THE INFLUENCE OF CALCIUM INTAKE DURING THE PERIOD OF SEXUAL MATURATION OF PULLETS ON ITS RETENTION AND EGGSHELL QUALITY OF LAYING HENS

Andrea Roztočilová[1], Ondřej Šťastník[1], Jakub Novotný[1], Leoš Pavlata[1], Ladislav Zeman[1], Eva Mrkvicová[1]

[1] Department of Animal Nutrition and Forage Production, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Abstract

The aim of the study was to determine the calcium requirements in preparation period of the sexual mature and egg-laying and evaluate development of calcium retention in the first laying period of the hybrid combination Bovans Brown hens. The experimental trial lasted from 14 to 30 weeks of hens age. The animals were fed *ad libitum* with a pelleted complete feed mixture with 42 g/kg of calcium content. The amount of calcium retained by pullets gradually increased along with feed intake depending on age and preparation for sexual maturity and was around 0.39 ± 0.19 g Ca in the first period. Ca retention increased up to an average of 0.85 g Ca from 8 day before laying the first egg. Retention dropped sharply on the day of laying the 1st egg and a negative Ca balance occurred. Shell output was 1.75 ± 0.21 g/day for the beginning of laying and subsequently increased to 2.23 ± 0.08 g/day during evaluate peak laying. The range weight of the first laid eggs was 45 ± 5 g. Percentage of eggshell move around an average of $10 \pm 0.37\%$ throughout the observed period. The daily calcium intake of pullets was 3.56 ± 0.26 g/day. With the beginning of laying, the daily intake increased to an average of 4.51 ± 0.43 g/day Ca. Before reaching sexual maturity, the calcium apparent digestibility of laying hens was relatively low, averaging around 22%, but the moment the hens began to lay, the apparent calcium digestibility began to increase to an average of 50%. Feeding a non-standard feed mixture with a higher calcium content than usual in mixtures for pullets did not have a negative effect on the finishing of animal development, the onset of sexual maturity, or deterioration of the observed parameters of eggs quality.

Keywords: Ca retention, quality of eggshells, Ca apparent digestibility, eggshell strength

INTRODUCTION

Layers belong to birds that lay eggs in long laying periods, during which they excrete a large amount of calcium in the shells. During the calcification they excretion in shells 2 to 3 g daily (Bar, 2009). The primary function of the shell is to protect the egg contents from external mechanical influences and microorganisms, which can affect the resulting quality and soundness of the eggs, or the hatchability of reproductive eggs (Nedomová *et al.*, 2013). Shell quality is very important for eggs producers. This quality is influenced by many factors, such as the age of the hens, the order of the eggs in the laying, nutrition, the health of the hen and mineral metabolism. Calcium affects eggshell most of the minerals contained in the feed. Calcium plays many important roles in the body. It participates in bone mineralization, maintenance of acid-base balance, blood clotting, coordination of neuromuscular excitability, and in laying hens it plays an important role in the formation of shells and subsequent influence on their quality (Kleyn, 2013; Zelenka, 2017). Increasing the intensity of laying requires increasing content of calcium in

the mixtures. Calcium content in feed mixtures for laying hens has doubled during the last 50 years. It was moved from values of 2.1% around 1956 to 4% in 2005 (Lukić, 2009). Requirement of calcium content in feed for hens changes during life depending on the current reproductive phase. Pullets has different calcium requirement compared to calcium requirement of laying hens (Zelenka, 2017). The changing values of the calcium content of the feed depending on the requirements of the animal are according by the NRC standard (NRC, 2005). Ca must be added to the feed ration during its preparations because in plants, are low contain. Inorganic minerals or other organic compounds are added to the feed mixture as a calcium sources (Kleyn, 2013).

Ground limestone or oyster shells are the most commonly used calcium sources (NRC, 2005). Individual ability of the animal to retain calcium from the source and further distribute it to the places of requirement is important factor. Understanding to the basic mechanisms of Ca retention and eggshell formation is essential for good nutrition of laying hens during their life and for preparing feed mixture with adequate contain of Ca according to requirements of hens. The aim of the study was to determine the calcium requirements in preparation period of the sexual mature and egg-laying and evaluate development of calcium retention in the first hens laying period.

MATERIALS AND METHODS

The experimental period evaluating feed intake and calcium retention in laying hybrids lasted from 14 to 30 weeks of age. This period was divided into the 3 period. The first period include time pre-laying period, when pullets were preparing for laying and finished their growing (from 14. to 18. week). Second period include days, when half of flock laying eggs (from 129 to 140 day of age). The third period is time, when all hens are mature and laying eggs every day (from 141 day of age to finished trial).

Laying Hens and Husbandry

Twelve pullets and later laying hens hybrid combination by the Bovans Brown were used for this experiment. They were breeding in individual balance cages located in the accredited experimental stables of the Mendel University in Brno. Animals were kept in balance cages. The cage has a length and height of 52 cm and a width of 30 cm. Each cage is equipped with a water system with nipples, an individual feeder, a slatted floor enabling individual collection of droppings and a hatching area for eggs.

