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Original Article

Orange Peel Oil Supplementation in Laying Quail Diet: Effects on Performance and Oxidative Stability of Egg Yolk

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ABSTRACT

The present study aimed to investigate the potential utilization of orange peel essential oil (OPEO), as a natural feed additive in poultry nutrition. The impact of incorporating OPEO into the diets of laying quails was examined with respect to various performance criteria, including feed consumption and efficiency, as well as egg production, weight, and shelf life. A total of 120 female egg quails, aged 16 weeks, were allocated into three main groups, and fed with control rations supplemented with OPEO at levels of 0 mg/kg (control group), 200 mg/kg, and 300 mg/kg. Throughout the study, egg production was monitored on a daily basis, feed consumption was recorded every two weeks, and egg weights were measured twice a week on consecutive days. Moreover, the levels of malondialdehyde, a lipid peroxidation marker, in the egg yolks were quantified. The results indicated that the inclusion of OPEO in quail rations did not exert a significant influence on feed consumption and efficiency. However, OPEO supplementation had a positive impact on egg production and weight, leading to substantial increases in both crucial performance parameters. Furthermore, the study elucidated a statistically significant impact of OPEO on the levels of malondialdehyde in the egg yolks. In summary, the incorporation of OPEO into quail diets showcased remarkable efficacy in significantly augmenting egg yield and weight, while leaving feed consumption unaffected. These results highlight the potential of utilizing OPEO as a natural feed additive to improve the performance and egg quality of laying quails in commercial production systems.

INTRODUCTION

The global demand for poultry products has been steadily increasing in response to the growing population and changing dietary preferences (FAO, 2013). To meet this rising demand, the development of innovative strategies to enhance animal productivity in the poultry industry has become imperative. Japanese quail (*Coturnix coturnix japonica*) has gained recognition as a model species in the meat and egg production industry due to its distinct characteristics, including small body size, fast growth rate, and short generation interval (Jeke *et al.*, 2018; Elsaidy *et al.*, 2021). Furthermore, Japanese quail farming offers significant advantages in developing countries as it provides a cost-effective source of animal protein, characterized by its low price, high-quality protein content, and economic value. However, intensive quail rearing practices can pose challenges, including reduced animal immunity, which in turn leads to decreased meat and egg production. Traditionally, the poultry industry has relied on the use of antibiotic growth promoters to mitigate such challenges. However, since the European Union banned the use of antibiotics in poultry feeding in

2006 due to concerns regarding antibiotic resistance and public health, there has been a growing need to explore alternative feed additives that can improve productivity and maintain animal health (EC, 2006). Consequently, researchers and industry professionals have shifted their focus towards finding effective and sustainable alternatives to antibiotic growth promoters (Gong *et al.*, 2014; Zeng *et al.*, 2015; Jamil *et al.*, 2022).

Fruit and vegetable processing methods generates substantial amounts of waste and by-products, resulting in environmental pollution and economic losses (Domínguez *et al.*, 2020). The principles of the circular economy advocate for the utilization of renewable biological resources to produce value-added products, including food and animal feed (Georganas *et al.*, 2020). Notably, vegetable and fruit processing residues contain a wide array of bioactive compounds that offer potential health benefits (Costa *et al.*, 2017). Consequently, their incorporation into animal diets not only addresses waste management challenges but also provides an avenue to develop value-added products (Truong *et al.*, 2019). Among the various residues, citrus peels and components derived from the fruit juice industry have emerged as viable candidates for evaluation as functional compounds. Citrus essential oils derived from citrus peels exhibit a broad spectrum of biological activities, including antimicrobial, antifungal, antioxidant, anti-inflammatory, and anxiolytic properties, making them promising candidates as food and feed additives (Singh *et al.*, 2021).

Previous studies have demonstrated the beneficial effects of essential oil mixtures containing citrus peel oils in broiler chickens, showing increased body weight and feed efficiency when compared to antibiotic-treated groups (Alçiçek *et al.*, 2003). Similar positive outcomes have been observed in layer quails, with improved egg yield and feed efficiency upon the addition of essential oil mixtures containing citrus peel oils (Cabuk *et al.*, 2014).

