



Physical-Chemical Characteristics of Designer and Conventional Eggs

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ABSTRACT

The objective of this study was to compare the internal and external quality of conventional and designer eggs. In total, 120 Tetra SL laying hens were randomly allotted to two treatments (K and P groups), with 12 replicates of five hens each. The control group (K) was fed a standard layer diet, producing conventional eggs, and the P group was fed a modified diet supplemented with 5% of an oil mixture, 0.5 mg organic selenium/kg, 200 mg lutein/kg and 200 mg vitamin E/kg, producing designer eggs. Treatments did not influence ($p>0.05$) egg weight or egg component weights. Designer eggs presented higher albumen proportion and lower eggshell proportion than conventional eggs ($p<0.01$). Relative to egg quality parameters, designer eggs presented higher HU values (83.99 vs. 80.81; $p=0.042$), more intense yolk color (14.38 vs. 8.70; $p<0.01$), and lower albumen pH (8.39 vs. 8.67; $p=0.007$). Feeding treatments and duration of egg storage did not significantly influence egg yolk lipid oxidation ($p>0.05$).

INTRODUCTION

Eggs is an inexpensive food item and frequently used in human nutrition. They are rich in high-quality proteins, as well as in vitamins and trace minerals (Sparks, 2006). Internal and external egg quality is influenced by various factors, such as production system (Ozbey & Esen, 2007; Yenice *et al.*, 2016), size and type of batteries (Leenestra *et al.*, 2014; Meng *et al.*, 2014), feeding management and feed composition (Imran *et al.*, 2015). By using modified feeds, designer eggs containing one or more functional ingredients, can be produced (Surai & Sparks, 2001; Shahriar *et al.*, 2008; Singh *et al.*, 2012).

The aim of this study was to verify the hypothesis that the internal and external quality of designer eggs, enriched with omega-3 fatty acids, selenium, vitamin E and lutein, was similar to that of conventional eggs. In order to verify the hypothesis, the physical-chemical properties of the egg yolk, albumen and eggshell were evaluated.

MATERIALS AND METHODS

Experimental design

In this study, 120 Tetra SL hybrid laying hens, with 31 weeks of age at the beginning of the experiment were evaluated for five weeks. Birds were randomly distributed in two treatment groups: control (K - conventional eggs) and experimental (P - designer eggs) groups, allotted to six cages (replicates) with five hens each, totaling 60 laying hens per group.

The diets were specially formulated and manufactured for this experiment. The ingredients and analyzed chemical composition of the



diets are shown in the Table 1. A standard-composition diet was fed to the control group, while the diet fed to the experimental group was supplemented with 5% oil mixture (fish oil, linseed oil, rapeseed oil, soybean oil), 0.5 mg/kg organic selenium, 200 mg/kg lutein, and 200 mg/kg vitamin E.

All birds were housed in the same facility, which microclimatic conditions were controlled to match the requirements of laying hens. Cages were equipped with automatic feeders and drinkers.

Eggs were manually collected daily. Egg quality parameters were evaluated on fresh eggs (one day after lay). Eggs were stored in a refrigerator at +4 °C for 28 days after lay to determine lipid oxidation (TBARS values).

Determination of external and internal egg quality

The external and internal quality of 60 eggs of the L class (eggs weighing 63-73 g) was evaluated. The external egg quality parameters analyzed in this study were: egg weight, egg shape index, eggshell strength and thickness, and eggshell weight. The following internal egg quality parameters were evaluated: albumen and yolk weights, yolk color, albumen height, Haugh units (HU), and albumen and yolk pH. Moreover, proportions of main egg components (albumen, yolk, and eggshell) were calculated.

Eggs and their main components were weighed on a digital scale (model PB 1502-S, Mettler Toledo, USA). Eggshell strength was measured using an automatic device (Eggshell Force Gauge Model-II, Robotmation Co., LTD, Japan). Eggshell thickness was measured using an electronic micrometer with a precision of 0.001 mm in the eggshell equator.

