

Effects of Dietary Soapwort Extract Supplementation on Laying Performance, Blood Biochemical Parameters, Fatty Acid Profile of Breast Meat and Antioxidative Potential of Liver and Heart Tissues in Cold Stressed Laying Japanese Quail

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Abstract

The purpose of this study was to evaluate the effects of dietary Soapwort Extract (SE) supplementation on laying performance, egg quality, some offal weights, some serum parameters, antioxidant status of liver and heart tissues and fatty acid composition of breast meat in chronic cold stressed Japanese quails. A total of sixty three 45-day-old Japanese quails were divided into three groups with three replicate. One group was fed with the corn-soybean based diet alone (control group) and the others were fed with the basal diet supplemented by 50 and 100 ppm SE. Cold stress was applied every night between 22.00 to 06.00 h as $7\pm 1^{\circ}\text{C}$. SE supplementation had no significant effect on laying performance, offal weights and egg quality parameters with the exception of egg and albumen weights. Serum glucose, triglyceride, uric acid and aspartate aminotransferase (AST) levels were reduced in SE-50 group. While supplementation of 100 ppm SE decreased both liver and heart MDA levels, both amounts of SE increased the liver GSH-Px enzyme activity. Supplementation of 100 ppm SE decreased the levels of oleic acid and MUFA in breast meat, and it significantly increased the linoleic, linolenic and arachidonic acid, PUFA, total omega 6 and the ratio of omega 6/omega 3. It is displayed that SE supplementation to diets of laying quails exposed to cold stress can alleviate the detrimental effects of oxidative stress with no affecting on performance parameters.

Keywords: Antioxidant, Cold stress, Fatty acid, Laying performance, Saponin, Soapwort extract

Soğuk Stresine Maruz Bırakılan Japon Bildircinlerinde Diyete İlave Edilen Çöven Ekstraktının Yumurtlama Performansı, Kan Biyokimyasal Parametreleri, Göğüs Eti Yağ Asidi Profili İle Karaciğer ve Kalp Dokusu Antioksidatif Potansiyeli Üzerine Etkisi

Özet

Bu çalışmada, karma yeme ilave edilen çöven ekstraktının (SE), kronik soğuk stresine maruz bırakılan Japon bildircinlerinde performans, yumurta kalitesi, bazı organ ağırlıkları, bazı serum parametreleri, karaciğer ve kalp dokularının antioksidan durumları ve göğüs eti yağ asidi profili üzerine etkileri araştırılmıştır. Toplam olarak 63 adet 45 günlük bildircin, 3 tekerrürlü 3 gruba ayrılmıştır. Gruplardan biri mısır-soya küspesine dayalı temel yem ile (kontrol grubu), diğer gruplar temel yeme 50 ve 100 ppm SE ilave edilen yemle beslenmişlerdir. Soğuk stresi her akşam 22.00-06.00 saatleri arasında kümes ısı $7\pm 1^{\circ}\text{C}$ 'ye düşürülerek uygulanmıştır. SE ilavesi performans, bazı organ ağırlıkları ile yumurta ve albümin ağırlığı hariç yumurta kalite parametrelerini etkilememiştir. Serum glikoz, trigliserit, ürik asit ve AST düzeyleri SE-50 grubunda düşmüştür. 100 ppm SE ilavesi karaciğer ve kalp dokularında MDA düzeylerini düşürürken, karaciğer GSH-Px enzim aktivitesi her iki SE grubunda artmıştır. 100 ppm SE ilavesi göğüs etinde oleik asit ve MUFA düzeylerini düşürürken, linoleik, linolenik ve arachidonik asit, PUFA, toplam omega 6 ile omega 6/omega 3 düzeylerini önemli ölçüde yükseltmiştir. Sonuç olarak; soğuğa maruz kalan yumurtacı bildircinlerde diyete ilave edilen SE'nin performans parametrelerini etkilemeden oksidatif stresin zararlı etkilerini azaltabileceği ortaya konulmuştur.

Anahtar sözcükler: Antioksidan, Soğuk stres, Yağ asidi, Yumurtlama performansı, Saponin, Çöven ekstraktı



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INTRODUCTION

During winter, the ambient temperature ranges from -5 to $+5^{\circ}\text{C}$ in many regions of the world, including Turkey. Such cold conditions negatively influence the performance of laying quails, increasing feed intake while decreasing egg production, egg weight, eggshell thickness and haugh unit ^[1]. Additionally, stressful conditions can provoke the creation of lipid peroxidation products especially in membranes and end up tissue damage ^[2].

Several plants have a worth value role in physiological and biochemical activities and reactions with the phytochemical contents of them. Therefore nowadays, contents of chemical matters of plants and their protective effects on disease are investigated ^[3]. Saponins which are found in many plants are glycosides containing a steroid or triterpenoid nucleus with one or more side chain of carbohydrates. Saponins have hemolytic effect when given intravenously. They have bitter taste and are distributed through the bark, leaves, stems, roots and flowers of the plants ^[4]. Recent years, the saponins containing plants and their effects on human and animal health are one of the most studied area. Several pharmacological properties are attributed to saponins such as immunological adjuvant ^[5], anticarcinogenic ^[6], hypocholesterolaemic ^[7], antifungal ^[8], anti-inflammatory and antioxidant ^[9,10]. Additionally, they are supplemented to poultry diets for decreasing the excrete ammonium ^[11].

