

## Laying, egg quality and blood profile of native hens

Adam Kraus<sup>1</sup>, Ondřej Krunt<sup>1</sup>, Lukáš Zita<sup>1\*</sup>, Karolína Machová<sup>2</sup>, Cyril Hrnčár<sup>3</sup>, Eva Chmelíková<sup>4</sup>

<sup>1</sup>Czech University of Life Sciences Prague, Faculty of Agrobiolgy, Food and Natural Resources, Department of Animal Science, Czech Republic

<sup>2</sup>Czech University of Life Sciences Prague, Faculty of Agrobiolgy, Food and Natural Resources, Department of Genetics and Breeding, Czech Republic

<sup>3</sup>Slovak University of Agriculture in Nitra, Faculty of Agrobiolgy and Food Resources, Insitute of Animal Husbandry, Slovak Republic

<sup>4</sup>Czech University of Life Sciences Prague, Faculty of Agrobiolgy, Food and Natural Resources, Department of Veterinary Sciences, Czech Republic

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The objective of this study was to assess egg quality parameters for the whole laying period depending on oviposition time and breed of Czech and Slovak native breeds of laying hens. Besides, to determine the differences between selected breeds in laying pattern, related to the oviposition place. Furthermore, biochemical blood parameters were measured at the end of the study. A total of 60 pullets at the age of 20 weeks were divided according to the breed. Each treatment consisted of 3 replications of 10 laying hens. The eggs were collected every day, at 6:00, 10:00 and 14:00 and the number of eggs was recorded for each oviposition time interval (from 14:00 to 5:59, from 6:00 to 9:59 and from 10:00 to 13:59). Moreover, the oviposition place (inside and outside the nest) and the number of eggs in particular place were recorded. In addition, blood samples were collected. Significantly heavier eggs were laid between 10:00 and 13:59 than between 6:00 and 9:59 h (52.44 vs. 51.39 g, resp.). Haugh units were highest in eggs from Czech golden spotted hens that were laid between 6:00 and 9:59 h and in eggs from Oravka hens that were laid between 6:00 and 9:59 h and between 10:00 and 13:59 h. Significantly lower content of yolk cholesterol was found in Czech golden spotted hens compared to Oravka hens (10.64 vs. 11.22 mg g<sup>-1</sup>, resp.). The Czech golden spotted hens had significantly higher level of glucose in blood serum than Oravka hens (16.47 vs. 14.03 mmol l<sup>-1</sup>, resp.). The Czech golden spotted hens, gene reserve of the Czech Republic, are not yet sufficiently described in scientific literature, which highlights the importance of this study.

**Keywords:** cholesterol, egg-laying, Czech golden spotted hen, Oravka hen, oviposition

### 1 Introduction

Nowadays, eggs belong to the most favourite animal products and the reasons for their popularity are numerous (Lesniewski & Stangierski, 2018). In terms of nutritional value, eggs represent a great source of all basic nutrients and apart from that dispose of many characteristics, which have a positive effect on human health status (Iannotti et al., 2014). Specifically, eggs contain high-quality proteins that are composed of balanced number of amino acids, such as histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine (Zaheer, 2015). Another important nutritional component of eggs are lipids. Polyunsaturated fatty

acids, including alpha-linolenic acid (omega-3) and linoleic acid (omega-6), are essential for health. One egg contains approximately 70 mg of omega-3 fatty acids. They are formed by metabolization of linoleic acid, arachidonic, alpha-linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA). EPA and DHA play an important role in prevention of cardiovascular diseases and furthermore have a positive impact against infections (Sparks, 2006). Cholesterol belongs among the substantial egg constituents. The average amount of cholesterol in one egg, precisely in one egg yolk is 200 mg. It is an important component as it influences the function of steroid hormones, vitamin D and works

\***Corresponding Author:** Lukáš Zita, Czech University of Life Sciences Prague, Faculty of Agrobiolgy, Food and Natural Resources, Department of Animal Science, Kamýcká 129, 165 00 Prague, Czech Republic, e-mail: [zita@af.czu.cz](mailto:zita@af.czu.cz)

as a precursor for bile to absorb and digest fat. Eggs are also a rich source of vitamins and minerals. Finally yet importantly, eggs are a source of antibodies IgY that are effective against bacterial and virus infections (Zaheer, 2015).

