Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2022.20078.1799

# The Effect of Herbal Product (NBS Superfood) Supplementation on Egg Quality Traits in Commercial Laying Hens

**Salah Mahdi Alsherify, Ahmad Hassanabadi, Saeed Zerehdaran & Hassan Nassiri Moghaddam**

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Poultry Science Journal 2022, 10(2): 251-267

**Keywords**
Energy
Laying hens
Herbal feed additive
Performance
Protein

**Corresponding author**
Ahmad Hassanabadi
hassanabadi@um.ac.ir

**Article history**
Received: April 03, 2022
Revised: July 15, 2022
Accepted: July 15, 2022

**Abstract**

The aim of this study was to evaluate the effect of a Herbal product (Nutrition Bio-Shield Superfood® (NBS)) supplementation in diets varying in metabolizable energy (ME) and crude protein (CP) contents on the internal egg quality and fatty acid profile of eggs in commercial laying hens. A total of 420 layer hens aged from 63-74 weeks were assigned to 10 treatments with 6 replicates in a completely randomized design with a 5×2 factorial arrangement with 5 dietary levels of NBS (0, 0.5, 1, 1.5, 2 g/kg diet) and 2 concentration of nutrients (standard and 5% diluted for ME and CP). The diluted diet significantly ($P < 0.05$) increased the shell thickness and shape index compared to the standard diet; however, it decreased shell weight and concentrations of margaric, elaidic, linoleic, docosahexaenoic, polyunsaturated fatty acids, n3, n6, and the ratio of n6:n3 fatty acids in egg yolk compared to the standard diet ($P \leq 0.05$). NBS supplementation linearly decreased egg yolk myristic, margaric, and ginkgoic acids concentrations ($P < 0.05$). In addition, a significant interaction effect was found between diet dilution and NBS supplementation on the haugh unit, shape index, albumen percentage, and egg yolk margaric acid concentration. In general, the reduction of energy and CP in the diet of laying hens by 5% of the standard had no adverse effect on egg quality parameters.

## Introduction

Herbal products are the most useful feed additives used in animal diets that have positive effects on animal health and production. Panda *et al.* (2000) noted that many herbs have been used for decades in animal nutrition because of their effect on feed intake, anthelmintic, coccidiostats, antimicrobial, and immune status. Recently, the European Union has adopted the use of many herbs in poultry nutrition as a viable alternative to other feed additives. According to the report by Markets and Markets (2019), the feed additives market has globally increased from USD 33.0 billion in 2018 to USD 44.3 billion by 2023. However, some additives such as antibiotics appeared to be harmful to the animals and then to people as a result of bacteria resistance and antibiotic residues (Swann *et al.*, 1969). Antibiotic growth promoters pose a threat to humans through meat or egg products, animal health, and the environment (Doeschate and Raine, 2006). As a result, the European Union banned the use of antibiotics in 2006, which was followed by many countries worldwide (Yakhkeshi *et al.*, 2011). Therefore, several feed additives such as probiotics, prebiotics, synbiotics, medical plants, and organic acids have been considered as alternatives to antibiotics.

Nutrition Bio-shield Superfood is a natural plant-based supplement that is extracted from wheat grains (NBS Organic Company, Turkey, 2019) containing water-soluble, except for B7 and B12, fat-soluble vitamins, minerals such as copper, zinc, iron, boron, phosphorus, potassium, sulfur, magnesium, calcium, and manganese, as well as omega-3 (n-3), omega-6 (n-6), and omega-9 (n-9) essential fatty acids (Bayat *et al.*, 2021). Plant-derived substances are one of the possible alternatives to antibiotics that can be used in animal feeding.

There is no available data about the effect of NBS product on laying hens performnce. But, wheat germ a similar feed additive contains valuable nutrients

Please cite this article as Salah Mahdi Alsherify, Ahmad Hassanabadi , Saeed Zerehdaran & Hassan Nassiri Moghaddam. 2022. The Effect of Herbal Product (NBS Superfood) Supplementation on Egg Quality Traits in Commercial Laying Hens. Poult. Sci. J. 10(2): 251-267.

such as vitamins, minerals, amino acids, and fibers (Brandolini and Hidalgo, 2012; Ghafoor *et al*., 2017). Also, Hammershøj and Johansen (2016) found that herbal materials positively affected the fatty acid (FA) composition of egg yolk with high amount of polyunsaturated fatty acids (PUFA), especially α-linoleic acid (Lopez-Bote *et al*., 1998). Fermented wheat germ had a good effect on poultry production and immunity (Mueller and Voigt, 2011). Hafeez *et al*. (2020) reported that herbal products improve nutrient digestibility such as crude protein (CP), fat and nitrogen-free extract (NFE). Kamran *et al*. (2008) stated that metabolizable energy (ME) and crude protein (CP) are the two main factors in poultry diets. Almeida *et al*. (2012) found that ME and CP levels affect egg production, egg mass, and feed intake in Hy-Line W-36 layers. But, some other studies did not show this effect (Jalal *et al*., 2006; Summers and Leeson,1993).

Beynen (2004) and Milinsk *et al*. (2003) reported that eggs fatty acids profile was influenced by the diet of laying hens. The levels of fatty acids can affect human health, whereas low level of saturated fatty acids (SFA) with high level of monounsaturated fatty acids (MUFA) and PUFA could decrease the risk of high cholesterol in consumers (Khosla and Hayes, 1992; Hopkins, 1992; Hayes *et al*., 1991; Grundy and Denke, 1990; Hegsted *et al*., 1965; Keys *et al*., 1965). In addition, unsaturated fatty acids have many beneficial effects on human health including anti-cancer, cardioprotective, reducing triglyceride and blood pressure, enhancing immunity and growth, and central nervous system maturation. Also, the ratio between omega-6 and omega-3 fatty acids has to be balanced in order to prevent health problems such as cancer and cardiovascular disease (Djuricic and Calder, 2021). Therefore, the appropriate ratio of omega-6 (n-6) and omega-3 (n-3) for human health is about 4-5/1 (Mariamenatu and Abdu, 2021). Hammershøj and Johansen (2016) showed that herbal materials affect the composition of fatty acids (PUFAs) especially n-3 to a higher amount in the egg yolk (Lopez-Bote *et al*., 1998). Since the diet represent about 70% of the total cost for poultry production (Kamran *et al*., 2010); thus, using low ME and CP diet in addition to the use of herbal product may reduce the cost and improve eggs internal traits. Therefore, the aim of this study was to evaluate the effect of using varying ME and CP levels with different supplementations of herbal product on the internal egg quality and fatty acid contents in laying hens from 63-74 weeks of age.

## Materials and Methods

### Experimental design, diets, and management

A total of 420 Hy-Line W-36 hens with an initial average BW of 1.6 kg starting at 63 wk of age were used in this study. The experiment was performed as a 5×2 factorial arrangement in a completely randomized design with 5 dietary levels of NBS (0, 0.5, 1.0, 1.5, 2.0 g/kg diet) and 2 dietary levels of ME and CP (standard and 5% reduced ME and CP) during 63-74 weeks of age for 12 wk. The birds were randomly allocated in 10 treatments with 6 replicate cages (experimental unit) of 7 birds each. The chemical composition of NBS supplement powder is presented in Table 1.

**Table 1.** Chemical composition of Nutrition Bio-Shield Superfood® powder[1].

| Composition | Amount (%) | Minerals | Amount (%) | Vitamins | Amount (mg/kg) |
|---|---|---|---|---|---|
| Moisture | 8.40 | Total phosphorus | 0.44 | B1 | 0.66 |
| Total ash | 1.80 | Potassium | 2.31 | B2 | 0.28 |
| Fiber | 11.26 | Sulfur | 0.28 | B3 | 2.70 |
| Digestible nutrients | 61.90 | Magnesium | 0.32 | B5 | 0.89 |
| Carbohydrate (g/100g) | 42.53 | Calcium | 1.67 | B6 | 0.89 |
| Gross energy (kcal/kg) | 4300 | Boron | 0.62 | C | 52.40 |
| Ether extract | 7.20 | Iron (mg/kg) | 241 | E | 0.97 |
| Crude protein | 20.60 | Manganese (mg/kg) | 49.80 | A (IU) | 530.0 |
| Sugar | 3.70 | Zinc (mg/kg) | 26.90 | D (IU) | 483.0 |
| Cellulose | 6.00 | Copper (mg/kg) | 13.6 | K (µg/kg) | 63.60 |
| Omega-3 fatty acids (mg/g) | 48.42 | | | | |
| Omega-6 fatty acids (mg/g) | 60.62 | | | | |
| Omega-9 fatty acids (mg/g) | 22.16 | | | | |

[1]Analyzed in Technology Development Center for Medicinal Plants. Department of Research and Development of Knowledge Based Green Drug Researchers Company.

Experimental diets (Table 2) were formulated based on the Hy-Line W-36 management guide for commercial layers (Hy-Line International, 2020). The complete feed mixture and the feed ingredients chemical compositions were determined based on the AOAC procedure (2019). Briefly, the samples of feed were ground and analyzed in the laboratory of Animal Science Department for dry matter, total ash, and CP based on the following methods (DM, 930.15), (Tash, 942.05), and (Kjeldahl, N × 6.25, 990.03), respectively. In addition, calcium and total phosphorus contents in the diets were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy instrument (ICP-OES). Cage dimensions were 60 × 60 × 40 cm for length, width, and height, respectively. Cages were equipped with a feeding trough and 2 nipple drinkers where the hens had access to feed and water ad libitum during the study. The temperature of rearing house was 16-20 °C, lighting program was 16 hL: 8hD, and relative humidity was 60% throughout the study. Wood partitions were used to prevent cross-feeding between the cages (replicates).

