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QUALITY PARAMETERS IN EGGS OF LAYER CROSS Hisex Brown AS AFFECTED BY THE OVIPOSITION TIME

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Abstract

Chicken eggs are valuable and chip source of the nutrients in human diets; this fact has propelled the interest toward the availability of this commodity and modification of its chemical composition in desirable directions. The morphology of the eggs is closely correlated with certain parameters of nutritive value and shelf life. The formation of eggs is a long process: the maturation of large yellow follicles in the ovarian hierarchy (until the ovulation) lasts for 7-10 days; the formation of egg in the oviduct (since ovulation to oviposition) takes 22.5-26.1 hours, depending on age and productivity level in parental hen. The quality of eggs is affected by multiple factors acting before the oviposition (breed and cross of chicken, individual physiological peculiarities, live bodyweight, laying rate, oviposition time, age, the regimes of management and nutrition, artificially induced moult, stresses, health status) and after the oviposition (conditions of collection, transportation, storage, washing and sanitary treatments, the effects of these factors being also depend on the initial egg quality formed before the oviposition. The optimization of egg quality requires the thorough knowledge on mechanisms and factors involved. In the study presented it was found out that oviposition can affect different parameters of egg quality though insignificantly with the exception of B2 content. The effects of the oviposition time on egg quality was studied on five treatments of commercial Hisex Brown layers (Hendrix Genetics BV, the Netherlands) since 210 to 450 days of age housed in cage batteries mounted in standard windowless poultry house (7 birds per cage) with constant lighting regime 14L: 10D with the onset of lighting at 5 am, setout at 7 pm. The eggs were collected during 5 periods of a day: 5-8 am (treatments 1), 8-10 am (treatment 2), 10-12 am (treatment 3). 0-2 pm Treatment 4), and 2-4 pm. Average weight of the "earliest" eggs (laid until 8 am) was significantly higher (by 2.1-2.5 g or 3.6-4.4 %, $p < 0.001$) as compared to later periods; absolute yolk weight was higher by 7.2-8.7 %, relative yolk weight by 1.0-1.4 % ($p < 0.001$); the resulting albumen/yolk ratio was lower by 4.2-8.0 % ($p < 0.01$-0.001). The late eggs (laid between 0 and 4 pm) featured better eggshell quality as indicated by increased average eggshell weight (by 3.2-6.7 %), eggshell thickness (by 1.8-5.7 %), and egg density (by 0.18-0.46 %). The shape index varied from 78.9 % (0-2 pm) to 77.7 % (2-4 pm). Chemical composition of the eggs was not significantly affected by oviposition time with the exception of the significantly higher concentrations of vitamin B_2 in yolk at early hours (5-8 am) as compared to later hours ($p < 0.05$-0.001). Earlier eggs (5-12 am) featured higher percentages of cracks and crackles while "evening" eggs featured the absence of extra-heavy eggs (>75 g); other external parameters of egg quality were generally similar in all treatments; however, egg weight tended to increase with hen age. The relative weight of the albumen was similar at 420 and 450 days of age, with the minimal index shape eggs laid in the interval 0-2 pm being found (with the exception of 210 and 450 days of age; $p < 0.05$-0.001). The increases in the concentration of total cholesterol in yolk was found at 270 and 420 days of hens' age (with general trend to increase with age). The age-related increase in the concentration of carotenoids in yolk was found at 360 days of age. As well as the trend to higher concentration of vitamin A in yolk.

Keywords: *Gallus gallus* L., laying hens, laying time, egg weight, yolk weight, shell weight, shell thickness, egg shape index, quality defects, chemical composition, vitamin B_2

Chicken eggs are traditionally considered an important and cheap source of nutrients for humans. The formation of large yellow follicles in the ovarian hierarchy (until the ovulation) lasts for 7-10 days, the formation of an egg in the oviduct (since ovulation to oviposition) takes 22.5-26.1 hours, depending on age and productivity level in the parental hen [1].

The quality of chicken eggs, including external (weight and egg shape; purity, thickness, weight, and strength of shell) and internal (weight of albumen and yolk, height and density of egg albumen, yolk color, Haugh units, chemical composition of albumen and yolk) characteristics [2], is influenced by a complex of various factors. The main part of them (breed, cross, individual characteristics, live weight, egg-laying capacity, egg-laying time, age, feeding and management conditions, forced molting, various stresses, the state of the bird's health) acts during egg formation, other factors (conditions of collection, transportation, storage, washing and processing of eggs) affect the already laid egg [3-7].

Many studies have found that the time of egg-laying affects the quality of eggs and the productivity of laying hens [8—10]. For example, in some works, it is shown [11-13] that the weight of eggs collected at 6^{00} was the largest. According to Harms [14], in a commercial herd of laying hens, the egg weight steadily decreased between 7^{45} and 15^{45}, after which it increased. In the experiments of Patterson [15], the largest weight was typical for eggs laid early in the morning, then (from 5^{00} to 18^{00}) it decreased. The work by Aksoy *et al.* [16] reported that eggs laid at 9^{00} had the largest weight, and after 15^{00} it was the smallest. However, in the experiment of Tůmová *et al.* [17], eggs laid between 10^{00} and 14^{00} were heavier than those laid at 6^{00}.

The time of laying plays an important role in the formation of the quality of the eggshell, since it is known that its weight linearly depends on the time the egg is in the oviduct (shell gland) after ovulation and, consequently, on the thickness and strength of the shell [17].

Most studies have noted that the quality of the shell is higher in eggs laid in the middle of the day [11-13, 18, 19]. According to some authors [13], the higher shell quality of "day" eggs is associated with a greater shell thickness and less elastic deformations. In such eggs, the proportion of the shell in the egg weight is slightly higher — 10.33 and 10.31% in eggs laid at 14^{00} and 10^{00}, respectively, compared with 10.03% in eggs laid at 6^{00} [18]. Similar results were also obtained in the work by Tůmová *et al.* [12]: in studies on three lines of the Dominant cross (Czech Republic), all shell quality parameters (absolute and relative weight, thickness, and strength) were significantly higher in eggs laid in the middle of the day (14^{00}). According to Harms [14], the shell weight of eggs laid before 7^{45} was significantly higher than in eggs laid between 7^{45} and 11^{45}. Then shell weight significantly increased by 12^{45} and remained high until the end of the day, excluding only the interval between 14^{45} and 16^{45}. Other authors [19], on the contrary, observed a tendency to decrease shell weight during the day, and especially strongly in ISA-Brown cross birds (Hendrix Genetics BV, the Netherlands).

