Omega-3 fatty acids reduce the negative effects of dexamethasone-induced physiological stress in laying hens by acting through the nutrient digestibility and gut morphometry

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ABSTRACT In this study, the effects of omega-3 fatty acids on egg production, nutrients digestibility, eggs yolk lipid peroxidation, and intestinal morphology in laying hens under physiological stress were investigated. Ninety-six 35-wk-old Lohmann LSL-Lite laying hens were used in 2×3 factorial arrangement with 2 levels of dexamethasone (**DEX**) (0 and 1.5 mg/kg of the diet) and 3 levels of omega-3 fatty acids (0, 0.24, or 0.48% of the diet) in a completely randomized design. At 41 wk of age, the stress groups were continuously fed with a DEX 1.5 mg/kg diet for 1 wk. Egg production, egg mass, feed intake, egg weight, and feed conversion ratio were recorded. In addition, the AME, digestibility of CP, crude fat (**CF**), and organic matter were measured during the stress induction period. At the end of 41 wk of age, malondialdehyde and cholesterol concentrations in the egg yolk and intestinal morphology were investigated. The results showed that egg production, egg mass ($P < 0.0001$), egg weight ($P = 0.043$), and BW ($P = 0.0005$) were lower in DEX layers. Feed intake was reduced by the interaction between DEX and omega-3 fatty acid ($P = 0.042$). Malondialdehyde value ($P = 0.002$) and cholesterol concentration ($P = 0.001$) in egg yolk increased by DEX administration. The combination of DEX administration and omega-3 fatty acids supplementation was found in the indices of intestinal morphology such as villus height and width and crypt depth ($P < 0.05$). Administration of DEX decreased the CP digestibility ($P < 0.0001$) and AME ($P = 0.006$). Digestibility of CF and AME in the group of 0.48% omega-3 fatty acids were higher ($P < 0.05$) than those of 0 and 0.24%. In conclusion, we found that dietary omega-3 fatty acids had beneficial effects on gut morphology and nutrient digestibility in laying hens under physiological stress. However, they could not alleviate the negative effects of physiological stress on performance.

Key words: digestion, egg yolk, glucocorticoid, laying hen, omega-3 fatty acid

INTRODUCTION

Some internal and external factors including high density, climate changes, nutritional constraints, fear, rapid growth rate, infection, and metabolic diseases can create chronic stress in birds (Breuner, 2011). Exposure to stressors, through a cascade mechanism, activates the physiological stress response (Romero, 2004). In this case, the hypothalamus–pituitary–adrenal (**HPA**) axis is activated, which leads to a rise in the level of corticosteroids that regulates metabolism. Corticosteroids are associated with endocrine changes, such as increased gluconeogenesis, mobilization of energy stores, and behavioral responses including inhibiting reproductive activities and increasing anxiety (Sapolsky et al., 2000). Transmission of the information concerning environmental conditions to the hypothalamic–pituitary–gonadal axis affects egg laying (Wang et al., 2017), oviduct weight, and growth rates at the end of this process (Hull et al., 2007). In addition to the role of stress on productive performance, it also can influence the digestive function. In this regard, Puvadolpirod and Thaxton (2000) showed that adrenocorticotropin hormone markedly decreased protein and carbohydrate digestibility of chickens which seems to correlate with their intestinal function.

Omega-3 and omega-6 fatty acids are the main constituents of the polyunsaturated fatty acid family as

well as the major components of cell membrane phospholipids of vertebrates. They play a primary role in various tissues, especially in brain development and function (Stillwell and Wassall, 2003). Moreover, they contribute to neurologic and psychological functions (Dacks et al., 2013).

Omega-3 fatty acids contribute to preventing violence and aggressive behaviors in human (De Vriese et al., 2004). Serotonergic system and HPA axis have important roles in the physiological stress responses, and omega-3 fatty acids reduce physiological stress responses through functional changes in the serotonergic system in rats (Du Bois et al., 2006) or the HPA axis in humans (Coiro et al., 2007)

In previous studies, different sources of omega-3 fatty acids have been used to improve the fatty acid profile of egg yolks in laying hens (Ao et al., 2015; Hoan and Khoa, 2016) and performance and immune response of broilers (Ibrahim et al., 2018).

As previously mentioned, experiencing stress is a common problem for laying hens reared under commercial conditions. This fact can disrupt their physiological homeostasis and laying performance. Nutritional manipulation is one of the principal strategies to optimize production during stress conditions (Scaramuzzi et al., 2006). Ongoing investigations on humans and rats have shown the antistress effects of omega-3 fatty acids (Ferraz et al., 2011; Pérez et al., 2013).

Dexamethasone (**DEX**) is a potent synthetic glucocorticoid and has been addressed in a previous study owing to its capability to simulate the effects of glucocorticoid in rats (Foucaud et al., 1998). So, in the present study, DEX was used to induce physiological stress in the laying hens.

