

ORIGINAL RESEARCH PAPER

EFFECTS OF DIETARY SUPPLEMENTATION ON EGG YOLK MINERAL AND CHOLESTEROL CONCENTRATIONS IN LAYING QUAILS

Vasile Macari¹, Liliana Rotari¹, Ana Macari¹, Nina Mija^{2*},
Daniela Paladi²

¹Technical University of Moldova, Faculty of Veterinary Medicine, MD-2012, Chisinau, Republic of Moldova

²Technical University of Moldova, Faculty of Food Technology, MD-2045, Chisinau, Republic of Moldova

*Corresponding author: nina.mija@toap.utm.md

Received: September, 05, 2025

Accepted: March, 25, 2026

Abstract: In this research, studies were conducted to determine the effect of supplementation of quails feed with ZooBioR, which is a bioproduct based on extracts from the cyanobacterium *Spirulina platensis* biomass. The quails tested belonged to the breed *Texas Super*. The eggs produced by quails had the following characteristics: egg mass – 12.92±1.30 g, yolk mass – 4.49±0.63 g, yolk/white ratio – 1:1.60, yolk pH – 6.43±0.21. Bioproduct ZooBioR, being rich in well assimilated nutrients (amino acids, phospholipids), it contributed to improving the nutritional value of the quail yolk. The mineral content of yolk was found to be enhanced – for P from 248.0 mg/100 g (control group) to 265.8 mg/100 g yolk (tested group), and the amount of Ca – from 24.5 mg/100 g (control group) to 28.6 mg/100 g yolk (tested group). The total amount of cholesterol does not differ much – from 1152.6 for the control group to 1088.1 mg/100 g yolk for the tested group. Instead, there are trends in the decrease of the free cholesterol fraction, respectively, from 1.9 to 0.3 mg/100 g yolk. The argument would be that the ZooBioR supplement, rich in phospholipids, contributed to the increase in esterified cholesterol fractions and decrease of free cholesterol. Consequently, the decrease in free cholesterol is a beneficial factor in reducing easily assimilated cholesterol from eggs. An increase in the HDL esterified cholesterol fraction was also recorded, from 427.5 (control group) to 457.1 mg/100 g yolk (tested group).

Keywords: calcium, egg quality traits, HDL, LDL, phosphorus, quail eggs, *Spirulina*, supplemented feed

INTRODUCTION

Birds raised in intensive industrial conditions of zootechnical farms are exposed to technological stress, which negatively affects their health and productive potential, and finally, the quality of the resulting poultry products [1, 2].

Feed based on ingredients with anti-stress, adaptive and bio-stimulatory components of natural origin is recommended as an effective remedy in strengthening the health of birds and for obtaining poultry products with high nutritional qualities [3 – 9].

In the poultry industry, there is a stable experience of using *Spirulina* preparations for physiological protective purposes in the process of raising hens and broilers [1], and quails [6, 10].

Supplementary, an effect of strongly reducing the egg cholesterol by addition in feed of the birds *Spirulina* algae were observed [6, 8]. Also, *Spirulina* as an ingredient of feed have multiple beneficial effects for laying birds like that: boosting the immune system, increase overall growth performance [1].

Of particular interest is the ZooBioR, a biologically active supplement, developed by the scientists of the Institute of Microbiology and Biotechnology, Chisinau, which contain *Spirulina platensis* biomass complex extracts as its key component and some mineral additives (Zn acetate, Na selenite) [10].

The addition of Zn salt can contribute to reducing the negative effect of technological stress, increasing feed efficiency of growing poultry [11]. The qualities of Se as an antioxidant can be beneficial in stabilization of oxidative processes, increasing the anti-microbial and anti-viral properties of poultry products [1].

In the estimation of the quality of consumption eggs, a controversial indicator is the cholesterol content. Cholesterol in its various forms (HDL, LDL and free cholesterol) significantly impacts cardiovascular health. High levels of LDL cholesterol contributes to the buildup of plaque in artery walls, a process called atherosclerosis [4, 6].

