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## The evaluation of dietary application of conjugated linoleic acid on performance, egg quality, blood parameters, antioxidant capacity and egg yolk cholesterol parameters in layer quails

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**ABSTRACT:** Conjugated linoleic acid (CLA) is a poly unsaturated fatty acid (FA) which is accepted as favorable for human health. The aim of this study is to ascertain the effects of CLA on performance, egg quality traits, egg yolk and albumin pH levels, blood serum biochemical parameters, egg yolk cholesterol content and blood antioxidant capacity in layer quails. 96 7-weeks-old female Japanese quails divided to 4 groups with 6 subgroups and the groups fed with basal diet supplied with 0 g/kg, 10 g/kg, 20 g/kg and 30 g/kg CLA respectively for 8 weeks. Our results indicated that CLA supplementation did not statistically affect performance, egg quality traits, egg pH levels, total antioxidant capacity in blood serum and yolk cholesterol content. Although dietary CLA did not significantly differ between groups for blood serum total protein, glycose, total cholesterol, triglyceride and high-density lipoprotein; lipoprotein lipase levels were significantly decreased in CLA supplemented groups ( $p<0.05$ ). In summary, our results have shown dietary CLA supplementation might affect lipid metabolism and enzyme activity in female Japanese quails.

**Keywords:** Conjugated linoleic acid, layer quail, performance, antioxidant, cholesterol

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## INTRODUCTION

Conjugated linoleic acid (CLA) which is an aggregate term for a blend of geometric (*cis, cis*; *cis, trans*; *trans, cis*; and *trans, trans*) and positional isomers of linoleic acid (12:2n-6;LA) with conjugated double bond system which can be either *trans* or *cis* configuration (Hur et al., 2007; Shokryazdan et al., 2017). Since its discovery in 1980s, many studies have also been focused on to investigate the benefits of CLA to human health such as immune modulation, fat mobilization in body, anticarcinogenic and antiatherogenic effects (Suksombat et al., 2006; Liu et al., 2016). It's estimated that a human weight around 70 kg should consume minimum 3 g of CLA/d to benefit its health favorable effects (Ahn et al., 1999). CLA is found in many animal products as beef and dairy, pork, marine products, poultry products and vegetable oils besides synthetically produced ones (Hur et al., 2007). Although the main natural resource of CLA is ruminant meat and milk, several researchers stated meat and eggs of poultry could be enriched with CLA by dietary supplementation (Du and Ahn, 2002; Bölükbaşı, 2006). Since CLA readily integrated into the fat fraction, poultry eggs stand out in this regard due to the yolk's 30-35 % lipid content (Suksombat et al., 2006).

In animal nutrition one of the main goals is to provide sustainable healthy and good quality livestock products for human consumption. CLA might be consider as a potential feed additive to reach this target due to its beneficial effects on human health along with cholesterol reducing and antioxidant activity in livestock metabolism (Hur et al., 2007). Ha et al. (1990) mentioned that  $\beta$ -hydroxyl acrolein moiety in CLA structure might be the reason of its antioxidative effect. Several studies have showed CLA had improved oxidative stability in mice (Ha et al., 1990), broiler chickens (Ko et al., 2004; Zhang et al., 2008a) and pigs (Joo et al., 2002). Although several researches have shown that dietary CLA supplementation decrease blood lipid parameters in rats (Kloss et al., 2005), geese (Zhang et al., 2008b) and broilers (Bölükbaşı, 2006), the cholesterol reducing effect of CLA is unclear for egg yolk. Hur et al., (2003) demonstrated that 1, 2.5 and 5% of dietary CLA supplementation significantly decreased cholesterol content in egg yolk after 5 weeks of feeding trial in layers. On the other hand, Szymczyk and Pisulewski, (2003) stated no difference observed on cholesterol level of yolk in commercial layers that fed with diets comprised 0, 5, 10, 15 or 20 g pure CLA/kg. Different

from those studies, Yin et al., (2008) observed CLA supplementation on White Leghorn and Brown Dwarf layer diets increased yolk cholesterol levels except for 2.5 % CLA in Brown Dwarf layers.

