POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 33(1): 49–58, Y. 2018

SELECTED MORPHOMETRIC PARAMETERS AND MINERAL DENSITY OF TIBIOTARSAL BONES IN GREEN-LEGGED PARTRIDGE COCKERELS AND CAPONS

Magdalena Zawacka[1], Daria Murawska[1], Anna Charuta[2], Michał Gesek[3], Tomasz Mieszczyński[1]

[1] Department of Commodity Science and Animal Improvement
University of Warmia and Mazury in Olsztyn, Poland
[2] Institute of Health
Siedlce University of Natural Sciences and Humanities, Poland
[3] Department of Pathological Anatomy
University of Warmia and Mazury in Olsztyn, Poland

Key words: caponization, bones, bone mineral density, testosterone.

Abstract

The aim of this study was to determine the effect of caponization on selected morphometric parameters and mineral density of tibiotarsal bones in Green-legged Partridge cockerels. The experiment was conducted on 200 cockerels. At 8 wks of age, 100 birds were surgically castrated. At 12 and 24 wks of age, blood samples were collected from 10 intact cockerels and 10 capons, and the birds were slaughtered. Tibiotarsal bones were dissected from individual birds. Age had a significant effect on tibia weight in both cockerels and capons ($P \leq 0.01$), whereas the effect of castration on this parameter was noted in older birds, at 24 wks of age (age x sex category interaction, $P \leq 0.01$). Tibiotarsal bones were longer in cockerels than in capons ($P \leq 0.01$). The tibiotarsal bones of capons had higher BMD values than the bones of cockerels ($P < 0.01$).

Address: Daria Murawska, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: +48 (89) 523 41 28; e-mail: daria.murawska@uwm.edu.pl

WYBRANE CECHY MORFOMETRYCZNE ORAZ GĘSTOŚĆ MINERALNA KOŚCI PISZCZELOWO-STĘPOWEJ KOGUTÓW I KAPŁONÓW ZIELONONÓŻKI KUROPATWIANEJ

***Magdalena Zawacka*[1], *Daria Murawska*[1], *Anna Charuta*[2], *Michał Gesek*[3], *Tomasz Mieszczyński*[1]**

[1] Katedra Towaroznawstwa Ogólnego i Doświadczalnictwa
Uniwersytet Warmińsko-Mazurski w Olsztynie, Polska
[2] Instytut Nauk o Zdrowiu
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach, Polska
[3] Katedra Anatomii Patologicznej
Uniwersytet Warmińsko-Mazurski w Olsztynie, Polska

Słowa kluczowe: kapłonienie, kości, gęstość mineralna kości, testosteron.

Abstrakt

Celem badań było określenie wpływu kastracji i wieku na wybrane cechy morfometryczne oraz gęstość mineralną kości piszczelowo-stępowej kogutów zielononóżki kuropatwianej. Materiał do badań stanowiło 200 kogutów, z których 100 poddano kastracji chirurgicznej w wieku 8. tygodni. W wieku 12. i 24. tyg. od 10 losowo wybranych kogutów i 10 kapłonów pobrano próby krwi, a następnie po uboju wypreparowano kość piszczelowo-stępową.

Stwierdzono, że wiek ptaków wpłynął istotnie na masę kości piszczelowo-stępowej kogutów i kapłonów ($P \leq 0.01$), natomiast wpływ kastracji ujawnił się u ptaków starszych, w wieku 24. tyg. (interakcja wiek × kategoria płciowa, $P \leq 0.01$). Kość piszczelowo-stępowa była dłuższa u kogutów, w porównaniu z kośćmi kapłonów, zarówno w 12., jak i w 24. tyg. ($P \leq 0.01$). Kości kapłonów wykazywały wyższe wartości BMD ($P < 0.01$).

Introduction

In Europe, the meat of capons (surgically castrated male chickens) is appreciated by consumers for its tenderness and flavor, and it is more expensive than meat from broiler chickens and organic chickens (Muriel Durán 2004, Franco et al. 2016). Caponized chickens of native breeds are most popular. Recent years have witnessed a growing interest in native chicken breeds which are well adapted to extensive egg and meat production systems (Padhi 2016). Male layer-type chicks of native breeds, which are considered "waste products" in the egg industry, are often used for capon production. The Green-legged Partridge is one of such breeds.

