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EFFECTS OF ORANGE PEEL OIL ON QUAIL (*COTURNIX COTURNIX JAPONICA*) GROWTH-PERFORMANCE, EGG QUALITY AND BLOOD PARAMETERS

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Abstract: In food industry, citrus peels are emerging as waste during processing of the fruits to juice or canned food. Thus, significant amounts of citrus peels are obtained each year all over the world. The aim of this study was to investigate the effects of orange peel oil on quail growth-performance, egg quality and blood parameters. First, essential oil was extracted from orange peel and then different amounts of essential oil (200, 400 and 600 ppm) were mixed with quail feed. Essential oil-containing and essential oil-free feeds were individually supplied to 3-5 day aged 36 quails during 9 weeks. Growth performance parameters, egg weight, shell thickness, shell strength, Haugh Units and some blood parameters of the quails were determined. The gas chromatography results revealed that limonene was the main volatile, aromatic and bioactive component of orange peel essential oil. Orange peel essential oil addition did not significantly affect growth performance of the quails. Moreover, essential oil addition influenced egg weight, b* values of yolk and a* values of albumen. Additionally, supplementation of 600 ppm essential oil increased monounsaturated fatty acids ratio and reduced the total saturated fatty acids ratio. In terms of blood parameters, essential oil addition affected quail blood parameters such as GLC, ALP, GOT, GPT, LDH values, while

ALB, TPROT, CH and TG values of the quails were not affected. In conclusion, orange peel oil may be used as valuable alternative natural additive for poultry feed.

Keywords*:* essential oil, limonene, egg quality, blood parameters, quail, feed.

INTRODUCTION

In food industry, after citrus fruit processing, citrus peels are obtained as waste. In this way, significant amounts of citrus peels are obtained each year in fruit industries throughout the world. D-limonene is one of the most common terpenes in nature and it is mostly found in the peels of citrus fruits such as orange, lemon, mandarin, lime, and grapefruit (Miller et al., 2008; Sun, 2007). Chromatographic measurements revealed that limonene was the main volatile, aromatic and bioactive component of orange peel essential oil. D-limonene is an aromatic and bioactive compound and it is commonly used as flavouring agent in fruit juices, soft drinks, baked goods, ice creams, and puddings. Additionally, d-limonene is listed in the Code of Federal Regulations as generally recognized as safe (GRAS) (Miller et al., 2008; Sun, 2007).

Both quail meats and eggs are considered as one of the most important protein sources of human diets. Quails have got rapid growth rate and egg maturity compared with the other poultry types. The age of the quail cut maturity and female egg maturity are 5-6 weeks. Moreover, quails are not only economic for the producers, but regarded as good nutritive products for the consumers. Quail eggs consist of approximately 25% dry matter, 14% crude protein, 11% crude fat and 1% ash. Additionally, egg fat contains 61-61% triglycerides, 33-34% phospholipids and 4-5% cholesterol. Also, quail egg is containing important ratio of group B vitamins, antioxidants and essential amino acids. In recent years, consumers have got some health concerns due to rapid living conditions, growing population and malnutrition. Hence, particularly poultry eggs are removed from human diets due to their cholesterol content. In addition to this, many consumers prefer poultry meat instead of red meat for various health and economic concerns.

In recent years, there is an innovative subject such as production of functional poultry feeds which might enhance poultry egg and meat quality. In this reason, poultry feeds were enriched with many additives such as antibiotics, antioxidants, prebiotics, probiotics, organic acids, herbs and herbal essential oils, various oils, vitamins, minerals and amino acids. In literature, essential oil supplementation to quail feed was reported by Denli et al. (2004) - herb essential oils, Cabuk et al. (2014) - herbal essential oil mixture, Luna et al. (2012) - thymol and isoeugenol, Labaque (2013) - thymol, Ciftci et al. (2013) - rosemary (*Rosmarinus officinalis* L.) oil, Mehdipour et al. (2013) - synbiotic and cinnamon, Yesilbag et al. (2013) - rosemary and oregano volatile oil mixture, and Olgun and Yildiz (2014) - essential oils mixture (thyme, black cumin, fennel, anise and rosemary).

