

Effect of dietary orange peel extract on physiological, biochemical, and metabolic responses of Japanese quail reared under low ambient temperature

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Abstract: The effects of dietary orange peel extract (OPE) supplementation on growth performance, carcass traits, serum biochemical parameters, antioxidant status of tissues, and fatty acid composition of breast meat in Japanese quails reared under low ambient temperature were investigated in the present study. A total of 108 quails of 15 days old were assigned to 3 groups with 3 replicates. Animals were fed with a corn/soy-based standard diet in the control group and a standard diet supplemented with either 100 or 200 ppm of OPE in the experimental groups (control, OPE-100, and OPE-200 groups, respectively). Room temperature was gradually lowered at night weekly from 14 °C to 8 °C to obtain chronic intermittent cold stress. OPE supplementation significantly increased live weight, live weight gain, and improved feed efficiency. OPE lowered triglyceride, total protein, glucose, total cholesterol, and uric acid levels in serum. The lowest malondialdehyde levels for liver and heart tissues were found in the OPE-100 group. Glutathione peroxidase activity and glutathione production in liver and heart tissues were found higher in the OPE groups. The lowest vitamin C levels for liver and heart tissues were observed in the control group. Feeding OPE resulted in increased accumulation of C20:2 ω -6, C22:6 ω -3, and total ω -3 fatty acids and decreased C18:0 level and total ω -6/ ω -3 ratio. These results suggest that OPE supplementation has positive effects against cold stress.

Key words: Antioxidant, cold stress, fatty acid, Japanese quail, orange peel extract

1. Introduction

Poultry are exposed to a great number of long- and short-term stressors (e.g., heat and cold stress, immune challenges, catching, transport), which may alter their internal homeostasis and oxidant/antioxidant balance, leading to oxidative stress, potentially having detrimental effects on performance and meat quality (1,2). Cold stress is a common problem in the poultry industry. Low ambient temperatures can cause an increase in feed intake, but also result in reduced growth, nutrient digestibility, and feed conversion efficiency (1,3). It also causes various physiological challenges. Long-term regulation of body in stress conditions causes increased levels of corticosterone due to the adrenal cortical hypertrophy in poultry. This hormone is responsible for the formation of glucose from the body's reserve of lipids and proteins (except for

carbohydrates) to protect the homeostasis mechanism of the body in this condition. On the other hand, extreme stress factors elevate lipid peroxidation and oxidative degradation of body tissues, especially polyunsaturated fatty acids, and increase the body's sensitivity to diseases (4,5).

It has been reported that birds reared in stress conditions need antioxidants in order to protect their tissues against lipid peroxidation damages, and also that ingestion of antioxidant additives by birds may alleviate these damages (6). Today essential oils extracted from different plants are the most popular supplements; they have been widely used because of their antioxidant capabilities (7). These natural feed additives are given to animals to improve their productive performance under normal or stress conditions (8).

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Orange peel contains high concentrations of phenols (9) and significant amounts of beta-carotene (10) and vitamin C (11). Vitamin C or polyphenols are enhanced antioxidant enzymes in red blood cells (6). Furthermore, orange peel components may counteract enzymatic lipid peroxidation (12).

The aim of this study was to evaluate the effect of dietary orange peel extract (OPE) supplementation on the performance, the carcass traits, and some blood parameters in Japanese quail reared under cold-stressed conditions.

2. Materials and methods

2.1. Experimental design and diet regimens

A total of 108 Japanese quails (*Coturnix coturnix japonica*) of 15 days old were obtained from a commercial company (Deva-Yum Co., Elazığ, Turkey) and divided into three experimental groups including 36 mixed-sexed quails in each. The experiment was carried out for 28 days. Each treatment group was further subdivided into three replicates of 12 birds per replicate. The birds were assigned to experimental groups at the beginning of the study with balanced sex ratios and initial live weights. The Firat

University Animal Experimentations Ethics Committee approved the study protocol (Protocol Number: 2014/130). The experiment was conducted at the Poultry Unit of Firat University. The presence and levels of OPE (Agromiks Food Additive Co., İzmir, Turkey) in diets were the main factors tested. The birds received either a basal diet or a basal diet supplemented with OPE at either 100 or 200 ppm of the diet (control, OPE-100, and OPE-200 groups, respectively). The birds were kept in wire cages in a temperature-controlled room. The temperature of the room is given in Table 1. The OPE was mixed prior to administration in a carrier (zeolite) to obtain stability and a homogeneous mixture. The concentrations of the volatile components in OPE are shown in Table 2. Diet and fresh water were offered ad libitum. Light was provided continuously (24 h) throughout the experiment. The composition of the basal diet is shown in Table 3. Fatty acid composition of the experimental diet is displayed in Table 4.