When comparing the morphological characteristics of eggs from laying hens of three breeds — Brown Leghorn, Oravka, and Brahma from 20 to 64 weeks of age with deep-litter housing (egg collection at 6^{00}, 10^{00}, and 14^{00} in the beginning, the middle, and the end of the productive period). The maximum weight of eggs was in the Brahma chicken breed at 6^{00}, and the relative weight, strength, and eggshell thickness was significantly higher at 14^{00} [20]. In brown Leghorns, egg-laying time did not have a significant effect on egg weight, relative weight, and the yolk index; the greatest shell thickness was noted at 6^{00}, and the maximum albumen index and Haugh units were at 14^{00}. In Oravka chickens, egg-laying time had no significant effect on egg weight, the relative weight of the albumen, yolk, and shell, the albumen and yolk index, Haugh units. At the same time, the formation of a thicker shell was registered at 14^{00} [20]. In another study [21], when eggs were collected at 10^{00} and 14^{00} in the first case, egg weight (65.25 vs. 63.94 g), absolute and relative albumen weight (40.91 g and 62.65% vs. 39.94 g and 62.42%), absolute yolk weight (16.56 vs. 16.35 g) and shell weight (7.78 vs. 7.64 g), egg shape index (76.72 vs 76.70%) and shell color (12.18 vs. 12.16 points) were higher, and the relative weight of yolk (25.40 vs. 25.60%) and shell (11.93 vs. 11.98%), Haugh units (76.60 vs. 76.70), shell thickness (0.51 vs. 0.54 mm) were lower.

Recent studies [22] have reported a significant effect of egg-laying time on the mineral content in the shell. Thus, the maximum calcium content (352 g/kg) was observed in eggs laid at 7^{30}, whereas at 15^{30}, this indicator was 2.84% lower. The content of phosphorus and magnesium in the shell increased with increasing egg-laying time: for 7^{30}, these indicators were 1.20 and 3.56 g/kg, respectively, for 15^{30} – 1.43 and 3.88 g/kg. Some authors [23] suggest that higher calcium content in the shell of morning eggs is associated with increased calcium deposition in the medullary bone at night.

According to some researchers [21, 24], the time of egg-laying affects the intensity of the shell color of colored eggs. Chickens lay the most colored eggs in the morning, less colored – in the late afternoon. In another report [24], the intensity of shell coloring decreased with the age of chickens, and the sequence number of an egg in the cycle (the number of eggs laid in a row) slightly affected shell color. Here, in the authors' opinion, there is a certain contradiction, since, as is known, the first egg in the cycle is laid by chickens early in the morning, and with an increase in the serial number, the laying is postponed to a later time of day [1]. In addition, according to many authors [25–28], the color of eggshell positively correlates with its thickness and strength, and these indicators, as already noted [12, 19], are highest in eggs collected at noon or the end of the day, but not in the morning.

There are data about the effect of egg-laying time on the cholesterol level in the egg, while some authors note an increase in its content in morning eggs [17], and others, on the contrary, in noon [29].

Thus, the analysis of scientific publications indicates that the time of egg-laying significantly affects the characteristics of egg quality. However, according to some indicators, the data are ambiguous and often contradictory, which is since when studying the effect of egg-laying time on egg quality characteristics, the authors do not always pay attention to the duration of the light or "subjective" day, and the time of the first light switch on in the lighting mode, which has a significant impact on the time of egg-laying by chickens [1]. In addition, it should be noted a certain genetically determined specificity of the effect of egg-laying time on some indicators of egg quality. This necessitates an in-depth study of morphological and biochemical parameters characterizing the quality of eggs, the mechanisms of their formation, and the conditions affecting these processes in different breeds.

In the presented work, it was found that morphological indicators of egg quality in Haisex Brown chickens were associated with the time of egg-laying, however, the observed effect was not the same for different traits. On average, over the entire observation period, eggs laid before 8^{00} had a higher egg weight, absolute and relative yolk weight. Eggs laid before noon had a greater number of shell defects, and those laid from 12^{00} to 16^{00} had a higher shell quality. No significant changes were detected in the chemical composition of the egg depending on the time of laying (except for vitamin B_2 content).

The work objective is to determine the quality indicators of eggs in the Haisex Brown egg cross hens depending on the time of egg-laying, starting from the peak productivity.

Materials and methods. The study was carried out on chickens of the commercial herd of Haisex Brown cross in the conditions of a typical windowless poultry house (vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm ARRTPI RAS, 2020). Among 210-day-old chickens of the same age, kept in KP-112LM cage batteries (Pyatigorskselmash, Russia), 7 birds per cage (cage area – 531 cm^2/bird) with a constant lighting mode of 14 h (light)/10 h (darkness) (turning on the light at 5^{00}, turning off at 19^{00}), a group of 490 laying hens was selected. The feeding and management conditions, which are not the subject of study in this experiment, were maintained following generally accepted and valid recommendations for the experimental period [30]. Eggs were collected at 5^{00}-8^{00}, 8^{00}-10^{00}, 10^{00}-12^{00}, 12^{00}-14^{00}, 14^{00}-16^{00} (groups 1, 2, 3, 4, 5 respectively). Accounting data were recorded in the age periods of 210 days, 270 days, 360 days, 420 days, and 450 days (in each group, 30 eggs were collected 3 days in a row — on days 210-212, days 270-272, days 360-362, days 420-422, and days 450-452).

