



Effect of Citrus Peel Essential Oil and Egg Storage Methods on the Quality of Quail Eggs (*Coturnix coturnix japonica*)

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Abstract. This study explores the potential of Citrus Peel Essential Oil (CPEO) as an alternative to Antibiotic Growth Promotor (AGP) due to its known antibacterial and antioxidant properties and examine the effects of different egg storage methods on quail (*Coturnix coturnix japonica*) eggs quality. The study employed 352 aged 17 weeks quails raised for 6 weeks period along with treatments. The treatments included P0 = basal feed (control), P0+ = basal feed + 0.15% zinc bacitracin (control positive), P1= basal feed + 0.05% CPEO, P2= basal feed + 0.10% CPEO, P3= basal feed + 0.15% CPEO, P4= basal feed + 0.20% CPEO, P5= basal feed + 0.25% CPEO, P6= basal feed + 0.30% CPEO. The eggs were stored under two conditions, refrigeration and room temperature for 14 days before egg quality testing. The results revealed that the use of CPEO as a feed additive gave significant effect ($P < 0.05$) on the yolk index, yolk color, and Haugh Unit, but did not significantly impact the albumin index ($P > 0.05$). Egg storage methods showed significant effect ($P < 0.05$) on Haugh Unit, albumin index, and yolk index but did not influence the yolk color. Interaction tests showed a significant interaction for the albumin index ($P < 0.05$), with no significant interaction effect observed for the yolk index, yolk color, and Haugh Unit ($P > 0.05$). The conclusion is level of essential oil improves quail egg quality, especially with refrigerated storage method.

Keywords: citrus peel essential oil, egg storing method, egg quality, quail eggs

1 Introduction

Quails have great potential as a livestock business in Indonesia, with fast growth and affordable maintenance needs. Feed quality and quantity play an important role in the optimal growth of quails. The main advantage possessed by quails is their ease of maintenance, which is largely due to their relatively small body size and ability to produce in a relatively short period of time. In addition, quails are also renowned for their ability to produce food products with high nutritional value, which play an important role in fulfilling people's daily animal protein needs. Therefore, quails have a very promising potential to be developed as an alternative source of high-quality food [1].

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Feed additives are a potential Antibiotic Growth Promotor (AGP) alternative to improve poultry performance since AGP has been banned due to its residues that can cause bacterial resistance that endangers livestock [2] and adversely affects the health of humans who consume it. Citrus is the largest orange genus of Rutaceae family. Citrus (*Citrus sinensis*) contains essential oil, also known as aetheric oil, volatile oil, whose main composition includes D-limonene as much as more than 68-98% [3]. Citrus peel essential oils (CPEO) had a positive impact on gut microbes responsible for feed digestion and maintaining gut health [4]. The use of CPEO as an alternative to antibiotics showed positive effects on gut microbes so that it has a positive effect on body weight gain and quail growth due to antimicrobial activity [5], antioxidant activity [6], and immune stimulation [7] of essential oils.

Egg quality degrades with the shelf life of the egg. The length of egg storage will determine the quality of the egg throughout storage. The longer the egg is stored, the more the quality and freshness of the egg deteriorates. Egg quality deterioration is caused by microbial spoilage, the environment and evaporation [8]. A focus on quail egg quality is important, especially in the context of storage duration and storage methods. Research suggests that storage duration and storage temperature can affect egg quality parameters, including texture, palatability and pH changes. According to research by [9], storage temperature is the main factor that needs to be considered. Storage of quail eggs in a refrigerator can provide additional benefits in maintaining egg quality and preventing the growth of pathogenic microorganisms [10]. It is expected that effect of combination between the use of CPEO and storage method might change the egg quality in interactive way. Therefore, the objective of this research is to examine the combination effects of CPEO and storage methods on egg quality in laying quails.