As previous findings regarding the detrimental effect of stress, we here hypothesized that omega-3 fatty acids may have an antistress effect in poultry. Therefore, we investigated if omega-3 fatty acids supplementation may reduce the adverse effects of physiological stress, thereby leading to increased egg production in laying hens. The present study aimed to evaluated the effects of omega-3 fatty acids on egg production, intestinal morphology, nutrients digestibility, yolk cholesterol, and lipid peroxidation in eggs of laying hens under physiological stress induced by DEX.

MATERIALS AND METHODS

Animal Ethics

All experimental procedures used were approved by the Animal Welfare Committee of the Department of Animal Science, University of Tehran.

Birds and Diets

Ninety-six 35-wk-old Lohmann LSL-Lite laying hens were obtained from a commercial layer farm and assigned to 6 treatments in a completely randomized design experiment with a factorial arrangement (2×3) and 4 replicates and 4 birds per each replicate. The treatments included 2 levels of DEX (0 and 1.5 mg/kg of the diet) and 3 levels of omega-3 fatty acids (control = 0.031, 0.24, or 0.48% of the diet). Salomega (Agritech Co. Ireland) was used as an omega-3 fatty acids source with graded levels of 0, 1.5, and 3% of diet, to supply the fatty acids desired in the experiment. Salomega is prepared using salmon oil, and corn cob is used as a carrier in this product. It contains 52% fat and about 17% total omega 3 fatty acids. The fat content and fatty acid profiles of salomega is presented in Table 1. Dexamethasone (DEX tablets; Iran Hormone Co., Iran) was dissolved in oil and mixed with a mash diet. The experimental diets were produced by first mixing all ingredients with the exception of DEX (Table 2, Lohmann laying hen's manual). Diets were divided into 2 parts. Each part was then supplemented, or not, with DEX and mixed again. The fatty acid profiles of the experimental diets are presented in Table 3. All birds were fed a standard isocaloric (2,620 kcal/kg) and isonitrogenous diet (18.5% protein) that was formulated to meet nutritional recommendations of laying hens. The birds were allocated in 24 cages equipped with 1 drinker and 1 feeder, from 35 to 41 wk of age. The diets and fresh water were offered ad libitum. The hens were maintained under similar conditions with a controlled photoperiod regimen (16L:8D cycle and temperature of 25°C $\pm$ 2°C).

From age of 35 to 41 wk, birds were fed basal diets supplemented with various levels of omega-3 fatty acids. At the beginning of the age of 41 wk, the stressed birds were

Table 1. Total fat content and fatty acids composition (%) of salomega.

Fatty acids	Reported[1] (%)	Measured(%)
DM	95.6	95.1
Total fat	52	51.3
Myristic acid (C14:0)	3.25	2.12
Palmitic acid (C16:0)	10.94	11.37
Stearic acid (C18:0)	2.97	2.88
Arachidic acid (C20:0)	0.30	0.38
Palmitoleic acid (16:1n7)	3.64	3.76
Oleic acid (C18:1 n9)	34.32	35.41
Eicosenoic acid(20:1n9)	3.76	3.46
Erucic acid(22:1n9)	0.60	0.23
Tetracosenoic acid(24:1n9)	0.37	0.16
Linoleic acid (C18:2 n6)	13.08	11.87
γ-linolenic acid (18:3n6)	0.10	ND
Eicosadienoic acid (20:2n6)	0.79	0.63
Eicosatrienoic acid (20:3n6)	0.19	0.93
Arachidonic acid (C20:4n6)	0.35	0.79
Alpha linolenic acid (C18:3 n3)	4.34	4.92
Eicosapentaenoic acid (C20:5 n3)	3.72	3.62
Docosapentaenoic acid (C22:5n3)	1.60	1.61
Docosahexaenoic acid (C22:6 n3)	4.61	5.43
Other fatty acids	10.83	10.43
Total of saturated fatty acids	17.92	16.75
Total of monounsaturated fatty acids	50.06	43.02
Total of polyunsaturated fatty acids	32.02	29.96
Total of omega-3 fatty acids	16.57	15.58
Total of omega-6 fatty acids	14.62	14.22

Abbreviation: ND, not detected.
[1]Fatty acid analysis of Salomega reported by Irish Agritech company (Compiled by: Nutrition Analytical Service, Institute of Aquaculture, University of Stirling).

Table 2. Ingredient and nutrient composition of basal diets.

