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Determination of vitamin D level in chicken eggs from conventional and free range systems

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Abstract

This study aimed to investigate the vitamin D levels and some quality features of eggs. A total of 150 eggs (75 conventional and 75 free-range) were selected for the study. The levels of 25-OH vitamin D were determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in egg yolks. Furthermore, we evaluated the weights of egg, yolk, albumen, and yolk color as well as shape index, shell thickness, and percentages of albumen, yolk, and shell. The 25-OH vitamin D levels were significantly higher ($P < 0.01$) in the conventional than in the free-range eggs. Furthermore, the weights of egg, albumen, yolk, percentage of yolk and yolk color were significantly higher ($P < 0.01$) in the conventional than in the free-range eggs. However, the shape index, percentage of albumen, percentage of shell and shell thickness were significantly higher ($P < 0.01$) in the free-range than the conventional eggs. It can be concluded that vitamin D levels in eggs can be related to yolk color. However, egg quality characteristics can also be influenced by various environmental factors.

Keywords: vitamin D; egg quality; rearing systems; chicken.

Practical Application: Yolk color is one of the significant egg quality parameters that impact on perspective of the consumer's demand. The yolk color tone is parallel with the 25-OH vitamin D level and darker egg yolks may contain more vitamin D. The vitamin D could act like the liver protector as an antioxidant agent. It can be concluded that vitamin D level in eggs can be related to yolk color.

1 Introduction

Poultry species (chicken, turkey, and quail, etc.) and eggs have become a great option for provision of protein needed for healthy and balanced nutrition of an increasing population. The protein qualtiy can be increased with foods of animal origin (Naeem et al., 2022). Chicken meat, and egg which are economically effective and contain high-quality proteins (Denli et al., 2016). Egg is an important source of nutrients because it contains protein, digestible fats, minerals (such as iron, phosphorus, copper, and magnesium), and valuable vitamins (Kishore et al., 2017). Recently, egg production has been an important focus. According to 2018 data, approximately 80 million tons of eggs have been produced in the world, with the first place taken by China who produces 31 million tons of eggs. This is followed by the United States, India, and Japan. An egg is considered important, since it meets about 18% of the vitamin needs of an adult human, considering that we take only 30% of the daily vitamin D requirement (Atapattu et al., 2013). Quality in the food sector was defined as the sum of the properties that affect the acceptability preference for a foodstuff by the consumer (Guesdon & Faure, 2004). The external quality features of egg include weight, shape index, shell thickness, shell weight, and shell ratio. The internal quality features of egg include yolk color tone, index, and proportions of white-yolk (Reu et al., 2008). Today, it is obvious that sun rays are not utilized sufficiently, given that foods that are enriched with vitamin D or supplementation of vitamin D are increasing. Vitamin D is among the fat-soluble vitamins and is also considered as a group of steroids that serve as hormones and hormone precursors, since they can be synthesized in an endogenously suitable biological environment. 25-hydroxy (25-OH) vitamin D is the most utilized parameter for showing vitamin D status (Atapattu et al., 2013, Al-Zohily et al., 2020). Vitamin D, which primarily regulates the balance of calcium and phosphate in the skeletal system, bone mineralization, plasma mineral balance, and bone development (by increasing reabsorption of calcium and phosphorus from the small intestine), as well as maintains proper calcium homeostasis (Popat et al., 2019; Karagöl & Atak, 2016). It also has anti-inflammatory and immunomodulatory effects, as well as possible effects on cytokine levels. Low levels of vitamin D have been associated with cancer, allergies, autoimmune diseases, insulin resistance, Type-2 diabetes, chronic kidney disease, and some infectious diseases (Mitri et al., 2011; Tellioglu & Basaran, 2013; Nielsen et al., 2019). This study aimed to determine the levels of 25-OH vitamin D and the differences in the conventional and free-range eggs in terms of some egg quality features that are related to vitamin D.