**Table 2.** Ingredients and nutrient composition of basal diets during 63 to 74 weeks, *as-fed basis*.

| Ingredients (%) | Standard | 5 % Diluted |
|---|---|---|
| Corn | 40.86 | 46.68 |
| Soybean meal (44% CP) | 22.44 | 19.38 |
| Wheat | 20.20 | 20.22 |
| Limestone | 10.54 | 10.54 |
| Vegetable oil | 3.28 | 0.38 |
| Dicalsium phosphate | 1.58 | 1.59 |
| Common salt | 0.26 | 0.26 |
| Sodium bicarbonate | 0.1 | 0.1 |
| Mineral premix[1] | 0.25 | 0.25 |
| Vitamin premix[2] | 0.25 | 0.25 |
| DL-Methionine | 0.24 | 0.26 |
| L-Lysine HCL | - | 0.09 |
| *Calculated analysis, %* | | |
| Metabolizable energy (kcal/kg) | 2823 | 2698 |
| Crude protein | 15.24 | 14.44 |
| Calcium | 4.32 | 4.32 |
| Available phosphorus | 0.41 | 0.41 |
| Digestible Lysine | 0.67 | 0.67 |
| Digestible Methionine | 0.45 | 0.45 |
| Digestible Met + Cys | 0.67 | 0.67 |
| *Determined analyses, %* | | |
| DM | 93.26 | 92.63 |
| Crude protein | 15.99 | 15.52 |
| Ash | 13.83 | 14.66 |
| Calcium | 3.36 | 2.91 |
| Total phosphorus | 0.45 | 0.55 |

[1]Provided per kg of diet: vitamin A (retinol), 8,800 IU; vitamin D3 (cholecalciferol), 3,300 IU; vitamin E (DL-*α*-tocopheryl acetate), 18.5 IU; vitamin K3 (menadione), 2.2 mg; vitamin B1 (thiamin), 2.2 mg; vitamin B2 (riboflavin), 5.5 mg; vitamin B3 (niacin), 28.0 mg; vitamin B5 (pantothenic acid), 6.6 mg; vitamin B6 (pyridoxine), 3.5 mg; vitamin B9 (folic acid), 0.7 mg; vitamin B12 (cyanocobalamin), 0.02 mg; biotin, 0.05 mg; antioxidant 1.0 mg.
[2]Provided (mg/kg of diet): Mn (manganese sulfate) 80.0, Fe (iron sulfate) 75.0, Zn (zinc sulfate) 64.0, Cu (copper sulfate) 6.0, Se (Sodium Selenite) 0.3.

**Sample Collection**

**Egg Quality**

Internal egg quality measurements were obtained every three weeks, for four periods during the study. During each period, the following measurements were conducted. Three eggs from each replicate (18 eggs per treatment) were collected for egg weight, specific gravity, and shape index measurements. Each of the eggs was weighed individually using a 0.001g digital scale (model GF 400, A&D Weighing, San Jose, CA, USA) and the average weight of them was recorded for each replicate. After that, the shape index was obtained from the same three eggs used previously by using 0.01 mm precision caliper (model

1116-150, Insize Co, Suzhou, China). The eggs height and width were measured and the shape index was calculated based on formula 1 (Asmundson and Baker, 1940). After taking the egg weight and the shape index, these three eggs were also used for measuring egg-specific gravity. The Archimedes law was used to calculate the specific gravity. The law states that any volume placed in water equals the weight of the fluid it displaces. The method was that the eggs were first weighed with the help of a scale with an accuracy of 0.001 g digital balance (model GF 400, A&D Weighing, San Jose, CA, USA). Then, with the help of copper wire, a small mesh with a minimum size was made and connected to the ring under the scale with the help of hook. Eventually, the egg was completely immersed in the mesh inside the beaker, then the weight of the egg (except for the mesh weight) was recorded as the weight in the water based on formula 2 (Hamilton, 1982; Asmundson and Baker, 1940).

(1) $$\text{Shape index} = \frac{\text{Egg width (mm)}}{\text{Egg length (mm)}} \times 100$$

(2) $$\text{Specific Gravity (S. G)} = \frac{\text{Egg weight in the air}}{\text{Egg weight in the air} - \text{Egg weight in the water}}$$

One egg was collected from each replicate (6 eggs per treatment) for measuring haugh unit (HU), yolk color index, shell thickness, yolk percentage, albumen percentage, and shell percentage. HU was measured based on formula 3 (Haugh, 1937); while yolk color index was measured by using DSM Broiler Fan (DSM Nutritional Products., Roche Basel, Switzerland). This fan has 8 color strips from 101 light colors to 108 dark colors. Shell thickness was measured 48 hours after washing the eggs by using a micrometer with an accuracy of 0.001 mm digital micrometer (model 293-240, Mitutoyo Co, Kanagawa, Japan) from three points, top, bottom, and the middle of the eggshell. Then, the average was considered as the thickness of the eggshell. Yolk, albumen, and shell percentage were obtained by using the descriptive method of Prochaska *et al*. (1996). First, yolk and albumen were separated from each other by using the egg yolk separator. With the use of a paper towel, additional albumen was removed from the yolk before taking the yolk weight. Second, shell weight was measured after rinsing with distilled water and then 48 hours of exposure to air. These parameters were weighed by using a digital scale with an accuracy of 0.001g balance (model GF 400, A&D Weighing, San Jose, CA, USA). Next, the albumen weight was measured by subtracting the yolk and shell weight from the whole egg weight. Then, the weight obtained for the yolk, albumen, and shell was divided by the weight of the whole egg and the result was expressed as a percentage of the total egg for each one individually.

(3) Haugh unit = $100 \times \log_{10}$ ([albumen height (mm) + 7.57] – [1.7 × egg weight (g)$^{0.37}$])

**Lipid extraction and fatty acid methylation**

Fatty acid methyl esters (FAME) were analyzed using the procedure described by Sukhija and Palmquist (1988) with some modifications. Yolk fatty acid content was obtained by taking one egg from each replicate (6 eggs per treatment; 60 eggs total) at the end of the experiment and placed on the chiller room (4 ºC) for further analysis. Each egg was broken and the yolk was separated from the albumen by yolk separator. The yolk was homogenized and about 2-3 mL sample was taken and placed in a small tube. The tubes with the yolk were transferred to the laboratory and placed in the freeze dryer for 24 h. Freeze-dried samples were ground to a fine powder and placed in small plastic bags (60 bags total) and thereafter kept in a deep freezer (-20 ºC) for further analysis.

Samples for fatty acid measurement were prepared by taking 10 mg of each sample and placed in a glass vial. Then, 0.5 mL of *n*-hexane was added to the vial. After that, 50 µL of 25% KOH/ methanol was added to them. Next, vials with the solutions vortexed for 2.5 min. Later on, the vial was placed on an ultrasonic bath with a temperature of 70 ºC for 10 min. After cooling, 1 mL of 0.88% NaCl was added to the vial and placed in the centrifuge at 2490 g for 10 min. Finally, about 2 µL from the top solution was withdrawn and placed on another vial for final measurements. A special instrument syringe was used to withdraw 1 µL from the final vial and placed in the device for the final reading.

**Determination of fatty acids profile**

Fatty acid methyl esters were analyzed by gas chromatography with flame ionization detection (CP-3800 Varian) using a fused silica capillary column by a Chrompack CP-Sil 88 TM (Varian Inc., Walnut Creek, CA, USA). Initially, the oven temperature was started at 140 °C for 5 min and then increased to 240 °C by 4 °C/min for 30 min. The carrier gas used was He with a flow rate of 1.0 mL/min. The temperature of both injector and detector was 260 °C. Basically, a comparison of sample peak retention times with the fatty acids of the FAME standard mixtures was used for identifying the common fatty acids (Sigma-Aldrich Chemie GmbH, Germany; Kiani and Gharooni, 2016).

**Statistical analysis**

Data were analyzed using PROC GLM of SAS software (SAS, 2012) for variance analyzes in a completely randomized design. The experiment was performed as a 2×5 factorial arrangement. The first factor was the diet dilution (standard and 5% diluted) and the second factor was the levels of NBS herbal product (0, 0.5, 1, 1.5, 2 g/kg diet). Duncan's multiple range test was applied to separate treatment means ($P \leq 0.05$). PROC REG was used to test linear and quadratic responses to increasing dietary levels of NBS.

**Results**

**Egg quality traits**

Table 3 shows the effect of the NBS herbal product supplementation in diets with varying ME and CP levels on egg quality traits in Hy-line W-36 laying hens during 63-65 weeks of age. For the first period (63-65 wk), both diet dilution and the NBS did not show any significant effect on the internal egg quality measurements. The interaction between diet dilution and the NBS herbal product had no significant effect on egg weight, egg specific gravity, percentage of shape index, shell thickness, yolk, albumen, shell, and yolk color index. However, the interaction effects of diet dilution and NBS supplementation showed a significant effect ($P < 0.05$) on the HU. So that, HU in the standard diet group was decreased as the NBS level increased and vice versa in the diluted diet group.

In the second period (66-68 weeks of age), diet dilution had a significant effect ($P < 0.05$) on the shell thickness. The 5% diluted diet significantly ($P < 0.05$) increased the shell thickness compared to the standard diet (30.12 vs 28.42 mm). Moreover, NBS supplementation and the interaction between diet dilution and NBS herbal product had a significant effect on the albumen percentage as shown in Table 4. However, diet dilution, NBS, and their interaction did not have significant effect on other internal egg quality measurements in this period.

In the third period of the study (69-71 weeks), diet dilution showed a significant effect ($P < 0.05$) on the shape index percentage; whereas the shape index in the group fed with a 5% diluted diet was significantly higher than standard diet fed birds (77.97 vs 77.09 %, respectively). The herbal product had no significant effect on the internal egg quality parameters. Shape index percentage was significantly affected ($P < 0.05$) by the interaction between diet dilution and NBS as shown in Table 5. However, no other effect was observed on the other parameters in this period.

During The last period (72 to 74 wks), NBS supplementation, diet dilution, and the interaction between diet dilution and the NBS did not have any significant effect on the internal egg quality parameters (Table 6).

