Original Paper

The Mineral Profile of the Keel Bone of Laying Hens in Alternative and Cage Housing Systems

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The aim of the study was to compare the keel bone profile of hens reared in cage and cage-free rearing systems. Experiments were carried out on a poultry farm on two alternative rearing systems and on one cage hall and measurements were carried out in laboratory conditions with Bovans Brown laying hens aged 32 to 47 weeks. The obtained results were evaluated using the statistical program SAS with version 8.2. In the set of 18 keel bone samples that we monitored, the average phosphorus content was measured as 15.19 $g.kg^{-1}$ with a variation of the measured values of 6.09, an average magnesium content of 0.49 $g.kg^{-1}$ with a variation of the measured values of 0.15, an average content of calcium 33.76 $g.kg^{-1}$ with a variation of the measured values of 14.78 and an average value of the ratio between calcium and phosphorus of 2.19 with a variation of the measured values of 0.09. Individual laying hen breeding systems did not have a statistically significant effect ($P > 0.05$) on the content of phosphorus, magnesium, calcium and the ratio between calcium and phosphorus in the keel bone of laying hens.

Keywords: laying hens, breeding system, calcium, phosphorus, keel bone

1 Introduction

In recent years, the inhabitants of Europe perceive health, welfare of animals and environmental issues more intensively. As a result, consumers in the European Union market demand "sustainable" food, i.e. healthy products as well as edible eggs, which also take into account the natural living conditions of animals. Based on the results of the European Union survey from 2016, the statements of individual respondents regarding animal welfare were evaluated. The results showed that more than 94% of Europeans preferred the protection of animals as very important.

More than 99% of respondents in Finland, Portugal and Sweden said that the well-being of animals and their natural environment in which they live during breeding is desirable. In other countries such as Hungary, Poland, Croatia, Slovakia and Bulgaria, up to 86–88% of respondents considered a calm breeding environment and the well-being of animals to be very important in the first place.

In countries such as Luxembourg, the Netherlands and Sweden, more than 59% of respondents are willing to buy even more expensive food and products that have been produced taking into account the welfare of animals, including laying hens. More specifically, when it comes to the attitude of Europeans to the good living conditions of laying hens, consumers distinguish between different breeding systems. They consider free-range breeding to be particularly important for good living conditions (Special Eurobarometer, 2016). The current shift from cages to alternative systems is the result of a social demand to improve the living conditions of animals, which is also supported by scientific evidence. Cage-free systems can offer layers to perform their natural activities (Nicol et al., 2017).

The citizens of the European Union are asking for better conditions for raising farm animals, conditions that take into account the natural environment and peaceful housing. On the basis of this requirement, pressure is created on food companies and on breeders of laying hens, because purchasing power prefers eggs from alternative breeding systems. The most important

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retailers include Tesco in Central Europe, Camst in Italy, Carrefour and Monoprix in France. It also includes large international brands such as Compass Group, Sodexo and Nestlé (CIWF, 2016a, 2017a).

Based on the European Citizens' Initiative "End the Cage Age", it is likely that breeders will exclusively use alternative housing systems for laying hens (and any other kind of livestock) and cage farming will be banned throughout the European Union. As a consequence of this fact, the country's politicians and egg producers must lead a smooth transition to an alternative system. A second European Union reference center for animal welfare, dedicated to poultry, will be a valuable contribution to support this transition. Producers will need to be guided in optimizing breed selection, housing and management to cope with some of the existing problems such as keel bone (carinae) damage, mortality and damaging coat in cage-free systems (Optimising Laying Hen Welfare in Cage-free Systems, 2021).

