Determination of selected biochemical parameters in blood serum and egg quality of Czech and Slovak native hens depending on the housing system and hen age

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ABSTRACT The objective of this study was to determine and evaluate the impact of the age and housing system on blood indicators (triacylglycerides, total cholesterol, aspartate aminotransferase, total proteins, albumin, glucose) and physical egg quality parameters (egg weight, shape index and surface area, eggshell proportion, thickness, strength, and color, albumen proportion and index, Haugh units, yolk proportion, index and yolk-to-albumen ratio) in selected native breeds of the Czech Republic (the Czech Golden Spotted hens) and Slovakia (the Oravka hens). Furthermore, the concentration of cholesterol in the yolk was determined. A total of 132 animals were used. There were 60 eggs collected from each breed at each monitored period for the evaluation of egg quality. Blood samples were taken by puncture of a wing vein. The assessments were made when the hens were of 34, 42, and 50 weeks old. Enriched cages and floor pens with litter were used as housing systems. The effects of breed, housing system, and age were observed. Furthermore, interactions among these factors were calculated. The significant effect of housing system was found in total cholesterol ($P = 0.098$) and aspartate aminotransferase ($P = 0.0343$) and the significant effect of age in total protein ($P = 0.0392$). The significant effect of breed ($P = 0.0199$), housing system ($P = 0.0001$), and age ($P = 0.0001$) was found in concentration of cholesterol in the yolk. Regarding the egg quality, the significant effect of breed ($P = 0.0001$) was found in eggshell color, albumen index and Haugh units, whereas the significant effect of housing system was found in egg weight ($P = 0.0002$), egg surface area ($P = 0.0003$), eggshell proportion ($P = 0.0460$), thickness ($P = 0.0216$), strength ($P = 0.0049$), and color ($P = 0.0009$). The significant effect of age was determined in all parameters except for the eggshell proportion and strength. The results represent an interesting comparison of changes in biochemical blood and egg quality parameters. It is necessary to further evaluate these indicators, especially in other genetic resources of hens, where the data are often nonexisting.

Key words: age, blood serum, egg quality, housing system, native breed

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INTRODUCTION

In general, blood serum parameters are reliable indicators of health status and reflect any physiological, nutritional, or even pathological changes that occur in the organism (Simaraks et al., 2004; Koronowicz et al., 2016). Both studies confirmed this statement using different genotypes of hens. Simaraks et al. (2004) used the Thai native hens, whereas Koronowicz et al. (2016) used Isa Brown, which belong to the group of commercial laying hybrid hens. These biochemical indicators simultaneously characterize the homeostasis of the internal environment of the animals, which has an effect not only on their health, but also on the production parameters (Pavlík et al., 2007). Glucose is the main energy source (Gallenberger et al., 2012), whereas triacylglycerols (**TAG**) represent another source of energy (Pillutla et al., 2005). Total cholesterol is a precursor of steroid hormones (Pavlík et al., 2007) and a simultaneously building component of cell membranes (Zhang et al., 2019). The adequate function of the liver can be detected from the activity of the aspartate aminotransferase enzyme (Mollahosseini et al., 2017). Total protein and

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albumin values reflect both protein utilization from the feed (Pavlík et al., 2007) and the level of hemoconcentration (Greene et al., 2013).

Eggs are one of the main poultry products and their final quality plays an important role for producers and for consumers as well (Hernandez et al., 2005). Many internal and external factors influence egg quality parameters. Genotype, housing system, and age are some of the most substantial ones and their significant effect was previously confirmed by numerous authors such as Hanusová et al. (2015), Kraus et al. (2019), and Sokołowicz et al. (2019). Moreover, the topic of housing systems is currently very actual because of growing concerns of the general public about the welfare and housing conditions of farm animals (Rahmani et al., 2019). As previously mentioned by Pavlík et al. (2007), biochemical blood indicators have an effect on health status of hens and according to Galli et al. (2018), ensuring good health status of hens positively affects final quality of eggs. The egg weight (**EW**) is an essential quality parameter for both, producers and consumers (Tolimir et al., 2017). The eggshell quality parameters are important because of several reasons. In the economical point of view, it is desirable to produce eggs with solid eggshells without cracks. Eggshell strength is influenced by other parameters such as egg shape, egg size, or eggshell thickness (Sapkota et al., 2017). Another function of the eggshell is protection against the contamination of egg internal content so in the food safety point of view, eggshell quality plays an important role as well (Vlčková et al., 2018). The quality of albumen and yolk concerns particularly consumers (Tolimir et al., 2017). The quality of both albumen and yolk is usually expressed by proportion and index (Zita et al., 2009; Hanusová et al., 2015; Kraus et al., 2019). Haugh units (**HU**) are essential albumen quality parameter that determines an overall quality of egg content and egg freshness (Narushin et al., 2020). The egg yolk is a great source of cholesterol and contains approximately 200 mg. The role of cholesterol in human nutrition is huge. It has a functional impact on steroid hormones, vitamin D, and it is also precursor for bile to absorb and digest fat (Zaheer, 2015). According to Pavlík et al. (2007), concentration of cholesterol in the yolk may be in relationship with concentration of cholesterol in blood. However, some authors claim the opposite (Shivaprasad and Jaap, 1977; Vogt et al., 1990). Both of these concentrations are associated with the hen-day egg production (Pavlík et al., 2007).

Nowadays, the use of native breeds of laying hens is still decreasing at the expense of commercial hybrids, which typically have a higher performance (Krawczyk et al., 2011). At the end of the 20th century, about 20% of farm animal breeds including poultry breeds became extinct (Anderle et al., 2014). In the case of poultry, maintaining native breed populations largely depends on small farmers (Krawczyk et al., 2011). The Czech Golden Spotted (**CGS**) hens (Anderle et al., 2014) and the Oravka (**OR**) hens (Hanusová et al., 2017) are included in native breeds of farm animals in the Czech Republic and Slovakia, respectively. Native breeds are valuable thanks to their adaptability to environmental conditions of specific regions and thanks to higher resistance against local diseases (Begli et al., 2010). In the absence of programs for the conservation of animal genetic resources, there would be a risk that many important fixed genes would be lost and could no longer be used in breeding work (Belew et al., 2016).

