparatus (Model: SGB-306, Şimşek Laborteknik Sağlık ve Laboratuvar Cihazları Sanayi Tic. Ltd. Şti., Ankara, Turkey) for 7 h at 70 °C. Maximum quantity of methanol was retrieved while remaining quantities were removed under reducing pressure and evaporation at 50 °C. Mortar and pestle were used to grind the dried aqueous and methanolic extracts to make fine powder. Consequently, the powdered extracts were thoroughly mixed using hand blender to make a homogenous mixture of both these extracts termed as GSE that was used for supplementation to the broiler diets.

Total phenolic content (TPC) was measured in the GSE by spectrophotometric method. For this purpose, the phenols of GSE were extracted into methanol/acetone/water adopting a procedure described by Chamorro et al. [6]. TPC was determined using Folin-Ciocalteu reagent assuming gallic acid as a standard, following the procedure described by Singleton and Rossi [11]. Absorbance was measured in Shimadzu UV-1601 (Shimadzu Corp., Kyoto, Japan) spectrophotometer at a wavelength of 750 nm. The TPC was 38.64 g gallic acid equivalent/100 g dry matter.

2.2. Experimental design

The study was conducted in a completely randomized design. A total of 240 male, 1-day-old Ross 308 broiler chicks were assigned to 4 experimental groups, each consisting of 4 replicates (16 replicates in total) having 15 birds in each replicate. One experimental group served as control group fed diets without GSE. Other groups received diets supplemented with 100, 200, or 400 ppm GSE thereby making a total of 4 experimental groups.

2.3. Diets and management

In this study, 16 floor pens were established following thorough cleaning and preheating the experimental room. The temperature of the experimental room was 32 °C before the arrival of chicks that was maintained during the 1st week of experiment. Later, 0.5 °C was reduced per day until a constant temperature of 24 °C was attained. A total of 240 healthy 1-day-old Ross 308 male broiler chicks were purchased from a local commercial hatchery (Garanti Tavukçuluk, A.Ş. Erzincan, Turkey). The chicks were randomly allocated to 16 floor pens as described in the experimental design, each pen representing an experimental unit. Each pen measured 1 m^2 floor space exclusive of the space occupied by feeder and drinkers. Floor feeders were used during the starter period and tube feeders thereafter until the end of experiment. Corn-soybean based basal diets were formulated for starter (day 1 to 14), grower (day 15 to 28), and finisher (day 29 to 42) periods according to the recommendations of NRC [12] to meet or exceed the nutrient requirements of broiler chickens (Table 1). Broiler chickens in control group were fed basal diets whereas other groups were fed basal diets supplemented with 100, 200, and 400 ppm GSE. *Ad libitum* feed and water were available to birds throughout the experiment.

Table 1. Ingredient and nutrient composition of basal diets for starter (day 1 to 14), grower (day 15 to 28), and finisher (day 29 to 42) phases.

Ingredients (%)	1–14 days	15–28 days	29–42 days	Nutrient composition, %	1–14 days	15–28 days	29–42 days
Corn	56.30	58.10	61.5	Crude protein	22.81	21.32	20.04
Soybean meal (48% CP)	31	28	25.85	Metabolizable energy (kcal/kg)	2930	3047	3111
Full-fat soybean meal	8	8	7	Crude fibre	2.96	2.90	2.85
Sunflower seed oil	-	1.5	2	Crude ash	7.21	7.08	7.01
Limestone	1.50	1.5	1	Calcium	1.05	0.96	0.88
Dicalcium phosphate	2.10	2	2	Available phosphorus	0.52	0.48	0.46
Salt	0.30	0.30	0.30				
DL-methionine	0.30	0.20	0.10				
L-lysine sulphate	0.25	0.15	-				
Vitamin min premix[1]	0.25	0.25	0.25				

[1]Supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 1,500 IU; vitamin E, 30 mg; vitamin K_3, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; niacin, 40 mg; calcium D-pantothenate, 10 mg; folic acid, 0.75 mg; d-Biotin, 0.075 mg; choline chloride, 375 mg; manganese, 80 mg; iron, 40 mg; zinc, 60 mg; copper, 5 mg; iodine, 0.4 mg; cobalt, 0.1 mg; selenium, 0.15 mg; and antioxidant 10 mg.

2.4. Growth performance

Pen body weights and feed intake were recorded weekly. Feed conversion ratio was calculated by dividing the feed intake with body weight gain. Growth performance related traits were adjusted in case of any mortality. The performance parameters of the study were presented at the 2nd International Animal Nutrition Congress [13].