**Fatty acids profile**

Saturated fatty acids (except C17:0) were not significantly affected ($P > 0.05$) by diet dilution. Also, no significant difference ($P > 0.05$) was observed among the five levels of the herbal product regarding the SFA C16:0, C17:0, C18:0, and C23: 0 except that myristic acid (C14:0) was significantly affected ($P < 0.05$) by the NBS levels where it was linearly decreased with increasing levels of NBS. C16:0 and C18:0 were not significantly affected by the interaction between diet dilution and the herbal product. However, the interaction was found to be significant ($P < 0.05$) for C14:0, C17:0, and C23:0 (Table 7).

As shown in Table 8, MUFA (C16:1, C17:1, C18:1n9c, and C20:1) were not significantly ($P > 0.05$) affected by diet dilution. However, it did have a significant effect on C18:1 n9c whereas the 5% diluted diet significantly ($P < 0.05$) decreased elaidic acid (C18:1n9t) compared to the standard diet (1.61 vs 1.28 mg FA/g egg yolk). None of MUFA was affected by the herbal product levels. The interaction between diet dilution and the herbal product was not significant in the case of C16:1 fatty acid; whereas the concentration of palmitoleic acid (C16:1) was linearly decreased with increasing levels of the herbal product. Also, linear and quadratic effect was found to be significant ($P \leq 0.05$) on the C17:1 fatty acid. However, no significant linear and quadratic trends were observed for other fatty acids (Table 8).

Diet dilution significantly decreased PUFA, specifically linoleic acid (C18:2n6c) and docosahexaenoic acid (C22:6n3) fatty acids compared to standard diet; egg yolks of hens fed with 5% diluted diet had significantly ($P < 0.05$) lower linoleic and docosahexaenoic acids compared to the egg yolks of hens fed with the standard diet (132.72 vs 118.23 mg FA/g egg yolk) and (4.79 vs 3.97 mg FA/g egg yolk), respectively. However, C18:3n6, C18:3n3, C20:2, C20:3n6, and C20:4n6 fatty acids were not significantly affected by diet dilution. In addition, 5% diluted diet had significantly ($P < 0.05$) lower PUFA, n3, and n6 FA compared to the standard diet (133.44 vs 149.97 mg FA/g egg yolk), (6.04 vs 7.20 mg FA/g egg yolk), and (129.02 vs 144.58 mg FA/g egg yolk), respectively; however, the n6/n3 ratio was significantly higher with the 5% diluted diet and low with the standard one (21.36 vs 20.08 mg FA/g egg yolk). The herbal product did not have any significant effect on the egg yolk PUFA concentrations, while the interaction between diet dilution and the NBS levels had a significant effect on arachidonic acid (C20:4n6) and docosahexaenoic acid (C22:6n3) fatty acids; however, no other fatty acids were affected by the interactions (Table 9).

**Table 3.** Effects of herbal product (NBS) in diets with varying energy and protein levels on egg quality traits in Hy-Line W-36 laying hens during 63 to 65 weeks of age[1]

| Treatments | | Egg weight (g) | ESG[1] (g. cm$^{-3}$) | Shape index (%) | Haugh unit | Yolk color index | Shell thickness (mm) | Yolk (%) | Albumen (%) | Shell weight (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet dilution | | | | | | | | | | |
| Standard | | 66.77 | 1.08 | 77.16 | 91.95 | 103.6 | 30.47 | 26.01 | 64.99 | 9.00 |
| 5% diluted | | 66.04 | 1.08 | 77.39 | 90.51 | 103.8 | 29.43 | 25.42 | 65.74 | 8.85 |
| *SEM* | | 0.06 | 0.00 | 0.04 | 0.08 | 0.02 | 0.07 | 0.05 | 0.05 | 0.03 |
| NBS (g/kg diet) | | | | | | | | | | |
| 0 | | 67.44 | 1.08 | 76.88 | 92.06 | 103.7 | 29.97 | 25.09 | 65.78 | 9.13 |
| 0.5 | | 65.48 | 1.08 | 77.90 | 90.19 | 103.7 | 30.28 | 25.67 | 65.02 | 9.31 |
| 1 | | 66.77 | 1.08 | 76.87 | 90.33 | 103.8 | 29.89 | 24.99 | 66.13 | 8.87 |
| 1.5 | | 66.18 | 1.08 | 77.58 | 89.72 | 103.7 | 29.25 | 26.40 | 64.94 | 8.66 |
| 2 | | 66.15 | 1.08 | 77.12 | 93.84 | 103.7 | 30.36 | 26.43 | 64.93 | 8.64 |
| SEM | | 0.15 | 0.01 | 0.09 | 0.20 | 0.06 | 0.17 | 0.12 | 0.12 | 0.07 |
| Dilution | NBS (g/kg diet) | | | | | | | | | |
| Standard | 0 | 68.05 | 1.08 | 76.59 | 95.83$^{a}$ | 103.7 | 30.00 | 25.55 | 65.52 | 8.93 |
| | 0.5 | 65.87 | 1.08 | 78.19 | 90.01$^{ab}$ | 103.3 | 29.94 | 26.33 | 64.44 | 9.23 |
| | 1 | 66.88 | 1.08 | 76.32 | 89.38$^{ab}$ | 103.7 | 30.56 | 24.27 | 66.81 | 8.92 |
| | 1.5 | 66.94 | 1.08 | 77.61 | 91.55$^{ab}$ | 104.0 | 29.56 | 27.09 | 64.11 | 8.81 |
| | 2 | 66.11 | 1.08 | 77.07 | 92.99$^{ab}$ | 103.5 | 32.28 | 26.84 | 64.07 | 9.10 |
| 5 % diluted | 0 | 66.83 | 1.08 | 77.16 | 88.30$^{b}$ | 103.7 | 29.95 | 24.63 | 66.04 | 9.33 |
| | 0.5 | 65.10 | 1.08 | 77.61 | 90.37$^{ab}$ | 104.0 | 30.61 | 25.01 | 65.61 | 9.39 |
| | 1 | 66.66 | 1.08 | 77.43 | 91.29$^{ab}$ | 104.0 | 29.22 | 25.72 | 65.46 | 8.83 |
| | 1.5 | 65.42 | 1.08 | 77.55 | 87.89$^{b}$ | 103.5 | 28.95 | 25.71 | 65.78 | 8.52 |
| | 2 | 66.19 | 1.08 | 77.18 | 94.69$^{ab}$ | 103.8 | 28.44 | 26.02 | 65.80 | 8.18 |
| *SEM* | | 0.29 | 0.01 | 0.18 | 0.39 | 0.11 | 0.34 | 0.23 | 0.23 | 0.14 |
| *P*- value | | | | | | | | | | |
| Dilution | | 0.358 | 0.865 | 0.461 | 0.317 | 0.178 | 0.338 | 0.231 | 0.136 | 0.426 |
| NBS | | 0.592 | 0.363 | 0.164 | 0.346 | 0.878 | 0.969 | 0.199 | 0.403 | 0.118 |
| NBS × dilution | | 0.965 | 0.504 | 0.504 | 0.046 | 0.182 | 0.719 | 0.355 | 0.270 | 0.235 |
| Linear | | 0.460 | 0.982 | 0.355 | 0.093 | 0.428 | 0.749 | 0.929 | 0.858 | 0.799 |
| Quadratic | | 0.537 | 0.755 | 0.362 | 0.068 | 0.440 | 0.755 | 0.637 | 0.707 | 0.828 |

[a–b]Values in the same column with different letters are significantly different ($P \leq 0.05$). [1]Each mean represents six observations. *SEM*: Standard error of the mean. [1] ESG: Egg specific gravity.

**Table 4.** Effects of herbal product (NBS) in diets with varying energy and protein levels on the interior egg quality traits in Hy-Line W-36 laying hens from 66 to 68 weeks of age[1]

| Treatments | | Egg weight (g) | ESG (g. cm$^{-3}$) | Shape index (%) | Haugh unit | Yolk color index | Shell thickness (mm) | Yolk (%) | Albumen (%) | Shell weight (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet dilution | | | | | | | | | | |
| Standard | | 65.93 | 1.08 | 77.41 | 90.02 | 103.3 | 28.42$^{b}$ | 25.95 | 65.01 | 9.04 |
| 5% diluted | | 66.05 | 1.08 | 77.72 | 92.65 | 103.4 | 30.12$^{a}$ | 25.43 | 65.37 | 9.20 |
| *SEM* | | 0.06 | 0.00 | 0.05 | 0.08 | 0.02 | 0.06 | 0.04 | 0.04 | 0.03 |
| NBS (g/kg diet) | | | | | | | | | | |
| 0 | | 65.02 | 1.08 | 77.86 | 90.96 | 103.6 | 29.94 | 25.29 | 65.42 | 9.30 |
| 0.5 | | 65.62 | 1.08 | 77.88 | 91.87 | 103.4 | 28.86 | 25.00 | 66.18 | 8.82 |
| 1 | | 65.81 | 1.08 | 77.59 | 92.55 | 103.3 | 28.61 | 25.90 | 65.24 | 8.86 |
| 1.5 | | 66.69 | 1.08 | 77.31 | 90.46 | 103.6 | 30.17 | 26.41 | 64.21 | 9.38 |
| 2 | | 66.82 | 1.08 | 77.20 | 90.84 | 103.3 | 28.78 | 25.85 | 64.90 | 9.25 |
| *SEM* | | 0.15 | 0.00 | 0.13 | 0.20 | 0.06 | 0.14 | 0.10 | 0.11 | 0.07 |
| Dilution | NBS (g/kg diet) | | | | | | | | | |
| Standard | 0 | 63.95 | 1.08 | 77.28 | 89.16 | 103.3 | 28.55 | 26.22 | 64.54$^{abc}$ | 9.24 |
| | 0.5 | 66.13 | 1.08 | 78.04 | 89.11 | 103.3 | 27.83 | 25.51 | 65.86$^{ab}$ | 8.63 |
| | 1 | 66.35 | 1.08 | 77.81 | 91.22 | 103.2 | 28.39 | 25.75 | 65.30$^{abc}$ | 8.96 |
| | 1.5 | 67.62 | 1.08 | 76.74 | 90.34 | 103.5 | 29.61 | 25.86 | 65.16$^{abc}$ | 8.98 |
| | 2 | 65.61 | 1.08 | 77.20 | 90.27 | 103.3 | 27.72 | 26.42 | 64.18$^{bc}$ | 9.40 |
| 5 % diluted | 0 | 66.09 | 1.08 | 78.44 | 92.77 | 103.2 | 31.33 | 24.35 | 66.29$^{ab}$ | 9.36 |
| | 0.5 | 65.10 | 1.08 | 77.72 | 94.63 | 103.5 | 29.89 | 24.49 | 66.50$^{a}$ | 9.01 |
| | 1 | 65.28 | 1.08 | 77.37 | 93.88 | 103.5 | 28.84 | 26.06 | 65.17$^{abc}$ | 8.77 |
| | 1.5 | 65.76 | 1.08 | 77.88 | 90.57 | 103.7 | 30.72 | 26.96 | 63.26$^{c}$ | 9.79 |
| | 2 | 68.02 | 1.08 | 77.20 | 91.41 | 103.3 | 29.83 | 25.29 | 65.62$^{ab}$ | 9.09 |
| *SEM* | | 0.29 | 0.01 | 0.25 | 0.41 | 0.12 | 0.28 | 0.21 | 0.21 | 0.14 |
| *P*- value | | | | | | | | | | |
| Dilution | | 0.880 | 0.455 | 0.600 | 0.093 | 0.448 | 0.029 | 0.201 | 0.389 | 0.373 |
| NBS | | 0.574 | 0.098 | 0.921 | 0.911 | 0.565 | 0.570 | 0.213 | 0.060 | 0.175 |
| NBS × dilution | | 0.291 | 0.463 | 0.835 | 0.833 | 0.792 | 0.878 | 0.150 | 0.050 | 0.302 |
| Linear | | 0.685 | 0.617 | 0.936 | 0.596 | 0.291 | 0.664 | 0.395 | 0.805 | 0.186 |
| Quadratic | | 0.936 | 0.720 | 0.914 | 0.548 | 0.351 | 0.713 | 0.595 | 0.904 | 0.138 |

