Laying hens under smallholder conditions: laying performance, growth and bone quality of tibia and femur including essential elements

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ABSTRACT The study aimed to assess laying performance, growth rate, and bone quality properties of tibia and femur bones of various genotypes of laying hens, including determining essential element composition at the end of the laying cycle in smallholder conditions. The study included three genotypes of laying hens; Czech golden spotted (**CGS**), White Leghorn (**LE**) and Dominant Partridge D300 (**D300**) hens. In total, 180 hens (60/genotype) were used in 3 replications (20 hens/replication). The eggs were collected to determine egg lay and hen-day egg production. Additionally, feed consumption was recorded to determine feed consumption per day or egg, resp. The mortality rate was recorded. Hens were individually weighed every 10 wk to analyze the growth performance and body weight changes during the laying cycle. The differences in performance characteristics were observed as significant in all studied parameters. The bone quality analysis consisted of the determination of bone weight, length, width, and fracture toughness. Furthermore, dry matter, ash, and selected elements, which included boron (B), calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), lead (Pb), and zinc (Zn) were assessed. Regarding the results of tibia and femur bones, the effect of genotype was determined as significant in all evaluated properties. In terms of element composition, all evaluated elements significantly differed among the genotypes in the tibia (with one exception of Cu) and in the femur (with one exception of Cd). In conclusion, our results showed that hens' performance, production quality, mortality and bone properties significantly differed among genotypes under smallholder conditions. Thus, every genotype needs to be carefully considered, when the rearing conditions are set.

Key words: calcium, femur, magnesium, phosphorus, tibia

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INTRODUCTION

The housing conditions of laying hens are nowadays a very actual topic, mainly due to growing concerns of the inexpert public about the welfare of farm animals (Rahmani et al., 2019). In the EU, conventional cages were banned in 2012 and had to be substituted by enriched cages. The allowed alternatives are; aviaries, litter housing, free-range housing, and organic systems, respectively (Dikmen et al., 2016). However, the problematics around the housing systems for laying hens persist because in some countries (e.g., France), many supermarkets have announced the end of selling eggs from enriched cages in the following years. Moreover, the percentage of non-cage systems use continuously increases in the EU, from 8% in 1996 up to 51% in 2019. Despite the fact that the number of non-cage housing systems is currently growing in EU, there are considerable differences among the countries. Alternative housing systems represent less than 10% in Poland or Spain and more than 85% in Austria, Germany, and the Netherlands (Gautron et al., 2021). Smallholder farming is common especially in developing countries, but at present, it arises as a possible alternative in developed countries (Shuma and Gurmessa, 2021).

Generally, the process of egg-laying represents a certain burden for the organism, which consequently deteriorates hens' health status during the life period (Bain et al., 2016). Specifically, skeletal integrity declines with the age of hens, which is especially due to the high demands of the organism on calcium for eggshell formation during the egg-laying period (Whitehead, 2004). Regarding welfare, bone fractures are a serious problem in laying hens

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(Council, 2010). Alternatively housed hens have higher bone strength than hens kept in cages (Leyendecker et al., 2005). On the other hand, the incidence of bone fractures is higher in alternative housing systems compared to cage housing systems (Sandilands, 2011). Not only the incidence of bone fractures but also osteoporosis (predominantly toward the end of the laying cycle) are one of the main welfare concerns regarding laying hens (Eusemann et al., 2018). When the birds have bone-connected problems, they decrease egg production, subsequently increase food intake and the mortality rises up (Riber et al., 2018). In terms of bone quality, there are also concerns over prolonged laying periods (Bain et al., 2016), which nowadays become more common due to higher production. The breeding programs are focused on high production (Liu et al., 2018), which usually results in health issues connected to the selection and breeding programs should take this problem into account (Bain et al., 2016). In addition, bone fractures cause economic losses (Clark et al., 2008).

As a complex material, bone consists of an inorganic part, an organic part, and water (Rodriguez-Navarro et al., 2018). Besides, bone is a living tissue that can be influenced by several factors, including body weight changes, physical activity, or calcium demand (Glimcher, 1998). Indeed, there are other significant factors, such as genotype, gender, nutrition, or the environment, that can influence bone properties and its development (Rose et al., 1996; Talaty et al., 2009). The bones of laying hens can be divided into 3 groups according to the relationship to egg formation − cortical bones, cancellous bones, and medullary bones. Cortical bones represent dense outer parts of bones. Cancellous bones can be generally found at the core of vertebral bones and the ends of the long bones, such as the femur. Medullary bones function as calcium storage for the formation of eggshells. Hence, egg production and egg quality (precisely eggshell quality) are closely related to bone quality and vice versa. The studies targeting specific elements' effect and function in hens' organisms are usually focused on macro minerals, such as calcium and phosphorus or vitamin D3, respectively. However, the importance of trace elements including zinc, manganese, and copper as enzymatic cofactors related to mineralization processes has been confirmed before (Pereira et al., 2020).