Quail eggs come to the forefront among the poultry eggs due to their great nutritional value, rich vitamin and mineral content (Tunsaringkarn et al., 2013). Studies on potential effect of CLA on layer and broiler quails are very limited. Aydin (2007) stated giving CLA alone with diet could create adverse effects on layer quails, and combination of CLA with 5% and 10% canola protect quail eggs against CLA-related pH changes in egg yolk by preserving the ratio of total unsaturated fatty acids and total saturated fatty acid. Aydin et al., (2006) reported that dietary 0.5% CLA supplementation significantly decreased body weight at the end of the study yet statistically improved breast muscle in male Japanese quail. Aydin and Cook (2004) observed 0.25 % dietary CLA supplementation increased egg size, yet 2 or 3% CLA had reduced egg proportions in Japanese quails. In the same study, all experiment groups except for the group fed with 0.25 % CLA had bigger liver sizes. No study has been conducted on effect of egg quality, antioxidant capacity and cholesterol content of egg yolk in layer quails.

In light of the above, the purpose of this study was to determine the outcomes of graded dietary levels of CLA (0, 10, 20 and 30 g/kg) on performance, egg quality, serum biochemical parameters, blood antioxidant capacity and egg cholesterol levels in layer Japanese quails.

## MATERIALS AND METHODS

### Birds, experimental diets and management

This research was approved by Selcuk University Faculty of Veterinary Ethics Committee of Experimental Animals Production and Research Center (2020/21). Furthermore, all procedures conducted in this study complied with the European directives for the use and care of animals in research (Directive 2010/63/EU).

A total of 96 7-weeks-old female Japanese quails (*Coturnix coturnix Japonica*) were split to 24 cages and divided into 4 experimental groups with 6 replicates non-specifically. The quails had *ad libitum* access to feed and water on 16 hours light and 8 hours dark program per a day. The animals fed with a commercial diet based on corn/soybean formulated to be isocaloric and isonitrogenous (Table 1) was manu-

factured in a local feed mill. CLA used in the study provided from a commercial company (Lutrell® Pure, BASF). The CLA supplement was a complex of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 18:2 isomers (11.8% of total FA composition was the *cis*-9, *trans*-11 isomer of CLA, 12.1% of total FA was the *trans*-10, *cis*-12 isomer of CLA, 16.5% of total FA was C16:0, 46.0% was C18:0 and 9.8% of total FA was C18:1 of the total fatty acid components) protected by the lipid encapsulation technique. The first group (Control) fed with a basal diet without supplementation. The experimental groups received 5 g/kg, 10 g/kg and 20 g/kg CLA supplementation in their diet respectively. During the trial, eggs were collected on a daily basis and weighed with 0.01 g scales to record average egg weight of each subgroup. In every two weeks, the quails weighed one at a time and feed intake of all birds in the cage were measured.

**Table 1.** Ingredients and calculated composition of the basal diets

Ingredient	%
Corn	50.60
Soybean Meal	25.62
Sunflowers Meal	10.50
Vegetable Oil	4.90
Limestone	6.00
Dicalcium Phosphate	1.56
Salt	0.40
Premix <sup>1</sup>	0.35
L-Lysine	0.25
DL-Methionine	0.22
<b>Total</b>	<b>100.00</b>
Chemical Composition	
Metabolizable Energy (kcal/kg)	2913
Crude Protein (%)	20.31
Ca %	2.72
Available P %	0.40
Lysine %	1.19
Methionine %	0.51

<sup>1</sup>Premix contains: Manganese: 60 mg; Zinc: 50 mg; Iron: 30 mg; Copper: 5 mg; Selenium: 0.1 mg; Vitamin A: 8.800 IU; Vitamin D3: 2.200 IU; Vitamin E: 11 mg; Nicotine acid: 44 mg; Cal-D-Pan: 8.8 mg; Riboflavin: 4.4 mg; Thiamine: 2.5 mg; Vitamin B12: 6.6 mg; Folic acid: 1 mg; D-Biotin: 0.11 mg; Coline: 220 mg.