Among the citrus essential oils, orange peel essential oil (OPEO) is particularly noteworthy. OPEO is concentrated in the colored (flavedo) part of the peel and pigment glands, constituting approximately 0.2-0.5% of the whole fruit. The primary component of OPEO, "limonene" (D-Limonene, 1-methyl-4-(1-methylethenyl) cyclohexane), represents approximately 95% of the oil and is considered safe for use in various industries, including food, cosmetics, perfumery, gum, beverages, and soap, and Generally Recognized as Safe (GRAS) additive (Hosni *et al.*, 2010; Farhat *et al.*, 2011).

While OPEO has recently been utilized as an additive in poultry rations, research investigating its effects is still limited. Previous studies have shown that the addition of OPEO to broiler diets at different concentrations resulted in increased body weight and feed conversion efficiency, with a positive correlation between OPEO concentration and performance (Aydın & Alçiçek, 2018). Furthermore, supplementing OPEO in the diet of heat-stressed chickens has been found to increase egg weight and shell thickness (Özek, 2012), while also improving egg quality in layer quails (Erisir *et al.*, 2015). Additionally, OPEO has been shown to positively impact serum parameters in both chickens (Alzawqari *et al.*, 2016) and layer quails (Sevim *et al.*, 2020).

In light of the extensive knowledge presented, the primary focus of this study was to investigate the impact of incorporating orange peel essential oil (OPEO), derived from the by-product of the fruit juice industry, into the feed of quails – an important poultry species raised for its meat and egg production. Specifically, this research aimed to comprehensively examine the effects of OPEO supplementation on key performance indicators of laying quails, including egg production, egg weight, feed consumption, feed efficiency, and egg shelf life. By conducting a systematic evaluation of the effects of OPEO on these parameters, this study contributes to a deeper understanding of its potential as a natural feed additive in enhancing the productivity and quality of poultry products.

MATERIALS AND METHODS

Animals

The animal model employed in this study consisted of 120 female laying quails (*Coturnix coturnix japonica*) at 16 weeks of age. The animals were housed under conditions of controlled lighting, following a 16-hour light and 8-hour dark cycle. Throughout the investigation, the quails were provided *ad-libitum* access to both feed and water. The ethical implications of the study were carefully addressed, and all experimental procedures were conducted in accordance with the guidelines set forth by the Manisa Celal Bayar University Animal Experiments Local Ethics Committee, adhering to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Decision No: 77.637.435).

Experimental procedure

A total of 120 female laying quails were divided into four subgroups, with each group consisting of 40 quails. Within each group, subgroups of ten quails were created. The quails were housed in multi-story layer quail cages, ensuring equal representation of each subgroup on each floor of the cages. This setup provided standardized housing conditions throughout the study.

The compound feed used in the laying quail trials was formulated with specific raw materials and nutrient compositions, as outlined in Table 1. The feed composition aimed to provide optimal nutrition for the quails during the study.

Table 1 – Ingredent and nutrient composition of the basal experimental layer quail diet (as fed).

Ingredent	
Corn	57.73
Soybean meal (0.48 CP)	19.18
Sunflower meal (0.37 CP)	8.33
Fish meal (0.70 CP)	2.00
Vegetable Oils	3.90
Ground limestone	6.96
Monocalcium phosphate	1.14
Salt	0.20
Vitamin-Mineral premix*	0.25
DL-methionine	0.32
Total	100.00
Nutrient Composition, %	
Dry matter	89.56
Crude Protein (CP)	18.00
Crude fiber	3.25
Crude ash	10.74
Ether extract	6.14
Methionine + cystine	0.82
Lysine	0.88
Calcium	3.00
Phosphorus, available	0.60
Metabolisable Energy, kcal/kg	2900

*Vitamin-mineral premix in 3.0 kg: Vitamin A 12000000 IU; Vitamin D3 3000000 IU; Vitamin E 30000 mg; Vitamin K3 5000 mg; Vitamin B1, 3000 mg; Vitamin B2 12000 mg; Vitamin B6 4000 mg; Vitamin B12 30 mg; Nicotine amide 55000 mg; Calcium--D-Pantothenate 15000 mg; Folic acid 2000 mg; D- Biotin 250 mg; Choline Chloride 600000 mg; Antioxidant 10000 mg; manganese 80000 mg; Iron 40000 mg; Zinc 60000 mg; Copper 5000 mg; Iodine 400 mg; Cobalt 100mg; Selenium 150mg.