There is a large number of factors, which have an impact on internal and external egg quality. Scientific studies usually focus their attention on the effect of breed (genotype, resp.), age of animals, nutrition (Tang et al., 2015) housing system (Zita et al., 2018), storage conditions (Vlčková et al., 2019) and microbial contamination (Krunť et al., 2021). However, there are other factors that affect egg quality and one of them is oviposition (Hrnčár et al., 2013; Tůmová et al., 2017; Shaker et al., 2019). According to Tůmová et al. (2017) time of the oviposition is an important factor and influences especially egg weight and eggshell quality parameters. Apart from the oviposition time, attention should be also directed towards oviposition place (Oliveira et al., 2019). Regarding the effect of genotype, the use of native breeds is still decreasing in favour of commercial hybrids, who achieve higher production. Therefore, breeding of native hens is dependent especially on small and hobby farmers (Krawczyk et al., 2011). The programs for the conservation of animal genetic resources also contribute significantly to preserve native breeds (Belew et al., 2016). Both, the Czech golden spotted and Oravka hens belong into genetic resources of the country of its origin, Czech Republic, and Slovakia (Kraus et al., 2021). Protection of native breeds is important because of the high adaptability and resistance of these animals in local conditions (Begli et al., 2010) and for keeping valuable genes (Belew et al., 2016). Biochemical blood parameters describe the health status and point out any changes in organism, which may have nutritional, physiological, or even pathological character (Koronowicz et al., 2016). These parameters may influence not only health of the animals, but also their production (Pavlík et al., 2007).

The main objective of this study was to assess egg quality parameters for the whole laying period depending on oviposition time and breed of Czech and Slovak native breeds of laying hens. Besides, to determine the differences between selected breeds in laying pattern, related to the oviposition place. Furthermore, measure and evaluate biochemical blood parameters at the end of the study.

## 2 Material and methods

The experiment was authorized by the Ethical Committee for Animal Experimentation of Czech University of Life Sciences Prague.

### 2.1 Animals and management

Two native breeds of hens were included in present study, the Czech golden spotted (CGS) hen and the Oravka (OR) hen. Floor pens with litter, which met the criteria set by Council Directive 1999/74/EC, were used as housing systems. The housing system design and equipment were made according to Kraus et al. (2021). A total of 120 pullets were obtained from the breeding facility at the age of 20 weeks and immediately divided according to the breed (60 pullets per breed). Each treatment consisted of 3 replications of 20 laying hens. The environmental conditions were controlled and maintained same for all animals. The temperature was kept between 18 °C and 20 °C and humidity between 50 and 60% throughout the whole study. Hens from 20 weeks of age were provided with 14 hours of light, which was regularly extended to 16 h from the 24 weeks of age. The lighting intensity was kept between 5–10 lx. Regarding the feeding, it was provided by commercial feed mixtures. Hens from the age of 20 weeks, feed mixture labelled as N1 was used and contained 16.71% of crude protein (CP) and 11.40 MJ of metabolizable energy (ME) and hens from the age of 42 weeks feed mixture labelled as N2 was used (15.41% of CP, 11.48 MJ of ME). Access to both, feed and water was *ad libitum* for the duration of the whole study.

