

Blood Plasma Mineral Profile and Qualitative Indicators of the Eggshell in Laying Hens in Different Housing Systems

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Abstract

The aim of this study was to compare the blood plasma mineral profile (Ca, P, K, Mg, Zn, Cu and Se) and egg-shell quality (eggshell weight, eggshell breaking strength and thickness) of laying hens housed in three different housing systems (traditional cage system, enriched cage system and deep litter system). In each housing system, 12 ISA Brown laying hens were observed during the laying period from week 22 to 75 of age. The effect ($p < 0.05$ and $p < 0.01$) of age and/or phase of the laying cycle on all mineral concentrations was determined. Eggshell breaking strength decreased ($p < 0.001$) with the age of birds. The results of this study indicate that the housing systems compared had no significant effect on the blood plasma mineral profile of laying hens under study and the values were within the physiological range. However, a significant effect of housing system on eggshell breaking strength and eggshell weight was found. Improved eggshell quality was obtained in most periods of the laying cycle in the enriched cage systems.

Blood minerals, egg-shell quality, standard cage, enriched cage, deep litter floor

Passage of the Council Directive 1999/74/EC has resulted in the replacement of traditional cages with enriched cages, litter technologies or aviaries to improve the welfare of laying hens. The shell quality remains one of the most important issues for the technology of further egg handling (Ledvinka et al. 2000). However, some authors pointed out that there are differences in eggshell quality and proportion of cracked eggs between different housing systems (Abrahamsson et al. 1995; Abrahamsson and Tauson 1997; Wall and Tauson 2002). Shell quality can be influenced by many factors including mineral nutrition. Calcium, magnesium and phosphorus are major inorganic constituents of avian eggshells (Cusack et al. 2003). Simons (1976) found small amounts of potassium, copper and zinc in the palisade layer of the eggshell. The presence of sodium, potassium, magnesium, zinc, and copper was confirmed also in the shell membranes (Wedral et al. 1974). The importance of minerals is reflected in changes of arrangement pattern of shell membrane fibres in relation to the structural composition of the eggshell, for example when using copper- and magnesium-deficient diets (Leach and Gross 1983). Traces of magnesium, potassium, copper and zinc were also found in the egg cuticle. Plasma mineral concentrations during the laying period can be influenced by many factors; such as laying rate and energy requirements (Suchý et al. 2001), partial quantitative feed restriction (Sahin and Kucuk 2001), mineral supplements (Eren et al. 2004), ambient temperature (Siegel 1995; Donoghue et al. 1990; Ching 1992; Belay and Teeter 1993; Večerek et al. 2002), production type (Suchý et al. 2004), age of hens (Cerolini et al. 1990; Gyenis et al. 2006), stress (Beisel 1982; Combs and Combs 1984; Tufft and Nockles 1991; Klasing 1998), exposure to heavy metals (Zralý et al. 2008), etc.

With regard to differences in eggshell quality between different housing systems we presumed also differences in the blood plasma mineral profile of hens. The objective of

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this study was to investigate the effects of different housing systems on plasma mineral profile in relation to eggshell quality. To our knowledge, studies comparing blood plasma mineral profile indicators among hens kept in these three systems have not yet been performed.

Materials and Methods

The experiments were performed in ISA BROWN pullets, kept in a hall with deep litter technology. The available area, complete diet, light-dark (L : D) cycle, temperature of housing, relative humidity of air changed according to technological instructions for ISA BROWN pullets. During the rearing period standard vaccinations were performed. At the age of 15 weeks, the animals were randomly divided into 3 of the following breeding systems:

Traditional cage housing system (S) – four-floor, total (available) area 550 cm²/bird (2 birds kept on 1 120 cm² – 32 × 35 × 45 cm), 2 nipple drinkers, belt feeder 15 cm/bird, device for claw shortening.

Enriched cage housing system (EE) according to Council Directive 99/74/EC – three-floor, total area 945 cm²/bird (8 birds kept on an area of 7 560 cm² – 180 × 42 × 45 cm), available area 643 cm²/bird, 6 nipple drinkers, belt feeder 20 cm/bird, nest (30 × 35 × 45 cm), perching area 15 cm/bird, devices for dustbathing and scratching, device for claw shortening.