The quails were divided into three main groups and fed three different diets throughout the experimental period. The groups included: (1) Control diet without orange peel essential oil (OPEO) (basal diet), (2) Basal diet with 200 mg/kg OPEO, and (3) Basal diet with 300 mg/kg OPEO. OPEO was mixed with a carrier and added at a rate of 1 kg per ton of basal diet. Care was taken to ensure thorough mixing to achieve homogenous distribution of OPEO in the ration.

Throughout the 8-week experimental period, the quails' egg production was recorded daily. Feed consumption and feed efficiency were assessed by weighing the feed every two weeks. Additionally, the weight of the eggs was measured twice a week on consecutive days, with a precision of 0.1 grams. Egg weights for the experimental groups were recorded starting from the second week of the experiment. Eggs were collected on two consecutive days each week for this purpose.

At the end of the 8th week of the experiment, 16 randomly selected eggs per diet treatment (4 from each subgroup) were stored at a temperature of +4°C for durations of 1, 15, and 30 days. To evaluate indicators of fatty acid oxidation and staling criteria, the levels of malondialdehyde (MDA) were measured. A total of 48 egg yolks, representative of each diet group, were used for MDA analysis. The concentration of MDA, an index of lipid peroxidation, was determined using the thiobarbituric acid reaction method, as described by Ohkawa *et al.* (1979).

Determination of Thiobarbituric Acid Reactive Substance (TBARS) Value

To determine the Thiobarbituric Acid Reactive Substance (TBARS) value, a TCA solution (6 ml) consisting of 7.5% TCA, 0.1% EDTA, and 0.1% Propyl gallate (dissolved in 3 ml ethanol) was added to each collected egg yolk. The samples were homogenized using a high-speed polytron for 15 seconds and subsequently filtered through Whatman No:1 filter paper. From the filtrate, 1 ml was transferred to a test tube, followed by the addition of 1 ml of 0.02 M TBA solution. The contents of the test tubes, which were glass test tubes with screw caps, were thoroughly mixed. The test tubes were then placed in a water bath at 100°C for 40 minutes, ensuring proper heating. Afterward, the tubes were promptly cooled in cold water for 5 minutes. Subsequently, the tubes were subjected to centrifugation at 2000 rpm for 5 minutes. The absorbance of the resulting supernatant was measured at a wavelength of 530 nm using a spectrophotometer. The TBARS value, expressed as mg malondialdehyde (MDA) per kg, was determined based on the absorbance readings obtained from the spectrophotometer. This measurement served as an indicator of lipid peroxidation in the egg yolks.

Statistical Analyses

The data obtained from the experiment were subjected to statistical analysis using the General Linear Model procedure implemented in the SAS software (SAS

Institute, 1991). To determine significant differences between the experimental groups, Duncan's multiple range tests were employed. A significance level of $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Feed Consumption, Feed Efficiency, and Body Weight

The influence of OPEO on feed consumption, feed efficiency, and final body weight of laying quails is summarized in Table 2. The daily feed consumption of quails in the control group was 30.75 g/quail, while it was 31.16 g/quail in the OPEO (200 mg/kg) group and 30.48 g/quail in the OPEO (300 mg/kg) group. These findings are consistent with previous studies in layer quails (Sevim *et al.*, 2020) and laying hens (Chowdhury *et al.*, 2008) that also reported no significant effect on feed consumption upon OPEO supplementation. However, Erisir *et al.* (2015) observed reduced feed consumption in layer quails supplemented with OPEO. The variation in results could be attributed to the OPEO content and dosage used in the studies, as well as the potential bitter taste of flavonoids in orange peel, which may affect the birds' appetite (Mason *et al.*, 1989; Burdock, 1997).

Table 2 – Effects of OPEO on feed consumption, feed efficiency and body weight in quails.

Treatments	Parameters		
	Feed Intake (g/quail)	Feed Efficiency (g feed/g egg)	Final Body Weight (g)
Control (OPEO-free)	30.75	2.51	279.80
OPEO 200 mg/kg	31.16	2.42	283.23
OPEO 300 mg/kg	30.48	2.45	283.35
SEM Pooled	0.37	0.03	0.363
Probability	0.4357	0.0886	0.4648

OPEO, Orange peel essential oil.

