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Analysis of bone mineralization, osteometric and mechanical properties in turkey hens at slaughter demonstrates a influence of housing system but not stocking density

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Abstract: *Analysis of bone mineralization, osteometric and mechanical properties in turkey hens at slaughter demonstrates a influence of housing system but not stocking density.* Stocking density and housing system during rearing may negatively influence skeletal development in poultry. However, in turkeys, the studies about the influence of those welfare factors on bone development are extremely limited. It this study, female Big-6 turkeys birds were raised from 7th to 16th week of age indoors (traditional system) or in mixed system where birds were allowed during the day to use veranda at two sticking densities: 3 birds per m^2 (33 kg of predicted final body weight per m^2 of floor space) or 4 birds per m^2 (44 kg of predicted final body weight per m^2 of floor space). The high stocking density significantly decreased only Young modulus of elasticity in tibia, while the positive effect of semi-open housing system was observed in tibia weight, bone mineralization and mechanical endurance, especially in indices describing bone fracture resistance (ultimate load, ultimate train and ultimate stress). Concluding, results of this experiment suggest that in turkeys bone quality is influenced more by housing conditions than by stocking density and open and semi-open rearing systems may have beneficial impact on bone development.

Key words: bone quality, turkey welfare, stocking density, housing system

INTRODUCTION

In modern fast-growing strains of meat poultry selection work and improvement of rearing conditions have resulted in a substantial shortening of the rearing period accompanied by upgrading of the carcass tissue composition. The rate of growth of the muscle tissue has increased more intensively than the rate of growth of other parts of the body. Due to the disproportionate growth of breast muscles, the shift of the bird's center of gravity towards the front disturbs the anatomically optimal distribution of body weight. Impairment of the functioning of the musculoskeletal system resulting from problems with maintaining balance, insufficient bone tissue growth rate and development induces problems in movement and may also lead to bone fracture during catching or transport, creating problems during processing (Rath et al. 2000).

High stocking density during rearing has been identified as one of the welfare factor that may negatively influence skeletal development in poultry, as shown in meat broiler chickens, slow-growing chickens, and lying hens of different age (Hall 2001, Bradsahw et al. 2002, Buijs et al. 2012). High stocking density affects the birds' locomotion ability through a higher severity of skeletal problems (Sorensen et al. 2000), including decreased braking strength of tibia (Škrbić et al. 2009). The high stocking density of growing birds can lead to heat accumulation in poultry house, increasing the risk of heat stress and hyperthermia (Jankowski et al. 2015). High stocking density also increases litter moisture, which promotes the prevalence of foot pad dermatitis.

Another factor that could directly affect the birds welfare and skeletal development is the type of rearing system (Rath et al. 2000). In open or semi-open housing systems birds have more opportunities to perform their natural behaviours because of the access to a more natural environment than in indoor systems (Ekstrand et al. 1997). Another positive aspects of that type of open/semi-open rearing system is the additional space available to the birds, which could lead to limit the litter moisture in the poultry house (Bassler et al. 2013). On the other side, in open or semi-open housing systems birds are exposed to adverse weather conditions (wind, sun radiation and rainfall), risk of infection or predator attack (Stadig et al. 2017).

However, in turkeys, the studies about the influence of housing system or stocking density on bone development are extremely limited. As the bone quality is directly related to the amount bone mineral material, bone spatial structure (geometry and cortical thickness) and mechanical strength of bones, the purpose of this study was to investigate the effect of stocking density and housing system, and its interaction, on tibia osteometric properties, densitometry, and mechanical strength of tibia in female turkeys.

MATERIAL AND METHODS

Animal breeding and experimental design

A total of 144 healthy Big-6 female turkeys were housed indoor during the initial period (up to 7th week of life) under standard turkey rearing conditions (litter maintenance system) and air temperature was set at the optimal level depending on the age. The turkeys had constant access to fresh water and were fed *ad libitum* with standard feed concentrates (Agropol, Motycz, Poland) for particular rearing periods (Table 1).