Barns are alternative breeding systems for laying hens that do not use cages and must provide (Article 4 of Council Directive 1999/74/EC): a maximum density of 9 laying hens.m^{-2} of usable area; either linear feeders providing at least 10 cm access to the hen, or circular feeders providing at least 4 cm to the hen; either continuous drinkers providing 2.5 cm access to the hen, or circular drinkers providing 1 cm to the hen; either one nest for every 7 hens, or 1 m^2 of nesting space for up to 120 hens (common nest); adequate perches providing at least 15 cm per hen; 250 cm^2 of litter for the laying hen, which must be a third of the ground surface; equal access to drinking and feeding facilities (applies to multi-level systems). All systems of laying hens and egg production comply with European regulations, but consumer distrust of eggs from caged hens is growing. This consumer demand has led to a strong market segmentation (80% of layers were caged in 2003 and 58% in 2018) (Gautron et al., 2022).

Egg production is influenced by the environment, mainly photoperiod, but also depends on the genetic component (England and Ruhnke, 2020). The existence of genetic variability in egg production has contributed to the current level of production in laying hens capable of laying more than 300 eggs per year. After intensive selection for sexual maturity and egg production up to 55 weeks of age, breeders are now also interested in persistence in laying, i.e. the ability of hens to have a longer laying period. Currently, laying hens are reared until approximately 72 weeks of age. Extending the laying period to 100 weeks with a total production of almost 500 eggs per hen would reduce their number (Bain et al., 2016).

Longer keeping of hens can have adverse consequences, such as molting, which leads to the absence of egg production for several weeks and to economic losses, because the hens are still fed but do not produce eggs. It is essential to maintain good bone quality in the laying hen, especially as the hen ages. The laying hen needs 2 to 2.5 g of calcium per day to form the egg shell. About 2/3 of this calcium is provided directly by the feed, the remaining 1/3 comes from the reserve by demineralization of renewable parts of the bones. The calcium from these bones is essential in the second part of the shell mineralization process, which occurs at night when the hen does not have access to feed, although the birds have had feed in the brood for several hours. The renewable portion of bone is capable of rapid absorption and repair, which can be optimized by dietary calcium sources (content, quality and particle size) (Whitehead, 2004). Even with a perfectly controlled feed, bone demineralization is a natural phenomenon that can also affect structural bone, ultimately leading to osteoporosis. This pathology, which can be prevalent in old layers, leads to bone fragility and keel fractures that seriously affect their welfare (Armstrong et al., 2020).

2 Material and methods

The subject of research was laying hens in three different breeding systems, their well-being, production, condition of keel bones, egg quality and breeding costs in the monitored interval after reaching the peak of egg production. The research was carried out on a selected poultry farm Babičkin dvor a.s. in Veľký Krtíš with Bovans Brown hybrids reared in three different housing systems, namely enriched cages with a condition of 30,892 layers, deep litter with a condition of 11,130 layers and aviaries with a condition of 27,958.

2.1 Breeding system in enriched cages on the farm

5ON04R cage breeding equipment is used on the farm. The cages are 4-story in 6 batteries. On the front side of the hall there are adapters for collecting eggs, and on the back side of the hall there is a device for removing droppings. The cages include a central power supply with a water control gauge on each floor and medication dispensers. The cage is equipped with a floor of 750 cm^2.layer^{-1} of which 600 cm^2 usable area, further the clear height of the cage 450 mm with a floor slope of 14% and the length of the feeding place 120 mm.layer^{-1}. In each cage there is a nest, a perch with a length of 150 mm.layer^{-1}, a space for digging and a device for grinding claws. Each laying hen has access to 2 watering holes. The height from the floor to the bottom row of cages in the hall is 350 mm.

The temperature in the hall is set from 20 to 21.5 °C.

The ventilation of the hall is solved by vacuum ventilation with a fan output of 5 $m^3.h^{-1}.kg^{-1}$ live weight of laying hens. The ventilation system is fully automated using sensors located inside the hall.

The light intensity during the laying period is at the level of 10 Lux and the length of the light day is set to 15 h.

2.2 Deep litter laying hen rearing system on the farm

Laying nests, feeders and nipple drinkers are placed in the center of the hall's slatted floor. Dry sand with a thickness of 3 cm is used for bedding. There are 9 laying hens per m^2 of the floor area of the hall. The droppings from the entire hall and from the litter area are removed at the end of the laying cycle after the hens have been removed. The grid floor is placed 500 mm above the floor, so that the accumulated droppings do not exceed the height of the grid at the end of the laying cycle after the hens are removed.

