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EFFECT OF CAPONIZATION ON BLOOD PARAMETERS AND TIBIA CHARACTERISTICS IN CROSSBRED CHICKENS DERIVED FROM CONSERVED BREED HENS AND MEAT ROOSTERS*

Dorota Wojtysiak[1♦], Jolanta Calik[2], Bartosz Leszczyński[3], Ewa Tomaszewska[4], Joanna Obrzut[2], Józefa Krawczyk[2], Dominik Panek[3], Siemowit Muszyński[5]

[1]Department of Genetics, Animal Breeding and Ethology, University of Agriculture in Krakow, Al. Mickiewicza 24/28, 30-059 Kraków, Poland
[2]Department of Poultry Breeding, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
[3]Department of Medical Physics, M. Smoluchowski Institute of Physics, Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Łojasiewicza 11, 30-348 Kraków, Poland
[4]Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-950 Lublin, Poland
[5]Department of Biophysics, Faculty of Environmental Biology, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland
♦Corresponding author: dorota.wojtysiak@urk.edu.pl

Abstract

The effect of caponization on the level of testosterone, biochemical bone markers as well as on the geometrical, densitometric, structural, mechanical and microstructural characteristics of the tibia of capons was the subject of this study. The study was conducted on 80 hybrids obtained from the crossing of Rhode Island Red (R-11) hens and meat roosters (Ross 308). Cockerels were surgically castrated at 8 week of age and then fattened until the 24th week of life. The results indicated that the serum testosterone (P<0.01), osteocalcin (P<0.05), and osteoprotegerin (OPG) (P<0.05) concentration were significantly lower, while the serum receptor activator of nuclear factor kappa-B ligand (RANKL) (P<0.01), RANKL/OPG ratio (P<0.05) and alkaline phosphatase concentrations (P<0.05) were significantly higher in capons. The tibia weight, the relative tibia weight, and the cross-sectional area of tibia were significantly higher (P<0.05) in cockerels, while capons had higher (P<0.05) body weight and transversal external diameter of tibia. Moreover, the bone ash, calcium and phosphorus content, bone mineral density and bone mineral content, and also yield load, ultimate load, work to fracture, as well as Young's modulus, bending moment, yield stress and ultimate stress were significantly lower (P<0.05) in capons. Caponization also caused a significant reduction in the percentage volume of bone (P<0.05) in the proximal epiphyses of the tibia, as well as changes in its microarchitecture (P<0.05) (fewer trabeculae and connections between trabeculae, thicker trabeculae and larger spaces between trabeculae). Concluding, caponization disrupts the homeostasis and microstructure of the tibia, and this results in a decrease in the mechanical strength of the tibia, making it more susceptible to deformation.

Key words: capons, cockerels, blood biochemical parameters, bone biomechanical markers, bone microstructure

Improving meat quality is a very important aspect of capon production. On the other hand, the loss of testicles through castration surgery results in androgen deficiency (Lin et al., 2012; Zawacka et al., 2017, 2018) that stimulates not only the reproductive system, metabolism and behaviour, but also the growth of muscles and bones (Miguel et al., 2008). In the case of bone, the major actions of androgen is to favor bone formation and to maintain trabecular bone mass and integrity (Chamouni and Oury, 2014). The loss of androgens increases the rate of bone remodelling by prolonging the lifespan of osteoclasts and shortening the lifespan of osteoblasts, which ultimately leads to loss of bone mass (Michael et al., 2005). Previous studies indicate that changes in bone metabolism as a result of rooster castration have a negative effect on the quality of long bone and, in particular, on the biomechanical parameters of bone (Tomaszewska et al., 2017; Chen et al., 2006 a, 2007; Lin et al., 2012; Muszyński et al., 2017; Kwiecień et al., 2019). However, the results of studies investigating the effects of caponization on the bone and blood parameters are inconclusive. Some studies found no differences in tibia length and weight (Muszyński et al., 2017; Kwiecień et al., 2019), while other studies showed that capon bones were lighter and shorter (Chen et al., 2014; Zawacka et al., 2018). In turn, Mahmud et al. (2014) noted significantly longer tibia in the caponized group. Similarly, for blood parameters, Lin et al. (2012) found that capons had higher plasma ionized calcium (Ca), inorganic phosphorus (P) and alkaline phosphatase (ALP) concentrations, while Kwiecień et al. (2019) observed no change in the values of these parameters between capons and roosters. There is also little information in the literature regarding changes in the microarchitecture of long bones as a result of rooster castration.