After the 7th week of life, the birds were individually weighed, and randomly allocated to four experimental

TABLE 1. Composition and nutritive value of the basal diet

Item	Age of birds (weeks)				
	1–3	4–6	7–9	10–12	13–16
Ingredient (%)					
Corn	15.10	15.10	25.00	25.00	25.00
Wheat	34.70	37.50	32.16	38.32	44.80
Soybean meal[1]	42.00	39.20	32.70	26.80	20.30
Soybean oil	1.50	1.50	3.80	4.00	4.50
Phosphate 1-calcium	1.66	1.66	1.46	1.16	0.85
Limestone	1.59	1.59	1.40	1.26	1.10
Sodium bicarbonate	0.04	0.04	0.04	0.04	0.04
Sodium chloride	0.31	0.31	0.31	0.31	0.31
Premix vita-min	0.50[2]	0.50[2]	0.50[3]	0.50[4]	0.50[5]
Concentrate protein-fat[6]	2.00	2.00	2.00	2.00	2.00
DL-methionine 99%	0.34	0.34	0.32	0.26	0.25
L-lysine-HCl 78%	0.26	0.26	0.26	0.25	0.25
L-threonine 99%	–	–	0.05	0.10	0.10
The nutritional value of 1 kg diet:					
**Metabolic Energy (kcal·kg^{-1})	2784	2744	3016	3070	3161
*Total protein (%)	27.2	24.81	21.98	20.57	18.50
*Crude fiber (%)	2.80	3.24	3.11	3.26	3.26
*Crude fat (%)	4.62	5.13	5.78	6.91	7.52
*Lysine (%)	1.76	1.53	1.32	1.22	1.12
*Methionine + Cysteine (%)	1.12	1.10	0.98	0.91	0.86
*Total calcium (%)	1.31	1.15	1.03	0.95	0.83
*Total phosphorus (%)	0.92	0.80	0.73	0.67	0.60
**Available phosphorus (%)	0.61	0.53	0.47	0.41	0.34
**Total calcium/available phosphorus	2.15	2.17	2.19	2.34	2.48

[1] 46% total protein in dry matter

[2] content of vitamins and minerals per 1 kg: Mn 60 mg, J 0.80 mg, Fe 50 mg, Cu 10 mg, Se 0.20 mg, vitamin A 15 000 UI, vitamin D_3 3 166 UI, vitamin E 60 UI, vitamin K_3 3.5 mg, vitamin B_1 2.3 mg, vitamin B_2 6.5 mg, vitamin B_6 4.2 mg, vitamin B_{12} 10.01 mg, biotin 0.13 mg, folic acid 1.2 mg, nicotinic acid 30 mg, pantothenic acid 17 mg, choline 40.30 mg;

[3,4] content of vitamins and minerals per 1 kg: Mn 60 mg, J 0.80 mg, Fe 50 mg, Cu 10 mg, Se 0.20 mg, vitamin A 14 100 UI, vitamin D_3 3 325 UI, vitamin E 40 UI, vitamin K_3 2.75 mg, vitamin B_1 1.9 mg, vitamin B_2 5.5 mg, vitamin B_6 3.6 mg, vitamin B_{12} 15.01 μg, biotin 0.11 mg, folic acid 1.00 mg, nicotinic acid 25 mg, pantothenic acid 14.5 mg, choline 20.00 mg;

[5] content of vitamins and minerals per 1 kg: Mn 60 mg, J 0.80 mg, Fe 50 mg, Cu 10 mg, Se 0.20 mg, vitamin A 12 460 UI, vitamin D_3 2 995 UI, vitamin E 32 UI, vitamin K_3 2.45 mg, vitamin B_1 1.74 mg, vitamin B_2 5.1 mg, vitamin B_6 3.36 mg, vitamin B_{12} 15.00 μg, biotin 0.11 mg, folic acid 0.92 mg, nicotinic acid 23 mg, pantothenic acid 13.5 mg, choline 12.30 mg;