### Egg quality characteristics, pH levels and yolk cholesterol determination

6 eggs were picked up from each subgroup for 3 days at the last week of the experiment. Each egg was weighed and an egg force reader (Egg Tester, Orka Technology, UT, USA) was used to determine egg shell strength. All collected eggs were broken on a flat glass plate a digital caliper was used to gauge the

length and width of albumen and diameter of yolk. The obtained values applied to calculate albumen index [(albumen height/average of albumen length and albumen width) x 100], yolk index [(yolk height/yolk diameter) x 100], and Haugh unit [(100 x Log(H + 7.57 - 1.7W<sup>37</sup>), in the formula H means albumen height, W means egg weight, were calculated.

36 eggs from each group were gathered during the 8<sup>th</sup> week of the study to determine egg yolk and albumen pH levels. The collected eggs were stored in a refrigerator for 14 days at +4°C. Albumens and yolks of the eggs were separated and stirred at 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> day of the storage. A digital pH meter (Hanna Instruments, USA) was used to determine yolk pH and albumin pH of the eggs.

The cholesterol level of quail egg yolk was measured by using the methods described by Hammad et al. (1996) and Kaya et al. (2003). 3 eggs were randomly picked up from each subgroup at the end of the study to determined yolk cholesterol. The collected eggs were hard boiled for 5 minutes. 0.1 g egg yolk samples were separated and blended with 4 ml isopropyl alcohol. The samples were centrifuged at 3000 rpm for 10 min. cholesterol level in the quail egg yolk was measured with using of a commercial cholesterol kit (Siemens Healthcare Diagnostics, Marburg, Germany).

### Serum biochemical and total antioxidant capacity examination

One quail was slaughtered at the end of 8 weeks' trial. Blood samples for the biochemical tests were collected from jugular vein and centrifuged at 3000 rpm for 10 min and stored at -24°C. Blood serum samples were measured to determine total cholesterol, total protein, glucose, lipase and high-density lipoprotein (HDL) with commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey). Total antioxidant capacity of blood serum was also measured with a commercial kit of same company.

### Statistical analysis

Results are given as mean ± standard deviation. Performance, egg quality traits, egg pH levels, serum biochemical parameters, serum antioxidant capacity and egg yolk cholesterol data were analyzed by ANOVA in Minitab (2000). The significant differences between groups were determined by using Duncan post hoc test. Differences with values of *P* < 0.05 were considered to be statistically significant.

## RESULTS

The effects of dietary CLA treatments on performance of layer quails are shown in Table 2. The changes in egg production, live weight gain, feed intake, egg weight and feed conversion rate between control and treatment groups were not statistically significant in the present experiment ( $p>0.05$ ). On the other hand, the average egg weight was not significantly but numerically higher in the quails fed with 10 g/kg CLA supplementation than control and other treatment groups.

Table 3 illustrates the effects of different levels of CLA on eggshell breaking strength, Haugh unit, albumin index, yolk index and egg shape index of quail eggs. No significant differences between control group and treatment groups regarding the egg quality trait by feeding with CLA-enriched diets to Japanese quails ( $p>0.05$ ).

Data illustrating the effect of dietary CLA supple-

mentation in different levels on the egg pH values in 1, 15 and 30 days after the experiment are presented in Table 4. The CLA intake in Japanese quails did not differ in pH levels significantly in our study ( $p>0.05$ ).

The results in Table 5 show the effect of dietary CLA on blood serum biochemistry status. Total protein, glucose, total cholesterol, triglyceride and HDL values didn't significantly differ between the groups because of CLA supplementation ( $p>0.05$ ). On the other hand, Lipoprotein lipase decreased significantly in the groups fed with CLA enriched diets ( $p<0.05$ ).

The effect of dietary CLA supplementation on blood serum total antioxidant capacity in Japanese quails are shown in Figure 1. CLA intake didn't differ total antioxidant capacity in blood serum between control and treatment groups ( $p>0.05$ ). Data in Figure 2 demonstrates the effect of dietary CLA supplementation in different levels on egg yolk cholesterol content. Yolk cholesterol levels weren't affected by dietary CLA ( $p>0.05$ ).