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Capon production is mainly based on native or locally adapted breeds of hens (Miguel et al., 2008; Shao et al., 2009; Amorim et al., 2016; Calik et al., 2017) which are usually characterized by small size. Therefore, it is increasingly common to use hybrids of pure hen lines and meat roosters for production of capons, which reach larger body sizes and their meat is characterized by high quality typical of native breeds (Wojtysiak et al., 2019; Calik and Obrzut, 2023). On the other hand, castration and the associated decrease in testosterone levels, coupled with a faster rate of weight gain, can lead to a skewed ratio of muscle to bone mass, and this can translate into structural and biomechanical bone parameters.

The study hypothesizes that caponization of chickens, resulting in the loss of testicles and androgen deficiency, may have significant effects on bone metabolism, blood parameters, and the biomechanical and microstructural characteristics of the tibia. Specifically, capons obtained from the crossing of Rhode Island Red hens and meat roosters may exhibit altered bone growth patterns, potentially affecting bone density, strength, and microarchitecture compared to non-caponized chickens. Additionally, the study aims to investigate potential differences in blood parameters related to bone metabolism between capons and cockerels. The findings from this research may provide valuable insights into the impact of caponization on skeletal health and could be relevant for optimizing poultry rearing practices and enhancing poultry welfare in the livestock industry.

The objective of the study was to determine the effect of caponization on the selected blood parameters as well as on the biomechanical and microstructural characteristics of the tibia of capons obtained by the crossing of Rhode Island Red (R-11) hens and meat roosters (Ross 308).

Material and methods

Ethical approval and veterinary treatments

The experiment was carried out in compliance with the European Union law (Directive 2010/63/UE, received in Poland by Legislative Decree 266/2015) of the European Parliament and of the Council on the protection of animals used for scientific purposes. All the experimental procedures conformed to the requirements of the Local Ethics Committee for Animal Experimentation (no. 1121/2014) in Kraków, Poland.

Animals and experimental design

The study was carried out on 80 hybrids obtained from the crossing of Rhode Island Red (R-11) hens and meat roosters (Ross 308). After weighing and individual marking, the chickens were randomly assigned into two groups: uncastrated roosters group and capons group (in each group 40 birds in 4 pens, 10 birds per pen). Cockerels were surgically caponized under local anesthesia by a veterinarian at 8 weeks of age. The birds were kept in the building of the Experimental Poultry Farm of the National Research Institute of Animal Production in Chorzelów. The animals were kept on litter, at a stocking rate of 5 birds/m^2, under the same environmental conditions. Throughout the entire period of rearing as well as fattening, the birds were fed *ad libitum* with unlimited access to water. Three-phase feeding was used in the experiment in accordance with AOAC (2000) procedures, namely: from 1 to 7 weeks of age – feed mixture I (dry matter: 88.38%, crude ash: 7.37%, crude protein: 19.30%, crude fat 2.23%, crude fiber: 2.34, and 11.92 MJ/kg of metabolizable energy), from 8 to 16 weeks of age – feed mixture II (dry matter: 87.86%, crude ash: 5.83%, crude protein: 18.66%, crude fat 2.54%, crude fiber: 2.54, and 12.05 MJ/kg of metabolizable energy), and from 17 to 24 weeks of age – feed mixture III (dry matter: 88.54%, crude ash: 3.96%, crude protein: 16.30%, crude fat 2.38%, crude fiber: 2.38, and 12.18MJ/kg of metabolizable energy). At the age of 24 weeks, 8 birds from the group of uncastrated roosters and 8 birds from the capon group (2 birds from each of the 4 pens), whose body weight was similar to the group average, were selected for slaughter. The birds were slaughtered using standard methods of killing by decapitation after electrical stunning. Immediately after slaughter, the birds were checked for castration success (removal of the testes).

Serum biochemical analysis

Blood was collected post-mortem from all slaughtered birds, and the serum was separated by centrifugation at 3000 rpm for 15 min at 4°C, and stored at −20°C until further analysis. The blood serum concentrations of osteocalcin (OC), osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL) were determined using commercial, chicken-specific, enzyme-linked immunosorbent assay (ELISA) kits: OC (E0471c; Elabscience Biotechnology Co., Ltd., Wuhan, China), OPG (MBS742348, MyBioSource, Inc., San Diego, CA, USA), RANKL (MBS269579, MyBioSource, Inc., San Diego, CA USA). All procedures were performed according to the manufacturer's protocols. Samples (n = 8 in each group) were analyzed in duplicate using an Epoch2 microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA) at a wavelength of 450 nm. Results were calculated using standard curves created in individual tests; the coefficient of determination of the standard curves was above 0.980.

Testosterone (T) level was analyzed in duplicate by radioimmunoassay (RIA) with the use of commercial Testo-RIA-CT kits supplied by DIAsource (DIAsource ImmunoAssays S.A., Louvain-la-Neuve, Belgium).