[6] concentrate protein-fat: protein – 65%, fat – 15%

* analysed values; ** calculated values

groups (N = 36 in each group) according to the experimental treatment: two indoor groups and two indoor + veranda groups. The birds in indoor groups were kept in indoor throughout the whole rearing period, and the stocking density was set to 3 birds per m^2 (33 kg of predicted final body weight per m^2 of floor space) in the "indoor 3" group and 4 birds per m^2 (44 kg of predicted final body weight per m^2 of floor space) in the "indoor 4" group. The birds in the indoor + veranda groups were allowed during the day to use veranda, however birds were confined to indoor pen at night. The stocking density was set to 3 birds per m^2 in the "indoor + veranda 3" group and 4 birds per m^2 in the "indoor + veranda 4" group. Feed and water also provided outdoors using trough feeders and water pans with reservoirs.

In the 16th week of life, 8 birds from each group (N = 32 in total) were randomly selected, weighted and slaughtered by cutting the carotid arteries. Immediately after slaughter, the tibiae from individual birds were dissected, cleaned from the remnants of adherent tissues, wrapped in gauze soaked in isotonic saline and frozen prior to analysis at a temperature of –25°C.

Bone analysis

The mechanical properties of right tibiae were determined using the three-point bending test on a Zwick Z010 universal testing machine (Zwick-Roell GmbH & Co., Germany) after overnight thawing at room temperature. The bone, placed on the supports with length span equal to 40% of total bone length, was loaded in the anterior-posterior plane with a displacement rate of 10 mm/min until fracture. Bone structural properties (stiffness, yield strength, ultimate strength, elastic energy and work to fracture) were determined on the basis of recorded force-displacement curves. Whole-bone material properties were calculated on the basis of determined structural properties and geometry of the bone diaphysis using engineering beam-theory equations (Muszyński et al. 2017). The ascertained structural properties included: elastic stress, elastic strain, Young modulus, ultimate strain and ultimate stress.

The bone diaphysis cross-sectional geometry was determined on the basis of osteometric measurements performed on the corresponding left tibiae. The measurements included determination of bone weight, length, and both external and internal diameters of the diaphysis cross-section (both in medial-lateral and anterior-posterior plane). The calculated geometric properties were: cortical cross section area, cortical index, mean relative wall thickness, diaphysis volume and the cross-sectional moment of inertia (Muszyński et al. 2017).

After evaluating the osteometric properties, the bones were subjected to the measurement of whole-bone mineral density (BMD) and bone mineral content (BMC). The analysis was performed using the dual-energy X-ray absorptiometry (DXA) method on a Discovery W densitometer (Hologic Inc., USA) equipped with a software with a Small Animals Studies option for investigation of bones from various types of animals. Also, the bone weight to length ratio (WLR), as the indicator of whole bone density, was calculated.

Statistical Analysis

The data were analysed using a 2 × 2 factorial design with the housing system and stocking density as the factors. The interaction between housing system and stocking density was added to the model. An individual animal was considered as the experimental unit. Whenever significant differences were found between treatments ($P < 0.05$), values were compared by Tukey's HSD test. Probability values with $0.05 < P < 0.1$ were described as trends.

RESULTS

The stocking density or housing system had no effect on birds final body weight (data not shown). The mean body weight in all groups was within the range of 11.0–11.5 kg.

However, there was an effect of housing system on bone length which decreased in birds reared in indoor + veranda system (Table 2). There was also an effect of housing system and stocking density interaction on bone length which decreased in birds reared in indoor at density of 4 birds/m^2. The decrease of tibia mean relative wall thickness in birds reared in the indoor + veranda system at lower density can be described as a trend. There was also a strong trend for reduction of diaphysis volume in tibiae of birds reared in greater stocking density. There was an effect of housing system on BMC and BMD, which were greater in tibiae of birds reared in the indoor + veranda system (Table 2). Bone mineral density was also dependent on stocking density (decreased in birds reared at density of 4 birds/m^2). No changes in other osteometric and densitometric properties were observed (Table 2).