Item (% diet)	Total omega-3 fatty acids (% of diet)		
	Control	0.24	0.48
Yellow corn	52.2	50.6	49.0
Soybean meal[1]	30.8	31.2	31.5
Bran wheat	2.5	2.5	2.5
Salomega	0	1.5	3
Canola oil	1.83	1.58	1.36
Dicalcium phosphate	1.53	1.51	1.52
Limestone	6.50	6.50	6.50
Oyster shell	3.66	3.66	3.66
Vitamin–mineral premix[2]	0.5	0.5	0.5
DL-Methionine	0.18	0.15	0.16
Sodium chloride	0.15	0.15	0.15
Sodium bicarbonate	0.15	0.15	0.15
Total	100	100	100
Calculated nutrients			
AMEn (kcal/kg)	2,620	2,620	2,620
CP (%)	18.5	18.5	18.5
Crude fiber (%)	2.64	2.67	2.71
Calcium (%)	4.25	4.25	4.25
Total phosphorus (%)	0.58	0.57	0.57
Available phosphorus (%)	0.43	0.43	0.43
Lysine (%)	1.03	1.02	1.02
Methionine (%)	0.48	0.44	0.44
Methionine + cysteine (%)	0.78	0.74	0.74
Measured nutrients			
CP (%)	17.33	19.58	18.09
Crude fat (%)	4.58	4.87	5.81
Total omega-3 fatty acid (%)	0.031	0.245	0.507

[1]Nondehulled soybean meal (44% CP).

[2]Vitamin and mineral premix supplied per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 2,000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; thiamin,1.8 mg; riboflavin, 6.6 mg; niacin, 30 mg; calcium pantothenate, 10 mg; vitamin B6, 3 mg; folic acid 1 mg; vitamin B12, 0.015 mg; biotin, 0.1 mg; choline, 500 mg; manganese oxide, 100 mg; ferrous sulfate, 50 mg; zinc oxide, 100 mg; copper sulfate, 10 mg; calcium iodate, 1 mg; sodium selenite, 0.2 mg.

continuously fed diets containing DEX (1.5 mg/kg diet) for 1 wk (end of 41 wk).

Performance

The feed intake, egg number, and egg weight were recorded during the stress period (week of 41), and then, the egg production, egg mass, daily feed intake,

Table 3. Fatty acids profiles (%) of the experimental diets.

Fatty acids	Total omega-3 fatty acids (% of diet)		
	Control	0.24	0.48
Myristic acid (C14:0)	-	2.536	3.027
Palmitic acid (C16:0)	15.872	14.709	14.767
Palmitoleic acid (C16:1)	-	0.720	1.225
Stearic acid (C18:0)	5.488	4.586	4.657
Oleic acid (C18:1 n9)	29.450	30.350	31.188
Linoleic acid (C18:2 n6)	46.915	41.127	36.387
Alpha linolenic acid (C18:3 n3)	2.275	3.151	3.263
Arachidic acid (C20:0)	-	0.944	1.849
Arachidonic acid (C20:4)	-	0.787	1.424
Eicosapentaenoic acid (C20:5 n3)	-	0.384	0.784
Docosapentaenoic acid (C22:5)	-	0.160	0.340
Docosahexaenoic acid (C22:6 n3)	-	0.546	1.089
Total of saturated fatty acids	21.360	22.775	24.300
Total of mono unsaturated fatty acids	29.450	31.070	32.413
Total of polyunsaturated fatty acids	49.190	46.155	43.287
Total of omega-6 fatty acids	46.915	41.914	37.811
Total of omega-3 fatty acids	2.275	4.241	5.476
Total of omega-6/total omega-3 fatty acids ratio	20.786	9.883	6.904

and feed conversion ratio (feed/egg mass) were calculated.

Malondialdehyde Assay and Cholesterol Values in the Yolk

To measure lipid peroxidation and egg yolk cholesterol concentration, at the last 2 d of 41 wk, 8 eggs per treatment were collected, cracked, and the yolks were separated from the albumen. The fresh yolks were weighted and then stored at $-20°C$ until performing further analyses. Yolk cholesterol concentration was determined based on the method proposed by Pasin et al. (1998). Briefly, 1 g of egg yolk was mixed with 9 mL NaCl solution (2%). The sample was then gently shaken for 2 h. A 1-mL solubilized yolk sample was further diluted 10-fold with 9 mL NaCl solution (2%) and used as a working sample. A cholesterol reagent kit (Zist Shimi, Tehran, Iran) was used to measure the amount of cholesterol. The samples were prepared by combining an additional amount of 1 mL enzyme reagent to 10 µL of working sample and standard solution. A blank was prepared by substituting 10 µL of deionized water with the working sample or standard solution. They were then vortexed for 30 s and left for 15 min into a water bath at 37°C. Absorbances were read at 505 nm using a spectrophotometer (Perkin Elmer Lambda25). To examine the lipid oxidation, yolk eggs were first stored in the refrigerator for 3 d. Next, the lipid peroxidation was estimated by spectrophotometric determination of malondialdehyde (**MDA**) using the method suggested by Botsoglou et al. (1994). In brief, 1 g of sample was transferred into a 15-mL centrifuge tube, and then, 4 mL of 5% aqueous trichloro acetic acid and 2.5 mL of 0.8% butylated hydroxy toluene in hexane were added.