2.5. Carcass characteristics

At the end of experiment, 3 broiler chickens were selected randomly from each replicate (12 birds/group, 48 birds in total). Birds were weighed, slaughtered by decapitation, feathers and viscera were removed, and carcasses were weighed. Heart, liver, gizzard, and spleen were weighed. Carcass yield was calculated as a percentage of slaughter weight.

2.6. Blood collection and serum separation

Blood samples were collected from each broiler chicken randomly selected for slaughtering (3 samples/replicate, 12/group, 48 in total). For this purpose, the needle of BD venipuncture (needle 22-gauge, 1-inch length) was inserted into the caudal tibial vein and blood (15 mL from each selected bird) was collected in the vacutainers. The blood samples were centrifuged immediately after clotting and sera were separated. These sera were used to analyse the biochemical and lipid profile.

2.7. Serum biochemical profile

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (Alb), and total protein (TP) levels were analysed in the sera by spectrophotometric method using commercial kits (Roche Diagnostics, İstanbul, Turkey). Measurements were taken using a spectrophotometric microplate reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., Winooski, VT, USA).

2.8. Serum lipid profile

Spectrophotometric method was employed to analyse the low-density lipoproteins (LDL), high-density lipoproteins (HDL), cholesterol (CHOL), and triglycerides (TG) in the sera. Commercial kits (Roche Diagnostics, İstanbul, Turkey) were used followed by measurements in a spectrophotometric microplate reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., Winooski, VT, USA).

2.9. Fatty acid composition of breast meat

Approximately 100 g breast meat samples were collected from each slaughtered broiler chicken (3 samples/replicate, 12/group, 48 in total) at the end of experiment. Fatty acid composition of breast meat was determined according to the procedure previously described by Sari et al. [14] with minor modification. Briefly, fat was extracted from each sample in Soxhlet apparatus using diethyl ether as solvent. Fatty acid methyl esters (FAMEs) were prepared using methylated boron trifluoride (35% methyl alcohol) after saponification with sodium hydroxide. FAMEs were separated by adding n-hexane, the mixture was vortexed and condensed under nitrogen gas, and finally filtered the condensate for analysis in GC-MS (Hewlett Packard 6890/5972 GCMS system, Santa Clara, CA, USA). Capillary column 100 m × 0.25 mm × 0.2 μm (HP88 capillary column, Agilent J&W, Santa Clara, CA, USA) was

used at initial temperature 120 °C and final temperature 230 °C, injection volume 1 μL, split ratio 1:50, and helium was used as carrier gas.

2.10. Statistical Analysis

Data were analysed using one-way ANOVA in a statistical software package SPSS version 22.0 (IBM Corp., Armonk, NY, USA) to assess the effect of dietary GSE on carcass characteristics, serum biochemical and lipid profile, and fatty acid composition of breast meat of broiler chickens. Each replicate served as an experimental unit. Percentage data were transformed to arcsine values although presented as percentage in results. Growth performance data were excluded due to low sample size. Shapiro–Wilk test was used to identify the nonnormalized traits and transformed using logarithmic or square root transformation method. Confidence interval was assumed at 95%. Duncan's multiple range test was used as post-hoc test to separate the significantly different means. Linear (L), quadratic (Q), and cubic (C) contrasts were applied to evaluate the response to increasing supplemental levels of GSE. Results were presented as mean ± SEM (pooled).

3. Results

Slaughter weight, hot carcass weight, carcass yield, and gizzard weight were not different among the treatments (Table 2). Birds fed 200 ppm GSE had greater liver weight in comparison with other groups (P = 0.004, L = 0.024, C = 0.010). Spleen weight increased linearly in broiler chickens fed increasing supplemental levels of GSE (L = 0.012).

Serum AST was different among the treatments (P < 0.001) that decreased linearly with increasing levels of dietary GSE supplementation (L < 0.001). Birds fed 200 or 400 ppm GSE had lower serum ALT followed by those fed 100 ppm GSE while highest in those reared on control diets (L < 0.001). Feeding broiler chickens 400 ppm GSE increased serum ALP followed by 200 and 100 ppm GSE that were even greater than those fed diets without supplemental GSE (L < 0.001). Broiler chickens fed supplemental GSE (100, 200, or 400 ppm) had higher serum Alb in comparison with those fed control diets (L < 0.001). Serum TP was lower in control group compared with other dietary treatments (L < 0.001) (Table 3).

