



Effect of dried orange peel on egg production and quality of laying hen

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ABSTRACT

Utilization of agricultural by-products in animal nutrition is a practice as old as the domestication of animals. The dried orange peel (DOP), by-product of citrus industry is a promising source of diversified bioactive ingredients with beneficial effects on health. Therefore, this study was carried out to assess the dietary effect of DOP on production and, internal and external egg quality characteristics of laying hens. A total of 60 layers of 25 weeks of age were segregated into three dietary treatment groups using a completely randomized design and reared up to 42 weeks. The dietary treatments were control (basal diet), 5% DOP (basal diet + 5% DOP), and 10% DOP (basal diet + 10% DOP). The study revealed that dietary DOP significantly decreased the feed consumption and feed conversion ratio of layers ($p < 0.05$) without having any significant effects on egg production, egg weight and egg mass. None of the exterior egg quality parameters were significantly affected by the dietary supplementation of DOP ($p > 0.05$). Among interior egg quality attributes, the albumen height, albumen index, yolk color, and Haugh unit were all considerably elevated through supplementation of 10% DOP ($p < 0.05$). Nevertheless, the yolk index was raised by both 5% and 10% DOP supplementation ($p < 0.05$). The relative weight of ovarian grape and oviduct did not affect by the supplementation of DOP with layer diet ($p > 0.05$). In summary, it may be said that layer diets can be supplemented with DOP up to 10% level to improve FCR, albumen index, yolk index, yolk color and Haugh unit without compromising egg production or exterior egg quality. Further studies are recommended with layer diets supplemented with DOP at levels higher than 10% to determine the ideal DOP dose during other production periods in various layer strains.

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Introduction

A transformation in eating habits has been observed recently, which may be related to an increase in overweight persons and consumer awareness of health issues. Eggs have gained popularity as a component of a balanced diet, because they are a superb source of high-quality, easily digestible protein and amino acids, and a decent source of both fat-soluble (A, D, E, and K), water-soluble B vitamins (Garza *et al.*, 2000) and cholesterol (200 to 300 mg/100 g). Recently,

several investigations have reported that egg cholesterol is not harmful to health because it increases both LDL and HDL cholesterol (Blesso *et al.*, 2013). This indicates that the primary predictor of heart disease, the total cholesterol to HDL ratio, is stable. Therefore, the daily consumption of eggs does neither increase serum cholesterol nor increases cardiovascular disease risk (Zaheer, 2015), while improving the atherogenic lipoprotein profile and insulin resistance in individuals with MetS (Blesso *et al.*, 2013).

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Egg production and eggshell quality are fundamental indicators for assessing the performance of laying hens and the financial viability of egg farms. The manipulation of layer diets to improve the egg production performance through the introduction of phyto-genic feed additives has been studied by several scientists at different production periods [Nazok et al., 2010 (25-37 weeks); Oyewole et al., 2018, (20 weeks); Sharmin et al., 2021 (26-42 weeks)]. These phyto-genic feed additives are risk-free and do not result in any negative side effects, such as antibiotic resistance. Along with other advantages like increased feed intake, phyto-genic also aid in promoting the gut's natural digestion processes and have a variety of antibacterial, antioxidant, and immune-stimulating effects. One of the most popular citrus fruit in the world is orange which contains a lot of phytochemicals (Hou et al., 2019; Satari and Karimi, 2018). According to M'hiri et al. (2015) and Singh et al. (2020), orange peel makes up between 40 and 50 % of the entire fruit mass and is collected after the pulp and juice have been taken off. These are commonly known as agro-industrial wastes, which can also be used as animal feed but cause environmental problems. (Casquete et al., 2015; Negro et al., 2016; Satari and Karimi, 2018). Orange peel is rich in pectin (20-30%, Chen et al., 2021) which can improve feed digestibility and lowers cholesterol levels by interfering with cholesterol absorption and by increasing cholesterol turnover (Kelley and Tsai, 1978). *Citrus sinensis* peel essential oil hold disinfecting, insecticidal, and antibacterial activities (Parmar and Kar, 2007; Prabuseenivasan et al., 2006). The peel of orange also rich in soluble sugars (46.241±0.015 g/100g), proteins (8.120±0.120 g/100g), and minerals (3.170±0.035 g/100g (M'hiri et al., 2015). The largest concentration of flavonoids (83.3 mg of catechin equivalent/g) and vitamin C (110.4 mg/100 g) are found in orange peels, which have been associated to antioxidant, anticancer, antiviral, and anti-inflammatory properties (Elkhatim et al., 2018; M'hiri et al., 2015). Goliomytis et al. (2018) revealed that supplementation of dried orange pulp at 9% level improved the oxidative stability of eggs and consequently the egg shelf life, while reduced the feed consumption, feed conversion ratio and laying rate. According to Nazok et al. (2010), adding dried citrus pulp at a level of 12% had no adverse effects on the productivity and egg quality of laying hens during the early stages of production. However, Oyewole et al. (2018) observed that layer chickens treated with 40% sun-dried citrus peel had increased body weight, feed intake, and egg weight without sacrificing the internal and exterior egg quality. The orange skin also contains considerable