This study is one of the first of its kind hence it focuses on the determination of element composition of tibia and femur bones of various laying hen genotypes in detail, which was not sufficiently studied in past, especially in smallholder conditions. The study aimed to assess laying performance, growth rate and bone quality properties of tibia and femur bones of various genotypes of laying hens, including determining essential element composition at the end of the laying cycle in smallholder conditions.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Czech University of Life Sciences Prague (approval document no. 07/2020).

Animals and Housing

The study included three genotypes of laying hens, specifically Czech golden spotted (**CGS**) and White Leghorn (**LE**) hens, which are native breeds of hens, and Dominant Partridge D300 hens (**D300**), which belong to the group of commercial hybrids.

CGS hens, which are gene reserves of the Czech Republic of lightweight type, lay creamy colored eggs with an average egg weight of around 57.5 g. CGS hens are capable of laying up to 110 eggs per laying cycle (Anderle et al., 2014). Hen-day egg production of CGS hens is lower compared to commercial hybrid hens and vary in different housing systems. Hen-day egg production of CGS hens housed in cages reaches around 24.49% and on litter around 30.85%. Live weight of adult CGS hens, who are typical representatives of lightweight type hens, can reach up to 2.0 kg (Zita et al., 2018). LE hens lay eggs with a white eggshell color (Hanusová et al., 2017) and an average egg weight of around 58.5 g (Pohle and Cheng, 2009). Goraga et al. (2012) stated that LE hens lay around 150 eggs during the laying cycle (from 18 to 60 wk of age). According to Pohle and Cheng (2009), hen-day egg production of LE hens may differ from 70 to 89%. However, Jones et al. (2001), state lower values, specifically from 56.88 to 73.38%. Another characteristic of this breed is its body constitution, body weight typically reaches values from 1.42 to 1.71 kg. All these parameters depend on many factors, mainly on hen age or housing system (Pohle and Cheng, 2009). D300 hens are commercial hybrid hens that were bred for high production. Laying cycle of D300 hens starts at the age of 19 wk and ends at the age of 78 wk. During the laying cycle, D300 hens are capable of laying around 320 white and creamy colored eggs with average weight of 62 g. Hen-day egg production can reach up to 95 % at its peak. Body weight of adult hens is between 1.90 and 2.00 kg (Dominant, 2022). Sexual maturity (age, when the first egg is laid) is dependent on the regulation of the hypothalamus-pituitary-gonadal axis (Yilmaz et al., 2015). Factors, such as genotype (Hassan and Abd-Alsattar, 2016), nutrition and light directly affect sexual maturity (Yilmaz et al., 2015).

In total, 180 hens (60/breed) were used in three replications (20 hens/replication). At the age of 20 wk, the hens of each genotype were transferred from the breeding facility into the experimental housing systems and divided into thirds, and kept separately because of the replication of the results. The sexual maturity (age at first egg) varied among the genotypes; CGS (23 wk), LE (26 wk), and D300 (21 wk). For the purpose of the study, the hens were housed until the age of 60 wk, when the study was terminated. Three D300 hens and one CGS hen died during the study. The total number of hens was adapted to the limited number of officially recognized CGS hens.

All hens were kept in an external experimental center of the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague. The litter housing system in open-sided houses was used, and all the animals were housed under the same conditions. The temperature and relative humidity of the environment were natural and corresponded to the season; the study took place from September 2020 to June 2021. The lighting regime was also natural, but artificial light was used when needed to maintain 16 h of light and 8 h of dark. Access to feed and water was ad libitum. All the requirements for housing of laying hens set by the European Commission Directive 1999/74/EC were met.

Feeding

Two feed mixtures for laying hens were used during the study because of the different component requirements of hens during the laying cycle. The feed mixture labeled as N1 was fed to the hens from the age of 20 wk to 40 wk and contained 156.7 g/kg of crude protein and 11.02 MJ of metabolizable energy. Feed mixture labeled as N2, which contained 150.0 g/kg of crude protein and 11.00 MJ of metabolizable energy, was fed to the hens from the age of 40 wk until the end of the study. Detailed composition of feed mixtures N1 and N2 are shown in Tables 1 and 2, respectively.

Performance, Growth Analysis, and Mortality

The eggs were collected every morning throughout the whole study. The eggs were collected to determine egg-lay, egg weight and hen-day egg production and calculated to the initial state. Also, feed consumption was recorded to determine parameters such as feed consumption per day or egg. For the purpose of growth analysis, hens were individually weighed every 10 wk (beginning at the age of 20 wk) to analyze the growth performance and body weight changes during the laying cycle. Last but not least, the mortality rate was recorded.