The main aim of the present study was to investigate the effects of inclusion of essential oil obtained from orange peels on quail growth performance, blood parameters and egg quality.

MATERIALS AND METHODS

Materials

A total of 36 quails (*Coturnix coturnix japonica*) at 3-5 days age were purchased from a commercial farmer in Bayramiç-Çanakkale province, Turkey. The quails were divided into 4 treatment groups. In each group the quails were placed individually in the cages.

The orange (*Citrus sinensis*) peel used in this study was obtained from local restaurants in Muğla province, Turkey. The orange peel oil (OPO) (containing 94,74% limonene) was obtained from fresh peel by hydro-distillation, using a Clevenger system with 150 g dry plant material and 1500 mL water. The oil was obtained after 3 h of distillation at

boiling temperature and stored at 4 ± 1°C in airtight glass vials covered with aluminium foil. The gas chromatography–mass spectrophotometry (GC–MS) analysis of the obtained essential oil was conducted at Mugla Sitki Kocman University (Mugla, Turkey) by using an Agilent GC (model 6 890) and an Agilent MS (model 5 973) equipped with a mass selective detector (MSD). Identification of the components in OPO was carried out with Wiley 275 MS data library. The GC results of the orange peel oil are given in Table1.

Table 1. Active ingredient content of orange (*Citrus sinensis*) peel oil

Active Ingredients	%	RT[a] (min)
E-2-Hexanal	0,015	2,750
1-Hexanol	0,043	3,216
α - pinene	0,761	4,902
3-Hexanol	0,045	5,216
4-Methyl-2-Penthanol	0,051	5,509
Beta-Phellandrene	0,323	5,989
Octanal	0,190	6,608
β-Pinene	2,397	6,723
3-Carene	0,461	7,374
D-Limonene	**94,742**	**8,221**
β-Ocimene	0,037	9,015
Gamma-Terpinene	0,096	9,343
1-Octanol	0,165	9,888
(4)-4-Carene	0,123	10,726
p-Menth-1-en-4-ol	0,225	14,952
Decanal	0,173	16,852
Cis-Citral	0,153	20,216

[a]*: Retention time.*

The diet was formulated to meet the nutrient requirements of the Japanese quails (24% CP and 2900 kcal/kg ME for starting and growing periods) according to the National Research Council (NRC, 1994) recommendations. OPO was added to the feed at 200 mg kg^{-1}, 400 mg kg^{-1} and 600 mg kg^{-1}. The control diet contained no OPO supplementation.

All of the other chemicals were purchased from Sigma-Aldrich and Merck.

Methods

Quails Performance Features

The specific growth rate (SGR) and feed conversion ratio (FCR) of the quails was monitored for 9 weeks. The SGR (1) and FCR (2) were calculated by using the following equations;

$$\% \mathbf{SGR} = [\ln(\text{last average weight}) - \ln(\text{first average rate}) / \text{number of trial days} \times 100] \quad (1)$$

$$\mathbf{FCR} = \frac{\text{feed consumption}}{\textit{weight gain}} \quad (2)$$

Quails Egg Quality Analysis

The shell thickness, yolk width, height, albumen width, height and length were measured by using digital calliper. Haugh Units (3) were calculated by using the following equation (Eisen et al., 1962).

$$Haugh\ Unit = 100 \log[Albumen\ height(mm) + 7.57 - 1.7\ Egg\ weight\ (g)^{0.37}] \quad (3)$$

A Texture Analyzer TA-XT2i (Stable Microsystems, Surrey, UK) was used to determine the egg shell strength and the measurements were done according to Xie et al. (2002). The puncture test parameters were 2 mm cylinder probe, weight head 50 kg, probe inlet velocity 2 mm/s, outlet velocity 2 mm/s. The puncture test results were calculated by TA-XT2i Software and the results were given as hardness values (N).

The colour values of the egg samples were determined with Minolta Colorimeter (Cr 400, Conica, Minolta, Japan).