Moreover, calcium (Ca), phosphorus (P), and alkaline phosphate (ALP) were determined in duplicate with an SP-830 plus spectrophotometer (Metertech Inc., Taipei City, Taiwan) using the original Alpha Diagnostics kits (Warsaw, Poland). The absorbance was measured at 450 nm for ALP, 570 nm for calcium and 340 nm for phos-

phorus. All procedures were performed in duplicate according to the manufacturer's protocols.

Bone analysis

Immediately after slaughter, the right and left tibia from each bird were manually defleshed of adhering tissues and frozen at –20°C. After thawing overnight at 7°C, the tibia were weighed and measured for length, after which the relative bone weight (bone weight to body weight ratio) was calculated. The left tibia was designated for densitometric and morphometric analysis, while the right tibia was designated for 3-point bending test, mineral composition and measurements of tibia weight, length, the relative bone weight, and mid-diaphysis geometry.

The measurements of bone mineral density (BMD) and bone mineral content (BMC) were performed using Norland XR 43 dual energy X-ray absorptiometry (DXA) system (Norland, Fort Atkinson, WI). BMD and BMC were analyzed for the whole bone.

The three-point bending test (mid-diaphyseal fragment of the tibia) was performed on a Zwick Z010 universal testing machine (Zwick-Roell GmbH & Co., Ulm, Germany) using TestXpert II 3.1 software (Zwick GmbH & Co. KG, Ulm, Germany) according to the methods described by Muszyński et al. (2017). A measuring head with an operational range of 10 kN was used in the study. Bones were placed horizontally on supports prior to measurement. In each case, a span corresponding to 40% of the total bone length was used and a constant load rate of 10 mm/min was applied until fracture. Next, from the obtained set of load-displacement curves the bone structural traits were determined: yield load, ultimate load, yield energy, work to fracture, and stiffness. To calculate tibia cross-sectional geometric properties the transversal external and internal as well as the anteroposterior external and internal diameters of the mid-diaphyseal cross-section were measured with a digital caliper. From these measurements, the following were calculated: cross-sectional area, mean relative wall-thickness, and cortical index (Muszyński et al., 2017). Finally, on the basis of measured diameters and structural traits material properties of the mid-diaphyseal fragment of the bone were calculated (Young modulus, yield strain, bending moment, yield stress, ultimate strain, ultimate stress) (Muszyński et al., 2017).

Mineral composition of bones

After strength tests, right tibia bones were mineralized in a muffle furnace (Czylok, Jastrzębie Zdrój, Poland) at 500°C for 12 hours to determine the ash content. The percentage ash was determined relative to the wet bone mass. The calcium (Ca) and phosphorus (P) content of the bones was determined by ICP-OES (inductively coupled plasma optical emission spectrometry) by using an Optima 7300 DV apparatus (Perkin Elmer, Boston, MA, USA), after prior mineralization of the bones in nitric acid.

Morphometric analysis – micro-CT imaging, image reconstruction, and visualization methods

In this research, a Skyscan 1172 Micro-CT Scanner (Skyscan Aartselaar, Belgium) was used to scan samples of the left proximal tibial head. The tomograph was equipped with an 8 W X-ray tube with 24 × 36 mm field of view. Imaging parameters were as follows: voltage, 80 kV; amperage, 100 μA; rotation step, 0.3°; detector resolution, 1000 × 666 px; and an aluminum filter (thickness, 0.5 mm). Time of the scan ranged from 3 to 5 h. The projection images were acquired over an angular range of 180° with a step of 0.3° resulting in the image pixel size equal to 14.97 μm. Obtained projections of bones were reconstructed using NRecon software (Skyscan, Bruker micro-CT, version 1.7.3.1) enabling the reconstruction by setting appropriate dynamic range, identical for all the samples (providing the same grayscale). Later, CTAn (Skyscan, Bruker micro-CT, version 1.16.1.0) was used. This software allowed for choosing the volume of interest (VOI) inside the bone (8 mm below the bone growth plate). Later, the binarization method was performed in order to assign the black or white colors to the image pixels by using appropriate threshold (the threshold value was chosen according to the grayscale histogram of a given sample). The final step in this stage of analysis was to calculate: percent bone volume (BV/TV) (BV – total volume of binarized objects within the VOI, TV – total volume of the VOI), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), connectivity density (Conn.Den) and structure model index (SMI). SMI involves a measurement of surface convexity. An ideal plate, cylinder and sphere have SMI values of 0, 3 and 4, respectively. Detailed methods of morphometric parameters calculations can be found in Bruker (2009) and Dempster et al. (2013). The last used software was CTVox (Skyscan, Bruker micro-CT, version 3.3.0 r1403), enabling the creation of three-dimensional visualizations of the examined samples thanks to the technique called volume rendering. The visualization was performed by modifying the transfer function in such a way that the low greyscale values (containing noise) were clipped and only the internal morphology of the sample was left.