Egg shape index was calculated according to the following equation: egg shape index (%) = egg width/egg length*100 (Panda, 1996). Yolk color, HU, and albumen height were determined using an automatic device (Egg Multi-Tester EMT-5200, Robotmation Co., LTD, Japan). Haugh unit (Haugh, 1937) was calculated using the following formula:

$$HU = 100 \log_{10} (H - 1.7 W^{0.37} + 7.57)$$

where H = height of the albumen (mm) and W = weight of egg (g)

Lipid oxidation

The oxidation of egg yolk lipids was determined in fresh eggs and in eggs stored for 28 days in a refrigerator at +4°C, as follows: 4 g of yolk was weighed in a test tube, and then 12 mL of 10% trichloroacetic acid were

Table 1 – Ingredients and analyzed chemical composition of the experimental diets.

Ingredient, %	Standard diet (K)	Modified diet (P)
Corn	40.81	46.22
Soybean meal	18.50	21.66
Toasted soybean	8.30	4.06
Triticale	15.00	-
Sunflower meal	1.65	5.93
Alfalfa	1.00	2.50
Livestock yeast	0.50	1.00
Limestone	9.36	10.31
Monocalcium phosphate	1.56	1.50
Salt for animals	0.32	0.64
Synthetic methionine	0.24	0.16
Vitamin E	-	0.04
Selenium	-	0.05
Lutein	-	0.10
Sunflower oil	2.26	-
Oil mixture ¹	-	5.00
Premix ^{2,3}	0.50	0.50
Total	100.00	100.00
Analyzed chemical composition (g/kg) ⁴		
Moisture	87	82
Crude protein	190	203
Crude fiber	40	52
Ash	139	170
Fat	52	69
Ca	41	50

¹The diet was supplemented with 5% oil (0.75% fish oil, 1% linseed oil, 2.00% rapeseed oil, 1.25% soybean oil)

²Premix diet K, content per kg: vitamin A 200000 UI, vitamin D₃ 500000 UI, vitamin E 10000 mg, vitamin K₃ 600 mg, vitamin B₁ 400 mg, vitamin B₂ 1000 mg, vitamin B₆ 1000 mg, vitamin B₁₂ 3000 µg, vitamin C 4000 mg, vitamin H 12 mg, vitamin B₃ 8000 mg, vitamin B₅ 2400 mg, vitamin B₉ 150 mg, vitamin B₄ 100000 mg, iodine 200 mg, manganese 18000 mg, zinc 14000 mg, cobalt 30 mg, iron 12000 mg, copper 1600 mg, selenium inorganic 50 mg, calcium 238 g, phytase 100000 FYT, canthaxanthin 500 mg, beta-apo-beta-carotenoic acid 300 mg, antioxidant (butylhydroxytoluene) 20000 mg

³Premix diet P, content per kg: vitamin A 200000 UI, vitamin D₃ 500000 UI, vitamin E 20000 mg, vitamin K₃ 400 mg, vitamin B₁ 420 mg, vitamin B₂ 900 mg, vitamin B₆ 540 mg, vitamin B₁₂ 2300 mg, B₉ 170 mg, Pantothenic acid 1400 mg, B₃ 5000 mg, vitamin H 17000 µg, vitamin B₄ 80000 mg, vitamin C 4500 mg, iodine 180 mg, manganese 14000 mg, zinc 12500 mg, iron 6000 mg, copper 1000 mg, selenium organic 50 mg, BHT 3400 mg, propyl gallate 1400 mg, canthaxanthin 600 mg, beta-apo-8'-Carotene-acid-Ethylester 200 mg

⁴Referential methods applied in the chemical analysis of the feed: HRN ISO 6496:200; HRN EN ISO 5983-2:2010; HRN EN ISO 6865:2001, Modified according to instructions of FOSS Fiber Cap manual; HRN ISO 5984:2004; HRN ISO 6492:2001, Modified according to instructions of extraction system ANKOM XT15; RU-5.4.2-11 (internal method)

added. The mixture was homogenized and centrifuged for 10 minutes at 10000 rpm and at 4°C. After centrifugation, 2.5 mL of supernatant was collected and mixed with 1.5 mL of thiobarbituric acid solution at pH 2.5. The test tubes were closed and immersed into water bath for 30 minutes at 90°C. After cooling, 1 mL of distilled water was added, and the mixture was centrifuged for 5 minutes at 6000 rpm and at 4°C. The content of colored product that occurred as a reaction



of products of lipid peroxidation with thiobarbituric acid was measured in a spectrophotometer at 534 nm. The obtained values were compared with standard curve prepared using standard malondialdehyde (MDA) tetrabutylammonium salt (Sigma-Aldrich, Switzerland), and expressed as $\mu\text{g MDA/g}$ of yolk. Lipid oxidation was determined in 20 fresh and 20 stored eggs, totaling 40 eggs.