Fatty Acid Composition

The lipid extraction of the egg samples was made according to Folch et al. (1957). The fatty acid methyl esters (FAME) were prepared according to ISO method 5 509 (ISO, 1978). Determination of the fatty acid methyl esters were performed using GC-MS (Shimadzu QP 2010, Kyoto, Japan) equipped with Mass Spectroscopy (MS) and column (100 m x 0,25 mm x 0,20 um, Restek, USA). Fatty acids were expressed as percentage of total methyl esters of fatty acids.

Blood collection and analyses

Three quails were randomly selected from each group and were decapitated, and the analyses were made on their blood samples. Prior to blood collection, the quails were fasted for 12 h. The blood samples were collected from the jugular vein on the last day of the trial, for use in biochemical analyses. The blood was then centrifuged at 4 000 rpm for 10 min to separate the serum for biochemical analyses. Biochemical indices, including glucose (GLU), total protein (TPROT), albumin (ALB) triglyceride (TRI), cholesterol (COL), alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) in serum were analysed using bioanalytical test kits (Bioanalytic Diagnostic Industry, Co) and measured by a Shimadzu spectrophotometer (PG Instruments, UK).

Statistical analyses

The results were represented as mean values with standard deviations. The data were analysed by ANOVA and the multiple comparisons of the means were accomplished with Tukey’s test. Statistical analysis was performed with Minitab v.17.1.0.

RESULTS AND DISCUSSION

The quail growth performance parameters are shown in Table 2. The initial weight of the control, OPO200, OPO400 and OPO600 groups were 31,78; 30,67; 33,44 and 28,33 g respectively. The final weight of the control, OPO 200, OPO 400 and OPO 600 groups were 166,33; 159,89; 170,22 and 161,89 g at the end of the eight-week period, respectively. There was no difference among the initial weight of the quails and similar results were observed for the final weight. Higher weight gain was observed for the OPO 400 group, though there was no difference among the groups, in terms of weight gain. Similar results were observed for the specific growth rate (SCR). Only in the feed conversion ratios of the groups there were statistically significant differences. The FCR values of the OPO supplemented groups were lower than that of the control group. The results indicated that orange peel oil supplementation did not influence weight gain and specific growth rate but it improved feed conversion ratio. In previous studies, it was reported that essential oil supplementation effects on performance parameters are inconsistent (Amerah & Ouwehand, 2016). Olgun and Yildiz (2014) reported that supplementing of essential oils from thyme, black cumin, fennel, anise and rosemary had no significant effect on performance parameters of quails. Ciftci et al. (2013) reported that rosemary oil supplementation did not affect the live weight gain and feed intake, while it improved the feed conversion ratio of quails. Luna et al. (2012) reported that feed supplementation with thymol or isoeugenol did not significantly affect growth rate and final body weight. Yesilbag et al. (2013) indicated that rosemary and oregano volatile oil

mixture supplementation did not affect body weight of quails, while it increased feed intake and improved feed efficiency. Denli et al. (2004) demonstrated that addition of thyme essential oil and flavomycin to quail diet led to significantly higher body weight gains and better feed efficiency as compared to that of control group. Mehdipour et al. (2013) reported the supplementing 200 mg cinnamonoil/kg and virginiamycin increased body weight gain of quails at 21–35-day experimental period. Our findings are close to literature findings.

Table 2. Performance parameters of quails fed with orange peel oil supplementation

	Control	OPO200	OPO400	OPO600
Initial Weight (g)	31,78±3,77	30,67±4,39	33,44±4,33	28,33±4,18
Final Weight (g)	166,33±12,31	159,89±18,39	170,22±14,38	161,89±20,59
Weight gain (g)	134,56±13,60	129,22±19,17	136,78±14,53	133,56±20,06
FCR	3,48±0,64a	3,32±0,90ab	2,53±0,45b	2,72±0,51ab
SGR	3,39±0,31	3,37±0,40	3,33±0,29	3,56±0,36