[abc]Values in the same column with different letters are significantly different ($P \leq 0.05$). [1]Each mean represents six observations. *SEM*: Standard error of the mean.

[1] ESG: Egg specific gravity.

**Table 5.** Effects of herbal product (NBS) in diets with varying energy and protein levels on the interior egg quality traits in laying hens (Hy-Line W-36) from 69 to 71 weeks of age[1]

| Treatments | | Egg weight (g) | ESG (g. $cm^{-3}$) | Shape index (%) | Haugh unit | Yolk color index | Shell thickness (mm) | Yolk (%) | Albumen (%) | Shell weight (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet dilution | | | | | | | | | | |
| Standard | | 65.64 | 1.08 | 77.09[b] | 90.04 | 103.6 | 28.09 | 26.02 | 65.10 | 8.88 |
| 5% diluted | | 65.45 | 1.08 | 77.97[a] | 92.13 | 103.7 | 29.10 | 24.93 | 65.13 | 9.11 |
| *SEM* | | 0.06 | 0.00 | 0.04 | 0.08 | 0.02 | 0.06 | 0.05 | 0.07 | 0.03 |
| NBS (g/kg diet) | | | | | | | | | | |
| 0 | | 64.91 | 1.08 | 77.11 | 91.66 | 103.5 | 29.05 | 25.31 | 65.24 | 9.45 |
| 0.5 | | 64.91 | 1.08 | 77.19 | 91.72 | 103.8 | 27.53 | 25.54 | 66.02 | 8.43 |
| 1 | | 65.86 | 1.08 | 77.75 | 89.22 | 103.6 | 28.86 | 25.63 | 65.31 | 9.06 |
| 1.5 | | 65.92 | 1.08 | 78.33 | 91.64 | 103.5 | 28.86 | 25.77 | 65.21 | 9.02 |
| 2 | | 66.13 | 1.08 | 77.27 | 91.18 | 103.8 | 28.67 | 25.12 | 63.80 | 9.01 |
| *SEM* | | 0.14 | 0.00 | 0.11 | 0.20 | 0.06 | 0.14 | 0.13 | 0.16 | 0.08 |
| Dilution | NBS (g/kg diet) | | | | | | | | | |
| Standard | 0 | 65.32 | 1.08 | 76.65[b] | 89.94 | 103.5 | 30.22 | 26.02 | 64.15 | 9.82 |
| | 0.5 | 65.18 | 1.08 | 77.39[b] | 89.70 | 103.8 | 27.39 | 26.61 | 64.89 | 8.50 |
| | 1 | 65.73 | 1.08 | 77.76[b] | 87.43 | 103.3 | 27.33 | 25.91 | 65.47 | 8.62 |
| | 1.5 | 66.75 | 1.08 | 76.60[b] | 94.84 | 103.5 | 27.72 | 25.78 | 65.51 | 8.72 |
| | 2 | 65.21 | 1.08 | 77.05[b] | 88.31 | 103.8 | 27.78 | 25.78 | 65.46 | 8.76 |
| 5 % diluted | 0 | 64.50 | 1.08 | 77.58[b] | 93.38 | 103.5 | 27.89 | 24.60 | 66.32 | 9.09 |
| | 0.5 | 64.64 | 1.08 | 76.98[b] | 93.74 | 103.8 | 27.67 | 24.48 | 67.16 | 8.36 |
| | 1 | 66.00 | 1.08 | 77.74[b] | 91.02 | 103.8 | 30.39 | 25.35 | 65.14 | 9.51 |
| | 1.5 | 65.08 | 1.08 | 80.07[a] | 88.45 | 103.5 | 30.00 | 25.75 | 64.92 | 9.33 |
| | 2 | 67.05 | 1.08 | 77.49[b] | 94.06 | 103.7 | 29.56 | 24.46 | 62.13 | 9.26 |
| *SEM* | | 0.28 | 0.01 | 0.21 | 0.40 | 0.12 | 0.28 | 0.25 | 0.33 | 0.15 |
| *P*- value | | | | | | | | | | |
| Dilution | | 0.801 | 0.746 | 0.036 | 0.177 | 0.601 | 0.156 | 0.070 | 0.969 | 0.306 |
| NBS | | 0.720 | 0.740 | 0.303 | 0.814 | 0.346 | 0.660 | 0.961 | 0.704 | 0.082 |
| NBS × dilution | | 0.621 | 0.658 | 0.036 | 0.107 | 0.532 | 0.138 | 0.822 | 0.363 | 0.146 |
| Linear | | 0.673 | 0.597 | 0.180 | 0.541 | 0.943 | 0.702 | 0.503 | 0.478 | 0.163 |
| Quadratic | | 0.874 | 0.502 | 0.235 | 0.558 | 1.000 | 0.666 | 0.485 | 0.350 | 0.175 |

[a-b]Values in the same column with different letters are significantly different ($P \leq 0.05$). [1]Each mean represents six observations. *SEM*: Standard error of the mean.
[1] ESG: Egg specific gravity.

**Table 6.** Effects of herbal product (NBS) in diets with varying energy and protein levels on the interior egg quality traits in laying hens (Hy-Line W-36) from 72 to 74 weeks of age[1]

| Treatments | | Egg weight (g) | ESG (g. cm$^{-3}$) | Shape index (%) | Haugh unit | Yolk color index | Shell thickness (mm) | Yolk (%) | Albumen (%) | Shell weight (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Diet dilution | | | | | | | | | | |
| Standard | | 68.32 | 1.08 | 78.72 | 89.37 | 104.2 | 29.04 | 25.49 | 65.53 | 8.98[a] |
| 5% diluted | | 67.72 | 1.08 | 78.23 | 90.90 | 104.1 | 27.54 | 25.59 | 65.79 | 8.62[b] |
| *SEM* | | 0.06 | 0.00 | 0.05 | 0.09 | 0.02 | 0.06 | 0.05 | 0.05 | 0.03 |
| NBS (g/kg diet) | | | | | | | | | | |
| 0 | | 67.80 | 1.08 | 77.64 | 90.31 | 104.1 | 28.36 | 25.92 | 65.41 | 8.68 |
| 0.5 | | 67.75 | 1.08 | 78.51 | 92.57 | 103.9 | 28.78 | 24.94 | 66.11 | 8.95 |
| 1 | | 68.77 | 1.08 | 79.42 | 88.36 | 104.3 | 27.17 | 25.19 | 66.23 | 8.58 |
| 1.5 | | 67.57 | 1.08 | 78.33 | 89.54 | 104.5 | 29.06 | 25.37 | 65.49 | 9.14 |
| 2 | | 68.22 | 1.08 | 78.46 | 89.90 | 103.9 | 28.11 | 26.28 | 65.07 | 8.65 |
| *SEM* | | 0.14 | 0.00 | 0.12 | 0.21 | 0.06 | 0.15 | 0.12 | 0.13 | 0.07 |
| Dilution | NBS (g/kg diet) | | | | | | | | | |
| Standard | 0 | 67.90 | 1.08 | 77.46 | 89.96 | 104.5 | 29.22 | 26.49 | 64.61 | 8.90 |
| | 0.5 | 67.73 | 1.08 | 79.85 | 91.18 | 103.8 | 30.00 | 24.71 | 66.10 | 9.19 |
| | 1 | 68.59 | 1.08 | 79.49 | 85.57 | 104.3 | 27.72 | 24.76 | 66.49 | 8.76 |
| | 1.5 | 68.48 | 1.08 | 77.90 | 91.97 | 104.5 | 29.22 | 25.32 | 65.60 | 9.09 |
| | 2 | 68.91 | 1.08 | 78.89 | 88.17 | 103.8 | 29.06 | 26.17 | 64.88 | 8.96 |
| 5 % diluted | 0 | 67.70 | 1.08 | 77.82 | 90.66 | 103.7 | 27.50 | 25.35 | 66.21 | 8.45 |
| | 0.5 | 67.78 | 1.08 | 77.17 | 93.96 | 104.0 | 27.56 | 25.17 | 66.12 | 8.71 |
| | 1 | 68.94 | 1.08 | 79.35 | 91.15 | 104.2 | 26.61 | 25.63 | 65.96 | 8.40 |
| | 1.5 | 66.67 | 1.08 | 78.76 | 87.10 | 104.5 | 28.89 | 25.42 | 65.39 | 9.20 |
| | 2 | 67.54 | 1.08 | 78.04 | 91.64 | 104.0 | 27.17 | 26.39 | 65.26 | 8.35 |
| *SEM* | | 0.28 | 0.01 | 0.25 | 0.43 | 0.12 | 0.31 | 0.25 | 0.26 | 0.14 |
| *P*- value | | | | | | | | | | |
| Dilution | | 0.425 | 0.849 | 0.400 | 0.371 | 0.328 | 0.095 | 0.860 | 0.686 | 0.050 |
| NBS | | 0.854 | 0.978 | 0.438 | 0.621 | 0.055 | 0.701 | 0.582 | 0.739 | 0.241 |
| NBS × dilution | | 0.862 | 0.970 | 0.355 | 0.365 | 0.127 | 0.954 | 0.848 | 0.845 | 0.749 |
| Linear | | 0.753 | 0.723 | 0.127 | 0.732 | 0.122 | 0.824 | 0.148 | 0.275 | 0.423 |
| Quadratic | | 0.787 | 0.735 | 0.156 | 0.823 | 0.138 | 0.832 | 0.113 | 0.218 | 0.440 |

[a-b]Values in the same column with different letters are significantly different ($P \leq 0.05$). [1]Each mean represents six observations. *SEM*: Standard error of the mean.
[1] ESG: Egg specific gravity.