The content of the tube was Ultra-Turraxed for 30 s at high speed and centrifuged at 3,000 g for 3 min, and the top hexane layer was discarded. The bottom aqueous layer was filtered (Whatman filter paper) and then taken to 5 mL volume using trichloro acetic acid. Three milliliter of 0.8% aqueous thiobarbituric acid was also added to the content. This mixture was heated in a water bath at 70°C for 30 min. After cooling down using water, the samples were submitted to spectrophotometry (Perkin Elmer Lambda25) at 521 nm for reading absorbance.

Intestinal Morphological Assay and Organ Weights

At the end of stress period (41 wk of age), 4 birds were randomly chosen per treatment and were weighed and euthanized. After opening the abdominal cavity, the liver, heart, and ovary were harvested and weighed separately. The weights of organs were expressed as a percentage of BW (%).

Histologic examination was determined as per the method described by Uni et al. (2001). At the end of

41 wk of age, 4 birds of each treatment were euthanized. Immediately after bird euthanasia, a 3-cm sample of the jejunum (midpoint between the bile duct entry and Meckel's diverticulum) was collected, flushed with PBS (pH = 7), and fixed in 4% formalin-buffered saline solution. Then, the segments were dehydrated and embedded in paraffin wax. For each segment, a 5-μm cross section was sectioned using a microtome, placed on a glass slide, and stained with hematoxylin and eosin. The histologic parameters including villus height and width and crypt depth were measured using a light microscope (Olympus CX31, Tokyo, Japan). The villus: crypt was determined as the ratio of villus height to crypt depth. Eight replicate measurements for each variable studied were taken from each bird, and the average values were used as an experimental unit in statistical analysis.

Digestibility Assay

Nutrient digestibility was investigated during stress period (week 41) for 1 wk in hens. Celite (5 g/kg) was added in all diets as an indigestible marker for calculations of AME and apparent digestibility coefficient for CP, crude fat, and organic matter (**OM**). The digestibility study included 4-d pre-experimental adaptation period followed by a 3-d collection period. During each 3-d collection period, pooled excreta samples from respective treatment cages (n = 4 per treatment) were collected daily. After contaminants such as feathers and other foreign materials were carefully removed, the excreta samples (n = 12 per treatment) were stored in air-tight containers at –20°C until later analysis.

Samples collected for each replicate during the 3-d collection period were mixed, and then, a uniform sample from each experimental unit was obtained for analyzes. Feed and excreta samples were dried at 55°C for 72 h, ground to pass through a 0.5-mm sieve and stored in air-tight containers at −20°C. All samples were subsequently analyzed for DM, protein, fat, and OM as per the standard methods of the Association of Official Analytical Chemists (2005). Gross energy (**GE**) was determined using an adiabatic oxygen bomb calorimeter (Parr Instrument Company, Mode l1261). Acid insoluble ash (**AIA**) concentrations in the feed and excreta were determined using the procedure described by De Coca-Sinova et al. (2011). The AME and apparent digestibility coefficients (**DC**) of CP, crude fat, and OM were calculated by using the following equations (Schneitz et al., 1998):

Statistical Analysis

Before ANOVA, the Shapiro-Wilk and Levene tests were used to test normality of residues and homogeneity of variances, respectively. Data were analyzed in a 2 × 3 factorial arrangement using the GLM procedure of SAS (version 9.4) to determine the main effects of DEX and omega-3 fatty acids and all 2-way interactions. Cage served as the experimental unit, and the number of replicates was 4 per treatment. When there was no interaction, the factors were analyzed separately. So for live BW and relative organ weight, MDA and cholesterol concentrations, and nutrient digestibility, only the main effects of DEX and omega-3 fatty acids were presented. If significant main effects or interactions were found, the Tukey's test was used to compare differences among the treatment means. For all statistical analyses, significance was declared at $P < 0.05$.

RESULTS

Performance

The effects of DEX and dietary omega-3 fatty acids supplementation on the performance of laying hens are shown in Table 4. Interaction effects of omega-3 fatty acids and DEX and the main effect of omega-3 fatty acids on egg production, egg mass, egg weight, and feed conversion ratio were not different ($P > 0.05$). Induction of physiological stress with DEX reduced egg production and egg mass ($P < 0.0001$) and increased feed conversion ratio ($P = 0.043$). The significant lower feed intake was noticed in all treatments with hens subjected to stress by DEX administration as compared with birds not subjected to stress. Moreover, stressed birds fed with diet containing 0.48% omega-3 had the lowest feed intake than the other groups ($P = 0.042$). Table 5 shows the results of DEX and omega-3 fatty acids on live BW and the relative weight of internal organs (% of live BW). Physiological stress induced by DEX decreases BW ($P = 0.0005$) and increases relative weight of the liver ($P = 0.042$). There was no significant difference in heart weight by DEX administration, although this parameter tended to be increased ($P = 0.063$). BW and relative weight of the liver and heart were not affected ($P > 0.05$) by the omega-3 fatty acids supplementation. Ovary weight was not affected by the experimental treatment.

$$\text{DC } (\%) = [100 - (100 \times \text{AIA diet / AIA fecal or ileal} \times \text{Nutrient fecal or ileal / Nutrient diet})]$$

$$\text{AME (kcal/kg)} = \text{GE diet} - (\text{GE feces} \times \text{AIA diet / AIA feces})$$

Table 4. Effects of experimental treatments on production performance in laying hens.