**The small letters show the differences among the treatments in the same row (p<0,05).*

The weight, shell thickness, shell strength and Haugh unit of the quail eggs are given in Table 3. In terms of egg weight, there were statistically significant differences while shell strength, shell thickness and Haugh units of the egg were not different (p<0,05). According to the results in Table 3, the weight of the quail eggs ranged between 8,10 and 9,31 g. In literature, weights of eggs obtained from quail fed with essential oil supplemented feed were reported as 11,48-12,17 g by Olgun and Yildiz (2014), 12,81-13,02 g by Luna et al. (2012), 11,07-11,70 g by Yesilbag et al. (2013). Our findings are quite lower than the literature findings. Additionally, the eggs of the control group had higher egg weight than the OPO supplemented groups, hence OPO supplementation negatively affected egg weights. So in our study, the quail egg weight was affected by orange peel oil supplementation, while opposite findings were reported by Olgun and Yildiz (2014), Luna et al. (2012) and Yesilbag et al. (2013). The shell thickness and strength values of the control group eggs were 0,20 mm and 11,18 N, respectively. On the other hand, the shell thickness and strength values of the OPO supplemented group eggs were 0,18-0,24 mm and 9,76-12,42 N, respectively. In literature, shell thickness values of quail eggs, when the quails were fed with essential oils (thyme, black cumin, fennel, anise and rosemary), were reported as 0,19-0,21 mm (Olgun & Yildiz, 2014). The researchers indicated that egg shell thickness was affected by essential oil mixture supplementation. On the other hand, Luna et al. (2012) reported that essential oil supplementation did not significantly affect the physical characteristics of eggs. The egg shell thickness and strength values of the eggs of the control group were 17,80 µm and 10,24 N, while the eggs of the rosemary and oregano volatile oil supplemented groups were 17,42-17,97 µm and 10,17-10,97 N, respectively (Yesilbag et al., 2013). In this study, it was observed that essential oil supplementation did not influence egg shell thickness and strength (Yesilbag et al. 2013). These literature findings are close to our results. Our findings are lower than the literature data. Haugh unit was defined as an indicator of the albumen quality for bakery industry (Sahin et al., 2008). The Haugh units of all samples were higher than 88. Yesilbag et al. (2013) reported that Haugh units of the control group eggs were 101.81 and rosemary and oregano volatile oil mixture supplemented group eggs were 101,49-103,67.

Table 3. Features of the egg of quail fed with orange peel oil supplementation*

	Weight (g)	Shell Thickness (mm)	Shell Strength (N)	Haugh Unit
Control	9,31 ± 1,30a	0,40 ± 0,38	11,18 ± 3,10	91,17 ± 2,14
OPO200	8,10 ± 0,70b	0,24 ± 0,04	12,42 ± 0,36	88,20 ± 1,98
OPO400	8,73 ± 0,74ab	0,18 ± 0,02	9,76 ± 0,99	92,87 ± 1,96
OPO600	8,36 ± 0,76b	0,20 ± 0,02	11,01 ± 1,74	93,63 ± 2,52

**The small letters show the differences among the treatments in the same column (p<0,05).*

The albumen and yolk colour values of the quail eggs are given in Table 4. The L, a* and b* values of the egg yolks were 50,38-53,19; 5,74-7,29 and 43,56-48,23 respectively. Besides, there were no differences between the L values of the treated and control samples, while significant differences were found for the a* and b* values. The L, a* and b* values of the albumen were 89,11-90,54; -2,60- -3,37 and 5,00-6,96, respectively. Similar to the yolk colour results, there were no differences between the L values of the treated and control samples, while significant differences were found for the a* and b* values in terms of albumen colour.

Table 4. Colour properties of the egg of quail fed with orange peel oil*

Yolk			
	L	**a***	**b***
Control	50,58 ± 3,83	5,74 ± 4,28	43,56 ± 5,08b
OPO200	53,19 ± 2,25	6,80 ± 4,12	48,23 ± 2,08a
OPO400	50,38 ± 2,32	5,96 ± 3,57	44,05 ± 2,99b
OPO600	52,36 ± 1,72	7,29 ± 3,37	47,20 ± 2,56ab
Albumen			
	L	**a***	**b***
Control	89,62 ± 2,64	-2,60 ± 0,83b	6,51 ± 2,34
OPO200	89,11 ± 2,20	-3,37 ± 0,67a	6,96 ± 2,55
OPO400	89,80 ± 1,34	-2,80 ± 0,60ab	5,58 ± 1,89
OPO600	90,54 ± 1,17	-2,90 ± 0,37ab	5,00 ± 1,15