2 Materials and Methods

The research was conducted by collecting *Citrus sinensis* oranges purchased from fruit stores around Malang City. The extraction process of essential oil from citrus peel was carried out at the Materia Medica Laboratory, Batu and the egg quality test was carried out at the Animal Feed Technology and Industry Laboratory, Faculty of Animal Husbandry, Universitas Brawijaya. This research was conducted in a farm located in Karang Ploso, Malang Regency, East Java, Indonesia.

2.1 Essential Oil Extraction

The procedure for making CPEO uses the maceration extraction method in UPT Materia Medika Laboratory. Citrus fruits were peeled and cut into small pieces using a fruit peeler so that only the outer yellow/green skin (flavedo) was collected. Maceration using technical n-hexane solution (ratio of sample to solvent 1:5) for 2 x 24 hours at room temperature with stirring approximately every 2 hours. The extraction results were filtered and separated filtrate and pulp with a filter cloth. The filtrate was evapo-

rated using a rotary evaporator with a speed of 150 rpm and a temperature of 60°C to remove n-hexane so that the CPEO was obtained.

2.2 Essential oil application on quail

Quail. This study used 352 quails *Coturnix coturnix Japonica* obtained from local poultry shop of 17 weeks old.

Cages and Equipments. The cages used during the study were 32 battery cages, with dimensions of 60 x 25 x 21 cm for each cage. Each cage was filled with 11 quails. Cages were made from wire. The tools used in the study included feed and drink containers, egg collection trays, excreta containers, 5 kg digital scales with an accuracy of 0.01 grams (used to weigh treatment feed, feed residue, and quail body weight), thermohydrometer, cage cleaning tools, buckets, stationery, knives, LED lights and plastic.

Feed Materials. The quail feed used was a self-formulated feed. The basal feed used is as shown in Table 1.

Table 1. Basal Feed Formulation

Composition	Ratio (%)	Total (kg)
Yellow corn	54.9	10.98
Soybean meal	23.0	4.6
Rice bran	4.00	0.8
Meat Bone Meal	6.00	1.2
Fishmeal	5.20	1.04
Methionine	0.40	0.08
Limestone	6.00	1.2
Salt	0.20	0.04
Premix	0.30	0.06
TOTAL	100	20

2.3 Methodology

This study used 352 quails *Coturnix coturnix Japonica* with 8 treatments and 4 replications, each experimental unit was filled with 11 quails. There design was Factorial having 2 factors. The main factors were levels of CPEO consisting of:

P0 : basal diet

P0+ : Basal diet + Zinc bacitracin 0.15%

P1 : Basal diet + 0.05% CPEO

P2 : Basal diet + 0.10% CPEO

P3 : Basal diet + 0.15% CPEO

P4 : Basal diet + 0.20% CPEO

P5 : Basal diet + 0.25% CPEO

P6 : Basal diet + 0.30% CPEO

The feed was provided 28 grams/day and water was available ad libitum. The experiment was last for 6 weeks period.

The second factors were methods of storage. There were two storage methods: room temperature storage with temperatures ranging from 28°C to 30°C and refrigerator storage with temperatures ranging from 4.8°C to -1°C. During storage, the temperature was monitored and recorded twice a day using a digital thermometer which was followed by an egg quality test on the 14th storage day.

On week 6 of research period, egg collection was carried out. The samples used were 15 eggs from each replicate unit in the treatment and were put in plastic clips that had been labelled according to the treatment. The results of the egg quality test calculations were averaged before being analyzed.

2.4 Qual Egg Quality Test

Yolk color. Measurement of yolk color using a Yolk Colour fan. The yolk color score has a color standard of 1-15, the higher the yolk color score, the better the egg quality.

Haugh Unit. Haugh Unit is a measurement to determine the quality of albumin by using a method found by Raymond Haugh. The measurement is done using a manual Haugh Unit tester [11].

Yolk Index. The yolk index is a quality index of egg freshness measured by the ratio of height and diameter of the yolk.

Albumin Index. Albumin index is a quality index of egg freshness measured from albumin height to albumin width and length.