The feed efficiency value, indicating the amount of feed consumed per unit of egg production, improved with the addition of OPEO to the ration, although the difference was not statistically significant. The feed efficiency values were 2.51 g feed/g egg in the control group, 2.42 g feed/g egg in the OPEO (200 mg/kg) group, and 2.45 g feed/g egg in the OPEO (300 mg/kg) group. These results are consistent with other studies that reported no significant change in feed efficiency with OPEO supplementation (Bozkurt *et al.*, 2012; Karabayir *et al.*, 2018; Sevim *et al.*, 2020). Conversely, studies conducted by Bozkurt *et al.* and Çabuk *et al.* (2006) demonstrated that adding an essential oil mixture to laying chicken feeds increased feed efficiency. These findings suggest that the impact of OPEO on feed efficiency may vary across species and depend on the specific formulation and dosage used.

There were no statistically significant effects of OPEO-containing feeds on the final body weight of the quails. The final body weights of quails were 279.80 g in the control group, 283.23 g in the OPEO (200 mg/kg) group, and 283.35 g in the OPEO (300 mg/kg) group. These findings align with previous studies that reported no significant changes in body weight with the addition of orange oil (Karabayir *et al.*, 2018; Sevim *et al.*, 2021) or orange-peel powder (Yildiz *et al.*, 2023) to the ration. The lack of significant effects on body weight suggests that OPEO supplementation within the studied doses does not exert substantial influence on the growth performance of laying quails.

In conclusion, feeding layer quails with diets containing different doses of OPEO did not significantly impact performance parameters. To improve performance quality, further research can explore broader dosage ranges of OPEO or combinations with different essential oil blends to enhance their potential healing effects.

Egg Production and Egg Weight

Significant effects were observed in egg production and egg weight due to the addition of OPEO to the layer quail diet. Supplementation of OPEO in the layer quail diet significantly increased egg production ($p<0.05$) and egg weight ($p<0.01$), as presented in Table 3. Egg production rates were 90.40% in the control group, 92.41% in the OPEO (200 mg/kg) group, and 90.88% in the OPEO (300 mg/kg) group. These findings are consistent with studies reporting positive effects of different doses of OPEO on egg production in poultry (Sevim *et al.*, 2020). Similarly, the addition of an essential oil mixture to the diets of laying chickens has been shown to increase egg production (Çabuk *et al.*, 2006; Bozkurt *et al.*, 2012). However, Özek *et al.* (2011) reported no increase in egg production with the addition of essential oil to hen diets.

Table 3 – Effects of OPEO on egg production and egg weight in quails.

Treatments	Parameters	
	Egg Production (%)	Egg Weight (g)
Control (OPEO-free)	90.40^{b}	12.25^{c}
OPEO 200 mg/kg	92.41^{a}	12.89^{a}
OPEO 300 mg/kg	90.88^{b}	12.45^{b}
SEM Pooled	0.55	0.04
Probability	0.0250	0.0001

^{a,b,c} Means with in column various superscript differ at $p<0.05$ for Egg Production and $p<0.0001$ for Egg Weight.

Egg weight was 12.25 g in the control group, 12.89 g in the OPEO (200 mg/kg) group, and 12.45 g in the OPEO (300 mg/kg) group. Although the 300 mg/kg OPEO dose resulted in slightly lower egg weight compared to the 200 mg/kg dose, it still yielded higher egg weight compared to the control group. Consistent with our findings, Bozkurt *et al.* (2012) reported increased egg weight with the addition of an essential oil mixture to laying hen diets. However, Karabayir *et al.* (2018) found a dose-dependent decrease in egg weight with OPEO supplementation, while Sevim *et al.* (2020) reported no effect on egg weight. These variations in results may be attributed to the specific doses and formulations used in the studies.