The experiments were realised accordance with the wording of Act no. 246/1992 Coll., on the protection of animals against cruelty, as amended and regulations. The technology and method of breeding corresponded to the regulation in decree number 208/2004 (Decree on minimum standards for the protection of farm animals), on minimum standards for the protection of farm animals. The temperature in the stable was 21–23 °C, the humidity around 50%. The light regime was set to 16 hours of light and 8 hours of darkness with a light intensity in the range of 5–10 lux in accordance with the technological instructions for this hybrids (Integra, 2018). The animals were checked daily. Furthermore, eggs were collected daily from individual hens. Once every three days (one balance period) the feed residues were weighed and all excreta were individually and quantitatively collected from individual animals. 37 balances were performed from all hens during the experimental trial.

Experimental Diets

Experimental feed mixture containing calcium (42.15 g/kg) was prepared before stocking the animals. It was used ultrafine limestone as a calcium source for experimental and granulation reasons. Chromium oxide indicator (5 g/kg) was added to the mixture. The mixture was prepared in accordance with the publication Recommended content of nutrients in feed mixtures and nutritional value of feed for poultry (Zelenka, Heger and Zeman, 2007). The feed components were shredded on a hammer mill to a particle size of 3 mm. The mixture was mixed in a Nautamix vertical homogenizer and pelleted using a BONSAI granulator from KOVO NOVÁK (Citonice, CZ) to a granule size of 3 mm. The detailed composition of the feed mixture per 1 kg is shown in Tab. I.

The individual components and the experimental feed mixture were chemically analysed in accordance with the official methods of analysis (Latimer, 2019). Sampling of the components and the mixture, including subsequent analyses, were carried out in accordance with the instructions for sampling and performing laboratory analyses resulting from Commission Regulation (EC) No. 152/2009 for the purposes of official feed control. Tab. II shows the results of the chemical analysis of the experimental feed mixture in 88% dry matter. Energy value of compound poultry feed was calculated:

MJ/kg of ME =

$= 0.1551 \times \%$ crude protein $+ 0.3431 \times \%$ crude fat $+$

$+ 0.1669 \times \%$ starch $+ 0.1301 \times \%$ total sugar

(expressed as sucrose).

The hens were fed *ad libitum*. The weight of feed and feed residues was measured during the 3 days period, and feed consumption was calculated from these. The pullets were stocked at the age of 13 weeks, habituation period to feed the pelleted feed mixture was started.

I: *Composition of experimental diet*

Components	Quantity in 1 kg of feed mixture	Units
Maize	400.00	g
Soybean meal extracted	260.00	g
Wheat (Vanesa)	184.50	g
Rapeseed oil	25.00	g
Calcium carbonate	100.00	g
Monocalcium phosphate	14.50	g
Cr_2O_3 (pa)	5.00	g
Sodium bicarbonate	2.50	g
Sodium chloride	2.50	g
Methionine (Rhodimet NP99)	2.50	g
Valine	0.50	g
L-lysine (78%)	0.50	g
L-threonine (98%)	0.50	g
Premix 11905 AG-NP-M [*]	2.00	g

* Premix composition (contents in 1 kg of feed mixture): Zinc 110 mg, manganese 120 mg, iron 60 mg, copper 15 mg, iodine 2 mg, selenium 0.3 mg, vitamin A 10.000 IU, vitamin D3 3,000 IU , vitamin C 104 mg, vitamin E 100 mg, vitamin B2 16 mg, vitamin K3 6 mg, vitamin B6 6 mg, vitamin B1 4 mg, vitamin B12 0.04 mg, Biotin 0.4 mg, Niacinamide 55 mg, Calcium Pantothenate 25.2 mg, Folic acid 4 mg, Choline chloride 240 mg, Betaine 120 mg, Butylhydroxytoluene 6 mg, Butylhydroxyanisole 1.2 mg

II: *Chemical composition of the experimental feed mixture*

Nutrient	Amount	Units
Dry matter	880.00	g/kg
ME_N[*]	11.50	MJ/kg
Crude protein*	171.00	g/kg
Crude fat	40.30	g/kg
Crude ash	126.20	g/kg
Crude fiber	33.40	g/kg
Calcium	42.15	g/kg
Lysine	8.27	g/kg
Methionine	4.98	g/kg
Non-phytate phosphorus	4.50	g/kg

ME_N = metabolizable energy; Crude protein (N x 6.25)

Eggs

Bovans Brown lay brown-shelled eggs. Eggs were collected, marked and weighed daily. Individual laying was thus evaluated. Subsequently the strength, weight and thickness of the egg shells were measured. The strength of the shells was determined by the destructive method using the Egg Force Reader (Orka Food Technology, Ltd., USA). This method determines the amount of force required to crash the shell, measured in Newton. After measuring the strength of the shells, the eggs were beaten and the shells were washed to remove the albumen. The shell was weighed and micrometrically in three places was measured thickness after drying the shell at laboratory temperature for 48 hours. The thickness was measured at both ends and in the equatorial line of the eggs. From the measured values, was calculated average thickness of the eggs. Furthermore, the proportion of the shell from the weight of the eggs was calculated. The laying intensity was calculated as (total number of laid eggs/number of feeding days) × 100.