Castration is a hormonal intervention which permanently influences metabolic processes in birds (Rikimaru et al. 2011, Adamski et al. 2016).

Due to reduced synthesis of sex steroids, caponization leads to increased fat deposition in the carcass, which was confirmed by post-mortem analyses, chemical analyses of muscles and histological analyses in castrated birds (Díaz et al. 2010, Gesek et al., 2017). Age and sex exert significant effects on bone tissue parameters during postnatal development (Charuta et al. 2013). Androgens promote bone formation, Ca and P retention (Johnson and Rendano 1984), whereas androgen deficiency is a risk factor for low bone mineral density (BMD) (Finkelstein et al. 1987). In poultry, adverse changes are most frequently observed in the tibia. Research shows that the volumetric mineral density of tibiotarsal bones in males of various poultry species decreases at different ages, e.g. at 4 wks of age in broiler chickens (Charuta et al. 2013) and at 9 wks of age in turkeys (Charuta et al. 2012).

Some studies (Lin and Hsu 2003, Chen et al., 2014) have revealed that castration affects the weight, length and histological parameters of bones. Muszyński et al. (2017) reported lower breaking strength of tibiotarsal bones in castrated males, which could be a risk factor in capon production. According to Manolagas et al. (2002), blood Ca is the substrate for bone ossification and mineralization, whereas decreased androgen concentrations contribute to osteoclast formation, bone erosion, and Ca and P release from bones. In contrast, Mahmud et al. (2014) found that caponization had no significant osteometric effects on almost all long bones. The results of studies investigating the effects of castration on the qualitative and quantitative parameters of bones are inconclusive, most likely due to differences in the origin and age of caponized birds. Testosterone levels also vary across breeds, which is another important consideration (Gryzińska et al. 2011, Murawska et al. 2015).

The objective of this study was to determine the effect of caponization and age on selected morphometric parameters and mineral density of tibiotarsal bones in Green-legged Partridge cockerels.

Materials and Methods

The experiment was conducted on 200 Green-legged Partridge cockerels. Day-old birds were weighed, marked with wing tags, and randomly distributed to 10 pens in the experimental center of the Department of Commodity Science and Animal Improvement of the University of Warmia and Mazury in Olsztyn, Poland. The birds were raised to 24 wks of age, and were fed commercial diets *ad libitum* (Table 1). At 8 wks of age, 100 birds were surgically castrated by a qualified veterinarian in accor-

dance with Commission Regulation (EC) No. 543/2008. The procedure was approved by the Local Ethics Committee in Olsztyn, Poland. The birds were divided into two sex categories (with 5 replications per group and 20 birds per replication).

Table 1

Composition of experimental diets. Composition of diets 1 and 2: ground cereal grain (cornmeal, ground wheat), soybean meal (line 40-3-2), calcium carbonate, monocalcium phosphate, sodium chloride

Nutritional value of diets	Diet 1 (weeks 1–8)	Diet 2 (weeks 9–24)
Metabolizable Energy [MJ kg^{-1}]*	11.64	11.94
Crude protein [%]	18.77	20.88
Crude fat [%]	2.81	3.64
Crude ash [%]	5.22	5.47
Crude fiber [%]	2.51	2.55
Lysine [%]	0.94	1.08
Met + Cyst [%]	0.72	0.75
Threonine [%]	–	0.80
Tryptophan [%]	–	0.24
Methionine [%]	–	0.52
Calcium [%]	0.77	0.87
Total phosphorus [%]	0.59	0.59
Sodium [%]	0.18	0.14
Mineral-vitamin premix [%]	1.0	1.0

* Metabolizable energy content estimated based on the percentages of selected ingredients determined analytically

Provided per kg of diet: microelements: Cu – 8.0 mg, Fe – 116.0 mg, Mn – 80.0 mg, Zn – 100.0 mg, J – 0.80 mg, Se – 0.20 mg. Vitamins: vitamin A (E 672) – 13200 IU, vitamin D_3 (E671) – 3120 IU, vitamin E – 68.0 mg, vitamin K_3 – 4.80 mg, vitamin B_1 – 2.2 mg, vitamin B_2 – 7.2 mg, vitamin B_6 – 5.0 mg, vitamin B_{12} – 44.0 mg, vitamin H (biotin) – 136 mcg, niacin – 44.0, Ca-D-pantothenate – 18.0 mg.