Malondialdehyde (MDA) Levels

The effect of OPEO supplementation on the oxidative stability of stored eggs was assessed by measuring malondialdehyde (MDA) levels, a marker of lipid peroxidation. The addition of OPEO in poultry rations resulted in improved egg quality, as evidenced by decreased MDA. Figure 1 illustrates the MDA levels in eggs stored for 1, 15, and 30 days. The MDA accumulation in the control and experimental groups did not differ significantly on the 1st day of storage. However, on the 15th and 30th days, the MDA accumulation in the egg yolk significantly decreased ($p<0.0001$). For instance, on the 15th day, the MDA accumulation in the control group was 0.179 mg/kg, while it was 0.153 mg/kg in the 200 mg/kg OPEO group and 0.147 mg/kg in the 300 mg/kg OPEO group. Similarly, on the 30th day, the MDA accumulation was 0.274 mg/kg in the control group, 0.207 mg/kg in the OPEO (200 mg/kg) group, and 0.180 mg/kg in the OPEO (300 mg/kg) group.

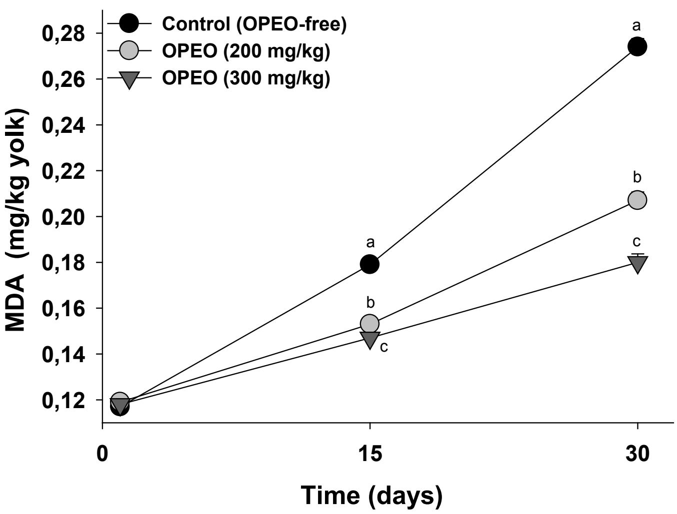

Figure 1 – The effect of OPEO on the accumulation of MDA (mg/kg yolk) in quail egg yolk during storage (1st, 15th, and 30th days). a,b,c means with in column varies superscript differ at p<0.0001.

These results can be attributed to the antioxidant properties of OPEO, which contains phenolic compounds such as Terpinolene and γ-Terpinene known for their inhibitory effects on lipid peroxidation (Ruberto *et al.*, 2000). These compounds reduce the chain length of lipid peroxyl radicals through their active methyl groups, exhibiting antioxidant behavior (Foti & Ingold, 2003; Sharififar *et al.*, 2010). The reduction in MDA levels observed in our study suggests that OPEO, through dietary supplementation, may have prevented the chain reaction involved in the oxidation of dietary lipids, thus enhancing oxidative stability in eggs. These results align with Selcuk & Sengul (2021), who reported decreased MDA values in quails' diets supplemented with lower doses of OPEO. Furthermore, the antioxidant effects of OPEO in poultry diets have been shown to improve both physiological mechanisms (Akbarian *et al.*, 2015) and the quality of animal-derived products (Bolukbasi-Aktas, 2017), supporting our study's results. The preservation of egg quality through the reduction of lipid peroxidation products further supports the potential application of OPEO as a natural antioxidant in the poultry industry.

CONCLUSIONS

In conclusion, our study highlights the significant contributions of orange peel essential oil (OPEO) as a natural additive in layer quail feeds. The addition of OPEO resulted in notable improvements in key yield characteristics such as egg production, egg weight, and egg shelf life. These findings demonstrate the potential of OPEO as a viable substitute for antibiotics in poultry nutrition, providing a natural and herbal growth promoter without the potential harmful residues associated with antibiotic use. Furthermore, the utilization of OPEO, a by-product of the juice industry, aligns with the principles of the circular economy, offering additional environmental and economic benefits. However, further research is necessary to investigate the effects of incorporating OPEO into animal feeds in different combinations and mixtures with other beneficial plant-based essential oils. These studies will enhance our understanding of the synergistic effects and optimal dosages to maximize the benefits of OPEO in poultry production.

By utilizing OPEO as a natural feed additive, the poultry industry can enhance productivity and product quality while reducing reliance on synthetic growth promoters. This research contributes to the development of sustainable and environmentally friendly strategies in poultry nutrition and supports the shift towards more natural and healthy production practices.

In conclusion, the findings of this study highlight the potential of OPEO as a promising feed additive in layer quail nutrition, promoting the sustainable production of high-quality poultry products.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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