Item		Egg production (%)	Egg mass (g/hen/day)	Egg weight (g)	Feed intake (g/hen/day)	Feed conversion ratio
Dexamethasone (mg/kg)						
0		98.115[a]	61.484[a]	62.683[a]	108.508[a]	1.765[b]
1.5		88.095[b]	54.086[b]	61.408[b]	98.718[b]	1.828[a]
SEM		1.056	0.657	0.426	0.566	0.021
Omega-3 fatty acids (%)						
Control (0.031)		94.940	58.689	61.823	106.190[a]	1.811
0.24		92.410	57.163	61.821	103.446[b]	1.817
0.48		91.964	57.502	62.492	101.202[b]	1.763
SEM		1.294	0.804	0.522	0.694	0.026
Dexamethasone × Omega-3 fatty acids						
0	Control (0.031)	97.916	61.140	62.463	110.922[a]	1.815
0	0.24	98.214	61.392	62.533	107.102[a]	1.745
0	0.48	98.214	61.921	63.053	107.500[a]	1.737
1.5	Control (0.031)	91.964	56.238	61.184	101.458[b]	1.808
1.5	0.24	86.607	52.935	61.110	99.791[b]	1.889
1.5	0.48	85.714	53.084	61.930	94.905[c]	1.789
SEM		1.830	1.138	0.739	0.981	0.036
Probability						
Dexamethasone		<0.0001	<0.0001	0.043	<0.0001	0.043
Omega-3 fatty acids		0.241	0.390	0.588	0.0003	0.287
Dexamethasone × Omega-3 fatty		0.181	0.190	0.979	0.042	0.142

[a–c]Means with different superscripts within a column are different at $P < 0.05$.

Table 5. Effects of experimental treatments on live BW and relative organ weight in laying hens.

Item	Live BW(g)	Heart	Liver	Ovary
Dexamethasone (mg/kg)				
0	1408.000[a]	0.451	2.153[b]	3.352
1.5	1244.000[b]	0.509	2.439[a]	3.740
SEM	25.691	0.020	0.090	0.258
Omega-3 fatty acids (%)				
Control (0.031)	1347.000	0.515	2.295	3.729
0.24	1309.000	0.462	2.258	3.709
0.48	1323.000	0.463	2.334	3.199
SEM	31.471	0.025	0.110	0.316
Dexamethasone	0.0005	0.065	0.042	0.306
Omega-3 fatty acids	0.695	0.264	0.889	0.429

[a,b]Means with different superscripts within a column are different at $P < 0.05$.

Malondialdehyde and Cholesterol Values in the Yolk

Table 6 shows the results of dietary DEX and omega-3 fatty acids on lipid oxidation of eggs stored for 3 d and cholesterol content in the egg yolk in laying hens. Dexamethasone increased MDA values ($P = 0.002$) and cholesterol content (mg/g of yolk and mg/yolk) ($P = 0.0008$, $P = 0.001$).

Intestinal Morphology

The effects of DEX and dietary omega-3 fatty acids supplementation on the morphometric traits of the jejunum of laying hens are shown in Table 7. The interaction between DEX and omega-3 fatty acids was significant on different morphologic indices. The interaction of DEX and omega-3 fatty acids increased the villus height ($P = 0.022$); there was no difference between birds under stress that fed a diet containing 0.48% omega-3 fatty acids and groups without stress in terms of villus height. The interaction of DEX and omega-3 fatty acids decreased the villus width ($P = 0.0009$). In this case, the birds under stress fed diets supplemented by 0.24 and 0.48% omega-3 fatty acids had less villus width comparing with the group under stress and without supplementation of omega-3 fatty acids. But, no difference was observed between the birds under stress fed diets

supplemented by 0.24 and 0.48% omega-3 fatty acids and other treatments.

The interaction of omega-3 fatty acids and DEX increased the crypt depth ($P < 0.0001$). Nonstressed and non–omega-3–supplemented group had the lowest crypt depth, but no difference was observed between this group and nonstressed birds fed diet supplemented by 0.24 omega-3 fatty acids. The birds under stress fed diet containing 0.24% omega-3 fatty acids had the highest crypt depth. The interaction between DEX and omega-3 fatty acids on villus: crypt showed that nonstressed birds and fed with a nonsupplemented omega-3 fatty acids diet had the highest villus: crypt ($P < 0.0001$). Hens under stress supplemented with high levels of omega-3 fatty acids (0.48%) had a greater villus-to-crypt ratio than other group under stress.