Nipple drinkers are located near the laying nests. The slatted area of the floor is used by laying hens to clean the runners when entering the nest.

Perches with a length of 150 mm are placed in the hall for one laying hen. There is a free space of 300 mm between the perches and the width of the space between the wall and the perches is 200 mm.

Egg collection is automated using collection conveyor belts. The eggs from the laying nests are rolled onto the egg belt and transported to the department on the central collection table, where the eggs are sorted.

The temperature in the hall is set between 20 and 22 °C.

The ventilation of the hall is solved by automated vacuum ventilation with a fan capacity of 5 $m^3.h^{-1}.kg^{-1}$ live weight of laying hens. The system is equipped with sensors in the hall.

The light intensity is 10 Lux and the daylight length is set to 15 h.

Removal of droppings from the hall after laying hens is carried out using universal loaders and subsequent cleaning using high-pressure washing equipment.

2.3 System of rearing laying hens in aviaries on the farm

This breeding system makes it possible to increase the number of laying hens per m^2. There are three-story aviary structures in the hall. In the aisles between the rows of the structure and below them, there is a litter of sand. The litter is used for raking laying hens and also as a dust bath. The area for one laying hen is 0.025 m^2.

The floor of the structure is made of wire mesh. Feeding troughs with a chain feed conveyor and drip watering troughs are located on each floor. Laying nests are located on the first and second floors, and there are perches between the nests. Laying nests are designed with an inclined surface for rolling eggs onto a collection belt covered with artificial grass to minimize the contact of the laid egg with droppings.

On each floor, under the slatted floor, there is a collection belt for the removal of droppings. Egg collection is automated. The eggs are transported by a central collection belt to a separate room where the eggs are placed on trays.

The temperature in the hall is set at 20 to 21.5 °C. Temperature regulation is automatic according to the set values.

2.4 Feeding of laying hens in the investigated systems

A complete feed mixture produced by the company Babičkin dvor a.s., Hontianske Nemce was used for feeding the laying hens, which the hens received *ad libitum* in all three rearing systems during the research (the composition of the feed mixture and the energy and nutrient content are shown in Table 1).

2.5 Indicators of the well-being of laying hens monitored in laboratory conditions

Individual indicators of the mineral profile of the keel of laying hens were monitored in laboratory conditions, namely calcium, phosphorus, magnesium and the ratio between calcium and phosphorus ($n = 6$ in each breeding system) at the age of 47 weeks of hens. The keel bone was obtained after killing the laying hens from the butchered body by separating the muscles. The keel bones were prepared for analysis after drying in an oven at a temperature of 105 ±2 °C, type HS 61 A and grinding to a fine powder using a Grindomix GM 200 knife grinder.

The prepared sample was mineralized by dry combustion in a muffle furnace. From the obtained ash, a solution was prepared using hydrochloric acid to determine the content of magnesium, calcium and phosphorus on a laboratory device, an atomic emission spectrometer with inductively coupled plasma (ICP-OES). ICP-OES introduces the sample solution by atomization into the plasma generated by the induction coil which is powered by a high-frequency current. The resulting thermal energy changes the elements of the sample and creates light. The spectrometer divides the generated light into characteristic spectra of individual elements and, based on the intensity of the spectra, determines the quality

Table 1 Composition of feed mixture, % and content of energy and nutrients in feed mixture HYD-10