Excreta Collection and Analysis

Excreta was quantitatively collected from all laying hens for each balance period and placed on Petri dishes. Feathers were removed from every collected excreta and subsequently deep-frozen and lyophilized. After that, excreta was ground and stored before subsequent laboratory chemical analysis. Calcium was measured after mineralization by using sulfuric acid and hydrogen peroxide on a VARIAN AA40 atomic absorption spectrometer (Australia) at a wave length of 422.7 nm. Chromium oxide content in excreta and feed samples was determined based on the method of Mandel, Turynek and Trávníček (1960). Chromium oxide content in the samples was calculated from the values of the thiosulphate used during the titration. Coefficient of apparent digestibly using the indicator method was calculated as: $100 - [(\% \ Cr_2O_3$ in the diet $\times \times \%$ nutrient in the excreta content) / (% Cr_2O_3 in the excreta content $\times \%$ nutrient in the diet)] $\times 100$.

Blood Samples

The blood from right wing vein were taken at the start and at the end of experiment. Blood was drawn into blood tubes. The obtained blood samples were left to stand for 6 hours at laboratory temperature. After were samples centrifuged at 3,000 rpm for 15 minutes. The blood serum samples were stored in a freezer (-20 °C) until the actual biochemical analyses.

Statistical Analysis

All the obtained data were processed in the MS Excel (USA) and subsequently evaluated in the TIBCO STATISTICA 14 (USA). A one-way analysis of variance (ANOVA) was used to determine the differences between the groups. To ensure evidential differences, Scheffé's test was applied and P < 0.05 was regarded as a significant difference.

RESULTS AND DISCUSSION

The experimental period was divided into three evaluated periods because pullets have different breeding and feeding requirements than laying hens. For design balanced adequate feed mixture covering the nutritional requirements of growing
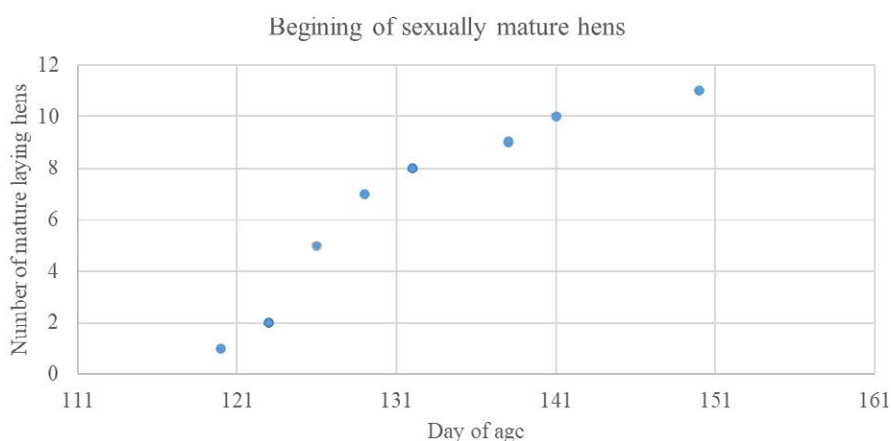
1: *The beginning of laying of individual hens*

pullets and hens which start laying, we need to understand the individuality of the animals. Laying hens have a higher nutrient requirement than pullets during preparing for sexual mature, when they are preparing a calcium reserve in the medial bone tissue and finished their grow. They also have a higher feed consumption. Pullets freely became sexually mature in the first evaluated period. The number of sexually mature laying hens in the balance days of age is shown in Fig. 1.

The term pullets means to young hens that learn to eat well, grow, but do not lay eggs at the age of 14–18 weeks in our experiment. Hens did not lay eggs until the 119th day of life. The first hen sexually matured and started laying eggs at the age of 120 days, which is about 6 days earlier than the technological instructions indicate (Integra, 2018). Half of the hens from the whole group laid in 129 days. All hens reached sexual maturity at the age of 150 days, which is around the 22nd week of life. Layers must grow well, their body weight curve must correspond to the technological instructions, so that they can produce quality eggs. Layers were weighed regularly. Live weight increased gradually as the pullets matured. At the beginning of the experiment, the weight ranged from 1064 to 1380 g. The measured weights almost correspond to the trend indicated in the technological instructions. For example, according to the instructions, the weight of laying hens at the age of 17 weeks should be in the range of 1476–1552 g (Integra, 2018). The layers in the experiment averaged 1448 ± 9.6 g at this age, but they reached 1549 ± 9.8 g at the end of week 18, which is in accordance with the technological instructions. The values found in our experiment are in accordance with the statement of Zelenka (2014), who wrote that a deviation of ±5% from the standard is permissible after the 13th week of the laying hen's age. Average weight gain for the experiment was 764.75 g, which corresponds to a daily gain of 3.64 g. The average daily feed intake per pullets increased depending on age and egg production. In the first evaluated