The information about the blood serum indicators and egg quality parameters of Czech and Slovak native hens is insufficient or even nonexistent. Thus, the main objective of this study was to determine some missing information, which would evaluate the impact of the hen age and housing system on blood indicators and physical egg quality parameters in selected native breeds.

MATERIALS AND METHODS

The Ethics Committee of the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic approved this research with animals.

Animals and Management

Two native breeds of hens were used in this study, the CGS hen and the OR hen. Each breed belongs to the genetic resources of animals in the country of its origin. The CGS hens come from the Czech Republic and the OR hens from Slovakia.

Enriched cages and floor pens with litter were used as housing systems. Both types of housing systems met the criteria set by Council Directive 1999/74/EC that defines minimum standards for the protection of laying hens. Used housing systems were designed to exactly satisfy above-mentioned criteria. The area in enriched cage per hen was 750 cm^2 (600 cm^2 usable area) and stocking density was 12 hens per cage. Furthermore, each cage was equipped with feed trough (12 cm per hen), 2 nipple drinkers, nest (150 cm^2), perches (15 cm per hen), plastic pad for raking, tray with dust for dust bathing, claw-shortening device, and egg collection trough (placed outside of the cage). Nest walls were made of plastic flaps, which were hung up from the ceiling of the cage. The ceiling was made of wire mesh. Nests were also equipped with plastic pad with artificial grass on the floor. The tilt of the floor in cages was 14%, which enabled the movement of eggs from nests into the egg collection trough. The area of floor pens with litter (maximum 9 hens per m^2 allowed in alternative housing systems) was adjusted to the number of hens placed inside the pens; the stocking density in one pen was 10 hens. Each floor pen was equipped with feed trough with 12 cm per hen (minimum allowed space is 10 cm per hen), 2 nipple drinkers, 2 nests (each 150 cm^2), perches (15 cm per hen). In terms of nest design, there were installed 2 nests in each pen (maximum allowed number of hens per nest is 7 hens). Nests were made of solid material and the entrances were made of plastic flaps. Floors in nests were also equipped with plastic pads with artificial grass (same as in enriched cages).

The floor in each nest was tilted by 14% to secure movement of eggs into the egg collection trough, which was placed outside of the nest. A straw litter bedding was used in floor pens.

A total of 132 pullets were obtained from the breeding facility and divided at the age of 17 wk in accordance with the breed (66 pullets per breed) and subsequently divided again in accordance with the housing system (36 hens per cage system and 30 hens per litter). Each treatment consisted of 3 replications of 12 laying hens in the cage system and of 3 replications of 10 laying hens in the litter system. The climate conditions were controlled and maintained on the same level in both housing systems. The temperature was kept between 18°C and 20°C and humidity between 50 and 60% throughout the whole study. From the age of 20 wk, the hens were provided with 14 h of light, which was regularly extended to 16 h from the age of 24 wk and remained unchanged until the end of the study. The intensity of lighting was set to 5–10 lx. The feeding was provided by different commercial feed mixtures in accordance with the age of the birds. Feed mixture N0, which was fed to pullets from 17 to 19 wk of age, contained 15.00% crude protein (**CP**) and 11.56 MJ of metabolizable energy (**ME**). From the age of 20 wk, hens were fed by commercial feed mixture N1 (16.66% CP, 11.40 MJ of ME) and from the age of 42 wk with a feed mixture N2 (15.37% CP, 11.48 MJ of ME). Access to feed and water was ad libitum during the whole study.

Blood and Yolk Cholesterol Concentration Analysis

Blood samples were taken by puncture of a wing vein between 7:00 and 8:00 AM from hens at the age of 34, 42, and 50 wk and were the subject of hematological and biochemical examination. Ten blood samples from each breed (5 from cages and 5 from litter) were collected in sterile syringes and then divided into 2 tubes, one was empty and the other contained sodium fluoride (**NaF**); the latter was used for glucose evaluation, only. Blood samples were centrifuged and the separated serum was stored at −20°C. Concentrations of TAG, total cholesterol (**CHOL**), aspartate aminotransferase (**AST**), total proteins (**TP**), albumin (**ALB**), and glucose (**GLU**) were determined in blood serum using commercial kits (Erba Lachema, s.r.o., CR) on the automatic analyzer XL – 200 (Erba Lachema s.r.o., CR).

Eggs for the assessment of cholesterol concentration in the egg yolk and for the assessment of selected quality parameters were collected at the same periods as blood samples. Twenty egg yolks from each breed (10 from cages and 10 from litter) at each of the monitored periods were used to determine the concentration of cholesterol in the yolk. Each yolk was evaluated separately as one sample and was evaluated in triplicate. Cholesterol was extracted with n-hexane and separated from fat by the saponification with potassium hydroxide in ethanolic solution. High-performance capillary gas chromatography (HRGC; Master GC, Dani Instruments S.p.A., Cologno Monzese, Italy) with the mass spectrometry and flame-ionization detectors was used for the determination of cholesterol content. Technical information and device settings were used as it follows. The length of a glass column was 1 m and internal diameter was 4 mm. The temperature was set to 300°C in detector, 290°C at injector, and 260°C in column. As a carrier gas was used argon, flow rate was 50 cm^3/min and internal standard Dotriacontane (Sigma, St. Louis, MO). The concentration of cholesterol in the yolk was calculated and expressed in mg/g.

Egg Quality Analysis

Sixty eggs were collected from each breed (30 from cages and 30 from litter) at each monitored period for the evaluation of egg quality parameters. 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 analysis, which was performed the following day (24 h after the egg collection). The evaluation of egg quality parameters, which included EW, egg shape index (**ESI**), egg surface area (**ESA**), eggshell proportion (**ESP**), eggshell thickness (**EST**), eggshell strength (**ESS**), eggshell color (**ESC**), albumen proportion (**AP**), albumen index (**AI**), HU, yolk proportion (**YP**), yolk index (**YI**), and yolk-to-albumen ratio (**YAR**) took place at the laboratory of the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague.