### 2.2 Egg quality and blood analysis

The collection of eggs for the purpose of the study started when hens were 24 weeks old and finished when hens were 64 weeks old. The eggs were collected every day, three times a day, at 6:00, 10:00 and 14:00 and the number of eggs was recorded for each oviposition time interval (from 14:00 to 5:59, from 6:00 to 9:59 and from 10:00 to 13:59). Furthermore, the oviposition place (inside and outside the nest) and the number of eggs in particular place were recorded. The design of the housing system was made according to Kraus et al. (2021). The eggs for egg quality analysis (50 eggs from each breed at each age) and for yolk cholesterol analysis were collected every four weeks and the collection of eggs was performed for 3 consecutive days to reach a required number of eggs for the analysis. After the collection, eggs were stored at 6 °C until the day of the analysis (24 h after the egg collection). The yolks for cholesterol analysis were randomly selected (5 egg yolks from each breed at each age) from the eggs, which were used for quality analysis. Each yolk was evaluated separately as one sample and was evaluated in triplicate. The evaluation of egg quality parameters included egg weight (EW), shape index (SI), eggshell reflectivity (ESR), thickness (EST), strength (ESST), surface (ESS), index (ESI) and proportion (ESP), yolk colour (YC), proportion (YP) and index (YI), cholesterol concentration in yolk (CH\_Y), albumen

proportion (AP) and index (AI), Haugh units (HU) and yolk to albumen ratio (YAR). All measurements and devices were used according to Kraus et al. (2021). Furthermore, the determination of eggshell index (ESI) was calculated according to Ahmed et al. (2005). The egg quality analysis took place at the laboratory of the Department of Animal Science and blood analysis took place at the Department of Veterinary Sciences of the Faculty of Agrobiological, Food and Natural Resources of the Czech University of Life Sciences Prague. The effect of age was not considered in this study, all evaluated parameters were evaluated for the whole observed period.

Biochemical blood analysis was performed from blood serum and the following parameters were studied: aspartate aminotransferase (AST), total protein (TP), albumin (ALB), glucose (GLU), triacylglycerol (TAG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triacylglycerol and high-density lipoprotein cholesterol ratio (TAG\_HDL), low-density lipoprotein cholesterol and high-density lipoprotein cholesterol ratio (LDL\_HDL), non-high-density lipoprotein cholesterol (Non\_HDL) and atherogenic index (ATI). Six birds per replication from each breed (in total, 36 animals) were randomly selected and slaughtered for the purpose of the blood analysis. Blood was collected into two types of tubes (each sample): the empty sterile tubes, which were used for all selected parameters apart from GLU and tubes with sodium chloride, which were used for the determination of GLU. The analysis of AST, TP, ALB, GLU, TAG, CHOL, HDL and LDL was made according to Kraus et al. (2021). Parameters, such as TAG\_HDL, LDL\_HDL was calculated as a ratio and Non\_HDL according to van Deventer et al. (2011) and ATI according to Salma et al. (2007).

### 2.3 Statistical analysis

The computer application SAS (SAS Inst. Inc., Cary, NC, USA) was used for the statistical analysis of the data. The effect of breed and oviposition time on each of egg quality parameters was assessed by the mixed model using the MIXED procedure of SAS:

$$Y_{ijkl} = \mu + B_i + OT_j + (B \times OT)_{ij} + A_{ijk} + e_{ijkl}$$

where:  $Y_{ijk}$  – the value of trait,  $\mu$  – the overall mean;  $B_i$  – the effect of breed (the CGS hens and the OR hens);  $OT_j$  – the effect of oviposition time (6:00, 10:00 and 14:00);  $(B \times OT)_{ij}$  – the effect of the interaction between breed and oviposition time;  $A_{ijk}$  – independent variable of the age;  $e_{ijkl}$  – the random residual error

The significance of the differences among groups was tested by Duncan's multiple range test. The

value of  $P \leq 0.05$  was considered as significant for all measurements.

Furthermore, the effect of breed on oviposition time and oviposition place was assessed. Although the data were repeatedly measured on two flocks of hens, they were evaluated as independent observations. Pearson's chi-square tests were used in intergroup comparisons of categorical variables. The relationship between the breed and the choice of nest for laying was tested first. This was followed by testing of the dependence of chosen place for laying and time for each breed separately.  $P$ -values lower than 0.05 were considered as statistically significant. The phi coefficient and Cramer's coefficient were used to estimate the degree of dependence for four-field and the six-field table, respectively. However, based on the nature of our data, there was no difference between the two coefficients. The calculations were performed using a statistical program Statistica 12 (StatSoft, Inc., Tulsa, Oklahoma).

## 3 Results and discussion

All results are shown in detail in attached tables and figure.