Broiler chickens receiving dietary GSE (200 or 400 ppm) had lowest serum LDL (L < 0.001) and CHOL (L < 0.001) followed by those fed 100 ppm GSE that was lower than those fed diets without supplementation whereas vice versa was true in case of serum HDL (L < 0.001). Serum TG levels were greater in control and 100 ppm GSE groups compared with 200 ppm GSE group which, in turn, were higher than 400 ppm GSE group (L < 0.001) (Table 4).

An increase in myristic acid (C14:0) was noted in breast meat of broiler chickens fed 200 ppm GSE compared to those fed control or 400 ppm GSE diets (P = 0.030 and Q = 0.008). Birds fed 100 or 200 ppm GSE had greater myristoleic acid (C14:1) in breast meat (P < 0.001). Supplemental GSE (200 ppm) increased palmitic acid (C16:0) in breast meat of broiler chickens in comparison with control of 400 ppm GSE groups (*P* = 0.016 and *Q* = 0.002). Palmitoleic acid (C16:1) concentration was greater in breast meat of broiler chickens fed 100 or 200 ppm supplemental GSE (P < 0.001). Groups receiving control or 400 ppm GSE had greater breast meat linoleic acid (n-6 C18:2; LA) concentration (P <0.001). α-linolenic acid (n-3 C18:3; ALA) in breast meat of broilers was greater in control group compared to those in 100 or 200 ppm GSE groups while broiler chickens fed 400 ppm GSE had greater ALA in comparison with those receiving 100 ppm GSE (P < 0.001). Eicosadienoic acid (n-6 C20:2) in breast meat was greater in broilers fed 200 or 400 ppm supplemental GSE in comparison with those fed 100 ppm GSE (P = 0.022 and C = 0.049). Supplementation of

Table 2. Carcass characteristics of broiler chickens fed supplemental GSE.

Items	Groups[1]				SEM	P < F	Contrasts[2]		
	Control	GSE 100	GSE 200	GSE 400			L	Q	C
Slaughter weight (g)	2561	2553	2612	2522	16.29	0.147	0.752	0.140	0.077
Hot carcass (g)	1935	1914	1952	1889	13.39	0.382	0.407	0.435	0.185
Carcass yield (%)	75.53	74.99	74.24	74.91	0.21	0.238	0.170	0.177	0.459
Liver (%)	1.80[b]	1.82[b]	2.10[a]	1.97[b]	1.04	0.004	0.024	0.115	0.010
Gizzard (%)	1.68	1.68	1.76	1.71	0.74	0.606	0.503	0.306	0.143
Spleen (%)	0.10	0.12	0.13	0.14	0.15	0.082	0.012	0.527	0.840

[1]GSE 100 = Grape seed extract 100 ppm; GSE 200 = Grape seed extract 200 ppm; GSE 400 = Grape seed extract 400 ppm

[2]L = Linear, Q = Quadratic, C = Cubic

[a, b]Means bearing different superscripts within the same row are significantly different (P < 0.05)

Table 3. Serum biochemical profile of broiler chickens fed supplemental GSE.

Items[1]	Groups[2]				SEM	P < F	Contrasts[3]		
	Control	GSE 100	GSE 200	GSE 400			L	Q	C
AST (U/L)	93.34[a]	84.57[b]	74.53[c]	64.43[d]	1.95	<0.001	<0.001	0.723	0.885
ALT (U/L)	58.67[a]	51.80[b]	41.15[c]	39.49[c]	1.43	<0.001	<0.001	0.076	0.053
ALP (U/L)	2350.33[c]	2598.41[b]	2631.90[b]	2749.53[a]	27.68	<0.001	<0.001	0.044	0.039
Alb (g/L)	8.94[b]	12.68[a]	12.56[a]	12.49[a]	0.28	<0.001	<0.001	<0.001	0.002
TP (mg/dL)	3.34[b]	3.60[a]	3.66[a]	3.62[a]	0.02	<0.001	<0.001	<0.001	0.491

[1]AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, Alb = Albumin, TP = Total protein
[2]GSE 100 = Grape seed extract 100 ppm, GSE 200 = Grape seed extract 200 ppm, GSE 400 = Grape seed extract 400 ppm
[3]L = Linear, Q = Quadratic, C = Cubic
[a, b, c, d] Means bearing different superscripts within the same row are significantly different (P < 0.05)

Table 4. Serum lipid profile of broiler chickens fed supplemental GSE.