The EW and the weight of individual egg components were measured by laboratory scale Ohaus (Model: Traveler TA502, Parsippany, NJ 07054) with 0.01 g precision. Egg shape index was calculated by the following formula: ESI (in %) = (egg width in mm/egg length in mm) × 100. An electronic sliding caliper (JOBI profi) with 0.01 mm precision was used for the measurement of width and length of egg and also of albumen and yolk respectively. The value of the ESA was determined by the following formula: ESA (in cm^2) = $3.9782 \times EW^{0.7056}$ in g. The proportions (in %) of the individual egg components (eggshell—ESP, albumen—AP, and yolk—YP) were calculated by the following formula: concrete egg component in g/EW in g × 100. The EST was measured by a digital micrometer (Digimatic Outside Micrometer, Mitutoyo Corporation, Japan) with 0.001 mm precision. The same device was used for the determination of the albumen and yolk height using different rack. The thickness was measured without eggshell membranes at the center of the eggshell. Regarding the measurement of EST, each eggshell was measured twice for more precise results. The ESS was determined by a device (Instron Universal Testing Machine; model 3342; Instron Ltd.), which calculates the force (in N/cm^2) required to crack the eggshell. The reflectometer (TSS QCR reflectometer, Chessingham Park, Dunnington, YORK YO19 5SE, England) was used for the determination of ESC in %. The higher value represents the lighter color of the

eggshell. Albumen index was calculated by the following formula: AI (in %) = (height in mm/average of length and width in mm) × 100. The formula HU = 100 × log (height of albumen in mm – 1.7 × $EW^{0.37}$ in g + 7.6) was used for the calculation of HU. Yolk index was calculated by the following formula: YI (in %) = (height in mm/average of 2 mutually vertical values of width in mm) × 100. Yolk-to-albumen ratio was calculated by the following formula: yolk weight in g/albumen weight in g.

Statistical Analysis

The computer application SAS was used for the statistical analysis of the data. The effect of breed, housing system, and age on each of biochemical indicators in blood serum, egg quality parameters, and concentration of cholesterol in the egg yolk was assessed by the mixed model using the MIXED procedure of SAS:

$$y_{ijkl} = \mu + B_i + HS_j + A_k + (B\times HS)_{ij} + (B\times A)_{ik} + (HS\times A)_{jk} + (B\times HS\times A)_{ijk} + e_{ijkl},$$

where y_{ijkl} is the value of trait, μ is the overall mean, B_i is the effect of breed (the CGS hens and the OR hens), HS_j is the effect of housing system (enriched cages and litter), A_k is the effect of the age of the hens (34, 42, and 50 wk), $(B \times HS)_{ij}$ is the effect of the interaction between breed and housing system, $(B \times A)_{ik}$ is the effect of the interaction between breed and the age of the hens, $(HS \times A)_{jk}$ is the effect of the interaction between housing system and the age of the hens, $(B \times HS \times A)_{ijk}$ is the effect of the interaction among the breed, housing system and the age of the hens and e_{ijkl} is 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.

RESULTS

The resulting values of biochemical indicators and of cholesterol concentration in the yolk are described in Table 1. Table 2 describes the results of the whole egg and eggshell parameters. The results of the albumen and yolk parameters are described in Table 3. Statistically significant interactions are discussed in detail in the text, but not described in tables.

Blood Serum Parameters and Yolk Cholesterol Concentration

Concentrations of TAG, CHOL, AST, TP, ALB, and GLU were observed in the blood serum. The effect of breed was calculated as nonsignificant in all of these indicators. The housing system significantly affected the concentration of CHOL (P = 0.0098) and AST (P = 0.0343), where lower values of both indicators were found on litter in most of the cases, whereas age had a significant (P = 0.0392) effect on the concentration of TP, which was lower at the end of the monitored period than at the beginning. The significant interaction between breed and housing system was calculated for GLU (P = 0.0374), but the interaction between breed

Table 1. Biochemical parameters in blood serum and concentration of cholesterol in the egg yolk.

			Parameter						
Breed	Housing system	Age (weeks)	TAG (mmol/L)	CHOL (mmol/L)	AST (μkat/L)	TP (g/L)	ALB (g/L)	GLU (mmol/L)	CH_Y (mg/g)
Czech Golden Spotted hens	Cages	34	5.27	3.69	3.835	56.90	19.85	17.35	11.59
		42	7.11	4.02	3.400	51.90	20.65	14.80	9.77
		50	7.76	2.41	2.854	46.18	17.76	14.69	9.88
	Litter	34	11.45	2.88	3.320	54.30	20.25	17.86	11.92
		42	3.68	2.43	2.873	45.10	16.17	12.98	10.62
		50	3.28	3.67	2.460	50.10	18.64	20.70	10.65
Oravka hens	Cages	34	3.50	4.11	3.238	52.30	20.34	16.07	11.19
		42	8.09	3.67	3.828	50.98	21.48	18.11	10.39
		50	5.08	3.26	3.584	50.36	20.30	16.00	9.89
	Litter	34	8.00	3.41	3.067	51.60	20.20	15.25	13.22
		42	4.96	2.30	3.083	43.53	19.15	13.01	12.09
		50	8.81	2.82	2.930	46.40	18.80	15.90	11.22
P-value	B		0.9895	0.7315	0.4765	0.4482	0.0767	0.4204	0.0199
	HS		0.6535	0.0098	0.0343	0.1563	0.0697	0.7906	0.0001
	A		0.7589	0.1784	0.2801	0.0392	0.2525	0.0940	0.0001
	B × HS		0.3664	0.3212	0.9223	0.5888	0.8476	0.0374	0.0406
	B × A		0.3449	0.4359	0.1738	0.7103	0.5744	0.1650	0.4096
	HS × A		0.0221	0.0050	0.8782	0.3556	0.0703	0.0101	0.9294
	B × HS × A		0.2397	0.1640	0.8364	0.5552	0.3506	0.4739	0.6110
SEM			0.644	0.129	0.114	1.009	0.333	0.467	0.196

P-value ≤ 0.05 means significant effect of concrete parameter.