The weight of eggs (g) was determined by individual weighing on laboratory scales with an accuracy of 0.1 g; the yield of eggs by categories (%) was determined by the results of weighing and inspection of eggs laid by chickens for 3 consecutive days, according to the current GOST ("Interstate standard GOST 31654-2012 "Food chicken eggs. Specifications". Moscow, 2013). Egg shape index (%) was measured using an indexometer during weighing; egg density (D, g/cm^3) — by weighing in distilled water and air in a sample of 30 eggs from each group (10 eggs, 3 days in a row) on the same scales (accuracy up to 0.01 g). D value was calculated as $D = W/(W - W1)$, where W is the weight of an egg in air (g), W1 is the weight of an egg in water (g). The weight of the albumen, yolk, shell, albumen/yolk weight ratio were estimated when separating and individually weighing the components in a sample of 30 eggs from each group (10 eggs, 3 days in a row) (laboratory scales, measuring accuracy up to 0.1 g). The albumen index (AI) was evaluated by the ratio of the height of the outer layer of dense albumen (measured with a micrometer with an accuracy of 0.01 mm) to the average diameter of its spreading. The formula for calculation is $AI = 2h/(d + D) \times 100\%$, where h is the height of dense albumen (mm), d and D are small and large diameters of the poured albumen (mm). Yolk index (YI) was evaluated by the ratio of the height of the yolk poured onto the horizontal surface (measured with a micrometer with an accuracy of 0.01 mm) to the average the diameter of its spreading. YI was calculated as $YI = 2h/(d_1 + d_2) \times 100\%$, where h is the height of the yolk (mm), d_1 and d_2 are small and large diameters of the poured yolk (mm). Haugh units were assessed according to a special table [32] based on the height of the albumen and the weight of the egg. The thickness of the shell (μm) was measured using a micrometer (accuracy up to 0.01 mm) in three parts of the egg (in the middle, at the blunt and point ends) with calculation of the average value for a sample of 30 eggs from each group (10 eggs, 3 days in a row). The intensity of the yolk coloring was individually assessed on the BASF color chart.

Total cholesterol, carotenoids, vitamins A, E and B_2 (μg/g) in the yolk, vitamin B_2 (μg/g) in the albumen, calcium (%) in the shell were determined in the sample of 5 eggs (combined sample) from each group 3 days in a row in the middle of each month during the observation period.

When measuring carotenoids (sum) and vitamins in egg yolk, single sample preparation was used (saponification of samples with 50% potassium hydroxide solution followed by extraction with diethyl ether) [32]. The mass fraction of vitamins A and E was determined by the normal-phase high-performance liquid chromatography (chromatographic system Knauer Advanced Scientific Instruments, Knauer Engineering GmbH Industrieanlagen & Co., Germany) following P 4.1.1672-03 “Guidelines for quality control and safety of biologically active food additives” (Moscow, 2003). Carotenoids were measured colorimetrically (photometer KFK-3-01, ZOMZ, Russia) using potassium bichromate for constructing a calibration graph with OD_{450} measurement (blue light filter). To quantify vitamin A and carotene, the 292 and 450 nm wavelengths were used where the absorption spectra of these substances practically do not overlap [33]. The components were separated on a column Luna 5 μ m Silica(2) 100 A New Column 150×4.6 mm (Phenomenex, USA), eluted with a mixture of hexane:isopropyl alcohol (98:2), the detection of vitamins A and E was performed at 292 and 324 nm, respectively, preparations Retinol Sigma of the category No. R 7632 were used as standards (Sigma-Aldrich, USA) and (+/–)-α-Tocopherol Fluka category No. 95240 (Fluka, Germany).

Water-soluble vitamin B_2 (riboflavin) in egg yolk and egg albumen was determined fluorometrically using a Fluorat-02-3M liquid analyzer (NPFNP Lumex, Russia). Sample preparation consisted of alcohol extraction (96% ethanol from albumen, 55% ethanol from yolk) followed by filtration through a paper mesoporous filter (“yellow stripe”). The fluorescence intensity of the obtained solutions was measured in ultraviolet rays, the concentration of riboflavin was calculated relative to the working standard solution of vitamin B_2.

To determine total cholesterol, the albumen was separated from the yolk. The yolk membrane was pierced, the yolk was poured into a glass container and frozen for 24 hours in the freezer at –20 °C. The frozen yolk was placed in a TFD series freeze dryer (Ilshinbiobase Co., Ltd., Korea) for 48 hours at –77.8°C and at a pressure of 5 mTorr, which made it possible to dry yolk, removing up to 97% of moisture, preserving biologically active substances. The dried yolk was crushed in a laboratory mortar, a subsample (100 mg) was mixed with Ringer's solution in a ratio of 1:100 and homogenized (B. Braun Melsungen AG, Germany) for 1 minute at 1500 rpm. The resulting liquid was centrifuged at 4000 rpm for 3 minutes. Cholesterol concentration was measured in the supernatant by the endpoint method on a BS-3000P semi-automatic biochemical analyzer (Sinnowa Medical Science & Technology Co., Ltd., China) with a flow cell, using the appropriate set of reagents of the company DIAKON-VET (Russia).

Calcium was determined following the adapted method (GOST 26570-95 "Fodder, mixed fodder and mixed fodder raw material. Methods for determination of calcium". Moscow, 2003) in an air-acetylene flame on an atomic absorption spectrophotometer (VARIAN, USA). The samples were prepared by the dry ashing technique. To prepare the standards, the state standard sample was used (Calcium ion GSO 7772-2000, EAA Eco-Analytica, Russia).

The data were processed by methods of variational statistics in Microsoft Excel. The tables show the means (M) and their standard errors (±SEM). The statistical significance of the differences between the groups was assessed by Student's t-criterion at $p < 0.05$.