**Table 2.** The effect of dietary CLA supplementation on production performance in Japanese quails.

Parameters	CLA0	CLA5	CLA10	CLA20	P-Value
Egg production (%)	77.59±1.92	73.59±3.45	77.87±2.69	80.43±2.81	0.395
Body weight gain (g)	42.50±6.57	47.08±2.79	40.71±2.31	39.42±4.32	0.623
Feed Intake (g)	26.52±0.46	25.93±0.55	26.61±0.53	25.22±0.18	0.148
Egg weight (g)	11.44±0.32	11.72±0.18	14.22±1.81	11.73±0.10	0.150
Egg mass (g/bird per day)	9.63±0.41	9.00±0.34	10.10±0.55	9.45±0.20	0.295
Feed conversion ratio (g/g)	2.77±0.08	2.90±0.11	2.66±0.09	2.67±0.05	0.192

CLA0, the basal diet; CLA5, 5 g CLA/kg diet; CLA10, 10 g CLA/kg diet; CLA20, 20 CLA/kg diet. Values are expressed as mean ± SE

**Table 3.** The effect of dietary CLA supplementation on egg quality traits in Japanese quails.

Parameters	CLA0	CLA5	CLA10	CLA20	P-Value
Eggshell breaking strength (N)	1344.87±52.05	1373.57±55.95	1396.62±38.76	1317.53±57.40	0.757
Haugh unit	87.59±0.71	86.11±0.72	86.91±0.60	85.66±1.34	0.493
Albumen Index	4.72±0.15	4.43±0.16	4.79±0.15	4.63±0.13	0.384
Egg yolk index	45.08±0.60	44.83±0.54	45.67±0.66	45.69±0.53	0.744
Egg shape index	75.84±0.49	76.86±0.55	76.94±0.69	77.26±0.63	0.598

CLA0, the basal diet; CLA5, 5 g CLA/kg diet; CLA10, 10 g CLA/kg diet; CLA20, 20 CLA/kg diet. Values are expressed as mean ± SE

**Table 4.** The effect of dietary CLA supplementation on egg quality traits in Japanese quails at 1<sup>st</sup>, 15<sup>th</sup> and 30<sup>th</sup> days after the study

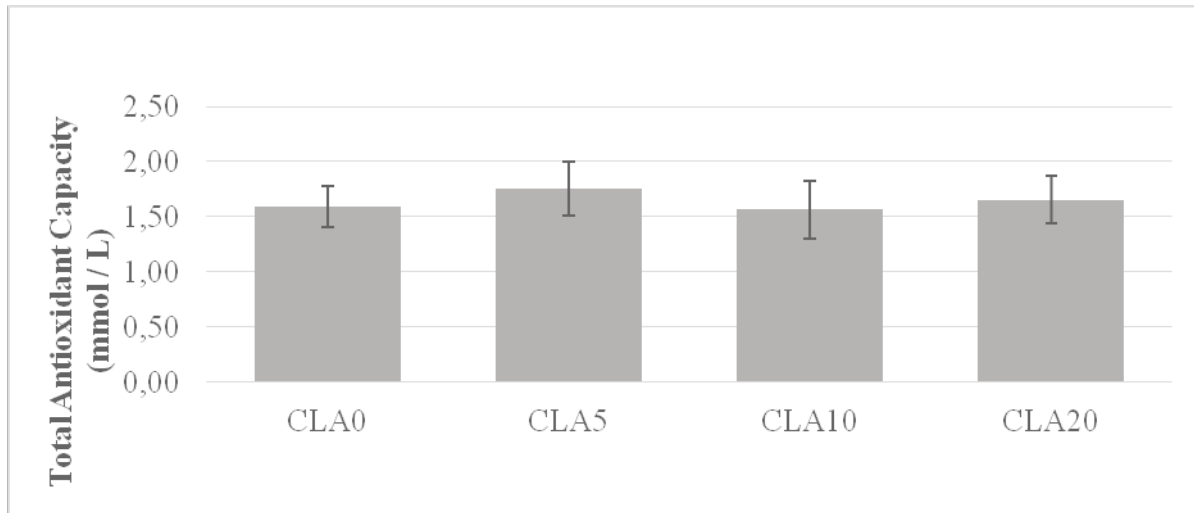
Days	Parameters	CLA0	CLA5	CLA10	CLA20	P-Value
1 <sup>st</sup> Day	Yolk pH	5.95±0.22	5.88±0.06	5.78±0.56	5.88±0.10	0.548
	Albumen pH	8.84±0.15	8.84±0.16	8.86±0.20	8.88±0.10	0.884
15 <sup>th</sup> Day	Yolk pH	6.27±6.27	6.26±0.17	6.23±0.23	6.35±0.24	0.547
	Albumin pH	9.16±0.05	9.11±0.12	9.15±0.04	9.12±0.13	0.549
30 <sup>th</sup> Day	Yolk pH	5.89±0.36	5.26±0.25	5.61±0.32	5.83±0.24	0.532
	Albumin pH	8.27±0.30	8.69±0.16	8.19±0.26	8.65±0.10	0.386