Abbreviations: A, age; ALB, albumin; AST, aspartate aminotransferase; B, breed; CHOL, cholesterol; CH_Y, cholesterol in egg yolk; GLU, glucose; HS, housing system; TAG, triacylglycerol;TP, total protein.

Table 2. Whole egg and eggshell quality parameters.

			Parameter						
Breed	Housing system	Age (weeks)	EW (g)	ESI (%)	ESA (cm^2)	ESP (%)	EST (mm)	ESS (N/cm^2)	ESC (%)
Czech Golden Spotted hens	Cages	34	48.22	76.24	72.92	9.27	0.312	40.00	59.01
		42	52.89	75.58	78.17	9.41	0.324	39.43	60.12
		50	53.74	75.22	79.11	9.19	0.293	35.57	58.63
	Litter	34	50.73	75.12	75.76	9.59	0.324	40.17	58.24
		42	54.04	74.29	79.44	9.78	0.338	43.06	59.95
		50	56.69	74.06	82.35	9.93	0.315	42.79	54.77
Oravka hens	Cages	34	49.61	74.79	74.49	9.47	0.326	41.56	40.78
		42	52.95	74.36	78.22	9.50	0.324	39.00	42.10
		50	53.76	73.18	79.13	9.57	0.289	39.17	42.98
	Litter	34	51.51	75.97	76.64	9.45	0.317	42.16	37.80
		42	53.06	75.57	78.35	9.24	0.317	37.23	41.86
		50	55.03	75.14	80.39	9.72	0.303	43.01	35.80
P-value	B		0.8804	0.4748	0.8581	0.7356	0.1101	0.8181	0.0001
	HS		0.0002	0.7106	0.0003	0.0460	0.0216	0.0049	0.0009
	A		0.0001	0.0282	0.0001	0.4726	0.0001	0.3681	0.0079
	B × HS		0.2078	0.0002	0.2035	0.0149	0.0096	0.0844	0.2162
	B × A		0.1410	0.8516	0.1352	0.4629	0.1282	0.0169	0.5133
	HS × A		0.2657	0.8721	0.2765	0.3296	0.0797	0.0217	0.0231
	B × HS × A		0.8812	0.8878	0.8661	0.8060	0.6746	0.2787	0.6955
SEM			0.244	0.171	0.275	0.051	0.002	0.391	0.620

P-value ≤ 0.05 means significant effect of concrete parameter.

Abbreviations: A, age; B, breed; ESA, egg surface area; ESC, eggshell color; ESI, egg shape index; ESP, eggshell proportion; ESS, eggshell strength; EST, eggshell thickness; EW, egg weight; HS, housing system.

and age was not significant in any of the monitored indicators. On the other hand, interactions between housing system and age were found for TAG ($P = 0.0221$), CHOL ($P = 0.0050$), and GLU ($P = 0.0101$). Furthermore, the concentration of cholesterol in the yolk was determined and was significantly affected by breed ($P = 0.0199$), housing system ($P = 0.0001$), age ($P = 0.0001$), and by interaction between breed and housing system ($P = 0.0406$).

The regular trends (increasing or decreasing) in blood serum parameters and in concentration of cholesterol in the yolk did not occur. The highest value of TAG was found in 34-week-old CGS hens kept on litter (11.45 mmol/L) and the lowest in 50-week-old CGS hens from the same housing system (3.28 mmol/L). The highest value of CHOL was determined in 34-week-old OR hens from cages (4.11 mmol/L), whereas the lowest in 42-week-old hens of the same breed from

Table 3. Albumen and yolk quality parameters.

			Parameter					
Breed	Housing system	Age (weeks)	AP (%)	AI (%)	HU	YP (%)	YI (%)	YAR
Czech Golden Spotted hens	Cages	34	61.17	9.89	86.95	29.55	47.15	0.49
		42	59.93	8.92	82.48	30.66	44.68	0.51
		50	58.81	7.57	77.95	32.01	43.86	0.55
	Litter	34	60.93	8.57	83.15	29.49	46.66	0.49
		42	59.42	8.18	79.10	30.80	43.57	0.52
		50	58.23	6.99	74.88	31.84	43.42	0.55
Oravka hens	Cages	34	60.27	9.62	85.78	30.26	47.14	0.50
		42	58.54	9.13	82.84	31.96	44.59	0.55
		50	57.97	7.34	76.89	32.46	42.39	0.56
	Litter	34	60.99	10.43	88.07	29.56	48.12	0.49
		42	60.01	10.11	85.63	30.75	44.52	0.51
		50	58.63	9.59	84.02	31.65	43.98	0.54
P-value	B		0.2678	0.0001	0.0001	0.1834	0.4124	0.1648
	HS		0.4155	0.3465	0.3465	0.1039	0.7766	0.1520
	A		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	B × HS		0.0255	0.0001	0.0001	0.1261	0.0078	0.0652
	B × A		0.9618	0.7883	0.7883	0.8013	0.2136	0.8875
	HS × A		0.8615	0.1918	0.1918	0.9750	0.2567	0.9291
	B × HS × A		0.7818	0.6863	0.6863	0.8435	0.7956	0.7555
SEM			0.157	0.129	0.129	0.145	0.163	0.004

P-value ≤ 0.05 means significant effect of concrete parameter.

Abbreviations: A, age; AI, albumen index; AP, albumen proportion; B, breed; HS, housing system; HU, Haugh units; YAR, yolk-to-albumen ratio; YI, yolk index; YP, yolk proportion.

litter (2.30 mmol/L). The highest value of AST occurred in 34-week-old CGS hens kept in cages (3.84 μkat/L), whereas the lowest in 50-week-old hens of the same breed kept on litter (2.46 μkat/L). The highest value of TP was found in 34-week-old CGS hens from cages (56.90 g/L) and the lowest in 42-week-old OR hens from litter (43.53 g/L). The highest value of ALB was observed in 42-week-old OR hens kept in cages (21.48 g/L) and the lowest in 42-week-old CGS hens kept on litter (16.17 g/L). The highest value of the last evaluated parameter of blood serum, which was GLU, was determined in 50-week-old CGS hens from litter (20.70 mmol/L) and the lowest in 42-week-old hens of the same breed from the same housing system (12.98 mmol/L). The highest value of cholesterol concentration in the yolk was found in 34-week-old OR hens kept on litter (13.22 mg/g), whereas the lowest in 42-week-old CGS hens kept in cages (9.77 mg/g).