Irrespective of stocking density, the indoor + veranda housing system positively influenced ultimate load (increase of approx. 6 10%,). Similarly, the ultimate strain and ultimate stress were significantly increased in birds from both groups reared in mixed housing system (an increase of 12.8% and 10.1%, respectively). The stocking density influenced the Young modulus, which was increased in groups housed at lower stocking density. There was also a trend in increasing the yield load of bones from birds housed in indoor + veranda system (Table 3). No other changes or trends in tibia mechanical properties were observed.

DISCUSSION

Despite the fact that stocking density has been identified as one of the main factors affecting turkey welfare and growth (Marchewka et al. 2013), to the best of our knowledge, only one study on the effect of stocking density on bone quality have been conducted previously (Jankowski et al. 2015). Similarly, there is only one previous study, where the effect of housing system on the growth and mechanical strength of bones in turkeys was analyzed (Burs et al. 2008).

In our study, high stocking density negatively influenced bone length in birds reared in indoor system, not affecting the bone weight. Also in both housing systems increased stocking density led to (a strong tendency for) reduction of bone diaphysis volume and had a negative effect on bone mineral den-

TABLE 2. The effect of housing system and stocking density on tibia osteometric and densitometric properties in 16-weeks-old female turkeys

Group	Osteometric properties							Densitometric properties		
	Bone weight (g)	Bone length (mm)	Cross section area (mm^2)	MRWT	Cortical index (%)	Diaphysis volume (cm^3)	Moment of inertia (mm^4)	WLR g/mm	BMC (g)	BMD (g/cm^2)
Indoor 3	78.5 ±7.3	202^b ±5.4	76.7 ±11.3	0.691 ±0.135	27.3 ±6.9	6.22 ±1.68	1059 ±209	0.377 ±0.035	7.81 ±1.07	0.273 ±0.043
Indoor 4	78.6 ±6.5	197^a ±3.0	76.7 ±7.2	0.678 ±0.095	29.6 ±3.1	5.95 ±0.81	1046 ±280	0.381 ±0.048	7.70 ±0.70	0.254 ±0.027
Indoor + veranda 3	75.1 ±10.4	199^b ±4.4	77.9 ±9.2	0.594 ±0.179	27.1 ±5.3	6.46 ±1.31	950 ±145	0.383 ±0.044	9.06 ±0.82	0.299 ±0.024
Indoor + veranda 4	76.6 ±6.5	200^b ±3.6	75.7 ±9.3	0.693 ±0.164	31.1 ±6.1	5.85 ±0.95	911 ±110	0.340 ±0.034	8.52 ±1.45	0.279 ±0.032
Significance *P*-value										
Housing system	0.033	0.528	0.863	0.448	0.733	0.138	0.250	0.240	0.010	0.046
Stocking density	0.934	0.030	0.566	0.426	0.123	0.051	0.840	0.194	0.386	0.068
(hs) × (sd)	0.552	0.017	0.462	0.073	0.659	0.355	0.704	0.116	0.574	0.860

Data given are mean ±standard deviation; $^{a, b}$ – mean values in columns with different letters differ significantly at $P < 0.05$.

(hs) × (sd) – interaction between housing system (hs) and stocking density (sd); MRWT – mean relative wall thickness; WLR – weight to length ratio; BMC – bone mineral content; BMD – bone mineral density 3 birds/m^2 – 33.0 kg of predicted final body weight per m^2 of floor space; 4 birds/m^2 – 44.0 kg of predicted final body weight per m^2 of floor space

TABLE 3. The effect of housing system and stocking density on tibia mechanical properties in 16-weeks-old female turkeys