Nutrients Digestibility

Table 8 shows the result of DEX and omega-3 fatty acids on the dietary ME and nutrient digestibility. Dexamethasone and omega-3 fatty acids had no significant effect on OM digestibility. Crude fat digestibility was not affected by DEX but was higher in the level of 0.48% omega-3 fatty acids compared with levels of 0 or 0.24% omega-3 fatty acids in the diets ($P = 0.020$). CP digestibility decreased with DEX ($P < 0.0001$). There was no significant difference in CP digestibility by consuming

Table 6. Effects of experimental treatments on MDA and cholesterol in laying hens.

Item	Yolk cholesterol (mg/g of yolk)	Yolk cholesterol (mg/yolk)	Malondialdehyde (μg/yolk)
Dexamethasone (mg/kg)			
0	11.434[b]	192.711[b]	0.367[b]
1.5	15.950[a]	259.021[a]	0.609[a]
SEM	0.813	12.808	0.048
Omega-3 fatty acids (%)			
Control (0.031)	13.762	229.417	0.509
0.24	14.237	237.310	0.537
0.48	13.076	210.870	0.417
SEM	0.996	15.687	0.059
Probability			
Dexamethasone	0.0008	0.001	0.002
Omega-3 fatty acids	0.713	0.485	0.349

[a,b]Means with different superscripts within a column are different at $P < 0.05$.
Abbreviation: MDA, malondialdehyde.

Table 7. Effects of experimental treatments on the morphology of the jejunum in laying hens.

Item		Villus height (μm)	Villus width (μm)	Crypt depth (μm)	Villus:crypt
Dexamethasone (mg/kg)					
0		1159.534	19.973[b]	183.415[b]	6.324[a]
1.5		1079.854	41.003[a]	221.251[a]	4.881[b]
SEM		30.260	2.865	4.011	0.182
Omega-3 fatty acids (%)					
Control (0.031)		1147.707[a]	40.529[a]	185.432[b]	6.185[a]
0.24		993.007[b]	26.752[b]	212.131[a]	4.683[b]
0.48		1218.367[a]	24.184[b]	209.661[a]	5.818[a]
SEM		37.061	3.509	4.903	0.221
Dexamethasone × Omega-3 fatty acids					
0	Control (0.031)	1267.922[a]	16.842[b]	159.092[e]	7.961[a]
0	0.24	1033.405[a,b]	23.512[b]	164.112[d,e]	6.290[b]
0	0.48	1177.275[a,b]	19.565[b]	227.520[b]	5.176[b,c]
1.5	Control (0.031)	1027.492[b]	64.215[a]	211.813[b,c]	4.851[c,d]
1.5	0.24	952.610[b]	29.992[b]	260.150[a]	3.667[d]
1.5	0.48	1259.460[a]	28.802[b]	191.802[c,d]	6.56[b]
SEM		52.412	4.963	6.935	0.312
Probability					
Dexamethasone		0.079	<0.0001	<0.0001	<0.0001
Omega-3 fatty acids		0.001	0.008	0.001	0.0009
Dexamethasone × Omega-3		0.022	0.0009	<0.0001	<0.0001

[a–e]Means with different superscripts within a column are different at $P < 0.05$.

omega-3 fatty acids, although this parameter tended to be increased ($P = 0.075$) in hens fed with 0.48% omega-3 fatty acids in the diet. Administration of DEX decreased ($P = 0.006$) AME, whereas omega-3 fatty acids increased this parameters ($P = 0.046$).

DISCUSSION

In previous works, supplementing laying hen's diet with omega-3 fatty acids for increasing omega 3 content of the egg have been reported; however, performance was not affected by omega-3 supplementation (Küçükersan et al., 2010; Al-Nasser et al., 2011; Zhang and Kim, 2014).

In this study, omega-3 fatty acids supplementation was used to reduce the adverse effects of physiological stress in laying hens. The combination of omega-3 fatty acids and DEX reduced feed intake in stressed birds.

Decreased feed intake and performance in laying hens and quails have been reported previously afetr the treatment with corticosterone and DEX (Wall and Cockrem, 2010; Liu et al., 2012; Berenjian et al., 2018). Activation of the HPA axis as a result of stressors can cause the secretion of the corticotropin-releasing factor, which is a potent anorexigenic peptide in birds (Richardson et al., 2000). Using omega-3 fatty acids in overweight and obese humans modulated hunger signals and postprandial satiety, in such a way that, omega-3 fatty acids can interact with various neuroendocrine factors that contribute in brain–gut loop signals related to energy metabolism and feed intake, such as insulin and leptin (Nettleton and Katz, 2005; Pérez-Matute et al., 2007).