Feed material		%
Rape extracted meal		5.00
Methionine 99%		0.132
Wheat 10.5%		44.058
Corn 8.3%		20.00
Feed sunflower oil		0.900
Soybean meal extracted 45%		19.30
Monocalcium phosphate		0.69
PRM N 0.3% Sigitrade*		0.30
Dolomitic limestone		4.50
Ground limestone I.		4.80
Feed salt		0.32
Energy and nutrients in compound feed	Unit of measure	Content
Metabolizable energy, MEN	$kJ.kg^{-1}$	11.148
Nitrogenous substances	$g.kg^{-1}$	168.92
Lysine	$g.kg^{-1}$	8.400
Methionine	$g.kg^{-1}$	4.150
Calcium	$g.kg^{-1}$	35.930
Phosphorus	$g.kg^{-1}$	5.340
Magnesium	$g.kg^{-1}$	6.140
Linoleic acid	$g.kg^{-1}$	12.954

* PRM N 0.3 Sigitrade – vitamin-mineral premix 0.3% containing vitamins A, E, D_3, B_1, B_2, B_6, B_{12}, folic acid, calcium pantothenate, choline chloride, niacin, antioxidant ethoxyquin and trace elements Cu, Mn, Zn, Se, Co, I and Fe

of individual types of elements and their quantitative concentration in the sample.

The ICPE-9800 series is made up of high-speed simultaneous systems, and another part is a large one-inch CCD that ensures low noise, and everything is made up of full-fledged software based on the ICPE solution. An ICPE-9820 was used for the measurement, which provides axial imaging and radial observation of the plasma in the vertical direction. This interesting dual imaging capability allows measurements to automatically switch between high sensitivity using axial imaging and high concentration using radial imaging, allowing elements to be analysed over a wide concentration range.

CCD stands for charge coupled component (charge coupled circuit). It is a designation for the electrical phenomenon of the transfer of electric charge along a surface in electronic (semiconductor) elements. The charge is transferred by means of a system of electrodes, to which mutually shifted synchronization signals are supplied. This phenomenon is used only in the only element that has become synonymous with it – in flat optical sensing elements used for recording image information. The charge, which is created after the illumination of the semiconductor due to the internal photoelectric phenomenon, is gradually pushed to the edge of the element, where it is electrically amplified and subsequently processed.

The statistical program SAS with version 8.2 was used for the statistical evaluation of the measured results of the established indicators. For the mathematical-statistical evaluation in the SAS program, the condition was the sorting of the data, which is understood as the sorting of the investigations according to the individual values of certain quantities. At the beginning of the mathematical-statistical calculations, the data were sorted in a certain order using the SAS program. A SAS statistical file was created from the data and, in the next step, a sorting procedure. On the basis of sorted data in the SAS program, mathematical and statistical calculations and evaluation of indicators according to ANOVA were carried out, descriptive characteristics according to laying systems such as SD – standard deviation, CV – coefficient of variation, Min. – minimum value and Max. – maximum values were used for the evaluation of the mineral profile of the keel bone of laying hens and the Scheffe test, statistical evidence of differences in the averages in the indicator between housing systems of hens at the level of evidence $P \leq 0.05$.

3 Results and discussion

Historically, animal welfare and well-being have been defined by negative experiences such as disease, hunger, thirst, stress or reduced fitness (Bracke and Hopster, 2006). In the last 40 years, most animal welfare research has focused on avoiding negative situations. People's interest in the field of experiences with positive states of animal well-being has a growing tendency (Hemsworth et al., 2015). The aforementioned developments in animal welfare science have led to the understanding that animal welfare and well-being cannot be achieved without experiencing and observing positive affective states such as comfort, health, pleasure and a sense of control (Mellor and Beausoleil, 2015).

In recent decades, the public has begun to observe and monitor changes in the housing of laying hens. There has been a gradual transition from conventional cage systems with an increase in the proportion of enriched cages that provide them with more space for movement, nest, perch and litter substrate and cage-free systems in some countries, initially in the European Union (EU

Directive 1999/74/EC) and subsequently outside Europe. For example as of March 2020, nearly 24% of all layers in the United States were kept in cage-free systems, up from 12% in 2016 and up from 4% in 2010 (Schuck-Paim et al., 2021).

3.1 Mineral profile of the keel of laying hens in cage and cage-free rearing systems

The mineral profile of the keel of laying hens in cage and cage-free housing systems is presented in Table 2 and the statistical evaluation in Table 3.