Statistical analysis

The normality of data distribution was tested using the Shapiro–Wilk test and the homogeneity of variance was tested by the Levene's test. For comparison of the means of the capon and cockerel groups, the Student's *t*-test was applied. In the case of abnormal distribution and/or unequal variance of data, the Mann–Whitney U-test was applied to determine significant differences between the examined groups. Statistical analysis was performed using Statistica 13 (Dell Software Inc., Aliso Viejo, CA, USA) and expressed as least square means and standard error of the means (SEM). A statistically significant difference between means was assumed when the probability value was less than 0.05 ($P<0.05$).

Results

Blood serum biochemical parameters

The results of the analysis of serum concentrations of selected hormones and bone turnover markers in roosters and capons are presented in Table 1. It was found that castration of roosters had a significant effect on decreasing the concentration of T ($P<0.01$), OC and OPG ($P<0.05$), while increasing the concentration of RANKL ($P<0.01$) and the RANKL/OPG ratio ($P<0.05$). For bone turnover markers such as Ca, P and ALP, caponization had no significant effect on Ca and P levels ($P>0.05$), but had a significant effect on increasing ALP levels ($P<0.05$).

Body weight and tibia geometric parameters

The results of measurements of body weight and geometric parameters of the tibia of roosters and capons are presented in Table 2. It is shown that caponization significantly contributes to the increase in body weight ($P<0.05$), with a concomitant decrease in the weight of the tibia and relative bone weight ($P<0.05$). Moreover, caponization had no effect on tibia length, anteroposterior internal and external diameter, transversal internal diameter, mean relative wall thickness and cortical index ($P>0.05$), but had a significant effect on an increase in the transversal external diameter ($P<0.05$), as well as a decrease in the cross-section area ($P<0.05$).

Bone densitometric parameters

Caponization had a significant effect ($P<0.05$) on both BMD and BMC reductions (Table 3). Moreover, the tibia of capons had a significantly lower ($P<0.05$) ash and total bone P and Ca content compared to cockerels, but the Ca:P ratio remained unchanged ($P>0.05$) (Table 3).

Tibia structural and material parameters

The results of the analysis of the effect of caponization on structural and material parameters of the tibia of cockerels and capons are presented in Table 3. The results obtained showed that caponization reduced the yield load, ultimate load and work to fracture values of the tibia ($P<0.05$). On the other hand, for parameters such as yield energy and stiffness, the differences were not statistically significant ($P>0.05$).

In the case of the tibia material parameters, significantly lower values of the Young modulus, bending moment, yield stress, and ultimate stress were noted in capons compared with cockerels ($P<0.05$). For the other material parameters (yield strain and ultimate strain), no significant differences were observed between capons and cockerels ($P<0.05$).

Table 1. Blood serum parameters of cockerels and capons

Items	Cockerel	Capon	SEM[1]	P value
T (ng/ml)	1.739	0.168	0.009	0.008
OC (ng/ml)	16.85	13.21	0.572	0.046
OPG (pg/ml)	75.19	61.85	1.086	0.039
RANKL (pg/ml)	362.53	1098.24	29.82	0.006
RANKL:OPG ratio	4.82	17.76	0.43	0.019
ALP (U/L)	328.6	464.2	24.27	0.048
Ca (mg/dl)	4.92	5.23	0.41	0.556
P (mg/dl)	2.46	2.53	0.23	0.093

Abbreviations: T – testosterone; OC – osteocalcin; OPG – osteoprotegerin; RANKL – receptor activator of nuclear factor kappa-B ligand; ALP – alkaline phosphate; Ca – calcium; P – phosphorus.

[1]Values are least square means and SEM.

Table 2. Body weight and geometrical parameters of tibia of cockerels and capons

Items	Cockerel	Capon	SEM[1]	P value
Body weight (g)	4100	4328	42.86	0.039
Tibia weight (g)	21.09	18.64	0.42	0.012
Relative tibia weight (%)	0.514	0.431	0.027	0.046
Tibia length (mm)	153.1	152.8	4.28	0.824
Transversal internal diameter (mm)	9.82	9.53	0.57	0.067
Transversal external diameter (mm)	6.59	7.01	0.18	0.023
Anteroposterior internal diameter (mm)	7.65	7.75	0.46	0.439
Anteroposterior external diameter (mm)	5.56	5.58	0.39	0.215
Cross-sectional area (mm^2)	30.292	27.159	1.09	0.047
Mean relative wall thickness (-)	0.449	0.384	0.042	0.083
Cortical index CI (%)	30.155	27.246	2.84	0.256

[1]Values are least square means and SEM.