Yolk fatty acid profile

Fatty acid profile was determined in 6 yolks per experimental group, totaling 12 egg yolks. Yolk samples (1 g) were homogenized, and fat was extracted using the method of Folch *et al.* (1957). All solvents used were ultrapure grade (Sigma-Aldrich, Schnelldorf, Germany). A volume of 100 mg/L butylated hydroxytoluene was added to the extraction solution (chloroform/methanol 2/1 vol/vol) as antioxidant. Fatty acids (FA) were then transmethylated by the base-catalyzed sodium-methoxide method of Christie (1982). Gas liquid chromatography was performed on a Shimadzu 2010 apparatus (Kyoto, Japan), equipped with a SP-2380 (Supelco, USA) type capillary column (30 m x 0.25 mm internal diameter, 0.20 μm film) and flame ionization detector. Characteristic operating conditions were: injector temperature of 270 °C, detector temperature of 300 °C, and helium flow of 28 cm/sec. The following oven temperatures were applied: from 80 to 205 °C at 2.5 °C/min, and 5 min at 205 °C; and from 205 to 250 °C at 10 °C/min and 5 min at 250 °C. In order to identify individual FA in the chromatogram, a FA standard mixture (Supelco 37 Component FAME Mix, CRM 47885) was used. The contents of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), as well as n-6 and n-3 polyunsaturated fatty acids (PUFA) are expressed as a percentage relative to total fatty acid content in the yolk lipids.

Egg selenium content

Egg Se content was determined in 10 yolks and 10 albumens per group, totaling 40 samples (20 yolks and 20 albumens). Laboratory equipment and glassware used for the analysis were immersed in a 10% solution of HNO_3 for 24 hours.

Samples weighing 1 g were analyzed. Each sample was transferred to a Teflon cuvette for destruction, and 8 mL HNO_3 and 4 mL H_2O_2 were added. After 15 minutes, cuvettes were closed and heated in a microwave oven (CEM, model Mars 5). After digestion for 25 min, the content of cuvette was transferred to a 50-mL flask, and the volume of the flask was

completed with distilled water. Then, 20 mL of the sample was removed from the flask and transferred to another 50-mL flask, and 20 mL of 36% HCl were added. The prepared samples were placed in a dryer for one hour at 90°C. After drying, the volume of the flask was completed with distilled water. When the samples reached room temperature, selenium content was read in a spectrometer (Perkin Elmer Optima 2100 DV0), according to Davidowski (1993).

Yolk vitamin E content

Vitamin E content was chemically analyzed in 5 yolks per experimental group, totaling 10 yolks. Firstly, 5 g of yolk were weighed and placed in a 50-mL Erlenmeyer flask, after which 15 mL of methanol were added. The mixture remained for 16 hours at room temperature in the dark, and then decanted from the Erlenmeyer flask into 50-mL glass recipient and allowed to precipitate. The supernatant was removed, and filtrated through a 0.45- μm PTFE microfilter into an 1-mL HPLC vial. The prepared sample was then analyzed by liquid chromatography (HPLC system Agilent Technologies 1200 Series with DAD detector). The specific conditions applied were: column - Agilent, Zorbax C18 (2.1x100), 1.8 μm ; 290 nm wavelength (UV area); mobile phase-100% methanol (HPLC cleanliness); tocopherol retention time of 2.707 min.

Yolk lutein content

Yolk lutein content was determined in 20 yolks (10 yolks per group). The method of Leeson & Caston (2004) was applied as follows: 0.5 g of yolk were weighed, and poured into a test tube, to which 5 mL acetone were added. The solution was strongly mixed in a vortex mixer for 30 s. Samples were left in the dark for 1 h, filtrated through 0.45- μm membrane filter, and then 1 mL of acetone extract was transferred to HPLC vial to evaporate slowly by heating in water bath at 40° C. The remnant was melted by adding 1 mL of hexane/ethyl-acetate solution (65:35, v/v) and mixed on a vortex. Lutein content was determined by passing the prepared samples through a RESTEK Viva C18 column (5 μm , 250x4.6 mm). Mobile phase consisted of a mixture of methanol and tetrahydrofuran (THF) 9:1 (v/v). Flow speed was 1 mL/min, duration of analysis was 20 minutes, and measurement wavelength was 450 nm. The volume of the injected sample was 20 μL . A standard lutein curve was prepared using standard lutein (ChromaDex, USA). All chemicals used were of HPLC quality.