Gray et al. (2013) reported that omega-3 fatty acids increase leptin levels and reduce appetite in patients with obesity (in humans). On the other hand, Houseknecht et al. (1998) stated that the cortisol

Table 8. Effects of experimental treatments on AME and nutrient digestibility in laying hens.

Item	Organic matter (%)	Fat (%)	Protein (%)	AME(kcal/kg)
Dexamethasone (mg/kg)				
0	67.531	77.866	33.521[a]	2746.466[a]
1.5	64.114	79.531	17.285[b]	2640.628[b]
SEM	1.206	1.655	2.217	23.374
Omega-3 fatty acids (%)				
Control (0.031)	64.668	76.095[b]	22.862	2644.112[b]
0.24	65.343	76.203[b]	22.567	2682.020[a,b]
0.48	67.566	83.798[a]	30.780	2754.508[a]
SEM	1.478	2.027	2.715	28.627
Probability				
Dexamethasone	0.078	0.485	<0.0001	0.006
Omega-3 fatty acids	0.367	0.020	0.072	0.046

[a,b]Means with different superscripts within a column are different at $P < 0.05$.

hormone stimulates the levels of leptin expression. So, high levels of triglycerides in the plasma, or the larger liver (due to more fat accumulation) of stressed layer hens can be attributed to the metabolic processes of corticosteroids or DXA. It can be concluded that feed intake in stressed birds is reduced owing to increased levels of leptin (as a satiety hormone) by the omega-3 fatty acids. In general, the synergistic effects of DEX and omega-3 fatty acids reduce the feed intake of birds.

The adverse effects of DEX on laying performance investigating by the present study are in agreement with other researches (Shini et al., 2009; Wang et al., 2013). The reduced productive performance in this article can primarily be due to the reduced feed intake. While reducing feed intake, the energy required to produce yolk precursors in the liver is not provided, resulting in the suppressed development of follicles and ovulation by reducing the availability of yolk precursors. Nevertheless, the cascade of physiological, metabolic, and behavioral changes induced by the secretion of glucocorticoids leads to shifting energy reserves from reproduction toward survival (Wingfield and Sapolsky, 2003).

In this study, the increased liver weight and decreased BW in stressed birds were in accordance with the previous ones (Shini et al., 2009; Wang et al., 2013). Similar to the results of this experiment, an elevated relative weight of the heart after DEX treatment has been reported (Qin et al., 2012). The decrease of BW after DEX administration indicates the enhanced protein catabolism in stressed laying hens. So, increased protein catabolism, decreased feed intake, as well as the allocation of energy and protein reserves for egg production has resulted in a reduction in the BW of laying hens. The high relative weight of the liver after administration glucocorticoids in this study and other studies indicate the liver hypertrophy due to increased activity of lipogenic enzymes and liver lipogenesis (Wang et al., 2013). An increase in the relative weight of the liver and heart after administration of DEX may also be owing to live BW loss.

Hemostasis of cholesterol metabolism is essential for maintaining health, welfare, and production in farm animals and consumers of meat and eggs. In birds, the liver is the principal and primary site for lipid metabolism. High levels of cholesterol in egg yolks of DEX-administered hens can be the result of increased cholesterol synthesis in the liver. It could be possible that the elevated level of cholesterol was transferred to the yolk from the liver. In line with our results, corticosterone has been reported to increase the accumulation of cholesterol in the liver of broiler chicks. This process is carried out by upregulating the expression of genes and transcription factors involved in cholesterol synthesis (Liu et al., 2016). Similarly, an increase in plasma cholesterol levels was observed in hamsters exposed to glucocorticoids (Solomon et al., 2011).

In the present study, the use of omega-3 fatty acids did not affect the level of egg yolk cholesterol. In agreement with the results of our study, the use of omega-3 fatty acids from the sources of flaxseed oil and fish oil in the diet of laying hens had no effect on egg yolk cholesterol (Küçükersan et al., 2010; Neijat et al., 2016). The lipid peroxidation in the egg yolk was evaluated by measuring the amount of MDA, which is an indicator of oxidative stress. In the present study, the administration of DEX increased the amount of MDA in the egg yolk. In line with the results of our investigation, Lin et al. (2009) showed that administration of 4 mg of corticosterone/kg of BW increases the content of MDA in the skeletal muscle of broiler chicks. In addition, injection of 4 mg of DEX/d in broiler chickens and laying hens for 7 d proved to increase lipid peroxidation in the plasma, liver, and egg yolk (Eid et al., 2006, 2008). Increasing MDA levels may be due to the elevated levels of lipid peroxidation in the yolk of eggs and also in the liver by DEX, where yolk precursors are synthesized and then transferred to the ovary (Eid et al., 2008). It can be stated that stress play an important role for enhancing the lipid peroxidation and reducing the stability of egg yolk lipids, which finally leads to decrease in egg quality during storage and egg shelf life for the consumers.

