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Research Article

Effects of Milk Thistle *(Silybum marianum)* Seed Supplementation to High-Calorie Basal Diets of Quails on Egg Production, Egg Quality Traits, Hatchability and Oxidative Stress Parameters

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Abstract

The purpose of this study was to examine the changes to occur in egg production, egg quality traits, hatchability, and oxidative stress parameters of laying quails fed with high calorie diets and effect of milk thistle (*Silybum marianum*) seed on these changes. A total of 75 45-day-old quails including 60 females and 15 males were used in the study. The quails were divided into 5 groups with three repetitions. 4 females and 1 male were used in each repetition. The groups of the study were arranged as following; control group (C) consuming corn-soybean based basal diet, oil group (SFO) in which 5% sun flower oil was added into basal diet, oil + milk thistle group (SFO+MT) in which 5% sun flower oil + 1% milk thistle seed was added into basal diet, syrup group (CS) in which 10% corn syrup was added into basal diet, and syrup + milk thistle group (CS+MT) in which 10% corn syrup + 1% milk thistle was added into basal diet. Total egg production was found to be significantly higher in SFO and SFO+MT groups of present study ($P<0.05$). Feed intake, total egg weight and feed conversion were found similar among the groups ($P>0.05$). The differences among the groups in albumen rate, yolk rate, dried shell rate and shape index were not statistically significant ($P>0.05$). The best yolk colour was obtained in SFO+MT and CS groups ($P<0.01$). There were no statistically differences among the groups in hatchability, hatchability of fertile and embrionic mortality ($P>0.05$), but the difference in examined fertility were statistically significant ($P<0.05$). Malonyl dialdehyde (MDA) values of blood, liver and heart tissues were similar among the groups of study ($P>0.05$). MDA level of kidney significantly increased in CS group, however addition of milk thistle seed into diet was reduced MDA level of kidney in CS+MT group ($P<0.01$). High calorie diets caused changes in antioxidant system in blood and tissues. Consequently, high-calorie basal diets especially addition of SFO improved only egg production and fertility rate. However feeding quails with corn syrup had significantly higher lipid peroxidation in kidney. Supplementation of milk thistle seed into CS group may protect kidney against free radical damage.

Keywords: *Quail, Egg yield, Hatchability, Milk thistle, High calorie diets, Oxidative stress*

Yüksek Kalorili Bıldırcın Karma Yemlerine Deve Dikeni *(Silybum marianum)* Tohumu İlavesinin Yumurta Verimi, Yumurta Kalite Özellikleri, Kuluçka Randımanı ve Oksidatif Stres Parametreleri Üzerine Etkisi