Results. Weight is one of the main indicators of the quality of incubation and food eggs. With the change in the weight of the egg, its quality also changes in many ways [3]. The results of the performed study showed (Table 1) that egg-laying time affected such an important indicator of egg quality as weight. For example, in all age periods and on average during the experiment, the weight of eggs laid from 5^{00} (the moment the light was turned on) to 8^{00} h (group 1) was significantly ($p < 0.01$-0.001) higher than later during the day. The exception was eggs from 450-day-old laying hens: the difference was insignificant, but the trend remained. On average, during the experiment, group 1 of eggs exceeded the others by weight by 2.1-2.5 g, or by 3.6-4.4% ($p < 0.001$). The obtained data are consistent with the results of other authors [11, 34-36], in whose studies eggs laid early in the morning had a higher weight than those laid during the rest of the day. In the present experiment, eggs collected at 8^{00}-10^{00}, 10^{00}-12^{00}, 12^{00}-14^{00}, and 14^{00}-16^{00} (groups 1, 2, 3, 4, 5) did not differ significantly in weight. However, a certain tendency to decrease the weight for egg collection from 8^{00} to 12^{00} and to increase after 12^{00} was observed. This contradicts the data of Patterson [15], according to which from 5^{00} to 18^{00} the weight of eggs decreased by 2–9 g. According to other data [16], eggs laid after 15^{00} also had the lowest weight.

1. Weight (g) **and categories of eggs of different laying times in Haisex Brown cross chickens of the commercial herd** ($n = 90$, M±SEM; vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm ARRTPI RAS, 2020)

Age of hens, days	Laying time				
	5^{00}-8^{00} (group 1)	8^{00}-10^{00} (group 2)	10^{00}-12^{00} (group 3)	12^{00}-14^{00} (group 4)	14^{00}-16^{00} (group 5)
			Egg weight, g		
210	56.6±0.33[a]	54.2±0.37	53.4±0.39	54.4±0.40	53.9±0.39
270	58.8±0.27[a]	56.1±0.35	55.7±0.66	56.3±0.40	55.6±0.37
360	61.4±0.42[ab]	59.4±0.42	58.8±0.39	59.6±0.42	58.8±0.38
420	61.0±0.47[a]	58.3±0.49	57.6±0.43	57.3±0.44	58.4±0.41
450	61.4±0.47	60.7±0.58	60.5±0.47	60.8±0.44	61.3±0.42
On average	59.8±0.20[a]	57.7±0.23	57.3±0.22	57.7±0.22	57.6±0.21
			Egg yield by category for 210-450 days, %		
Higher	0.2	0.5	0.2	0	0
Selected	11.8	6.4	5.3	6.0	8.0
First	76.0	62.7	63.1	64.4	60.5
Second	10.7	29.1	29.6	28.9	30.4
Third	0	0.2	0.5	0	0.2
Breakage and check	1.3	1.1	1.3	0.7	0.9

[a, b] Differences between group 1 and other groups (for each age and on average by the experiment) are statistically significant, respectively, at $p < 0.001$ and $p < 0.01$.

The yield of eggs by category directly depended on the weight of eggs. For example, in group 1 (5^{00}-8^{00}), the proportion of eggs of the selected and first category for the period of 210-450 days was higher by 3.8-6.5 and 11.6-15.5%, in group 2 lower by 18.2-19.7%, respectively, than in other groups. Other groups in the yield of eggs of different categories did not differ much.

In groups 4 and 5, where eggs were laid between 12^{00}-14^{00} and 14^{00}-16^{00}, the number of damaged eggs was less by 0.4-0.6 and 0.2-0.4%. This is due to the better quality of the eggshells laid after 12^{00}, as evidenced by such indicators of the quality of the eggshells as relative weight, thickness, and density of eggs (Table 2). Both in all age periods and on average during the experiment in groups 4 and 5, these indicators of eggshell quality were significantly higher ($p < 0.001$-0.05) than in the groups of eggs laid before 12^{00}. The worst indicators of shell quality were observed in groups 1 and 2 (5^{00}-8^{00} and 8^{00}-10^{00}). The obtained data are consistent with the results of studies [12, 18, 20], where it was noted that eggs laid at 14^{00} had a higher relative weight of the shell and its thickness in comparison with eggs obtained at the beginning of daylight (6^{00}). The authors suggested that the weight of the shell tended to increase towards the last egg in the cycle. A later study by Tůmová et al. [37] indicates the dependence of the weight of the shell and, consequently, its strength on the duration of the egg in the eggshell gland of the oviduct.

2. Morphological parameters of eggs of different laying times in Haisex Brown cross chickens of the commercial herd (n = 30, for D n = 90, M±SEM; vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm ARRTPI RAS, 2020)