Items[1]	Groups[2]				SEM	P < F	Contrasts[3]		
	Control	GSE 100	GSE 200	GSE 400			L	Q	C
CHOL (mg/dL)	137.17[a]	129.65[b]	121.90[c]	119.68[c]	1.32	<0.001	<0.001	0.088	0.400
TG (mg/dL)	50.43[a]	48.34[a]	43.51[b]	37.42[c]	1.13	<0.001	<0.001	0.234	0.843
LDL (mg/dL)	42.10[a]	37.28[b]	31.28[c]	30.82[c]	0.79	<0.001	<0.001	<0.001	0.007
HDL (mg/dL)	81.58[c]	86.37[b]	96.94[a]	99.49[a]	1.26	<0.001	<0.001	0.241	0.002

[1]LDL = Low-density lipoproteins, HDL = High-density lipoproteins, CHOL = Cholesterol, TG = Triglycerides
[2]GSE 100 = Grape seed extract 100 ppm, GSE 200 = Grape seed extract 200 ppm, GSE 400 = Grape seed extract 400 ppm
[3]L = Linear, Q = Quadratic; C = Cubic
[a, b, c] Means bearing different superscripts within the same row are significantly different ($P < 0.05$)

400 ppm GSE in broiler diets increased the breast meat arachidonic acid (n-6 C20:4; AA) compared with control group and 100 ppm GSE group (P = 0.021 and L = 0.003). Supplemental 400 ppm GSE increased eicosapentaenoic acid (n-3 C20:5; EPA) in breast meat of broiler chickens compared with those fed 100 ppm GSE (P = 0.015 and Q = 0.008). Broiler chickens fed 100 or 200 ppm supplemental GSE exhibited greater docosahexaenoic acid (n-3 C22:6; DHA) concentration in breast meat (P < 0.001). Dietary supplementation of 100 ppm GSE increased total monounsaturated fatty acids (∑MUFA) in breast of broiler chickens compared with control and 400 ppm GSE groups (P = 0.028 and Q = 0.011). Total polyunsaturated fatty acids (∑PUFA), PUFA: saturated fatty acids ratio (PUFA/SFA), and total n-6 PUFA were higher in breast meat of broilers fed control diets or diets supplemented with 400 ppm GSE (P < 0.001). In breast meat of broiler chickens fed 100 ppm GSE, n-3 PUFA were lower compared to those in control or 400 ppm GSE groups (P = 0.044 and Q = 0.035) (Table 5).

4. Discussion

Our results are partially in agreement with the previous studies that reported no or limited effects on carcass and organ yields in response to various grape processing industry by-products [7,15,16]. In contrast, Brenes et al. [17] reported an increase in relative liver weight at day 21 in broiler chickens fed GSE, however, liver yield remained unaffected while other organ yields increased in broiler chickens at day 42. The discrepancies in findings of different studies might be attributed to the difference in composition of diets, polyphenolic content of GSE, and strains of the broiler chickens used. Carcass characteristics are largely dependent on growth performance of poultry including broiler chickens that yield heavier body parts and organs attributed to relief from the stressful conditions under the influence of polyphenols in GSE. Usually, the studies that report greater carcass characteristics in response to supplementation of feed additives in poultry diets also report an improvement in growth performance. Growth performance indicators remained unaffected

Table 5. Fatty acid composition of breast meat of broiler chickens fed supplemental GSE.