Table 3. Densitometric, structural and material parameters of tibia of cockerels and capons

Items	Cockerel	Capon	SEM[1]	P value
Densitometric parameters				
BMD (g/cm^2)	0.334	0.293	0.017	0.028
BMC (g)	5.51	4.76	0.14	0.043
Ash (%)	49.7	45.2	0.42	0.012
Ca (mg/g)	238.5	203.7	5.26	0.039
P (mg/g)	82.9	76.2	1.37	0.019
Ca:P ratio	2.88	2.67	0.12	0.128
Structural parameters				
Yield load (N)	338.2	218.8	12.35	0.019
Ultimate load (N)	447.6	297.6	32.47	0.037
Yield energy (mJ)	169.4	132.2	16.84	0.256
Work to fracture (mJ)	529.6	385.6	69.31	0.020
Stiffness (N/mm)	366.1	228.2	28.53	0.084
Material parameters				
Young modulus (GPa)	11.512	7.085	0.832	0.024
Yield strain (-)	1.147	1.357	0.165	0.341
Ultimate strain (-)	2.299	2.533	0.091	0.527
Bending moment (N·m)	5.178	3.326	0.213	0.016
Yield stress (MPa)	127.652	81.694	14.622	0.042
Ultimate stress (MPa)	168.607	112.332	12.851	0.032

Abbreviations: BMD – bone mineral density; BMC – bone mineral content; Ca – calcium; P – phosphorus. [1]Values are least square means and SEM.

Table 4. Microarchitectural parameters of trabecular bone in the proximal tibial head of cockerels and capons

Items	Cockerel	Capon	SEM[1]	P value
BV/TV (%)	8.656	6.964	0.271	0.016
Tb.Th (mm)	0.148	0.195	0.008	0.025
Tb.Sp (mm)	1.734	2.208	0.023	0.041
Tb.N (mm^{-1})	0.372	0.266	0.017	0.012
Conn.Den (mm^{-3})	4.958	1.436	0.094	0.018
SMI (–)	0.701	0.578	0.032	0.047

Abbreviations: BV/TV – bone volume; Tb.Th – trabecular thickness; Tb.Sp – trabecular separation; Tb.N – trabecular number; Conn.Den – connectivity density; SMI – structure model index. [1]Values are least square means and SEM.

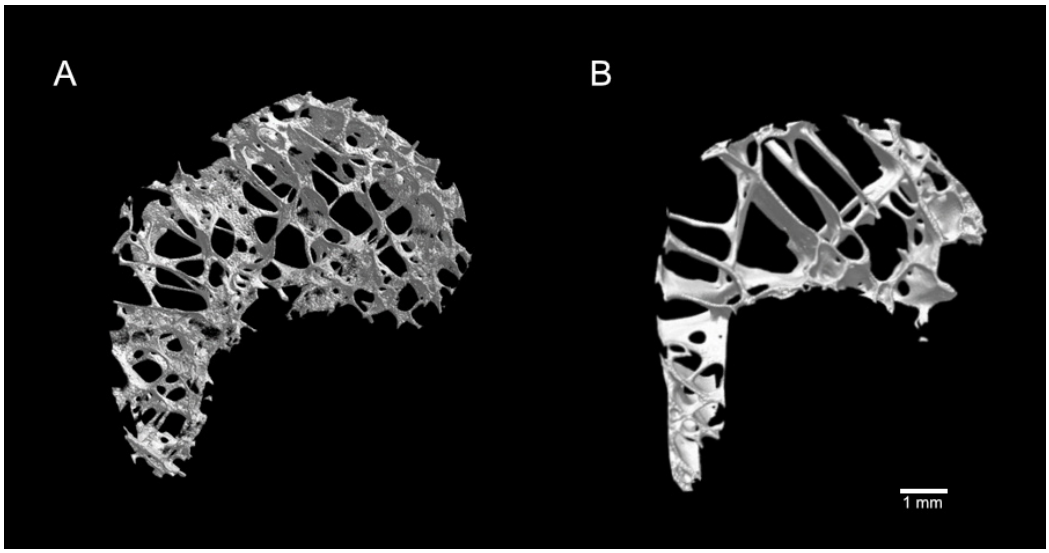


Figure 1 A–B. 3D model of trabecular bone fragments from the proximal tibial head of cockerel (A) and capon (B) made by volume rendering using SkyScan's CTvox software

Morphometric analysis

The bone microarchitectural parameters are presented in Table 4 and Figure 1 A, B. It was observed that the trabecular bone of the proximal tibial epiphyses is composed of internal rods or plates that form a 3D branching network, while in capons the trabecular network had a looser mesh (larger spaces, fewer connections between the trabeculae), and the bone trabeculae were thicker and mainly took the form of plates.