Saponin-rich and economically important taxa of *Gypsophyllia sp. is a* perennial, herbaceous plant from Caryophyllaceae family which named as "Çöven" by public and are used to make "Tahini Halvah", "Foam Halvah", "Turkish Delight", "Herbal Cheese" and "Bread of Çöven", also to product detergent as fabrication, fire extinguisher, liquor, soap and to obtain commercial saponin ^[12,13]. These plants notified as diuretic, expectorant, acne remover are used to polish gold at jewellery sector and exported to abroad ^[13]. Saponins are organic compounds which are water soluble carbohydrates ^[14].

It is well known that use of plant materials are due to the antioxidant, antimicrobial, carminative substances content that activate the organism from the detrimental effects of stressful conditions. The effects of SE on laying performance, egg quality, carcass parameters, fatty acid profile of breast meat, some biochemical parameters and antioxidant status of liver and heart tissues of laying quails reared at low ambient temperature was studied.

MATERIAL and METHODS

Sixty three, 45-day-old and 199 g mean body weight Japanese quail (*Coturnix Coturnix Japonica*) were obtained from a commercial seller in Elazig province of Turkey. The experiment was conducted at the Poultry Unit of Veterinary

Faculty of Firat University, after the local ethic committee approval (Official form date and number: 25.02.2015 and 2015/08). The quails were divided into three groups of 21 female each with three replicate. Animals were adapted to experimental conditions until they were the hen-day 5%. Cold stress was applied every night between 22.00 to 06.00 h as $7\pm 1^{\circ}\text{C}$. During the day, $20\pm 2^{\circ}\text{C}$ thermo-neutral ambient temperature was provided. The relative humidity of the environment was 60-65%. One group was fed with the basal diet alone (control group) and the others were fed with the basal diet supplemented by 50 and 100 ppm Soapwort Extract (SE-50 and SE-100 groups respectively). Chemical composition of feed ingredients and Soapwort extract were analyzed according to the Association of Official Analytical Chemists ^[15] procedures and crude fiber was determined by the methods of Crampton and Maynard ^[16]. The carbohydrate level in the Soapwort extract was determined by the method of Lane and Eynon ^[17]. The metabolisable energy (ME, kcal/kg) was calculated according to Carpenter and Clegg ^[18] = $53 + 38 \text{ B}$ formula [B= (crude protein %) + (2.25) (ether extract %) + (1.1) (starch %) + (sugar %)]. The amount of saponin within Soapwort extract was determined using the method described by Lalitha et al. ^[19]. The chemical composition of the SE used in the experiment is shown in Table 1. The quails were fed with isonitrogenic and isocaloric diets according to the National Research Council ^[20] recommendations are given at Table 2. Diets and fresh water was provided for *ad libitum*. A photoperiod of 16 h/day was maintained. All birds were kept under standard laying cages with 7 birds per cage under the same environmental conditions. The experiment was continued 42 days.

Feed intake, feed conversion ratios (FCR) of the groups were weekly determined. Egg production was daily determined and all eggs were weighted individually. Additionally, for determining the egg quality parameters, eggs were collected once a week after 2nd week of the experiment. The next day, the collected eggs were evaluated for the weights of egg, shell, albumen and egg yolk as well as shell thickness, yolk color and shape index. Egg shells were washed under tap water gently and dried in the air after 24 h and then evaluated. Approximately, 360 eggs were evaluated at each dietary treatment for egg quality parameters.

At the end of the experiment, 6 quails from each group, were randomly selected and slaughtered with decapitation in order to collect blood samples. Serum was separated and stored at -20°C until analysis. Following slaughtering, offal weights were evaluated in accordance with Institute of Turkish Standards Rules ^[21]. Liver, heart and spleen percentages were calculated from whole body (slaughter weight). *M. pectoralis profundus* of breast were obtained and stored (-20°C) for fatty acid composition analyses. Blood samples were centrifuged at 4.000 rpm for 10 min, the serums were separated. Serum glucose,

Table 1. The chemical composition of the soapwort extract, %**Tablo 1.** Çöven ekstraktının kimyasal bileşeni, %

Analysis	Result, %
Dry matter	90.73
Crude protein	2.23
Ether extract	1.22
Crude cellulose	0.83
Ash	6.10
Carbohydrates	36.35
Saponin	44.00

Table 2. Ingredients and chemical composition of standard diet**Tablo 2.** Bazal diyetin kompozisyonu ve bileşimi

Feed Ingredients (g/kg)		Chemical Composition (g/kg)	
Maize	528.0	Dry matter	907.0
Soybean meal (48% CP)	190.0	Crude protein	200.0
Sunflower meal (36% CP)	115.0	Ether extract	63.0
Fullfat soybean	60.0	Crude fiber	52.5
Vegetable oil	34.0	Crude ash	92.2
Dicalcium phosphate	15.10	Sugar	49.0
Calcium carbonate	53.3	Starch	335.0
Salt	2.4	Calcium**	25.0
Sodium bicarbonate	1.0	Available Phosphorus**	8.4
DL-Methionine	0.1	Sodium**	1.8
L-Lysine	0.5	Methionine+Cystine**	7.0
L-Threonine	0.3	Lysine**	10.0
Vitamin-Mineral Premix*	0.3	Threonine**	7.7
		Tryptophan**	2.5
		ME, kcal/kg***	2938
Total	1000.0		