**The small letters show the differences among the treatments in the same column (p<0,05).*

The fatty acid composition of the eggs of the OPO supplemented and control groups are shown in Table 5. The major saturated fatty acids of all egg samples were palmitic and stearic, while the major unsaturated fatty acids were oleic and linoleic acids. The total saturated fatty acid ratio of the eggs of the OPO600 quail group when compared with the other egg groups had lower ratio. Additionally, the MUFA ratio was higher than that of other egg groups, while PUFA ratio was lower. As a result, the 200 and 400 ppm OPO supplementation did not affect the fatty acid composition of the eggs, but 600 ppm OPO supplementation affected especially the oleic acid ratio of the eggs. Bolukbasi et al. (2010) reported that bergamot oil supplementation led to an increase in eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and n-3 concentration, while a decrease in the n-6/n-3 ratio of the egg yolk was observed. Our findings are differing from literature findings. These differences might be explained with the fact that different types of oils supplementation may lead to different changes in fatty acid composition of the eggs. Previous studies reported that differences of the fatty acid composition of the eggs depended on different oils supplemented to feed (Ceylan et al., 2011; Aydin & Cook, 2004; da Silva et al., 2009).

Table 5. Fatty acid composition (%) of the egg of quail fed with orange peel oil

Fatty Acid	Name	Control	OPO200	OPO400	OPO600
C14:0	Miristic acid	0,47	0,20	0,43	0,44
C16:0	Palmitic acid	21,41	22,71	22,14	18,91
C16:1	Palmitoleic acid	5,42	5,13	4,64	3,69
C17:0	Heptadecanoic acid	0,23	0,24	0,25	0,18
C17:1	cis-10-Heptadecanoic acid				
C18:0	Stearic acid	8,66	8,44	9,15	8,39
C18:1n9	Oleic acid	43,39	40,31	44,43	51,84
C18:2n6	Linoleic acid	15,99	17,76	16,30	12,99
C18:3n6	gamma-Linolenic acid	0,25	0,60	0,33	0,25
C18:3n3	alpha-linolenic acid	0,53	0,62	0,57	0,51
C20:1n9	cis-11-eicosenoic acid	0,17	0,20	0,08	0,14
C20:2n6	cis-11,14-Eicosadienoic acid				
C20:3n3	cis-11,14,17-Eicosatrienoic acid				
C20:5n3	cis-5,8,11,14,17-Eicosapentaenoic acid	1,15	1,25	0,99	1,34
	Total	**97,67**	**97,46**	**99,31**	**98,68**
	Total saturated	**30,77**	**31,59**	**31,97**	**27,92**
	Mono unsaturated	**48,98**	**45,64**	**49,15**	**55,67**
	Polyunsaturated	**17,92**	**20,23**	**18,19**	**15,09**
	Total unsaturated	**66,90**	**65,87**	**67,34**	**70,76**

The effects of OPO on quail's biochemical features are presented in Table 6. In parallel with the value of essential oils groups, serum GLU level was significantly higher in the OPO 600 group ($p<0,05$). Serum TPROT, ALB, CHO and TG levels showed no significant differences between treatment groups ($p>0,05$). Liver enzymes such as ALP and GOT increased with inclusion of OPO in the diets and showed significant differences compared with control groups ($p<0,05$). The GPT and LDH levels were significantly lower in OPO 400 and OPO 600 groups ($p<0,05$).