### 3.1 External and internal egg quality regarding the breed and oviposition time

Egg and eggshell quality parameters are presented in Table 1, while yolk and albumen quality parameters are presented in Table 2. The effect of breed, oviposition time and interaction of these two factors are shown in both tables for each parameter. Breed significantly affected ESR, EST, ESI, ESP (Table 1), YC, YP, CH\_Y, AP, AI, HU and YAR (Table 2). The effect of oviposition time was calculated as statistically significant in EW, SI, ESR, EST, ESS, ESI, ESP (Table 1), YC, YI, AP, AI, HU and YAR (Table 2). The interaction between breed and oviposition time was significant in ESR, EST, ESST, ESI, ESP (Table 1), YC, YP, AP, HU and YAR (Table 2).

### 3.2 Hens' oviposition regarding the time, place, and breed

The percentage of laid eggs regarding the oviposition time and place and the interaction between oviposition time and oviposition place of CGS hens is shown in Table 3 and of OR hens in Table 4. The statistically significant interaction between oviposition time and oviposition place was found only in CGS hens (Table 3). The preference of oviposition place regarding the breed and ratio between total numbers of laid eggs in each place are presented in Figure 1. Statistically significant difference between the oviposition places (inside the nest and outside the nest) was found only in CGS hens.

**Table 1** Egg and eggshell quality parameters regarding the breed and oviposition time

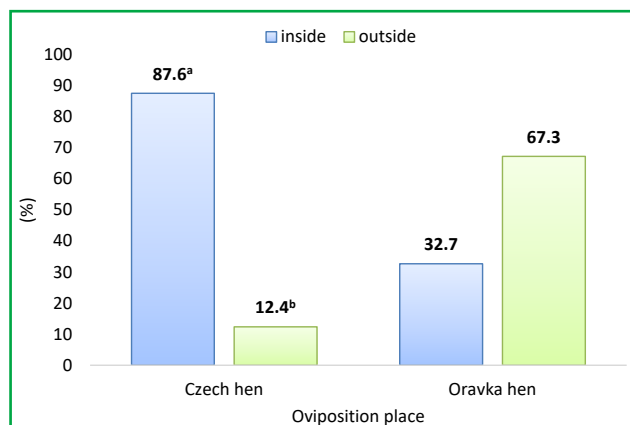
Item		Parameter							
Breed	oviposition time	EW (g)	SI (%)	ESR (%)	EST (mm)	ESST (N cm <sup>-2</sup> )	ESS (cm <sup>2</sup> )	ESI (g 100 cm <sup>-2</sup> )	ESP (%)
CGS		52.05	74.68	56.81 <sup>a</sup>	0.316 <sup>b</sup>	38.94	77.19	7.61 <sup>b</sup>	9.53 <sup>b</sup>
OR		51.78	74.53	39.89 <sup>b</sup>	0.319 <sup>a</sup>	39.75	76.90	7.72 <sup>a</sup>	9.69 <sup>a</sup>
	6	51.92 <sup>ab</sup>	74.90 <sup>a</sup>	47.41 <sup>b</sup>	0.322 <sup>a</sup>	39.57	77.06 <sup>ab</sup>	7.80 <sup>a</sup>	9.78 <sup>a</sup>
	10	51.39 <sup>b</sup>	74.43 <sup>b</sup>	49.73 <sup>a</sup>	0.311 <sup>b</sup>	38.97	76.45 <sup>b</sup>	7.51 <sup>c</sup>	9.46 <sup>b</sup>
	14	52.44 <sup>a</sup>	74.50 <sup>b</sup>	47.91 <sup>b</sup>	0.319 <sup>a</sup>	39.50	77.62 <sup>a</sup>	7.67 <sup>b</sup>	9.59 <sup>ab</sup>
CGS	6	52.07	74.76	55.19 <sup>b</sup>	0.324 <sup>a</sup>	39.33 <sup>ab</sup>	77.23	7.81 <sup>a</sup>	9.78 <sup>a</sup>
	10	51.21	74.63	59.32 <sup>a</sup>	0.305 <sup>c</sup>	37.65 <sup>c</sup>	76.25	7.36 <sup>d</sup>	9.28 <sup>c</sup>
	14	52.87	74.66	55.93 <sup>b</sup>	0.319 <sup>b</sup>	39.84 <sup>ab</sup>	78.10	7.64 <sup>c</sup>	9.52 <sup>ab</sup>
OR	6	51.77	75.04	39.63 <sup>c</sup>	0.320 <sup>ab</sup>	39.81 <sup>ab</sup>	76.89	7.78 <sup>ab</sup>	9.79 <sup>a</sup>
	10	51.57	74.23	40.13 <sup>c</sup>	0.317 <sup>b</sup>	40.28 <sup>a</sup>	76.66	7.66 <sup>c</sup>	9.63 <sup>ab</sup>
	14	52.01	74.33	39.90 <sup>c</sup>	0.319 <sup>b</sup>	39.15 <sup>b</sup>	77.14	7.71 <sup>bc</sup>	9.66 <sup>ab</sup>
<i>P</i> -value									
B		0.2756	0.3744	0.0001	0.0336	0.0578	0.2828	0.0005	0.0001
OT		0.0018	0.0489	0.0019	0.0001	0.4017	0.0020	0.0001	0.0001
B × OT		0.1202	0.1952	0.0156	0.0001	0.0011	0.1203	0.0003	0.0031
SEM		0.116	0.081	0.310	0.001	0.174	0.130	0.015	0.020