Feed intake and body weight were determined at weekly intervals. The weight gain and feed conversion ratios of birds were then calculated.

A total of six quails (3 males and 3 females) from each group with an average body weight near the group average

Table 1. Temperature degrees of the room (°C).

Days	Times of day	
	From 2200 to 0600 hours	From 0600 to 2200 hours
15–22	14	26
22–29	12	24
29–36	10	22
36–43	8	22

Table 2. The concentrations of the volatile components in orange peel extract (%).

Analysis	Result*
Limonene	92.31
Beta-myrcene	3.25
Alpha-pinene	1.41
Linalool	0.89
Sabinene	0.61
Delta-3-carene	0.22
Octanal	0.21
Undefined	1.10

*: Obtained by GC-MS analysis.

Table 3. Ingredients and chemical composition of standard diet (g/kg).

Ingredients	g/kg	Calculated analyses, g/kg	
Maize	400.0	Dry matter	894.1
Wheat	90.0	Crude protein	241.0
Soybean meal (48% CP)	290.0	Crude cellulose	33.8
Corn gluten	115.0	Ether extract	63.0
Vegetable oil	40.0	Crude ash	62.5
DL-Methionine	3.4	Calcium	10.0
Dicalcium phosphate	29.1	Available phosphorus	4.9
Ground limestone	10.0	Sodium	1.8
L-Lysine hydrochloride	3.3	Methionine + cysteine	10.9
L-Threonine	0.9	Lysine	14.1
L-Tryptophan	0.9	Threonine	9.6
NaHCO ₃	1.0	Tryptophan	3.7
Salt	3.0	ME, kcal/kg	3121
Vitamin-mineral mix*	3.4		
Zeolite**	10.0		
Total	1000		

*: Vitamin premix supplied per kg: Vitamin A 15,500 IU; vitamin D₃ 3500 IU. Mineral premix supplied per kg: Mn 120 mg; Fe 40 mg; Zn 100 mg; Cu 16 mg; Co 200 mg; I 1.25 mg; Se 0.30 mg.

** : Control group, 1000 g zeolite; OPE-100 group, 10 g OPE + 990 g zeolite; OPE-200 group, 20 g OPE + 980 g zeolite.

Table 4. Fatty acid composition of basal diet (%).

Fatty acids	%
C16:0	11.40
C16:1 ω7	0.67
C18:0	3.12
C18:1 ω9	24.19
C18:2 ω6	54.95
C18:3 ω3	4.24
C20:1 ω9	0.56
C20:2 ω6	0.12
C20:5 ω3	0.39
C22:2	0.18
C22:5	0.18
ΣSFA	14.52
ΣMUFA	25.42
ΣPUFA	60.06
Σ ω-6	55.07
Σ ω-3	4.63
Σ ω-6/ω-3	11.89

were slaughtered at the end of the study (43rd day). To obtain serum, collected blood samples were centrifuged at $2260 \times g$ for 5 min. Following slaughtering, carcass features were evaluated in accordance with Turkish Standards Institute rules (13).

For the fatty acid analyses of chicken meat, *M. pectoralis profundus* of the breast was obtained and stored at -20°C until analysis.