Özet

Bu araştırmada yüksek kalorili yemlerle beslenen yumurtacı bıldırcınlarda yumurta verimi, yumurta kalite özellikleri, kuluçka performansı ve oksidatif stres parametrelerinde meydana gelebilecek değişimler ve bu değişimler üzerine deve dikeni (*Silybum marianum*) tohumunun etkisinin incelenmesi amaçlanmıştır. Araştırmada, 45 günlük yaşta 60 adet dişi, 15 adet erkek olmak üzere toplam 75 adet bıldırcın kullanılmıştır. Araştırma 5 grup ve her grupta 3 tekerrürden oluşturulmuştur. Her bir tekerrürde 4 dişi ve 1 erkek bıldırcın kullanılmıştır. Deneme grupları; mısır-soya esasına dayalı temel karma yemi tüketen kontrol grubu (C), temel diyete % 5 ay çiçeği yağı ilave edilen yağ grubu (SFO), temel diyete %5 ay çiçeği yağı + %1 deve dikeni ilave edilen yağ + deve dikeni grubu (SFO+MT), temel diyete %10 mısır şurubu ilave edilen şurup grubu (CS) ve temel diyete %10 mısır şurubu + %1 deve dikeni ilave edilen şurup + deve dikeni grubu (CS+MT) şeklinde oluşturulmuştur. Çalışmada, toplam yumurta verimi SFO ve SFO+MT gruplarında önemli ($P<0.05$) derecede yüksek bulunmuştur. Yem tüketimi, toplam yumurta ağırlığı ve yemden yararlanma yönünden gruplar birbirine benzer bulunmuştur ($P>0.05$). Yumurta akı ve sarı oranı, kurutulmuş kabuk oranı ve şekil indeksi yönünden gruplar arasında fark bulunamamıştır ($P>0.05$). En iyi sarı rengi SFO+MT ve CS gruplarında belirlenmiştir ($P<0.01$). Kuluçka randımanı, çıkış gücü ve embriyonik ölüm açısından gruplar arasında fark saptanmazken ($P>0.05$), döllülük açısından gruplar arasında önemli ($P<0.05$) fark tespit edilmiştir. Kan, karaciğer ve kalp dokularının Malonil dialdehid (MDA) değerleri benzer bulunmuştur ($P>0.05$). Böbrek MDA değeri CS grubunda önemli olarak artarken, diyete deve dikeni tohumu ilavesi CS+ MT grubunda böbreğin MDA değerini azaltmıştır ($P<0.01$). Yüksek kalorili diyetler kan ve dokularda antioksidan sistemde değişikliğe sebep olmuştur. Sonuç olarak, yüksek kalorili diyetler özellikle SFO ilavesi sadece yumurta üretimini ve döllülük oranını iyileştirmiştir. Diğer taraftan mısır şurubu tüketimi böbrekte lipit peroksidasyonunu artırmıştır. CS gruplarında deve dikeni tohumu ilavesi böbreği serbest radikal hasarına karşı koruyabilir.

Anahtar sözcükler: *Bıldırcın, Yumurta verimi, Kuluçka, Deve dikeni, Yüksek kalorili diet, Oksidatif stres*

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INTRODUCTION

Poultry products constitute a significant source in order to meet protein deficit of increasing population in the world. Quail takes an important place in poultry farming because it has high reproduction rate and fertility, short reproduction period, highfarming per unit area and high feed conversion ratio, easy farming, products especially rich in protein and minerals, and also is fondly consumedby people [1].

Natural and reliable alternative sources have attracted attention of researchers after growth factors like antibiotics, hormones etc. and productive additive substances were prohibited in the animal feeding area. In this context, alternative supplements such as organic acids, probiotics, prebiotics, bioenzymes, aromatic plants, and essential oils have acquired currency. Aromatic plants and essential oils are used in alternative medicine for many centuries and made a research subject by scientists in all fields because they are natural and contain several active components. Aromatic plants and essential oils have been conveniently used as alternatives to these synthetic growth factors in farm animal diet [2,3].

Milk thistle *(Silybum marianum* L. Gaertn.) is a plant belonging to Asteraceae family. Its seeds have been used for protecting liver against diseases of liver and gallbladder, and intoxication; and also for treatment of cases such as mushroom poisoning, snake bite, bug bites since 2000. Extracts acquired from milk thistle seeds contain plenty of silymarin. Silymarin is chemically composed of isomer flavanolignans named as silybin (silybinin), isosilybin, silychristin, silydianin, and dehydrosilybinin [4]. Basic component of silymarin which is thought to be responsible for its biological activity is silybin; however, it is also thought that other flavanolignans found in silymarin can have a role in this biological activity [5].

In the studies conductedup to the present, positive results have been obtained from feed supplements with various antioxidant and immune system promoting characteristics added into diets. Some of these supplements were banned because they are synthetic and there are also difficulties in supplying some of them; therefore researchers direct to alternative supplements [6]. In this regard; aromatic plants and essential oils have been used by humans for centuries and become a research subject for scientists in every field because they are natural and contain numerous active components [2,3].

The aim of this study was to examine the changes to occur in egg production, egg quality traits, hatchability, and oxidative stress parameters of laying quails fed with high calorie diets and effect of milk thistle seed on these changes.

