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EFFECT OF ORIGIN AND FLOCK SIZE ON PERFORMANCE AND BONE QUALITY OF HENS OF LOCALLY ADAPTED BREEDS

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Abstract

Production results, morphometric indices and mineralisation levels of long bones, as well as selected blood parameters of locally adapted breeds of hens were compared according to flock size. Birds were assigned to 4 experimental groups differentiated by breed: Sussex (S-66) and Leghorn (H-22) and herd sizes: 50 and 200 pieces. During the experiment, production results were monitored, and in the 64th week of rearing, femur, tibia and humerus bones were dissected from birds in each group, weighed and measurements were taken: length and width. The Seedor Index (SI) and the percentage of bones in relation to body weight were calculated. The content of calcium (Ca), phosphorus (P) and crude ash (CA) was examined in the bones. Blood was also collected from 64-week-old hens, in which levels of Ca, P, pyridinoline and deoxypyridinoline were determined. The origin of the hens and the interaction of experimental factors (breed x herd size) influenced the production results. Analysis of bone morphometric indicators showed differences between races in terms of weight and width in the case of humerus and length and Seedor index (SI) in all three types of bones. In the conducted studies, Sussex hens (S-66) were characterized by worse production results. Shorter bones and a higher Seedor Index (SI) were also found in hens of this breed, which may indicate higher bone density and strength compared to the Leghorn (H-22) breed. No effect of herd size on the parameters tested was observed. However, similar studies should be carried out, taking into account the rearing of these breeds with access to paddocks and ecological.

Keywords: hens of locally adapted breeds, herd size, productivity, bone quality

Introduction

Modern rearing of laying and meat hens brings with it a major welfare problem associated with bone damage, resulting in pain, higher mortality and economic losses (Heerkens et al., 2016; Candelotto et al., 2017). Bone damage also reduces the mobility of birds and has a negative impact on productivity, feed utilization and egg quality (Heerkens et al., 2016; Casey-Trott et al., 2017). Intensive rearing methods that limit the movement of hens lead to bone loss and increase the tendency to bone fragility – osteoporosis (Sharma et al., 2021). Recent studies indicate that despite raising animal welfare standards during rearing, the percentage of birds with fractures is very high and can range from 20 to 96% in commercial flocks (Toscano et al., 2020). There are also reports in the scientific literature of a high frequency of bone damage in hens reared in alternative systems (Wilkins et al., 2011; Heerkens et al., 2016). Although birds have greater mobility in these support systems, which generally stimulates the formation of strong bones, they often break as a result of, for example, collisions with poultry house equipment (nests, feeders, drinkers, perches) or falls when moving between perches or levels in the building, as well as damage and bone deformations caused by excessive load when birds squat on the perch (Stratmann et al., 2015; Heerkens et al., 2016). In turn, the research of Kolakshyapati et al. (2019) showed that the hens' access to the paddock also had no beneficial effect on bone quality.

In Poland, usually domestic and locally adapted breeds of chickens are recommended for alternative rearing, which use paddocks better than commodity hybrids, and are less susceptible to adverse weather conditions. Indigenous and locally adapted breeds of hens are used for organic farming (Sosnówka-Czajka et al., 2017), as well as for the production of capons (Calik et al., 2017) and pulards (Krawczyk et al., 2019), niche products considered exclusive and distinguished by their very good quality (Kuźniacka et al., 2017). Hence, there is a need to research bone quality in hens of native and locally adapted breeds, not only because of their welfare, but also food safety (leaving bone chips in meat) and economic losses.

Most studies on skeletal development in poultry are carried out on the long bones: tibia, femur and arm, as they contribute to overall skeletal stability (Souza et al., 2017). A characteristic feature of skeletal health is bone mineralization, which is strongly correlated with their structural strength (Ogunwole et al., 2018; Ma et al., 2020). Rath et al. (2000) indicate the relationship between bone strength and bone growth and weight. According to Almeida Paz and Bruno (2006), one of the most important parameters of bone is mineral density, which can be determined using, among others, bone mineral composition, fracture strength or Seedor index (SI). The authors state that this feature of the condition of the skeleton depends on many factors such as: age, sex, diet or the amount of egg production. Many studies have also found differences in skeletal properties depending on the origin of birds (Onbaşilar et al., 2016; Mabelebele et al.,2017; Sharma et al., 2021; Skomorucha and Sosnówka-Czajka, 2021).