2.2. Chemical analyses

Serum glucose, triglyceride, total cholesterol, uric acid, and total protein concentrations were measured using a biochemical analyzer (Architect i2000) at the Firat University Faculty of Medicine, Department of Biochemistry. Chemical composition of basal feed ingredients (dry matter, crude protein, ash, and ether extract) was analyzed according to AOAC procedures (14) and crude fiber was determined by the methods of Crampton and Maynard (15). Extraction of lipids from the tissue specimens was performed according to the method of Hara and Radin (16). Vitamin C was analyzed according to the method of Omayya et al. (17). Preparation of fatty acid methyl esters was analyzed according to the method of Christie (18). The fatty acid methyl esters were analyzed in a gas chromatograph with a Macherey-Nagel capillary column. During the analysis, column heat was maintained at $120\text{--}220^\circ\text{C}$, injection heat was maintained at 240°C , and detector heat was maintained at 280°C . The column heat program was regulated to 220°C from 120°C ; the heat increase was set to $5^\circ\text{C}/\text{min}$ until reaching 200°C and to $4^\circ\text{C}/\text{min}$ from 200 to 220°C , and it was then held at 220°C for 8 min. Nitrogen was the carrier gas and the detector was a flame-ionization detector. Malondialdehyde (MDA) levels of the liver and heart were spectrophotometrically measured by using the method described by Placer et al. (19). Superoxide dismutase (SOD) activities of the liver and heart were measured using xanthine and xanthine oxidases to generate superoxide radicals, which react with nitroblue tetrazolium (NBT), by the methods of Sun et al. (20). The glutathione peroxidase (GSH-Px) activity was determined according to Lawrence and Burk (21). The glutathione (GSH) contents of the liver and heart were measured at 412 nm by the method of Sedlak and Lindsay (22).

2.3. Statistical analysis

All performance, carcass, blood, oxidative stress, and meat quality parameters were analyzed by orthogonal polynomial contrast of variance procedures and multiple comparisons were performed by Tukey-HSD multiple-range test. Viability rates were compared with the chi-square test. All analyses were determined by using SPSS for Windows (23). Results were considered significant at $P < 0.05$.

3. Results

The effects of dietary OPE on the performance of Japanese quails reared under low ambient temperature are shown in Table 5. The highest finishing live weight was seen in the OPE-100 group ($P < 0.05$). For the fourth week (days 36–43) of the experiment, a quadratic decrease of feed conversion ratio, a quadratic increase of daily live weight gain, and a linear decrease of feed intake were seen with OPE supplementation ($P < 0.05$). When the viability table was examined (Table 6), viability rates of the control, OPE-100, and OPE-200 groups were found as 76.7%, 90.0%, and 93.3%, respectively. There was no statistical significance among the groups ($P > 0.05$). Carcass yield was linearly increased by OPE supplementation ($P < 0.05$) and the other carcass traits were similar among the groups ($P > 0.05$), as shown in Table 7.

Based on examination of Table 8, under low ambient temperature OPE supplementation linearly decreased triglyceride, total cholesterol, and uric acid levels of serum taken at the end of the study ($P < 0.05$). Serum glucose and total protein levels were also affected by OPE supplementation ($P < 0.05$).

A quadratic decreased effect of dietary OPE supplementation was observed for MDA levels in the heart ($P < 0.001$) as shown in Table 9. For MDA levels of liver tissue quadratic and linear decrease was observed with OPE supplementation ($P < 0.001$). Dietary OPE supplementation caused linear and quadratic increase in GSH-Px activity and linear increase in GSH production of the liver ($P < 0.01$, $P < 0.05$). For heart tissue, linear and quadratic increase in GSH production and linear increase in GSH-Px activity were observed ($P < 0.05$, $P < 0.01$). However, SOD production of the liver and heart was similar in all groups ($P > 0.05$). The lowest vitamin C level of the liver ($P < 0.05$) and heart ($P < 0.01$) were observed in the control group. Dietary OPE supplementation caused linear and quadratic increase in the vitamin C level of liver tissue ($P < 0.05$) and a linear increase in the vitamin C level of heart tissue ($P < 0.001$).

Effects of OPE supplementation under low ambient temperature on fatty acid profiles of breast muscle are given in Table 10. As shown there, C20:2 $\omega 6$ ($P < 0.05$), C22:6 $\omega 3$ ($P < 0.05$), total polyunsaturated fatty acid (PUFA; $P < 0.05$), and total ω -3 ($P < 0.001$) were linearly increased, and C18:0 ($P < 0.05$) and total ω -6/ ω -3 ($P < 0.001$) fatty acid levels were linearly decreased by OPE supplementation. The other fatty acids, total saturated fatty acids, and total monounsaturated fatty acid ratios in different groups were found to be similar to each other ($P > 0.05$).

Table 5. Effect of orange peel extract (OPE) supplementation on performance of Japanese quails reared under low ambient conditions.