* Provided per kg of diet: retinol, 2.64 mg; cholecalciferol, 0.04 mg; dl-*α*-tocopherol-acetate, 11 mg; riboflavin, 9.0 mg; pantothenic acid, 11.0 mg; vitamin B₁₂, 0.013 mg; niacin, 26 mg; choline, 900 mg; vitamin K, 1.5 mg; folic acid, 1.5 mg; biotin, 0.25 mg; iron, 30 mg; zinc, 40 mg; manganese, 60 mg; copper, 8 mg; selenium, 0.2 mg; ** Calculated; *** Calculated, ME (kcal/kg) = 53+38 B used formula. B= (Crude protein, %) + (2.25) (Ether extract, %) + (1.1) (Starch, %) + (Sugar, %)

triglyceride, total, LDL and HDL cholesterol, uric acid and AST concentrations were measured using a biochemical analyzer (Olimpus AU-600) at University of Firat, Faculty of Medicine, Department of Biochemistry. Malondialdehyde (MDA) level of liver and heart was spectrophotometrically measured with the procedures described by Placer et al.^[22]. SOD activity of liver and heart was measured using xanthine and xanthine oxidases to generate superoxide radicals which react with nitroblue tetrazolium (NBT) by the methods of Sun et al.^[23]. The GSH-Px activity was determined according to Lawrence and Burk^[24]. The GSH content of the liver and heart was measured at 412 nm by the method of Sedlak and Lindsay^[25].

Extraction of lipids from the breast meat was per-

formed according to the method of Hara and Radin^[26]. For the preparation of methyl esters, lipid extract in a hexane: isopropanol phase was placed in 30-mL experiment tubes. Five milliliters of 2% methanolic sulfuric acid was added and the mixture was vortexed. This mixture was left to methylate in a 50°C incubation for 15 h. It was then cooled at room temperature, and 5 mL of 5% sodium chloride was added and mixed. The fatty acid methyl esters that were produced were extracted with 5 mL of hexane. The hexane phase was then removed with a pipette and treated with 5 mL of 2% KHCO₃. The solvent in the methyl ester-containing mixture was evaporated at 45°C with a nitrogen flow and dissolved with 1 mL of hexane. All the mixture containing the solvent and hexane was then placed in 2- mL closed autosampler vials and analyzed^[27].

All data were analyzed by analysis of variance procedures and significant differences were further subjected to Tukey HSD multiple range tests by using SPSS 11.5 for Windows^[28]. The results were considered as significant when P<0.05, P<0.01 and P<0.001.

RESULTS

Supplementation of 50 ppm SE significantly decreased (P<0.05) egg weight in fourth week and 100 ppm SE significantly decreased the feed intake in third week when compared with control group (Table 3). Addition of 100 ppm SE caused significant reductions in egg weight (P<0.01) and albumen weight (P<0.001) when compared with control group (Table 4). Soapwort supplementation had no effect on the offal weights and percentages (Table 5).

Serum total cholesterol, HDL and LDL cholesterol levels were similar among groups (Table 6). However, dietary supplementation of 50 ppm SE reduced the serum glucose, triglyceride, uric acid and AST levels (P<0.05) in comparison with the control group. In addition, 100 ppm SE significantly reduced the only AST level when compared with the control group.

The essential effect of SE was seen on antioxidant status of liver and heart tissue as shown at Table 7. The supplementation of 100 ppm SE significantly decreased both liver and heart MDA levels (P<0.05), and both amounts increased the liver GSH-Px enzyme activity (P<0.01) as compared with control group.

The other essential data was obtained on fatty acid profile of breast meat of quails subjected to cold stress as shown at Table 8. The essential fatty acid deposition was significantly increased by SE supplementation. Although oleic acid (C18:1 ω₉) and MUFA were decreased, linoleic acid (C18:2 ω₆), *α*-linolenic acid (C18:3 ω₃) and arachidonic acid (C20:4 ω₆) which called essential fatty acids, PUFA, total omega 6 and total omega 6/omega 3 were increased by 100 ppm SE supplementation.

Table 3. Effect of soapwort extract supplementation on laying performance of laying quails reared under low ambient condition

Tablo 3. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bildircinlarda yumurtlama performansına etkisi

Weeks	Control	SE-50	SE-100	SEM	P
Egg Weight, g					
1	11.27	11.60	10.72	0.37	NS
2	12.29	12.33	11.53	0.34	NS
3	12.56	11.80	11.99	0.20	NS
4	14.01 ^a	12.90 ^b	13.33 ^{ab}	0.21	*
5	13.88	13.34	12.92	0.20	NS
6	14.04	13.83	13.44	0.14	NS
0-6	13.01	12.63	12.32	0.17	NS
Feed Intake, g/bird/day					
1	31.82	28.77	30.06	0.88	NS
2	35.03	33.84	32.31	1.15	NS
3	41.42 ^a	37.84 ^{ab}	34.79 ^b	1.24	*
4	37.62	33.77	34.45	1.57	NS
5	38.53	37.67	34.79	1.22	NS
6	39.92	41.05	40.04	0.55	NS
0-6	37.39	35.49	34.41	0.86	NS
Egg Production, %					
1	29.36	32.53	30.16	1.22	NS
2	36.05	40.81	40.13	3.43	NS
3	61.79	57.82	60.88	5.45	NS
4	68.48	67.12	70.06	4.14	NS
5	74.49	77.32	81.63	2.90	NS
6	82.31	84.69	92.51	2.92	NS
0-6	58.75	60.05	62.56	2.90	NS
Feed Conversion Ratio, g feed intake x female number/egg production x egg weight					
1	9.61	7.62	9.29	0.53	NS
2	7.90	6.72	6.98	0.58	NS
3	5.33	5.54	4.76	0.53	NS
4	3.92	3.90	3.68	0.21	NS
5	3.71	3.65	3.29	0.15	NS
6	3.45	3.50	3.21	0.09	NS
0-6	4.89	4.67	4.46	0.17	NS