One of the factors influencing the health and welfare of birds is the size of the flock (Marin et al., 2014). Depending on the size of the group, bird behaviour and social relations in the flock are formed (Rodenburg and Koene, 2007; Sosnówka-Czajka et al., 2007; Marin et al., 2014), which may affect stress levels, feed intake and productivity (Vits et al., 2005; Rodenburg and Koene, 2007; Marin et al., 2014), and thus also on bone development and quality. Widowski et al. (2017) report that hens cluster together when performing certain behaviours, resulting in higher densities in certain areas of the henhouse, while other areas of the house are largely unoccupied. High bird densities lead to reduced activity and insufficient space availability associated with a higher frequency of injuries, especially wing fractures (Marchewka et al., 2013).

Hence, the aim of the study was to compare production results and morphometric indicators and the level of mineralization of the arm, femoral and tibial bones, as well as selected blood parameters of locally adapted breeds: Leghorn (H-22) and Sussex (S-66) depending on the size of the herd.

Material and methods

The experiment was carried out on the experimental Poultry Farm of IZ PIB. The experimental material consisted of a total of 1000 locally adapted hens covered by the genetic resourcesconservation program. The birds came from ZD IZ PIB Chorzelów Sp. z o. o. 18-week-old hens were assigned to 4 experimental groups differentiated by breed: Sussex (S-66) and Leghorn (H-22) and herd sizes: 50 and 200 pieces in the herd. Each group consisted of 4 subgroups. The birds were reared on litter with a stocking density of 9 pcs./m^2 to 64 weeks of age. They were fed at will with standard feed mixtures prepared on the basis of concentrates intended for laying hens (Table 1). Throughout the experiment, the birds had free access to drinking troughs with water. During bird rearing, the air temperature was at the level of 33-31° in the first days and was gradually reduced to about 16 ° C in the 11th week of rearing, while the humidity was maintained at 65%. During the laying period, a light program was used with 16 hours of light and 8 hours of darkness.

Table 1. Ingredient composition and nutritive value of diets (%)

Item	%
Ground maize	58,1
Wheat bran	4,00
Soybean meal	15,00
Rapeseed cake	3,00
Maize DDGS	7,00
Dried alfalfa	2,00
Mineral-vitamin premix	1,00
Ground limestone	8,60
Monocalcium phosphate	1,00
NaCl	0.25
DL-methionine	0.05
Nutrients per kg	
Crude protein (%)	15,8
Metabolisable energy (MJ)	11,2
Lysine (g)	7,0
Methionine (g)	3,3
Calcium (g)	34,1
Phosphorus (g)	3,4

Provided per kilogram of diet: vit. A 10000 IU; vit. D_3 2500 IU; vit. E 25 IU; Mn (manganese oxide) 85 mg; Mn (dimanganese chloride trihydroxide) 15 mg; Cu 15 mg; Fe 70 mg; Zn 80 mg; I 1.5 mg; Se 0.2 mg; Ca min. 0.84 g; At 1.57 g; crude protein 1.45 g; crude fibre 0.002 g; ash 6.60 g; crude fat 0.072 g; bald 0.3 g; methionine 1.5 g; ME 19.08 kcal.