These observations were confirmed by morphometric analysis, where it was shown that caponization significantly contributed to a decrease in BV/TV ($P<0.05$), and an increase in Tb.Th and Tb.Sp ($P<0.05$). Capons were also characterized by significantly lower Tb.N, as well as lower values of Conn.Den and SMI compared to roosters ($P<0.05$).

Discussion

The interest in capon production is primarily related to obtaining high-quality meat with specific taste and dietary qualities, as well as to obtain higher body weight gains (Diaz et al., 2010; Calik et al., 2017; Calik and Obrzut, 2023). Body weight in birds, like other quantitative traits, depends on both genetic and environmental traits, as well as interactions between them. A number of previous studies – as shown in the present study – have concluded that caponization contributes to the increase in body weight of capons compared to roosters (Kwiecień et al., 2015, 2019; Muszyński et al., 2017; Calik et al., 2020; Hossen et al., 2021; Calik and Obrzut 2023), which is undoubtedly beneficial from the breeder's point of view. In other studies, Shao et al. (2009), Symeon et al. (2012), Franco et al. (2016) and Murawska et al. (2019) did not show a significant effect of caponization on body weight. Chen et al. (2007) and Shao et al. (2009) suggested that the controversy regarding body weight of capons, as shown in many studies, may be related to the age of the birds at which the castration procedure was performed, nutrition, duration of rearing, as well as to the different genetic background of the birds.

It is worth remembering that a high ratio of muscle to bone mass can result in growth disorders, bone overload, and consequently deformation and even bone fractures (Tatara et al., 2004).

Unlike previous studies (Lin and Hsu, 2003; Duran, 2004; Chen et al., 2006 a, 2007; Lin et al., 2012; Muszyński et al., 2017), where there were no differences in tibia weight between capons and roosters, the tibia bones of capons were lighter in the work presented here. These results are consistent with the studies of Chen et al. (2014) and Zawacka et al. (2018). Moreover, in our study, as in previous work (Lin and Hsu, 2003; Duran, 2004; Chen et al., 2006 a; Lin et al., 2012; Muszyński et al., 2017), the relative tibia weight of capons was significantly lower compared to roosters, and this indicates that the tibia of capons are subjected to greater overload.

Bones as structural entities, in addition to supporting and protecting internal organs, also play an important metabolic role as a reservoir of ions, such as Ca and P, and of growth factors stored in the bone matrix (Hardy and Fernandez-Patron, 2020). The greatest increase in bone mass occurs during the body's period of growth and sexual maturation, and is therefore closely related to the development of the sex glands and their activity. Androgens, including testosterone, play an important role here (Clarke and Khosla, 2009). Sinnesael et al. (2011) showed that androgen receptors – expressed in osteoblasts, osteoclasts, osteocytes, and pluripotential mesenchymal bone marrow stroma – affect bone function, bone gain, metabolism and help prevent bone loss. As expected, the results of the presented work showed that caponization causes a drastic decrease in testosterone levels, which is consistent with previous studies by Chen et al. (2006 a), Shao et al. (2009), Symeon et al. (2010), Lin et al. (2012), Zawacka et al. (2017, 2018) and Kwiecień et al. (2019). It has been well recognized that lowering androgen levels disrupts the balance between bone formation and resorption and contributes to bone loss (Falahati-Nini et al., 2000; Fink et al., 2006; Sinnesael et al., 2011; Rył et al., 2019). Bone formation is a function of osteoblasts, while osteolysis is a function of osteoclasts (Takayanagi et al., 2000). The process of predominant importance in the loss of bone mass is the activation of osteoclasts. Previous studies indicated that a key role in osteoclast maturation, bone homeostasis, and bone turnover is played by the RANKL/RANK/OPG system, which includes osteoprotegerin (OPG), receptor activator of nuclear factor kappa-B (RANK) and receptor activator of nuclear factor kappa-B ligand (RANKL) (Yasuda, 2021; Marcadet et al., 2022). RANKL/RANK signaling regulates osteoclast formation, activation and survival in normal bone modelling and remodelling and in a variety of pathologic conditions characterized by increased bone turnover (Kohli and Kohli, 2011). Factors such as low level of gonadal hormones, that can induce bone resorption act on osteoblasts to induce RANKL expression, which then binds to its receptor RANK on the surface of osteoclast progenitor cells, inducing preosteoclast differentiation into multinucleated, fully-functional osteoclasts (Michael et al., 2005). On the other hand, OPG, which is secreted by osteoblasts and osteogenic stromal stem cells, protects bone from excessive bone resorption by binding to RANKL and preventing it from interacting with RANK (Yasuda, 2021). Thus, the RANKL/OPG ratio is a major determinant of bone health and reflects the balance between bone formation and resorption (Kohli and Kohli, 2011). In our study, castration of roosters resulted in decreased OPG levels and increased RANKL levels, as well as the RANKL/OPG ratio. On this basis, it can be concluded that the lack of sex steroids during intensive skeletal development in capons disrupts bone homeostasis, contributing to the predominance of resorption processes over bone formation, which in the long run may lead to bone loss and