**Table 7.** Effects of herbal product (NBS) in diets with varying energy and protein levels on SFA[1] content of egg yolk (mg FA/g egg yolk) in laying hens (Hy-Line W-36) from 63 to 74 weeks of age[2]

| Treatments | | C14:0 | C16:0 | C17:0 | C18:0 | C23:0 | SFA |
|---|---|---|---|---|---|---|---|
| Diet dilution | | | | | | | |
| Standard | | 3.81 | 262.4 | 1.78$^{a}$ | 91.23 | 9.22 | 368.4 |
| 5% diluted | | 3.90 | 255.3 | 1.50$^{b}$ | 81.23 | 8.44 | 350.3 |
| *SEM* | | 0.04 | 0.27 | 0.02 | 0.16 | 0.05 | 0.32 |
| NBS (g/kg diet) | | | | | | | |
| 0 | | 5.11$^{a}$ | 266.0 | 2.01 | 92.99 | 8.91 | 375.0 |
| 0.5 | | 4.12$^{ab}$ | 272.1 | 1.50 | 89.48 | 9.18 | 376.4 |
| 1 | | 3.54$^{b}$ | 269.8 | 1.60 | 86.12 | 8.86 | 369.9 |
| 1.5 | | 2.99$^{b}$ | 238.9 | 1.48 | 79.71 | 8.24 | 331.3 |
| 2 | | 3.46$^{b}$ | 247.3 | 1.64 | 82.85 | 8.97 | 344.2 |
| *SEM* | | 0.10 | 0.69 | 0.06 | 0.39 | 0.12 | 0.80 |
| Dilution | NBS (g/kg diet) | | | | | | |
| Standard | 0 | 4.90$^{ab}$ | 255.5 | 2.00$^{a}$ | 98.79 | 9.07$^{ab}$ | 370.3 |
| | 0.5 | 4.65$^{ab}$ | 276.3 | 1.78$^{abc}$ | 95.50 | 9.79$^{ab}$ | 388.0 |
| | 1 | 3.58$^{ab}$ | 304.3 | 1.95$^{ab}$ | 98.64 | 10.16a | 418.6 |
| | 1.5 | 3.14$^{b}$ | 258.2 | 1.79$^{abc}$ | 88.45 | 9.16$^{ab}$ | 360.8 |
| | 2 | 2.78$^{b}$ | 217.6 | 1.40$^{abc}$ | 74.78 | 7.91$^{ab}$ | 304.5 |
| 5 % diluted | 0 | 5.32$^{a}$ | 276.5 | 2.02$^{a}$ | 87.20 | 8.74$^{ab}$ | 379.8 |
| | 0.5 | 3.59$^{ab}$ | 268.0 | 1.23$^{bc}$ | 83.46 | 8.56$^{ab}$ | 364.8 |
| | 1 | 3.50$^{ab}$ | 235.3 | 1.25$^{bc}$ | 73.60 | 7.57$^{ab}$ | 321.2 |
| | 1.5 | 2.85$^{b}$ | 219.5 | 1.16$^{c}$ | 70.98 | 7.31$^{b}$ | 301.8 |
| | 2 | 4.28$^{ab}$ | 277.0 | 1.93$^{ab}$ | 90.92 | 10.02$^{ab}$ | 384.1 |
| *SEM* | | 0.21 | 1.37 | 0.12 | 0.79 | 0.24 | 1.59 |
| *P*- value | | | | | | | |
| Dilution | | 0.834 | 0.685 | 0.050 | 0.090 | 0.140 | 0.442 |
| NBS | | 0.019 | 0.683 | 0.141 | 0.624 | 0.828 | 0.663 |
| NBS × dilution | | 0.389 | 0.179 | 0.044 | 0.231 | 0.057 | 0.182 |
| Linear | | 0.002 | 0.259 | 0.046 | 0.140 | 0.410 | 0.881 |
| Quadratic | | 0.009 | 0.349 | 0.074 | 0.179 | 0.362 | 0.922 |

[abc]Values in the same column with different letters are significantly different ($P \leq 0.05$).

[1] SFA: Saturated fatty acids (C14:0 =Myristic acid; C16:0 =Palmitic acid; C17:0 =Margaric acid; C18:0 =Stearic acid; C23:0 =Tricosylic acid )

[2]Each mean represents six observations.

*SEM*: Standard error of the mean.

**Table 8.** Effects of herbal product (NBS) in diets with varying energy and protein levels on monounsaturated fatty acids (MUFA)[1] content (mg FA/g egg yolk) in laying hens (Hy-Line W-36) from 63-74 weeks of age[1].

| Treatments | | C16:1 | C17:1 | C18:1n9t | C18:1n9c | C20:1 | MUFA |
|---|---|---|---|---|---|---|---|
| Diet dilution | | | | | | | |
| Standard | | 25.41 | 0.84 | 1.61[a] | 402.6 | 2.19 | 430.4 |
| 5% diluted | | 27.75 | 0.79 | 1.28[b] | 383.3 | 2.15 | 413.1 |
| *SEM* | | 0.08 | 0.02 | 0.02 | 0.30 | 0.02 | 0.31 |
| NBS (g/kg diet) | | | | | | | |
| 0 | | 30.17 | 0.93 | 1.35 | 408.7 | 2.23 | 441.1 |
| 0.5 | | 27.74 | 0.76 | 1.59 | 403.8 | 2.18 | 433.9 |
| 1 | | 26.29 | 0.78 | 1.44 | 394.4 | 2.21 | 422.9 |
| 1.5 | | 24.02 | 0.73 | 1.24 | 369.1 | 2.08 | 395.1 |
| 2 | | 24.66 | 0.88 | 1.61 | 388.7 | 2.15 | 415.8 |
| *SEM* | | 0.21 | 0.06 | 0.06 | 0.74 | 0.06 | 0.77 |
| Dilution | NBS (g/kg diet) | | | | | | |
| Standard | 0 | 29.05[a] | 0.93 | 1.46 | 416.3 | 2.18 | 418.6 |
| | 0.5 | 26.27[ab] | 0.80 | 1.77 | 414.4 | 2.24 | 417.0 |
| | 1 | 27.51[ab] | 0.84 | 1.83 | 432.0 | 2.41 | 434. 7 |
| | 1.5 | 24.23[ab] | 0.81 | 1.43 | 402.4 | 2.27 | 404.7 |
| | 2 | 19.99[b] | 0.80 | 1.53 | 347.8 | 1.86 | 350.1 |
| 5 % diluted | 0 | 31.29[a] | 0.94 | 1.25 | 401.0 | 2.28 | 403.2 |
| | 0.5 | 29.22[a] | 0.70 | 1.42 | 393.3 | 2.12 | 395.4 |
| | 1 | 25.07[ab] | 0.65 | 1.04 | 356.9 | 2.00 | 358.5 |
| | 1.5 | 23.82[ab] | 0.64 | 1.02 | 335.8 | 1.89 | 337.4 |
| | 2 | 29.34[a] | 0.94 | 1.69 | 429.6 | 2.44 | 432.2 |
| *SEM* | | 0.42 | 0.10 | 0.11 | 1.49 | 0.12 | 1.54 |
| *P*- value | | | | | | | |
| Dilution | | 0.158 | 0.539 | 0.011 | 0.353 | 0.742 | 0.431 |
| NBS | | 0.136 | 0.366 | 0.273 | 0.771 | 0.963 | 0.711 |
| NBS × dilution | | 0.217 | 0.682 | 0.225 | 0.135 | 0.123 | 0.127 |
| Linear | | 0.050 | 0.019 | 0.577 | 0.212 | 0.456 | 0.533 |
| Quadratic | | 0.105 | 0.024 | 0.634 | 0.213 | 0.383 | 0.681 |

[a-b]Values in the same column with different letters are significantly different ($P \leq 0.05$).
[1]MUFA: C16:1= Palmitoleic; C17:1=Ginkgolic; C18:1n9t =Elaidic; C18:1n9c =Oleic; C20:1= Eicosenoic.
[2]Each mean represents six observations.
*SEM*: Standard error of the mean.