MATERIAL and METHODS

Management

The study was conducted atthe Poultry Unit, Faculty of Veterinary Science, Fırat University. A total of 75 45-day-old quails including 60 females and 15 males were used in the study. The quails were divided into 5 groups with three repetitions. 4 females and 1 male were used in each repetitions.The groups of the study were arranged as following; control group (C) consuming corn-soybean based basal diet, oil group (SFO) in which 5% sun flower oil was added into basal diet, oil + milk thistle group (SFO+MT) in which 5% sun flower oil + 1% milk thistle seed was added into basal diet, syrup group (CS) in which 10% corn syrup was added into basal diet, and syrup + milk thistle group (CS+MT) in which 10% corn syrup + 1% milk thistle was added into basal diet. The experiment was conducted under the protocol which was approved by Firat University Animal Use Local Ethical Committee (No: 2015/101). All groups were given diet and water as *ad libitum*. Rations used in the study were arranged according to standards of National Research Council [7] and given in *Table 1*. The milk thistle (*Silybum marianum*) was provided from a commercial company (Naturoil Food and Chemical Industry Company Limited). The chemical composition of milk thistle was given in *Table 2*. Quails were kept in special laying cages and in a room with temperature between 15 and 25°C. A lighting program including daylight of 16 hours and dark program of 8 h was applied during the laying period. Records of egg production in quails were taken as beginning from the period of 5% egg production quails-days. For this purpose, eggs were collected in the same hour of every day by counting them, daily egg production (%) was determined by dividing number of the obtained eggs into number of quails in that day. Diets were weighed and given daily, remaining diet in feeders were weighed weekly and daily feed consumption was determined with the difference between them. Feed conversion ratio was calculated by using values of egg production, egg weigh, and feed consumption. As from the 3rd week of the study, a total of 250 eggs including 50 eggs from each group (including sub-groups) were cracked in order to determine internal quality traits after external quality traits were determined. Fertility rate, hatchability, and hatchability of fertile eggs, were determined by incubating eggs collected for 3 weeks as from the 3rd week of the study. Blood, liver, kidney, and heart samples were taken during 6 animals from each experimental group were slaughtered by using decapitation method at the end of the study.

Chemical Analysis

After blood samples were taken into tubes containing heparin and whole blood was separated for determination of glutathione (GSH) and glutathione peroxidase (GSH-Px), remaining blood samples were centrifuged at 3.000 rpm

Table 1. *Ingredients and nutrient composition of experimental diets (g/kg)*
Tablo 1. *Araştırma diyetinin bileşimi ve besin madde içeriği (g/kg)*

Experimental Groups	C	SFO	SFO+MT	CS	CN+MT
Ingredients, g/kg					
Corn	530.0	444.5	440.4	453.1	450.0
Wheat bran	85.0	112.0	105.0	29.0	21.9
Soybean meal (48% CP)	192.0	188.4	190.0	224.8	225.0
Corn gluten (43% CP)	90.0	113.0	112.5	90.0	90.0
Vegetable oil	25.0	65.	65.0	25.0	25.0
Milk thistle seed	-	-	10.0	-	10.0
Corn syrup	-	-	-	100.0	100.0
DL-Methionine	1.7	1.7	1.7	1.9	1.8
Dicalcium phosphate	18.0	17.1	17.1	18.0	18.1
Ground limestone	51.7	51.7	51.7	51.6	51.6
$NaHCO_3$	1.0	1.0	1.0	1.0	1.0
Salt	2.6	2.6	2.6	2.6	2.6
Vitamin-Mineral Mix*	3.0	3.0	3.0	3.0	3.0
Nutritional Composition, g/kg					
Dry matter	904.0	908.0	908.0	906.0	906.0
Crude protein	170.0	170.0	170.0	170.0	170.0
Crude fiber	525.0	525.0	525.0	525.0	525.0
Ether extract	630.0	630.0	630.0	630.0	630.0
Calcium	25.1	25.0	25.0	25.0	25.1
Available phosphorus	4.1	4.0	4.1	4.0	4.1
Sodium	1.8	1.8	1.8	1.8	1.8
Meth+Sist	7.6	7.6	7.6	7.6	7.6
Lysine	8.1	8.1	8.2	8.6	8.6
Threonine	6.4	6.4	6.4	6.5	6.5
Tryptophan	2.0	2.0	2.0	2.1	2.1
ME, kcal/kg**	2750	2900	2900	2900	2900