CLA0, the basal diet; CLA5, 5 g CLA/kg diet; CLA10, 10 g CLA/kg diet; CLA20, 20 CLA/kg diet. Values are expressed as mean ± SE

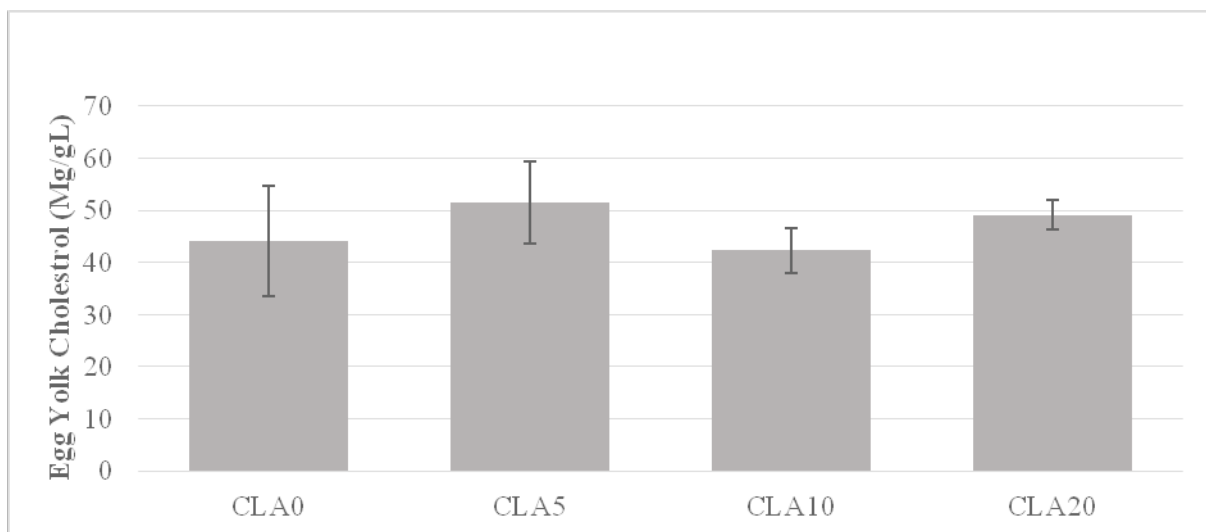
**Table 5.** The effect of dietary CLA supplementation on blood serum biochemical parameters in Japanese quails.

Parameters	CLA0	CLA5	CLA10	CLA20	P-Value
Total protein (g/dl)	3.40±0.18	3.70±0.20	3.26±0.26	3.35±0.16	0.471
Glycose (mg/dl)	345.92±10.54	344.67±11.69	325.33±10.36	348.33±6.75	0.351
Total cholesterol (mg/dl)	221.42±18.87	232.00±11.47	200.42±11.77	211.33±15.13	0.470
Triglyceride (mg/dl)	657.00±3.34	649.67±4.83	657.00±62.71	665.67±5.94	0.479
HDL (mg/dl)	166.25±42.46	230.56±44.34	207.30±43.69	197.40±43.45	0.765
Lipoprotein lipase(U/l)	15.08±0.89 <sup>b</sup>	12.00±0.46 <sup>a</sup>	11.50±0.38 <sup>a</sup>	12.08±0.50 <sup>a</sup>	0.0003

CLA0, the basal diet; CLA5, 5 g CLA/kg diet; CLA10, 10 g CLA/kg diet; CLA20, 20 CLA/kg diet. Values are expressed as mean ± SE. <sup>a-b</sup> Means within a row with unlike superscripts are different ( $p < 0.05$ )



CLA0, the basal diet; CLA5, 5 g CLA/kg diet; CLA10, 10 g CLA/kg diet; CLA20, 20 CLA/kg diet.