SE-50: Supplemented 50 ppm Soapwort Extract (SE); SE-100: Supplemented 100 ppm SE; P: Statistical significance, SEM: Standard error mean, NS: No significant, * P<0.05, ^{ab} Mean values with different superscripts within a row differ significantly

DISCUSSION

Previously, saponin-rich plant species are recognized as antinutritional feed which should be processed before using as a feed supplement [29]. Nowadays saponins are considered as health beneficial natural food components due to their anticarcinogenic [6], hypocholesterolemic [7,30], hepatoprotective adjuvant [4,5], antiviral, antifungal [8],

Table 4. Effect of soapwort extract supplementation on egg quality collected once a week for the last four weeks of laying quails reared under low ambient condition

Tablo 4. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bildircinlarda denemenin son dört haftası boyunca haftada bir kez toplanan yumurtaların kalitesine etkisi

Weeks	Control	SE-50	SE-100	SEM	P
Examined egg weight, g	13.67 ^a	13.39 ^{ab}	13.17 ^b	0.06	**
Shell weight, g	1.07	1.05	1.04	0.01	NS
Shell thickness, mm	2.64	2.57	2.63	0.01	NS
Yolk color	9.43	9.41	9.69	0.05	NS
Egg yolk weight, g	4.27	4.15	4.17	0.03	NS
Albumen weight, g	7.01 ^a	6.98 ^a	6.68 ^b	0.03	***
Shape index, %	77.15	77.62	76.69	0.24	NS

SE-50: Supplemented 50 ppm Soapwort Extract (SE); SE-100: Supplemented 100 ppm SE; P: Statistical significance, SEM: Standard error mean, NS: No significant, ** P<0.01, *** P<0.001, ^{ab} Mean values with different superscripts within a row differ significantly

Table 5. Effect of soapwort extract supplementation on some offal weights and percentages of laying quails reared under low ambient condition

Tablo 5. Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bildircinların bazı organ ağırlıklarına ve oranlarına etkisi

Traits	Control	SE-50	SE-100	SEM	P
Slaughter Weight, g	268.00	260.33	260.00	2.52	NS
Liver Weight, g	7.71	8.61	7.58	0.28	NS
Liver Ratio, %	2.87	3.30	2.91	0.10	NS
Heart Weight, g	2.23	2.06	2.28	0.08	NS
Heart Ratio, %	0.83	0.79	0.88	0.03	NS
Spleen Weight, g	0.12	0.12	0.12	0.01	NS
Spleen Ratio, %	0.04	0.04	0.05	0.00	NS

SE-50: Supplemented 50 ppm Soapwort Extract (SE); SE-100: Supplemented 100 ppm SE; P: Statistical significance, SEM: Standard error mean, NS: No significant

antiinflammatory and antioxidant [9,10] properties which are shown *in vitro* and *in vivo* animal tests. Saponins are used as a feed supplement in livestock for reducing the emission of ammonia from animal excreta due to their surfactant activity [11]. Preliminary studies have shown that saponin supplements have variable effects on performance of studied animals. Rowland et al. [11] have obtained that supplementing 31 and 155 ppm saponin yucca powder to the diets of laying hens increased the egg production performance and lowered the house ammonia amount while supplementing 465 ppm decreased egg production performance. Likewise, Aslan et al. [31] reported that dietary supplementation of laying hens by 100 ppm Deodorase induced positive effects on egg production, enhanced antioxidant capacities and decreased glycemia and cholesterolemia. However, Kutlu et al. [30] have obtained that supplementing 30, 60 and 120 ppm saponin yucca extract into the laying hen diets had no effects on feed intake, egg production rate, feed conversion ratio, daily body

Table 6. Effect of soapwort extract supplementation on some serum metabolites of laying quails reared under low ambient condition**Tablo 6.** Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda bazı serum metabolitlerine etkisi

Traits	Control	SE-50	SE-100	SEM	P
Glucose, mg/dL	251.00 ^a	198.67 ^b	243.67 ^a	8.79	*
Triglyceride, mg/dL	107.67 ^a	77.00 ^b	97.83 ^{ab}	5.06	*
Total Cholesterol, mg/dL	176.00	182.17	173.67	5.07	NS
HDL Cholesterol, mg/dL	90.83	103.83	92.83	6.77	NS
LDL Cholesterol, mg/dL	66.67	56.33	62.50	2.15	NS
Uric Acid, mg/dL	5.10 ^a	3.76 ^b	4.80 ^{ab}	0.33	*
AST, U/L	272.50 ^a	212.50 ^b	222.83 ^b	8.18	*

SE-50: Supplemented 50 ppm Soapwort Extract (SE); SE-100: Supplemented 100 ppm SE; AST: Aspartate aminotransferase; P: Statistical significance, SEM: Standard error mean, * P<0.05, ^{ab} Mean values with different superscripts within a row differ significantly

Table 7. Effect of soapwort extract supplementation on antioxidant status of liver and heart tissues in laying quails reared under low ambient condition**Tablo 7.** Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda karaciğer ve kalp dokusu antioksidan durumuna etkisi