Parameter	Laying time				
	5^{00}-8^{00} (group 1)	8^{00}-10^{00} (group 2)	10^{00}-12^{00} (group 3)	12^{00}-14^{00} (group 4)	14^{00}-16^{00} (group 5)
			Aged 210 days		
Weight:					
albumen^{A}					
g	37.1±0.47	36.5±0.50	36.0±0.37	36.7±0.42	35.3±0.46
%	64.2	65.7	65.3	65.5	64.8
yolk^{B}					
r	14.6±0.16	13.3±0.18	13.1±0.13	13.1±0.16	13.0±0.16
%	25.3	23.9	23.8	23.4	23.9
shell^{C}					
g	6.1±0.07	6.0±0.10	6.0±0.08	6.2±0.09	6.2±0.11
%	10.5	10.4	10.9	11.1	11.3
Egg shape index, %^{D}	79.6±0.24	78.6±0.24	79.3±0.29	79.4±0.22	77.9±0.26
Egg density, g/cm^{3E}	1.091±0.001	1.089±0.001	1.091±0.001	1.094±0.001	1.097±0.001
Albumen index, %^{F}	12.5±0.36	13.4±0.28	13.5±0.32	13.8±0.34	13.2±0.36
Yolk index, %^{G}	47.9±0.42	48.7±0.50	48.2±0.41	49.7±0.47	48.4±0.56
Shell thickness, rm ^{H}	378±4.4	365±4.7	377±4.9	386±5.2	391±6.6
Albumen/yolk weight ratio^{I}	2.5±0.05	2.8±0.06	2.7±0.04	2.8±0.05	2.73±0.05
Yolk color intensity, score^{J}	2.7±0.12	2.5±0.11	2.8±0.15	2.9±0.17	2.5±0.12
Haugh units^{K}	94.0±0.76	95.8±0.65	96.1±0.66	96.1±0.63	94.6±0.84
			Aged 270 days		
Weight:					
albumen^{A}					
g	36.4±0.30	35.7±0.25	35.7±0.31	35.5±0.32	34.9±0.40
%	62.2	63.6	63.5	63.3	61.6
yolk^{B}					
g	16.0±0.27	14.6±0.16	14.5±0.17	14.5±0.16	15.6±0.38
%	27.4	26.0	25.8	25.8	27.5
shell^{C}					
g	6.1±0.05	5.8±0.09	6.0±0.16	6.1±0.09	6.2±0.11
%	10.4	10.4	10.7	10.9	10.9
Egg shape index, %^{D}	79.7±0.27	79.6±0.24	79.7±0.24	80.4±0.27	79.0±0.27
Egg density, g/cm^{3E}	1.088±0.0001	1.087±0.0010	1.090±0.0020	1.093±0.0010	1.093±0.0010
Albumen index, %^{F}	12.5±0.34	13.4±0.37	13.5±0.45	13.1±0.46	12.4±0.36
Yolk index, %^{G}	46.2±0.43	47.1±0.52	45.5±0.54	46.7±0.53	46.3±0.47
Shell thickness, rm ^{H}	372±4.0	365±5.7	383±7.3	388±6.1	391±6.7
Albumen/yolk weight ratio^{I}	2.3±0.05	2.5±0.03	2.5±0.04	2.5±0.04	2.4±0.04
Yolk color intensity, score^{J}	3.7±0.13	3.3±0.12	3.4±0.12	3.1±0.09	3.0±0.10
Haugh units^{K}	94.3±0.93	96.6±0.75	95.1±0.58	95.0±0.93	94.1±0.85
			Aged 360 days		
Weight:					
albumen^{A}					
g	38.2±0.31	37.3±0.22	37.0±0.32	37.5±0.27	37.6±0.28
%	62.1	62.8	62.6	62.7	63.1
yolk^{B}					
g	17.0±0.25	16.0±0.15	15.7±0.16	15.6±0.16	15.3±0.20
%	27.6	26.9	26.6	26.1	25.7
shell^{C}					
g	6.3±0.09	6.1±0.07	6.4±0.11	6.7±0.09	6.7±0.09
%	10.3	10.3	10.8	11.2	11.2
Egg shape index, %^{D}	78.5±0.24	78.7±0.25	78.5±0.25	79.5±0.29	77.7±0.23
Egg density, g/cm^{3E}	1.086±0.001	1.089±0.001	1.095±0.001	1.097±0.001	1.096±0.001
Albumen index, %^{F}	11.3±0.28	10.6±0.37	11.5±0.45	10.4±0.35	11.7±0.36
Yolk index, %^{G}	47.0±0.52	46.4±0.46	46.7±0.36	46.2±0.43	46.6±0.37
Shell thickness, rm ^{H}	372±4.4	372±3.4	386±7.4	403±5.1	399±4.9
Albumen/yolk weight ratio^{I}	2.3±0.06	2.3±0.03	2.4±0.04	2.4±0.04	2.5±0.05
Yolk color intensity, score^{J}	3.3±0.10	3.4±0.10	3.6±0.15	3.3±0.12	3.3±0.11
Haugh units^{K}	92.1±0.90	90.3±1.13	92.5±1.21	89.7±1.11	93.1±0.98
			Aged 420 days		
Weight:					
albumen^{A}					
g	37.8±0.29	36.4±0.27	35.8±0.31	35.9±0.25	36.7±0.29
%	62.0	62.2	62.0	62.2	62.5
yolk^{B}					
g	17.0±0.18	16.0±0.19	15.7±0.16	15.4±0.20	15.6±0.18
%	27.9	27.4	27.1	26.7	26.5

Continued Table 2

shell^{C}					
g	6.2±0.09	6.1±0.09	6.3±0.12	6.4±0.09	6.4±0.07
%	10.1	10.4	10.9	11.0	11.0
Egg shape index, %^{D}	77.4±0.24	77.5±0.25	77.6±0.26	78.0±0.27	77.1±0.26
Egg density, g/cm^{3E}	1.089±0.001	1.089±0.001	1.092±0.001	1.094±0.001	1.089±0.001
Albumen index, %^{F}	11.4±0.37	11.5±0.37	11.1±0.40	11.4±0.46	11.3±0.40
Yolk index, %^{G}	46.6±0.55	46.4±0.40	46.5±0.51	46.5±0.44	46.0±0.37
Shell thickness, rm ^{H}	370±5.5	368±5.1	385±7.1	393±5.2	383±4.4
Albumen/yolk weight ratio^{I}	2.2±0.03	2.3±0.03	2.3±0.03	2.3±0.04	2.4±0.04
Yolk color intensity, score^{J}	3.3±0.10	3.3±0.10	3.3±0.10	3.1±0.12	3.2±0.08
Haugh units^{K}	92.8±1.10	92.5±0.99	91.0±1.18	91.2±1.22	88.5±2.93
			Aged 450 days		
Weight:					
albumen^{A}					
g	38.1±0.34	38.1±0.35	37.8±0.41	37.9±0.30	38.5±0.27
%	62.3	62.9	62.6	62.4	62.9
yolk^{B}					
gr	16.9±0.18	16.3±0.12	16.3±0.22	16.3±0.26	16.2±0.14
%	27.6	26.9	27.0	26.9	26.5
shell^{C}					
g	6.2±0.09	6.2±0.08	6.3±0.09	6.5±0.08	6.5±0.11
%	10.1	10.2	10.4	10.7	10.6
Egg shape index, %^{D}	76.7±0.26	76.2±0.25	77.0±0.26	77.3±0.27	77.0±0.30
Egg density, g/cm^{3E}	1.089±0.001	1.090±0.001	1.086±0.001	1.091±0.002	1.092±0.001
Albumen index, %^{F}	10.1±0.32	9.7±0.32	9.7±0.40	9.1±0.44	9.8±0.27
Yolk index, %^{G}	46.7±0.39	45.9±0.34	46.0±0.5	45.9±0.5	47.1±0.41
Shell thickness, rm ^{H}	364±4.4	376±4.3	376±4.5	380±7.6	379±6.8
Albumen/yolk weight ratio^{I}	2.3±0.04	2.3±0.03	2.3±0.05	2.3±0.05	2.4±0.03
Yolk color intensity, score^{J}	3.3±0.09	3.4±0.10	3.4±0.10	3.3±0.10	3.5±0.10
Haugh units^{K}	88.7±1.05	87.4±1.12	87.1±1.32	84.9±1.89	88.4±0.85
			On average		
Weight:					
albumen^{A}					
g	37.5±0.16	36.8±0.16	36.5±0.17	36.7±0.16	36.6±0.19
%	62.5	63.4	63.3	63.2	63.0
yolk^{B}					
g	16.3±0.12	15.2±0.12	15.0±0.12	15.0±0.12	15.1±0.14
%	27.2	26.2	26.0	25.8	26.0
shell^{C}					
g	6.2±0.03	6.0±0.04	6.2±0.05	6.4±0.04	6.4±0.05
%	10.3	10.4	10.7	11.0	11.0
Egg shape index, %^{D}	78.4±0.12	78.1±0.12	78.4±0.13	78.9±0.13	77.7±0.12
Egg density, g/cm^{3E}	1.089±0.0004	1.089±0.0004	1.091±0.0006	1.094±0.0006	1.093±0.0006
Albumen index, %^{F}	11.6±0.17	11.7±0.19	11.9±0.22	11.5±0.23	11.7±0.18
Yolk index, %^{G}	46.9±0.21	46.9±0.21	47.0±0.22	47.0±0.23	46.9±0.21
Shell thickness, rm ^{H}	371±2.0	369±2.1	382±2.8	390±2.7	389±2.7
Albumen/yolk weight ratio^{I}	2.3±0.02	2.4±0.02	2.5±0.02	2.5±0.02	2.5±0.02
Yolk color intensity, score^{J}	3.2±0.05	3.2±0.06	3.3±0.06	3.2±0.06	3.1±0.05
Haugh units^{K}	92.3±0.45	92.5±0.51	92.4±0.60	91.4±0.63	91.7±0.71