amount of xanthophyll which can improve the yolk color, a very important factor of egg quality (Chowdhury et al., 2008). Based on the above research findings, this study was therefore designed to examine the implications of incorporation of dried orange peel (DOP) on egg production performance and egg quality of Shavar-579 layers at 25 to 42 weeks of production. Although almost all egg quality characteristics remain optimum naturally during peak production period, we chose this production period to perceive if dietary treatments can affect the egg production percentage or egg quality characteristics of layers.

Material and Methods

Preparation of experimental dried orange peel (DOP)

Following removal from the fruits, fresh orange peels were dried in the sun using the procedure previously described by Farahmandfar et al. (2019). The samples had been dried until the moisture level reached 10%. After drying, the peels were grinded using a kitchen grinder to pass through a 0.15 mm sieve. The dried orange peel (DOP) was then tightly packed into a zip lock bag and maintained at room temperature until used. By withdrawing a corresponding amount of the basal diet, the DOP will be added. The DOP was analyzed in triplicate for crude protein (CP), ether extract (EE), moisture and ash, as described by the Association of Official Analytical Chemists (AOAC 2002). The proximate composition data of DOP is presented as Table 1.

Table 1: Chemical composition of dried orange peel and experimental feed

Ingredients	Amount (%)
Moisture	9.72±0.05
Crude protein	7.16±0.05
Crude fiber	12.67±0.03
Ash	5.07±0.10
Fat	5.62±0.05

Mean ± SD of triplicate determinations

Experimental diets and management

The experiment was conducted in a temporary layer shed constructed on the premises of the Atish Dipankar University of Science and Technology. For this experiment, 18-week old Shavar-579 pullets were purchased from a vendor and were housed in an open-sided caged house equipped with trough feeders and trough drinkers. At 25 weeks, 60 birds in total were randomly divided into three dietary treatment groups with five replicates and four birds per replicate, and they were reared for up to 42 weeks of age. Layers of this age were chosen for

the trial in accordance with the studies of Nazok *et al.* (2010), Dahiya *et al.* (2016), Oyewole *et al.* (2018) and Sharmin *et al.* (2021). The dietary treatments were control (basal diet), DOP 5% (basal diet + 5% DOP), and DOP 10% (basal diet +10% DOP). A commercial layer diet served as basal diet containing 2800 kcal ME, 18.5 % crude protein, 5% crude fiber, 3% crude fat, 12% moisture, 3.5% Calcium and 0.40 % available Phosphorus. The additives were added at the cost of equal amount of basal diet (Ahmed *et al.*, 2016; Aqil, 2016; Wan *et al.*, 2020). Layers were supplied feed in troughs placed in front of each cage two times every day with ad libitum water. A 16-hour photoperiod and 8 h darkness was maintained during the experimental period. Environmental temperature and humidity were recorded twice a day (morning and afternoon) using a digital temperature humidity meter. The experimental house had a temperature range of 26 to 30 °C and a relative humidity value of 52 to 63% throughout the study period. Strict hygiene and sanitation measures were implemented at the experimental house. The waterers were cleaned every morning with tap water, and the feeders were cleaned once a week. In addition, waterers were meticulously cleaned and detergent-washed once a week.

Performance parameters

Body weight (g/bird), feed intake (g/day/bird) and laying percentage (%) were monitored throughout the experimental period. Body weight was measured at the beginning and the end of the experimental period. Feed intake was recorded daily and average daily feed intake was calculated for the overall experimental period. Eggs were collected and weighed every week to calculate the hen-day egg production and feed conversion ratio. Average hen-day production was computed by dividing the total number of eggs by the total number of hen-days, which is used to describe egg production. Egg mass was calculated using the following formula followed by Toye *et al.* (2012):

Egg mass = egg production (%) x mean egg weight (g)

Feed conversion ratio was calculated as the ratio between feed consumption and egg mass on a replicate basis.