Deep litter housing system (DL) – available area 2 000 cm²/bird (20 birds kept on an area of 40 000 cm² – 200 × 200 × 180 cm), tube feeder 5 cm/bird, round drinker 2cm/bird, deep litter made of wood shavings.

All of the housing technologies were situated in the same building with central system of ventilation and temperature regulation. For each technology, experimental group consisting of 12 birds were established with the mean body weight of 1,300 ± 50 g. Throughout the study, the hens were fed a complete diet for laying hens containing 875 g·kg⁻¹ of dry matter, energy content ME_N 11.1 MJ·kg⁻¹, content of nitrogen substances 170.7 g·kg⁻¹, Ca 35.9 g·kg⁻¹ and P 6.3 g·kg⁻¹. A constant light-dark (L : D) cycle (15 : 9, switching on at 4.00 h, switching off at 19.00 h) was maintained in all three technologies as recommended in technological instructions for ISA BROWN hens. The temperature of housing was in the range from 18 to 22 °C; relative humidity ranged from 65 to 70%. No red mite and other parasite or viral infection was found during experimental period. Local Ethics Committee approved the experimental protocol.

Collection of blood samples

Blood samples (5 ml) of all hens in experimental groups were collected from the brachial vein of hens at the age of 22, 47 and 75 weeks, always between 07:00 and 08:30 h. Heparin was used as anticoagulant. Two ml of blood of all samples were centrifuged and the separated plasma was stored at -20 °C until analyzed. Three ml of whole blood were used for Se analysis. Blood sampling was performed randomly in hens kept in standard, enriched and deep litter technology.

Biochemical indicators

In blood plasma the following elements were measured: calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), zinc (Zn), and copper (Cu); selenium concentration (Se) was measured in whole blood. The minerals were analyzed with commercially available kits Bio-La-Tests made by Pliva-Lachema, Inc., Czech Republic, with COBAS MIRA S (Roche, CH). Selenium concentration was analysed by atomic absorption spectrometry (AAS). Samples of whole heparinised blood were mineralized in a closed system using a microwave (MLS-1200, Milestone, Italy) digestion technique with HNO₃ and H₂O₂. Samples were evaporated and the mineral residue was dissolved in water to which 20% HCl was added. Selenium was then determined with Solar 939 AA Spectrometer (Unicam, UK) using a hydride AAS technique.

Determination of the eggshell quality

Eggs of average size were selected on days of blood collection for the respective technologies (eggs that were too big or too small and eggs with damaged or absent eggshell were not included in the analysis). The eggs were marked with a pencil; group designation and serial number were written on each egg to avoid confusion. The eggs were analyzed immediately after the collection. Eggshells were weighed with an accuracy of 0.1 g with laboratory scales after washing with warm water and drying at room temperature for one week (egg membranes were not removed). The eggshell strength (N/cm²) was determined using the Egg Crusher EGC (VEIT Electronic, CZ). Eggshell thickness (mm) is expressed as an average value measured by Digimatic Outside Micrometer (Mitutoyo, JPN) at both poles and in the equator of the egg.

Statistical evaluation

The data are expressed as means ± SEM. Changes in minerals were analyzed by repeated measures ANOVA for factors housing technology as independent variable and age of hens as dependent variable. Egg quality was analyzed by two-way ANOVA for factors housing technology as independent variable and age of hens as dependent variable. ANOVA was followed by post-hoc Fisher's LSD test for pairwise comparisons, where appropriate. All statistical analyses were performed by the Statistica 7.0 statistical software (StatSoft Inc., Tulsa, USA). The overall level of significance was defined as $p < 0.05$.

Results

Plasma calcium significantly increased from the beginning to the end of the experiment ($F(2,66) = 12.616, p < 0.001$) in all housing systems (Fig. 1). At 22 weeks of age, the highest plasma calcium concentrations were found in the birds housed on deep litter floor, whereas at 47 weeks of age this housing system provided the lowest plasma calcium levels. At 47 weeks of age a significantly higher feed intake was found in the DL group ($145.6 \text{ g}\cdot\text{bird}^{-1}\cdot\text{day}^{-1}$ vs. S – $119.1 \text{ g}\cdot\text{bird}^{-1}\cdot\text{day}^{-1}$ and EE – $123.3 \text{ g}\cdot\text{bird}^{-1}\cdot\text{day}^{-1}$). No differences in plasma Ca concentrations, feed intake and daily eggshell production were found between the groups S and EE (Table 1), particularly at 47 weeks of age. At the end of experiment, the lowest Ca concentrations were found in laying hens housed in enriched housing system. No significant differences in calcium concentrations were found between the housing systems. The lowest eggshell production was found in the DL group in all the periods evaluated (Table 1).