Note. A-K — parameters, a-e — groups.

*, **, *** Differences between groups are statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Significant differences in indicators between groups:

for 210 days: A — *ab, de; B — ***ab, ac, ad, ae; D — *bd, **ab, ce, ***ae, de; E — *ad, cd, de, **bd, ***ae, be, ce; F — *ab, ac, **ad; G — *dc, **cd; H — **bd, be; I — *ab, ae, **ac, ***ad; K — *ac, ad;

for 270 days: A — *ad, **ae; B — *be, ce, de, ***ab, ac, ad; C — *bd, **ab, cd; D — *ae, bd, be, ce, ***de; E — *bc, cd, ce, **ad, ae, ***bd, be; G — *bc; H — *ad, ae, **bd, be; I — **ab, ac, ad; J — *ab, ce, ***ad, ae; K — *be;

for 360 days: A — *ab, ac; B — **ab, be, ***ac, ad, ae; C — *bc, cd, ce, **ad, ae, ***bd, be; D — *ad, ae, bd, cd, ce, **be, ***de; E — *ab, ***ac, ad, ae, bc, bd, be; F — *be, de; H — ***ad, ae, bd, be; I — *ae, **be; K — *de;

for 420 days: A — *ae, ce, de, **ab, ***ac, ad; B — *bd, ***ab, ac, ad, ae; C — *bd, be; D — *de; E — *ac, bc, ce, **ad, bd, de; H — *be, **ad, bd; I — *be, **ad, bd;

for 450 days: C — *ad, ae, bd, be; D — *bc, be, **bd; E — *ac, ae, cd, **bc, ***ce; G — *be; I — *be; J — differences between groups are not significant; differences between groups in egg weight are not significant.

on average: A — **ab, ad, ae, ***ac; B — ***ab, ac, ad, ae; C — **ae, bc, cd, ce, ***ab, ad, bd, be; D — *be, cd, **ad, ***ae, bd, ce, de; E — *ce, **ac, bc, cd, ***ad, ae, bd, be; H — *cd, **ac, ***ad, ae, bc, bd, be; I — **ab, bc, bd, be, ***ac, ad, ae; J — *ce.

The albumen and yolk content in eggs also depended on the time of laying. In eggs laid before 8^{00}, the absolute weight of the albumen and yolk on average was the highest; in the other periods of the day, it significantly ($p < 0.001$-0.01)

decreased. However, the relative weight of the albumen in eggs collected from 8^{00} to 14^{00} increased on average from 62.5 to 63.4%, and at the end of the day (14^{00}-16^{00}) decreased slightly to 63.0%. In 420- and 450-day-old laying hens, the relative weight of the albumen does not change depending on the time of egg-laying. The relative weight of the yolk decreased on average by the end of the day (14^{00}-16^{00}) from 27.2 to 25.8%. A change in the weight of the albumen and yolk, depending on the time of egg-laying, entails a significant ($p < 0.01$–0.001) increase in the ratio of albumen and yolk weight (on average from 2.3 to 2.5). Thus, eggs laid early in the morning have more yolk and less albumen than in other periods of the day. Consequently, the nutritional value and yield of dry matter of eggs laid in the period from 5^{00} to 8^{00} are higher than those laid in the rest of the day, which serves as a positive factor for deep processing of eggs.

The increase in the absolute weight of the albumen, yolk, and, consequently, the weight of eggs laid before 8^{00}, in the authors' opinion, can be explained by the fact that the first eggs in the cycle after a pause in oviposition (from one to several days) are laid by chickens early in the morning.

On average, throughout the experiment, the egg-laying time did not affect such indicators of egg quality as the albumen index and Haugh units, but in young birds (210-day-old), the albumen in eggs laid before 8^{00} is more liquid than in other batches. In this group, the albumen index was significantly lower ($p < 0.01$-0.05) than in eggs of groups 2, 3, 4 by 0.9, 1.0, and 1.3%, respectively and Haugh units – by 1.8, 2.1, and 2.1 units, respectively, which is important during egg incubation since dense albumen affects the embryonic development of poultry and incubation results deteriorate by 1-5% [38]. However, from the point of view of consumer qualities, eggs with a high albumen density have a better amino acid composition and taste, as well as foaming ability [39].