**Figure 1.** The effect of dietary CLA supplementation on blood serum total antioxidant capacity in Japanese quails.

CLA0, the basal diet; CLA5, 5 g CLA/kg diet; CLA10, 10 g CLA/kg diet; CLA20, 20 CLA/kg diet.

**Figure 2.** The effect of dietary CLA supplementation on egg yolk cholesterol content in Japanese quails



## DISCUSSION

CLA supplementation to the diets of layer Japanese quails didn't affect the animals' performance in the present experiment ( $p>0.05$ ). Our results agree with previous reports that have reported no detrimental effect on performance of laying hens (Meluzzi et al., 2003; Shang et al., 2004; Alvarez et al., 2005; Kim et al., 2007; Koronowicz et al., 2016) and quails (Aydin et al., 2006) fed with CLA. Besides several researchers informed that layer hens fed with CLA enriched diets also had adverse effect on egg weight, egg production, feed consumption and feed conversion rate than the animals fed with basal diet (Ahn et al., 1999; Szymczyk and Pisulewski, 2003; Shang et al., 2004; Yin et al., 2008). It has been reported that certain amounts of CLA could be an intense inhibitor of body fat accumulation, therefore CLA might decrease feed intake and feed conversion rate in mice rats and chickens (Szymczyk et al., 2001). In our study, the average egg weight of the quails fed with 10 g/kg CLA supplementation was higher than the control and other treatments numerically. Dietary CLA applications have been reported to reduce egg weight and egg production in some research in poultry (Suksombat et al., 2006; Yin et al., 2008). Contrarily, Aydin et al., 2006 reported that dietary CLA at levels of 0.5 % improved average egg weights statistically at 3<sup>rd</sup> and 4<sup>th</sup> weeks of their experiment in Japanese quails. These might be due to promoting effect of certain amount of dietary CLA supplementation on thyroid hormone and estradiol which affect positively on egg weight and performance (Liu et al., 2016).

Egg quality traits were not affected with the treatments compared to the control group ( $p>0.05$ ). Similar to our findings, Vashan et al. (2008) couldn't also report any significant differences between the layer hens fed with 0 %, %4 and %10 safflower oil, rich with linoleic acid. Furthermore, other researchers have mentioned dietary CLA treatments were ineffective on egg Haugh unit in hens (Suksombat et al., 2006; Liu et al., 2016). Whereas, Kim et al. (2007) recorded hens fed with 2 CLA-enriched diet had lower Haugh units but higher egg yolk index than control and other CLA treatments. (Alvarez et al., 2005) also noted dietary %2 CLA supplementation showed highest values in albumen height and Haugh unit in hens. The egg quality alterations in the hens fed with %2 CLA enriched diet might be related with the changes in yolk water content and fat composition in vitelline membrane by virtue of ion movement through the membrane (Alvarez et al., 2005; Kim et al., 2007).

pH of yolk and albumin are important measures for egg quality in poultry sector along with Haugh Unit, albumin and yolk index (Lee et al., 2016). No effect of dietary CLA concentration on pH levels of yolk and albumin were observed during storage at +4°C for 30 days ( $p>0.05$ ). Correlatively, Alvarez et al. (2005) also informed CLA addition to hens' diet didn't statistically affect albumen and yolk pH. Whereas, it has been reported that discoloration of egg yolk and albumen or undesirable pH changes can be seen when the chicken were fed with CLA, supplemented with low fat diets. Probable cause of this result could be modification of the levels of mono-unsaturated fatty acids and saturated fatty acids in the eggs of animals fed with CLA-enriched diets (Aydin, 2007). Another study on hen eggs indicated that yolk pH was higher in the groups fed with CLA diets than control group (Ahn et al., 1999). In the related study, it is estimated that the ion movement from yolk and albumen through the yolk membrane in the eggs of hens fed with CLA was higher than control as a possible cause of elevated pH in yolk. This might be a result of *cis-trans* arrangements of CLA that increased permeability of yolk membrane.