**Table 9.** Effects of herbal product (NBS) in diets with varying energy and protein levels on PUFA content of egg yolk in laying hens (Hy-Line W-36) from 63-74 weeks of age[1].

| Treatments | | C18:2n6c | C18:3n6 | C18:3n3 | C20:2 | C20:3n6 | C20:4n6 | C22:6n3 | PUFA | n3 | n6 | n6/n3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet dilution | | | | | | | | | | | | |
| Standard | | 132.7[a] | 0.83 | 2.41 | 1.22 | 1.81 | 9.22 | 4.79[a] | 150.0[a] | 7.20[a] | 144.6[a] | 20.08[b] |
| 5% diluted | | 118.2[b] | 0.73 | 2.07 | 1.01 | 1.62 | 8.44 | 3.97[b] | 133.4[b] | 6.04[b] | 129.0[b] | 21.36[a] |
| *SEM* | | 0.17 | 0.03 | 0.03 | 0.02 | 0.03 | 0.05 | 0.04 | 0.18 | 0.04 | 0.18 | 0.05 |
| NBS (g/kg diet) | | | | | | | | | | | | |
| 0 | | 131.2 | 0.90 | 2.42 | 1.16 | 1.76 | 8.91 | 4.27 | 147.7 | 6.69 | 142.8 | 21.35 |
| 0.5 | | 125.8 | 0.66 | 2.15 | 1.09 | 1.61 | 9.18 | 4.65 | 142.4 | 6.80 | 137.3 | 20.19 |
| 1 | | 126.8 | 0.77 | 2.24 | 1.16 | 1.68 | 8.86 | 4.45 | 143.1 | 6.69 | 138.1 | 20.65 |
| 1.5 | | 117.1 | 0.89 | 2.06 | 1.02 | 1.63 | 8.24 | 4.06 | 132.4 | 6.12 | 127.9 | 20.90 |
| 2 | | 126.8 | 0.74 | 2.32 | 1.18 | 1.90 | 8.97 | 4.49 | 143.3 | 6.81 | 138.4 | 20.33 |
| *SEM* | | 0.43 | 0.07 | 0.07 | 0.06 | 0.07 | 0.12 | 0.09 | 0.46 | 0.11 | 0.45 | 0.13 |
| Dilution | NBS (g/kg diet) | | | | | | | | | | | |
| Standard | 0 | 135.1 | 0.94 | 2.59 | 1.35 | 2.00 | 9.07[ab] | 4.54[abc] | 152.2[ab] | 7.13 | 147.1 | 20.63 |
| | 0.5 | 133.4 | 0.72 | 2.29 | 1.21 | 1.73 | 9.79[ab] | 5.17[ab] | 151.4[ab] | 7.46 | 145.6 | 19.52 |
| | 1 | 145.0 | 0.90 | 2.53 | 1.32 | 1.93 | 10.16a | 5.38[a] | 164.0[a] | 7.91 | 158.0 | 19.98 |
| | 1.5 | 133.4 | 0.99 | 2.40 | 1.08 | 1.77 | 9.16[ab] | 4.82[abc] | 150.8[ab] | 7.22 | 145.3 | 20.13 |
| | 2 | 116.7 | 0.60 | 2.22 | 1.11 | 1.58 | 7.91[ab] | 4.07[abc] | 131.5[ab] | 6.29 | 126.8 | 20.15 |
| 5 % diluted | 0 | 127.4 | 0.85 | 2.25 | 0.94 | 1.53 | 8.74[ab] | 4.00[abc] | 143.3[ab] | 6.25 | 138.5 | 22.17 |
| | 0.5 | 118.2 | 0.49 | 2.02 | 0.96 | 1.51 | 8.56[ab] | 4.13[abc] | 133.5[ab] | 6.15 | 128.8 | 20.94 |
| | 1 | 108.6 | 0.56 | 1.96 | 0.96 | 1.40 | 7.57[ab] | 3.53[bc] | 122.36[b] | 5.49 | 118.2 | 21.52 |
| | 1.5 | 100.8 | 0.74 | 1.71 | 0.95 | 1.49 | 7.31[b] | 3.30[c] | 113.9[b] | 5.01 | 110.4 | 22.04 |
| | 2 | 135.9 | 0.85 | 2.41 | 1.25 | 2.21 | 10.02[ab] | 4.91[abc] | 154.2[ab] | 7.32 | 149.1 | 20.36 |
| *SEM* | | 0.86 | 0.13 | 0.14 | 0.11 | 0.15 | 0.24 | 0.18 | 0.92 | 0.22 | 0.90 | 0.26 |
| *P*- value | | | | | | | | | | | | |
| Dilution | | 0.042 | 0.264 | 0.062 | 0.074 | 0.217 | 0.140 | 0.012 | 0.039 | 0.015 | 0.044 | 0.018 |
| NBS | | 0.779 | 0.331 | 0.736 | 0.893 | 0.791 | 0.828 | 0.802 | 0.787 | 0.868 | 0.776 | 0.520 |
| NBS × dilution | | 0.098 | 0.290 | 0.576 | 0.590 | 0.162 | 0.057 | 0.080 | 0.089 | 0.149 | 0.090 | 0.697 |
| Linear | | 0.606 | 0.508 | 0.459 | 0.850 | 0.118 | 0.410 | 0.961 | 0.484 | 0.705 | 0.466 | 0.611 |
| Quadratic | | 0.620 | 0.564 | 0.467 | 0.851 | 0.106 | 0.362 | 0.866 | 0.563 | 0.737 | 0.547 | 0.654 |

[abc]Values in the same column with different letters are significantly different ($P \leq 0.05$).

[1]Each mean represents six observations.

PUFA: Polyunsaturated fatty acids (C18:2n6c= Linoleic; C18:3n6 =γ-Linolenic; C18:3n3 =α-Linolenic; C20:2 =Eicosadienoic; C20:3n6 =Eicosatrienoic; C20:4n6 =Arachidonic; C22:6n3 =Docosahexaenoic ).

*SEM*: Standard error of the mean.

## Discussion

### Egg quality traits

Diet dilution, NBS herbal product, and their interaction during the study period (12 weeks) had no significant effects on the egg quality parameters. The ME and CP levels in this study had no significant effect on the internal and external egg quality parameters during the whole study period except for the shell thickness in the second period (66-68 weeks) and the shape index percentage in the third period (69-71 weeks). This means that feeding hens with 5% diluted diet (5% low ME and CP) in this study had approximately the same results compared to the standard diet. Therefore, having good egg quality parameters including egg weight, egg specific gravity, shape index percentage, HU, yolk color index, shell thickness, and the percentage of yolk, albumen, and shell with reduced ME and dietary CP levels is a good benefit for egg producers. In several studies, the egg quality parameters were not affected by the dietary level of ME, CP, or both in agreement with our findings (Jalal *et al*., 2006; Summers and Leeson 1993). Torki *et al*. (2016) reported that using different levels of CP in the laying hens' diet did not significantly affect egg weight, HU, yolk color, shell weight, shell thickness, and shape index, which is in line with the current results.

In another study, Ahmad and Balander, (2003) found that using different ME and CP levels had no remarkable effect on shell thickness and egg-specific gravity in the laying hens aged 64 weeks. In addition, using different levels of ME and CP did not affect HU, shape index, and egg components (albumen, yolk, and shell) percentage in the laying hens (Scappaticcio *et al*., 2021; Lombardi *et al*., 2020). Also, using different ME and CP levels in the laying hens diet did not show any significant effect on the egg weight, egg-specific gravity, shell thickness, yolk color, and the percentages of shell, albumen, and yolk (Mikulski *et al*., 2020; White *et al*., 2021). Similarly, Hassan *et al*. (2013) showed that varying levels of ME and CP in the diet of laying hens had no significant effect on HU and yolk color which agrees with our results.

Moreover, Zhang *et al*. (2017) found that feeding diets containing 16.5% CP with 2633 kcal ME yielded an egg weight of 63-64 g in hens aged 63-68 weeks; while in the current study, feeding 5% diluted diet with 2698 kcal ME and 14.44% CP resulted in higher egg weight with approximately 66 g with the same aged laying hens. In addition, the shell percentage was approximately close to what they reported. The result of this study agreed with the result found by Samiullah *et al*. (2017) regarding egg weight and shell percentage; however, shell thickness, HU, and yolk color values were found to be higher than that of this study.

Herbal product in this study did not have significant effect on the egg quality parameters mentioned earlier except for the albumen percentage and yolk color index in the second (66-68 weeks) and fourth (72-74 weeks) periods, respectively. These results agree with the results reported by Sosnówka–Czajka and Skomorucha (2021) who found that feeding hens with diets varying in ME and CP levels supplemented by different herbal extracts had no significant effect on the egg weight, HU, yolk, and shell percentages, shell thickness, and shape index percentage. In addition, Tao *et al*. (2021) reported that using different levels of feed additives in the laying hen diet did not show any significant effect on the egg quality parameters including egg weight, shell thickness, HU, yolk color, and yolk percentage. In another study, it was found that shape index, shell, yolk, and albumen percentage were not affected by the feed additives as well (Lombardi *et al*., 2020).

The interaction between diet dilution and NBS herbal product had no significant effect on most of the egg quality parameters; however, HU in the first experimental period (63-65 weeks), albumen percentage in the second period (66-68 weeks), and the shape index percentage in the third period (69-71 weeks) were significantly affected by the interaction effect. HU is a very important factor that has to be taken under consideration when evaluating eggs quality. Because HU is a method of measuring the freshness of the eggs based on the correlation between the albumen height and the egg weight. The internal egg quality standard depends on the HU in the egg industry (Keener *et al*., 2006). Thus, as long as the HU is good and high, the internal egg quality parameters are good as well; which was observed in this study. In general, there are several factors that affect egg quality characteristics (internal or external) such as strain, diet, age, housing system, type of disease, environmental temperature, storage time, time of oviposition, and quality of water (Anderson *et al*., 2004; Ahmadi and Rahimi, 2011).

### Fatty acids profile

The saturated fatty acid concentration in egg yolks was not affected by the ME and CP levels in this study. This means that reducing ME and CP in the diet of laying hens by 5% has no significant effect on the SFA profile. In agreement with our results, Hassan *et al*. (2013) reported that different ME and CP levels in the diet of laying hens have no significant effect on the SFA specifically, myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0).