In the set of 18 keel bone samples that we monitored, the average phosphorus content was measured as 15.19 $g.kg^{-1}$ with a variation of the measured values of 6.09, an average magnesium content of 0.49 $g.kg^{-1}$ with a variation of the measured values of 0.15, an average content of calcium 33.76 $g.kg^{-1}$ with a variation of the measured values of 14.78 and an average value of the ratio between calcium and phosphorus of 2.19 with a variation of the measured values of 0.09. The interpreted results are in Table 2.

The breeding system of laying hens had no statistically significant effect (P >0.05) on the content of phosphorus, magnesium, calcium and the ratio between calcium and phosphorus in the keel of hens (Table 3).

3.1.1 Phosphorus in the keel bone of laying hens

The average values of phosphorus content in the keel bone of laying hens (Table 4) showed a tendency to decrease in the order of cage > litter > aviaries. The average content of phosphorus in the keel of hens placed in the cage system was found to be 17.35 $g.kg^{-1}$, which was the highest value of all observed average values of phosphorus in breeding systems. Fluctuation of the measured data of the phosphorus content expressed by the standard deviation was SD = 4.69. In the breeding system on deep bedding, the average phosphorus content was found to be 15.60 $g.kg^{-1}$ with the variation of the measured data expressed by the standard deviation SD = 8.42. The difference in average of phosphorus content in the keel bone between the bedding and the cage system was not statistically significant (P >0.05).

Table 3 Statistical evidence of the influence of the breeding system on the monitored parameters of the keel bone

Parameter of the keel bone	*F*-test
Phosphorus ($g.kg^{-1}$)	0.93-, P >0.05
Magnesium ($g.kg^{-1}$)	0.56-, P >0.05
Calcium ($g.kg^{-1}$)	0.94-, P >0.05
The ratio between calcium and phosphorus	1.07-, P >0.05

- and P >0.05 means a statistically insignificant influence of the breeding system

The average phosphorus content of 12.62 $g.kg^{-1}$ was recorded in the aviaries, which was the lowest value of all monitored average phosphorus values in breeding systems. Fluctuation of the measured data of the phosphorus content expressed by the standard deviation was SD = 4.29. The difference in the average phosphorus content in the keel bone between aviaries and litter and between aviaries and cages was not statistically significant (P >0.05). The range of maximum phosphorus content data ranged from 19.51 $g.kg^{-1}$ for aviaries to 27.57 $g.kg^{-1}$ for litter and minimum data from 7.48 $g.kg^{-1}$ for litter to 12.03 $g.kg^{-1}$ for cages.

3.1.2 Magnesium in the keel of laying hens

The average values of magnesium content in the keel of hens (Table 4) indicated a tendency to decrease in the order of cage > litter > aviaries. The average magnesium content in the keel of hens placed in the cage system was found to be 0.52 $g.kg^{-1}$, which was the highest value of all observed average magnesium values in the rearing systems. The fluctuation of the measured data of the magnesium content expressed by the standard deviation was SD = 0.12. In the breeding system on litter, the average magnesium content was found to be 0.51 $g.kg^{-1}$ with the variation of the measured data expressed by the standard deviation SD = 0.21. The difference in the average content of magnesium in the keel between the litter and the cage system was not statistically significant (P >0.05). The average magnesium content of 0.43 $g.kg^{-1}$ was recorded in the aviaries,

Table 2 Basic statistical evaluation of monitored parameters of the keel bone

Parameter of the keel bone	*n*	Mean	SD
Phosphorus ($g.kg^{-1}$)	18	15.19	6.09
Magnesium ($g.kg^{-1}$)	18	0.49	0.15
Calcium ($g.kg^{-1}$)	18	33.76	14.78
The ratio between calcium and phosphorus	18	2.19	0.09

n – number of samples; SD – standard deviation

Table 4 Statistical evaluation of the content of phosphorus, magnesium and calcium in the keel bone of laying hens in individual housing systems