Fatty acids %	Groups[1]				SEM	P < F	Contrasts[2]		
	Control	GSE 100	GSE 200	GSE 400			L	Q	C
C12:0	0.04	0.04	0.04	0.04	0.01	0.874	0.650	0.522	0.797
C14:0	0.76^{b}	0.83^{ab}	0.90^{a}	0.78^{b}	0.02	0.030	0.461	0.008	0.199
C14:1	0.18^{b}	0.24^{a}	0.28^{a}	0.17^{b}	0.01	<0.001	0.697	<0.001	0.135
C16:0	24.72^{b}	27.03^{ab}	27.77^{a}	24.89^{b}	0.43	0.016	0.717	0.002	0.556
C16:1	5.56^{b}	7.85^{a}	8.35^{a}	5.68^{b}	0.27	<0.001	0.628	<0.001	0.437
C18:0	7.78	7.62	8.36	9.11	0.33	0.387	0.118	0.500	0.763
C18:1	31.45	33.63	30.24	31.47	0.63	0.297	0.554	0.707	0.076
C18:2 n-6	21.00^{a}	16.78^{b}	17.35^{b}	20.79^{a}	0.44	<0.001	0.987	<0.001	0.503
C18:3 n-6	0.29	0.31	0.34	0.30	0.01	0.234	0.536	0.131	0.202
C18:3 n-3	1.75^{a}	1.33^{c}	1.42^{bc}	1.60^{ab}	0.04	0.001	0.237	<0.001	0.197
C20:0	2.44	0.22	0.26	0.23	0.55	0.398	0.187	0.325	0.633
C20:1	0.55	0.64	0.67	0.57	0.02	0.226	0.662	0.047	0.713
C20:2 n-6	0.41^{ab}	0.34^{b}	0.43^{a}	0.46^{a}	0.01	0.022	0.059	0.094	0.049
C20:3 n-6	0.48	0.52	0.62	0.58	0.02	0.164	0.065	0.345	0.359
C20:4 n-6	1.12^{b}	1.18^{b}	1.37^{ab}	1.62^{a}	0.07	0.021	0.003	0.502	0.927
C20:5 n-3	0.47^{ab}	0.31^{b}	0.42^{ab}	0.58^{a}	0.03	0.015	0.076	0.008	0.371
C22:6 n-3	0.24^{b}	0.37^{a}	0.42^{a}	0.27^{b}	0.02	<0.001	0.343	<0.001	0.396
∑SFA	36.17	36.07	37.67	35.42	0.51	0.465	0.889	0.300	0.231
∑MUFA	38.10^{b}	42.79^{a}	39.94^{ab}	38.39^{b}	0.63	0.028	0.709	0.011	0.098
∑PUFA	25.74^{a}	21.15^{b}	22.39^{b}	26.19^{a}	0.50	<0.001	0.447	<0.001	0.341
∑UFA	63.83	63.93	62.33	64.58	0.51	0.465	0.889	0.300	0.231
PUFA/SFA	0.73^{a}	0.59^{b}	0.59^{b}	0.74^{a}	0.02	<0.001	0.729	<0.001	0.963
UFA/SFA	1.81	1.78	1.67	1.83	0.03	0.374	0.831	0.182	0.259
n-6	23.28^{a}	19.13^{b}	20.13^{b}	23.75^{a}	0.46	<0.001	0.446	<0.001	0.420
n-3	2.93^{a}	2.54^{b}	2.88^{ab}	3.01^{a}	0.06	0.044	0.182	0.035	0.095
n-6/n-3	8.01	7.63	7.05	7.89	0.14	0.065	0.432	0.026	0.176

[1]GSE 100 = Grape seed extract 100 ppm; GSE 200 = Grape seed extract 200 ppm; GSE 400 = Grape seed extract 400 ppm
[2]L = Linear, Q = Quadratic, C = Cubic
[a, b, c] Means bearing different superscripts within the same row are significantly different (P < 0.05)

through the experimental duration in the present study (data not shown) that might be the reason behind no improvement in carcass characteristics [13].

Limited literature is available describing the effect of dietary GSE on serum biochemistry in broiler chickens. Liver-related enzymes, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and gamma enzymes, such as glutamyl transferase (GGT), are leaking into the circulation from hepatocellular cells and are the determining enzymes of hepatocellular damage. Aminotransferases are sensitive indicators of liver cell damage and acute hepatocellular diseases. In the majority of acute hepatocellular diseases, ALT is greater than or equal to AST [18]. The AST, ALT, ALP, Alb, and TP are enzymes and proteins secreted or made by liver expressed in blood. An increase in serum AST, ALT, Alb, and TP levels whereas a decline in serum ALP levels occurs with increasing age of broilers [19]. In the present study, serum biochemical traits among the treatments remained within the reference values reported by Meluzzi et al. [19] for broiler chickens. An upward trend in serum AST, ALT, and ALP but a downward trend in serum Alb and TP take place under certain disease conditions of liver. However, supplemental GSE lowered the serum AST and ALT levels

while augmented the serum ALP levels in broiler chickens in comparison with those in control group. This indicates the hepatoprotective effect of supplemental GSE rich in polyphenols manifested by opposing the changes in enzyme levels secreted by liver unlike the control group. Our findings disagree with those of Abu-Hafsa and Ibrahim [7] who reported that dietary grape seed had no effect on plasma AST, ALT, Alb, and TP of broilers. Serum TP comprises of numerous proteins involved in maintaining the blood volume, pH (buffering), transporting hormones and drugs, and blood clotting. Importantly, certain blood proteins including Alb and immunoglobulins are vital for inflammatory and immune responses [20]. In the present study, supplemental GSE increased serum Alb and TP level in broiler chickens compared with control group. This might be indicative of immunomodulatory effect of GSE. Future research with immune related proteins might present the true picture of immunomodulatory effect of GSE in broiler chickens.