2.5 Data Analysis

The first study on the effect of essential oil addition used 8 treatments with 4 replications, resulting in a total of 32 units, each consisting of 11 quails. The research data obtained were then analyzed using the experimental method of Completely Randomized Design (CRD) factorial pattern and analyzed by statistical analysis using variance analysis (ANOVA). If there are significantly different results then continued using Duncan's test.

3 Results and Discussion

3.1 Effect of citrus peel essential oil addition on quail egg quality

Eggs that had been treated with CPEO for 6 weeks through feed were tested for quality including yolk color, haugh unit, yolk index, and albumin index. Based on the results recorded in the Table 2, the addition of CPEO to quail feed showed a significant impact ($P < 0.05$) on yolk color, haugh unit, and yolk index. Addition of essential oil at different levels did not show significant impact ($P > 0.05$) on albumin index.

Table 2. Effect of CPEO addition on quail egg quality.

Treatment	Yolk Color	Haugh Unit	Yolk Index	Albumin Index
P0	5.85±0.53 ^{bc}	89.58±4.16 ^a	0.48±0.08 ^{ab}	0.14±0.05
P0+	6.04±0.59 ^d	89.38±3.75 ^a	0.48±0.09 ^a	0.15±0.05
P1	5.82±0.64 ^{cd}	91.81±3.99 ^{ab}	0.49±0.08 ^{ab}	0.14±0.04
P2	5.23±0.49 ^a	92.53±6.10 ^b	0.49±0.07 ^{ab}	0.13±0.04
P3	5.38±0.28 ^{ab}	92.00±4.84 ^{ab}	0.51±0.11 ^{abc}	0.13±0.04
P4	5.76±0.39 ^{abc}	92.57±4.46 ^b	0.52±0.11 ^{bc}	0.14±0.04
P5	5.46±0.13 ^{ab}	93.72±3.93 ^b	0.54±0.09 ^{cd}	0.15±0.04
P6	5.38±0.53 ^{ab}	94.30±3.13 ^b	0.56±0.07 ^d	0.14±0.03

Means with different superscripts in the same column differ ($P < 0.05$)

The treatment showed a significant effect ($P < 0.05$) on yolk color because citrus peel essential oil contains natural pigments that contribute to the color of the resulting yolk. Citrus peels are composed of natural pigments: lipid-soluble carotenoids and water-soluble yellow pigments. β -Carotene, lycopene, and anthocyanins are the primary pigments in citrus fruits [12]. Along with these findings, research by [13] reported that essential oils with higher darkness had an impact on darker yolk color intensity. This illustrates that yolk color characteristics can be influenced by the quality of essential oils used in feed. In addition, the treatment can improve the absorption of dyes in the feed. Citrus peel essential oil can improve enzyme performance and inhibit the metabolism of pathogenic bacteria in digestion, so that yellow color-forming pigments can be absorbed. [14] explained that citrus peels contain essential oil compounds that can increase the permeability of cell membranes, causing the release of enzymes and the process of bacterial respiration to be inhibited. Essential oils are also able to disrupt the structure of bacterial proteins causing proteins to denature in the digestive tract which causes the absorption of carotene and xanthophyll pigments in feed to be more optimal.

The higher the yolk index and haugh unit values, the better the egg quality. Citrus peel essential oil contains antioxidant compounds such as flavonoids, limonene, and terpenoids [15]. These antioxidants work by protecting cells from oxidative damage, which is damage caused by free radicals that can damage cell structures and other

important components if not immediately neutralized so that cell membranes and components in the egg can be damaged, which ultimately reduces the quality of the yolk. When eggs are produced, the antioxidant effect helps protect the cell and tissue structure of the yolk. Antioxidants also help maintain the integrity of the yolk membrane, extending egg shelf life [16]. This is important because a stronger and undamaged membrane gives the yolk a round and stable shape, which in turn increases the Haugh unit value and yolk index. In other words, the presence of antioxidants helps the yolk maintain its density and shape, resulting in a higher index value.