Indicator	*n*	Breeding system											
		enriched cages				deep litter				aviaries			
		mean	SD	min.	max.	mean	SD	min.	max.	mean	SD	min.	max.
Phosphorus (g.kg^{-1})	6	17.35[a]	4.69	12.03	22.67	15.60[a]	8.42	7.48	27.57	12.62[a]	4.29	7.99	19.51
Magnesium (g.kg^{-1})	6	0.52[a]	0.12	0.38	0.67	0.51[a]	0.21	0.32	0.81	0.43[a]	0.11	0.32	0.62
Calcium (g.kg^{-1})	6	38.95[a]	11.55	26.31	51.96	34.91[a]	20.43	15.14	64.20	27.41[a]	10.23	15.49	43.84
The ratio between calcium and phosphorus	6	2.23[a]	0.07	2.13	2.29	2.19[a]	0.12	2.02	2.33	2.15[a]	0.08	2.02	2.25

n – number of samples; SD – standard deviation; min. – minimum value; max. – maximum value, the same letters in the superscript for averages in a row mean a statistically insignificant difference *P* >0.05

which was the lowest value of all monitored average magnesium values in breeding systems. Fluctuation of the measured data of the phosphorus content expressed by the standard deviation was SD = 0.11. The difference in the average content of magnesium in the keel between aviaries and bedding and between aviaries and cages was not statistically significant (*P* >0.05). The range of maximum magnesium content data ranged from 0.62 g.kg^{-1} for aviaries to 0.81 g.kg^{-1} for litter and minimum data from 0.32 g.kg^{-1} for litter and likewise for aviaries to 0.38 g.kg^{-1} in cages.

3.1.3 Calcium in the keel of laying hens

The average values of calcium content in the keel of hens (Table 4) indicated a tendency to decrease in the order of cage > litter > aviaries. The average calcium content in the keel of hens placed in the cage system was found to be 38.95 g.kg^{-1}, which was the highest value of all observed average calcium values in breeding systems. The fluctuation of the measured data of the calcium content expressed by the standard deviation was SD = 11.55. In the breeding system on litter, the average calcium content was found to be 34.91 g.kg^{-1} with the variation of the measured data expressed by the standard deviation SD = 20.43. The difference in the average content of calcium in the keel bone between the litter and the cage breeding system was not statistically significant (*P* >0.05). The average calcium content of 27.41 g.kg^{-1} was recorded in the aviaries, which was the lowest value of all monitored average calcium values in breeding systems. The fluctuation of the measured data of the calcium content expressed by the standard deviation was SD = 10.23. The difference in the average content of calcium in the keel between aviaries and bedding and between aviaries and cages was not statistically significant (*P* >0.05). The range of maximum calcium content data ranged from 43.84 g.kg^{-1} for aviaries to 64.20 g.kg^{-1} for litter andminimum data from 15.14 g.kg^{-1} for litter to 26.31 g.kg^{-1} for cages.

3.1.4 The ratio of calcium to phosphorus in the keel bone of laying hens

The average values of the ratio of calcium to phosphorus in the keel of laying hens (Table 4) indicated a narrowing tendency in the order of cage > litter > aviaries. The average value of the ratio of calcium to phosphorus in the keel of hens placed in the cage system was found to be 2.23, which was the widest ratio of all monitored calcium to phosphorus ratios in the rearing systems. The fluctuation of the measured data of the ratio of calcium to phosphorus expressed by the standard deviation was SD = 0.07. In the bedding system, the average ratio of calcium to phosphorus was found to be 2.19 with a variation of the measured data expressed by the standard deviation SD = 0.12. The difference in the average ratio of calcium to phosphorus in the keel bone between the litter and the cage rearing system was not statistically significant (*P* >0.05). An average of calcium to phosphorus ratio of 2.15 was recorded in the aviaries, which was the lowest value of all monitored average calcium to phosphorus values in breeding systems. The fluctuation of the measured data of the ratio of calcium to phosphorus expressed by the standard deviation was SD = 0.08. The difference in the average ratio of calcium to phosphorus in the keel bone between aviaries and bedding and between aviaries and cages was not statistically significant (*P* > 0.05). The range of maximum calcium to phosphorus ratio data ranged from 2.25 for aviaries to 2.33 for litter and minimum data from 2.02 for litter and also for aviaries to 2.13 for cages.