period without laying, the average feed intake per pullets and day was 63.72 ± 6 g/day. Average feed intake of 90.41 ± 2 g/day/hen was in the period of starting of sexual maturity. Feed consumption was the highest (99.71 g/day/hen ± 5 g) of all three evaluated periods in the third evaluated period. Our results do not confirm the hypothesis that a higher level of calcium with the use of fine limestone as a Ca source could lead to a decrease in feed intake by laying hens, as reported by Zelenka (2022) and Araújo *et al.* (2011) who reported lower feed intake when Ca levels increased from 3.5 to 4.2% in the late laying phase. Lichovníková and Zeman (2008) also used fine limestone as a source of calcium in their experiment. The calcium content in the feed ration of the first group 36.8 g/kg and in the second group 34.4 g/kg, and they state that the intake of feed, and therefore of calcium, was rather influenced by the type of technology used. In non-enriched cages, feed intake was higher compared to enriched cages, and thus calcium intake was also higher (P < 0.001). The amount of calcium that the laying hens received with feed and then actually retained in the body, depending on the age during the experiment, is shown in Fig. 2.

Daily calcium intake was 3.56 ± 0.26 g/day at the beginning of the experiment. Daily intake increased to an average of 4.51 ± 0.43 g/day Ca from -9 before first egg to day 15 after egg laying

In theperiod of full laying (18th to 84th day), the daily intake was around 4.91 ± 0.45 g/day Ca. The Fig. 2 shows the average retained amount of calcium in the body of laying hens after minus the calcium excreted in the excreta, in the eggshell and in the eggs contents. Daily calcium retention is best expressed by a third degree polynomial with the equation $y = 5E\text{-}06x^3 - 0.0002x^2 - 0.0219x + 0.0581$ ($R^2 = 0.7128$). The amount of calcium retained by pullets gradually increased along with feed intake depending on age and preparation for sexual maturity and was around 0.39 ± 0.19 g Ca in the first period. Ca retention increased up to an average of 0.85 g Ca from 8 day before laying
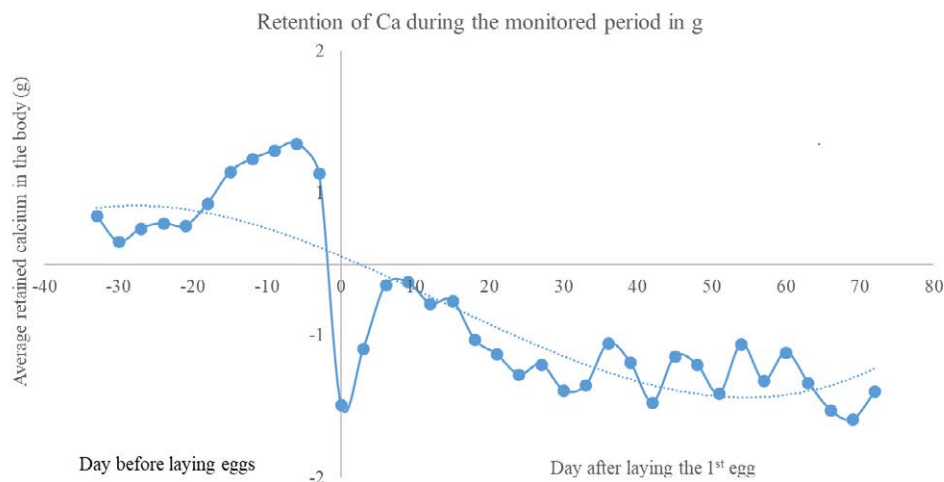
2: *Average daily retained amount of calcium by hens*
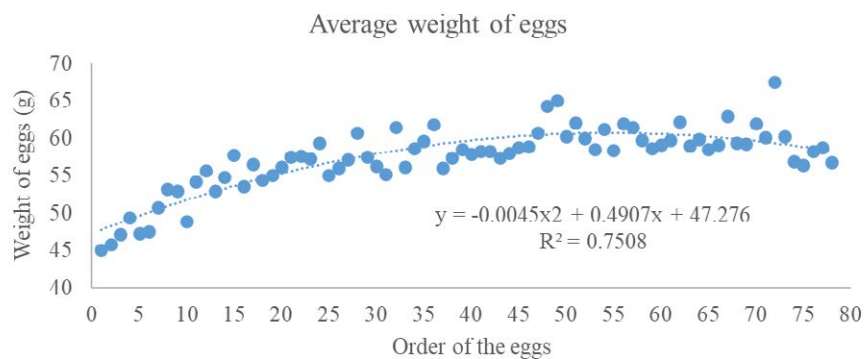
3: *Average eggs weight depending on the order of the eggs*