Traits	Control	OPE-100	OPE-200	SEM	P - mean effects of diets		
					Linear	Quadratic	Combined
Live weight, g							
Day 15	41.61	41.66	41.61	0.79	NS	NS	NS
Day 22	72.78	75.44	77.71	1.64	NS	NS	NS
Day 29	109.77	116.60	117.47	2.38	NS	NS	NS
Day 36	148.06	155.03	157.76	2.48	NS	NS	NS
Day 43	170.84 ^b	184.20 ^a	179.80 ^{ab}	1.59	NS	NS	*
Daily live weight gain, g/bird							
Days 15–22	4.45	4.83	5.16	0.19	NS	NS	NS
Days 22–29	5.28	5.88	5.68	0.19	NS	NS	NS
Days 29–36	5.47	5.49	5.76	0.14	NS	NS	NS
Days 36–43	3.25 ^b	4.17 ^a	3.15 ^b	0.17	NS	*	*
Days 15–43	4.61 ^b	5.09 ^a	4.93 ^{ab}	0.10	NS	NS	*
Daily feed intake, g/bird							
Days 15–22	12.59	12.14	12.90	0.24	NS	NS	NS
Days 22–29	18.28	18.25	19.79	0.49	NS	NS	NS
Days 29–36	25.13	25.26	25.24	0.21	NS	NS	NS
Days 36–43	26.81 ^a	26.19 ^a	24.28 ^b	0.22	**	NS	**
Days 15–43	20.71	20.46	20.55	0.23	NS	NS	NS
Feed conversion ratio, g feed/g gain							
Days 15–22	2.83	2.51	2.50	0.09	NS	NS	NS
Days 22–29	3.46	3.10	3.48	0.11	NS	NS	NS
Days 29–36	4.59	4.60	4.38	0.14	NS	NS	NS
Days 36–43	8.25 ^a	6.28 ^c	7.70 ^b	0.41	NS	**	**
Days 15–43	4.49	4.01	4.16	0.10	NS	NS	NS

P: Statistical significance; SEM: standard error of the mean; NS: nonsignificant; *: P < 0.05; **: P < 0.01; ^{a,b}: mean values with different superscripts within a row differ significantly.

Table 6. Effect of orange peel extract (OPE) supplementation on mortality and viability rates of Japanese quails reared under low ambient conditions.

Days	Control	OPE-100	OPE-200	Chi-square
15–22	4	-	2	-
22–29	2	1	-	-
29–36	1	2	-	-
36–43	-	-	-	-
Total	7	3	2	-
Mortality rate	23.3	10.0	6.7	-
Viability	76.7	90.0	93.3	χ^2 : 4.038 P: 0.133

Table 7. Effect of orange peel extract (OPE) supplementation on carcass characteristics of Japanese quails reared under low ambient conditions.

Traits	Control	OPE-100	OPE-200	SEM	P - mean effects of diets		
					Linear	Quadratic	Combined
Slaughter weight, g	165.30	172.70	172.33	3.04	NS	NS	NS
Carcass weight, g	108.28	118.59	119.01	3.29	NS	NS	NS
Carcass yield, %	66.61	68.62	69.06	2.22	*	NS	NS
Liver weight, g	4.89	4.92	4.68	0.17	NS	NS	NS
Liver ratio, %	3.00	2.84	2.71	0.12	NS	NS	NS
Heart weight, g	1.66	1.74	1.92	0.06	NS	NS	NS
Heart ratio, %	1.02	1.01	1.11	0.04	NS	NS	NS
Spleen weight, g	0.11	0.12	0.09	0.01	NS	NS	NS
Spleen ratio, %	0.07	0.07	0.04	0.01	NS	NS	NS

P: Statistical significance; SEM: standard error of the mean; NS: nonsignificant; *: P < 0.05.

Table 8. Effect of orange peel extract (OPE) supplementation on biochemical parameters of Japanese quails reared under low ambient conditions.

Traits (mg/dL)	Control	OPE-100	OPE-200	SEM	P - mean effects of diets		
					Linear	Quadratic	Combined
Glucose	291.67 ^a	256.83 ^b	264.67 ^{ab}	6.17	NS	NS	*
Triglyceride	91.50 ^a	70.33 ^{ab}	54.00 ^b	6.25	*	NS	*
Total cholesterol	176.67 ^a	138.17 ^b	143.53 ^b	6.45	*	NS	*
Uric acid	5.18 ^a	3.58 ^b	3.68 ^b	0.30	*	NS	*
Total protein	2.73 ^{ab}	2.85 ^a	2.47 ^b	0.07	NS	NS	*

P: Statistical significance; SEM: standard error of the mean; *: P < 0.05; ^{a,b}: mean values with different superscripts within a row differ significantly.