In this experiment, the treatment with DEX and omega-3 fatty acids had a significant effect on the intestinal morphology parameters of the laying hens. In line with our findings, Chang et al. (2015) also showed a decrease in the villus height and an increase in the crypt depth of broiler chickens injected with different concentrations of DEX. Similarly, Deng et al. (2012) observed the reduction in the villus height and the villus height:crypt depth in the ileum and cecum of the laying hens under thermal stress. Quaroni et al. (1999) reported that glucocorticoids can regulate the continuous proliferation, migration, differentiation, and maturation of stem cells in crypt. Villus height has a positive correlation with the surface area of absorption (Hu and Guo, 2008). However, increased crypt depth owing to villus atrophy reduces the surface area absorption (Pacha, 2000). The deeper crypt caused by the proliferation of enterocytes acts as a response to the rapid turnover of the intestinal epithelium, which is the consequence of inflammation, pathogens, or different stresses (Xu et al., 2003). A shorter villus and deeper crypt reduce nutrient absorption capacity and ultimately affect animal performance.

To our knowledge, the results of various experiments conducted to study the effect of omega-3 fatty acids on intestinal morphology are distinct, and only a little information is available on this purpose. In a study, the use of 3.2% of fish oil in broiler diets reduced the villus height–to–crypt depth ratio and decreased absorption (Aziza et al., 2014). The addition of fish oil in the diet of rabbits did not affect the morphology of ileum in terms of crypt depth and villus height (Rodríguez et al., 2017). The use of red seaweeds supplement (as a source of omega-3 fatty acids) in the diet of laying hens increased the villus height, the crypt depth, the villus width, and the surface of the villus (Kulshreshtha et al., 2014). The increased villus height and crypt depth by using omega-3 fatty acids is linked to the healthy renovation of epithelial cells and active cellular mitosis. There are evidence to prove

the presence of an optimal microbial population in the gastrointestinal tract can contribute to the proper regeneration of epithelial cells and reduce the inflammation caused by pathogens and toxins (Baurhoo et al., 2007). Polyunsaturated fatty acid have been reported to reduce gastrointestinal inflammation by eliminating harmful microbes, increase the adhesion of certain strains of *Lactobacillus* bacteria to cells and also increase the survival of this bacterium (Bentley-Hewitt, 2010). The positive effects of omega-3 fatty acids in the stressed group receiving 0.48% omega-3 in this study were probably because of the improvement of the digestive environment, the intestinal microbial population, and the reduction of intestinal inflammation. This finding suggests that the requirements for omega-3 fatty acid increased in stressed birds for improving the gut environment and repairing the intestinal epithelium.

The present findings are consistent with the effects of heat stress on the digestibility of nutrients. It has been shown that heat stress reduces the digestibility of nutrients in broiler chickens and Japanese quail (Sahin et al., 2002; de Souza et al., 2016). Continuous infusion of adrenocorticotropin hormone at 8 IU/kg BW/d for 7 d caused decreases digestion of DM, proteins, GE, and carbohydrates of the broiler chickens (Puvadolpirod and Thaxton, 2000). Reduction of digestion in these studies may be caused by incomplete digestion owing to inadequate levels of digestive enzymes, decreased various digestive enzymes activity, and the changed digestive environment. The supplementation of omega-3 fatty acids in this study improved the digestibility of crude fat and ME in laying hens. Results regarding the effect of omega-3 fatty acids on digestibility of nutrients are different and contradictory. Aziza et al. (2013) reported that the use of camelina or flaxseed meal reduced protein digestibility and AME of the diet. These researchers stated that the presence of antinutrients in these compounds could reduce digestibility. In another study, soy oil supplementation and protein level in laying hen diets improved the digestibility of protein and fat (Dänicke et al., 2000).

In general, improving the digestibility of crude fat and ME in this study can be owing to the positive effects of omega-3 fatty acids on the intestinal morphology, improved digestion and intestinal absorption, and higher energy efficiency. The increase in villus height is paralleled by an increase in digestive and absorptive functions and expression of brush border enzymes (Caspary, 1992).

In conclusion, dietary supplementation of omega-3 fatty acids showed to improve the gut health, morphometric characteristics of the intestine, and digestibility of nutrients in laying hens under physiological stress induced by exogenous DEX. However, the cumulative effect of DEX and omega-3 fatty acids could reduce the feed intake. The use of omega-3 fatty acids could not improve the negative effects of stress on laying performance as tested at dosage in this research. Further research needs to be conducted to provide a better understanding of the beneficial effects of omega-3 to alleviate the adverse effects of stress on poultry.

DISCLOSURES

The authors declare no conflicts of interest.

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