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2 Materials and methods

A total of 150 eggs (based on the operating licence) purchased from the market were used for this study. The study protocol was approved by Firat University Non-invasive Ethics Committee (permission no: 2020/05-09). The eggs were brought to laboratory and kept in the refrigerator for a day. The eggs were divided into 2 groups: 75 conventional eggs and 75 free-range eggs. The eggs were weighed, while the width and length of the eggs were measured with a digital caliper (Tresna, 0-300 mm, USA) to determine the shape index. The shape index value was obtained by dividing the egg width by the egg length and multiplying the result by one hundred. All eggs were broken and their yolks were separated from the albumen. The yolk was passed through a filter paper to avoid the introduction of albumen residues. Both the yolk and albumen were weighed after this process. The yolk color was determined using Roche yolk color fan. The shells of each egg were cleaned under water and air-dried for a day to determine the Shell characteristics. The eggshell was weighed and thickness was measured a micrometer (Mitutoyo, Model No 2050-08, Japan). Yolk colors were scored with Roche yolk color fan. Shape index [(width/length) x 100], percentage of albumen [(albumen weight/egg weight) x 100], percentage of yolk [(yolk weight/egg weight) x 100], and percentage of shell [(shell weight/egg weight) x 100] were calculated. When the eggs broken in this study were examined in terms of quality features, the yolk of each egg was collected in 50 mL falcon tubes and mixed with the help of glass baguette and then the 25-OH vitamin D analysis was performed. The extraction was done according to the method described by Hara & Radin (1978). In brief, 2 g of egg yolk samples were taken and BHT hexane/isopropyl alcohol solution was added. This mixture was homogenized for 30 s (Bosch GWS750 Stuttgart, Germany) and the homogenate was centrifuged at 3500 rpm and 4 °C for 20 min. The supernatant was then transferred into a glass tube. 10% methanolic potassium hydroxide solution was added and mixed with the extract. The samples were kept at 85 °C for 15 min. The tubes were cooled to room temperature and mixed with distilled water. All samples were equalized with n-hexane in order to extract of non-saponified lipophilic molecules. This mixture was inverted three times in the sealed tubes and then stored at room temperature for 24 h until phase separation. After the phase was transferred into glass tubes and stored at 37 °C for 48 hours. 1 mL acetonitrile/methanol mixture was added to the residue and transferred into vials for analysis. A Shimadzu LC system (LC-20AT, Japan) interfaced to an SCIEX Triple Quad 3500 (Rotakim, Turkey) LC-MS/MS system equipped with Turbo V source and Electrospray Ionization probe was used to determine the levels of 25-OH vitamin D. The IC3450rp HPLC column (50 x 3 mm) was used for analysis of the vitamin. A mixture of acetonitrile/methanol (60% + 40%, v/v) was used as the mobile phase, and the flow rate was set at 1 mL/min. Each sample (20 µL) was injected into the device. The standard of 25-OH vitamin D (Sigma-Aldrich Co., Germany) was prepared in methanol for calibration. The values expressed as µg/L.

2.1 Statistical analysis

SPSS 22 for Windows (IBM Corp., Armonk NY, USA) was used for the data analysis. Independent Sample Test was used to analyze the data regard levels of vitamin D. After the test of normality, egg and albumen weight, percentage of albumen, and shell thickness were analyzed by Mann-Withney U test. The shape index, yolk and shell weight, and percentage of yolk and shell were analyzed by Independent Sample t Test. Mann-Withney U test was used to compare yolk color values obtained with scoring. The yolk color score was expressed as median, minimum, and maximum values. Data are expressed as mean and standard error mean (SEM). $P < 0.05$ was considered statistically significant.

3 Results

Some quality characteristics of eggs are summarized in Table 1. The weights of egg, albumen, and yolk in conventional eggs were significantly ($P < 0.05$) higher than those of free-range eggs. The shape index and shell thickness decreased significantly ($P < 0.05$) in conventional eggs. In addition, percentages of albumen and shell in free-range egg were significantly higher ($P < 0.05$) than those in conventional eggs. However, the percentage of yolk in free-range egg was significantly ($P < 0.05$) lower than that of conventional eggs. The yolk color scoring of the eggs are given in Table 2. The yolk color score in conventional eggs was significantly ($P < 0.05$) higher (median 12) than that of free-range eggs (median 9). In addition, the levels of 25-OH vitamin D are given in Table 3. Also, 25-OH vitamin D level in conventional eggs was significantly ($P < 0.05$) higher (1.28 µg/L) than in free-range eggs (0.75 µg/L).

Table 1. Some quality characteristics of the eggs.

Traits	Conventional	Free-range	P value
Egg weight (g)	66.14 ± 0.28^a	60.66 ± 0.12^b	< 0.001
Shape index (%)	73.36 ± 0.31^a	78.90 ± 0.27^b	< 0.001
Albumen weight (g)	38.09 ± 0.26^a	36.30 ± 0.20^b	< 0.001
Yolk weight (g)	18.89 ± 0.16^a	15.66 ± 0.13^b	< 0.001
Shell weight (g)	6.26 ± 0.05	6.14 ± 0.05	> 0.05
Percentage of albumen (%)	57.58 ± 0.28^a	59.85 ± 0.31^b	< 0.001
Percentage of yolk (%)	28.57 ± 0.23^a	25.83 ± 0.22^b	< 0.001
Percentage of shell (%)	9.46 ± 0.07^a	10.12 ± 0.08^b	< 0.001
Shell thickness (mm)	0.417 ± 0.002^a	0.438 ± 0.003^b	< 0.001

a,b: Different letters in the same column show differences between groups. Values are expressed as mean ± standard error. $P < 0.05$ is statistically significant.