the first egg, Retention started to decrease 3 days before egg laying, and on the day of laying the 1st egg, it dropped sharply and a negative Ca balance occurred. During the laying period, the levels of retained calcium fluctuated and the calcium balance was still negative, the individuality of the animal, its individual management of calcium, could be seen. From the 12th day after laying, the negative balance deepened until the 33rd day by an average of 0.61 ± 0.22 g Ca. After that, the negative calcium balance decreased slightly and increased again from the 42nd day. Subsequently, the negative balance decreased and increased within the alternating trial balance, balance, always after a period, which may refer to calcium metabolism and the creation of sufficient reserves, or their depletion. Pullets excreted an average of 2.64 ± 0.12 g/day of Ca in their excreta during the first period when they did not lay eggs. This was more than in other periods. In the second and third periods, the laying hens were already laying eggs, and thus the release of calcium in the excreta in both periods slightly decreased to 2.40 ± 0.24 g/day and 2.49 ± 0.14 g/day.

Shell output was 1.75 ± 0.21 g/day for the beginning of laying and subsequently increased to 2.23 ± 0.08 g/day during evaluate peak laying. The results indicate that the organism slowly responds to the available level of calcium in the feed ration and its need by the organism to produce eggshell and the creation of reserves of the element in bone. Our values of calcium excreted in the shell during laying correspond to the statement that the eggshell contains about 2.3 g of Ca, which comes from diet and bone reserves (Bar, 2009) and with the statement of Clunies *et al.* (1992), which reported 2.17 g Ca when fed 45 g/kg Ca content in eggshell. The average weight of the eggs depending on the order of the eggs is shown in Fig. 3. The average weight of the first laid eggs was 45 ± 5 g. The weight gradually increased from the first day to the 36th day, averaging around 54 ± 4 g. Egg weights were lower from day 37 to day 49, then fluctuated depending on egg order. The weight of the eggs at the end of the monitored period averaged around 58 ± 1.3 g. The development of the eggs weight over the entire monitored period is best expressed by a second degree polynomial with the equation $y = -0.0045x^2 + 0.4907x + 47.276$ and the achieved determination index $R^2 = 0.7508$.

The eggs weights in our experiment are in accordance with the study by Tůmová and Gous
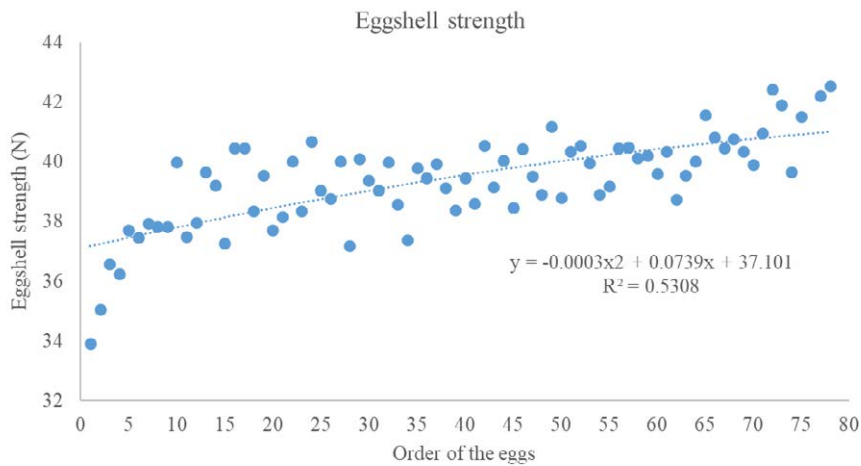
4: *Average eggshell strength by order of eggs*
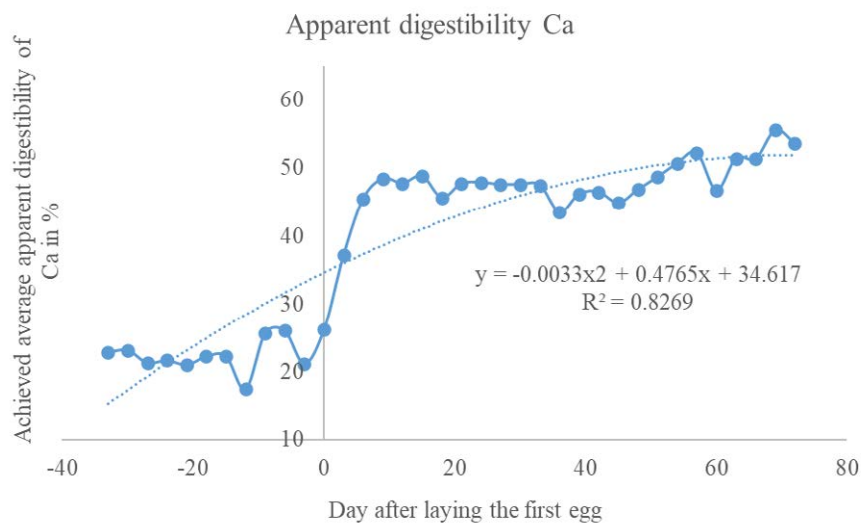
5: *Average calcium apparent digestibility depending on the day until egg laying*