Statistical analysis

The effects of the dietary treatments on the weight of fresh eggs and their main components, proportions of the main egg components, external and internal quality parameters of fresh eggs, yolk fatty acid profile, and selenium, lutein, and vitamin E contents were analyzed according to the following model:

$$y_{ijklm} = m + T_i + e_{ij}$$

where:

y_{ij} = estimated parameter

m = intercept;

T_i = fixed effect of treatment i ($i = T_1; T_2$);

e_{ij} = residual error.

Data were submitted to analysis of variance and means were compared by the multiple comparison test of Scheffe, at 5% significance level, using SAS software (SAS Institute Inc., 2000).

RESULTS AND DISCUSSION

Egg weight, and egg component weights and proportions are shown in Table 2. Egg weight and the weights of the main egg components were not statistically different ($p > 0.05$) between conventional and designer eggs. However, when main egg components proportions were compared, designer eggs presented significantly lower albumen and eggshell proportions ($p < 0.01$) than conventional eggs (60.97% vs. 62.05%, and 12.09% vs. 12.72%, respectively). Yolk proportion of both evaluated groups were similar ($K = 26.31\%$ and $p = 25.86\%$; $p > 0.05$).

Table 2 – Egg weight and weight and proportions of main egg components (\bar{x}).

Parameter	Conventional eggs	Designer eggs	p value
Egg weight, g	67.18	67.46	0.996
Albumen weight, g	41.13	41.86	0.086
Albumen proportion, %	60.97 ^B	62.05 ^A	0.002
Yolk weight, g	17.75	17.44	0.299
Yolk proportion, %	26.31	25.86	0.151
Eggshell weight, g	8.30	8.16	0.409
Eggshell proportion, %	12.72 ^A	12.09 ^B	0.008

^{A, B} $p < 0.01$

Novak & Scheideler (2001) studied the effect of the dietary addition of flax seed on the quality of chicken eggs. The authors state that increasing levels of flaxseed in the feed reduced yolk proportion. These results are considered to be beneficial, as eggs with smaller yolks contain less cholesterol compared with those with larger yolks.

Table 3 shows the external and internal quality parameters of conventional and designer eggs. No statistically significant difference in shape index was detected between conventional and designer eggs ($p > 0.05$). Castaldo and Maurice (1988) state high levels of dietary fat affect calcium absorption and eggshell formation. Layers fed diets with high vegetable oil inclusion presented reduced calcium absorption and eggshell thickness (Brake, 1993). Flaxseed compounds, such as phytic acid, may slow down the absorption of various minerals, including calcium, which is required for calcification of the eggshell (Ahmad, 2010). In addition, phytoestrogens present in flaxseed compete with endogenous estrogens in the metabolism, and may reduce intestinal calcium absorption (Novak and Scheideler, 2001) and vitamin D3 activation, leading to reduced eggshell quality.

Yenice *et al.* (2016) determined a shape index of 77.74%, eggshell proportion of 12.68%, eggshell strength of 2.70 kg/cm² and HU of 70.10 in table eggs produced by caged layers. Compared with these results, the values obtained in the conventional eggs (K group) in the present study for egg shape index and eggshell proportion are slightly lower, while HU and eggshell strength are better.

Conventional eggs presented significantly stronger eggshells (3.101 kg/cm²) compared with designer eggs (2.774 kg/cm²; $p = 0.043$). In agreement with the obtained eggshell thickness values, although not statistically significant ($p = 0.090$), conventional eggs presented thicker eggshell than designer eggs (0.410 mm vs. 0.394 mm, respectively). Yan *et al.* (2014) determined 0.309-0.387 mm eggshell thickness and 3.618-4.850 kg/cm² eggshell strength in chicken eggs, and a medium-strong correlation of 0.319 between these two parameters ($p < 0.01$). The positive impact of eggshell thickness on eggshell strength was also observed in the present study, although the values of these two parameters are noticeably lower than those reported by Yan *et al.* (2014). Obe *et al.* (2014) determined eggshell thickness values of 0.47 mm in free-range layers, and of 0.38 mm in caged layers. Gül *et al.* (2012) reported numerically lower eggshell thickness and strength values in the eggs of laying hens fed diets with rapeseed oil compared with those fed a control diet (6% oil addition - 0.38 mm and 1.82 kg/cm², and control group - 0.39 mm and 2.17 kg/cm²; $p > 0.05$). Our eggshell thickness results are consistent with these findings.