Statistically significant differences in the interaction between housing system and age in TAG showed that the highest value of TAG had 34-week-old hens from litter (9.73 mmol/L) and the lowest value had 42-week-old hens from litter (4.32 mmol/L) and 34-week-old hens from cages (4.38 mmol/L). The significant effect of this interaction was found also in CHOL, where the highest value of CHOL had 34- and 42-week-old hens from cages (3.90 and 3.84 mmol/L) and the lowest value had 42-week-old hens from litter (2.36 mmol/L). The last parameter, where the interaction between housing system and age was found as statistically significant was GLU. The highest value of GLU was found in 50-week-old hens from litter (18.30 mmol/L) and the lowest value in 42-week-old hens from litter (12.99 mmol/L). Glucose was also significantly affected by the interaction between breed and housing system, where the highest level of GLU was determined in CGS hens from litter (17.18 mmol/L) and the lowest in OR hens from litter (14.72 mmol/L). Statistically significant effect of the interaction between breed and housing system was found in concentration of cholesterol in the yolk. The highest value was found in OR hens kept on litter (12.18 mg/g) and the lowest in CGS hens kept in cages and on litter (10.41 and 11.06 mg/g) and in OR hens kept in cages (10.49 mg/g).

Egg Quality Parameters

Regarding the assessment of egg quality, these parameters were observed: EW, ESI, ESA, ESP, EST, ESS, ESC, AP, AI, HU, YP, YI, and YAR. The significant effect ($P = 0.0001$) of breed was determined in ESC, AI, and HU, whereas the effect of housing system was determined as significant in EW ($P = 0.0002$), ESA ($P = 0.0003$), ESP ($P = 0.0460$), EST ($P = 0.0216$), ESS ($P = 0.0049$), and ESC ($P = 0.0009$). The significant effect of age was discovered in all evaluated parameters apart from the ESP and ESS. The significant interaction between breed and housing system was discovered in ESI ($P = 0.0002$), ESP ($P = 0.0149$), EST ($P = 0.0096$), AP ($P = 0.0255$), AI ($P = 0.0001$), HU ($P = 0.0001$), and YI ($P = 0.0078$). The only significant interaction between breed and age was found in ESS ($P = 0.0169$). The interaction between housing system and age was calculated as significant in ESS ($P = 0.0217$) and ESC ($P = 0.0231$). All interactions among breed, housing system, and age were nonsignificant.

The heaviest eggs had 50-week-old CGS hens from litter (56.69 g) and the lightest eggs had 34-week-old CGS hens from cages (48.22 g). The highest value of ESI was found in eggs from 34-week-old CGS hens kept in cages (76.24%), whereas the lowest in eggs from 50-week-old OR hens kept in cages (73.18%). The highest value of ESA was determined in eggs from 50-week-old CGS hens from litter (82.35 cm^2) and the lowest in eggs from 34-week-old hens of the same breed, but from cages (72.92 cm^2). The highest value of EP had eggs from 50-week-old CGS hens kept on litter (9.93%), whereas the lowest had eggs from hens of the same age and breed kept in cages (9.19%). The highest value of EST was found in eggs from 42-week-old CGS hens from litter (0.338 mm) and the lowest in eggs from 50-week-old OR hens from cages (0.289 mm). The highest value of ESS had eggs from 42-week-old CGS hens from litter (43.06 N/cm^2) and the lowest had eggs from 50-week-old hens of the same breed from cages (35.57 N/cm^2). The highest value of ESC was observed in eggs from 42-week-old CGS hens kept in cages (60.12%), whereas the lowest in eggs from 50-week-old OR hens kept on litter (35.80%). The highest value of AP was found in eggs from 34-week-old CGS hens from cages (61.17%), whereas the lowest in eggs from 50-week-old OR hens from cages (57.97%). The highest value of AI was determined in eggs from 34-week-old OR hens kept on litter (10.43%) and the lowest in eggs from 50-week-old CGS hens also kept on litter (6.99%). The highest value of HU had eggs from 34-week-old OR hens from litter (88.07) and the lowest had eggs from 50-week-old CGS hens from the same housing system (74.88). The highest value of YP was found in eggs from 50-week-old OR hens from cages (32.46%), whereas the lowest in eggs from 34-week-old CGS hens from litter (29.49%). The highest value of YI was determined in eggs from 34-week-old OR hens from litter (48.12%) and the lowest in eggs from 50-week-old OR hens from cages (42.39%). The highest value of YAR was observed in eggs from 50-week-old OR hens kept in cages (0.56) and the lowest in eggs from 34-week-old CGS hens from both housing systems and from OR hens kept on litter (0.49).

On top of that, several trends occurred in most of the observed parameters. Values of EW regularly increased with the age of the hens in both housing systems and in both breeds. With the increasing EW, the ESA increased, so the trend was exactly the same. The increasing trend was also determined in YP and thus in YAR. The opposite trend was found in ESI, AP, AI, HU, and YI. The trend in ESP was regular only in eggs from the CGS hens kept on litter and in eggs from the OR hens kept in cages, where the values constantly increased with the age. The rest of the values varied irregularly. A certain trend was detected also in EST, where the lowest values were for eggs from

the oldest hens and in ESC, where the highest values were found in eggs from 32-week-old hens in three of four groups.