During the rearing of hens, production results were recorded: laying capacity, egg weight and feed intake (g/pc) and feed utilisation (g/1 egg and g/1 kg of eggs). At the 64th week of rearing, 10 birds per group were randomly selected, in which the right femur, tibia and humerus were dissected after being weighed and slaughtered. After being cleaned of soft tissues, the bones were weighed and measurements were made using an electronic caliper: length and width (at the longest and narrowest point), and the Seedor Index was calculated according to the *formula SI = bone weight (mg)/bone length (mm),* indicating bone density (Souza et al., 2017). The percentage of prepared bones in relation to body weight was also calculated. The content of calcium (Ca), phosphorus (P) and crude ash (CA) was also examined in the prepared bones. The bones were burned in a muffle furnace in three stages: at 600ºC for 21 h, after which the temperature was increased to 850ºC within 2 hours and the samples were kept at this temperature for 1 hour. The resulting ash was homogenized. The weighed quantity of ash was diluted at room temperature in 100 ml flasks in 0,6 mol/l HCl. The resulting solution was analyzed for Ca content by atomic absorption spectrometry and for P content by spectrophotometric method (wavelength 700 nm). Crude ash was analysed according to the Coutand et al. method. (2008). The ratio of Ca to P was also calculated.

At 64 weeks of rearing, blood was also collected from 10 birds in the group to determine pyridinoline, deoxypyridinoline, calcium (Ca) and phosphorus (P). Blood was drawn in the morning from the pterygoid vein using a vacuum blood collection kit. After collection, the blood was centrifuged (MPW-52 centrifuge) and the plasma separated by centrifugation was pipetted into Eppendorf tubes. Ca and P analysis in bird blood was performed using the Mindray BS-120 biochemical analyzer and Alpha Diagnostics' reagents and methodology. Pyridinoline and deoxypyridinoline were determined by enzyme-linked immunosorbent assay (EIA) on Mindray MR-96 A ELISA reader using the Chicken Pyridinoline ELISA Kit (E0246Ch Ref.) and the Chicken Deoxypiridinoline Crosslinks ELISA Kit (E0248Ch Catalog No.) manufactured by Bioassay Technology Laboratory (BT laboratory, Shanghai, China). The research was conducted with the consent of the First Local Ethical Committee for Animal Experiments in Krakow (No. 58/2017).

The results were statistically compiled using two-factor ANOVA analysis. Significant differences in means between experimental groups were determined by the Duncan test. The effects were considered significant at the probability of $p<0.05$ and $p<0.01$. Statistica 12 was used for statistical calculations (StatSoft Inc., 2011, USA).

Results

The effect of hen breed on laying capacity, egg weight and feed conversion per kg of eggs was found at $p<0.05$, $p<0.01$ and $p<0.05$, respectively (Table 2). There was also an interaction of experimental factors in the case of egg weight ($p<0.05$), feed utilisation per egg ($p<0.05$) and 1 kg eggs ($p<0.01$). Bird mortality in flocks of 200 birds was 0.2% higher compared to groups with fewer chickens.

There was a difference in the weight and width of the arm bone between the Leghorn and Sussex hens at $p<0.05$ (Table 3). Leghorn chickens had longer bones ($p<0.05$ and $p<0.01$) and lower Seedor index (SI) at $p<0.01$ and $p<0.05$. In the case of tibia, the interaction of experimental factors and its effect on the Seedor Index (SI) was found at $p<0.05$. Herd size had no effect on bone morphometric indicators.

The content of ash and minerals in the bones of both breeds was at a similar level, regardless of the size of the herd (Table 4). There was also no influence of experimental factors on the tested blood parameters (Table 5).

Table 2. Average performance results of hens

Item	Group				Pooled SEM	Breed (A)	Flock size (B)	AxB
	Sussex S-66		Leghorn H-22					
	Sx-50	Sx-200	Lg-50	Lg-200				
Egg production (%)	54,05	50,30	59,88	56,76	1,28	<0.05	NS	NS
Egg weight (g)	55,62a	55.54ac	60.03b	59.47bc	0,72	<0.01	NS	<0.05
Feed intake (g) per bird/day	140,09	131,63	131,92	137,23	1,53	NS	NS	NS
Feed conversion (g) per egg	255,95	269.77 A	223,84b	251,29	6.57	NS	NS	<0.05
Feed conversion (kg) per kg of eggs	4.69A	4.80Aa	3.75B	4.19b	0.11	<0.01	NS	<0.01
Mortality (%)	0,5	0,71	0,5	0,72	-	-	-	-

