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Original article

Effects of caponization and age on the histology, lipid localization and fibre diameter in muscles of Rhode Island Red cockerels

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Abstract

Poultry scientists are constantly studying different breeds of cockerels that would be suitable for capon meat production. Capon meat, although not yet very popular, is characterized by exceptional taste qualities that could appeal to many customers. Obtaining the appropriate palatability, structure and tenderness of capon meat is possible thanks to the reduction in androgen levels following the castration of roosters. Surgical or chemical castration affects the metabolism of fats, thus increasing their accumulation in the abdominal cavity, subcutaneous tissue and muscles. The main aim of our research was histological evaluation and analysis of the concentration and distribution of adipose tissue in muscles in Rhode Island Red cockerels and capons. In addition, we analysed the diameter of the pectoral muscle fibre. The experiment was performed on 200 Rhode Island Red cockerels; the testes were removed at 8 weeks of age. At 12, 16, 20, 24 and 28 weeks of age, 6 cockerels and 6 capons were slaughtered, and samples from the pectoral and thigh muscles were evaluated. Differences in the accumulation of adipose tissue with muscular atrophy ($p < 0.05$) were observed in thigh muscles, with higher amounts in capons than in cockerels. All examined locations in the pectoral and thigh muscles of capons (around the blood vessels, in the perimysium, in the endomysium, and in the sarcoplasm) showed much higher concentrations of lipids compared to the levels in cockerels. The diameters of the pectoral muscle fibres were different ($p < 0.05$) at 12 and 16 weeks of age, and the diameters of the giant fibres were different ($p < 0.05$) at 12 and 20 weeks of age, with higher values in cockerels. The high concentration of lipids in the skeletal muscles of Rhode Island Red capons is impressive. These dual-purpose cockerel breeds can be a source of high-quality meat.

Keywords: caponization, dual-purpose cockerels, fat, histology, muscle



Introduction

Currently, one day old cockerels of laying and general purpose breeds are most often euthanized due to little interest in meat production (Choo et al. 2014, Murawska et al. 2019). This is mainly due to a relatively long rearing period and high feed consumption per kilogram of body weight gain (compared to broiler chickens), which adversely affects production efficiency (Murawska and Bochno 2007, Koenig et al. 2012). Moreover, in some chicken breeds, roosters become aggressive as they reach sexual maturity, which poses a problem for breeders and producers (Essien et al. 2012). Undesirable behavioral changes can be eliminated by surgical or chemical castration (Quaresma et al. 2017). Caponization is carried out to produce meat with exceptional flavor (Díaz et al. 2010, Calik et al. 2015, Adamski et al. 2016, Amorim et al. 2016). Consumers' preferences change over time in response to both increasing awareness of animal welfare and socio-cultural factors affecting their food choices (Long and Blok 2017, Bosona and Gebresenbet 2018). Local restrictions together with ethical reasons determine the method of caponization, either chemical or surgical (Rahman et al. 2004, Sirri et al. 2009, Chen et al. 2010). In the UE a capon is a male hen, surgically castrated before reaching sexual maturity and slaughtered at the age of at least 140 days (20 weeks), and after the procedure the capons must be fattened for a minimum of 77 days (Regulation EC No. 543/2008 of 16 June 2008). Although the procedure of castration of birds has been known and used in various countries for centuries, the effects of a permanent decrease in the production of testosterone and other androgens have not been fully understood (Chen et al. 2014).

Most often accumulation of abdominal, subcutaneous, and intramuscular fat is the result of low androgen levels – the effect of testes removal. High lipid levels in muscular tissue change the flavour, texture, and tenderness of meat (Amorim et al. 2016). Studies concerning capon production describe fat accumulation (Chen et al. 2005), chemical analysis of the meat (Sirri et al. 2009) and intramuscular fat content (Symeon et al. 2010, Sinanoglou et al. 2011). Androgen deficiency after testes removal also influences muscle fibre diameter. Changes in fibre diameter associated with androgen deficiency have been established between capons and cockerels in Taiwan country chickens (Lin and Hsu 2002), Green-legged Partridges (Calik et al. 2015, Gesek et al. 2017) and Leghorns (Gesek et al. 2019b) over different fattening periods. Other research included analysis of histopathological changes in pectoral and thigh muscles and the localization and accumulation of lipids in pectoral and thigh muscles in Green-

-legged Partridge and Leghorn capons and cockerels (Gesek et al. 2017, 2019b) and histopathological changes in internal organs in Leghorn capons and cockerels (Gesek et al. 2019c).

Currently in Europe, the meat from capons of selected traditional breeds is appreciated by some consumer groups for its tenderness and flavor, but it is more expensive than meat from broiler chickens and organic chickens (Duran 2004, Franco et al. 2016). Most native bird breeds have been caponized, such as Castellana Negra chickens (Miguel et al. 2008), Taiwan country chickens (Chen et al. 2006), Nara chickens (Rahman et al. 2004), Extremena Azul chickens (Duran 2004), Green-legged Partridges, Yellow-legged Partridges, and Barred Rock chickens (Calik 2014, Calik et al. 2015, Kwiecień et al. 2015, Gesek et al. 2017). Gesek et al. (2019b) also examined laying-type Leghorn capons and cockerels and reported that unwanted cockerels are a potential source of high-quality capon meat. In poultry, the most valuable muscles in terms of consumption include the pectoral muscles and thigh muscles. In recent years a number of meat defects have been observed in broiler chickens, especially the pectoral muscle, such as wooden-breast, white stripping or spaghetti meat (Baldi et al. 2019, Pertacci et al. 2019, Lebednikaite et al. 2023, Valenta et al. 2023). It should be emphasized that in the case of cockerels and capons of general-purpose and laying breeds, little attention has been paid to the issue of morphological assessment of these muscles.