At 12 and 24 wks of age, blood samples were collected from 10 intact cockerels and 10 capons, and the birds were slaughtered (electrical stunning followed by cutting the jugular vein). Blood samples were collected from 10 randomly selected birds from each treatment to determine testosterone levels. Blood was collected into test tubes containing heparin. Freshly collected blood was centrifuged twice (MPW-350R centrifuge, MPW MED INSTRUMENTS; 5 minutes, 10000 rpm), and each time the supernatant was transferred to 1.5 ml Eppendorf Safe-Lock micro test tubes with an Eppendorf automatic electronic pipette. Plasma samples were frozen at -72°C in 1.5 ml Eppendorf Safe-Lock micro test tubes in the Kaltis 390 ultra low temperature laboratory freezer. Testosterone levels were analyzed by radioimmunoassay (RIA) with the use of commercial kits supplied

by DIAsource TESTO – RIA – CT (DIAsource ImmunoAssays S.A., Belgium). Serum Ca and P concentrations, and alkaline phosphatase activity were analyzed using an automatic blood chemistry analyzer and Roche testing kits (COBAS MIRA plus, Roche Diagnostics, Rotkreuz, Switzerland).

Live body weight (BW) was determined before slaughter. Carcasses were chilled for approximately 18 h at a temperature of 4°C, and carcass dissection was performed. Tibiotarsal bones were dissected from individual birds. The length of right bones was measured with a caliper accurate to 1 mm. The structure of bone tissue (BMD – Bone Mineral Density, cortical/compact and cancellous/spongy bone combined) was determined by computed microtomography using the SkyScan 1174 scanner (Bruker – SkyScan, Belgium) and the following software: CT-Analyzer, Nrecon, CT Vox and DataViewer. The data were analyzed using Nrecon 1.6.9.18, CTAnalyser 1.14.4.1+, CTVox 2.1.0 r741 and DataViewer 1.5.1.2 packages. The values of BMD were determined for a Hounsfield units (HU) range of –1000 to 0. The analyzed area was the proximal metaphysis of the tibiotarsal bone.

The statistical analysis involved the determination of arithmetic means ($\overline{x}$) and standard deviations (SD). The data were analyzed by two-way ANOVA (age × sex category; A × B: 2 × 2). The results were processed using Statistica 2010 software.

Results

At 12 wks of age, Green-legged Partridge cockerels and capons were characterized by similar average body weight of 1194 g and 1206 g, respectively, which increased to 2030.6 g and 2067.6 g at 24 wks of age. Caponization had no significant effect on the body weights of birds, which were influenced only by age ($P \leq 0.01$, Table 2).

At 12 wks of age, tibia weight was similar in cockerels and capons (10.01 g and 9.79 g, respectively), whereas at 24 wks of age, it was higher in cockerels (15.38 g vs. 13.17 g, $P \leq 0.01$, Table 2). Age had a significant effect on tibia weight in both cockerels and capons ($P \leq 0.01$), whereas the effect of castration on this parameter was noted in older birds, at 24 wks of age (age x sex category interaction, $P \leq 0.01$, Table 2).

Tibia length was affected by both the age of birds and castration ($P \leq 0.01$, Table 2). Cockerels had longer tibiotarsal bones than capons, both at 12 and 24 wks of age ($P \leq 0.01$, Table 2).

Table 2

Body weights and selected tibia parameters in 12- and 24-week-old Green-legged Partridge cockerels and capons (mean ± SD)

Item	Sex category	Age [wks]		P-value		
		12	24	age	sex category	A · S interaction
Body weight [g]	cockerels	1194.40	2030.8	0.000	0.241	0.227
		±80.20	±107.845			
	capons	1206.00	2067.80			
		±48,44	±40.95			
Tibia weight [g]	cockerels	10.01	*15.38	0.000	0.005	0.023
		±0.43	±0.76			
	capons	9.79	13.17			
		±0,27	±0,48			
Tibia length [cm]	cockerels	*12.46	*14.88	0.000	0.028	0.670
		±0.13	±0.34			
	capons	11.48	13.98			
		±0.34	±0.20			
Bone mineral density (BMD) [g cm^{-3}]	cockerels	0.98	1.80	0.000	0.000	0.247
		±0.09	±0.11			
	capons	*1.62	*2.32			
		±0.15	±0.14			