Table 1. Composition of N1 feed mixture for laying hens.

N1 feed mixture			
Composition (%)		Phosphorus	5.04 g
Barley	5.00	Sodium	1.60 g
Wheat	53.52	Potassium	6.63 g
Corn	10.00	Chlorine	1.90 g
Wheat bran	5.00	Magnesium	1.47 g
Soybean meal (47 %)	15.50	Sulphur	1.69 g
DL-methionine	0.10	Iron	87.87 mg
Mono-calcium phosphate	0.45	Manganese	67.48 mg
Fodder limestone	9.00	Zinc	80.41 mg
Fodder salt	0.25	Copper	12.06 mg
Lysine HCL (98%)	0.08	Iodine	0.90 mg
Rapeseed oil	0.80	Selenium	0.27 mg
VN UCH 304*	0.30	Cobalt	0.36 mg
Chemical analysis of the N1 feed mixture (kg of diet)		Vitamin A	8252 IU
Metabolizable energy	11.02 MJ	Vitamin D	2352 IU
Dry matter	888.90 g	Tocopherol	26.31 mg
Ash	115.38 g	Vitamin K	1.50 mg
Crude protein	156.70 g	Thiamine	5.41 mg
Fat	28.48 g	Riboflavin	5.37 mg
Carbohydrates	37.46 g	Pyridoxine	5.67 mg
Fiber	30.45 g	Vitamin B12	10.68 μg
Starch	426.57 g	Biotin	0.17 mg
Lysine	7.79 g	Folic acid	0.81 mg
Threonine	5.43 g	Niacin	43.32 mg
Methionine	3.35 g	Pantothenic acid	14.91 mg
Linoleic acid	10.98 g	Choline	1213.40 mg
Sulphur amino acids	6.27 g		
Calcium	35.60 g		

*Mineral/vitamin commercial premix.

Table 2. Composition of N2 feed mixture for laying hens.

N2 feed mixture			
Composition (%)		Phosphorus	5.00 g
Barley	5.00	Sodium	1.55 g
Wheat	55.34	Potassium	6.29 g
Corn	10.00	Chlorine	1.90 g
Wheat bran	5.00	Magnesium	1.44 g
Soybean meal (47 %)	13.30	Sulphur	1.64 g
DL-methionine	0.10	Iron	88.46 g
Mono-calcium phosphate	0.39	Manganese	68.30 mg
Fodder limestone	9.70	Zinc	79.99 mg
Fodder salt	0.25	Copper	11.69 mg
Lysine HCL (98%)	0.12	Iodine	0.90 mg
Rapeseed oil	0.50	Selenium	0.27 mg
VN UCH 304*	0.30	Cobalt	0.35 mg
Chemical analysis of the N2 feed mixture (kg of diet)		Vitamin A	8252 IU
Metabolizable energy	11.00 MJ	Vitamin D	2352 IU
Dry matter	889.00 g	Tocopherol	26.53 mg
Ash	120.54 g	Vitamin K	1.50 mg
Crude protein	150.00 g	Thiamine	5.44 mg
Fat	25.54 g	Riboflavin	5.33 mg
Carbohydrates	35.69 g	Pyridoxine	5.62 mg
Fiber	29.94 g	Vitamin B12	10.70 μg
Starch	435.93 g	Biotin	0.17 mg
Lysine	7.48 g	Folic acid	0.80 mg
Threonine	5.08 g	Niacin	43.71 mg
Methionine	3.24 g	Pantothenic acid	14.81 mg
Linoleic acid	10.626 g	Choline	1171.20 mg
Sulphur amino acids	6.04 g		
Calcium	38.11 g		

*Mineral/vitamin commercial premix.

Bone Quality Analysis

The bone quality analysis consisted of the determination of bone weight, length, width, and breaking strength. Furthermore, dry matter, ash, and eleven selected elements, which include boron (B), calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), lead (Pb), and zinc (Zn) were assessed. The analysis was performed on the tibia and femur bones. Bones were taken from 7 randomly chosen hens (that were slaughtered at the age of 60 weeks) from each replication and each genotype from the right leg. It means that 42 bones (21 tibia bones and 21 femur bones) from each genotype were analyzed in total.