Table 6. Blood parameters of quail fed with orange peel oil*

Parameters	Control	OPO200	OPO400	OPO600
ALB	0,20±0,08	0,22±0,03	0,12±0,05	0,26±011
GLC	311,2±64,5b*	337,9±25b	346,1±50,6b	553,2±97,8a
TPROT	9,51±2,10	9,18±1,16	9,45±2,14	10,33±2,74
CH	223,3±38,4	200±32,3	187,8±37,3	280,7±70,3
TG	150,3±24,3	123,46±11,03	146,35±6,77	128,72±13,24
ALP	177,38±3,98b	193,7±34,4b	304,52±8,76a	317,9±30,9a
GOT	37±10,36ab	24,83±1,78b	41,38±3,7a	36,60±1,84ab
GPT	24,36±3,05b	34,45±2,12a	28,17±2,41ab	26,11±1,95b
LDH	221,5±20,2a	188,51±9,96a	52,2±17,6b	85,86±4,78b

**The small letters show the differences among the treatments in the same row ($p<0,05$).*

The massive use of antibiotics as growth promoters and as initiators of bacterial infections in feed lead to phenomena of resistance to antibiotics, and the utilization of

chemicals can be harmful to animals, consumers and environment (Alderman & Hastings, 1998). Hence, much attention has been given to natural products in order to replace antibiotics in poultry farming. One such possibility of natural products is the use of essential oils. They are obtained from many plant materials such as flowers, buds, seeds, leaves, fruits (Brenes & Roura, 2010). The present study aimed to evaluate the possible effects of OPO, which is obtained by fruit juice industry waste, as growth promoter and to improve the quail health status. In this context, earlier studies were attempted to determine citrus peel essential oil effects on growth performance under stressful conditions of quails (Ciftci et al., 2013). In the present study, it was aimed to determine the effects of different concentrations of OPO on growth performance, serum biochemical parameters, egg fatty acid composition.

It has been reported that ALP, GOT, GPT and LDH are remarkable values for the determination of liver damage in birds (Mahmoud et al., 2012). In the present study there was an increase in ALP and GOT activities of birds fed with OPO 400 and OPO 600 groups. Based on this result, we can say that OPO has hepatoprotective effect when used in diet at limited levels. Similar results were obtained by Denli et al. (2005) who used 10 g/kg flavomycin in quial diets. However, quails fed with OPO 400 and OPO 600 diets tended to decrease serum GPT and LDH levels compared to control groups. AST, ALT and LDH usually appear in serum, when there is damage on the liver and muscle tissues caused by excessive stress (Scholl et al., 2006; Ozyurt et al., 2006). A decrease in enzyme activity may be due to the protective effect of OPO on cells, tissues and organs. Vahdatpour and Babazadeh (2016) reported no damage in liver enzymes when kefir was used in quail diets. In the present study, serum GLC concentration was statistically found higher in the OPO 600 group compared with the other treatments. However, earlier studies on broiler chicks found no differences in serum GLC level when the birds were fed with black seed and cumin seed supplemented diets (Alimohamadi et al., 2014). Also, a study on quails supported the findings of Alimohamadi et al., (2014), when coriander extract was used in poultry drinking water (Hosseinzadeh et al., 2014). High rate of OPO used in the present study may have caused the breakdown of glucose mobilization in the tissues and may lead to migration from the tissues directly to the blood. Serum total CH and TG levels were decreased in the OPO 200 and OPO400 groups. Similar to the current results cholesterol lowering effects of essential oils were reported in laying hens (Ali et al., 2007; Radvan-Nadia et al., 2008). It is known that plants are effective at lowering cholesterol due to the phytosteroids they contain and this hypocholesterolaemic effect of essential oils in chickens may be related with the inhibitory effects of active substances in essential oils on hepatic 3- hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (Crowell, 1999). Results achieved from this study agree with Al-Kassie (2009) who reported that when 200 ppm of essential oil derived from *Cinnamomum verum* were added to a standard diet of broiler chicks for 42 days; a significant increase in total proteins was observed. In the present study, the increase in TPROT level was not statistically different among the treatment groups, but slightly higher values were observed when OPO was used in the quail diets.

CONCLUSION

Orange peel oil supplementation did not negatively affect quail growth performance, but improved feed conversion ratio. On the other hand, OPO addition led to a decrease in egg weight, while egg shell thickness and strength were not affected. Moreover, 600 ppm OPO supplementation decreased the total saturated acids ratio of the quail eggs. The analysis of blood biochemical parameters revealed that OPO supplementation to quail had a hepatoprotective effect.

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