(2012), which reported an average egg weight of 56.6 g for young laying hens under the age of 29 weeks. The changing average strength of the eggshell depending on the order of the eggs is shown in Fig. 4. The first laid eggs had an average strength of around 33.92 ± 6 N. The strength increased until the 17th egg to 40 ± 3.88 N. Subsequently, the strength oscillated around 39 ± 0.94 N until the 70th day after egg laying. The strength increased slightly to 42 ± 0.17 N towards the end of the evaluated period, but if we continued to observe the strength of the eggs for a longer time, the strength would tend to decrease. The development of shell strength is best expressed by a second degree polynomial with the equation $y = -0.0003x^2 + 0.0739x + 37.101$ with the determination index $R^2 = 0.5308$.

The percentage of shell in the first laid eggs was around $9.93 \pm 1.1\%$. Subsequently, the shell percentage was around an average of $10 \pm 0.37\%$ throughout the observed period. Comparing our shell % results with the results from Nascimento *et al.* (2014) experiment, it can be stated that we

recorded a higher shell percentage when we fed 4.1% Ca. Nascimento *et al.* (2014) reported an average shell percentage of $8.36 \pm 0.05\%$ when fed a diet with a calcium level of 4.45%. Similarly, in shell thickness, Nascimento *et al.* (2014) reported a shell thickness of 0.36 ± 0.002 mm, which was the same shell thickness achieved in our first laid eggs and subsequently increased to 0.43 ± 0.16 mm over the entire monitored period. Zelenka (2022) states, while monitoring the management of the organism of laying hens with calcium during starvation for one day, there was a good state of the shell on the day of starvation and a significant deterioration of the shell in their experiment were the next day. The reason was that on the first day of starvation, the laying hens had a sufficient reserve of calcium in their bodies and bones. The next day, the reserves were already exhausted and the organism did not have time to supplement the deficit sufficiently from the feed, and to cover the current requirement of the element for the formation of the shell, which caused its lower strength of eggshell. Summer

and Leeson (1993) did not observe changes in shell strength when including levels of 3.4 and 3.8% Ca. Similarly, Rao *et al.* (2003) state that when evaluating feeding levels of Ca 32.5, 35, 37.5, 40, 42.5 and 45 g/kg, they did not notice an improvement in shell characteristics. Bar (2009) reported that eggshell formation directly affects Ca utilisation. From the calcium content in the feed mixture, feed consumption and balance digestibility, its digestibility for the evaluated balances was calculated depending on the day from the egg being laid. The results are shown in Fig. 4.

Calcium apparent digestibility is calculated depending on the order of days since egg laying. The obtained values can be used to calculate the theoretical calcium deposition by hens during the entire experiment. That is, from the completion of the development of pullets through the beginning of egg laying to full laying. Digestibility during individual periods is best expressed by a second degree polynomial with the equation $y = -0.0033x^2 + 0.4765x + 34.617$. The equation for the evaluated period has a relatively high index of determination ($R^2 = 0.8269$), which means that approximately 82.69% of the points lie on the theoretical line. The values of digestibility achieved during the first period where pullets were preparing for the sexual maturity, were lower $21.52 \pm 1.68\%$ Ca. The values of Ca apparent digestibility were scattered during the second period, including the days before the onset and the onset of sexual maturity with the beginning of laying. The Ca apparent digestibility began to gradually with onset of sexual maturity approached increase to $24.80 \pm 2.08\%$ after day zero, i.e. the first egg laying, and subsequently the digestibility increased to an average of $47.63 \pm 4.33\%$ Ca. In the third period of full laying, the digestibility ranged on average around $46.50 \pm 1.39\%$ Ca in the first half of the observed period. In the second half of the third monitored period from the 48th day after egg laying to the 72nd day after egg laying, the digestibility increased to an average of $50.79 \pm 2.77\%$ Ca. Thomas and Ravindran (2010) monitored mineral digestibility in chicks at the beginning of growth from hatching. They state that the highest digestibility of minerals was recorded on day 3 for calcium, specifically on day 5 of hatching age. Subsequently, they gradually decreased until the 7th day and were unchanged from the 7th day to approximately the 14th day. The digestibility is influenced by many factors, among which are the own source of the mineral substance and its ability to dissolve in the water environment, the age of the animal and the current productivity (Kleyn, 2013; Čermák, 2000). It is in accordance with our results, when before reaching sexual maturity, the calcium digestibility of laying hens was relatively low, averaging around 22% percent, but the moment the hens began to lay, the calcium digestibility began to increase to an average of 45%. Calcium digestibility fluctuated slightly from an average achieved digestibility of 46% to 50% in the full laying period. Our results are comparable to utilization reported in the literature, considering that most literature focuses on the peak of laying, not the development of apparent digestibility from young hens, through the onset of laying and the beginning of the peak of laying. Common (1943), as well as Hurwitz and Griminger (1960), found between 25 and 35% calcium apparent digestibility in pullets fed a 2.7% calcium mixture. Apparent digestibility increased to 60% on the same feeding after the start of laying. In an experiment by Rao and Roland (1989), digestibility ranged between 58 and 63% at 3% Ca in feed for laying hens. Lichovníková (2007) tested different sources of calcium and found the highest digestibility coefficients when feeding a mixture of 50% ground limestone and 50% calcium grit.