Factors that affect egg index are various, feed ingredient is one of them. Albumin level is mainly influenced by the protein content in the ration, so that the ration protein affects the viscosity of the egg. Albumin index is a freshness quality measured by the thickness of albumin divided by the diameter of albumin [17]. The main ingredient to determine the height of albumin and the formation of ovomucin lies in protein consumption. This is in accordance with the opinion of [18], the higher the protein consumption, the greater the formation of ovomucin, so the higher the albumin index.

3.2 Effect of egg storage method on quail egg quality

The results of research on the effect of storage methods, room temperature and refrigerator temperature, can be seen in the Table 3. Treatment with different storage methods had a significant effect ($P < 0.05$) on haugh unit, yolk index, and albumin index. However, the treatments did not have a significant effect ($P > 0.05$) on yolk color. The best treatment with the highest notation and the best value is owned by the treatment with refrigerator temperature storage method. The results of this study are in line with [19] who found that storage duration significantly influenced air cell height, weight loss, Haugh Unit (HU) and shell thickness but not yolk color.

Table 3. Effect of citrus peel essential oil addition on quail egg quality.

Treatment	Yolk Color	Haugh Unit	Yolk Index	Albumin Index
Refrigerator	5.64±0.58	95.32±2.84 ^b	0.58±0.04 ^b	0.16±0.03 ^b
Room Temperature	5.59±0.47	88.65±3.03 ^a	0.43±0.05 ^a	0.12±0.04 ^a

Means with different superscripts in the same column differ ($P < 0.05$)

Egg formation occurs within the oviduct, an environment high in carbon dioxide (CO_2). Consequently, freshly laid eggs contain dissolved CO_2 in the albumin, which gradually diffuses through the eggshell to the outside environment during storage. Carbon dioxide suppresses the growth of aerobic bacteria by influencing both the lag phase and growth rate of these microorganisms. While CO_2 aids in slowing microbial growth, antioxidants offer protection against oxidative damage from free radicals. This combined effect is advantageous in prolonging egg shelf life, particularly during the initial storage phase when CO_2 levels begin to decrease.

The HU value is an important parameter in evaluating egg quality, with good quality eggs generally having a high HU value [20]. Refrigerator temperatures ranging

from 4.8oC to -1oC can inhibit the evaporation of water and CO₂ as found by [21]. During storage, CO₂ passes through the pores of the eggshell, the pH of the albumin increases, the complex forming capacity of ovomucin and lysozyme deteriorates, and the viscosity and Haugh Units decreases. Evaporation is also affected by temperature and relative humidity [22], so it is recommended to store quail eggs at refrigerator temperature rather than at room temperature.

The longer the eggs are stored, the more the yolk index and albumin index values decrease. During storage of eggs, the yolk index decreases as a result of a progressive weakening of the vitelline membranes, reduction of the total solids, a progressive transition of yolk which is caused mainly by osmotic diffusion of water from the albumin. According to [23] normally during egg storage, the vitelline membrane will break so that the yolk will melt and its height will decrease. The CO₂ in the egg also comes out a lot resulting in an increase in acidity, as well as evaporation so that the weight of the egg decreases and albumin becomes more diluted [22]. However, the study states that the index value of quail yolk stored at room temperature is significantly different from the value recorded at refrigerator temperature which is also in line with [24]. This is due to the ability of refrigerator temperature to inhibit the evaporation process of water and CO₂. The study also showed that the phenomenon of evaporation is influenced by temperature and relative humidity factors, in line with previous research presented by [22].