a ,b – values in rows with different letters differ significantly (p<0.05).
A, B – values in rows with different letters differ highly significantly (p<0.01).
Pooled SEM – Pooled standard error of mean.
Sx-50 –flock of Sussex hens – 50 birds.
Sx-200 –flock of Sussex hens – 200 birds.
Lg-50 –flock of Leghorn hens – 50 birds.
Lg-200 –flock of Leghorn hens – 200 birds.

Table 3. Body weight and bone morphometric parameters of hens at 64 weeks of age

Item	Group				Pooled SEM	Rasa Breed (A)	Flock size (B)	AxB
	Sussex S-66		Leghorn H-22					
	Sx50	Sx200	Lg50	Lg200				
Body weight (g)	2161,43	2327,14	2158,57	2140,00	50,59	NS	NS	NS
Humerus								
Bone weight (g)	5,80	5,99	4,75	5,19	0,19	<0.05	NS	NS
Relative bone weight (%)	0,27	0.26	0.22	0.25	0.01	NS	NS	NS
Length (mm)	77,76	77,71	80,88	80,95	0.57	<0.01	NS	NS
Diameter (mm)	6,32	6,58	6,27	6,17	0.06	<0.05	NS	NS
SI (mg/mm)	74,59	77,04	58,63	64,23	2.42	<0.01	NS	NS
Femur								
Bone weight (g)	9,95	9,70	9,33	9,23	0,24	NS	NS	NS
Relative bone weight (%)	0,47	0.44	0.43	0.44	0.01	NS	NS	NS
Length (mm)	85,73	85,86	87,85	88,40	0.56	<0.05	NS	NS
Diameter (mm)	7,59	7,67	7,52	7,23	0.09	NS	NS	NS
SI (mg/mm)	115,97	119,56	105,76	104,40	1,96	<0.01	NS	NS
Tibia								
Bone weight (g)	11,58	12,06	11,05	11,19	0,31	NS	NS	NS
Relative bone weight (%)	0,54	0,52	0.51	0.53	0.01	NS	NS	NS
Length (mm)	123,30	123,76	125,69	127,77	0,80	<0.05	NS	NS
Diameter (mm)	6,16	6,45	6,18	6,20	0.07	NS	NS	NS
SI (mg/mm)	93,81	97.43 A	89,74	86.36 b	1,52	<0.05	NS	<0.05

* For explanations see Table 2.
SI – Seedor index.

Table 4. Crude ash (CA), calcium (Ca) and phosphorus (P) and Ca to P ratio in bones of hens at 64 weeks of age

Item		Group				Pooled SEM	Rasa Breed (A)	Flock size (B)	AxB
		Sussex S-66		Leghorn H-22					
		Sx50	Sx200	Lg50	Lg200				
Femur	PS (%)	35,56	37,35	35,42	35,31	1,09	NS	NS	NS
	Ca (g/kg)	142,00	150,33	139,67	140,00	4,68	NS	NS	NS
	P (g/kg)	62,07	65,57	61,57	61,23	1.87	NS	NS	NS
	Ca:P	2,29	2,29	2,26	2,29	0.01	NS	NS	NS
Tibia	PS (%)	36,87	37,55	37,78	37,13	0,93	NS	NS	NS
	Ca (g/kg)	147,67	150,00	150,00	150,00	3,78	NS	NS	NS
	P (g/kg)	64,93	65,97	66,07	65,23	1,57	NS	NS	NS
	Ca:P	2,28	2,29	2,29	2,29	0.01	NS	NS	NS
Humerus	PS (%)	42,84	42,90	45,80	44,92	1,29	NS	NS	NS
	Ca (g/kg)	172,33	172,00	183,33	178,33	5,54	NS	NS	NS
	P (g/kg)	75,33	75,43	79,97	77,87	2,33	NS	NS	NS
	Ca:P	2,29	2,29	2,29	2,29	0.01	NS	NS	NS

[*] For explanations see Table 2.