The bones were de-fleshed without boiling, subsequently individually packed in plastic bags, and frozen at −20°C until the analysis. The bones were thawed for 24 h and cleaned from all excessive tissue before the analysis. The length and width (in the middle of the bone) were measured 3 times for each bone by an electronic sliding caliper (DIN 862; IP54; Shut Geometrical Metrology; Gröningen, Netherlands) with 0.01 mm precision. To determine fracture toughness, the Instron device (Instron Universal Testing Machine; model 3342; Instron Ltd.; Norwood, MA), which calculates the force (in N) required to break the bone, was used. The 50-kg-load cell at 50-kg-load range with a crosshead speed of 50 mm/min with bone supported on 3.35-cm span according to Shafer et al. (2001). After the determination of fracture toughness, the bones were dried for 24 h at 105°C and weighed on a digital laboratory scale Ohaus (Model: Traveler TA502, Parsippany, NJ) with 0.01 g precision. Broken bones were subsequently used for the elemental composition analysis.

Bone composition and determination of elements content were made as follows. The bone dry matter content was determined by oven drying at 105°C. The ash content was determined by oven burning at 550°C. The ashed samples were then treated with concentrated HCl and HNO_3 acids and the elemental compositions were analyzed using ICP-OES iCAP 7000 (Thermo Fisher Scientific, Waltham, MA); the limit of detection (**LD**) was calculated using the equation: LD = 3.29 $\sigma0$ (where $\sigma0$ is a blank sample standard deviation). The samples and standards were matrix matched. Several procedural blanks were included throughout the analysis.

The analysis of basic bone quality properties was carried out in the laboratory of the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague, while the analysis of element composition in the laboratory of the Department of Soil Science and Soil Protection of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague.

Statistical Analysis

The data were analyzed by statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, 2012). The data were tested for normality with univariate plot standard procedure of SAS and subsequently subjected to a one-way ANOV, with genotype (CGS, LE, D300 hens) as main effect using the GLM procedure of SAS. The data of performance parameters (egg lay, hen-day egg production, feed consumption per feeding day and feed consumption per egg) and bone quality properties were determined using Duncan's test and mortality with non-parametric Kruskal-Wallis test. The value of $P \leq 0.05$ was considered statistically significant. The results in the tables show the average values of each treatment and the standard error of the mean (**SEM**).

RESULTS

Performance Parameters

Performance parameters, precisely egg lay, egg weight, hen-day egg production, feed consumption per feeding day, and feed consumption per egg of selected hen genotypes, are presented in Table 3. Figure 1 displays egg lay curve of selected genotypes from 20 to 60 w, of age throughout the season. Statistically significant differences among the genotypes in performance characteristics were found in all evaluated parameters. D300 hens had significantly the highest egg lay, hen-day egg production (both parameters jointly with LE hens), egg weight, feed consumption per feeding day and mortality, and the lowest feed consumption per egg. On the other hand, the CGS hens had significantly the lowest egg lay, egg weight, hen-day egg production, and feed consumption per feeding day, and the highest feed consumption per egg. LE hens had the lowest mortality.

Growth Rate

Figure 2 displays body weight gains of observed hen genotypes (D300, CGS, and LE) in 10 wk intervals from 20 to 60 wk of age. D300 hens had the highest average body weight throughout the study, followed by LE hens (with the only exception at 30 wk of age, where CGS hens were heavier than LE hens). CGS hens had the lowest body weight from the beginning until the end of the study (as mentioned above, with the only exception at 30 wk of age). All observed genotypes reached the peak (the highest body weight) at the end of the monitored period, at the age of 60 wk. Life weight of all included genotypes gradually increased from the beginning (20 wk of age) to the end (60 wk of age) of the studied period.

Bone Quality Properties

Basic bone quality properties including fracture toughness, length, width, and weight were analyzed, and differences among the selected genotypes were calculated. The results are presented in Table 4 for tibia bone and Table 5 for femur bone. Regarding the tibia bone, the effect of genotype was determined as significant in all evaluated properties. In femur bones, all properties were significantly affected by genotype. Significantly the highest fracture toughness values were found in tibia bones of LE and D300 hens and in femur bones of D300 hens. On the other hand, the lowest values were calculated for CGS hens for tibia bones and CGS and LE hens for femur bones. An identical state was observed in bone weight. The differences in bone length and width among the genotypes were statistically significant for both observed bones.

Table 3. Performance characteristics of selected hen genotypes.