ultimately osteoporosis. These suppositions may also be confirmed by the lower level of osteocalcin in the serum of capons shown in the present study. OC is an important marker of bone formation. It is primarily produced by osteoblasts, osteocytes, odontoblasts, and hypertrophic chondrocytes. Falahati-Nini et al. (2000) suggest that the decrease in serum OC levels in the setting of acute sex steroid deficiency likely reflects apoptosis of osteoblasts and osteocytes. Moreover, *in vitro* studies indicate that T has antiapoptotic effects on mature osteoblastic cells (Manolagas, 2000). The predominance of resorption processes over bone formation in capons is also evidenced by the higher levels of ALP activity in capons shown in this study, which is consistent with previous studies by Chen et al. (2006 a) and Lin et al. (2012). In contrast, Lin and Hsu (2003), Chen et al. (2007), Zawacka et al. (2018) and Kwiecień et al. (2019) found that caponization had no effect on the blood ALP concentration. ALP is a glycoprotein found on the surface of osteoblasts and reflects the activity of these cells in bone metabolism (Bonnick et al., 2013). Elevated ALP is caused by high bone turnover during bone growth or degeneration, if the resorption rate is greater than the rate of bone formation (Mukaiyama et al., 2015) and this results in loss of bone mass. Chen et al. (2006 b) point out that androgen deficiency also leads to an increase in Ca release from bone, as reflected by an increase in serum Ca levels. In contrast, Lin and Hsu (2003), Chen et al. (2006 a, 2007, 2014), Zawacka et al. (2018) and Kwiecień et al. (2019), similar to what was shown in the present study, reported no effect of rooster castration on serum Ca levels. Previous studies also indicate that caponization of roosters has an effect on increasing serum/plasma P levels (Chen et al., 2006 b, 2007). Also, Zawacka et al. (2018) observed an increase in the value of this parameter in capons compared to roosters, but only in individuals at 12 weeks of age. On the other hand, the authors did not note such correlations in older 24-week-old individuals. Similarly, the present study found no differences in serum P levels between roosters and capons, which is consistent with the studies of Lin and Hsu (2003), Chen et al. (2006 a) and Kwiecień et al. (2019). In our study the caponization significantly decreased ash content in tibia. These changes were also accompanied by a significant decrease in Ca and P content. Importantly, however, the Ca:P ratio remained unchanged. These results are partly consistent with those obtained by Lin and Hsu (2003), who noted lower levels of bone Ca in the tibia of capons relative to roosters. In addition, Lin et al. (2012) reported lower ash, Ca and P content in the tibia of capons. Chen et al. (2006 b), Muszyński et al. (2017), and Kwiecień (2019), on the other hand, showed that caponization decreased tibia's ash and P content. Mauras et al. (1999) noted that gonadal androgens can directly influence calcium fluxes and bone calcium resorption/deposition in males. These authors also suggested that the reduced bone ash, calcium and phosphorus contents in males with reduced testosterone levels may be the result of increased bone calcium loss and decreased kinetic markers in the bone calcium deposition.

The changes in tibial mineralization of capons demonstrated in the present study are also reflected in densitometric characteristics. Thus, the tibia bones of capons were characterized by lower levels of both BMD and BMC. In contrast to our study, Zawacka et al. (2018) show that the tibia of capons had higher BMD values than the bones of cockerels. Muszyński et al. (2017), on the other hand, noted no significant effect of caponization on the total MBD and total BMC values of the tibia. On the other hand, when analyzing densitometric parameters separately for the tibial shaft and epiphysis, the authors found that demineralizing effect of caponization is especially substantial in the bone midshaft area (lower values of BMD and BMC).

The mechanical properties of bones are important for the ability of skeletons to support movement and provide protection for internal organs. Therefore, deterioration of the densitometric, geometric and structural characteristics of the bone, can have a significant impact on bird welfare (Danek-Majewska et al., 2023; Nadziakiewicz et al., 2023). Previous work by Muszyński et al. (2017) indicates that caponization significantly affects the diameter of tibial shafts which translates into an increase in the cross-sectional area of tibial shafts in capons compared to roosters, and this may be related to the body's adaptation to increased mechanical work of the bone. On the other hand, these authors, as demonstrated in the present study, did not note an effect of caponization on parameters reflecting physiological bone maturity, namely mean relative wall thickness and cortical indices. Kwiecień et al. (2019), on the other hand, found no changes in both the diameters and cross-sectional area of the tibial shafts of roosters and capons. In the present study, however, capons showed a larger horizontal external diameter of the tibial shafts which resulted in a decrease in the bone cross-sectional area compared to the tibial shafts of roosters. The observed differences in the geometric parameters of the bones, especially in the size of the cross-sectional area, may be due to the fact that the body's adaptation to altered conditions after castration involves not only the bones but also the muscles, which in everyday life are responsible for most of the stresses to which the skeleton is subjected (Ferretti et al., 2001).