Table 5. P, Ca, pyridinoline and deoxypyridinoline concentrations in blood plasma of hens at 64 weeks of age

Item	Group				Pooled SEM	Rasa Breed (A)	Flock size (B)	AxB
	Sussex S-66		Leghorn H-22					
	Sx50	Sx200	Lg50	Lg200				
P (mmol/l)	0,940	1,176	1,510	1,262	0.09	NS	NS	NS
Ca (mmol/l)	3,632	3,560	4,268	4,166	0.17	NS	NS	NS
Pyridinoline (ng/ml)	63,16	47,58	55,90	55,89	13,43	NS	NS	NS
Deoxypyridinoline (ng/ml)	11,99	13,75	9,93	10,12	3,52	NS	NS	NS

* For explanations see Table 2.

Discussion

In the families of hens covered by the genetic resources conservation program, a large variation is observed, among others, in terms of production parameters (Calik, 2011). This is confirmed by our own research: Leghorn hens were characterized by better laying, higher egg weight and better feed efficiency per 1 kg of eggs compared to Sussex hens. Differences in the production results of hens of different origins were also obtained by Calik (2009), Singh et al. (2009) and Onbasılar et al. (2015). In turn, Vits et al. (2005) and Bovera et al. (2014) showed a significant impact of flock size on the production results of birds, which, however, is not confirmed by their own research. Similarly, Widowski et al. (2017) found no effect of group size on laying, egg weight, or bird weight. The authors showed higher feed/bird/day intake in smaller flocks. In another study comparing laying hens in groups of 8, 10, 20 and 40 head, Wall (2011) found no differences between the groups. In turn, Habig and Distl (2013) noted the lack of effect of flock size on egg weight, and Bovera et al. (2014) on the body weight of birds, which is consistent with own research. Other results were obtained by Marin et al. (2014), who found the dependence of body weight on the number of laying hens in the herd.

Morphometric measurements of long bones, such as weight, length and width, were used as indicators of bone quality in hens. Casey-Trott et al. (2017) report that wider bones are directly correlated with greater fracture strength. Rayan et al. (2013) showed differences in tibia mass and width between Hy-Line Brown and Hy-Line W-36 hens. Also Onbaşılar et al. (2016) found different weights and diameters of the tibia and femur of Lohmann Brown Classic (LB) and Lohmann LSL Classic (LW). LB birds were characterized by thicker and heavier bones compared to LW hens. However, the authors found no differences in the length of the examined bones. In our own studies, the breed of hens had a significant impact on the weight and width of the examined bones only in the case of the humerus, while differences in length were observed in all three types of bones. Sussex hens had wider and heavier humerus and shorter humerus, femur and tibia bonescompared to Leghorn hens. In turn, Skomorucha and Sosnówka-Czajka (2021) found no differences in the width as well as in the length of the humerus, femoral and tibial bones, comparing 64-week-old Sussex and Leghorn hens. In their own studies, Sussex hens had a higher Seedor Index (SI), which may indicate higher bone density and strength (Ogunwole et al., 2018). A study in mammals has shown that bone density is a hereditary trait (Boskey et al., 1999) and that genetic defects can cause bone weakness. In poultry, hens with brown plumage have been shown to have higher bone density and strength than hens with white plumage (Riczu et al., 2004; Habig and Distl, 2013). In our own research, it was found that origin can influence bone characteristics, leading to greater strength, also in hens with similar plumage.