Traits	Control	SE-50	SE-100	SEM	P
MDA (nmol/g protein)					
Liver	4.92 ^a	4.12 ^{ab}	3.43 ^b	0.23	*
Heart	4.59 ^a	3.62 ^{ab}	2.46 ^b	0.31	*
GSH (nmol/g protein)					
Liver	0.07	0.09	0.07	0.00	NS
Heart	0.10	0.10	0.10	0.00	NS
GSH-Px (U/g protein)					
Liver	0.09 ^b	0.11 ^a	0.12 ^a	0.01	**
Heart	0.12	0.13	0.12	0.00	NS
SOD (U/g protein/mL)					
Liver	30.02	28.97	28.09	0.83	NS
Heart	70.04	66.88	68.43	1.86	NS

SE-50: Supplemented 50 ppm Soapwort Extract (SE); SE-100: Supplemented 100 ppm SE; MDA: Malondialdehyde; GSH: Glutathione; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; P: Statistical significance, SEM: Standard error mean, NS: No significant, * P<0.05, ** P<0.01, ^{ab} Mean values with different superscripts within a row differ significantly

weight gain, white and yolk weights, shell thickness and shape index but reduced yolk cholesterol content in a dose related. In the present study SE supplementation had no effect on performance parameters except reductions in feed intake at the third week and egg weight at the fourth week. The reduced egg weights within four week may depend on reduced feed intake seen at the previous week. The exciting data was obtained from the collected eggs for determining the external and internal egg quality parameters through the last four week of the experiment. Addition of 100 ppm SE caused significant reductions in egg weight and albumen weight when compared with control group. There were no differences in examined ofal

Table 8. Effect of soapwort extract supplementation on fatty acid profile of breast meat in laying quails reared under low ambient condition**Tablo 8.** Çöven ekstraktı ilavesinin soğuk şartlarda yetiştirilen yumurtacı bıldırcınlarda göğüs eti yağ asidi profiline etkisi

Fatty Acids	Control	SE-50	SE-100	SEM	P
C16:0	21.74	20.97	19.30	0.55	NS
C16:1 ω7	3.27	2.71	3.16	0.18	NS
C18:0	17.29	17.67	18.43	0.83	NS
C18:1 ω9	33.15 ^a	35.01 ^a	27.52 ^b	1.34	*
C18:2 ω6	16.49 ^b	16.09 ^b	20.18 ^a	0.57	*
C18:3 ω3	0.35 ^b	0.31 ^b	0.49 ^a	0.03	*
C18:3 ω6	0.95	0.88	0.91	0.03	NS
C20:4 ω6	3.85 ^b	3.44 ^b	6.92 ^a	0.53	*
C24:1	0.55	0.57	0.66	0.07	NS
C22:6 ω3	2.36	2.35	2.43	0.25	NS
ΣSFA	39.03	38.64	37.73	0.35	NS
ΣMUFA	36.97 ^a	38.29 ^a	31.34 ^b	0.35	***
ΣPUFA	24.00 ^b	23.07 ^b	30.93 ^a	1.05	***
Σ ω-6	21.29 ^b	20.41 ^b	28.01 ^a	0.90	***
Σ ω-3	2.71	2.66	2.92	0.14	NS
Σ ω-6/ω-3	7.86 ^b	7.67 ^b	9.59 ^a	0.50	*

SE-50: Supplemented 50 ppm Soapwort Extract (SE); SE-100: Supplemented 100 ppm SE; C16:0: Palmitic acid; C16:1 ω7: Palmitoleic acid; C18:0: Stearic acid; C18:1 ω9: Oleic acid; C18:2 ω6: Linoleic acid; C18:3 ω3: α-Linolenic acid; C18:3 ω6: γ-Linolenic acid; C20:4 ω6: Arachidonic acid; C24:1 ω9: Nervonic acid; C22:6 ω3: Docosa hexaenoic acid; SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid; P: Statistical significance, SEM: Standard error mean, NS: No significant, * P<0.05, *** P<0.001 ^{ab} Mean values with different superscripts within a row differ significantly

weights and percentages in the present study. Similarly to present study, in some studies carried out with saponin extract supplementation to livestock diets indicated no differences were determined in performance parameters such as feed intake, growth or laying performance while in some studies either adverse effects or positive effects were observed. So it can be concluded that variable effects may be seen by saponin feeding experiments. Heywang and Bird [32] and Anderson [33] who reported that supplementing alfalfa saponin over 0.15% and 0.1%, respectively to diets of broilers had negative effects on growth performance. In the same study of Anderson [33] reported that 0.3% saponins to layer hens diet caused a reduction on egg production but this reduction was temporary and by the time egg production has returned to the normal/or higher levels. Similarly, in laying hens, Heywang et al. [34] obtained that adding 0.29 and 0.40% saponins from alfalfa to diets caused a reduction on feed intake and egg production without any effect on egg weight. In another study with growing chicks, Jenkins and Atwal [35] reported that supplementing different saponin sources with different amounts as 0.1% to 0.9%, they obtained that gypsophila saponins and quillaja saponins reduced growth

performance and feed consumption but sarsaponin had no effect on same parameters. Whitehead et al.^[36] reported that the effect of supplementing saponin to diets on the performance of laying hens was dose dependent and no effects were seen with 0.1% saponin while negative effects were seen with 0.4-0.5% saponin on performance and liver lipid concentrations, and egg production rate. Cheeke et al.^[37] reported that the depressed feed intake by the bitter taste of saponin is caused the negative effects on performance. Additionally, saponins has interaction with some vitamins and trace minerals such as zinc and this influences the metabolic and digestive enzymes^[35,38,39].

