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Original Scientific Paper

Nutritional strategy in production vitamin D fortified eggs

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A B S T R A C T

Vitamin D is a liposoluble vitamin that regulates calcium homeostasis and is important for animal and human health. Lack of vitamin D in humans can cause rickets, osteomalacia, and decreased immunity. In laying hens, vitamin D deficiency negatively affects egg production (decrease in egg production, thin-shelled eggs, poor feed conversion rate, etc.). Farm animal requirements for vitamin D depend on age, sex, health status and production category. Foods that contain vitamin D in human nutrition include salmon, red meat, liver, tuna and eggs, but due to an unbalanced diet, people often cannot meet their needs in this way. In animals, the daily needs can be met by adding vitamin D through premixes into the feed. The Department of Nutrition and Botany, Faculty of Veterinary Medicine, University of Belgrade, conducted research on a commercial laying hen farm, where an active form of vitamin D was added through premix into the feed of 20-week-old laying hens. The nutritional reference value for vitamin D is 5 µg/day, and an enriched egg contained an average of 2.56 µg/100 g of egg mass. By consuming food or feed enriched with vitamin D, various diseases can be prevented, and it is easier to meet the daily needs of both humans and animals.

1. Introduction

Chicken eggs belong to the group of products of animal origin that are most often consumed around the world. According to the reports of the Food and Agriculture Organization (FAO), the consumption of eggs increased sharply in the period from 2000 to 2018 (by as much as 50%) (*FAO*, 2020). The demand for chicken eggs is expected to continue to grow due to their widespread acceptance by consumers and population growth (*FAO*, 2018). The perfect balance and variety of nutrients, together with high digestibility and an affordable price, put the egg at the top of the list of foods used in human nutrition. Eggs are considered as nutritionally the best source of protein (*Domingo*, 2014; *Lesnierowski and Stangierski*, 2018). They contain an optimal ratio of essential amino acids, fatty acids, fat-soluble vitamins (A, D, E, K) and B group vitamins (B1, B2, B5, B6, B7, B9, B12, choline). In addition to the above, they contain minerals: calcium, iron, magnesium, phosphorus, selenium, sodium and zinc, as well as antioxidants that reduce free radicals produced by cellular metabolism (*Rodriguez-Gonzalo et al.* 2017). Table 1 shows the chemical composition of the whole edible hen's egg.

With the increase in egg production for the world market (*FAO*, 2020), consumer awareness of egg quality is increasingly developing (*Duman et al.*, 2016), which includes information on the origin of

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the product, type of production, nutrition of the laying hen, animal welfare and environmental conditions on the farm. For these reasons, ways to improve and adapt development to market requirements are being explored, while respecting all relevant regulations and meeting consumer expectations for high-quality products (*Gracia et al*., 2014). Consumer culture, traditions, lifestyles and attitudes about egg consumption are carefully considered in order to meet the demands and expectations set by the market (*Patricia and Hester*, 2017). Factors affecting the quality of chicken eggs are very complex and are mainly generated in the production phase (*Zaheer*, 2015). Research by the European Consumer Association indicates that table eggs are increasingly recognized as a quality product, highlighting the most important parameters: nutritional value and sensory characteristics.

Table 1. The basic chemical composition of the whole edible hen's egg

Nutrient	g/100g
Protein	12.56
Fat	9.51
Carbohydrate	0.72
Moisture	76.15
Ash	1.06

However, the egg still faces long-standing recommendations by nutritionists aimed at limiting egg consumption in order to reduce the incidence of cardiovascular disease. Human nutrition in developed countries is specific because it involves an excessive intake of protein, cholesterol, saturated fatty acids and n-6 polyunsaturated fatty acids (PUFA), while the intake of n-3 PUFA, fibre and antioxidants is insufficient. Such an unbalanced diet is partly responsible for the high frequency of illness and the occurrence of chronic diseases, of which cardiovascular diseases are the leading cause of death in the world (*Miranda et al.,* 2015). But most experimental, clinical and epidemiological studies have concluded that there is no evidence of a correlation between dietary cholesterol provided by eggs and an increase in total plasma cholesterol. Egg remains a food product of high nutritional quality for adults, including the elderly, and children (*Godbert et al.,* 2019). Today, great efforts are invested in the development of innovative technologies, which create a product with added value (enriched product or functional food). In addition to increasing the content of desirable functional ingredients in the product (eggs), they also have a positive effect on animal health, improve performance and, thus, affect the sustainability and quality of the product (*Wang et al*., 2017). The most common functional ingredients or nutrients used in food fortification (meat and eggs) are: omega-3 fatty acids, selenium, vitamin E, vitamin A, vitamin D and others. These listed ingredients are of interest since they are factors in the biggest problems of the modern lifestyle (*Kralik and Lovreković*, 2012).