Measurements of egg quality parameters

All the egg quality parameters were assessed in the Pharmacy lab of Atish Dipnagar University of Science and Technology. To determine the external and internal egg quality twenty eggs (four eggs/replication) were collected randomly from each group after 12 weeks of supplementation (37 weeks of age) according to Chowdhury *et al.* (2008)

and weighed individually. Egg weight was recorded first and then egg length and width were measured using a slide calipers, and used to compute of the egg shape index (SI = width/length x 100), according to Panda (1996).

After the eggs were broken, the components of the eggs (albumen, yolk, shell) were manually separated and a tripodmicrometer was used to measure the height and width of the yolk and albumen. The albumen index was computed using the equation reported by Doman *et al.* (2016) and yolk index was calculated using the formula followed by Sharmin *et al.* (2021) and Nazok *et al.* (2010).

$$\text{Albumen index} = \left(\frac{\text{Albumen height}}{(\text{Albumen length} + \text{Albumen width})/2} \right) \times 100$$

Yolk index = height of the yolk/ width of yolk x 100.

Eggshell thickness was determined using a shell thickness meter after washing and removal of shell membranes. Eggshell weight was determined after air drying according to (Englmaierová *et al.*, 2014). The surface area of each egg was determined using the equation reported by Thompson *et al.* (1985): Egg surface area=4.67 x (egg weight)^{2/3}. The formula for eggshell index calculation was - Eggshell index = (shell weight/shell surface) x 100 (Ahmed *et al.*, 2005).

A Roche yolk color fan was used to measure yolk color. The albumen and yolk were also weighed to determine their ratio (component weight X 100/absolute egg weight). Haugh unit was calculated from the records of albumen height and egg weight using this formula proposed by Carter (1975): HU = 100 log₁₀(H - 1.7W^{0.37} + 7.56); Where, HU = Haugh unit, H = height of albumen (millimeters), and W = egg weight.

Determination of relative weight of reproductive organ

At 42 weeks 10 birds per group (two/replication) were slaughtered to collect the reproductive organ (ovarian grape and oviduct). The relative weight of ovarian grape or oviduct (ovarian grape or oviduct weight X 100/reproductive system weight) were determined and the follicles around each ovary were numbered.

Statistical analysis

All data were analyzed by using SPSS 16.0 (Statistical Package for the Social Sciences, IBM Corp., IBM SPSS Statistics for Windows, Version 16.0, Armonk, NY, USA) to compute analysis of variance (ANOVA). Each pen was considered an

experimental unit for production performance, whereas a group of four eggs served as the experimental unit for egg quality parameters and two birds served as the experimental unit for relative weight of reproductive organ. Duncan's Multiple Range Test (Duncan, 1955) was done to compare the treatment means at 5% level of significance (Steel and Torrie, 1980).

Results and Discussion

Proximate composition of dried orange peel

Table 1 displays the estimated composition of dried orange peel (DOP).

Table 2. Effects of dried orange peel (DOP) on the Production performance of layer chickens (25 to 42 weeks)

Performance parameter	Dietary treatments ²			SEM ³	p-value
	Control	DOP 5.0%	DOP 10%		
Initial body weight (g)	1424.0	1432.00	1433.60	12.97	0.67
Final body weight (g)	1598.8	1607.4	1603.60	10.69	0.58
Average daily feed intake (ADFI) (g)	105.18 ^a	103.22 ^b	103.15 ^b	0.52	0.03
Hen day egg production (%)	90.95	92.45	93.70	2.06	0.42
Egg weight (g)	54.11	54.57	55.40	0.55	0.22
Egg mass (g)	49.25	50.45	51.88	1.16	0.19
FCR (egg mass)	2.15 ^a	2.05 ^b	1.99 ^b	0.06	0.02

Same letters within a row indicate non-significant and different letters indicate statistically significant at 5% level ($p < 0.05$). ¹ Values represent the means of five replications with four birds per replication. ² Control; DOP 5%, Basal diet+5% dried orange peel; DOP 10%, Basal diet+10% dried orange peel; ³ SEM, Pooled standard error.

According to its nutritional composition, DOP has 9.72% moisture, 7.16% crude protein, 12.67% crude fiber, 5.07% ash, and 4.02% fat. The result of crude protein is almost similar (7.15%) to the findings of Olabinjo *et al.* (2017), however lesser than that of 11% reported by Egbuonu and Osuji (2016). The crude fiber contents of the experimental DOP (12.67%) was also comparable to the finding of Olabinjo *et al.* (2017), but lesser than that of 13.99% reported by Egbuonu and Osuji (2016). The concentration of fat and ash was comparable with findings of Egbuonu and Osuji (2016). Different cultivars could be too responsible for the compositional diversity.