Statistically significant effect of interaction between breed and housing system was found in ESI. The highest value of ESI had eggs from the CGS hens kept in cages and eggs from the OR hens kept on litter (75.68 and 75.56%) and the lowest had eggs from the OR hens kept in cages (74.11%) and eggs from the CGS hens kept on litter (74.49%). Also ESP was significantly affected by the interaction between breed and housing system. The highest value was found in eggs from the CGS hens kept on litter (9.76%) and the lowest in eggs from the same breed kept in cages (9.29%). Last eggshell parameter that was significantly influenced by this interaction was EST, where the highest value was determined in eggs from the CGS hens kept on litter (0.326 mm) and the lowest in eggs from the CGS hens kept in cages (0.310 mm) and in eggs from the OR hens kept on litter and in cages (0.312 and 0.313 mm). The significant interaction between breed and age was calculated in ESS. The highest value was calculated in eggs from 34- and 50-week-old OR hens (41.86 and 41.09 N/cm^2) and in eggs from 42-week-old CGS hens (41.25 N/cm^2) and the lowest in eggs from 42-week-old OR hens (38.11 N/cm^2). This parameter was also significantly influenced by the interaction between housing system and age. The highest value of ESS was found in eggs from 50-week-old hens kept on litter (42.90 N/cm^2) and the lowest in eggs from 50-week-old hens kept in cages (37.37 N/cm^2). Statistically significant interaction between housing system and age was calculated in ESC, where the highest value had eggs from 42-week-old hens from cages and litter (51.11 and 50.90%) and the lowest had eggs from 50-week-old hens from litter (45.29%). The interaction between breed and housing system was determined as significant in AP, where the highest value had eggs from the CGS hens kept in cages and from the OR hens kept on litter (59.97 and 59.88%) and the lowest had eggs from the OR hens kept in cages (58.93%). This interaction was found as significant also in AI. The highest value was found in eggs from the OR hens kept on litter (10.04%) and the lowest in eggs from the CGS hens kept on litter (7.91%). Also HU were significantly influenced by the interaction between breed and housing system. The highest value of HU was calculated in eggs from OR hen kept on litter (85.91) and the lowest in eggs from CGS hens kept on litter (79.04). The last egg quality parameter that was significantly affected by the interaction between breed and housing system was YI, where the highest value had eggs from OR hens kept on litter (45.54%) and the lowest had eggs from CGS hens kept on litter and OR hens kept in cages (44.55 and 44.70%).

DISCUSSION

Blood Serum Parameters and Yolk Cholesterol Concentration

The concentration of TAG was significantly influenced only by the interaction between the housing system and the age of the hens. Gyenis et al. (2006) found a significant effect of genotype on the concentration of TAG and simultaneously described an extreme increase of TAG at the age of 17 wk (from concentrations between 0 and 5 mmol/L to concentrations between 15 and 20 mmol/L) because of the change of feed mixture. The concentration of CHOL in blood serum was significantly influenced by the housing system ($P = 0.0098$) and by the interaction between housing system and age ($P = 0.0050$). The results indicate that litter housing system may be more suitable than cages for selected native breeds (2.92 vs. 3.53 mmol/L). The interaction between housing system and age showed the highest value of CHOL in blood serum of 34-week-old hens kept in cages (3.90 mmol/L), which is associated with the higher level of stress. The level of CHOL in blood serum of hens kept in cages dropped in the next monitored period (50 wk of age) to 2.83 mmol/L. This finding may indicate that the hens got used to the housing system. The level of CHOL decreased when comparing the beginning and the end of the monitored period, which corresponds with the findings from Suchý et al. (2001) and Burnham et al. (2003). However, average values were higher (5.26–7.19 mmol/L) than the values discovered in this study (2.30–4.11 mmol/L). On the other hand, Suchý et al. (1999) and Pavlík et al. (2007) noted that the highest rise of CHOL concentration occurred in the middle of the laying period. Such a trend in CHOL concentration was found only in the CGS hens from cages. The dynamics of changes in CHOL concentration during the laying period may have been caused by stress (Puvadolpirod and Thaxton, 2000) and by laying intensity (Suchý et al., 1999). Monitoring of the activity of the enzyme AST is closely related to energy, protein, and fat metabolism. Aspartate aminotransferase represents changes in the permeability of liver cell membranes and hence the functionality of the liver parenchyma (Goncalves et al., 2010). Aspartate aminotransferase was significantly influenced only by the housing system ($P = 0.0343$), where higher values were found in hens from cages in comparison with hens from litter (3.46 vs. 2.96 μkat/L). Higher concentration of AST means a higher load on the liver cells. Goncalves et al. (2010) point out that laying intensity is a factor that significantly influences the liver function. The consistent stress load results in increased AST activity and simultaneously in increased concentration of CHOL and GLU in the blood serum of cage-housed hens, which suggests that stress occurs in cage housing system in the long-term point of view (Everds et al., 2013). Values of AST and CHOL were significantly higher in the blood serum of hens kept in cages than in that of hens kept on litter. Values of GLU were not higher significantly but were higher in cage housed hens numerically. A higher concentration of AST and CHOL refers to higher level of catecholamines (dopamine and epinephrine), which was determined higher in the blood serum of hens from cages with lower production than in that of hens with higher production (Cheng at al., 2001). Total