Table 2. The yolk color scoring of the eggs.

Groups	Median	Minimum	Maximum	P value
Conventional[a]	12	9	13	< 0.001
Free-range[b]	9	8	11	

a,b: Different letters in the same column show differences between groups.

Table 3. Levels of 25-OH vitamin D in the eggs.

	25-OH Vitamin D (µg/L)	P value
Conventional (n = 75)	1.28 ± 0.08^a	< 0.001
Free-range (n = 75)	0.75 ± 0.04^b	< 0.001

a,b: Different letters in the same column show differences between groups. significant. The data are expressed as mean ± standard error. $P < 0.05$ is statistically significant.

4 Discussion

Eggs are important in human nutrition because of their nutrient value and low cost.Poultry eggs are an important dietary component worldwide owing to their high nutritional value and ease of digestio (Zhang et al., 2022) . It contains proteins, carbohydrates, lipids and essential fatty acids. Also, eggs are rich in micronutrients, especially fat-soluble vitamins (vitamins A, D, and E). Each component has a specific function to human nutrition requirements (Pereira et al., 2014). Vitamins are essential nutrients that perform specific and vital functions for health (Zang et al., 2011). Vitamin D can be converted into an active form after ingestion from animal foods (Li et al., 2022). It regulates calcium absorption, serum calcium and phosphorus level to maintain bone structure (Devkate et al., 2019). There are two major sources of vitamin D for humans: those synthesized endogenously via by exposing the skin to sunlight and those obtain via dietary intake (Guo et al., 2017). However, there are some limitations for the *in vivo* synthesis of vitamin D such as ultraviolet radiation, indoor lifestyle, environment, and individual factors (skin pigmentation and age) (Nair & Maseeh, 2012). It is known that vitamin D deficiency is common in Europe. Dietary intake of vitamin D may become more important due to these factors. For this purpose, eggs are enriched for vitamins through the diet of the producing hen. These eggs are also known as functional eggs. On the other hand, it is reported that other environmental factors, such as housing systems, have an impact on vitamin D content (Schmid & Walther, 2013; Guo et al., 2017). There are limited data on the vitamin D content of retail eggs from the different production systems. In this study, eggs from conventional system had higher amount of vitamin D than free-range eggs. In contrary to our findings, Guo et al. (2017) reported that outdoor production systems are likely to yield higher amounts of vitamin D in egg yolks. However, this case might not be a consistent, due to dietary effects and genotype. Matt et al. (2009) found that the vitamin D level of conventional eggs was higher than that of organic eggs (outdoor) and this in agreement with our findings. This case is thought can be caused by feed content and additives at conventional system. Yolk color is one of the significant egg quality parameters that impact on perspective of the consumer's demand (Bovšková et al., 2014). Yolk color is primarily determined by the content and profile of pigmented carotenoids present in the laying hens' diet (Hernandez et al., 2005). Carotenoids are yellow, orange and red pigments that soluble in fats (Bovšková et al., 2014). In addition, yolk color is also influenced by the genotype of laying hens and their age (laying period) (Kim et al., 2014; Sokołowicz et al., 2019). In this study, we discussed the relation of yolk color tone alteration with vitamin D content of the yolk. Based on the results of this study, 25-OH vitamin D levels and some egg quality parameters appear to be different between conventional and free-range systems. According to this study, the yolk color tone is parallel with the 25-OH vitamin D level and darker egg yolks may contain more vitamin D. Yolk color depends on also liver's activity in hens. Beyond the mentioned factors above that affect yolk color; stress and cage breeding have an impact on yolk color (Saleh et al., 2021). On the other hand, the antioxidant trait of vitamin D is much greater than taht of vitamin E (Mokhtari et al., 2017). The vitamin D could act like the liver protector as an antioxidant agent therefore, the yolk color may be ameliorated or be darker with the aid of vitamin D. However, this case must be discussed in considering multiple parameters such as genotype, hen age, and diet formulation.

5 Conclusion

The 25-OH vitamin D levels were significantly higher in the conventional eggs. Furthermore, the weights of egg, albumen, yolk, percentage of yolk and yolk color were significantly higher in the conventional eggs. However, the shape index, percentage of albumen, percentage of shell and shell thickness were significantly higher in the free-range eggs. It can be concluded that vitamin D level in eggs can be related to yolk color. However, egg quality characteristics can also be influenced by various environmental factors.

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