In the present study, serum total cholesterol, HDL and LDL cholesterol levels were similar among groups. However, dietary supplementation of 50 ppm SE reduced the serum glucose, triglyceride, uric acid and AST levels in comparison with the control group. In addition, 100 ppm SE significantly reduced the only AST level when compared with the control group. Inconsistent with the present study, the lowering ability of saponins on serum cholesterol has also been reported for both human and animals^[7,39,40]. Consistent with this study, Yang et al.^[41] observed that the green tea saponins had no effect on the serum cholesterol levels of broilers. Also, Whitehead et al.^[36] reported that dietary saponins reduced the liver cholesterol and plasma triglyceride levels without any effect on plasma cholesterol (HDL, LDL, total) levels in broilers. Similarly to our results, in a study conducted in rats, steroidal saponins contained *Yucca schidigera* supplementation reduced the plasma triglyceride, uric acid and glucose levels^[42]. Increased serum uric acid, AST, glucose levels of control group in the present study could be due to cold conditions. Under stress condition, some metabolic alterations occur because of the subsequent secretion of corticosterone (CS). CS promotes gluconeogenesis for endogenous glucose production from glycogen stores, or by synthesis *in vivo* from gluconeogenic precursors such as amino acids and the others. Increasing serum glucose level of the quail under cold condition may be associated with effects of CS on carbohydrate metabolism^[43]. On the other hand, CS induces the catabolism of structural protein to free amino acids. This action causes to uric acid excretion^[44]. Prolonged stress also induces lipolysis and causes to increase in free fatty acid concentration and serum triglycerides^[43]. Increased triglyceride level of the study could be clarified with this metabolism. Likewise, thermal stress influences blood chemistry values of northern Bobwhite quails^[45].

The body also has the regular antioxidant system, but additional exogenous antioxidants have synergetic and beneficial effects to body defense system. Most of the plant bioactive compounds have antioxidant properties. Soapwort saponins have *in vitro* antioxidant activity also proved in a study carried out by Arslan and Celik^[9]. Kucukkurt et al.^[46] reported that saponin contained *Agrostemma githago* L. and *Saponaria officinalis* L. extracts

enhanced the antioxidant status and decreased the incidence of lipid peroxidation in blood samples of rats exposed to X-radiation. Sur et al.^[10] investigated that antioxidant mechanism of tea saponins was occurred by xanthine and xanthine oxidase pathway in rats. Consistent with above findings, the supplementation of 100 ppm SE significantly decreased both liver and heart MDA levels, and both amounts increased the liver GSH-Px enzyme activity as compared with control group.

It is well known that poultry meat is particularly preferred due to its relatively high content of polyunsaturated fatty acids with its low content of cholesterol, but meat chemical compositions differ among poultry species and influence by nutritional factors^[41]. In the present study the essential fatty acid deposition of breast meat was significantly increased by 100 ppm SE supplementation. It is the worth value that arachidonic acid, alfa-linolenic acid and linoleic acid ratio of breast meat were 1.8, 1.4 and 1.2 fold much more deposited respectively in SE 100 group than control group. Although oleic acid (C18:1 ω9) and MUFA were decreased, PUFA, total omega 6 and total omega 6/omega 3 were increased by 100 ppm SE supplementation. The fat metabolism altering effects of saponins from different sources were previously studied. Recently, Rohaida et al.^[47] reported that saponin contained Candle Nut Kernel Meal supplementation to broiler diets at the rate of 2% were increased linolenic acid (LNA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DPA) of breast meat, whereas linoleic acid (LA) and the ratio of ω-6:ω-3 fatty acids were decreased when compared to control. Supporting to our results, in a lamb study, Brogna et al.^[48] observed that supplementing Quillaja saponin increased arachidonic acid (C20:4 ω6) and ω-3:ω-6 (LNA/LA) level of longissimus dorsi muscle but they did not observe any difference on SFA, MUFA and PUFA levels. Afrose et al.^[40] also, reported that Karaya saponin supplementation reduced saturated fatty acid levels due to the decrease in palmitic (C16:0) and stearic (C18:0) acids levels in the thigh and breast muscle of broilers. However they observed the total MUFA as well as PUFA were improved in response to treatment with Karaya saponin was primarily caused by the increase in oleic (C18:1) and linoleic (C18:1) acids levels in thigh and breast muscle. The researches connected these results that adding karaya saponin to the diet of broilers, caused a significant reduction in the content of cholesterol and triglycerides in the broiler meat.

The results of this study demonstrated that both amounts of SE supplementation, especially 100 ppm, to diets of laying quails exposed to cold stress prevented the detrimental effects of oxidative stress without any negative effect on performance. Furthermore, SE supplementation increased the essential fatty acid deposition of breast meat and the altering effect of SE on fatty acid profile of deposited fats may be needed to a detailed study in unstressed birds.