B – breed, OT – oviposition time, EW – egg weight, SI – shape index, ESR – eggshell reflectivity, EST – eggshell thickness, ESST – eggshell strength, ESS – eggshell surface, ESI – eggshell index, ESP – eggshell proportion, CGS – Czech golden spotted hen, OR – Oravka hen; SEM – standard error of the mean; *P*-value ≤0.05 means significant effect of concrete parameter. Values marked with different superscript letters for each parameter are significantly different

**Table 2** Yolk and albumen quality parameters regarding the breed and oviposition time

Item		Parameter							
Breed	oviposition time	YC (point)	YP (%)	YI (%)	CH_Y (mg g <sup>-1</sup> )	AP (%)	AI (%)	HU (point)	YAR
CGS		6.28 <sup>a</sup>	31.20 <sup>b</sup>	45.37	10.64 <sup>b</sup>	59.28 <sup>a</sup>	8.66 <sup>b</sup>	81.64 <sup>b</sup>	0.53 <sup>b</sup>
OR		6.13 <sup>b</sup>	31.68 <sup>a</sup>	45.50	11.22 <sup>a</sup>	58.63 <sup>b</sup>	9.34 <sup>a</sup>	83.65 <sup>a</sup>	0.54 <sup>a</sup>
	6	6.17 <sup>b</sup>	31.51	44.72 <sup>c</sup>	11.02	58.72 <sup>b</sup>	8.18 <sup>c</sup>	79.65 <sup>c</sup>	0.54 <sup>a</sup>
	10	5.97 <sup>c</sup>	31.22	46.03 <sup>a</sup>	10.95	59.33 <sup>a</sup>	9.52 <sup>a</sup>	84.74 <sup>a</sup>	0.53 <sup>b</sup>
	14	6.46 <sup>a</sup>	31.59	45.56 <sup>b</sup>	10.82	58.82 <sup>ab</sup>	9.29 <sup>b</sup>	83.56 <sup>b</sup>	0.54 <sup>a</sup>
CGS	6	6.31 <sup>b</sup>	31.62 <sup>abc</sup>	44.56	10.57	58.60 <sup>c</sup>	7.76	78.03 <sup>c</sup>	0.54 <sup>ab</sup>
	10	5.93 <sup>c</sup>	30.66 <sup>d</sup>	45.97	10.76	60.07 <sup>a</sup>	9.37	84.59 <sup>a</sup>	0.51 <sup>c</sup>
	14	6.60 <sup>a</sup>	31.32 <sup>c</sup>	45.57	10.60	59.16 <sup>b</sup>	8.85	82.30 <sup>b</sup>	0.53 <sup>b</sup>
OR	6	6.04 <sup>c</sup>	31.40 <sup>bc</sup>	44.88	11.47	58.83 <sup>bc</sup>	8.60	81.26 <sup>b</sup>	0.54 <sup>ab</sup>
	10	6.01 <sup>c</sup>	31.77 <sup>ab</sup>	46.09	11.15	58.59 <sup>c</sup>	9.68	84.89 <sup>a</sup>	0.55 <sup>a</sup>
	14	6.33 <sup>b</sup>	31.86 <sup>a</sup>	45.54	11.04	58.48 <sup>c</sup>	9.73	84.81 <sup>a</sup>	0.55 <sup>a</sup>
<i>P</i> -value									
B		0.0125	0.0006	0.3939	0.0323	0.0001	0.0001	0.0001	0.0001
OT		0.0001	0.0891	0.0001	0.8188	0.0028	0.0001	0.0001	0.0307
B × OT		0.0382	0.0011	0.6195	0.6746	0.0001	0.0915	0.0236	0.0004
SEM		0.029	0.067	0.076	0.132	0.069	0.055	0.205	0.002