Group	Structural properties					Material properties				
	Yield load (N)	Ultimate load (N)	Elastic energy (mJ)	Work to fracture (J)	Stiffness (N.mm)	Young modulus (GPa)	Yield strain (%)	Ultimate strain (%)	Elastic stress (MPa)	Ultimate stress (MPa)
Indoor 3	528 ±56	708 ±60	270 ±36	1.36 ±0.32	520 ±101	5.31 ±1.02	1.33 ±0.49	2.97 ±0.58	62.0 ±11.6	84.5 ±17.2
Indoor 4	505 ±45	696 ±115	288 ±53	1.36 ±0.26	558 ±38	4.73 ±0.69	1.11 ±0.16	2.65 ±0.29	61.4 ±7.3	84.8 ±14.9
Indoor + veranda 3	558 ±45	793 ±78	283 ±41	1.44 ±0.07	526 ±65	5.67 ±0.68	1.21 ±0.18	3.11 ±0.34	66.7 ±8.1	98.2 ±8.1
Indoor + veranda 4	534 ±53	753 ±48	270 ±51	1.43 ±0.16	527 ±84	4.97 ±1.03	1.25 ±0.37	3.23 ±0.38	67.3 ±9.9	88.3 ±9.2
Significance *P*-value										
Housing system	0.092	0.018	0.333	0.328	0.653	0.348	0.937	0.015	0.119	0.071
Stocking density	0.176	0.360	0.514	0.962	0.460	0.049	0.440	0.466	0.991	0.306
(hs) × (sd)	0.982	0.621	0.891	0.923	0.488	0.866	0.282	0.125	0.856	0.276

Data given are mean ±standard deviation; [a, b] – mean values in columns with different letters differ significantly at $P < 0.05$.

(hs) × (sd) – interaction between housing system (hs) and stocking density (sd); MRWT – mean relative wall thickness; WLR – weight to length ratio; BMC – bone mineral content; BMD – bone mineral density 3 birds/m^2 – 33.0 kg of predicted final body weight per m^2 of floor space; 4 birds/m^2 – 44.0 kg of predicted final body weight per m^2 of floor space sity. This might be caused by the bone shape, as bone length was measured in a straight line. In meat type birds tibia deformations are common (Bradshaw et al. 2002) and high stocking density can additionally lead to increased tibia curvature (Buijs et al. 2012). This suggestion is supported by the lack of a influence of stocking density on tibia weight as well as other determined osteometric indices of bone quality (mean relative wall thickness, cross section area, moment of inertia) and bone mineral content. This is also in agreement with other studies performed on poultry, showing that stocking density generally is not influencing bone mineral content or bone ash percentage in broiler chickens (Tablante et al. 2003, Baitshotlhi et al. 2014, Vargas-Galicia et al. 2018).

Moreover, in our study, stocking density did not influence bone mechanical properties in turkeys, except Young modulus. However, Young modulus is strongly dependent (cube exponent) by the bone length (Muszyński et al. 2017, Tomaszewska et al. 2018). Our results are in partially agreement with the previous study examining the effect of stocking density on bone quality in turkeys. The study performed on 18-weeks old male meat-type turkeys housed in indoor system showed that, like in presented study, some indices characterizing bone diaphysis geometry (cortical index, mean relative wall thickness) or bone densitometry characteristic (bone volumetric density and bone ash percentage) were not affected by the stocking density (Jankowski et al. 2015). On the other hand, increased stocking density negatively affected tibia, its length and weight, bone diaphysis cross-sectional area, moment of inertia and bone breaking strength (Jankowski et al. 2015). However, that study examined the effect of both stocking density and heat stress temperature and, as the effects of stocking density and temperature were confounded, it is not possible to conclude how stocking density alone affected bone quality.

In chickens, birds welfare is influenced more by housing conditions than by stocking density (Dawkins et al. 2004). Likewise, in our study showed that in turkeys housing system exerts greater influence on bone properties than stocking density. Moreover, our results about the influence of housing system on bone quality are generally in correspondence with findings of similar study performed on turkey-toms raised from 7th to 22nd week of age indoors or in semi-open system under a shelter with access to open-air runs (Burs et al. 2008). Like in presented study, the semi-open system positively influenced tibia weight and the values of ultimate strain, while the yield strain and elastic energy were not affected by housing system (Burs et al. 2008). The main discrepancy between both studies is that in our female turkeys the effect of housing system on tibia yield and ultimate load was observed. This could be result of increased bone mineral content observed in tibias of turkeys reared in open housing system. However, this could be only speculated, as bone densitometry parameters were not examined in that previous study.