Production performance

Table 2 displays the results of the effect of DOP on the productivity of laying birds.

The final body weight of the experimental chickens was not significantly ($p > 0.05$) affected by the dietary treatments. In agreement to our results, Nazok *et al.* (2010) found no appreciable impact on final body weight (at 37 weeks of age) of layer chicken when fed dry citrus pulp up to a 12% level. Chowdhury *et al.* (2008) also found no significant effects of dried orange skin on body weight of layer when fed at 40 g/kg level. However, dietary supplementation of 5.0% and 10.0% DOP

significantly reduced ($p < 0.05$) the feed intake of layer chicken. Contrary to our results, Nazok *et al.* (2010) and Chowdhury *et al.* (2008) did not find any significant changes in feed intake. Due to increasing crude fiber content in diet containing DOP may be responsible for lower feed intake of layers. Additionally, orange peel-based diets are less tasty than controls, which may also be to reason for the decrease in feed consumption (Ani *et al.*, 2015).

Egg production, egg weight, and egg mass did not alter significantly when 5.0% and 10.0% DOP were added to the diet of laying hens ($p > 0.05$). Results of the present study are also in conformity with the findings of the previous researches of Chowdhury *et al.* (2008), Florou-Paneri *et al.* (2001) and Karunajeewa (1978). According to Florou-Paneri *et al.* (2001), adding dried citrus pulp to the diet of laying quails up to 6% did not have a negative impact on egg production. Karunajeewa (1978) also discovered that feeding laying hens 5% citrus pulp did not have an impact on egg production. On the contrary, Nazok *et al.* (2010) reported significantly lower egg production, while higher egg weight and egg mass in chickens fed 8, 12 and 16% citrus pulp. Oyewole, *et al.* (2018) reported significantly higher egg weight in chicken fed 10 to 40% sundried sweet orange fruit peel meal (SOPM). The

study's findings showed that the feed conversion ratio was significantly lower in the supplemented groups compared to control ($p < 0.05$) which indicated that utilization of feed for egg mass production was significantly improved with the addition of 5.0% and 10.0% level of DOP in laying hen diet. The citric acid contents of orange peel may increase the digestibility of protein and fibre (Atapattu and Nelligawatta, 2005) and therefore improve the FCR (Wan et al., 2020). Contrary to our result Nazok et al. (2010) found significantly higher FCR in the dried citrus pulp (DCP) supplemented group.

Egg quality parameters and reproductive characteristics

The effects of DOP on external and internal egg quality parameters are represented in Table 3. It

was observed that none of the external egg quality parameters (shape index, shell thickness, shell surface area and egg shell index) of the layers were affected by the dietary supplementation of DOP ($p > 0.05$). In agreement to our results, Nazok et al. (2010) also found no effect of dietary DCP on shell thickness, egg shell index, shell weight and egg score. Oyewole, et al. (2018) also discovered that sweet orange peel meal (SOPM) had no appreciable impact on shell thickness. The height for the inner thick albumen is a benchmark of albumen quality, and the Haugh unit is the result of this measurement (Haugh, 1937; Scott and Silversides, 2000). In the current research, the albumen height and albumen index were higher in the 10% DOP supplemented group compared to control and 5% DOP group ($p < 0.05$), however the albumen weight did not differ significantly.

Table 3. Effects of dried orange peel (DOP) on external and internal egg quality traits

Performance parameter	Dietary treatments ²			SEM ³	p-value
	Control	DOP 5.0%	DOP 10%		
External egg quality traits					
Shape index (%)	79.97	81.23	81.07	0.87	0.27
Shell thickness (mm)	0.44	0.44	0.45	0.01	0.11
Shell (%)	12.86	12.75	12.56	0.15	0.09
Egg surface area (cm ²)	66.81	68.08	68.78	0.46	0.32
Egg shell index (g/100cm ²)	19.26	18.74	18.26	0.33	0.24
Internal Egg quality					
Albumen height (cm)	0.60 ^b	0.63 ^b	0.70 ^a	0.01	0.19
Albumen index	8.44 ^b	8.59 ^{ab}	8.90 ^a	0.20	0.03
Albumen weight (g)	31.26	32.30	32.08	0.24	0.17
Yolk weight (g)	12.93	13.52	13.90	0.11	0.15
Yolk index	42.98 ^b	43.92 ^a	43.77 ^a	1.11	0.04
Yolk color score	7.60 ^b	8.82 ^{ab}	9.10 ^a	0.13	0.02
Haugh unit	79.20 ^b	80.71 ^b	85.10 ^a	0.81	0.01

Same letters within a row indicate non-significant and different letters indicate statistically significant at 5% level ($p < 0.05$).