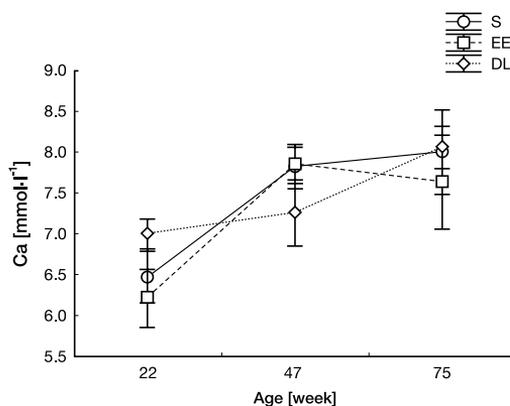


Fig. 1. Plasma calcium concentrations in laying hens kept in traditional (S, $n = 12$), enriched (EE, $n = 12$) and deep litter (DL, $n = 12$) housing system during experimental period. Data represent mean \pm SEM.

Table 1. Egg shell production and feed intake of hens in traditional (S, $n = 12$), enriched (EE, $n = 12$) and deep litter (DL, $n = 12$) housing system during experimental period

	Technology	week 22	week 47	week 75
Egg shell production ($\text{g}\cdot\text{hen}^{-1}\cdot\text{day}^{-1}$)	S	5.14	5.81	4.60
	EE	5.25	5.78	4.83
	DL	3.89	4.66	2.22
Feed intake ($\text{g}\cdot\text{hen}^{-1}\cdot\text{day}^{-1}$)	S	117.3	119.1	111.5
	EE	124.7	123.3	115.5
	DL	126.2	145.6	89.4

Eggshell breaking strength (Table 2) significantly decreased with the age of birds ($F(2,66) = 93.041, p < 0.001$). A significant influence of housing system on eggshell strength was detected ($F(4,66) = 3.302, p < 0.05$). The eggshell strength was lower ($p < 0.05$) in the group S compared to EE at 22 weeks of age. Also eggshell production at 22 weeks of

age was lower for S, and higher plasma Ca content was observed in this group. At 75 weeks of age, a higher eggshell strength was found in the birds housed in the traditional ($p < 0.05$) and enriched ($p < 0.01$) cages compared to those housed on deep litter.

Age had an effect on eggshell weight ($F(2,66) = 63.042, p < 0.001$). For traditional cages, eggshell weight gradually increased throughout the experiment, although an increase ($p < 0.01$) was confirmed only for the period from 22 to 47 weeks of age (Table 2). For the enriched cages and deep litter, an increase ($p < 0.01$) was observed from the beginning of the 47th week of age, and was followed by a decrease till the end of the experiment.

Table 2. Qualitative indicators of egg shell of hens in traditional (S, n = 12), enriched (EE, n = 12) and deep litter (DL, n = 12) housing system during experimental period. Superscripts (a, b) represent significant differences between the groups at $p < 0.05$.

	Technology	n	week 22	<i>p</i>	week 47	<i>p</i>	week 75	<i>p</i>
Shell breaking strength (N·cm ⁻²)	S	12	38.78 ± 1.21 ^a		33.97 ± 0.81		25.76 ± 0.94 ^a	
	EE	12	44.83 ± 1.56 ^b		34.73 ± 1.74		27.50 ± 1.61 ^a	
	DL	12	42.74 ± 1.98 ^{ab}		35.17 ± 1.69		20.38 ± 2.12 ^b	
Shell thickness (mm)	S	12	0.411 ± 0.003		0.403 ± 0.003 ^a		0.325 ± 0.005 ^a	
	EE	12	0.417 ± 0.007		0.417 ± 0.004 ^b		0.343 ± 0.009 ^a	
	DL	12	0.405 ± 0.006		0.407 ± 0.005 ^{ab}		0.378 ± 0.009 ^b	
Shell weight (g)	S	12	5.97 ± 0.063		6.31 ± 0.061 ^a		6.38 ± 0.073	
	EE	12	6.19 ± 0.112		6.66 ± 0.680 ^a		6.4 ± 0.145	
	DL	12	6.00 ± 0.103		6.61 ± 0.116 ^b		6.28 ± 0.146	

The decrease was significant ($p < 0.01$) only for the deep litter technology. Effects of housing technology on eggshell weight were also confirmed ($F(4, 66) = 3.705$, $p < 0.05$). At 47 weeks of age, higher values were found for deep litter ($p < 0.05$) and enriched cages ($p < 0.01$) compared to traditional cages. Egg weight was also higher deep litter and enriched cages (EE, DL) than for the technology S.