Calcium levels in the blood of our pullets were around $4.79 \, \text{mmol/l} \pm 1.31 \, \text{mmol}$. As sexual maturity was reached and egg-laying began, calcium levels began to increase to $6.44 \pm 0.26 \, \text{mmol/L}$, but more sampling would be required to eliminate the effect of time of egg-laying for accurate interpretation.

Feeding a non-standard feed mixture with a higher calcium content than usual in mixtures for pullets did not have a negative effect on the completion of animal development, the onset of sexual maturity, or deterioration of the observed parameters of eggs quality. All monitored parameters were in accordance with the technological manual of the hybrid. Retention of calcium received from the feed mixture increased before sexual maturity, sharply decreased on the day the first egg was laid, and during the laying period it moved in a negative calcium balance. On the contrary, the apparent digestibility before the start of laying was lower, but it increased significantly with the laying of the first egg and increased slightly during the laying. Which shows that the organism has individually adapted the amount of calcium available in the feed mixture and its need by the organism to maintain homeostasis of the element. Therefore, new studies evaluating individual feed consumption, calcium retention and eggs production with modern hybrids used for eggs production are needed.

CONCLUSION

The coefficients of retention and apparent digestibility of calcium varied depending on the age, the stage of sexual maturity and the intake of the feed mixture. The organism of the laying hens has adapted to the requirements of the calcium for produce the eggshell, but with a certain delay.

REFERENCES

ARAÚJO, J. A., SILVA, J. H., COSTA, F. G. *et al.* 2011. Effect of the levels of calcium and particle size of limestone on laying hens. *Revista Brasileira De Zootecnia*, (40): 997–1005.

BAR, A. 2009. Calcium transport in strongly calcifying laying birds: Mechanisms and regulation. *Comparative Biochem. and Physiol. Part A: Molecular & Integrative Physiology*, 152(4): 447–469.

ČERMÁK, B. 2000. *Basics of nutrition and feeding of farm animals* [in Czech: *Základy výživy a krmení hospodářských zvířat*]. České Budějovice: Jihočeská univerzita.

COMMON, R. H. 1943. Observations on the mineral metabolism of pullets. *The Journal of Agricultural Science*, 33(4): 213–220. DOI: https://doi.org/10.1017/S0021859600007188

EUROPEAN COMMISSION. 2009. *COMMISSION REGULATION (EC) No. 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (Text with EEA relevance) Text with EEA relevance*. Available at: http://data.europa.eu/eli/reg/2009/152/2020-11-16 [Accessed: 2022, December 1].

CLUNIES, M., PARKS, D. and LEESON, S. 1992. Calcium and Phosphorus Metabolism and Eggshell Formation of Hens Fed Different Amounts of Calcium. *Poultry Science*, 71(3): 482–489.

CZECH REPUBLIC. 1992. *Act of Czech National Council. Act no. 246* from day 15. April 1992 Coll. Act for the Protection of Animals Against Cruelty. In: *Collection of laws of the Czech Republic*. Available at: https://eagri.cz/public/web/mze/legislativa/pravni-predpisy-mze/tematicky-prehled/Legislativa-MZe_uplna-zneni_zakon-1992-246-viceoblasti.html [Accessed: 2022, November 15].

HURWITZ, S. and GRIMINGER, P. 1960. Observations on the calcium balance in laying hens. *Journal of Agricultural Science*, (54): 373–377. DOI: https://doi.org/10.1017/S0021859600021316

INTEGRA. 2018. Technological instructions for cage housing systems Bovans Brown [in Czech: Technologický návod klecový systém Bovans Brown]. *Hendrix Genetics* [online]. Available at: https://www.integrazabcice.cz/cs/produkty/bovans-brown-cz/ [Accesed: 2022, November 11].