Myristic acid (C14:0) was the only SFA that was significantly affected by the NBS herbal product levels. The concentration of myristic acid in the egg yolks was decreased as the NBS level increased. However, other SFAs (C16:0, C17:0, C18:0, and C23:0) were not affected by the NBS levels in the

diet of laying hens. Most of the SFA including myristic acid (C14:0), margaric acid (C17:0), tricosylic acid (C23:0) were significantly affected by the interaction between diet dilution and the herbal product. Also, it was found that the concentrations of fatty acids (C14:0, C17:0, and C23:0) were linearly decreased with increasing the level of NBS in both standard and 5% diluted diets. Throughout the literature, there is no available report about the effect of using NBS herbal product on the egg yolk fatty acids composition in laying hens to be compared with the current study.

There is an argument about the strain effect on fatty acids profile in egg yolk. Scheideler *et al*. (1998) approved that laying hen strain affected the content of fatty acids such as oleic, stearic, palmitic, and linoleic acids in eggs when the different strains are fed with flaxseed in diet; however, another report by Ahn *et al*. (1995) showed that strain has no effect on the fatty acids composition in eggs when hens fed an α-linolenic acid enriched diet. We found that SFAs of C14:0, C16:0, and C18:0 were higher than the values reported by Altuntaş and Aydin (2014) when they used Hy-line laying hens aged 80 wks. Another study has been done by Galobart *et al*. (2001) reported that using α-tocopheryl acetate (vitamin E in doses of 0 and 200 mg/kg diet) and rosemary extract (RE; in doses of 0, 500, 1000 mg/kg diet) with an ME and CP levels of 2893 kcal and 15.79%, respectively (which were close to the ME and CP levels in this study) in the diet of Lohmann laying hens gave lower values of C14:0, C16:0, C17:0, and C18:0 fatty acids compared to our study; however, vitamin E and RE at the levels of 200 and 1000 mg/kg, respectively gave approximately close value to C17:0 fatty acid in our study which was 1.8 mg/g yolk.

Diet dilution (5% low ME and CP) had no effect on MUFA except for the elaidic acid (C18:1n9t). It was found that elaidic acid in the egg yolks of the hens fed with the standard diet was significantly ($P < 0.05$) higher than that in the 5% diluted diet group (1.61 vs 1.28 mg/g egg yolk) respectively. Elaidic acid value in the 5% diluted diet was close to the results found by Altuntaş and Aydin (2014). Hassan *et al*. (2013) reported that using different ME and CP levels in the diet of laying hens did not significantly affect the MUFA specifically, palmitoleic acid (C16:1) and oleic acid (C18:1n9c) which is similar to the results of our study. Also, NBS herbal product had no significant effect on the egg yolk MUFA concentration in this study. In addition, the interaction between diet dilution and the herbal product had no significant effect on the MUFA, except for the palmitoleic acid (C16:1). This fatty acid was significantly decreased with increasing NBS levels in both levels of dietary ME and CP (standard and 5% diluted diet). Altuntaş and Aydin (2014) showed that both C18:1n9c and C20:1 fatty acids had slightly higher values compared to the current study. Galobart *et al*. (2001) reported that using vitamin E and RE (as stated above) in the diet of Lohmann laying hens resulted in lower values of C18:1n9c and C20:1 fatty acids compared to our study; however, C16:1 fatty acid of both vitamin E and RE- free diet showed similar result to the current study. In addition, C16:1 fatty acid concentration was decreased with increasing levels of vitamin E and RE which is in agreement with our findings, as the increasing NBS levels lead to a decrease in C16:1 fatty acid value.

Polyunsaturated fatty acids including linoleic acid (C18:2n6c) and docosahexaenoic acid (C22:6n-3) were significantly affected by diet dilution. Hens fed with the standard diet had significantly higher linoleic and docosahexaenoic acids compared to hens fed with 5% diluted diet in the egg yolks. However, this result disagreed with the results obtained by Souza *et al*. (2008) who found that feeding the hens with the standard diet provided eggs with poor α-linolenic acid (18:3n-3) and docosahexaenoic acid. Hassan *et al*. (2013) found that different ME and CP levels in the diet of laying hens did not have any significant effect on the PUFA specifically, α-linolenic acid (C18:3n3) and arachidonic acid (C20:4n6) which is similar to the results of our study.

PUFA in this study was not affected by dietary supplementation of the herbal product. The interaction between diet dilution and the herbal product significantly affected arachidonic (C20:4n6) and docosahexaenoic (C22:6n3) fatty acids concentrations in the egg yolks. Kiani and Gharooni (2016) reported that both arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6 n-3) were the most available long-chain PUFA (LC-PUFA) in conventional eggs. Most of these fatty acids level were higher than that reported by Altuntaş and Aydin (2014) except for C22:6n3 fatty acid which had lower value compared to our study. Galobart *et al*. (2001) reported that using vitamin E and RE in the diet of Lohmann laying hens gave higher values of C18:2n6c, C18:3n6, C18:3n3, and C22:6n3 fatty acids and lower values of C20:2, C20:3n6, C20:4n6 compared to our study. Fatty acid values in the current study were higher than the study done by Wang *et al.*, (2000) with the following fatty acids: C14:0, C16:0, C17:0, C18:0, C16:1, C18:1n9c, C20:1, C18:2n6, C18:3n3, C20:4n6, C22:6n3. Sattler *et al*. (1991) and Lepage and Roy (1986) reported that using the FAME method leads to high fatty acids values because of low lipids concentrations.

## Conclusion

This study showed that ME and CP level in laying hens' diets could be reduced by 5% below the standard at 63 to 74 weeks of age without adverse effects on egg quality parameters, SFA, and MUFA except for the elaidic acid (C18:1n9t); whereas it was

significantly increased in the standard diet compared to the 5% diluted diet. Similarly, PUFA, specifically linoleic acid and docosahexaenoic acid were significantly higher in the eggs produced by feeding the standard diet compared to the 5% diluted diet. Even, the diluted diet increased eggshell thickness and egg shape index compared to the standard diet. The dietary supplementation levels of the herbal product had no significant effect on internal and external egg traits. In addition, the herbal product supplementations did not have any significant effect on the MUFA, PUFA, and SFA except for myristic acid, while the interaction between diet dilution and herbal products significantly affected myristic, margaric, and tricosylic acids of the SFA, palmitoleic acid of the MUFA, and arachidonic and docosahexaenoic fatty acids of the PUFA.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**References**


Ahmad HA & Balander RJ. 2003. Alternative feeding regimen of calcium source and phosphorus level for better eggshell quality in commercial layers. Journal of Applied Poultry Research, 12: 509-514. DOI: 10.1093/japr/12.4.509

Ahmadi F & Rahimi F. 2011. Factors affecting quality and quantity of egg production in laying hens: a review. World applied Sciences Journal, 12: 372-384.

Ahn DU, Sunwoo HH, Wolfe FH & Sim JS. 1995. Effects of dietary α-linolenic acid and strain of hen on the fatty acid composition, storage stability, and flavor characteristics of chicken eggs. Poultry Science, 74: 1540-1547. DOI: 10.3382/ps.0741540

Almeida VR, Dias AN, Bueno CFD, Couto FAP, Rodrigues PA, Nogueira WCL & Faria Filho DE. 2012. Crude protein and metabolizable energy levels for layers reared in hot climates. Brazilian Journal of Poultry Science, 14: 203-208. DOI: 10.1590/S1516-635X2012000300007

Altuntaş A & Aydin R. 2014. Fatty acid composition of egg yolk from chickens fed a diet including marigold (*Tagetes erecta* L.). Journal of Lipids, 2014: DOI: 10.1155/2014/564851

Anderson KE, Tharrington JB, Curtis PA & Jones FT. 2004. Shell characteristics of eggs from historic strains of single comb white leghorn chickens and the relationship of egg shape to shell strength. International Journal of Poultry Science, 3: 17-19.

AOAC. 2019. Official methods of analysis. 21th ed. Arlington (VA): Association of Official Analytical Chemists.

Asmundson VS & Baker GA. 1940. Percentage shell as a function of shell thickness, egg volume, and egg shape. Poultry Science, 19: 227-232. DOI: 10.3382/ps.0190227

Bayat A, Khalkhali A & Mahjoub AR. 2021. Nutrition Bio-Shield Superfood: Healthy and live herbal supplement for immune system enhancement. International Journal of Nutrition and Food Engineering, 15: 6-9.

Beynen AC. 2004. Fatty acid composition of eggs produced by hens fed diets containing groundnut, soya bean or linseed. NJAS-Wageningen Journal of Life Sciences, 52: 3-10. DOI: 10.1016/S1573-5214(04)80026-5

Brandolini A & Hidalgo A. 2012. Wheat germ: not only a by-product. International Journal of Food Sciences and Nutrition, 63: 71-74. DOI: 10.3109/09637486.2011.633898

Djuricic I & Calder PC. 2021. Beneficial Outcomes of Omega-6 and Omega-3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. Nutrients, 13: 2421. DOI: 10.3390/nu13072421

Doeschate RAHM & Raine H. 2006. History and current use of feed additives in the European Union: legislative and practical aspects. Avian Gut Function in Health and Disease, edited by G.C. Perry, CAB International, chapter 1, 3-12.

Galobart J, Barroeta AC, Baucells MD, Codony R & Ternes W. 2001. Effect of dietary supplementation with rosemary extract and α-tocopheryl acetate on lipid oxidation in eggs enriched with ω3-fatty acids. Poultry Science, 80: 460-467. DOI: 10.1093/ps/80.4.460

Ghafoor K, Özcan MM, AL-Juhaımı F, Babıker EE, Sarker ZI, Ahmed IA & Ahmed MA. 2017. Nutritional composition, extraction, and utilization of wheat germ oil: A review. European Journal of Lipid Science and Technology, 119(7):1600160. DOI: 10.1002/ejlt.201600160

Grundy SM & Denke, MA. 1990. Dietary influences on serum lipids and lipoproteins. Journal of Lipid Research, 31: 1149-1172. DOI: 10.1016/S0022-2275(20)42625-2

Hafeez A, Sohail M, Ahmad A, Shah M, Din S, Khan I, Shuiab M, Nasrullah, Shahzada W, Iqbal M & Khan RU. 2020. Selected herbal plants showing enhanced growth performance, ileal digestibility, bone strength and blood metabolites in broilers. Journal of Applied Animal Research. 48: 448-53. DOI: 10.1080/09712119.2020.1818569

Hamilton RMG. 1982. Methods and factors that affect the measurement of egg shell quality, Poultry Science. 61: 2022-2039. DOI: 10.3382/ps.0612022

Hammershøj M & Johansen NF. 2016. The effect of grass and herbs in organic egg production on egg fatty acid composition, egg yolk colour and

sensory properties. Livestock Science, 194: 37-43. DOI: 10.1016/j.livsci.2016.11.001

Hassan MR, Choe HS, Jeong YD, Hwangbo J & Ryu KS. 2013. Effect of dietary energy and protein on the performance, egg quality, bone mineral density, blood properties and yolk fatty acid composition of organic laying hens. Italian Journal of Animal Science, 12: 60-65. DOI: 10.4081/ijas. 2013.e10

Haugh RR. 1937. The Haugh unit for measuring egg quality. United States egg and poultry magazine, 43: 522-555.