** Provided per kg of diet: retinol, 2.64 mg; cholecalciferol, 0.04 mg; dl-a-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, ME (kcal/kg) = 53+38 B used formula. B = (% Crude protein) + (2.25) (%Ether extract) + (1.1)(% Starch) + (% Sugar); Group control: C, Group sun flower oil: SFO, Group sun flower oil + milk thistle: SFO+MT, Group corn syrup: CS, Group corn syrup + milk thistle: CN+MT*

Table 2. *The chemical composition of milk thistle seed (%)*
Tablo 2. *Deve dikeni tohumunun kimyasal bileşimi (%)*

Chemical Composition	(%)
Dry Matter	94.29
Moisture	5.71
Crude protein	14.96
Crude fiber	31.3
Ether extract	25.48
Ash	5.63
Carbohydrates	16.92

for ten minutes and their plasma was used for Malonyl dialdehyde (MDA) determination. Remaining erythrocyte packages were washed with 0.9% NaCl 3 times and used for catalase (CAT) assay. MDA and GSH levels and CAT and GSH-Px activities were determined in liver, kidney, and heart tissues. While MDA level was determined based on the method of Placer et al.[8], GSH level was determined based on method of Chavan et al.[9]. While the method of Matkovics et al.[10] was used for measurement of GSH-Px activity, Aebi [11] method was used to measure CAT activity.

Statistical Analysis

After test of normality, all data including performance, egg quality, hatchability and oxidative stress parameters were subjected to one-way analysis of variance (anova). Significant differences were then subjected to Tukey HSD test. All analyses were performed by using Statistical Packages for the Social Sciences for Windows [12]. The results were considered as significant when P values were less than 0.05.

RESULTS

The hen-day egg production, feed intake, total egg weight, feed conversion and egg quality parameters of treatment groups are shown in *Table 3*. Total egg production was found to be significantly higher in SFO+MT and SFO groups of present study ($P<0.05$). There was not any significant difference in total egg production of the other control, CS and CS+ MT groups of the study. Feed intake, total egg weight and feed conversion were found similar among the groups ($P>0.05$). Some egg quality parameters in laying quails fedhigh-calorie diet are also shown in *Table 3*. The differences among the groups in albumen rate, yolk rate, dried shell rate and shape index were not statistically significant ($P>0.05$). The best yolk colour was obtained in SFO+MT and CS groups ($P<0.01$). C group had lower value in this parameter and SFO and CS+MT groups were similar to other groups ($P>0.05$).

The effect of dietary milk thistle seed on some hatchability traits in laying quails fedhigh-calorie diet are shown in *Table 4*. There were no statistically differences among the groups in hatchability, hatchabilitiy of fertile and embrionic mortality ($P>0.05$), but the difference in examined fertility were statistically significant ($P<0.05$). When fertility rate was examined, it was found that higher fertility rate was obtained in CS and CS+MT group. Addition of silybum marianum in to diet did not affect neither fertility rate nor other hatchability traits.

The effect of dietary milk thistle seed on oxidative stress parameters of different tissues in laying quails fed high-calorie diet are shown in *Table 5*. According to *Table 5*, MDA values of blood, liver and heart tissues were similar among the groups of study ($P>0.05$). MDA level of kidney significantly increased in CS group, however addition of milk thistle seed into diet was reduced MDA level of kidney

Table 3. *Effects on some performance and egg quality parameters of milk thistle (Silybum marianum) seed supplementation to high-calorie basal diets of quails*
Tablo 3. *Yüksek kalorili bıldırcın karma yemlerine deve dikeni tohumu (Silybum marianum) ilavesinin bazı performans ve yumurta kalite parametrelerine etkisi*