protein in blood serum was significantly ($P = 0.0392$) influenced by the age of the hens and the higher values were found in the blood serum of younger hens. Other observed factors did not affect proteinemia values. These findings are in accordance with the results of Pavlík et al. (2007), who also found no changes in TP in blood serum in different housing systems, but in contrast to our results, the differences among the values of TP were very slight depending on the age of the hens. The values varied between 52 and 56 g/L, whereas the data of this study showed variability in TP values from 43.53 to 56.90 g/L. These differences may be caused by the use of different hen genotypes. Two native breeds of hens (the CGS hen and the OR hen) were used in this study, whereas Pavlík et al. (2007) used a commercial hybrid Isa Brown. The decrease of TP with the age of the hens could be caused by the quality of the protein contained in the feed mixture, especially by the content of essential amino acids (Pavlík et al., 2007). The higher value of TP means better health condition of the animal (Marono et al., 2017). The effect of all observed factors on ALB was calculated as nonsignificant. Cerolini et al. (1990) similarly did not find any significant effect of genotype but found a significant effect of age on ALB concentration in the blood serum. These distinctions may be connected with the age of hens, when the observations were made (at 18, 30, 36, 58, and 67 wk of age) as well as with the used genotypes (Warren (ISA) and Golden-Comet (Hubbard)). As mentioned previously, this study used CGS hens and OR hens at the age of 34, 42, and 50 wk. In addition, the trends of ALB depending on age considerably differ. Findings from Cerolini et al. (1990) show an obvious increase of the ALB with the age of the hens, whereas findings of this study show very inconsistent concentrations of blood serum ALB. The results from the study by Gyenis et al. (2006) confirm the increasing trend of ALB concentration in blood serum with the age of the hens. Because there have been no major changes in blood GLU levels, which are considered as a main source of readily available energy (Nasrel-din et al., 1988), proteins cannot be assumed to serve as an alternative source of energy. The level of GLU in blood serum was significantly influenced by the interaction between breed and housing system ($P = 0.0374$) and by the interaction between housing system and age ($P = 0.0101$). The combination of housing system with breed had a significant effect on the concentration of GLU in blood serum. The highest value of this interaction had CGS hens kept on litter (17.18 mmol/L) and the lowest had OR hens kept on litter (14.72 mmol/L), whereas the values of both breeds kept in cages differed slightly (15.61 vs. 16.73 mmol/L). This may indicate a different demand on energy utilization and the level of glycemia in relation to the body constitution of concrete breed and its physical activity in concrete housing system. Regarding the interaction between housing system and age, in context of the cage housing system and age, the stress affected the concentration of GLU, which decreased linearly with the age. This trend was also observed in the concentration of CHOL, which may indicate that hens are getting used to the concrete housing system. The age did not significantly affect the level of GLU, which means that the prompt energy required for egg laying was sufficiently covered from the feed. The results from the study by Pavlík et al. (2007) also show a nonsignificant effect of the housing system on glycemia. However, on the contrary to the findings of this study, Onbasilar and Aksoy (2005) discovered the effect of age as being significant. The dynamics of changes in GLU concentration during the monitored period showed higher average values than reported by Pavlík et al. (2007). The concentration of GLU varied between 12.98 and 20.70 mmol/L and reached average value around 16 mmol/L, whereas Pavlík et al. (2007) determined values which varied between 12.5 and 14 mmol/L. In most of the groups of laying hens, glycemia decreased in the middle of the monitored period, at 42 wk of age, whereas Pavlík et al. (2007) discovered a decrease in glycemia at 75 wk of age and Onbasilar and Aksoy (2005) at 56 wk of age. The critical thing was, as can be seen from blood GLU and TAG values, hens' age of 42 wk, when hens that were kept in cages experienced an increase in TAG, which is accompanied by an increase of glycemia and AST activities in the OR hens. Total protein values suggest a certain blood dilution. Statistically, these findings showed a significant difference in both glycemic and TAG values depending on age and housing system. Therefore, it can be stated that hens' age of 42 wk means a significant energy burden for caged laying hens, which means a compensation from fat resources, because the supply of ready energy is insufficient.

The concentration of cholesterol in the yolk was significantly affected by breed ($P = 0.0199$), housing system ($P = 0.0001$), age ($P = 0.0001$), and by the interaction between breed and housing system ($P = 0.0406$). Basmacioğlu and Ergül (2005) found the significant effect of genotype on concentration of cholesterol in the yolk. In addition, Rizzi and Chiericato (2010) add that the higher concentration of cholesterol in the yolk is typical for eggs from native breeds in comparison with commercial hybrids, which is caused by lower laying intensity of native breeds. Zemková et al. (2007) confirm that both, housing system and age, significantly influence the concentration of cholesterol in the yolk. Matt et al. (2009) simultaneously determined that higher values of cholesterol in the yolk concentration are in eggs from alternative housing systems (489 mg/100 g) and lower in eggs from cages (341 mg/100 g), which is in accordance with the results of this study (11.62 vs. 10.45 mg/g). Statistically significant interaction between breed and housing system in the concentration of cholesterol in egg yolk may be caused by the fact that the effect of breed and housing system were determined as significant even separately. Therefore, the interaction was calculated as significant. According to Griffin (1992), the level of egg yolk cholesterol is very resistant to change. The present review argues that because of the particular mechanisms involved in yolk formation. Yolk precursors are synthesized in the liver

of the laying hens and transported in the plasma to the ovary, where they are taken up into the developing follicles by receptor-mediated endocytosis. Therefore, the cholesterol content of the yolk is primarily dependent on the cholesterol content of triglyceride-rich lipoproteins. The concentration of cholesterol in the yolk may be in relationship with the concentration of cholesterol in blood (Pavlík et al., 2007). On the other hand, Vogt et al. (1990) claim the opposite.

Egg Quality Parameters

A large number of authors, such as Hanusová et al. (2015), Samiullah et al. (2017), and Sokołowicz et al. (2019), previously studied the factors that influence egg quality, including genotype, housing system, and age of hens and confirmed their effect. Most of previously determined results were also found in this study.