Regardless of the age of laying hens (except for 210- and 450-day-olds), eggs laid from 12^{00} to 14^{00} had the maximum value of the shape index. On average, during the observation period in this group, this indicator was significantly higher ($p < 0.05$-0.001) than in other groups. In all age periods and on average during the experiment, the lowest shape index was observed in eggs laid from 14^{00} to 16^{00}.

According to the chemical analysis (Table 3), on average, during the experiment period, no significant differences were found between the egg groups in terms of moisture content, calcium in the shell, carotenoids, vitamins A and E in the yolk, and vitamin B_2 in the albumen. However, in terms of vitamin B_2 content in the yolk, eggs laid from 5^{00} to 8^{00} in all age periods (except for 450 day-olds, when a slight superiority was in favor of eggs laid from 8^{00} to 10^{00}) and on average during the experiment period significantly ($p < 0.05$–0.001) exceeded eggs laid in other periods of the day. Tůmova et al. [2] report a significant effect of the time of egg-laying on the content of calcium in the shell: in eggs laid at 7^{30}, its content was 35.2% and laid at 15^{30} – only 34.2%. In the present study, this pattern was not confirmed.

The authors' observations revealed a tendency to an insignificant increase in total cholesterol content in the yolk of eggs laid from 5^{00} to 8^{00}, on average during the observation period. Tůmova *et al.* [17] also recorded an unreliably higher cholesterol content in the yolk of early morning eggs compared to daytime eggs. At the same time, this contradicts the results of Abdalla *et al.* [29], who, on the contrary, noted a significantly lower amount of total cholesterol in morning eggs than in daytime eggs, calculated both per unit yolk weight (12.53 vs 16.23 mg/g) and per egg (176.63 vs. 221.14 mg/egg).

3. Chemical composition of eggs of different laying times in Haisex Brown cross chickens of the commercial herd (by n = 15 in 3 combined samples for each period, M±SEM; vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm ARRTPI RAS, 2020)

Parameter	Laying time				
	5^{00}-8^{00} (group 1)	8^{00}-10^{00} (group 2)	10^{00}-12^{00} (group 3)	12^{00}-14^{00} (group 4)	14^{00}-16^{00} (group 5)
			Aged 210 days		
Content of:					
moisture, %	75.76	75.43	75.75	76.18	76.09
calcium in the shell, %[A]	36.91±0.30	36.91±0.33	37.66±0.10	37.27±0.18	36.34±0.07
in the yolk, μg/g:					
carotenoids [B]	6.29±0.03	6.52±0.10	6.28±0.02	6.08±0.09	6.28±0.01
vitamin A [C]	4.68±0.14	4.78±0.09	4.78±0.30	4.75±0.11	4.68±0.03
vitamin E [D]	52.91±2.21	52.82±1.91	54.65±1.98	53.62±3.01	53.79±1.68
vitamin B 2 [E]	5.33±0.07	4.43±0.16	3.63±0.15	4.20±0.15	3.98±0.01
cholesterol, mmol/l [F]	42.1±0.10	43.1±1.25	40.1±1.24	38.7±0.94	42.8±1.12
in the albumen of vitamin B2, μg/g[G]	3.33±0.08	3.76±0.01	3.37±0.20	3.61±0.17	3.73±0.08
			Aged 270 days		
Content of:					
moisture, %	75.90	75.48	75.98	75.37	74.79
calcium in the shell, %[A]	35.92±0.35	36.75±0.20	36.51±0.13	36.47±0.23	36.15±0.46
in the yolk, μg/g:					
carotenoids [B]	2.60±0.03	2.54±0.07	2.54±0.07	2.45±0.06	2.86±0.03
vitamin A [C]	5.45±0.14	5.39±0.08	5.17±0.05	4.88±0.15	5.36±0.12
vitamin E [D]	110.26±2.25	107.47±2.65	108.98±2.10	105.32±1.01	105.29±1.21
vitamin B 2 [E]	5.58±0.26	5.49±0.02	4.50±0.18	4.72±0.06	5.16±0.03
cholesterol, mmol/l [F]	82.9±2.42	74.4±3.10	78.1±1.76	75.2±2.71	74.7±2.14
in the albumen of vitamin B2, μg/g[G]	3.64±0.16	4.11±0.11	4.07±0.05	4.16±0.17	3.87±0.19
			Aged 360 days		
Content of:					
moisture, %	75.76	75.43	75.75	76.18	76.09
calcium in the shell, %[A]	36.77±0.36	37.14±0.23	37.14±0.21	36.59±0.42	36.96±0.13
in the yolk, μg/g:					
carotenoids [B]	9.43±0.71	9.37±0.09	10.2±0.27	9.63±0.09	10.09±0.32
vitamin A [C]	5.16±0.04	5.20±0.13	5.44±0.12	5.70±0.35	5.16±0.21
vitamin E [D]	49.23±2.39	47.27±2.86	48.53±0.93	51.78±0.76	55.74±1.55
vitamin B 2 [E]	5.04±0.32	4.91±0.08	4.58±0.19	4.51±0.28	4.19±0.18
cholesterol, mmol/l [F]	63.7±4.72	69.7±7.56	52.8±4.85	56.6±5.47	51.3±4.77
in the albumen of vitamin B2, μg/g[G]	3.42±0.24	3.18±0.16	3.30±0.41	3.32±0.30	3.18±0.24
			Aged 420 days		
Content of:					
moisture, %	74.52	76.07	75.53	75.43	74.35
calcium in the shell, %[A]	35.82±0.16	36.67±0.19	36.48±0.18	36.20±0.26	36.60±0.44
in the yolk, μg/g:					
carotenoids [B]	4.44±0.15	4.66±0.23	4.34±0.20	4.50±0.06	4.31±0.06
vitamin A [C]	5.38±0.35	5.75±0.08	5.70±0.20	5.42±0.31	5.46±0.12
vitamin E [D]	68.97±3.32	71.81±0.71	76.15±1.53	73.23±3.23	71.43±8.55
vitamin B 2 [E]	5.67±0.12	4.70±0.19	5.55±0.12	4.57±0.24	4.77±0.07
cholesterol, mmol/l [F]	83.3±8.38	76.9±9.98	61.9±4.52	67.1±4.49	68.2±5.54
in the albumen of vitamin B2, μg/g[G]	3.91±0.28	4.38±0.18	4.51±0.15	4.19±0.22	4.32±0.13
			Aged 450 days		
Content of:					
moisture, %	74.83	75.65	75.25	76.06	75.66
calcium in the shell, %[A]	37.26±0.05	37.04±0.20	36.44±0.32	37.18±0.11	36.79±0.35
in the yolk, μg/g:					
carotenoids [B]	3.47±0.33	3.11±0.32	3.84±0.29	3.52±0.23	3.60±0.13
vitamin A [C]	4.64±0.06	5.20±0.20	5.29±0.10	5.21±0.23	5.40±0.18
vitamin E [D]	64.23±1.74	69.39±3.64	76.30±0.64	63.54±0.60	64.34±1.22
vitamin B 2 [E]	5.07±0.21	5.13±0.09	4.81±0.46	4.21±0.08	4.71±0.22
cholesterol, mmol/l [F]	30.7±0.61	31.9±1.00	30.2±0.88	28.5±0.69	30.5±2.5
in the albumen of vitamin B2, μg/g[G]	4.15±0.41	4.19±0.44	3.61±0.31	3.80±0.29	3.43±0.24