As for the structural parameters of the tibia, the three-point bending test conducted in the presented work indicates a decrease in the mechanical strength of the tibia of the capons, as evidenced by a decrease in the load-bearing capacity of capons' bones in the elastic region (decrease in yield load and ultimate load), and also a decrease in the bone fracture strength. Significant reduction in the yield strength of capon's bones corresponds to the earlier study of Muszyński et al. (2017). Similarly, Kwiecień et al. (2019) suggest that capon bones are more susceptible to a greater risk of permanent bone deformation, as the threshold value of the load resulting in plastic bone deformation decreased, which may lead to bone

damage. Chen et al. (2007), and Lin et al. (2012), in turn, showed that the fracture strength of tibia decreased after caponization. On the other hand, Chen et al. (2006 b) and Muszyński et al. (2017) found no effect of caponization on the breaking strength of tibia.

The differences in geometric and structural parameters of the tibia demonstrated in the present study were reflected in the material parameters of the bone calculated from them. Thus, similar to what was shown in an earlier study by Muszyński et al. (2017), the lower values of Young modulus and yield stress, as found in the present study, indicate a decrease of elastic endurance of the tibia. Also, the lower values of bending moment and ultimate stress of the tibia bones of capons compared to roosters correspond to previous studies by Chen et al. (2006 a). Muszyński et al. (2017) suggest that the decreased values of ultimate stress of bones show that they can withstand much lower mechanical stress before fracture.

Changes in bone metabolism are also reflected in its microarchitecture. Given that the trabecular bone is more porous than the cortical bone, and also has a greater surface area and an increased number of bone cells that make it more metabolically active than the densely-packed cortical bone (Oftadeh et al., 2015), the focus of the present study is on assessing the microarchitecture within the proximal tibial epiphysis. The general functional role of the trabecular bone is to provide strength and transfer external load away from the joint and toward the cortical bone. Additionally, it acts as a reservoir in regulating the concentration of calcium and other mineral ions throughout the body (Kivell, 2016). The microtomographic analysis carried out in the present study showed that in capons the arrangement of bone trabeculae formed a looser mesh. There were also microstructural differences in the shape, thickness and number of trabeculae. Thus, in capons the bone trabeculae were thicker and less numerous. Plate-shaped trabeculae predominated, in contrast to rooster bones where rod-shaped trabeculae were also observed. These observations, as well as the decrease in BV/TV in capons demonstrated in the present study, indicate a rapid loss of the bone trabeculae, which most likely occurs through the complete removal of some structural elements, leaving those more distant from each other and less well connected. Also, the lower Conn.Den values, which indicate the number of connections between trabecular structures per unit volume in capons, recorded in the work presented here, may indicate a weakening of the trabecular bone structure. Keaveny and Yeh (2002) believe that the disruption of connections between structural elements causes a disproportionate loss of strength, which the increased thickness of the remaining trabeculae can only partially compensate for. According to the authors, the loss of bone trabeculae may result from two mechanisms. The first biological one is related to a decrease in the rate of bone formation (Chamouni and Oury, 2014), which in the case of capons can be evidenced by lower T levels and a lower RANKL/OPG ratio as well as lower OC levels, resulting in some trabeculae becoming so thin that normal osteoclast resorption breaks through the entire trabecula. This causes perforation of the bone trabeculae, and this leads to early trabecular loss. In addition, disruption of the trabecular mesh effectively removes the surface area where osteoblasts could then replace the resorbed bone. The second mechanism causing bone trabecular atrophy is mechanical overload, which leads to trabecular rupture, and this results in the disruption of the trabecular mesh and subsequent loss of trabeculae. It seems that in the case of capons, the two mechanisms are not mutually exclusive. One factor is, of course, reduced testosterone levels, the other is higher body weight and, more importantly, lower relative tibia weight.

Conclusion

In conclusion, caponization of roosters disrupts bone homeostasis, leading to an imbalance between bone resorption and formation, as evidenced in the altered microarchitecture of the proximal tibial epiphyses, characterized by fewer but thicker bone trabeculae and increased trabecular spacing. Moreover, changes in geometrical, structural and material parameters indicate a reduction in the mechanical strength of the capons' tibia. Consequently, the capons' bones may become more susceptible to deformation or fracture.

Conflict of interests statement

The authors declare that they have no conflict of interests regarding the publication of this article.

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