The significant effect of housing system on EW was previously confirmed by the number of authors including Lewko and Gornowicz (2011) and Kraus et al. (2019). Unlike the results of this study (53.51 g from litter vs. 51.86 g from cages), both of these authors found that EW is higher in eggs from cages than in eggs from litter. The difference of the results may be caused by genotypes, which were used. This study, in contrast with the studies of Lewko and Gornowicz (2011) and Kraus et al. (2019), was made with native breeds of hens, which reach better results in noncage systems. The significant effect of age was confirmed by Zita et al. (2009) and Sokołowicz et al. (2019). The significant effect of age on ESI was also found by Yilmaz Dikmen et al. (2017). Sokołowicz et al. (2018) calculated a nonsignificant interaction between genotype and housing system in ESI unlike in this study, where the interaction was calculated as significant. Sirri et al. (2018) confirmed the statistically significant effect of age on ESA, but did not observed the effect of housing system on this parameter, which was significant in this study. However, Kraus et al. (2019) determined the significant effect of housing system on ESA with higher values in eggs from cages than in eggs from litter. These results are in contrast with the results of this study, where the values of ESA were determined higher on litter in comparison with cages (78.82 vs. 77.01 cm^2). Also these results may be influenced by different genotypes used in these studies (native breeds vs. commercial hybrids). Regarding the eggshell parameters, Lewko and Gornowicz (2011) found the significant effect of age on ESP; the results showed highest ESP in eggs from free range (9.93%), followed by eggs from cages (9.03%) and from litter (8.77%). This study showed opposite results, the ESP was higher in eggs from litter than from cages (9.62 vs. 9.40%). The study from Ketta and Tůmová (2014) included a breed of CGS hens, but did not find any significant effect of housing system. Nevertheless, they found the significant interaction between genotype and housing system, which was also determined in this study. Sokołowicz et al. (2018) confirmed the significant effect of housing system on EST using different housing systems (organic, litter, and free range), whereas Kraus et al. (2019) confirmed this finding using same housing systems (cages and litter) and determined same results as this study (EST was higher in eggs from litter than in eggs from cages). In terms of this study, this may be again in relationship with the used native breeds and their higher suitability in noncage systems. Moreover, Sirri et al. (2018) confirmed the significant effect of age on EST. Sokołowicz et al. (2018) determined the significant interaction between genotype and housing system, which corresponds with the results of this study. Moreover, Sokołowicz et al. (2018) confirmed the significant effect of housing system on ESS. On the other hand, Yilmaz Dikmen et al. (2017) did not find this factor as significant. Differences of these studies may be caused by the comparison of different housing systems (organic, litter, and free range vs. conventional cages, enriched cages and free range). Nevertheless, Zita et al. (2009) calculated the significant interaction between genotype and age in ESS and confirmed the findings of this study. Furthermore, Yilmaz Dikmen et al. (2017) and Kraus et al. (2019) determined the interaction between housing system and age in ESS. Both of these studies used Lohmann Brown hens (commercial hybrid). Yilmaz Dikmen et al. (2017) calculated this interaction as statistically significant, which is in accordance with the results of this study. However, Kraus et al. (2019) did not find this interaction as significant. The differences between results of Yilmaz Dikmen et al. (2017) and Kraus et al. (2019) may be caused by the length of each study, because the used hen genotype was the same. Statistically significant effect of genotype on ESC was determined by Sokołowicz et al. (2019), who used native breeds and commercial hybrid. The effect of housing system on ESC was also confirmed by Samiullah et al. (2015) and Kraus et al. (2019), but the results from Sokołowicz et al. (2018) showed the opposite. Statistically significant effect of age on ESC confirmed various authors, such as Zita et al. (2009), Samiullah et al. (2015), and Kraus et al. (2019). The significant interaction between housing system and age in ESC was calculated by Kraus et al. (2019), which corresponds with findings of this study. Regarding the assessment of internal egg parameters, statistically significant effect of genotype on AI was not confirmed by Zita et al. (2009), who used commercial hybrids and neither by Hanusová et al. (2015), who used 2 breeds including OR hens. Statistically significant interaction between genotype and housing system was calculated in AI also by Ledvinka et al. (2012). Statistically significant effect of genotype on HU was previously determined by authors such as Zita et al. (2009), Sokołowicz et al. (2018), and Sokołowicz et al. (2019). The significant interaction between genotype and housing system was determined in HU by Sokołowicz et al. (2018), which is in accordance with the results of this study. Furthermore, statistically significant effect of age on AP, AI, HU, YP, and YI was found by Yilmaz Dikmen et al. (2017). Statistically significant interaction between genotype and housing system in AP was determined by Svobodová et al. (2014),

who also included CGS hens. Unlike the results of this study, Ledvinka et al. (2012) calculated the interaction between genotype and housing system in YI as nonsignificant. The effect of age on YAR was found as significant also by Kraus et al. (2019).

Kraus et al. (2019) determined same trends in EW, ESI, ESA, EST, and HU, whereas Zita et al. (2009) found the same trends in AP, AI, YP, and YI. Moreover, the findings from Kraus et al. (2019) confirm irregular trends in ESP and Zita et al. (2009) in ESS. Sokołowicz et al. (2019) found the increasing trend in ESC in contrast to the results of this study. The use of the different hen genotypes may be the reason why the results vary. Van den Brand et al. (2004) also found the increasing trend of YAR, but the increase was not regular as in the results of this study. The difference may be caused by the higher number of monitored periods.

CONCLUSION

The consistent stress load could cause an increased AST activity and simultaneously an increased concentration of CHOL and GLU in the blood serum of cage-housed hens, which suggests that stress may occur in cage housing system in the long-term point of view. Values of GLU were not higher significantly but were higher in cage-housed hens numerically. The supportive statement of increasing level of catecholamines (dopamine and epinephrine) results in higher concentration of AST and CHOL, which could indicate the truthfulness of the assumption that stress affects blood serum concentrations of observed parameters in terms of housing system. The higher value of TP means a better health condition of the animal. The combination of housing system with breed had a significant effect on the concentration of GLU in the blood serum. This may indicate a different demand on energy utilization and the level of glycemia in relation to the body constitution of concrete breed and its physical activity in concrete housing system. Regarding the interaction between housing system and age, in context of the cage housing system and age, the stress affected the concentration of GLU, which linearly decreased with the age. This trend was also observed in the concentration of CHOL, which may indicate that hens are getting used to the concrete housing system. Statistically significant interaction between breed and housing system in concentration of cholesterol in the egg yolk may be caused by the fact that the effect of breed and housing system were determined as significant even separately. The relationship between concentration of cholesterol in the blood serum and concentration of cholesterol in the yolk was previously confirmed by some authors, but some authors disproved this statement.

In terms of egg quality, the results showed that the litter housing system is more suitable for used native breeds (CGS and OR hens). When comparing the litter and cage housing systems, significantly higher values were determined in eggs from litter in the most important egg quality parameters such as EW, EST, and ESS. Higher values were found also in eggs from litter in other important quality parameters including AI, HU, and YI. The values of these parameters were higher only numerically, not statistically.

The obtained results are an interesting comparison of the dynamics of changes in biochemical blood and egg quality parameters of Czech and Slovak original hen breeds during the laying cycle housed in 2 different systems. This study is one of the first of its kind because it focuses on the evaluation of biochemical blood indicators of laying hens, which have not been sufficiently studied in the past. Moreover, these indicators were complexly examined and determined for the first time in both of these national breeds, the CGS hens and the OR hens. Indeed, it is necessary to further evaluate these indicators, especially in other genetic resources of hens, where the data are often nonexisting.

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DISCLOSURES

The authors declare no conflicts of interest.

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