Parameter	Genotype			P-value	SEM
	CGS	LE	D300		
Egg lay (eggs)	75.43^{b}	129.58^{a}	132.44^{a}	0.0001	1.345
Egg weight (g)	52.78^{c}	56.20^{b}	59.30^{a}	0.0001	0.145
Hen-day egg production (%)	27.54^{b}	45.26^{a}	53.22^{a}	0.0001	0.545
Feed consumption per feeding day (g)	113.97^{c}	151.73^{b}	162.42^{a}	0.0015	1.113
Feed consumption per egg (g)	500.79^{a}	446.69^{b}	340.07^{c}	0.0001	3.247
Mortality (%)	1.67^{b}	0.00^{c}	5.00^{a}	0.0001	0.148

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different ($P \leq 0.05$)

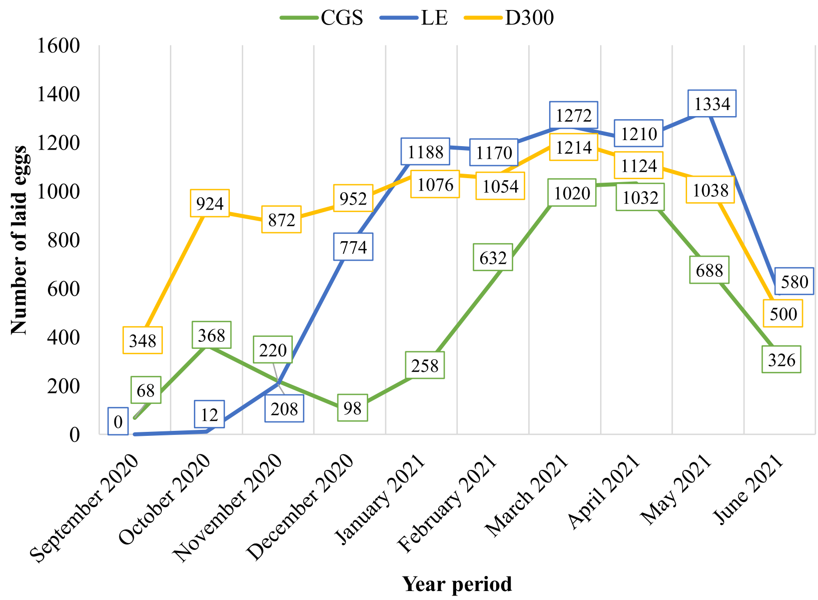

Figure 1. Egg lay from 20 to 60 weeks of age throughout the observed period. Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Chemical and Element Composition of Bones

The content of dry matter, ash, and selected macro and micro-elements (B, Ca, Cd, Cu, Fe, Mg, Mn, Na, P, Pb, and Zn) is shown in Table 6 (for tibia bones) and in Table 7 (for femur bones). These tables display the differences among observed genotypes in the properties mentioned above of tibia and femur bones in detail. Statistically, the highest amount of dry matter was found in bones from D300 and CGS hens for both tibia and femur and, therefore, the lowest in bones from LE hens. The effect of genotype on ash content was found to be significant only for femur bones, and the highest value was detected in bones from LE hens, while the lowest in bones from D300 and CGS hens. In terms of element composition of the tibia, D300 hens had significantly highest amounts of Ca, Mn, P, and Zn and the lowest amounts of Fe, Mg, Na, and Pb (jointly with LE hens). CGS hens had the highest amounts of Fe (jointly with LE hens), Mg, Na, and Pb and the lowest amounts of Ca, Mn (jointly with LE hens), P, and Zn (jointly with LE hens). The effect of genotype showed identical results for femur as for tibia in the following elements: B, Cd, Fe, Mn, Pb, and Zn. Moreover, for the femur, D300 hens had significantly the highest amounts of Ca, Cu, and P and the lowest amounts of Mg and Na. CGS hens had significantly the highest amounts of Mg and Na and the lowest amounts of Cu (all jointly with LE hens). Last but not least, LE hens had statistically the lowest amounts of Ca and P. The elements not mentioned in this section did not statistically differ among the genotypes.

DISCUSSION

Performance Parameters

The effect of genotype, specifically, comparison of commercial hybrids with native breeds of laying hens on performance parameters, was subject to several studies (Ershad, 2005; Rizzi, 2020; Özentürk and Yildiz, 2021). The results of the present study are in accordance with the