Vitamins are a group of complex diverse organic compounds essential for the development of basic physiological and metabolic processes in humans and animals. These are physiologically active food micro-ingredients required in minimal amounts for health, life support, growth and reproduction. In case of deficiency (insufficient amount in food, insufficient synthesis), signs of deficit and health disorders are manifested.

Vitamin D (calciferol) belongs to a group of compounds with anti-rachitic activity, the most important of which are ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Calciferol is necessary for optimal growth and reproduction, as well as for maintaining a normal state of health, while a possible function in the immune status of animals is also indicated (*Clark et al*., 2021). The main function of calciferol is to, together with calcitonin and parathormone, ensure the optimal concentration of calcium in the blood necessary for normal physiological processes in which calcium is involved. Calciferol affects resorption (gut), deposition and mobilization (bones), as well as calcium excretion (kidneys) (*Jokić et al.*, 2024).

Poultry have higher requirements for calciferol than do other animal species. Actively laying hens, due to the high content of calcium in the eggs, have significantly higher needs for vitamin D compared to the needs for life support in non-laying hens. The calciferol requirements for laying hens, 1500-1800 IJ/kg of feed, were determined on the basis of the material balance and biological nutritional trials. This amount meets the hen's needs for optimal growth, ossification, reproduction and normal health maintenance. Apart from the absolute amounts of calciferol in the diet necessary to meet the needs of the laying hen, the form is also unusually important. Namely, cholecalciferol is a physiologically more active form of the vitamin than ergocalciferol. In poultry, the relative biological activity of ergocalciferol is only 28% compared to cholecalciferol. Most feeds used in the diet of laying hens contain small amounts of calciferol, although provitamins are widely distributed. Cereal feeds, as well as industrial by-products, are extremely poor sources of calciferol or do not contain it at all. Conversion of ergosterol to ergocalciferol occurs only in the dead parts of plants exposed to UV rays. Foods of animal origin are a relatively poor source, and only fishmeal and fish oil from marine fish contain extremely large amounts of cholecalciferol. Considering the role of calciferol in the regulation of calcium and the importance of calcium in the process of muscle contraction and neuromuscular excitability, paresis and paralysis occur in cases of deficiency. This occurs especially in animals that have extremely high calcium needs, such as laying hens, in which, if experiencing a deficit, the inability to stand and paralysis can occur (cage paralysis of laying). Also, apart from the above, a lack of calcium causes a drop in carrying capacity, eggs with shell deformities (thickness, mass, firmness, shape) or eggs without a shell. In poultry, poor feathering occurs with feather pigmentation disorder (achromotrichia) (*Šefer and Sinovec*, 2008).

Due to the low concentration of vitamin D in feed, in the animal feed industry, vitamin D is added to feed mixtures for laying hens through vitamin-mineral premixes. The vitamin D additive used in these vitamin-mineral premixes is present in one of two basic forms: conventional vitamin D3, at levels in feed of at least 500,000 I.U./g of vitamin D3 and 1,25-dihydroxycholecalciferol – the active form of vitamin D3. Metabolism of the active form of vitamin D3 in laying hens takes place in the kidneys, where, bypassing the metabolic processes in the intestines and liver, it enters the bloodstream directly. The addition to feed of the active form of vitamin D3 enables its proper absorption, which, facilitates better assimilation of Ca and P, improvement of carrying capacity, and transfer and deposition of vitamin D into the egg mass (*Šefer and Sinovec*, 2008).

Eating two eggs a day covers about 10% of people's vitamin needs. It is important to note that the content of fat-soluble vitamins (vitamins A, D, E and K) in the yolk largely depends on the diet of the hen.

2. Materials and methods

The research was carried out in cooperation with the Department of Nutrition and Botany of the Faculty of Veterinary Medicine, University of Belgrade, and the company Fish Corp 2000, by adding the active form of vitamin D3 through a premix, to mixtures for laying hens for 4 weeks. The study lasted six months and was carried out on a commercial egg-laying hen farm in Srbobran, Serbia, with a capacity of 13,000 hens, 20 weeks old. Vitamin-mineral premix, in floury form and intended for use at a level of 1% in the finished feed mixture for egg-laying hens, contained: Vitamins: 1,000,000 I.J. Vitamin A (3a672a), 200,000 I.J. Vitamin D3 (3a671), 11.25 mg 25-hydroxycholecalciferol (3a670a), 1,500 mg Vitamin E (3a700), 200 mg Vitamin K3 (3a711), 200 mg Vitamin B1 (3a821), 400 mg Vitamin B2/riboflavin, 200 mg Vitamin B6 (3a831), 1.2 mg Vitamin B12 /cyanocobalamin, 2,000 mg Vitamin C/ascorbic acid, 10 mg Biotin (3a880), 2,000 mg Niacin (3a314), 500 mg Calcium D-pantothenate (3a841), 40 mg Folic acid (3a316), 50,000 mg Choline chloride (3a890); Microelements: 5,000 mg iron as iron (II) sulphate monoch. (3b103), 1,500 mg copper as copper (II) sulphate pentah. (3b405), 6,000 mg zinc as zinc oxide (3b603), 8,000 mg manganese as manganese sulphate monoch. (3b503), 50 mg iodine as calcium iodate (3b202), 20 mg cobalt as cobalt carbonate (3b302), 15 mg selenium as sodium selenite (3b801).