*– values in columns (cockerels an capons of age group) differ significantly

Caponization had a significant effect on the mineral density of tibiotarsal bones. The tibiotarsal bones of capons had higher BMD values than the bones of cockerels, both at 12 and 24 wks of age ($P < 0.01$, Table 2). BMD values increased with age, from 0.098 g/cm^2 and 0.0162 g cm^{-2} in 12-wk-old cockerels and capons, respectively, to 0.180 g cm^{-2} and 0.232 g cm^{-2} in 24-wk-old birds ($P < 0.01$, Table 2).

Age significantly influenced the blood concentrations of Ca and P, and the activity of alkaline phosphatase ($P \leq 0.01$, Table 3). At 12 wks of age, blood Ca concentration reached 13.90 mg dl^{-1} in cockerels and 10.67 mg dl^{-1} in capons ($P < 0.01$), and blood P levels were determined at 6.92 mg dl^{-1} in cockerels and 8.44 mg dl^{-1} in capons ($P < 0.01$). At 24 wks of age, blood Ca concentration was comparable in cockerels and capons (9.38 mg dl^{-1} and 9.00 mg dl^{-1}, respectively), and a similar trend was noted in blood P concentration (cockerels – 3.83 mg dl^{-1}, capons – 3.85 mg dl^{-1}). Caponization increased the blood concentrations of Ca and P, but only at 12 wks of age (age × sex category interaction, $P \leq 0.01$, Table 3). The surgical procedure had no influence on the activity of alkaline phosphatase (Table 3).

Table 3

Effect of caponization on selected blood parameters and testosterone levels in Green-legged Partridge cockerels and capons (mean ± SD)

Item	Sex category	Age [wks]		P-value		
		12	24	age	sex category	A · S interaction
Ca [mg dL^{-1}]	cockerels	*13.91	9.38	0.000	0.021	0.027
		±0.17	±0.18			
	capons	10.67	9.00			
		±0.16	±0.17			
P [mg dL^{-1}]	cockerels	6.92	3.83	0.000	0.025	0.003
		±0.062	±0.076			
	capons	*8.44	3.85			
		±0.086	±0.748			
Alkaline phosphatase [U L^{-1}]	cockerels	756.3	523.2	0.000	0.321	0.247
		±0.04	±0.06			
	capons	804.3	476.8			
		±0.02	±0.01			
Testosterone [ng ml^{-1}]	cockerels	*0.40	*1.86	0.000	0.000	0.247
		±0.04	±0.06			
	capons	0.13	0.11			
		±0.02	±0.01			

*– values in columns (cockerels an capons of age group) differ significantly

The effectiveness of surgical castration was confirmed by an analysis of blood testosterone levels, which increased with age and were significantly ($P < 0.01$) higher in cockerels than in capons (Table 3).

Discussion

The present study investigated whether surgical castration influences morphometric parameters and the mineral density of tibiotarsal bones in Green-legged Partridge cockerels and capons. Testosterone stimulates the activity of osteoblasts in the bone formation process, as well as the ossification of long bones. Therefore, the absence of sex steroids in cockerels could affect bone metabolism (Mahmud et al. 2014, Muszyński et al. 2017). Our findings indicate that the castration-induced decrease in blood testosterone levels had no effect on the body weights of Green-legged Partridge cockerels and capons, but it significantly affected the analyzed morphometric parameters and mineral density (BMD) of tibiotarsal bones (Table 2).