Egg yolk cholesterol originates from serum blood cholesterol of birds, but due to complex metabolic transformations, serum blood cholesterol concentration is not closely associated with the concentration of yolk cholesterol [12]. Cholesterol content in the yolk eggs is influenced by genetic factors, diet composition, lay intensity, layer age and the growing microclimate [5].

Multiple studies have demonstrated the influence of the nutrients contained in the food on the amount and profile of the cholesterol fractions accumulated in the yolk egg [4, 5, 7 – 9, 11, 13, 14]. Thus, cholesterol levels in egg yolk can be strongly influenced by antioxidant compounds or fatty acid composition and lecithin of lipids in feed [7, 13].

This article aims to estimate the effect of supplementing feed with ZoobioR, *Spirulina* based preparation, on the quality and nutritional traits of quail eggs.

MATERIALS AND METHODS

Scientific research was conducted on laying quails at the quail poultry farm „MIHAILORINA-COM”, Rașcani district, Republic of Moldova located at 47°50' N latitude and 27°50' E longitude.

In the study, 200 quails (Texas Super breed) of 8 weeks old were used, these being at the beginning of the egg-laying cycle. Four groups, each containing 50 birds were formed.

Quails were kept in cages (45 × 50 × 28 cm) in a closed space, with a lighting period of 18/6 hours. During the 4 months of the study, water and feed were supplied *ad libitum*. The ZooBioR supplement, *Spirulina platensis* biomass extracts, were used in the diets of the four treatment groups at levels of 0, 10, 15, and 20 mg·kg⁻¹ of feed.

After the 4 months of treatment the quail eggs were collected from each group and weighed individually with a balance (HJW-600, with 0.01 mg sensitivity, UWE, New Taipei City-Taiwan). The shape index was calculated by using the following equation (1), [15].

$$\text{Shape index} = (\text{egg width} / \text{egg length}) \times 100 \quad (1)$$

The surface area, expressed as centimeters squared (cm²), of each egg was estimated using the equation of Carter (2), [16]:

$$\text{Surface area [cm}^2\text{]} = 3.9782 \times W^{0.7056} \quad (2)$$

where W is the egg weight in grams.

Eggs were broken onto a glass table individually. The height of the yolk and albumen was quantified with a vernier caliper (Mitutoyo, no. 2050S-19, 0.01-20 mm, Kawasaki, Japan). Egg internal quality parameters were calculated with these measurements by using the following equation (3), [9]:

$$\text{Yolk index} = (\text{yolk height} / \text{yolk diameter}) \times 100 \quad (3)$$

Egg yolk color of treated eggs was scored by using Roche yolk color fan [17] on a glass plate. A pH-meter for semi-solid products was used in the determinations (model HI 98161, with 0.001 units' sensitivity, HANNA, Temse, Belgium).

For mineral and cholesterol evaluation, six eggs randomly selected from each group were used. The amount of minerals was determined after dry mineralization of samples and application of classical arbitration methods. Identification and quantification of phosphorus in the mineralized samples were made with ammonium molybdate-vanadate reagent, iron – sulfosalicylic acid (20 %) and calcium – Trilon B solution (0.1 N), respectively.

The method for determination of cholesterol fractions included several steps: separation of the yolk serum from the fresh yolk, consecutively sedimentation of HDL, and then sedimentation of LDL, and finally, separation and spectrophotometrically determination of free cholesterol. Separation of the yolk serum from the fresh yolk: using high methoxyl pectins gel (1 %) [18];

Sedimentation of the HDL fraction: serum treatment with phosphotungstic acid (4 %) and MgCl₂ (2M) was added, followed by centrifugation at 10.000 rpm [19];

Sedimentation of the LDL fraction: serum treatment with zincum sulfate (15 %), followed by centrifugation at 10.000 rpm [20];