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REFERENCES

- Sahin N, Sahin K, Onderci M:** Vitamin E and selenium supplementation to alleviate cold-stress-associated deterioration in egg quality and egg yolk mineral concentrations of Japanese quails. *Biol Trace Elem Res*, 96, 179-189, 2003. DOI: 10.1385/BTER:96:1-3:179
- Kovacs P, Juranek I, Stankovicova T, Svec P:** Lipid peroxidation during acute stress. *Pharmazie*, 51, 51-53 1996.
- Wallace RJ, Oleszek W, Franz C, Hahn I, Baser KH, Mathe A, Teichmann K:** Dietary plant bioactives for poultry health and productivity. *Br Poult Sci*, 51, 461-487, 2010. DOI: 10.1080/00071668.2010.506908
- Moghimpour E, Handali S:** Saponin: Properties, methods of evaluation and applications. *Ann Res Rev Biol*, 5, 207-220, 2015. DOI: 10.9734/ARRB/2015/11674
- Fleck JD, Kauffmann C, Spilki F, Lencina CL, Roehe PM, Gosmann G:** Adjuvant activity of *Quillaja brasiliensis* saponins on the immune responses to bovine herpesvirus type 1 in mice. *Vaccine*, 24, 7129-7134, 2006. DOI: 10.1016/j.vaccine.2006.06.059
- Xiao X, Bai P, Bui Nguyen TM, Xiao J, Liu S, Yang G, Hu L, Chen X, Zhang X, Liu J, Wang H:** The antitumoral effect of Paris saponin I associated with the induction of apoptosis through the mitochondrial pathway. *Mol Cancer Ther*, 8, 1179-1188, 2009. DOI: 10.1158/1535-7163.MCT-08-0939
- Sidhu GS, Oakenfull DG:** A mechanism for the hypocholesterolaemic activity of saponins. *Br J Nutr*, 55, 643-649, 1986. DOI: 10.1079/BJN19860070
- Coleman JJ, Okoli I, Tegos GP, Holson EB, Wagner FF, Hamblin MR, Mylonakis E:** Characterization of plant-derived saponin natural products against *Candida albicans*. *ACS Chem Biol*, 5, 321-332, 2010. DOI: 10.1021/cb900243b
- Arslan I, Celik A:** Saponin rich fractions (SRPs) from soapwort show antioxidant and hemolytic activity. *APCBEE Procedia*, 7, 103-108, 2013. DOI: 10.1016/j.apcbee.2013.08.019
- Sur P, Chaudhuri T, Vedasiromoni JR, Gomes A, Ganguly DK:** Antiinflammatory and antioxidant property of saponins of tea (*Camellia sinensis* (L) O. Kuntze) root extract. *Phytother Res*, 15, 174-176, 2001. DOI: 10.1002/ptr.696
- Rowland LO, Pleyler JE, Bradley JW:** *Yucca schidigera* extract effect on egg production and house ammonia levels. *Poult Sci*, 55, 2086-2093, 1976.
- Baytop T:** Türkiye'de bitkiler ile tedavi, geçmişte ve bugün. İstanbul Üniversitesi Yayınları, No: 3255, Eczacılık Fakültesi Yayınları, No: 40, 520s. İstanbul, 1984.
- Özçelik H, Yıldırım B:** Türkiye çövenlerinin (*Gypsophila* L. ve *Ankyropetalum Fenzl* spp.) ekonomik önemi, kullanım olanakları ve korunması üzerine düşünceler. *SDU Fac Forestry J*, 12, 57-61, 2011.
- Cheeke PR:** Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. *J Anim Sci*, 77, 1-10, 2000. DOI: 10.1007/978-94-015-9339-7_25
- AOAC:** Official Methods of Analysis Association of AOAC International. 17th ed., AOAC International Maryland, 2000.
- Crampton EW, Maynard LA:** The relation of cellulose and lignin content to nutritive value of animal feeds. *J Nutr*, 15, 383-395, 1983.
- Lane JH, Eynon L:** Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. *J Soc Chem Ind Trans*, 42, 32-36, 1923.
- Carpenter KJ, Clegg KM:** The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. *J Sci Food Agric*, 7, 45-51, 1956. DOI: 10.1002/jsfa.2740070109
- Lalitha T, Seshadri R, Venkataraman LV:** Isolation and properties of saponins from *Madhuca butyracea* seeds. *J Agric Food Chem*, 35, 744-748, 1987. DOI: 10.1021/jf00077a024
- National Research Council:** Nutrient Requirements of Poultry, 9th ed., National Academic Press, Washington, DC, 1994.
- Anonymous:** Poultry carcass-rules for carcass dissecting. Institute of Turkish Standards, TS 5890, Ankara, Turkey, ICS 67.120.10; 67.120.20, 2009.
- Placer AZ, Linda LC, Johnson B:** Estimation of product lipid peroxidation (malonyldialdehyde) in biochemical systems. *Anal Biochem*, 16, 359-364, 1966. DOI: 10.1016/0003-2697(66)90167-9
- Sun Y, Oberley LW, Li Y:** A simple method for clinical assay of superoxide dismutase. *Clin Chem*, 34, 497-500, 1988.
- Lawrence RA, Burk RF:** Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Comm*, 71, 952-958, 1976. DOI: 10.1016/0006-291X(76)90747-6
- Sedlak J, Lindsay RH:** Estimation of total protein bound and non-protein sulfhydryl groups in tissue with Ellman reagent. *Anal Biochem*, 25, 192-205, 1968. DOI: 10.1016/0003-2697(68)90092-4
- Hara AR, Radin NS:** Lipid extraction of tissues with a low-toxicity solvent. *Anal Biochem*, 90, 420-426, 1978. DOI: 10.1016/0003-2697(78)90046-5
- Christie WW:** Gas chromatography and lipids. The Oil Press, Glasgow, 302, 1992.
- SPSS Inc:** SPSS for Windows Release 11.5 (6 Sep. 2002), Standard Version, Copyright SPSS Inc, Chicago, 2002.
- Hostettmann K, Marston A:** Saponins. Cambridge: Cambridge University Press, UK, 1995.
- Kutlu HR, Gorgulu M, Unsal I:** Effects of dietary *Yucca schidigera* powder on performance and egg cholesterol content of laying hens. *J Appl Anim Res*, 20, 49-56, 2001. DOI: 10.1080/09712119.2001.9706736
- Aslan R, Dundar Y, Eryavuz A, Bulbul A, Kucukkurt I, Fidan AF, Akinci Z:** Effects of different dietary levels of *Yucca schidigera* powder (Deodorase) added to diets on performance, some hematological and biochemical blood parameters and total antioxidant capacity of laying hens. *Rev Med Vet*, 156, 350-355, 2005.
- Heywang BW, Bird HR:** The effect of alfalfa saponin on the growth, diet consumption, and efficiency of diet utilization of chicks. *Poult Sci*, 33, 239-241, 1954. DOI: 10.3382/ps.0330239
- Anderson JO:** Effect of alfalfa saponin on the performance of chicks and laying hens. *Poult Sci*, 36, 873-876, 1957. DOI: 10.3382/ps.0360873
- Heywang BW, Thompson CR, Kemmerer AR:** Effect of alfalfa saponin on laying chickens. *Poult Sci*, 38, 968-971, 1959. DOI: 10.3382/ps.0380968
- Jenkins KJ, Atwal AS:** Effects of dietary saponins on fecal bile acids and neutral sterols, and availability of vitamins A and E in the chick. *J Nutr Biochem*, 5, 134-135, 1994. DOI: 10.1016/0955-2863(94)90084-1
- Whitehead CC, McNab JM, Griffin HD:** The effects of low dietary concentrations of saponin on liver lipid accumulation and performance in laying hens. *Br Poult Sci*, 22, 281-288, 1981. DOI: 10.1080/00071688108447887
- Cheeke PR, Powley JS, Nakae HS, Arscott GH:** Feed preference responses of several avian species fed alfalfa meal, high-and low-saponin alfalfa, and quinine sulfate. *Can J Anim Sci*, 63, 707-710, 1983. DOI: 10.4141/cjas83-080
- Southon S, Johnson IT, Gee JM, Price KR:** The effect of *Gypsophila* saponins in the diet on minerals status and plasma cholesterol concentration in the rat. *Br J Nutr*, 59, 49-55, 1988. DOI: 10.1079/