The housing system had no effect on blood plasma phosphorus concentrations. Plasma phosphorus concentrations were decreasing in all the housing systems from the beginning to the end of the experiment (Fig. 2). During the laying period significant changes in factor time were found ($F(2,66) = 30.051$, $p < 0.001$). Fisher's post hoc test showed a decrease ($p < 0.01$) in week 22 for cage systems.

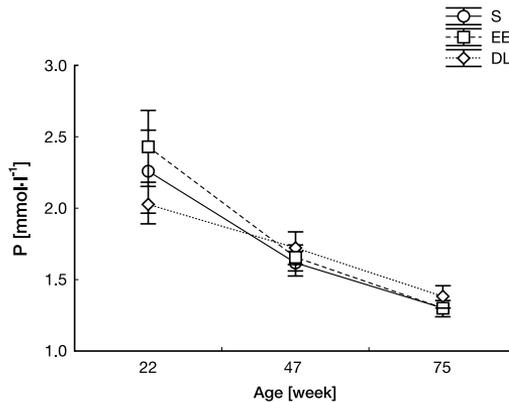


Fig. 2. Plasma phosphorus concentrations in laying hens kept in traditional (S, n = 12), enriched (EE, n = 12) and deep litter (DL, n = 12) housing system during experimental period. Data represent mean ± SEM.

The age had a significant effect on potassium concentrations ($F(2,66) = 6.036$, $p < 0.01$). Potassium levels were increasing from the beginning of the experimental period to 47 weeks of age for all the housing systems (Fig. 3). A decrease ($p < 0.05$) followed again till 75 weeks of age for the enriched cages and deep litter. Only for the traditional cages plasma potassium levels were increasing till the end of the experimental period. The housing system also affected blood plasma potassium concentrations ($F(2,33) = 5.341$, $p < 0.01$). The lowest concentration ($p < 0.05$) was found for deep litter at 22 weeks of age

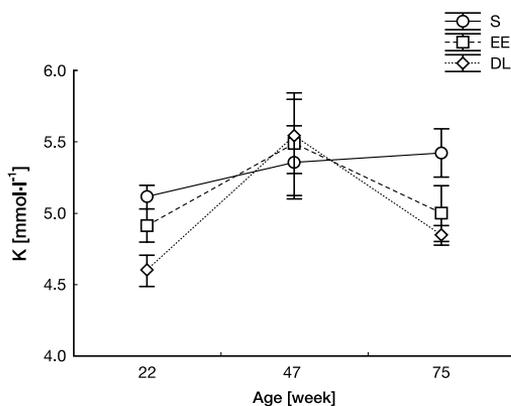


Fig. 3. Plasma potassium concentrations in laying hens kept in traditional (S, $n = 12$), enriched (EE, $n = 12$) and deep litter (DL, $n = 12$) housing system during experimental period. Data represent mean \pm SEM.

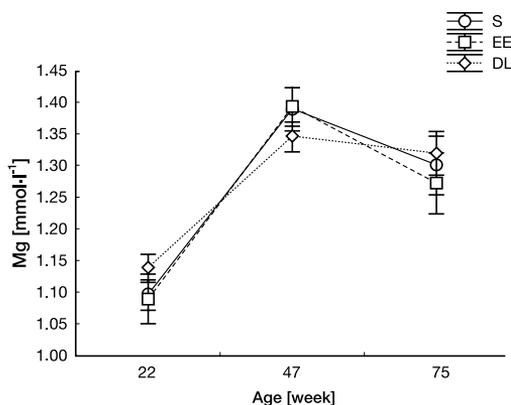


Fig. 4. Plasma magnesium concentrations in laying hens kept in traditional (S, $n = 12$), enriched (EE, $n = 12$) and deep litter (DL, $n = 12$) housing system during experimental period. Data represent mean \pm SEM.

of birds. A significant difference ($p < 0.05$) was also found at 75 weeks of age between the traditional cages and deep litter.