3.3 Interaction between Citrus Peel Essential Oil and Storage Method on Quail Egg Quality

The results of research on the interaction between CPEO and treatment method on quail egg quality can be seen in the Table 4 and Table 5. The results showed a significant interaction between the dose of CPEO in quail feed and egg storage method (refrigerator and room temperature) on albumin index. This significant interaction indicates that the effect of CPEO on albumin index is not constant, but depends on storage conditions. This means that although the addition of CPEO can affect albumin quality, this effect can be amplified or reduced depending on the storage temperature. The study by [25] which applied antioxidant-rich grape extract to poultry, was shown to increase the haugh unit of eggs which directly proves that albumin quality improved. This suggests that bioactive compounds in feed can improve egg quality through antibacterial and antioxidant mechanisms. The results of this study reinforce the importance of the interaction between feed and storage method. The use of essential oils in feed contributes to the improvement of albumin quality by inhibiting microbial growth, while storage at refrigeration temperature helps slow down the oxidation and deterioration process. Given the results obtained at low temperatures, the storage of eggs at refrigeration temperature represents an effective way of preventing bacterial growth [26]. Moreover, the refrigeration temperature enables to reduce the loss of the vitelline membrane integrity and the access of bacteria to yolk in eggshell. These results suggest that the integration of CPEO in quail feed, especially in combination with proper storage, can significantly improve egg quality, both in terms of bird health and final product quality.

Table 4. Interaction of CPEO treatment and refrigerator storage method on quail egg quality

Variable	REFRIGERATOR TEMPERATURE							
	P0	P0+	P1	P2	P3	P4	P5	P6
Albumin Index	0.13±0.03 abcd	0.14±0.02 abcde	0.14±0.01 abcde	0.16±0.03 ede	0.16±0.02 cde	0.18±0.02 de	0.19±0.02 ^c	0.17±0.01 ede
Yolk Index	0.54±0.03	0.56±0.03	0.56±0.04	0.54±0.03	0.61±0.01	0.62±0.02	0.62±0.02	0.63±0.02
Yolk Color	5.95±0.60	6.31±0.55	5.95±0.67	5.24±0.71	5.20±0.28	5.63±0.29	5.43±0.17	5.45±0.50
Haugh Unit	91.81±3.29	92.21±2.08	94.74±1.96	97.27±2.05	96.04±2.60	96.42±2.08	97.10±1.89	96.97±1.46

Means with different superscripts in the same column differ (P < 0.05)

Table 5. Interaction of CPEO treatment and room temperature storage method on quail egg quality

Variable	ROOM TEMPERATURE							
	P0	P0+	P1	P2	P3	P4	P5	P6
Albumin Index	0.15±0.02 abcde	0.16±0.03 bcde	0.14±0.02 abcde	0.10±0.01 ^a	0.10±0.08 ^a	0.11±0.01 ab	0.12±0.01 abc	0.12±0.01 abc
Yolk Index	0.42±0.03	0.40±0.03	0.43±0.02	0.43±0.02	0.41±0.03	0.42±0.03	0.47±0.02	0.50±0.03
Yolk Color	5.75±0.55	5.76±0.71	5.69±0.29	5.23±0.50	5.55±0.56	5.90±0.21	5.50±0.47	5.31±0.68
Haugh Unit	87.35±2.08	86.55±2.05	88.88±2.08	87.80±0.46	87.96±2.96	88.71±4.79	90.33±1.55	91.64±1.91

Means with different superscripts in the same column differ (P < 0.05)

Storage conditions can affect the stability of bioactive compounds in CPEO. Inappropriate storage processes can lead to degradation of active compounds, thereby reducing the effectiveness of CPEO in improving overall egg quality. Therefore, although CPEO showed potential in improving albumin quality, the interaction with storage method did not have a significant impact on yolk index, yolk color and Haugh Unit, indicating that the factors affecting each parameter are more complex and require further research to understand the deeper interactions.

4 Conclusion

In conclusion, this study demonstrates the efficacy of citrus peel essential oil (CPEO) as a viable alternative to AGP in enhancing the quality of quail eggs. The antibacterial and antioxidant properties of CPEO contribute to better overall egg quality, while its pigment compounds also enhance yolk pigmentation, resulting in more visually appealing eggs. Refrigerator temperature effectively preserves egg quality by reducing microbial growth, oxidation, and nutrient loss, resulting in a better Haugh Unit and yolk and albumin indices than room temperature storage. Thus, incorporating 0,3%

CPEO into quail feed using refrigerator temperature to storage the eggs may contribute to improved egg quality and shelf life, promoting better production practices in quail farming. Further research might be needed to evaluate the practical use of this product in commercial farms as reference on the use of natural AGP resulting in healthier egg products.

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