Performance Parameters	C	SFO	SFO+MT	CS	CS+MT	P-Significance
Hen-Day Egg Production (egg production/100 female birds/day)						
0-14	51.19±1.19	50.59±5.18	63.09±10.73	52.97±9.90	46.42±8.24	NS
15-28	55.95±6.76	81.54±8.05	77.38±10.40	70.83±9.83	64.88±12.45	NS
29-42	69.64±6.76	91.07±1.03	92.87±4.49	83.33±6.29	74.40±11.86	NS
43-56	78.57±9.16	89.28±1.03	89.23±2.72	85.11±4.16	85.71±7.14	NS
Total	63.83±3.58^{b}	78.12±2.60^{a}	80.65±5.51^{a}	73.06±4.39^{ab}	67.85±1.03^{b}	*
Feed Intake(g/bird/day)						
0-14	25.34±0.72	27.44±1.77	28.00±1.39	28.64±2.30	27.35±0.47	NS
15-28	29.93±1.40	30.83±0.65	32.00±0.38	32.04±0.45	30.85±0.28	NS
29-42	29.78±1.80	32.71±1.59	31.29±1.15	31.77±1.09	31.05±0.70	NS
43-56	30.11±2052	30.33±1.39	29.65±0.54	29.60±0.75	30.78±0.31	NS
Total	28.79±1.27	30.32±1.33	30.28±0.72	30.51±0.14	30.01±0.31	NS
Total Egg Weight (g)						
0-14	11.78±0.28	11.90±0.46	11.28±0.20	11.80±0.24	12.08±0.20	NS
15-28	11.65±0.67	12.20±0.13	11.47±0.16	11.92±0.21	12.20±0.25	NS
29-42	11.92±0.62	12.71±0.30	12.24±0.20	12.65±0.24	12.41±0.40	NS
43-56	11.92±0.40	12.71±0.62	12.24±0.30	12.65±0.20	12.41±0.24	NS
Total	12.16±0.09	12.99±0.62	12.38±0.29	12.75±0.05	12.50±0.27	NS
Feed Conversion (g feed intake x female number/egg production x egg weight)						
0-14	4.20±0.13	4.55±0.53	3.93±0.80	4.58±0.63	4.87±0.98	NS
15-28	4.59±0.30	3.09±0.07	3.60±0.42	3.79±0.34	3.89±0.81	NS
29-42	3.58±0.47	2.82±0.11	2.75±0.13	3.01±0.32	3.36±0.49	NS
43-56	3.21±0.06	2.67±0.10	2.71±0.05	2.73±0.22	2.89±0.14	NS
Total	3.70±0.16	2.98±0.13	3.03±0.30	3.27±0.28	3.53±0.26	NS
Egg Parameters						
Albumen rate (AR), %	52.52±0.85	51.91±0.32	51.98±1.28	53.03±0.49	52.13±0.29	NS
Yolk rate (YR), %	31.29±0.21	31.41±0.10	32.29±0.85	30.64±0.21	30.74±0.31	NS
Dried shell rate(DSR), %	8.08±0.06	7.93±0.03	8.10±0.16	8.21±0.06	7.86±0.07	NS
Shape index, (SI)	77.15±1.38	76.67±0.46	77.67±0.42	78.65±0.39	78.45±0.41	NS
Yolk colour	8.09±0.18^{b}	8.39±0.10^{ab}	8.78±0.16^{a}	8.75±0.11^{a}	8.65±0.15^{ab}	**

Data were given as Mean ± SEM, ***NS:*** *Not statistically significant; * P≤0.05, ** P≤0.01; ^{a,b} Mean values wirth different superscripts within a row differ significantly. AR = (Albumen weight/Egg weight)*100, YR = (YYolk weight/Egg weight)*100, DSR = (Shell weight/Egg weight)*100, SI = (Egg with/Egg length)*100*

Table 4. *Effects on some hatchability traits of milk thistle (Silybum marianum) seed supplementation to high-calorie basal diets of quails*
Tablo 4. *Yüksek kalorili bıldırcın karma yemlerine deve dikeni (Silybum marianum) tohumu ilavesinin bazı kuluçka özellikleri üzerine etkisi*