BJN19880008

39. Southon S, Wright AJA, Price KR, Fairweather-Tait SJ, Fenwick GR: The effect of three types of saponin on iron and zinc absorption from a single meal in the rat. *Br J Nutr*, 59, 389-396, 1988. DOI: 10.1079/BJN19880048

40. Afrose S, Hossain MS, Maki T, Tsujii H: Hypocholesterolemic response to Karaya saponin and *Rhodobacter capsulatus* in broiler chickens. *Asian-Aust J Anim Sci*, 23, 733-741, 2010. DOI: 10.5713/ajas.2010.90481

41. Yang CJ, Yang IY, Oh DH, Bae IH, Cho SG, Kong IG, Uganbayar D, Nou IS, Choi KS: Effect of green tea by-product on performance and body composition in broiler chicks. *Asian-Aust J Anim Sci*, 16, 867-872, 2003. DOI: 10.5713/ajas.2003.867

42. Kucukkurt I, Dundar Y: Effects of dietary *Yucca schidigera* supplementation on plasma leptin, insulin, iodated thyroid hormones and some biochemical parameters in rats. *Rev Med Vet*, 7, 362-367, 2013.

43. Johnson JS: Heat stress alters animal physiology and post-absorptive metabolism during pre- and postnatal development. *Graduate Theses and Dissertations*, Paper: 13982, 2014.

44. Virden WS, Kidd MT: Physiological stress in broilers: Ramifications on nutrient digestibility and responses. *J Appl Poult Res*, 18, 338-347, 2009. DOI: 10.3382/japr.2007-00093

45. Dabbert CB, Lochmiller RL, Teeter RG: Thermal stress influences clinical chemistry values of northern bobwhite (*Colinus virginianus*). *Comp Haematol Int*, 6, 120-122, 1996. DOI: 10.1007/BF00426054

46. Kucukkurt I, Ince S, Enginar H, Eryavuz A, Fidan AF, Kargioglu M: Protective effects of *Agrostemma githago* L. and *Saponaria officinalis* L. extracts against ionizing radiation-induced oxidative damage in rats. *Rev Med Vet*, 162, 289-296, 2011.

47. Rohaida AR, Alimon AR, Sazili AQ: Fatty acid composition of breast and thigh muscles of broilers fed diets supplemented with candle nut kernel meal subjected to different heat treatments. *Mal J Anim Sci*, 17, 47-60, 2014.

48. Brogna DM, Nasri S, Salem HB, Mele M, Serra A, Bella M, Priolo A, Makkar HP, Vasta V: Effect of dietary saponins from *Quillaja saponaria* L. on fatty acid composition and cholesterol content in muscle Longissimus dorsi of lambs. *Animal*, 5, 1124-1130, 2011. DOI: 10.1017/S1751731111000048