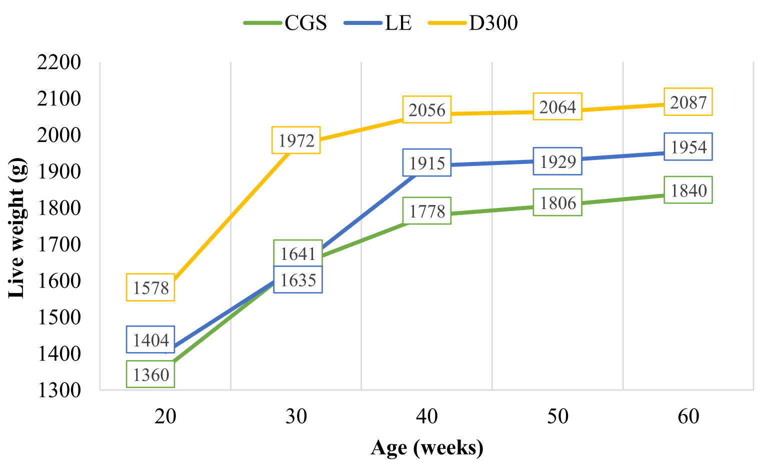

Figure 2. Growth rate of selected hen genotypes from 20 to 60 weeks of age. Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Table 4. The effect of hen genotype on basic tibia properties.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Fracture toughness (N)	185.77[b]	228.87[a]	218.12[a]	0.0463	11.010
Length (mm)	109.19[b]	117.63[a]	116.29[a]	0.0018	1.227
Width (mm)	6.99[b]	7.64[a]	7.51[a]	0.0391	0.133
Weight (g)	7.14[b]	8.46[a]	9.58[a]	0.0034	0.341

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different ($P \leq 0.05$)

Table 5. The effect of hen genotype on basic femur properties.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Fracture toughness (N)	224.08[b]	239.69[b]	272.31[a]	0.0491	17.169
Length (mm)	76.71[b]	78.69[ab]	81.03[a]	0.0483	0.748
Width (mm)	7.71[ab]	7.30[b]	7.90[a]	0.0414	0.104
Weight (g)	5.80[b]	7.02[a]	8.00[a]	0.0039	0.310

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different ($P \leq 0.05$)

generally known fact that high-productive commercial hybrids, reach higher egg lay, egg weight, hen-day egg production, and therefore lower feed consumption per egg in comparison with native breeds (Özentürk and Yildiz, 2021). The differences of these parameters might be supported by the different age of sexual maturity, which was the lowest in D300 hens. However, the findings of our study showed that D300 hens (commercial hybrid) did not reach their full performance potential according to the technological manual of the hybrid (Dominant, 2022), and compared to native breeds, they had a significantly worst mortality rate. This may raise questions about the suitability of commercial hybrids in smallholder conditions in terms of health or welfare. Sokołowicz et al. (2018) recorded the mortality of various genotypes (including commercial hybrids and native breeds) across various housing systems. Likewise, they found out that commercial hybrids compared with native breeds had significantly

Table 6. Properties and element composition of tibia bone regarding the genotype.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Dry matter (%)	85.57[a]	78.790[b]	85.010[a]	0.0001	0.671
Ash (%)	49.89	48.284	48.618	0.4781	0.564
Boron (mg/kg)	4.21	4.13	4.19	0.8645	0.028
Calcium (g/kg)	229.03[c]	253.37[b]	281.85[a]	0.001	3.643
Cadmium (mg/kg)	0.13	0.14	0.14	0.9321	0.007
Copper (mg/kg)	16.30	19.32	19.05	0.0505	0.565
Iron (mg/kg)	95.95[a]	89.81[a]	68.75[b]	0.0099	3.952
Magnesium (g/kg)	3.32[a]	3.20[ab]	3.15[b]	0.0414	0.291
Manganese (mg/kg)	8.51[b]	9.35[b]	11.59[a]	0.0001	0.329
Sodium (g/kg)	6.52[a]	6.09[b]	5.67[c]	0.0001	0.650
Phosphorus (g/kg)	95.54[c]	107.78[b]	114.62[a]	0.0001	1.344
Lead (mg/kg)	13.96[a]	5.320[b]	7.25[b]	0.0015	1.081
Zinc (mg/kg)	214.43[b]	222.54[b]	292.26[a]	0.0001	6.758

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different ($P \leq 0.05$)

Table 7. Properties and element composition of femur bone regarding the genotype.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Dry matter (%)	80.79[a]	77.479[b]	82.870[a]	0.0005	0.598
Ash (%)	42.61[b]	47.134[a]	44.408[b]	0.0020	0.546
Boron (mg/kg)	4.27	4.33	4.35	0.9112	0.034
Calcium (g/kg)	234.36[b]	215.86[c]	265.79[a]	0.0001	3.477
Cadmium (mg/kg)	0.14	0.13	0.13	0.3765	0.003
Copper (mg/kg)	14.69[b]	15.12[b]	20.18[a]	0.0001	0.639
Iron (mg/kg)	127.03[a]	134.36[a]	91.51[b]	0.0002	4.853
Magnesium (g/kg)	3.41[a]	3.40[a]	3.10[b]	0.0001	0.333
Manganese (mg/kg)	11.13[b]	11.45[b]	15.54[a]	0.0001	0.408
Sodium (g/kg)	6.28[a]	6.15[a]	5.32[b]	0.0001	0.749
Phosphorus (g/kg)	99.92[b]	89.01[c]	109.64[a]	0.0001	1.424
Lead (mg/kg)	15.98[a]	4.76[b]	9.23[b]	0.0001	0.866
Zinc (mg/kg)	264.46[b]	247.84[b]	344.61[a]	0.0001	7.800