Attia et al. (2020) conducted an experiment with laying hens aged 60 to 72 weeks that were fed feed mixtures with three different proportions of calcium 3.5, 4.0 and 4.5%. At the age of 72 weeks of laying hens, 6 laying hens were randomly selected from each monitored group, whose keel bones were analysed for selected parameters of mineral substances.

Their results of the calcium content in the order of the mentioned proportions of calcium in the feed 30.6, 32.7 and 32.7 $g.kg^{-1}$ despite the fact that they used the tibia for the analysis are approximately the same as our results for the analysed keel bone. About the same results of phosphorus content of 12.8, 14.6 and 14.7 $g.kg^{-1}$ and calcium to phosphorus ratio of 2.41, 2.25 and 2.23 of these authors are also compared to ours found in the keel. In order to clarify the relationship between the observed mineral substances in the bones of laying hens and the formation of eggshells, it is necessary to start from already known knowledge and point out contradictory or unclear ones that are the subject of further investigation.

Casey-Trott et al. (2017) investigated the proportion of damaged keel bones of hens in enriched cages and aviaries during the laying cycle. Laying hens of different ages were included in the research. In the first category they put laying hens at the age of 30 weeks, in the second category hens at the age of 50 weeks and in the third category hens at the age of 70 weeks. In the evaluated results, they found that both the age of hens (P <0.001) and the housing system (P <0.001) have an effect on the occurrence of keel bone fractures. Our study did not confirm their results.

Another study investigated keel damage in laying hens in Greece. Experiments were carried out with layers that were housed in enriched cages on deep litter and in a free range system. They used the palpation method to assess damage to the keel bone. Their obtained results pointed to the fact that the effect of housing has an effect on keel bone damage. The highest incidence of damage was recorded in the free-range housing system (50%), followed by the enriched cage system (24%), and in third place were systems with deep bedding (7%) (Dedousi et al., 2020).

Saraiva et al. (2019) in their results report a significant influence (P <0.001) of individual housing systems on keel bone damage. Analyzing the results, it was concluded that the highest proportion 60.4% of deformed keel bones was in free range, followed by enriched cages with a 54.2% share of damaged keel bones and, last but not least, deep bedding with a 53.5% share of keel bone damage.

Let's start from the knowledge that calcium and phosphorus are among the basic nutrients and play an important role in the fulfillment and management of important biological processes. They participate in mineralization and bone development in the form of hydroxyapatite (99% calcium, 80% phosphorus) (Veum et al., 2010).Calcium and phosphorus balance is maintained by absorption in the small intestine, reabsorption and excretion by the kidneys, and storage and mobilization from bone. In laying hens, calcium and phosphorus are involved in the formation of eggshells during the laying period. The chemical composition of the eggshell of laying hens is estimated to be 94% calcium carbonate, 1% magnesium carbonate, 1% calcium phosphate and 4% organic matter (Stadelman, 2000). An asynchronous increase in calcium content with increased eggshell weight has been implicated in the deterioration of eggshell quality due to aging hens (Bolukbasi et al., 2005). Phosphorus affects the deposition of calcium during bone formation and thereby affects the calcium content of the eggshell (Ahmad and Balander, 2003). In fact, eggshell contains little phosphorus, but this element plays a very important function in the formation and metabolism of eggshells (Rao et al., 1992). Most of the calcification of eggshells is carried out during the night in the dark, when calcium is mostly not available to the organism of the laying hen. Then the laying hens mobilize it from the bones, about 20 to 40% (Comar and Driggers, 1949).