Spectrophotometric determination of free cholesterol in the *n*-hexane extract: extraction with *n*-hexane, then recording the molecular absorption spectrum. The extraction of free cholesterol was carried out using serum and *n*-hexane in a ratio of 1:1 (v/v) [21]. The spectra analysis has been done at double beam spectrophotometer (model UV-Vis 1800, spectral bandwidth – 1 nm, Remma, Paris, France) in the wavelength range of 200 - 450 nm. Equation used (4) for calculation of free cholesterol was:

$$A = \varepsilon_{\lambda} \cdot C \cdot l \quad (4)$$

where, A is absorbance at 250 nm, $\epsilon_{\lambda} = 1.824 \cdot 10^4 \text{ L} \cdot (\text{mol} \cdot \text{cm})^{-1}$ – molar extinction of cholesterol, C – cholesterol concentration in the tested sample, $\text{mol} \cdot \text{L}^{-1}$, l – thickness of the optical cell, cm [19].

Software STATISTICA version 7.1 was used to perform the one-factor analysis of variance. Results were shown as means with their standard deviation. Statistical significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

The ingredient and chemical composition of the experimental feeds for tested quails are presented in Table 1. The basic diet consisted of a mixture of cereals enriched with minerals and vitamins. Basic diet was rich in proteins and carbohydrates. The other three diets were supplemented with ZoobioR preparation, which have multiple promoting components: soluble peptides, aminoacids, phospholipids, proteoglycans, and carotenoids [10].

Table 1. *Ingredients of basal feed and chemical composition of feed of control group*

Ingredients	Quantity [$\text{g} \cdot \text{kg}^{-1}$]	Composition of feed	Quantity [%]
Maize	280	Protein	18.1
Wheat	280	Fat	2.81
Soybean meal	120	Carbohydrates	46.87
Sunflower meal	160	Fiber	2.8
DCP ¹	140	Dry substances	76.3
Vitamin and mineral premix Vitafort ²	20	Metabolizable energy [$\text{kcal} \cdot \text{kg}^{-1}$]	2710.2

1 – DCP feed grade dicalcium phosphate, 18 %.

2 – Vitamin and mineral premix Vitafort contains: calcium, phosphorus, vitamin A, vitamin D, vitamin E, vitamin K, vitamin B1, vitamin B2, vitamin B6, vitamin B12, pantothenic acid, nicotinic acid, folic acid, biotin, choline chloride, zinc, copper, iron, manganese, selenium, iodine, betaine, methionine, enzyme mixture.

Results of effects of dietary treatment on egg traits are presented in Table 2. The egg weight of control and 10, 15 and 20 $\text{mg} \cdot \text{kg}^{-1}$ treatment groups were silently affected by dietary treatments. Thus, dietary 15 $\text{mg} \cdot \text{kg}^{-1}$ ZooBioR supplement increased the egg weight with 0.56 g/egg against control group. Respectively, an increase in the egg surface area (of about 0.73 cm^2), the large diameter (of about 1.92 mm) and the small diameter (of about 0.59 mm) was recorded. At the same time, the shape of the egg after fortified treatment tends to become rounder, the shape index increasing from 77.64 (control group) to 80.17 (20 $\text{mg} \cdot \text{kg}^{-1}$ feed).

The inclusion of the different amounts of ZooBioR supplement continuously affects yolk index and color index of egg yolks when compared with the control group. The increase in yolk index could be explained by the improvement of the physiological vitality of quails with the addition of biologically active components in the feed [1, 22]. The increase in the intensity of the rich orange colour with increasing amounts of carotenoid-rich supplement is a signal of the possible accumulation of carotenoids in the egg yolk [14, 17]. The variations in the pH values are not significant and do not exceed 0.08 units pH for egg white and 0.10 for egg yolk within the groups.

Supplementation of quail feed with ZooBioR interferes in the process of mineral accumulation in quail egg yolks. The biologically active components of the preparation

(phospholipids, carotenoids) increase the physiological activity of birds, which induces the progressive accumulation of minerals at the egg formation stage [3].