B – breed, OT – oviposition time, YC – yolk colour, YP – yolk proportion, YI – yolk index, CH\_Y – cholesterol concentration in yolk, AP – albumen proportion, AI – albumen index, HU – Haugh units, YAR – yolk to albumen ratio, CGS – Czech golden spotted hen, OR – Oravka hen; SEM – standard error of the mean; *P*-value ≤0.05 means significant effect of concrete parameter. Values marked with different superscript letters for each parameter are significantly different



**Figure 1** Preference of oviposition place regarding the breed and percentage of laid eggs values marked with different superscript letters for each parameter are significantly different ( $P$ -value  $\leq 0.05$ )

### 3.3 Biochemical blood parameters regarding the breed

Table 5 displays biochemical blood parameters of CGS hens and OR hens. Statistically significant differences between CGS and OR hens in blood serum were found in GLU, TAG, CHOL, HDL, LDL, NonHDL. The rest of the evaluated parameters did not differ significantly.

### 3.4 External and internal egg quality regarding the breed and oviposition time

The effect of oviposition time on EW was previously studied by authors such as Samiullah et al. (2016) or Tůmová & Ledvinka (2009). However, the contrary to the results of this study, Samiullah et al. (2016) found out that the heaviest eggs are laid early in the day and Tůmová & Ledvinka (2009) stated that the heaviest eggs were laid between 14:00 and 5:59. The significant effect of oviposition time on SI and ESS was determined, which is in accordance with the study from Tůmová et al. (2017). Furthermore, YI and AI were also significantly affected by oviposition time, where higher values were observed at morning eggs (laid between 6:00 and 9:59). Tůmová & Ebeid (2005) confirmed a significant effect of oviposition time on both parameters and found the same trend for YI, but on the other hand, different for AI, where they found the highest value of AI in eggs that were laid between 10:00 and 13:59 h.

In our study, the CH<sub>Y</sub> content was affected just by breed, while oviposition time had no significant effect, which is in accordance with Tůmová & Ebeid (2005). Oppositely, Abdalla & Ochi (2018) found lower CH<sub>Y</sub> content in the morning eggs. Genotype (or breed) has a direct impact on several egg quality parameters including concentration of cholesterol in egg yolk (Rizzi & Chiericato 2010; Kraus et al., 2021). Similarly, Yang et

**Table 3** Percentage of laid eggs regarding the oviposition time and place in Czech golden spotted hens (%)

Oviposition time (OT)	Oviposition place (OP)	Number of eggs (%)
14:00 – 5:59	inside	34.7 <sup>ab</sup>
	outside	4.9 <sup>b</sup>
6:00 – 9:59	inside	11 <sup>b</sup>
	outside	2.8 <sup>b</sup>
10:00 – 13:59	inside	41.9 <sup>a</sup>
	outside	4.7 <sup>b</sup>
<i>P</i> -value		
OT × OP		0.0042

values marked with different superscript letters for each parameter are significantly different ( $P$ -value  $\leq 0.05$ )