Values represent the means of five replications with four eggs per replication. ² Control; DOP 5%, Basal diet+5% dried orange peel; DOP 10%, Basal diet+10% dried orange peel; ³ SEM, Pooled standard error.

Contrary to our results, Nazok et al. (2010) found no effect of citrus pulp on albumen height. Chowdhury et al. (2008) reported no effects of orange skin on albumen weight and albumen index. However, Oyewole et al. (2018) found higher albumen weight in eggs of chicken fed diet supplemented with 10-40% SOPM. When compared to the control group, dietary supplements of 5.0% and 10.0% DOP elevated the yolk index ($p < 0.05$). However, the yolk weight did not affect by dietary treatments ($p > 0.05$). In partial agreement with our results, Oyewole et al. (2018) reported significantly higher yolk weight, yolk height and

yolk index when chicken were supplemented with 10% SOPM. Conversely, Chowdhury et al. (2008) found no significant effects of orange skin on yolk weight and yolk index. In the present study, the yolk color and Haugh unit were significantly higher in the 10% DOP supplemented group compared to control ($p < 0.05$). In agreement to our results Chowdhury et al. (2008) found higher yolk color in eggs of layers fed 40 g/kg orange skin. Contrary to our results, Nazok et al. (2010) found no effect of citrus pulp on egg color and Haugh unit. For the egg industry and consumer choice, yolk color is an important aspect of egg quality. Consumers prefer

golden-orange colored, pigmented egg yolks in the majority of countries (Grashorn, 2016). The peel of orange contains considerable amount of xanthophyll (Chen and Yen, 1995; Chowdhury et al., 2008) which may be responsible for higher yolk color in the supplemented group. The height of the egg white serves as the basis for the Haugh unit, which

values the protein content of egg whites (albumen). Higher the Haugh unit score the better the egg quality. In this experiment higher albumen height and Haugh unit in the supplemented group indicated that dietary DOP has a positive effect on egg quality.

Table 4. Effects of dried orange peel (DOP) on oviduct and ovarian grape relative weights¹

Performance parameter	Dietary treatments ²			SEM ³	p-value
	Control	DOP 5.0%	DOP 10%		
Ovarian grape					
Relative weight	3.10	2.94	3.05	0.09	0.45
No. of follicles	15	16	16	0.36	0.37
Oviduct relative weight	4.30	4.58	4.31	0.33	0.26

¹ Values represent the means of five replications with two birds per replication. ² Control; DOP 5%, Basal diet+5% dried orange peel; DOP 10%, Basal diet+10% dried orange peel; ³ SEM, Pooled standard error.

Dietary supplementation of DOP had no measurable effects on relative weight of ovarian grape and oviduct, and no. of follicles (Table 4). We were unable to discover any previous research to compare with our experiment about the dietary effects of citrus by-products on the relative weight of ovarian grape and oviduct.

Conclusion

The results of this study revealed that inclusion of 5.0% and 10.0% DOP in layer diets could improve the feed conversion ratio while lowering feed intake without having any negative effects on the body weight, egg production, egg weight, egg mass, external egg quality parameters and reproductive characteristics. In addition, treatment with 10.0% DOP increased the albumen height, albumen index, yolk index, yolk color and Haugh unit, which indicate a positive effect on internal egg quality. In summary, it is therefore recommended that, with a balanced diet farmer can use DOP up to 10% to improve the food conversion and internal egg quality characteristics. From a perspective of practicality, this study suggested further investigation for determination of optimum inclusion dosage of DOP in the layer diets to achieve the desired beneficial outcome.

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Conflict of interest

No potential conflict of interest was reported by the authors.

Authors contribution

The experiment was developed and designed by Sonia Tabasum Ahmed. The proximate analysis was carried out by Md. Masudul Hasan and Samira Islam Resmi. Measurements of the parameters affecting egg quality were conducted by Md. Wahidul Islam and Mahfuzur Rahman. Sonia Tabasum Ahmed contributed in the drafting of the manuscript, whereby all authors critically revised for noteworthy intellectual value and then approved the final manuscript.

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