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different ($P \leq 0.05$)

highest mortality in a litter housing, where the Hy-line Brown hens had 2.4%, Rhode Island Red hens 0.6%, Greenleg Partridge hens 0.4%, and Sussex hens 0%. The authors observed similar results in organic housing, where commercial hybrids had statistically the highest mortality (Greenleg Partridge hens had 3.3%, Araucana and Rhode Island Red hens 6.7%, and Hy-line Brown hens 10%). These findings may be related to the previously mentioned concern that commercial hybrids are less suitable for alternative housing systems than native breeds, who are better adapted to these housing systems and local conditions, respectively (Kraus et al., 2021).

Growth Rate

The flock's live weight and weight uniformity are essential factors for egg production. The body weight of hens changes because of the sexual and physical maturity during life (Lacin et al., 2008). Leeson et al. (1997) observed body weight changes of four different strains of Leghorn hens and found out that body weight constantly increased from 22 to 66 wk of age, which corresponds to our findings, where the life weight of hens of all observed genotypes constantly increased during the monitored period (from 20 to 60 wk of age). Also, the growth and individual weights of LE hens are very similar between the study of Leeson et al. (1997) and ours. When comparing the growth curves, the trend was very balanced among the selected genotypes. Regarding the body weight, CGS hens had the lowest body weight compared to D300 and LE hens, which may be caused by genetics, because CGS hens are typical representatives of the lightweight breed (Kraus et al., 2021).

Bone Quality Properties

Considering the bone quality, scientific literature often focuses on the effect of nutrition (Świątkiewicz et al., 2010) or housing system (Tactacan et al., 2009). However, several studies, such as (Riczu et al., 2004; Sharma et al., 2021), observed the effect of genotype.

Sharma et al. (2021) observed the genotype's effect on quality properties of the tibia bone, also confirmed that genotype significantly influenced bone length and fracture toughness. Unlike our study, the authors did not confirm the significant effect of genotype on tibia weight, which can be attributed to comparing different genotypes between the studies. Significant differences in tibia length and weight might refer to different average body weights and sizes of each genotype at the end of the study when the sampling was conducted. The study of Riczu et al. (2004) compared fracture toughness of femur bones between brown and white egg-laying hens and concluded that brown egg-laying hens had a significantly higher fracture toughness (30.68 kg) than white egg-laying hens (19.54 kg). Furthermore, they confirmed the significant effect of genotype on femur weight, which is also in accordance with our findings. Vice versa they did not confirm this effect for femur length. Regardless of some differences among studies or evaluated bones, it is evident that genotype belongs to factors that influence bone properties, such as fracture toughness, weight, or size.

Chemical and Element Composition of Bones

Similarly to our results, the effect of genotype on dry matter and ash content in the tibia of laying hens was observed by Silversides et al. (2012), who confirmed that genotype significantly influenced both of these properties. The authors compared the following genotypes: Lohmann White, Lohmann Brown, Cross (Rhode Island Red × Barred Plymouth Rock) and H&N White. The authors observed that Cross had the highest value of dry matter and ash (10.645 g; 4.436 g) and H&N White and Lohmann White had the lowest (6.604 and 7.015 g; 0.636 and 0.640 g). Moreover, the content of dry matter and ash in tibia bone was studied by Yalcin et al. (2001), who analyzed the effect of strain in broiler chickens and concluded that this factor significantly influenced the content of dry matter and ash. Sharma et al. (2021) found the same results in the effect of genotype on tibia ash content (nonsignificant effect of genotype). However, for femur bones, the ash content was calculated as significant among the genotypes. The differences between the results of tibia and femur bones might be caused by the different composition of the bones. The tibia belongs among the most mineralized group of bones and therefore is often used as an indicator of overall skeletal mineralization (Rose et al., 1996; Talaty et al., 2009), so, when bone quality is measured, tibias are typically used (Min et al., 2019; Sibanda et al., 2020; Teng et al., 2020). Moreover, it is in accordance with our results, where a higher amount of ash was found in the tibia than in the femur in all genotypes.