During embryonic and juvenile development, important structural and trabecular bone is formed that contains highly organized hydroxyapatite crystals. After the completion of structural bone deposition (Hudson et al., 1993), at approximately 18 weeks of age before the laying period, the increased content of circulating estrogen has an effect on the positive development of modular bone, which is localized in the pneumatic and long bones (Whitehead, 2004). Hydroxyapatite crystals are abundantly present in medullary bone (Dacke et al., 1993), which ensures the intensive course of anabolic and catabolic processes of hydroxyapatite during egg formation. Bone resorption releases calcium and phosphorus into the circulation in the form of ionized calcium (iCa^{2+}) and inorganic phosphate [PO_4^{3-} (Pi)], which are either used for eggshell formation or excreted from the body of the laying hen. In the absence of eggshell calcification, medullary bone is remineralized (Kerschnitzki et al., 2014) and absorbed during eggshell calcification (Van de Velde et al., 1984) through increased osteoclast activity (parathyroid hormone effect) and the bioactive form of vitamin D_3, 1, 25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] (Taylor and Belanger, 1969).

When parathyroid hormone binds to the parathyroid hormone receptor 1 (PTH1R) on osteocytes (Zhao et al., 2002), receptor activator of nuclear factor-kappa B ligand (RANKL) is secreted and interacts with receptor activator of nuclear factor-kappa B (RANK) on osteoclasts that stimulate bone resorption. In addition, parathyroid hormone increases the activity of vacuolar-type adenosine triphosphatase (V-ATPase) of osteoclasts, which causes intracellular acidification necessary for bone breakdown (Liu et al., 2016). Eggshell calcification increases osteoclast activity up to ninefold (Van de Velde et al., 1984) and may contribute to bone thinning as osteoclasts resorb structural bone after medullary bone depletion. Dysregulation of medullary bone remodeling may stimulate the bone thinning process in old laying hens, which show increased bone expression of the resorption marker carbonic anhydrase 2 (CA2) and vitamin D_3 receptor (VDR), as well as decreased expression of accretion proteins, e.g. as collagen type 1 alpha 1 (COL1A1), compared to younger layers (Gloux et al., 2020). To maintain the daily supply of calcium in laying hens, it is important to regulate the homeostasis of this element.

The eggshell is formed at night, and that is why a stable supply of calcium is very important during the mentioned term, when the laying organism depends on the reduced pH of the intestine to thoroughly dissolve the coarse limestone retained in the stomach (Scanes et al., 1987). This condition occurs through stimulation of the activity of the myocardial form of the enzyme H^+/K^+-ATPase in the proventriculus (Guinotte et al., 1995) and the subsequent secretion of hydrochloric acid (Guinotte et al., 1993).

Improving the skeletal health of laying hens in relation to good living conditions requires a deep understanding of the regulatory systems that control calcium and phosphorus utilization and how they change with age. Further research is needed to understand the mechanism for maintaining skeletal health and egg production during changes throughout the laying cycle (Sinclair-Black et al., 2023), not just the period after peak laying, as our research was intended to address. It is also important to know the influence of the breeding system. The results of our experiment show that the results of the content of calcium, phosphorus, magnesium and the ratio of calcium to phosphorus were comparable and the tendency of their decrease and narrowing of the ratio was indicated starting from the cages, in the order of litter and aviary.

4 Conclusions

The following conclusion emerged from the evaluated results of the research on the influence of cage and cage-free systems of laying hens on the mineral profile of the keel bone of hens at the age of 47 weeks:

- The influence of breeding in cages, on deep bedding and in aviaries was not statistically confirmed on the parameters of the mineral profile of the keel bone of laying hens. The content of phosphorus, magnesium, calcium and the ratio between calcium and phosphorus were comparable under the influence of cage and cage-free systems of laying hens.
- In conclusion, we can conclude that the question of the good living conditions of laying hens and their improvement in order to determine the appropriate breeding system in connection with the quality of their production is a very complex issue due to the multifactorial influence, which requires further research to adopt best practices from the aspect of resource-based parameters, management and animal.

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