Continued Table 3

		On average			
Content of:					
moisture, %	75.35	75.61	75.65	75.84	75.40
calcium in the shell, %[A]	36.54±0.18	36.90±0.10	36.85±0.15	36.74±0.15	36.57±0.15
in the yolk, μg/g:					
carotenoids [B]	5.24±0.66	5.24±0.67	5.44±0.72	5.24±0.67	5.43±0.70
vitamin A [C]	5.06±0.11	5.26±0.10	5.28±0.11	5.19±0.13	5.21±0.09
vitamin E [D]	69.12±5.90	69.19±5.42	72.92±5.70	69.50±5.27	70.06±5.23
vitamin B_2 [E]	5.34±0.11	4.93±0.11	4.61±0.19	4.44±0.09	4.56±0.12
cholesterol, mmol/l [F]	60.54±3.20	59.20±3.30	52.60±2.40	53.22±2.50	53.50±2.50
in the albumen of vitamin B_2, μg/g[G]	3.69±0.13	3.92±0.14	3.77±0.16	3.82±0.12	3.70±0.13

N o t и e. A-E — parameters, a-e — groups.

*, **, *** Differences between groups are statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Significant differences in indicators between groups:

for 210 сут: A — *ab, ac, **cd, ce; B — *ab, ad, bc, be, cd, de, **bd; E — *be, cd, ce, **bc, ***ab, ac, ad, ae; F — *bd, de, **ad; G — ***ab, be;

for 270 сут: B — ***ae, be, ce, de; C — *ad, bc, de, **bd; E — **ac, ad, ***bc, bd, be, de; F — *ab, ae; G — *ab, ac, ad;

for 360 сут: B — *bc, ce; C — *ac; D — *ae, be, de, cd, **ce; E — *ae, **be, ***de;

for 420 сут: A — *ac, **ab; B — *de; D — *bc; E — *cd, **bc, ***ab, ad, ae, ce; F — *ac;

for 450 сут: A — *ac, cd, de, **be, ***ab, ae; C — *ab, ad, **ae, ***ac; D — ***ac, ad, ae; E — *de, **ad, ***bd; F — *ad, cd;

on average: E — *ab, be, **ac, bd, ***ad, ae.

So, in laying hens of the Haisex Brown cross, with a constant lighting mode (turning on the light at 5^{00}, turning off at 19^{00}) and cellular content, the egg-laying time significantly affects the morphological indicators of egg quality. In eggs laid up to 8^{00}, on average during the observation period, the average weight is 2.1-2.5 g or 3.6-4.4% higher, the absolute weight is 7.2-8.7%, and relative yolk weight is 1.0-1.4% and, consequently, the albumen/yolk weight ratio is 4.2-8.0% lower. Eggs laid from 12^{00} to 16^{00} have better shell quality, as evidenced by higher shell weight (by 3.2-6.7%), its greater thickness (by 1.8-5.7%), and higher egg density (by 0.18-0.46%). The egg shape index varied curvilinearly, its maximum value (78.9%) was in eggs laid from 12^{00} to 14^{00}, the minimum (77.7%) — in eggs laid at the end of the day (from 14^{00} to 16^{00}). Eggs laid before noon had a greater number of shell defects (breakage and check), and in the evening batches, there were no eggs of the highest category. Within each age, the indicators between the groups differed little. However, the egg weight in each group generally increased with age (for example, in the group of eggs collected at 5^{00}-8^{00} from 56.6±0.33 to 61.4±0.47). In 420- and 450-day-old laying hens, the relative weight of the albumen does not change depending on the time of egg-laying. In a young (210-day-old) bird, the albumen of eggs laid before 8^{00} is more liquid. Regardless of age (except for 210- and 450-day-olds), eggs laid from 12^{00} to 14^{00} had the maximum value of the shape index. In all age periods and on average during the experiment, the lowest shape index was observed in eggs laid from 14^{00} to 16^{00}. The time of oviposition did not affect the chemical composition of eggs, except for vitamin B_2 in the yolk; in eggs, collected from 5^{00} to 8^{00}, this indicator significantly exceeded that of eggs collected at a later time (except for the 450-day-old laying hens that have a slight advantage in favor of eggs laid from 8^{00} to 10^{00}). The increased cholesterol content in the yolk was noted in eggs of 270- and 420-day-old birds (in general, it increased with the age of the bird), carotenoids — in 360-day-old laying hens. The content of vitamin A in the yolk also tended to increase depending on the age of chickens. The experimental data obtained will allow optimizing the egg collection schedule, selection and use of packaging containers to reduce damage to marketable products. In breeding programs, early egg-laying can serve as a sign that ensures the best quality of food and incubation eggs. In further experiments, the authors plan to study the effect of egg-laying time on the growth, development, and homogeneity of embryos, the results of incubation, and the subsequent productivity of poultry.

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