From our observation, no significant differences were observed in blood serum biochemical parameters ( $p>0.05$ ) except for Lipoprotein lipase ( $p<0.05$ ). In addition, HDL levels in blood serum were not statistically significant, but numerical higher in the layer quails fed with CLA supplementation. Dietary CLA supplementation is accepted to have plasma and abdominal lipid lowering effects by inhibiting endogenous fatty acid production in the body (Lin et al., 2001). Several researches also demonstrate dietary CLA supplementation have increased blood HDL content in broiler (Szymczyk et al., 2001; Bölükbaşı, 2006) and layer chickens (Yin et al., 2008; Wang et al., 2019) and geese (Zhang et al., 2008b). Japanese quails submitted to CLA in our study had lower lipoprotein lipase activities in blood serum than the control group. Lipoprotein lipase is an extracellular enzyme that is synthesized mostly in skeletal muscle and adipose tissues. The enzyme mostly hydrolyze triglycerides in the structure of chylomicrons and lipoproteins to glycerols and free fatty acids (Zhang et al., 2007). The product used in the study contains *trans*-10 *cis*-12 isomers of CLA acknowledged to reduce lipid accumulation in adipocytes by inhibiting lipoprotein lipase activity in metabolism (Kim et al., 2002). Similar to our findings, there are several studies noted lipoprotein lipase inhibiting effect of CLA

in broilers (Zhang et al., 2007), hamsters (Zabala et al., 2006) and cultured 3T3-L1 adipocytes (Lin et al., 2001).

In the present study, CLA intake didn't statistically differ blood serum TAS concentrations between groups ( $p>0.05$ ). The effect of dietary CLA to antioxidant defense system in the metabolism is still not clear (Zhang et al., 2008a). Several studies have also shown dietary CLA decreased lipid peroxidation in broiler meat (Zhang et al., 2008a; Liu et al., 2017) and hen eggs (Hur et al., 2003). Zhang et al., (2008a) indicated dietary CLA could show antioxidant effect both boosting the antioxidant enzyme activity and inhibiting the reactive oxygen species chain reactions. On the contrary, Ko et al. (2004) reported broiler chickens fed with the diet containing 1.5% CLA improved hepatic catalase activity significantly but other antioxidant enzymes were not affected. Leung and Liu (2000) reported that despite, *trans*-10, *cis*-12-CLA isomer indicated to have stronger free radical scavenging capacity, *cis*-9, *trans*-11-CLA could have prooxidant properties. The reason that we couldn't find any antioxidant effect of dietary CLA supplementation in the study might be due to the balance of the *trans*-10, *cis*-12-CLA (12.1% of total fatty acid components) and *cis*-9, *trans*-11-CLA (11.8% of total fatty acid components) in the product.

In our study, dietary CLA at the level of either 10, 20 and 30 mg/kg in diet didn't induce alterations in egg yolk cholesterol content ( $p>0.05$ ). The effects of

dietary CLA intake on egg yolk cholesterol levels are debatable. Similar to our results, Szymczyk and Pi-sulewski (2003) also reported CLA supplementation in layer diets didn't differ in egg yolk cholesterol level between groups. Wang et al. (2019) indicated that CLA addition didn't modify the levels of egg yolk cholesterol on day 28, yet caused a reduction in yolk cholesterol content on day 56. Besides, Hur et al. (2003) added the level of egg yolk cholesterol was decreased significantly in the groups fed with CLA supplemented diets for 5 weeks in layers. Yin et al., (2008) reported dietary CLA intake caused an elevation of yolk cholesterol amount in different breed of layer hens. The discrepancies between the outcomes of researches might be due to fatty acid composition CLA, experimental conditions, doses in diet or animal type.

## CONCLUSION

The present study gave some evidence that dietary CLA supplementation might affect lipid metabolism and enzyme activity in female Japanese quails. More detailed studies are needed on correlation between metabolic lipid metabolism and egg lipid profile after dietary CLA supplementation in layer quails. Further studies should also aim to investigate the immunostimulant effects of CLA in poultry.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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