Sharma et al. (2021) report that bone mineralization is strongly correlated with their structural strength and thus fracture resistance. According to the authors, there are genetic differences in the mineralization and strength of growing long bones. Skomorucha and Sosnówka-Czajka (2021) noted the effect of origin on the level of crude ash (PS), phosphorus (P) and the size of the Ca:P ratio in the arm bone of Sussex and Leghorn hens. However, the authors did not find any differences in the level of PS, P and Ca in the other long bones examined, i.e. tibia and femur. Also Rayan et al. (2013) found no significant differences in tibial calcium and phosphorus levels between Hy-Line W-36 and Hy-Line Brown hens. Also in our own studies, the effect of breed on the level of PS, Ca, P and the Ca:P ratio in the long bones of 64-week-old hens was noted. Sharma et al. (2021) report that bone strength correlates with laying capacity and ash levels: the lower the laying capacity, the greater the ash level and the greater the bone strength. In their own study, Leghorn hens had higher egg production compared to Sussex hens, while the percentage of ash in the bones was at a similar level for both breeds. Sosnówka-Czajka et al. (2007) report that in smaller flocks birds show greater motor activity, which has a positive effect on bone mineral density (Jahja et al., 2013; Widowski et al., 2017).

In our own research, however, no effect of herd size on the mineralization of long bones was found.

In our own research, the content of calcium (Ca) and phosphorus (P) in the blood was also determined as an assessment of the state of mineral management directly related to the condition of bones. Homeostasis of Ca and P is important for optimal bone mineralization (Li et al., 2020) and in maintaining bone strength (Rath et al., 2000). Li et al. (2020) report that low serum phosphorus levels lead to the activation of osteoclasts, which results in greater bone resorption. In turn, pyridinoline and deoxypyridinoline are amino acids released from the cross-linked bone structure during collagen degradation during bone resorption (Regmi et al., 2017). In our own studies, no influence of experimental factors on the evaluated blood parameters was observed. Also, Skomorucha and Sosnówka-Czajka (2021) found no differences in the level of calcium, pyridinoline and deoxypyridinoline in the blood of chickens of the three studied breeds.

In summary, the origin of the hens had a significant impact on the production results: laying, egg weight and feed efficiency per 1 kg of eggs. The interaction of experimental factors (breed x herd size) and its effect on egg weight and feed efficiency per 1 egg and 1 kg of eggs was also found. Analysis of bone morphometric indicators showed differences between races in terms of weight and width in the case of humerus and length and Seedor index (SI) in all three types of bones.

In our own studies, Sussex hens (S-66) were characterized by worse production results. Shorter bones and a higher Seedor Index (SI) were also found in hens of this breed, which may indicate higher bone density and strength compared to the Leghorn (H-22) breed. In the experiment, no effect of herd size on the tested parameters was observed. However, similar studies should be carried out, taking into account the rearing of these breeds with access to paddocks and ecological.

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EFFECT OF ORIGIN AND FLOCK SIZE ON PERFORMANCE AND BONE QUALITY OF HENS OF LOCALLY ADAPTED BREEDS

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SUMMARY

Comparison was made of the production results, morphometric indices, mineralization level of long bones, and selected blood parameters of locally adapted breeds according to flock size. Birds were allocated to 4 experimental groups differing in breed: Sussex (S-66) and Leghorn (H-22), and flock size: 50 and 200 birds. Production results were monitored throughout the experiment. At 64 weeks of rearing, femoral, tibial and humeral bones were dissected from the birds of each group, weighed and measured for length and width. Seedor index (SI) and bone percentage in relation to body weight were calculated. The bones were analysed for the content of calcium (Ca), phosphorus (P) and crude ash. Blood was also collected from 64-week-old hens to determine the levels of Ca, P, pyridinoline and deoxypyridinoline. Origin of the hens and the interaction of treatments (breed × flock size) had an effect on production results. Analysis of bone morphometric indices showed that the breeds differed in bone weight and width in the case of humeral bone, and in bone length and Seedor index in the case of all three bone types. Sussex hens (S-66) were characterized by poorer performance. The same hens also had shorter bones and a higher Seedor index, which could indicate that they had greater bone density and strength compared to Leghorns (H-22). Flock size was observed to have no effect on the analysed parameters. A similar experiment should be conducted in which the hens of these breeds will have access to an outdoor area and be reared organically.

Keywords: hens of locally adapted breeds, flock size, productivity, bone quality