Although albumen height was numerically higher in designer eggs than in conventional eggs, the difference was not statistically significant (7.42 mm



and 6.95 mm, respectively, $p=0.052$). Designer eggs had significantly higher ($p=0.042$) Haugh unit values (83.99) than conventional eggs (80.81). Kralik *et al.* (2017) state that HU values range between 70.36 and 71.33, which are lower compared with the results obtained in the present study (80.81 and 83.99 for conventional and designer eggs, respectively). Oke *et al.* (2014) found that eggs produced in different rearing systems presented 66.86-71.41 HU, with higher values obtained in younger than in older layer flocks. Hanusova *et al.* (2015) point out that the internal quality of eggs depends on their freshness and, therefore, it is the best to consume eggs immediately after lay. Papas *et al.* (2005) show that HU significantly decreases from 88.5 in fresh eggs to 75.62 in eggs stored for 14 days. During egg storage, albumen thickness is gradually reduced due to the degradation of mucin threads, consequently reducing HU values (Kralik *et al.*, 2014).

The results show that the yolk color of designer eggs was significantly more intense than that of conventional eggs (14.38 and 8.70, respectively; $p<0.001$). This large difference may be explained by the different composition of the diets, as the diet of the experimental group was supplemented with lutein, which is a pigment.

Conventional eggs presented significantly higher ($p=0.007$) albumen pH values than designer eggs (8.67 and 8.39, respectively), while similar yolk pH values were determined in both egg groups (5.99 and 5.98; $p=0.734$). Scheideler *et al.* (2010) found that albumen pH of fresh eggs ranges from 7.6 to 8.5, and increased to 9.7 after 2 weeks of storage, while yolk pH increased from 6.0 in fresh eggs to 6.9 in stored eggs. Kralik *et al.* (2015) reported that albumen and yolk pH depend on laying hen age, with higher values observed in younger (8.94 and 7.6, respectively) than in older hens (8.85 and 6.1, respectively). Quality and extended freshness of eggs guarantee their sale (Kralik *et al.*, 2012).

Table 3 – Comparison of external and internal quality parameters of fresh eggs (\bar{x}).

Indicator	Conventional eggs	Designer Eggs	<i>p</i> value
Egg shape index, %	75.65	76.12	0.349
Eggshell strength, kg/cm ²	3.101 ^A	2.774 ^B	0.042
Eggshell thickness, mm	0.410	0.394	0.090
Albumen height, mm	6.95	7.42	0.052
Haugh units	80.81 ^B	83.99 ^A	0.042
Yolk color	8.70 ^B	14.38 ^A	<0.001
Albumen pH	8.67 ^A	8.39 ^B	0.007
Yolk pH	5.99	5.98	0.734

^{A, B} $p<0.05$

This study showed that yolk lipid oxidation, expressed as μg MDA/g yolk, increased during storage. In conventional eggs, the determined values were 0.605 μg MDA/g in fresh eggs, and 0.727 μg MDA/g in stored eggs (Figure 1), while designer eggs tended to present higher MDA values, of 0.680 μg MDA/g of yolk in fresh eggs and 0.764 μg MDA/g in stored eggs. However, these differences were not statistically significant ($p>0.05$).