At 24 weeks of age, the tibiotarsal bones of Green-legged Partridge cockerels were longer and heavier than the bones of capons. In a study of male Taiwan country D × L_2 cockerels, CHEN et al. (2006b) noted lower weight of tibiotarsal bones in capons, whereas the length of the analyzed bones was not affected by castration. In an experiment performed on male Single Comb White Leghorns (CHEN et al. 2007), caponization decreased the length of tibiotarsal bones but had no influence on bone weight. The results of our study and the findings of other authors point to different responses of cockerels of various breeds to reduced testosterone levels. It should also be noted that Taiwan country D × L_2 cockerels (CHEN et al. 2006b) were caponized at 10 wks of age, Single Comb White Leghorns (CHEN et al. 2007) were caponized at 12 wks of age, and Green-legged Partridge cockerels analyzed in this study were surgically castrated at 8 wks of age.

In the current study, capons were characterized by higher BMD values than cockerels, both at 12 and 24 wks of age. In a study of Polbar chickens caponized at 8 wks of age, no significant differences in the mineral density of tibiotarsal bones were found between intact males and capons (MUSZYŃSKI et al. 2017). It should be stressed, however, that in the cited study, caponization had a beneficial influence on the body weights of birds, which was not observed in the present experiment. The above results suggest that male birds of various breeds may differ in their susceptibility to changes resulting from decreased synthesis of steroid hormones.

CHEN et al. (2006a) demonstrated that caponization increased total blood Ca concentrations. However, in a study by CHEN et al. (2007), caponization had no influence on total blood Ca levels. In the current study, an increase in the blood concentrations of Ca and P was observed in 12-wk-old capons, i.e. 4 wks after castration, whereas 24-wk-old cockerels and capons (16 wks after castration) were characterized by similar blood Ca levels (Table 3). The significant decrease in blood Ca concentrations, noted in capons 4 wks after castration, resulted from declining testosterone levels. The stimulation of osteoclast formation and the release of Ca from bones contributed to the stabilization of blood Ca levels, which were comparable in 24-wk-old cockerels and capons.

LIN AND HSU (2003) analyzed Taiwan country cockerels (TLRL native chicken Taishi No. 13) and found no differences in blood Ca concentrations between 28-wk-old cockerels and capons; capons had only higher blood P levels. According to LIN AND HSU (2003), caponization increases blood ionized Ca concentrations, but not total Ca levels. CHEN et al. (2006a) reported that caponization did not affect plasma alkaline phosphatase levels, which is consistent with our findings.

The results of this study indicate that caponization exerted a significant effect on the analyzed morphometric parameters and mineral density of tibiotarsal bones in Green-legged Partridge cockerels. However, further research involving a higher number of parameters is needed to determine whether the observed changes could pose a risk in capon production.

Tranlaslated by ALEKSANDRA POPRAWSKA

Accepted for print 18.01.2018

References

ADAMSKI M., KUŹNICKA J., BANASZAK M., WEGNER M. 2016. *The analysis of meat of Sussex cockerels and capons (S11) at different ages*. Poult. Sci., 95: 125–152.

CHARUTA A., COOPER R.G., PIERZCHAŁA M., HORBAŃCZUK J.O. 2012. *Computed tomographic analysis of tibiotarsal bone mineral density and content in postnatal turkeys (Meleagris gallopavo) as influenced by age and sex*. Czech. J. Anim. Sci., 57: 572–578.

CHARUTA A., DZIERZĘCKA M., KOMOSA M., KALINOWSKI Ł., PIERZCHAŁA M. 2013. *Age- and sex-related differences of morphometric, densitometric and geometric parameters of tibiotarsal bone in Ross broiler chickens*. Folia Biol. (Kraków), 61: 211–220.

CHEN K.L, TSAY S.M, LEE T.Y., CHIOU W. S. 2006a *Effects of caponization and different exogenous androgen on the bone characteristics of male chickens. Poult. Sci.*, 85: 1975–1979.

CHEN K.L., CHANG M.H., TSAY S.M., HURNG H.Y., CHIOU P.W.S. 2006b. *Effects of caponization on bone characteristics and histological structure in chicken*s. Asian-Aust. J. Anim. Sci., 19(2): 245–251.

Chen K.L., TSAY S.M., LO D.Y., KUO F.J., WANG J.H., CHI W.T., C. CHU, CHIOU P.W.S. 2007. *Effects of caponization and testosterone on bone and blood parameters of SCWL male chickens. Poult. Sci.,* 86: 1754–1759.