Hatchability Parameters	C	SFO	SFO+MT	CS	CS+MT	P-Significance
Fertility*(n/100 eggs set)*	73.95±3.91^{b}	78.32±3.42^{ab}	71.67±1.51^{b}	91.64±1.17^{a}	82.57±4.92^{a}	*
Hatchability*(commercial chicks/100 eggs set)*	57.30±4.61	62.19±3.87	58.21±6.64	77.27±6.74	64.25±3.28	NS
Hatchability of fertile*(chicks/100 fertile egg)*	78.48±9.71	79.97±7.56	80.91±7.76	84.18±6.51	78.74±8.09	NS
Embrionic mortality *(n/100 fertile eggs)*	21.50±7.72	20.01±5.56	19.08±5.62	15.80±4.51	21.24±6.15	NS

Data were given as Mean ± SEM, ***NS:*** *Not statistically significant; * P≤0.05, ^{a,b} Mean values wirth different superscripts within a row differ significantly*

Table 5. *Effects on oxidative stress parameters of milk thistle (Silybum marianum) seed supplementation to high-calorie basal diets of quails*
Tablo 5. *Yüksek kalorili bıldırcın karma yemlerine deve dikeni tohumu (Silybum marianum) ilavesinin oksidatif stres parametrelerine etkisi*

Stress Parameters	C	SFO	SFO+MT	CS	CS+MT	P-Significance
Blood						
MDA nmol/ml	1.70±0.15	1.84±0.14	1.68±0.07	1.85±0.10	1.46±0.07	NS
GSH mmol/g protein	39.96±1.07	43.69±3.31	37.10±1.52	35.46±2.98	40.01±2.31	NS
GSH-Px U/g Hb	72.68±5.46^{a}	73.81±4.06^{a}	60.69±2.50^{b}	59.18±3.32^{b}	61.75±2.65^{b}	**
CAT k/g Hb	16.66±1.30^{b}	22.42±1.18^{ab}	27.37±1.81^{a}	25.00±3.44^{ab}	26.65±2.15^{a}	*
Liver						
MDA nmol/g tissue	19.71±1.26	17.50±0.68	18.60±1.74	21.35±1.39	17.58±0.68	NS
GSH mmol/g protein	3.90±0.09^{b}	3.22±0.35^{b}	5.92±0.64^{a}	4.17±0.25^{b}	3.25±0.24^{b}	***
GSH-Px U/g protein	4.66±0.25	4.98±0.31	5.76±0.81	5.88±0.53	5.21±0.28	NS
CAT k/g protein	118.38±7.05^{b}	157.44±17.02^{ab}	151.42±6.86^{ab}	134.92±9.43^{b}	189.25±8.77^{a}	**
Kidney						
MDA nmol/g tissue	24.73±2.57^{b}	30.02±1.56^{b}	32.84±3.68^{ab}	43.40±4.05^{a}	24.96±1.20^{b}	**
GSH mmol/g protein	4.45±0.32^{a}	3.18±0.15^{bc}	2.65±0.06^{c}	4.09±0.40^{ab}	3.11±0.22^{bc}	***
GSH-Px U/g protein	4.86±0.24^{a}	3.94±0.20^{ab}	3.65±0.25^{ab}	3.93±0.51^{ab}	2.98±0.19^{b}	**
CAT k/g protein	68.77±2.40^{a}	50.89±3.37^{b}	53.51±3.51^{b}	56.05±3.56^{b}	53.42±1.35^{b}	**
Heart						
MDA nmol/g tissue	12.95±1.03	14.35±0.39	13.19±0.68	11.95±0.81	12.77±0.89	NS
GSH mmol/g protein	21.41±1.15^{a}	17.69±0.94^{ab}	20.05±1.00^{ab}	17.40±1.35^{ab}	16.26±1.31^{b}	*
GSH-Px U/g protein	34.96±2.27	32.97±3.48	37.15±3.84	30.96±4.05	26.08±2.12	NS
CAT k/g protein	46.51±2.07^{a}	45.61±4.28^{ab}	42.99±2.89^{ab}	32.95±2.90^{b}	38.89±2.77^{ab}	*