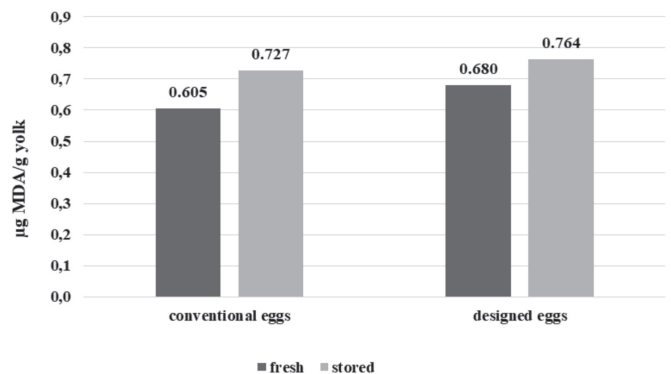


Figure 1 – Lipid oxidation in fresh and stored conventional and designer eggs

The trend of greater lipid oxidation in designer compared to conventional eggs may be due to the higher levels of unsaturated fatty acids, which are more susceptible to oxidation, in the designer eggs (Dunn-Hurrocks *et al.*, 2011). Although designer eggs contained antioxidants, their greater lipid oxidation relative to conventional eggs may be a result of the higher dietary inclusion of oils, which contained greater levels of unsaturated fatty acids susceptible to oxidation. Dunn-Horrocks *et al.* (2011) reported that the inclusion of fish and linseed oils in layer diets resulted in higher TBARS values, consequently negatively influencing egg quality and shelf life. Gül *et al.* (2012), in study of the influence of the dietary addition of different rapeseed oil levels on the quality and omega-3 fatty acid profile of eggs, reported that increasing rapeseed oil levels increased TBARS values. Those authors obtained MDA values ($\mu\text{g/g}$) of 7.53 $\mu\text{g/g}$ and 16.68 $\mu\text{g/g}$ in eggs stored for 21 days laid by hens fed 0 and 16% rapeseed oil, respectively. However, the research of Mohiti-Asli *et al.* (2008) confirmed that the supplementation of vitamin E and selenium in layer diets had favorable influence on lipid oxidation during storage, with eggs enriched with vitamin E and selenium presenting lower TBARS values than conventional eggs.

Table 4 presents n-3 PUFA, selenium, lutein, and vitamin E levels of designer and conventional eggs. The results show that the contents of the mentioned



compounds were significantly higher ($p < 0.001$) in designer eggs compared with conventional eggs. According to Gajčević *et al.* (2009), increasing selenium levels in the feed from 0.2 ppm to 0.4 ppm proportionally increased Se content in the yolk from 573 to 783 ng Se/g, and in albumen from 171 to 345 ng Se/g. Herkel *et al.* (2016) reported that the dietary addition of 3% pumpkin (E1) or linseed (E2) oils increased total n-3 PUFA levels in the eggs from 1.43% (control group) to 4.72% (E1) and 6.80% (E2).

The addition of lutein in layer feed increases its content in the egg yolks, according to Leeson and Caston (2004). Those authors demonstrated that the inclusion of 375 ppm lutein to a diet based on corn and soybean meal increased egg lutein content from 0.3 mg to 1.5 mg/60 g egg.

Table 4 – Levels of n-3 PUFA, selenium, lutein, and vitamin E in conventional and designer eggs (\bar{x}).

*per 100 g of yolk	Conventional eggs	Designer eggs	p value
n-3 PUFA (mg)	366.53 ^B	876.62 ^A	<0.001
Selenium – albumen (μ g)	5.3 ^B	14.3 ^A	<0.001
Selenium - yolk (μ g)	38.7 ^B	66.2 ^A	<0.001
Lutein (mg)	1.24 ^B	10.49 ^A	<0.001
Vitamin E - yolk (mg)	1.25 ^B	1.98 ^A	<0.001

^{A, B} $p < 0.05$

In a study on egg enrichment with vitamin E and selenium, Gjorgovska *et al.* (2011) reported that the addition of vitamin E to layer diets at 100 (E1) or 230 mg/kg of feed (E2) significantly increased yolk vitamin E content compared with the control group (K), where hens were fed 30 mg vitamin E/kg, obtaining values of 1.68 mg/yolk (K), 2.90 mg/yolk (E1) and 5.58 mg/yolk (E2). Pal *et al.* (2002) found that the vitamin E content of egg yolks doubled when dietary vitamin E level was increased from 55 to 110 IU/kg of feed.

CONCLUSIONS

Based on the results of this study, it is concluded that the modification of layer diets for the purpose of producing eggs enriched with various nutrients (omega-3 fatty acids, selenium, vitamin E, and lutein) has no negative impact on the physical-chemical indicators of table eggs.

Moreover, in comparison with conventional eggs, designer eggs presented better egg yolk color, albumen height, and HU values, as well as more favorable albumen and yolk pH values. Although the enriched diet had a negative effect on eggshell strength, the other evaluated eggshell quality parameters of the designer eggs were not significantly different ($p > 0.05$)

from those of the conventional eggs and were not lower than the optimal values for table eggs.

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