CHEN S.Y., LI T.Y., TSAI C.H., LO D.Y., CHEN K.L. 2014. *Gender, caponization and exogenous estrogen effects on lipids, bone and blood characteristics in Taiwan country chickens*. J Anim. Sci., 85: 305–312.

DÍAZ O., RODRÍGUEZ L., TORRES A., COBOS A. 2010. *Chemical composition and physico-chemical properties of meat from capons as affected by breed and age*. Span. J. Agric., 8: 91–99.

FINKELSTEIN JS, KLIBANSKI A, NEER RM, GREENSPAN SL, ROSENTHAL DI, CROWLEY JR W. 1987. *Osteoporosis in men with idiopathic hypogonadotropic hypogonadism*. Ann. Intern. Med., 106: 354–361.

FRANCO D., PATEIRO M., ROIS D., VÁZQUEZ J.A., LORENZO J.M. 2016. *Effects of caponization on growth performance, carcass and meat quality of Mos Breed capons reared in free-range poduction system*. Ann. Anim. Sci. 16(3): 909–929.

GESEK M., ZAWACKA M., MURAWSKA D. 2017. *Effects of caponization and age on the histology, lipid localization and fiber diameter in muscles from Greenleg Partridge cockerels*. Poult. Sci., 96(6): 1759–1766.

GRYZIŃSKA M., STRACHECKA A., KRAUZE M. 2011. *Concentration of testosterone in blood serum in roosters of the Polbar breed depending on age*. Annales UMCS, Zootechnica, 29(4): 46–50.

JOHNSON A.L., RENDANO V.T. 1984. *Effects of castration, with and without testosterone replacement, on leg bone integrity in the domestic fowl*. Am. J. Res., 45: 319–325.

LIN C.Y., HSU J.C. 2003. *Comparison of some selected growth, physiological and bone characteristics of capon, slip and intact birds in Taiwan Country chicken cockerels*. Asian-Aust. J. Anim. Sci., 1: 50–56.

MAHMUD M., SHABA P., GANA J., YISA H., NDAGIMBA R. 2013. *Effects of surgical caponisation on growth, carcass and some haematological parameters in cockerel chicken*s. Sokoto J. Vet. Sci., 11: 57–62.

MANOLAGAS S.C., KOUSTENI S., JILKA R.L. 2002. *Sex steroids and bone*. Recent. Prog. Horm. Res., 57: 385–409.

MURAWSKA D., ZAWACKA M., BORKOWSKI R., MIKA M., MICHALIK D., GESEK M. 2015. *Age-related changes in the growth rate and blood testosterone concentrations in Rhode Island Red and Leghorn roosters*. VIth International Scientific Symposium for PhD Students and Students of Agriculture Colleges, Bydgoszcz – Ciechocinek, Poland, 17–19 September, pp. 98.

MURIEL DURÁN A. 2004. *The effect of caponization on production indices and carcass and meat characteristics in free-range Extremeña Azul chickens*. Span. J. Agric. Res., 2(2): 211–216.

MUSZYŃSKI S., KWIECIEŃ M., TOMASZEWSKA E., SWIETLICKA I., DOBROWOLSKI P., KASPEREK K., JEŻEWSKA-WITKOWSKA G. 2017. *Effect of caponization on performance and quality characteristics of long bones in Polbar chickens*. Poult. Sci., 96: 491–500.

OURSLER M.J., KASSEM M., TURNER R., RIGGS B. L., SPELSBERG T. C. 1996. *Regulation of bone cell function by gonadal steroids*. In: *Osteoporosis*. Eds. R. Marcus, D. Feldman, J. Kelsey. Academic Press, San Diego, CA, pp. 237–60.

PADHI M.K. 2016. *Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance*. Scientifica, article ID 2604685, 2604685, doi:10.1155/2016/2604685.

PEDERSON L., KREMER M., JUDD J., PASCOE D., SPELSBERG T., RIGGS B. L., OURSLER M.O. 1999. *Androgens regulate bone resorption activity of isolated osteoclasts in vitro*. Proc. Natl. Acad. Sci. U.S.A, 96: 505–510.

RIKIMARU K., SHIJI O., KOMASTU M., ISHIZUKA J. 2009. *Effects of caponization on meat quality of Hinai-jidori chicken*. J Poult. Sci., 46: 345–350.