Data were given as Mean ± SEM, ***NS:*** *Not statistically significant; * P≤0.05; ** P≤.0.01; *** P≤0.001;* ***MDA:*** *Malondialdehyde,* ***GSH:*** *Glutathione,* ***GSH-Px:*** *Glutathione peroxidase,* ***CAT:*** *Catalase;* ^{a,b,c} *Mean values wirth different superscripts within a row differ significantly*

in CS+MT group (P<0.01). MDA levels of SFO groups slightly increased but not significant (P>0.05). The differences in GSH levels of blood, GSH-Px activities of liver and heart in the examined groups were not significant (P>0.05). GSH-Px activities of blood in control and SFO groups were higher than the other treatment groups (P<0.01), CAT activity of blood was found lower in control group (P<0.05). GSH level of liver in SFO+MT (P<0.001) and also CAT activity in CS+MT group were significantly higher (P<0.01). GSH levels and GSH-Px, CAT activities of kidney (P<0.01), in addition, CAT activities of heart were higher in control group (P<0.05). Addition of milk thistle seed into diet significantly reduced GSH level (P<0.001) and GSH-Px (P<0.01) activity of kidney and also GSH level of heart (P<0.05).

DISCUSSION

Table 3 also shows the effect of MT supplementationto diet on some performance and egg quality traits in laying quails fed with high calorie diets. When total hen-day egg production was examined, it was determined that egg production significantly increased in SFO+MT and SFO groups. Even though high calorie diets increased egg production compared to C group, this increase was found to reach significant levels in oil groups. While acquired egg production values were similar to those reported by Kaplan et al.[13] and Silici et al.[14], they were lower than values reported by Yörük et al.[15], Kaplan et al.[16], and Erişir et al.[17]. It was determined that addition of MT did not make any contribution to egg production. The fact that egg production was higher in oil added groups compared to CS group was associated with extra-caloric and extra-metabolic effect of oils [18]. Different from this study, in the study of Midilli et al.[19] it was reported that addition of 1.5% and 3% SFO into basal diet did not affect egg production. Daily feed consumption in study groups varied between 28.78 g and 30.51 g, and the difference between the groups was statistically insignificant *(Table 3)*. While feed consumption was expected to decrease depending on energy content in groups consuming high calorie diet Griffiths et al.[20] similar results were achieved in all groups of this study and thus it was determined that quails use the extra energy they consumed for egg production and accordingly egg production increased in parallel with energy consumption. Furthermore, the fact that they used extra energy, they produced due to extra-caloric and extra-metabolic effects of oils, for egg production did not caused a significant difference in feed consumption also in these groups. While the obtained feed consumption values were similar to those reported by Silici et al.[14] and Erişir

et al.[17], they were higher than those reported by Kaplan et al.[13] and Kaplan et al.[16] and lower than values reported by Yörük et al.[15]. In their study, Midilli et al.[19] did not found any difference between control group and groups with addition of 1.5% and 3% SFO in terms of daily feed consumption. Some related studies have revealed that addition of essential oils into basal diets increased feed consumption [21,22]. Egg weigh varied between 12.16 and 12.99 g in the study. There was no difference between the groups in terms of egg weight. While the studies Whitehead et al.[23] and Lelis et al.[24] have emphasized that essential oils added into diet increase egg weight due to essential fatty acids they contain, it was thought that the increase in number of eggs in these groups is a factor inhibiting the increase of egg weight. Egg weight values found in this study were similar to egg weight values reported by Silici et al.[14] Yörük et al.[15] and Karabayır et al.[25] and were higher than those reported by Kaplan et al.[13], Kaplan et al.[16] and Erişir et al.[17]. Feed conversion values in the study varied between 2.98 and 3.70. Despite a significant difference in egg production, no difference was found between the groups in terms of feed conversion. This result is thought to be caused by small differences in parameters of egg weight and feed consumption which are effective in the calculation of feed conversion ratio. While acquired feed conversion ratios were found similar to values reported by Kaplan et al.[13] Silici et al.[14] Yörük et al.[15] and Erişir et al.[17] they were higher than those reported by Kaplan et al.[16]. When some parameters belonging to egg quality traits in the study were examined, it was determined that the groups were similar in terms of albumen, yolk, and shell rates, and shape index and there was a significant improvement in all study groups regarding color of yolk compared to C group. It could be asserted that addition of MT into diet increased color of yolk. This situation can vary depending on xanthophyll or carotene contents of supplements used [26].