The housing system was found to have no influence on blood plasma magnesium levels in laying hens (Fig. 4). On given dates the values for the different housing systems showed no marked differences. Differences were found for factor time ($P(2,66) = 44.972$, $p < 0.001$). Magnesium levels significantly increased from the beginning of the experiment to 47 weeks of age ($p < 0.01$) for all the housing systems. The subsequent decrease of mean values at 75 weeks of age in all the groups was not significant.

A similar tendency was observed in plasma zinc levels (Fig. 5). An effect was found again in the age of laying hens ($P(2,66) = 38.948$, $p < 0.001$). Plasma zinc levels increased from the beginning of the experiment to 47 weeks of age ($p < 0.01$) in all the housing systems, and the increase was followed by a non-significant decrease in mean values at 75 weeks of age in all the groups. The highest values were found for the traditional cages during the entire experimental period, whereas the lowest mean values were found for deep litter. No significant effect of housing systems on plasma zinc levels was found.

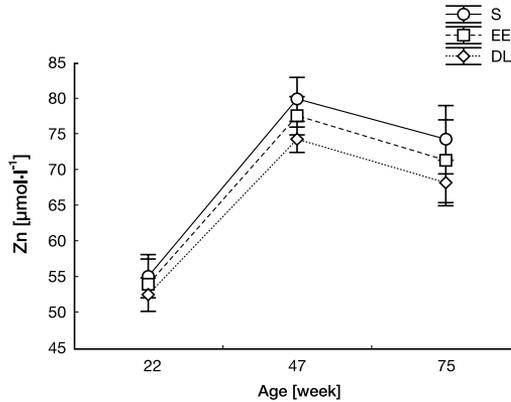


Fig. 5. Plasma zinc concentrations in laying hens kept in traditional (S, $n = 12$), enriched (EE, $n = 12$) and deep litter (DL, $n = 12$) housing system during experimental period. Data represent mean \pm SEM.

The age of hens had an effect on blood plasma copper concentrations ($F(2,66) = 6.653$, $p < 0.01$). Mean values increased in all the housing systems from 22 weeks of age (Fig. 6), and the increase was for enriched cages ($p < 0.01$) and deep litter ($p < 0.05$). It was followed by a non-significant decrease in values. The housing system had no effect on plasma copper levels.

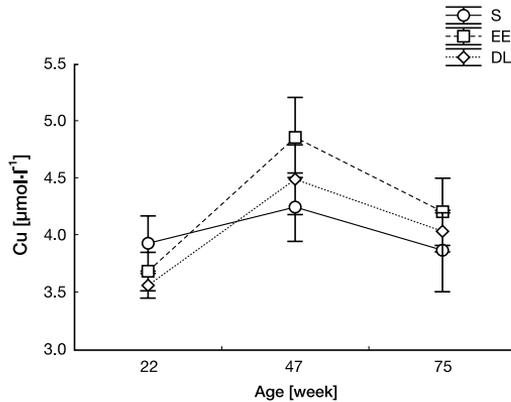


Fig. 6. Plasma copper concentrations in laying hens kept in traditional (S, $n = 12$), enriched (EE, $n = 12$) and deep litter (DL, $n = 12$) housing system during experimental period. Data represent mean \pm SEM.

The highest blood selenium levels in laying hens were determined at the beginning of the experiment at 22 weeks of age (Fig. 7). During the experiment, a decrease was found at 47 weeks of age ($F(2, 66) = 131.27$, $p < 0.001$) and subsequent testing confirmed differences ($p < 0.001$) between all the housing systems. No significant decreases were found until the end of experiment. Housing technology was confirmed to have an effect ($F(4, 66) = 3,8.27$, $p < 0.05$) on blood Se levels. Blood selenium levels were found to be higher ($p < 0.05$) in laying hens housed in traditional cages than in those housed in the enriched cages.