Regarding the elemental composition of bones, scientific studies usually focus on nutrition (Olgun and Aygun, 2016) or the housing system in connection with movement (Krunt et al. 2021). However, our study analyzed the elemental composition of bones from a different perspective and focused on the effect of genotype. Scientific literature concerning the effect of genotype on elemental composition of bones is limited, but other effects, such as nutrition (Jing et al., 2018) or housing system (Newman and Leeson, 1998) were previously studied. Jing et al. (2018) observed the content of Ca and P, while Newman and Leeson (1998) only the content of Ca. Many studies previously confirmed the essential role of Ca, P, and Mg for bone quality across the animal species, from rats (Takahara et al., 2000), through rabbits (Krunt et al., 2021) to poultry (Shastak and Rodehutscord, 2015). In the present study, the effect of genotype resulted in significant differences of Ca, P, and Mg content in tibia and femur bones. In laying hens, demand for Ca is high, mainly during the peak production period and also towards the end of the laying cycle, when the efficiency of Ca absorption from feed decreases (Al-Batshan et al., 1994). Approximately 20 to 40% of Ca needed for eggshell formation is supplied from bones, representing a specific bone integrity burden (Mueller et al., 1964). Bone quality is in close relationship with egg production and subsequent egg quality. Therefore, the selection for high production negatively influences bone quality. Negative correlations between bone fracture toughness and egg production and bone fracture toughness and eggshell thickness were determined (Bishop et al., 2000). Bone quality is not defined only by the content of Ca, but also by the content of P, which is in close relationship with Ca and is essential for bone structure. Especially, the ratio between Ca and P is crucial because the relationship between Ca and P is inverse, which means that the more P is in the blood, the less Ca there is and contrariwise (Copp, 1957). Furthermore, P plays a key role in eggshell formation (Taylor, 1965). Wei et al. (2021) determined differences in the element content of fractured and nonfractured keel bones, including all elements as in our study. The authors found significant differences in B, Ca, Cu, Na, P, and Pb content among observed groups of birds with results of: higher amount of Ca (154,840.10 vs. 110,095.10 mg/kg), P (76,904.19 vs. 62,448.86 mg/kg), Na (1,430.35 vs. 1,068.37 mg/kg) and lower amount of B (2.46 vs 3.59 mg/kg), Cu (0.86 vs 1.20 mg/g), and Pb (0.97 vs. 2.26 mg/kg) in nonfractured keel bones compared to fractured ones. In general, Ca and P are mutually influenced, and Mg is closely connected to them, while Mg is an antagonist to Ca (Shastak and Rodehutscord, 2015). Krunt et al. (2021), who studied Ca, P, and Mg content in the tibia and femur bones of rabbits in various housing systems, highlighted the importance of Mg for bone quality, specifically for fracture toughness, and concluded that Mg could be a key player in the determination of bone fracture toughness. Considering the element Mg, it is essential for the metabolism of cells and bone development (Shastak and Rodehutscord, 2015).

From generally less discussed elements affecting bone quality, B belongs among the important ones because it interacts with Ca, Mg, and vitamin D. The amount of B in bones depends on the amount of B received from feed (Chapin et al., 1998). Nevertheless, Cu, Fe, Mn, and Zn are also important for bone quality hence they participate in bone-related metabolic processes (Palacios, 2006). For example, a deficiency of Mn may cause several bone abnormalities (Spears, 2019). Osteoporosis is a risk factor affecting bone quality, which is also influenced by Na. High intake of Na from feed negatively influences Ca metabolism, respectively its excretion from organism (Teucher and Fairweather-Tait, 2003).

Cd (Rani et al., 2014) and Pb are major environmental pollutants (Angelidis et al., 2011). Cd accumulates upon exposure in several organs (e.g., brain, kidney, and liver), including bones (Nordberg, 2009), which belong among the most critical target organs influenced by Cd exposure. The unfavorable effect of Cd on bone quality is characterized by a higher occurrence of fractures and decreased level of mineralization in comparison with a standard state (Sughis et al., 2011). Toxicity of Cd causes disorders in bone cells' metabolism and absorption (and excretion) of Ca in the intestines and kidneys, which leads to a lack of Ca and, therefore, to bone abnormalities and defects (Chen et al., 2011). Similarly, Pb can accumulate in bones (Angelidis et al., 2011), and its deposition is highly enduring thus it forms stable complexes with P (Agrawal, 2012). When the concentration of Pb in bones is high, the degree of mineralization dramatically decreases, which can lead to osteoporosis or to bone weakness (Álvarez-Lloret et al., 2014).

CONCLUSIONS

Significantly LE and D300 hens had the highest values of fracture toughness for tibia bones and for femur bones D300 hens. Regarding the egg lay and hen-day egg production, statistically LE and D300 hens had the highest values of both parameters. The differences among the genotypes in the majority of bone properties (including element composition) were found to be statistically significant. D300 hens had the best results in terms of egg lay, hen-day egg production, and bone strength, but from the mortality point of view, this genotype had statistically the worst results. It might indicate that native breeds are better adapted to local environmental conditions and smallholder housing conditions.

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DISCLOSURES

We declare that there has been no conflict of interest.

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