Bone mineral phase is the dominant factor determining the bone ultimate endurance while the bone elastic properties are also depended on bone organic phase, mostly collagen matrix. In our study, most of the mechanical indices characterizing the bone mechanical endurance (ultimate load, ultimate strain, ultimate stress) were influenced by the housing system while the yield load was the only parameter describing the bone elasticity which was housing system dependent.

The mechanisms by which the semi--open housing system may have improved bone mineral density and mineral content are not clear. One of the possible factor responsible for the change in bone mineralization may be a different physical activities of turkeys in both housing systems. Martrenchar et al. (1999) showed that stocking density had little effect on male turkey activity except more frequent disturbances of resting birds by other birds at the high density (4 birds per m^2 of floor space, the same as in our study). Thus, it is possible that the physical activity of our turkeys as the same in both stocking densities. However, despite the same floor space, our birds reared in semi-open system might have been forced to perform more physical activity (for example, due to the weather conditions), which increased their bones' mineralization (Rath et al. 2000). Moreover, it has been shown that light intensity and light source affect activity levels and bone health in poultry (Bailie et al. 2013). The provision of sunlight UVA may lead to increase exploratory and foraging behaviours as these wavelengths play an important role in avian colour vision (Maddocks et al. 2001), while UVB wavelengths are involved in the synthesis of vitamin D, which stimulate the absorption of calcium from the gut (Stanford 2006). Nevertheless, it is not certain whether the birds housed in semi-open system actually took more steps than the indoor birds as physical activity had not been monitored. This should be further investigated.

CONCLUSIONS

In conclusion, findings of this experiment suggest that in turkeys bone quality is influenced more by housing conditions than by stocking density and open and semi-open rearing systems may have beneficial impact on bone development and its mechanical strength compared to conventional housing system. However, the mechanisms by which this occurs are unknown and further research is needed to understand the interactions among stocking density, rearing system and turkey behaviour in order to improve birds health and welfare.

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Streszczenie: *Analiza mineralizacji kości, właściwości osteometrycznych i mechanicznych u indyczek w zależności od obsady i systemu utrzymania.* Gęstość obsady ptaków na m^2 powierzchni oraz system utrzymania podczas chowu mogą wpływać na rozwój szkieletu ptaków. W przypadku indyków badania dotyczące wpływu tych czynników na rozwój kości są niezwykle ubogie. Celem przedstawionych badań była ocena wpływu systemu utrzymania i obsady indyków, na wskaźniki mineralizacji i wytrzymałości mechanicznej kości ptaków. Badaniami objęto indyczki Big-6 w okresie pomiędzy 7. a 16. tygodniem życia. Ptaki utrzymywano w pomieszczeniach zamkniętych (system tradycyjny) lub w systemie mieszanym, w którym ptaki mogły w ciągu dnia korzystać z wybiegu, w dwóch zagęszczeniach: obsada 3 ptaki na m^2 (33 kg przewidywanej końcowej masy ciała na m^2 powierzchni) lub 4 ptaki na m^2 (44 kg przewidywanej końcowej masy ciała na m^2 powierzchni). Zagęszczenie obsady znacznie zmniejszyło jedynie moduł Younga sprężystości w kości piszczelowej, natomiast pozytywny wpływ półotwartego systemu utrzymania obserwowano w masie kości piszczelowej, mineralizacji kości i wytrzymałości mechanicznej, zwłaszcza w indeksach opisujących odporność kości na złamanie. Podsumowując, wyniki tego eksperymentu sugerują, że u indyków jakość kości zależy bardziej od warunków utrzymania niż od obsady, a otwarte i półotwarte systemy hodowlane mogą mieć korzystny wpływ na rozwój kości.

Słowa kluczowe: jakość kości, dobrostan indyków, gęstość obsady, system utrzymania

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