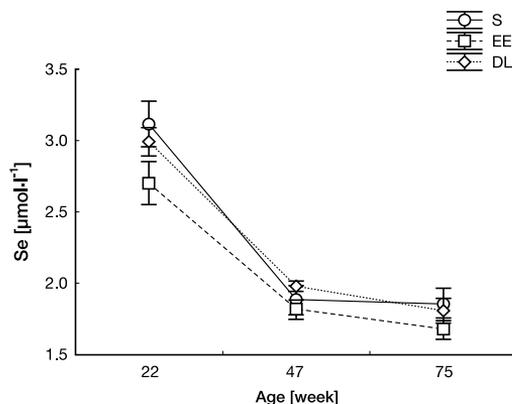


Fig. 7. Plasma selenium concentrations in laying hens kept in traditional (S, n = 12), enriched (EE, n = 12) and deep litter (DL, n = 12) housing system during experimental period. Data represent mean \pm SEM.

Discussion

None of the blood plasma minerals evaluated, except for potassium, was significantly influenced by the housing systems. This was to a certain extent caused by the uniform diets for all groups of birds under study because dietary levels of macro- and microelements significantly affect plasma mineral levels (Sooncharernying and Edwards 1989; Bain 1992; Petrovič et al. 2006).

A considerable increase in plasma Ca levels at the beginning of laying period and subsequent gradual increase was also observed by Cerolini et al. (1990), Gyenis et al. (2006), Filizciler et al. (2002), and Strakova et al. (1994). On the contrary, Burnham et al. (2003) described an increase in calcium levels before the laying period and a decrease in calcium levels during the laying. Also Eren et al. (2004) reported decreasing plasma calcium levels in laying hens from 22 to 28 weeks of age; however, these changes were not significant. Suchy et al. (2001) and Kurtoglu et al. (2001) reported mean Ca levels similar to the mean values found in our experiment. Our values also fall within the physiological range as described by Belay and Teeter (1996). Lower values as compared with our results were reported by Koelkebeck and Odom (1995), Sahin et al. (2002b), and Eren et al. (2004).

For hens housed in cages, higher eggshell production was found to be associated with lower plasma calcium levels, and vice versa. Increasing plasma Ca levels were associated with decreasing eggshell strength and thickness. This finding to a certain extent corresponds with the results of Rezac et al. (2000), who determined the highest plasma Ca levels in laying hens producing eggs with damaged shells. On the other hand, Hester et al. (1980) reported that a decrease in blood plasma Ca levels had no significant effect on eggshell quality. Other authors also reported that plasma calcium content does not correlate with eggshell quality (Buss and Stout 1981; Jerabek et al. 1993). Higher plasma Ca levels at 22 weeks of age in DL could have been caused by a potential intake of litter as described by Millan et al. (2003), who found the effect of a diet with higher fibre content on blood chemistry in birds, e.g. increased plasma calcium concentrations. In deep litter technology, the lowest eggshell production was found, but given the free housing the hens could have eaten the eggs which might have biased the result of eggshell production. Feed intake was also very irregular during the experimental period.

The lowest eggshell strength, thickness and weight were found in eggs produced in traditional cage technologies at the beginning and in the middle of the experimental

period. The highest mean values of these indicators were found in the enriched cages. Bain (1992) in accordance with results of this study reported a thinning of the eggshell with an increasing number of laying hens per area unit. Leyendecker et al. (2002) and Pistekova et al. (2006), unlike other researchers, found no significant differences in eggshell strength between cages and deep litter. These authors reported higher eggshell weight for traditional cages than for deep litter, which does not correspond with this study's results, either.

Mean values of plasma phosphorus in laying hens of all groups decreased from the beginning of the laying cycle to the end at 75 weeks of age, which corresponds with the results of Strakova et al. (1994) and Eren et al. (2004). On the contrary, Filizciler et al. (2002) and Suchy et al. (2004) found a gradual increase in plasma phosphorus levels in laying hens up to 38 weeks of age. Suchý et al. (2004) reported a subsequent decrease of values with varying tendencies. The values we obtained from all the housing systems ranged within mean values reported e.g. by Jerabek et al. (1993), Koelkebeck and Odom (1995), Belay and Teeter (1996), Kaya et al. (2001), Suchy et al. (2001), Sahin et al. (2002a), and Thiemel and Jelinek (2004). Substantially higher values were published by Eren et al. (2004), whereas lower values were reported by Kurtoglu et al. (2001). Boorman and Gunaratne (2001) reported that there is in fact no relationship between plasma phosphorus levels and eggshell weight, which is in accordance with our results given the decrease in plasma P levels and varying changes in eggshell weight during the laying period.