**Table 4** Percentage of laid eggs regarding the oviposition time and place in Oravka hens (%)

Oviposition time (OT)	Oviposition place (OP)	Number of eggs (%)
14:00 – 5:59	inside	9.5
	outside	21.8
6:00 – 9:59	inside	9.9
	outside	18.1
10:00 – 13:59	inside	13.3
	outside	27.4
<i>P</i> -value		
OT × OP		0.4826

values marked with different superscript letters for each parameter are significantly different ( $P$ -value  $\leq 0.05$ )

al. (2013) confirmed the differences in CH<sub>Y</sub> between breeds and added that CH<sub>Y</sub> is dependent also on other factors, such as EW, laying intensity or age of hens. Kraus et al. (2021) also compared differences in CH<sub>Y</sub> between CGS and OR hens and discovered the lower cholesterol content (11.06 mg g<sup>-1</sup>) in eggs from CGS hens housed on deep litter compared to eggs from OR hens (12.18 mg g<sup>-1</sup>), which is quite similar to our results (10.64 vs. 11.22 mg g<sup>-1</sup>). Consumption of eggs is generally being linked with a higher risk of cardiovascular diseases, especially because of the cholesterol content. Moreover, the problematics around the impact of cholesterol consumption is still controversial. Specifically, Shin et al. (2013), stated that there is no connection between egg consumption and cardiovascular diseases. On the other hand, Zhuang et al. (2021) concluded that intake of cholesterol is associated with higher all-cause, cardiovascular diseases, and even with cancer mortality.

**Table 5** Biochemical blood parameters regarding the breed

Parameter	Breed		P-value	SEM
	CGS	OR		
AST ( $\mu\text{kat l}^{-1}$ )	2.76	2.83	0.7242	0.093
TP (g/l)	46.45	43.47	0.3218	1.479
ALB (g l <sup>-1</sup> )	17.48	18.14	0.4671	0.444
GLU (mmol l <sup>-1</sup> )	16.47 <sup>a</sup>	14.03 <sup>b</sup>	0.0498	0.652
TAG (mg dl <sup>-1</sup> )	262.85 <sup>b</sup>	274.22 <sup>a</sup>	0.0318	24.356
CHOL (mg dl <sup>-1</sup> )	114.79 <sup>a</sup>	105.65 <sup>b</sup>	0.0321	4.556
HDL (mg dl <sup>-1</sup> )	168.18 <sup>a</sup>	135.89 <sup>b</sup>	0.0401	18.853
LDL (mg dl <sup>-1</sup> )	82.24 <sup>b</sup>	89.08 <sup>a</sup>	0.0461	4.529
TAG_HDL	2.40	2.18	0.7794	0.375
LDL_HDL	0.72	0.72	0.9973	0.062
Non_HDL (mg dl <sup>-1</sup> )	13.88 <sup>b</sup>	20.72 <sup>a</sup>	0.0313	13.995
ATI	0.90	0.87	0.7886	0.060

CGS – Czech golden spotted hen, OR – Oravka hen; SEM – Standard Error of the Mean; AST – aspartate aminotransferase, TP – total protein, ALB – albumin, GLU – glucose, TAG – triacylglycerol, CHOL – cholesterol, HDL – high-density lipoprotein cholesterol, LDL – low-density lipoprotein cholesterol, TAG\_HDL – triacylglycerol high-density lipoprotein cholesterol ratio, LDL\_HDL – low-density lipoprotein cholesterol high-density lipoprotein cholesterol ratio, Non\_HDL – non high-density lipoprotein cholesterol, ATI – atherogenic index. Values marked with different superscript letters for each parameter are significantly different ( $P$ -value  $\leq 0.05$ )