Hayes KC, Pronczuk A, Lindsey S & Diersen-Schade D. 1991. Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. The American Journal of Clinical Nutrition, 53: 491-498. DOI: 10.1093/ajcn/53.2.491

Hegsted DM, Mcgandy RB, Myers ML & Stare FJ. 1965. Quantitative effects of dietary fat on serum cholesterol in man. American Journal of Clinical Nutrition, 17: 281-295.

Hopkins PN. 1992. Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. The American Journal of Clinical Nutrition, 55: 1060-1070. DOI: 10.1093/ajcn/55.6.1060

Hy-Line International. 2020. Hy-Line W36 Commercial Management Guide. Hy-Line International, West Des Moines, IA.

Jalal MA, Scheideler SE & Marx D. 2006. Effect of bird cage space and dietary metabolizable energy level on production parameters in laying hens. Poultry Science, 85: 306-311. DOI: 10.1093/ps/85.2.306

Kamran Z, Sarwar M, ahr-un-Nisa, Nadeem MA & Mahmood S. 2010. Effect of low levels of dietary crude protein with constant metabolizable energy on nitrogen excretion, litter composition and blood parameters of broilers. International Journal of Agriculture and Biology, 12: 401–405.

Kamran Z, Sarwar M, Mahr-un-Nisa, Nadeem MA, Mushtaq T, Ahmed T, Babar ME & Mushtaq MM. 2008. Effect of low levels of dietary protein on growth, protein utilisation and body composition of broiler chicks from one to twenty-six days of age. Avian Biology Research, 1: 19-25. DOI: 10.3184/175815508X336639

Keener KM, McAvoy KC, Foegeding JB, Curtis PA, Anderson KE & Osborne JA. 2006. Effect of testing temperature on internal egg quality measurements. Poultry Science, 85: 550-555. DOI: 10.1093/ps/85.3.550

Keys A, Anderson JT & Grande F. 1965. Serum cholesterol response to changes in the diet: II. The effect of cholesterol in the diet. Metabolism, 14: 759-765. DOI: 10.1016/0026-0495(65)90002-8

Khosla P & Hayes KC. 1992. Comparison between the effects of dietary saturated (16:0), monounsaturated (18:1), and polyunsaturated (18: 2) fatty acids on plasma lipoprotein metabolism in cebus and rhesus monkeys fed cholesterol-free diets. The American Journal of Clinical Nutrition, 55: 51-62. DOI: 10.1093/ajcn/55.1.51

Kiani A & Gharooni MH. 2016. Comparative Analysis of Fatty Acid Composition of Yolk Lipids in Indigenous and Conventional Chicken Eggs. Iranian Journal of Applied Animal Science, 6: 401-405.

Lepage G & Roy CC. 1986. Direct transesterification of all classes of lipids in a one-step reaction. Journal of Lipid Research, 27: 114-120. DOI: 10.1016/S0022-2275(20)38861-1

Lombardi P, Addeo NF, Panettieri V, Musco N, Piccolo G, Vassalotti G, Nizza A, Moniello G & Bovera F. 2020. Blood profile and productive performance after partial substitution of maize grain with ancient wheat lines by-products in organic laying hens' diet. Research in Veterinary Science, 133: 232-238. DOI: 10.1016/j.rvsc.2020.09.035

Lopez-Bote CJ, Arias RS, Rey AI, Castano A, Isabel B & Thos J. 1998. Effect of free-range feeding on n− 3 fatty acid and α-tocopherol content and oxidative stability of eggs. Animal Feed Science and Technology, 72: 33-40. DOI: 10.1016/S0377-8401(97)00180-6

Mariamenatu AH & Abdu EM. 2021. Overconsumption of omega-6 polyunsaturated fatty acids (PUFAs) versus deficiency of omega-3 PUFAs in modern-day diets: The disturbing factor for their “balanced antagonistic metabolic functions” in the human body. Journal of Lipids. 17: 2021. DOI: 10.1155/2021/8848161

Markets and Markets. 2019. Feed Additives Market. Report Retrieved from https://www.marketsandmarkets.com/Market-Reports/feed-additives-market-870.html

Mikulski D, Jankowski J, Mikulska M & Demey V. 2020. Effects of dietary probiotic (Pediococcus acidilactici) supplementation on productive performance, egg quality, and body composition in laying hens fed diets varying in energy density. Poultry Science, 99: 2275–2285. DOI: 10.1016/j.psj.2019.11.046

Milinsk MC, Murakami AE, Gomes STM, Matsushita M & De Souza NE. 2003. Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids. Food Chemistry, 83: 287-292. DOI: 10.1016/S0308-8146(03)00094-3

Mueller T & Voigt W. 2011. Fermented wheat germ extract-nutritional supplement or anticancer drug? Nutrition Journal, 10: 1-6. DOI: 10.1186/1475-2891-10-89

Nutrition Bio-Shield, NBS Super Food. 2019. https://nbsorganik.com.tr/en/ or www.nbs-superfood.com

Panda AK, Reddy MR, Rao SR, Raju MV & Praharaj NK. 2000. Growth, carcass characteristics, immunocompetence and response to Escherichia coli of broilers fed diets with various levels of probiotic. Archiv für Geflügelkunde, 64: 152-156.

Prochaska JF, Carey JB & Shafer DJ. 1996. The effect of L-lysine intake on egg component yield and composition in laying hens. Poultry Science, 75: 1268-1277. DOI: 10.3382/ps.0751268

Samiullah S, Omar AS, Roberts J & Chousalkar K. 2017. Effect of production system and flock age on eggshell and egg internal quality measurements. Poultry Science, 96: 246-258. DOI: 10.3382/ps/pew289

SAS (Statistical Analyses System). 2012. SAS/STAT Software, Version 9.4. Cary (NC): SAS Institute Inc.

Sattler W, Puhl H, Hayn M, Kostner GM & Esterbauer H. 1991. Determination of fatty acids in the main lipoprotein classes by capillary gas chromatography: BF3/methanol transesterification of lyophilized samples instead of Folch extraction gives higher yields. Analytical Biochemistry, 198: 184-190. DOI: 10.1016/0003-2697(91)90526-Y

Scappaticcio R, García J, Fondevila G, de Juan AF, Cámara L & Mateos GG. 2021. Influence of the energy and digestible lysine contents of the diet on performance and egg quality traits of brown-egg laying hens from 19 to 59 weeks of age. Poultry Science, 100: 101211. DOI: 10.1016/j.psj.2021.101211

Scheideler SE, Jaroni D & Froning G. 1998. Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. Poultry Science, 77: 192-196. DOI: 10.1093/ps/77.2.192

Sosnówka - Czajka E & Skomorucha I. 2021. Effect of supplementation with dried fruit pomace on the performance, egg quality, white blood cells, and lymphatic organs in laying hens. Poultry Science, 100: 101278. DOI: 10.1016/j.psj.2021.101278

Souza JG, Costa FGP, Queiroga, RCRE, Silva JHV, Schuler ARP & Goulart CC. 2008. Fatty acid profile of eggs of semi-heavy layers fed feeds containing linseed oil. Brazilian Journal of Poultry Science, 10: 37-44.

Sukhija PS & Palmquist DL. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry, 36: 1202-1206. DOI: 10.1021/jf00084a019

Summers JD & Leeson S. 1993. Influence of diets varying in nutrient density on the development and reproductive performance of White Leghorn pullets. Poultry Science, 72: 1500-1509. DOI: 10.3382/ps.0721500

Swann MM, KL FH, Howie JW, Lucas IA, Millar EL, Murdocj JC, Parsons JH & White EJ. 1969. Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine. Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine.

Tao Y, Wang T, Huang C, Lai C, Ling Z, Zhou Y & Yong Q. 2021. Production performance, egg quality, plasma biochemical constituents and lipid metabolites of aged laying hens supplemented with incomplete degradation products of galactomannan. Poultry Science, 100: 101296. DOI: 10.1016/j.psj.2021.101296

Torki M, Nasiroleslami M & Ghasemi HA. 2016. The effects of different protein levels in laying hens under hot summer conditions. Animal Production Science, 57: 927-934. DOI: 10.1071/AN15463

Wang Y, Sunwoo H, Cherian G & Sim JS. 2000. Fatty acid determination in chicken egg yolk: a comparison of different methods. Poultry Science, 79: 1168-1171. DOI: 10.1093/ps/79.8.1168

White D, Adhikari R, Wang J, Chen C, Lee JH & Kim WK. 2021. Effects of dietary protein, energy and b-mannanase on laying performance, egg quality, and ileal amino acid digestibility in laying hens. Poultry Science, 100:101312. DOI: 10.1016/j.psj.2021.101312

Yakhkeshi S, Rahimi S & Gharib Naseri K. 2011. The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. Journal of Medicinal Plants, 10: 80-95.

Zhang YN, Zhang HJ, Wang J, Yue HY, Qi XL, Wu SG & Qi GH. 2017. Effect of dietary supplementation of organic or inorganic zinc on carbonic anhydrase activity in eggshell formation and quality of aged laying hens. Poultry Science, 96: 2176-2183. DOI: 10.3382/ps/pew490