Despite the varying tendencies, the average values of plasma potassium levels gradually increased from the beginning until 47 weeks of age, which does not correspond with findings of Strakova et al. (1994), who observed a significant reduction in plasma potassium levels during the laying cycle. On the other hand, Koelkebeck and Odom (1995) reported no significant changes in plasma potassium levels under ambient temperature. Mean values similar to those received in this study were reported by Suchy et al. (1989; 2001), Gezen et al. (2005), and Gyenis et al. (2006). Lower mean plasma potassium levels were reported by Koelkebeck and Odom (1995). The results of this experiment correspond to a certain extent with the findings of Strakova et al. (1994), who noted increased magnesium levels during the laying period. Slight changes in plasma potassium levels during the laying period were reported by Eren et al. (2004). Mean values found in this study correspond with the range of values reported for instance by Suchy et al. (2001) and Kurtoglu et al. (2002). Lower values were published by Koelkebeck and Odom (1995), Kurtoglu et al. (2001), and Thiemel and Jelinek (2004).

Filizciler et al. (2002) reported a similar range of plasma Cu levels compared to our results (3.56 - 4.86 $\mu\text{mol}\cdot\text{l}^{-1}$). Copper levels increased during the experiment from 22 to 47 weeks of age. From 47 to 75 weeks of age, a decrease in plasma Cu levels was observed in all the housing systems. Also Filizciler et al. (2002) reported increasing plasma copper levels from the beginning of the laying period. Plasma zinc levels considerably increased from 22 to 47 weeks of age in all the housing systems. From 47 weeks of age to the end of the experiment, plasma zinc levels decreased in these technologies. A similar increase in zinc concentration was reported by Jankowski et al. (2003). Opposite tendencies were observed by Filizciler et al. (2002) when evaluating zinc concentrations during the period from 26 to 38 weeks of age. Decreased plasma zinc levels in laying hens in all the housing systems in the second half of the experiment were within the physiological range. Changes in plasma zinc levels during the experimental period were also associated with circulation of vitellogenin in relation to the reproductive status (Mitchell and Carlisle 1991). Kaya et al. (2001) found a positive correlation between plasma zinc concentrations and egg production. These authors reported a range of values similar to the mean values obtained in this experiment.

Mean blood selenium concentrations in laying hens decreased for all housing systems from the beginning to the end of the experiment. Petrovič et al. (2006) reported mean blood selenium levels in laying hens during the laying period of $1.7 \mu\text{mol}\cdot\text{l}^{-1}$, while the values in this experiment ranged from 1.60 to $3.11 \mu\text{mol}\cdot\text{l}^{-1}$.

The results of this study indicate that the housing systems compared had no significant influence on the blood plasma mineral profile in laying hens and values were within the physiological values. However, the age of laying hens or the phase of the laying cycle had a certain effect on changes in mineral levels. Better qualitative indicators of eggshell were found in most time periods of the laying cycle for the enriched cages. In several cases, these differences were significant.

Minerální profil krevní plazmy a kvalitativní indikátory skořápky u nosnic v různých technologických systémech ustájení

Cílem práce bylo porovnat ukazatele minerálního profilu krevní plazmy (Ca, P, K, Mg, Zn, Cu a Se) a kvality skořápky (hmotnost, pevnost a tloušťka skořápky) u nosnic ustájených ve třech různých systémech (tradiční klecový systém, obohacený klecový systém a hluboká podestýlka). V každém ze systémů ustájení bylo sledováno 12 nosnic hybridní kombinace ISA Brown v průběhu snáškového cyklu od 22. do 75. týdne věku. Byl zaznamenán průkazný vliv ($p < 0,05$ a $p < 0,01$) věku (fáze snáškového cyklu) na koncentraci všech analyzovaných minerálních prvků. Pevnost vaječné skořápky se snižovala ($p < 0,001$) s věkem zvířat. Na základě dosažených výsledků lze konstatovat, že srovnávané technologie ustájení signifikantně neovlivnily sledované ukazatele minerálního profilu krevní plazmy nosnic a pohybovaly se v rozmezí fyziologických hodnot. Ačkoli byl zaznamenán průkazný vliv technologie ustájení na pevnost a hmotnost skořápky. Nejvyšší kvalita vaječné skořápky byla zaznamenána ve většině případů v obohacené klecové technologii.

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