The content of vitamin D3 in eggs was determined by the 02I.01.022 method (Ložnjak Švarc et al. 2021).

3. Results and discussion

With conventional nutrition, egg-laying hens contain 1 µg of vitamin D3 per 100 g of egg mass. Based on the statement of the local Serbian Zdravlje Health Cooperative, an egg can be declared as rich in vitamin D provided that 100 g of the product contains a minimum of 30% of the nutritional reference value (NRV) for vitamin D. Given that the NRV for vitamin D is 5 µg, an egg rich in vitamin D should contain a minimum of 1.5 µg of vitamin D in 100 g of egg mass. Addition of the active form of vitamin D via premix to layers for 4 weeks resulted in a product that contained an average of 2.56 µg of vitamin D3 per 100 g of egg mass, shown in Figure 1. These eggs contain more than 50% of the NRV for vitamin D and can be declared as a Vitamin Egg.

Daily consumption of vitamin D-enriched eggs produced by hens that receive appropriate pre-mix is important for maintaining optimal health, especially for people with mobility difficulties and reduced outdoor time. Eggs enriched with vitamin D can provide adequate intake of the vitamin, which is important in a state of stress or illness (*Persia et al*., 2013).

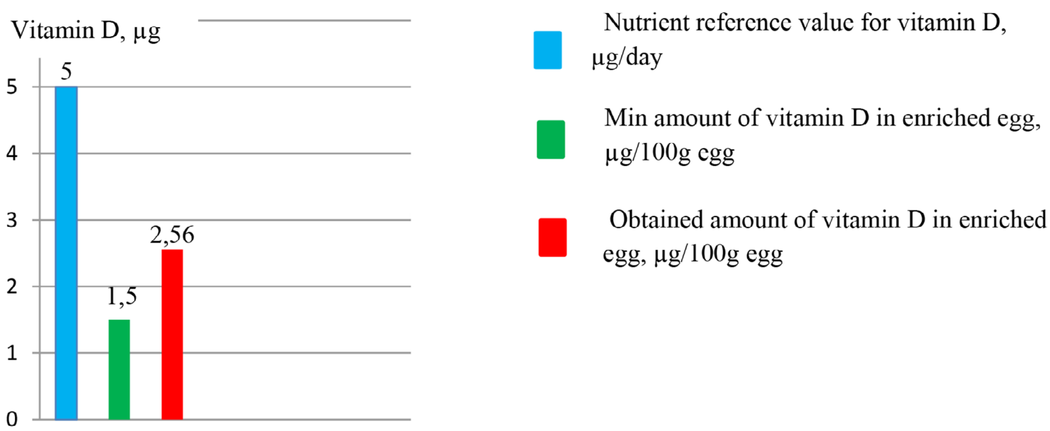

Figure 1. Nutritional reference value for vitamin D, minimum amount of vitamin D in an enriched egg and mean vitamin D level per 100g of egg mass after hens received vitamin-mineral premix.

Finnish scientists from the Agricultural Research Center came to similar conclusions. They fed 30-week-old hens a conventional diet and a diet supplemented with vitamin D. Hens that consumed feed with 3.5 times more vitamin D produced eggs with seven times more vitamin D3 than eggs from hens fed a standard diet. They also observed that increased supplementation of this vitamin has an effect on the health of chickens: healthy reproductive system, properly formed egg shell, maintenance of strong bones and a well functioning immune system (*Matilla et al.*, 1999).

Multiple studies in the UK have shown that adding vitamin D3 to the hen diets results in a significant improvement in the vitamin D content of eggs (*Browning and Cowieson*, 2014). These studies have shown that yolk saturation with vitamin D is achieved within about 6 weeks of consuming fortified feed, and that the total vitamin D content of eggs can potentially increase by up to 78% *(Matilla et al.*, 2003).

4. Conclusion

For centuries, eggs have been considered a food of high nutritional value for humans. It is predicted that their consumption will continue to grow in the future, given the increasing number of consumers who are starting to adopt a meat-free diet (vegetarians) or who are significantly reducing their meat intake. Demands are increasing as consumer awareness of the quality of the food they eat is growing.

The Vitamin Egg is a product of the collaboration between the Department of Nutrition and Botany, Faculty of Veterinary Medicine, University of Belgrade and the company Fish Corp 2000. With these joint efforts that aim to reduce the difference between the intake of vitamins through food and human nutritional needs, eggs enriched with vitamin D (2.56 µg/100g of egg mass) were obtained, which contain more than 50% of the nutritional reference value for vitamin D (5 µg/day).

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