4. Discussion

In the present study, the effects of OPE supplementation on performance, mortality rates, carcass traits, serum parameters, antioxidant status of the liver and heart, and fatty acid profiles of breast muscle were investigated in Japanese quails reared under low ambient temperatures. Under cold-stressed conditions, chicks fed an OPE-supplemented diet had increased live weight, live weight gain, and improved feed efficiency when compared to those fed the nonsupplemented control diet. Similar results were obtained by Alcicek et al. (24) from broilers and Abd El Latif et al. (25) from Japanese quails such that there were such improvements in performance parameters with

herbal products used as feed additive. Such improvement may be attributed to the properties of essential oils that could act not only as antibacterial, antiprotozoal, and antifungal agents but also as antioxidants (26). The positive effects of these additives may also be attributed to the biological function or pharmacological activities of these extract components (limonene, beta-myrcene, alpha-pinene).

Supplementation of OPE to the diet did not affect the carcass traits in the present study. However, it has been reported that addition of anise oil (7) and essential oil mix (Herbomix) (24) to diets had positive effects on the carcass yield in broilers.

Table 9. Effect of orange peel extract (OPE) supplementation on antioxidant status of liver and heart tissues in Japanese quails reared under low ambient conditions.

Traits	Control	OPE-100	OPE-200	SEM	P - mean effects of diets		
					Linear	Quadratic	Combined
MDA (nmol/mL)							
Liver	5.07 ^a	3.30 ^b	3.95 ^b	0.86	***	***	***
Heart	4.40 ^a	3.12 ^b	3.86 ^b	0.16	NS	***	**
GSH (nmol/mL)							
Liver	0.09 ^b	0.13 ^a	0.13 ^a	0.01	*	NS	*
Heart	0.22 ^b	0.37 ^a	0.33 ^a	0.02	*	*	*
GSH-Px (U/g Hb)							
Liver	0.09 ^b	0.14 ^a	0.14 ^a	0.01	**	*	**
Heart	0.28 ^b	0.45 ^a	0.46 ^a	0.03	**	NS	**
SOD (U/Hb)							
Liver	35.72	37.91	35.70	1.23	NS	NS	NS
Heart	114.21	128.45	122.49	4.64	NS	NS	NS
Vitamin C (mg/dL)							
Liver	0.82 ^b	1.10 ^a	1.03 ^a	0.04	*	*	*
Heart	0.63 ^b	0.92 ^a	0.96 ^a	0.05	***	NS	**

P: Statistical significance; SEM: standard error of the mean; NS: nonsignificant; *: P < 0.05; **: P < 0.01; ***: P < 0.001; ^{a,b}: mean values with different superscripts within a row differ significantly.

In the present study, cold stress conditions had increased glucose, triglyceride, and cholesterol levels in the serum taken at the end of the study, but dietary OPE had a positive effect on these parameters. Our results are in agreement with the findings of Priya and Gomathy (27). The increased level of serum glucose in cold-stressed birds could result from their increasing energy demand to keep themselves warm. In addition, the increased glucose level might result from the increased cortisol activity. Kucuk et al. (3) indicated that vitamin C and E supplementation to diets of laying hens that were exposed to low ambient temperature decreased serum glucose, triglyceride, and cholesterol levels. Glucose production causes the production of nonprotein nitrogen, decreases the incorporation of glucose carbon into protein, and increases uric acid excretion (28). In parallel with these findings, increased serum uric acid levels of the control group obtained in the present study may be associated with the presence of cold stress. We observed that birds kept under cold stress had higher levels of total protein and dietary OPE at 200 ppm had a positive effect on total blood

protein level. Similarly, Aarif and Mahapatra (4) indicated that cold stress increased the total serum protein level of broad-breasted white turkeys reared under low ambient temperature in both sexes.

The increments in MDA levels of liver and heart tissues in the current study might be due to cold-stressed conditions. Cold stress often causes damage to cell membranes. It is well known that environmental stress causes an increased production of free radicals, thus resulting in increased levels of MDA (4,6). Supplementation of OPE to the diet reduced the MDA levels in liver and heart tissues under cold-stressed conditions in this study. Similarly, Kucuk et al. (3) indicated that supplementation of vitamins C and E, which have antioxidant properties, decreased the serum MDA levels of laying hens that were exposed to low ambient temperature. In this study, OPE supplementation had positive effects on antioxidant enzyme activities of the liver and heart. Similarly, Akbarian et al. (26) recorded that the dietary supplementation of orange peel essential oil significantly changed the GSH-Px and SOD activities as compared to the control group. It is possible that the

Table 10. Effect of orange peel extract (OPE) supplementation on the fatty acid composition of lipids isolated from breast meat under low ambient conditions (%).