Endogenous triglycerides synthesized from feed carbohydrates in poultry are released into the blood as very low density lipoproteins (LDL). In order for triglycerides in the blood to be used by body tissues, it must first enter the cell. Triglycerides synthesized in the liver can only leave the liver as LDL. In this study, dietary GSE decreased the serum CHOL, TG, and LDL levels whereas an increase in serum HDL level was noted in broiler chickens. These findings are in line with those of Abu-Hafsa and Ibrahim [7] who reported that supplemental grape seed reduced the plasma total lipids, TG, and CHOL in broilers. Similar results were reported by Farahat et al. [21] in terms of CHOL and LDL levels whereas HDL levels remained unaffected in broiler chickens fed dietary GSE. In addition, reduction in CHOL and LDL in hypercholestrolemic animal models and humans following consumption of polyphenols has been reported in several studies [22–24]. In contrast, Chamorro et al. [6] reported that plasma CHOL, TG, LDL, and HDL remained unaffected in 21-day-old broiler chickens fed increasing levels of dietary GSE. The differences in result might have arisen from the fact that 21-day-old broiler chickens have low levels of circulating CHOL than older birds [25]. Polyphenols are capable of binding CHOL thereby increase the excretion CHOL through excreta [26]. Moreover, polyphenols increase the expression of cholesterol 7-alpha hydroxylase enzyme that regulates the bile synthesis and homeostasis of cholesterol [27]. In addition, polyphenols inhibit the activity of hydroxyl-3-methyl-glutaryl-CoA, a rate-limiting enzyme in CHOL synthesis [28]. Consequently, blood CHOL and oxidation of LDL are lowered and HDL levels surge. In the present study, lowering of serum CHOL, LDL, and TG, and increase in HDL levels indicate the antiatherosclerotic effect of supplemental GSE.

The most important fatty acid for human health is n-3 polyunsaturated fatty acids (PUFA). An insufficient intake of n-3 PUFA, particularly docosahexaenoic acid (DHA = 22: 6n-3), adversely affects brain growth and functional parameters in children. Although research has focused on producing more nutritious meat products, scientists have been cautious about the effects of manipulation of lipid composition on meat quality [29]. No literature is available reporting the changes in fatty acid composition of breast meat of broiler chickens fed supplemental GSE. In this study, MUFA levels were greater in breast meat of broiler chickens fed diets supplemented with 100 ppm GSE. On the other hand, PUFA (including n-3 and n-6) were greater in broiler chickens fed control diets or 400 ppm GSE supplemented diets. In general, essential fatty acids such as ALA, AA, EPA, and DHA were greater in breast meat of broiler chickens fed supplemental GSE (100, 200, or 400 ppm) despite no difference in UFA levels among the treatments. This shows that dietary GSE improved the antioxidant activity and oxidative stability in addition to reduction in lipid peroxidation of fatty acids. Brenes et al. [17] demonstrated that absorption of polyphenols occurs in the intestine that is expressed in the body tissues of broiler chickens when fed GSE. In addition, it was reported that antioxidant activity (in terms of TPC) and lipid stability (in terms of thiobarbituric acid reactive substances (TBARS)) increased in breast and thigh muscles of broiler chickens in response to dietary grape polyphenols [9]. Similarly, dietary supplementation of grape polyphenols increased the free radical scavenging activity (in terms of inhibition of 1,1- diphenyl-2-picrylhydrazyl (DPPH)) in breast meat of broiler chickens [9]. Moreover, dietary grape pomace concentrate reduced the lipid peroxidation (in terms of malondialdehyde levels) in breast meat of broilers [15,21]. These might be the possible mechanisms by which the dietary GSE managed to improve the essential fatty acid composition of breast meat in broiler chickens in the present study.

In conclusion, this study showed that supplemental GSE might not improve the carcass and organ yields. However, dietary GSE may improve the serum biochemical and lipid profile that adds to the better health status of broiler chickens. In addition, supplementation of GSE in broiler chickens improved the fatty acid composition of breast meat to varying extent despite no effect on overall UFA concentration in the breast meat. PUFAs act out of their nutritional value as functional foods to prevent noninfectious diseases such as obesity, cardiovascular and degenerative disorders. This requires further elucidation in terms of a comparative evaluation of fatty acid composition of diets and that of the breast meat that may present the true picture.

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

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