Table 2. Effects of dietary treatments on egg characteristics of laying quail
(mean±SD)

Egg characteristics	Treatment group [mg·kg ⁻¹]			
	Control	10	15	20
Egg mass [g]	12.92±0.96	13.09±0.55	13.48±0.56	13.36±0.68
Surface area [cm ²]	24.20±1.53	24.42±0.89	24.93±1.04	24.78±1.06
Big diameter [mm]	33.41±1.48	33.39±1.12	35.33±1.45	32.33±1.58
Small diameter [mm]	25.94±0.73	25.87±0.57	26.53±0.93	25.92±0.65
Shape index	77.64±9.12	77.48±10.04	75.05±14.43	80.17±9.25
White height [mm]	4.69±0.79	4.84±0.94	4.88±0.90	4.83±1.03
Yolk height [mm]	9.34±1.38	9.61±0.52	10.18±0.63	10.11±1.22
White weight [g]	6.50±0.59	6.78±0.24	6.80±0.43	6.85±0.44
Yolk weight [g]	4.49±0.56	4.38±0.41	4.71±0.58	4.55±0.36
Yolk index	23.87±1.05	22.98±0.52	24.71±0.91	25.16±1.10
Color index of yolk	6.6±1.8	6.8±1.1	8.0±1.2	9.3±1.4
pH of white egg	8.96±0.05	9.03±0.13	8.89±0.17	9.04±0.04
pH of yolk egg	6.43±0.21	6.46±0.18	6.34±0.27	6.48±0.14

Supplementation of quail feed with ZooBioR interferes in the process of mineral accumulation in quail egg yolks. The biologically active components of the preparation (phospholipids, carotenoids) increase the physiological activity of birds, which induces the progressive accumulation of minerals at the egg formation stage [3]. The addition of 15 and 20 mg·kg⁻¹ of feed of ZooBioR in basal diet increased the content of phosphorus in egg yolk with 6.7 and 12.8 %, and calcium with 14.3 and 16.4 %, respectively, as compared with the control diet (Figure 1). As it was mentioned above, preparation ZooBioR contains Zn acetate as an addition. Ramadan *et al.* [23] has reported that zincum and iron contained in feed act as synergists, which contributes to the fortification of egg yolk with iron. However, no strong correlation was recorded between the effect of supplementation on the presence of iron in egg yolk in the series of tested samples ($p \geq 0.05$).

ZooBioR being a bio-product, based on extracts from the cyanobacterium *Spirulina platensis* biomass, also introduced some modifications in the cholesterol profile of tested quail eggs (Table 3).

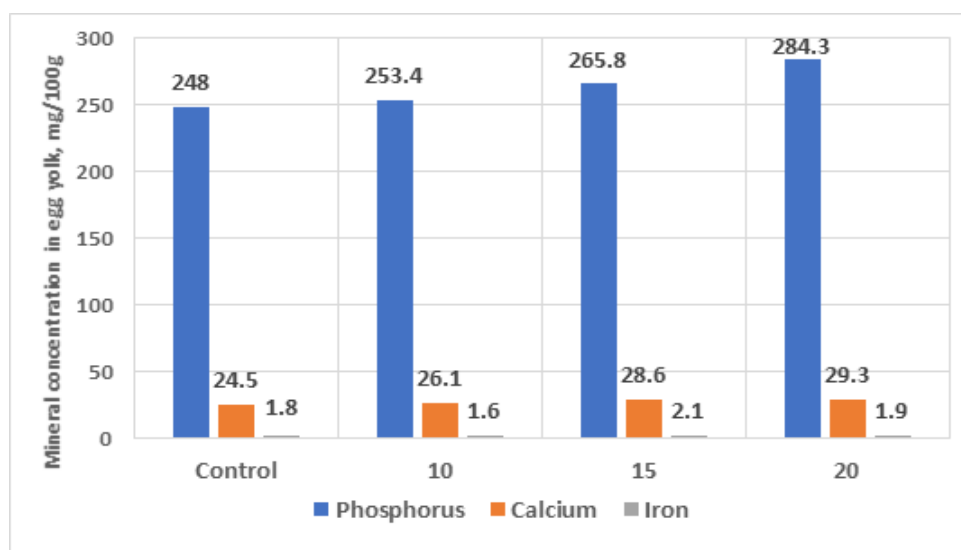


Figure 1. Effect of ZooBioR supplementation ($\text{mg}\cdot\text{kg}^{-1}$) on mineral concentration of egg yolk in treatment quails