Fatty acids	Control	OPE-100	OPE-200	SEM	P - mean effects of diets		
					Linear	Quadratic	Combined
C16:0	17.94	17.63	17.22	0.28	NS	NS	NS
C16:1 ω7	1.40	1.90	1.18	0.17	NS	NS	NS
C18:0	30.47 ^a	30.27 ^a	28.65 ^b	0.37	*	NS	*
C18:1 ω9	1.74	1.55	1.62	0.05	NS	NS	NS
C18:2 ω6	28.84	28.16	27.80	0.59	NS	NS	NS
C18:3 ω3	0.55	0.77	0.57	0.07	NS	NS	NS
C20:2 ω6	12.77 ^b	12.62 ^b	14.62 ^a	0.35	*	NS	*
C20:3 ω6	0.56	0.42	0.43	0.04	NS	NS	NS
C20:4 ω6	-	0.41	0.49	0.02	-	-	-
C24:0	0.69	0.68	0.75	0.03	NS	NS	NS
C24:1	0.66	0.78	1.06	0.07	NS	NS	NS
C22:5	0.81	0.95	0.93	0.04	NS	NS	NS
C22:6 ω3	3.57 ^b	3.86 ^b	4.68 ^a	0.15	*	NS	*
ΣSFA	49.10	48.58	46.62	0.53	NS	NS	NS
ΣMUFA	3.80	4.23	3.86	0.24	NS	NS	NS
ΣPUFA	47.10 ^b	47.19 ^b	49.52 ^a	0.50	*	NS	NS
Σ ω-6	42.17	41.61	43.34	0.46	NS	NS	NS
Σ ω-3	4.12 ^b	4.63 ^b	5.25 ^a	0.14	***	NS	***
Σ ω-6 / ω-3	10.24 ^a	8.99 ^b	8.26 ^b	0.27	***	NS	**

SFA: Saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; P: statistical significance; SEM: standard error of the mean; NS: nonsignificant; *: P < 0.05; **: P<0.01; ***: P<0.001; ^{a,b}: mean values with different superscripts within a row differ significantly.

antioxidant compounds within the essential oils are being utilized by the cells, thus sparing the intracellular antioxidant system. Vitamin C levels of liver and heart tissues were found to be higher in the OPE groups than the control group in this study. This may be explained by the vitamin C concentration of the OPE supplemented to the diets.

In our study, fatty acid composition of breast meat was determined, and feeding OPE resulted in increased accumulation of ω-3 fatty acids in breast muscle lipids and increased amounts of desaturation and elongation products such as C20:2 ω6 and C22:6 ω3, while no statistical differences in total or index values were observed with OPE. Supplementing OPE also decreased the C18:0 level and total ω-6/ω-3 ratio of breast meat. An increase

has been shown in the demand for foods with enhanced levels of functional fatty acids, such as long-chain ω-3 fatty acids and conjugated linoleic acids, due to their biological roles in cells. In addition, a high ω-6/ω-3 ratio has been reported to promote the pathogenesis of many diseases, including cardiovascular disease, cancer, osteoporosis, and inflammatory and autoimmune diseases, whereas increased levels of ω-3 PUFAs (a lower ω-6/ω-3 ratio) exert suppressive effects (29). In our study, supplemental OPE lowered the ω-6/ω-3 ratio of breast meat, which is preferred by nutritionists. Similarly to our study, Bölükbaşı et al. (30) observed that bergamot oil added to feeds of layer hens significantly increased eicosapentaenoic acid, docosahexaenoic acid, and ω-3 concentrations and decreased the ω-6/ω-3 ratio in egg yolk. On the other

hand, Aksu Elmali et al. (8) stated that supplementation of vegetable extracts to quail ration did not have a significant effect on fatty acid composition.

It is concluded that there is a definitive effect of OPE on the performance, antioxidant, and biochemical parameters in Japanese quails reared under cold-stressed conditions. These effects of dietary OPE in response to cold stress help birds to overcome stressful conditions. Furthermore, it is

evident from this study that OPE supplementation to the diets of birds can also help them thrive well under cold climatic conditions.

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