The two-way interaction between breed and oviposition time was found for ESR. In general, breed or hybrid genotype affect the ESR (Kraus & Zita, 2019). Moreover, in terms of oviposition time, Samiullah et al. (2016) observed the trend, where hens laid darker eggs in the morning. Furthermore, statistically significant interactions of breed and oviposition time were calculated for EST, ESST and ESI. These results reflect the real value of the whole eggshell and due to that are valuable (Tyler & Geake, 1961) and important in terms of cracks occurrence, because Kibala et al. (2015) found positive correlation between EST and ESST. Concerning ESI, the highest value means smaller crystals of CaCO<sub>3</sub> and higher breaking strength (Ahmed et al., 2005). Similarly to our results, Samiullah et al. (2016) stated the reduction in the EST in eggs, which were laid later in the morning and connected these results with the time that eggs remain in the shell gland and does not necessarily mean of extra calcite. On the other hand, Tůmová & Ebeid (2005) did not observe eggshell parameters as significant in connection with oviposition time. Beside other parameters, Hrnčár et al. (2013) studied the effect oviposition on ESP in Brown Leghorn, Oravka and Brahma hens and did not found any significant effect of oviposition time on ESP with the exception of eggs from Brahma hens, which had significantly lowest value ESP when laid between 14:00 and 5:59. However, the housing was the same, differences in YC could have a link with immune response due to carotenoids, which provide these actions to support immune system (Moller et al., 2000). The oviposition time could be affected by stress

in individuals, which proves a delay of laying eggs, which influences an internal quality (Reynard & Savory, 1999). Tůmová et al. (2017) also found significant interaction (B × OT) for YP and AP. They observed an increase of the values with the time of oviposition in Bovans and Moravia hybrid strains. These results of HU are in accordance with results from Hrnčár et al. (2013), who found lower values in eggs from Brahma hens that were laid between 6:00 and 9:59 h. Vice versa, Tůmová & Ebeid (2005) discovered higher values of HU in the afternoon eggs.

### 3.5 Hens' oviposition regarding the time, place and breed

Results of eggs laid into nests are important thanks to their connection with better hatchability of chickens (Keeling, 2004) or higher status of food safety, because the most of bacteria comes from the litter on the floor (Brandl et al., 2014). Basically, it can be expected that the most of eggs will be laid during the morning. However, some delay in the timing of oviposition can occur, when stress occurs (Reynard & Savory, 1999) or when there is not enough space to lay eggs synchronously. This can also end in adaptation of hens and laying later or choosing a different place to oviposit (Villanueva et al., 2017). These authors also found differences between brown and white laying hybrids, which varied in the live weight as well as our hens (OR hens are heavier than CGS hens). Another factor, which can affect the preference of place to oviposit, is a natural tendency of hens to nest in groups to avoid predators (Riber, 2012).

### 3.6 Biochemical blood parameters regarding the breed

GLU and TAG are energy sources, where GLU is considered as the main energy source. Significant differences between CGS and OR hens may be simply caused by different body constitution or different physical activity of particular breed (Kraus et al., 2021). Regarding the cholesterol, Andrews et al. (1968) stated that its origin in eggs is in blood serum. Blood serum cholesterol was influenced by age of hens and housing system in study of Kraus et al. (2021), who proved the negative effect of cage housing of native breeds with an impact on blood and yolk cholesterol. Zita et al. (2018) calculated the correlation between concentration of cholesterol in egg yolk and in blood serum, but the result was non-significant. The role of cholesterol is also important because it is a precursor of steroid hormones (Kraus et al., 2021). Furthermore, the cholesterol fractions (LDL and HDL) can be used for determination of the onset of CVD (Fernandez and Webb, 2008). Non-HDL was previously confirmed to be predictive of CVD as well (Packard & Saito, 2004) and is considered as a superior predictor of CVD compared to LDL (Blaha et al., 2008).

## 4 Conclusions

The effects of breed, oviposition time and their interaction on egg quality were determined as significant in most of the evaluated quality parameters. The preference of CGS hens to lay eggs inside the nest was also confirmed. Based on this finding, we can assume that CGS hens showed less risky nest behaviour regarding the egg quality. It can be also summarised that egg quality varied independently with time of oviposition. The effect of breed on blood serum parameters was calculated as statistically significant especially in cholesterol connected parameters. The blood serum data may help to acquire more complete information about these native breeds. The uniqueness and originality of this study is highlighted using native breeds, the Czech golden spotted and the Oravka hens, which are insufficiently explored from this point of view.

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