Table 3. Effect of feed supplementation on cholesterol concentration in quail's eggs (mean \pm SD)

Item	Treatment group [$\text{mg}\cdot\text{kg}^{-1}$]			
	Control	10	15	20
Esterified cholesterol: [mg/100 g]				
HDL	427.5 \pm 0.3	415.0 \pm 0.5	457.1 \pm 0.3	430.9 \pm 0.3
LDL	723.2 \pm 0.6	659.8 \pm 0.4	643.6 \pm 0.5	687.1 \pm 0.6
Total esterified cholesterol: [mg/100 g]	1150.7	1074.8	1100.70	1118.0
Free cholesterol [mg/100 g]	1.9 \pm 0.1	0.5 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.1
Total cholesterol [mg/100 g]	1152.6 \pm 0.6	1075.2 \pm 0.7	1101.0 \pm 0.6	1118.2 \pm 0.5

The addition of the supplement decreased the total amount of cholesterol by an average of 54.47 mg/100 g yolk compared to the control group, or by 4.73 %. The total cholesterol reduction in egg yolk was probably due to the effect of antioxidants present in feed (organic Se and *Spirulina*) [14].

The amount of free cholesterol in egg yolks has also steadily decreased. It is a positive trend, because free cholesterol is that component, which when being consumed is fastly absorbed into the blood and can easily trigger cardiovascular diseases [24]. Hypothetically, the phospholipids, present in ZooBioR in quite large quantities, about 27.5 mg/100 g, contributed to the etherification of cholesterol and, respectively, in the decrease of the amount of free cholesterol.

A decrease in the amount of LDL, on average by 59.7 mg/100 g of yolk, was also attested. Compared to other foods, eggs do not have a high proportion of LDL, because eggs contain less saturated fat than other food products, the presence of which correlates with an increased level of LDL [7].

When comparing the three groups of supplemented eggs, it was observed that a greater amount of HDL was accumulated in eggs that were obtained when the concentration of the supplement in the feed was 15 $\text{mg}\cdot\text{kg}^{-1}$ feed – 457.1 mg/100 g yolk. The increase in

the amount of HDL compared to the control group by 29.6 mg/100 g yolk can also be attributed to physiological changes in the composition of the blood serum of the birds, which were manifested in strengthening the capacity of HDL to acquire free cholesterol presence [24].

CONCLUSIONS

Theoretical and experimental investigations included in this study focused on the evaluation of the quality of quail eggs, obtained as a result of supplementing the feed with ZooBioR, a complex of organic extracts developed on the basis of *Spirulina platensis*. The experimental treatment included three levels of supplementation of quails feed, which were 10, 15 and 20 mg·kg⁻¹ of feed for a period of 123 days and industrial conditions of maintenance.

The biologically active compounds of ZooBioR (amino acids, phospholipids, carotenoids) positively influenced the physiology of quails, which resulted in increased egg quality.

Egg weight, egg diameters, egg shape index, yolk index, white weight obtained from the fortified feed groups increased slightly compared to the control group. The color of the egg yolk has become more intense.

The best values of mineral accumulation in egg yolk were recorded for phosphorus and calcium. Phospholipids and phosphates contained in ZooBioR preparation facilitate the transition of the free cholesterol fraction to the esterified cholesterol fraction. The decrease in the amount of free cholesterol and the increase in the amount of HDL in eggs treated with ZooBioR could bring health benefits to people who consume them.

In summary, according to the experimental results, the quality of quail eggs as determined by performance indicators and health potential was higher when the tested ZooBioR supplement had a concentration of 15 mg·kg⁻¹ feed.

ACKNOWLEDGMENTS

The research was supported by Institutional Project. subprogram 020405 “Optimizing food processing technologies in the context of the circular bioeconomy and climate change”. Bio-OpTehPAS, being implemented at the Technical University of Moldova.

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