Table 4 illustrates the effect of milk thistle supplementation to diet on some hatchability traits in laying quails fed with high calorie diet in the study. As is seen in *Table 4*, a significant difference was determined between the groups in terms of fertility. In CS and CS+MT groups, fertility was found to be the highest. It was seen that addition of MT into ration decreased the fertility rate from 78.32% determined in SFO group to 71.67% in SFO+MT group and the fertility rate from 91.64% determined in CS group to 82.57% in CS+MT group. The fertility rates determined in the study was lower than fertility rates reported by İpek et al.[27] and Silici et al.[14]. Similarity was found between the groups in terms of hatchability, hatchability of fertile eggs, and embryonic mortality in the study. Hatchability and hatchability of fertile eggs found in the study was lower than values reported by Silici et al.[14] and İpek et al.[27]. Embryonic mortality rate was determined to be quite higher than embryonic mortality rate reported by İpek et al.[27]. In this study, fertility increased in groups fed with high calorie diets and especially in CS group. This result supports the result that high energy has a challenging effect on fertility [28]. It was thought that while sharing of energy in oil groups was towards egg production more, it promoted mating and sperm quality in sugar groups. Additionally, it was observed that MT added into diet did not have a positive effect on fertility and hatchability traits. Being different from this study, Şimşek et al.[28] emphasized that oils of cinnamon and rosemary added into ration increased fertility depending on their bioactive characteristics, some active components or mineral and vitamin contents.

Oxidative stress may be caused by excess Reactive oxygen species (ROS) production and/or deficient antioxidant capacity [29,30]. ROS produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation, and DNA damage. As a result of the degradation of lipid peroxides, MDA forms and is used as an indicator for lipid peroxidation [31]. In this study, CS caused oxidative stress with statistically significant increase of MDA and decrease of antioxidants (GSH, GSHPx, CAT) in kidney tissue of quails. On the other hand, SFO caused a statistically insignificant increase in MDA and decreases in antioxidants in kidney. Liu et al.[32] reported that high concentration of poly-unsaturated fatty acid in the diet made membrane susceptible to peroxidative degradation and increased oxidative stress. In a study conducted similar to our results, insignificant differences was found in serum MDA of ducks fed with SFO diet [32]. It is known that *Slybum marianum* extracts and active substances like *silymarin* show antioxidant characteristics and inhibit oxidative stress in various tissues such as liver, kidney, brain of rats [5,33-35]. It was determined that it increased in kidneys of diabetic rats; while the use of milk thistle extracts and *silymarin* decreased Thiobarbituric acid reactive substances (TBARS) values in a statistically significant way, the decreased SOD, CAT, and GSHPx activities increased [34,36]. In this study, the use of both CS and MT enabled MDA levels to become normal in kidney. The fact that MDA level return to normal with addition of MT indicated that the addition of MT is beneficial for kidney. However, since antioxidants levels were lower in CS+MT and SFO+MT groups, antioxidants could remain insufficient in case of using MT longer than 60 days. Silybin and silymarin are effective in returning the increased TBARS and lipid hydroperoxide levels, caused by Arsenic, Gentamicin, and Aflatoxin in kidney, and the decreased nonenzymatic and enzymatic antioxidants to normal, and are suggested for decreasing nephrotoxicity [29,31,33]. According to results of this study, we can suggest that MT is nephroprotective in poultry as well due to its antioxidant characteristics.

Consequently, high-calorie diets could be used to obtain higher egg production and fertitility rate in quail production. However these diets caused changes in anti-

oxidants in blood and tissues in the birds. Particularly, in quails feeding with corn syrup significantly increased lipid peroxidation in kidney. Supplementation of milk thistle seed into corn syrup groups may protect kidney against free radical damage, on the contrary the additive was not able to any success on performance and hatchability parameters.

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