KLEYN, R. 2013. *Chicken nutrition: a guide for nutritionists and poultry professionals.* Packington: Context Publications. ISBN 978-1-899043-42-2

LATIMER G. W. and ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS INTERNATIONAL. 2019. *Official methods of analysis of AOAC international.* 21st Edition. Gaithersburg, Maryland: AOAC International.

LICHOVNIKOVA, M. and ZEMAN, L. 2008. Effect of housing system on the calcium requirement of laying hens and on eggshell quality. *Czech Journal of Animal Science*, 53(4): 162–168. DOI: https://doi.org/10.17221/375-CJAS

LICHOVNÍKOVÁ, M. 2007. The effect of dietar calc ium source, concentration and particle size on calcium retention, eggshell quality and overall calcium requirement in laying hens. *British Poultry Science*, 48: 71–75. DOI: https://doi.org/10.1080/00071660601148203

LUKIC, M., PAVLOVSKI, Z. and SKRBIC, Z. 2009. Mineral nutrition of modern poultry genotypes. *Biotechnology in Animal Husbandry*, 25(5-6-1): 399–409. DOI: https://doi.org/10.2298/BAH0906399L

MANDEL, L., TURYNEK, V. and TRÁVNÍČEK, J. 1960. Iodometric method for determining chromium oxide, used as an indicator in digestibility experiments [in Czech: Jodometrická metoda stanovení kysličníku chromitého, použitého jako indikátoru při pokusech stravitelnosti]. *Animal production*, 5: 645–652.

NATIONAL RESEARCH COUNCIL. 2005. *Mineral Tolerance of Animals.* Second Revised Edition. Washington, DC: The National Academies Press. DOI: https://doi.org/10.17226/11309

NASCIMENTO, G. R., MURAKAMI, A., GUERRA, A. *et al.* 2014. Effect of different vitamin D sources and calcium levels in the diet of layers in the second laying cycle. *Brazilian Journal of Poultry Science*, 16(2): 37–42. DOI: https://doi.org/ 10.1590/1516-635x160237-42

NEDOMOVÁ, Š., BUCHAR, J. and STRNKOVÁ, J. 2013. Mechanical Behaviour of Ostrich's Eggshell at Compression. *Acta Univ. Agric. et Silvic. Mendelianae Brun.*, 61(3): 729–734.

RAO, S. V. R., PANADA, A. K., RAJU, M. V. L. N., SUNDER, G. S. and PRAHARAJ, N. K. 2003. Requirement of calcium for commercial broilers and white leghorn layers at low dietary phosphorus levels. *Animal Feed Science Technology*, 106(1–4): 199–208. DOI: https://doi.org/10.1016/S0377-8401(02)00296-1

RAO, K. S. and ROLAND, D. A. 1989. Influence of dietary Ca level and particle size of Ca source on in vivo Ca solubilization. *Poultry Sci.*, 68(11): 1499–1505.

SUMMER, J. D. and LEESON, S. 1993. Influence of Pre-Lay Treatment and Dietary Protein Level on the Reproductive Performance of White Leghorn Hens. *Journal of Poultry Science*, (72): 1705–1713. DOI: https://doi.org/10.3382/ps.0721705

TUMOVÁ, E. and GOUS, R. M. 2012. Interaction of hen production type, age, and temperature on laying pattern and egg quality. *Poulry Science*, 91(5): 1269–1275. DOI: https://doi.org/10.3382/ps.2011-01951

THOMAS, D. and RAVINDRAN, V. 2010. Mineral Retention in Young Broiler Chicks Fed Diets Based on Wheat, Sorghum or Maize. *Asian-Australasian Journal of Animal Sciences*, 23(1): 68–73. DOI: https://doi.org/10.5713/ajas.2010.90129

ZELENKA, J. 2022. Calcium in poultry nutrition [in Czech: Vápník ve výživě drůbeže]. *Drůbežář – hydinár*, 16(1): 10–11.

ZELENKA, J. 2017. Calcium in poultry nutrition [in Czech: Vápník ve výživě drůbeže]. *Krmivářství*, 21(2): 21–23.

ZELENKA, J. 2014. *Poultry nutrition and feeding* [in Czech: *Výživa a krmení drůbeže*]. 1st Edition. Olomouc: Agriprint s.r.o. ISBN 978-80-87091-53–1

ZELENKA, J., HEGER, J. and ZEMAN, L. 2007. *Recommended content of nutrients in compound feed and nutritional value of feed for poultry* [in Czech: *Doporučený obsah živin v krmných směsích a výživná hodnota krmiv pro drůbež*]. Brno: MZLU.

Contact information

Andrea Roztočilová: andrea.roztocilova@mendelu.cz (corresponding author)
Ondřej Šťastník: ondrej.stastnik@mendelu.cz
Jakub Novotný: jakub.novotny@mendelu.cz
Leoš Pavlata: leos.pavlata@mendelu.cz
Ladislav Zeman: ladislav.zeman@mendelu.cz
Eva Mrkvicová: eva.mrkvicova@mendelu.cz