Our aim for this study was to determine whether a dual-purpose breed of chicken, such as Rhode Island Red, can be a potential source of high-quality meat. Thus, the two purposes of this present study were to analyse the occurrence of morphological lesions in skeletal muscles from Rhode Island Red (a dual-purpose breed of chicken) capons and cockerels, and to analyse lipid accumulation and concentration and fibre diameter of the pectoral muscles.

Materials and Methods

Two hundred one-day-old Rhode Island Red cockerels were weighed, marked with wing tags, and randomly distributed in pens at the experimental centre of the Department of Commodity Science and Animal Improvement of the University of Warmia and Mazury in Olsztyn. The birds were fed commercial diets *ad libitum*, and the feed composition was the same as that presented in our earlier studies with Green-legged Partridge cockerels and capons (Zawacka et al. 2017).

At 8 weeks of age, the birds were divided into 2 groups (2 x 100), with 6 replications per group (15-17

birds per replication). 100 birds were then surgically castrated by a qualified veterinarian under general anesthesia (0.2 ml Bioketan i.m., Vetoquinol, Gorzów Wielkopolski, Poland; castrations were via an incision between the last 2 ribs on both sides). The procedure was approved by the Local Ethics Committee in Olsztyn, Poland (number 16/2014, Poland). From 12 weeks of age, at 4-week intervals, 6 intact cockerels and 6 capons (1 bird per replication) were selected randomly (birds \pm 15% of average body weight) and slaughtered (electrical stunning followed by cutting the jugular vein). When the necropsy showed that the capon's testes had not been fully removed, the bird was eliminated from the experiment.

During necropsy, samples of pectoral (*pectoralis major*) and thigh (*musculus semimembranosus*) muscles from 6 cockerels and 6 capons at each slaughter age were fixed in 10% neutralized formalin and embedded in paraffin blocks. Haematoxylin and eosin staining was used on muscle sections of 5 μ m. Oil Red O staining (Bio-Optica, Milan, Italy) to detect lipids was used on frozen sectioned pectoral and thigh muscle tissue (8 μ m thickness), also collected during necropsy and submerged in a 30% saccharose solution with sodium azide (3 g/1000 ml solution). Each section was imaged using a MIDI 3DHISTECH Panoramic Scanner (3DHISTECH, Budapest, Hungary). Panoramic Viewer software (3DHISTECH, Budapest, Hungary) was used to measure fibre diameter (minimum of 100 fibres in each muscle section). Lipid concentration was evaluated using a three-point scale (+, low lipid content; ++, moderate lipid content; and +++, high lipid content) in four locations: around vessels, in the perimysium between fascicles, in the endomysium between fibres and in the sarcoplasm of muscle fibres (Gesek et al. 2019a).

Statistical analyses

The results were processed statistically in the Statistica 13.0 PL program (StatSoft Inc., Tulsa, OK, USA). Data were tested for normality using the Kolmogorow-Smirnow test ($p < 0.05$). The research hypothesis was assessed with regard to histopathological changes by chi-square test (the Fisher test for 2x2 tables – Fisher exact test; significant at $p \leq 0.05$). The results for the diameter of the muscle fibres ($n=6$: number of birds in each group; in each pectoral muscle 100 measurements of fibres were taken x 6 age groups x 2 experimental groups – capons and cockerels) were presented as mean \pm standard deviation (SD). Significant differences were determined using Duncan's multiple range test with the level of significance set at $p < 0.05$. The results were validated using two-way (A x G) or one-way (A or G)

ANOVA. The differences among groups of chicken's age (A) or caponization (group G) were estimated using the following model:

$$Y_{ij} = \mu + Y_i + e_{ij},$$

where:

- Y_{ij} – is the observation of dependent variable;
- μ – is the overall mean;
- Y_i – fixed effect of chicken's age or caponization (group) = ($Y_i = \mu_i - \mu$);
- μ_i – mean for the i -group;
- e_{ij} – is the random residual error.

Fixed effects of chicken's age (A) and caponization (group G) and their interaction were included in the model. The following model was used:

$$Y_{ij} = \mu + A_i + G_j + (A \times G)_{ij} + e_{ij},$$

where:

- Y_{ij} – is the observation of dependent variable;
- μ – is the overall mean;
- A_i – is the effect of chicken's age (A);
- G_j – is the effect of caponization (group) (G);
- $(A \times G)_{ij}$ – is the interaction term of A and G;
- e_{ij} – is the random residual error.

Fixed effects of chicken's age (A) and group (castration, G) and their interaction were included in the model. The following model was used:

$$Y_{ij} = \mu + A_i + G_j + (A \times G)_{ij} + e_{ij},$$

where:

- Y_{ij} – is the observation of dependent variable;
- μ – is the overall mean;
- A_i – is the effect of chicken's age (A);
- G_j – is the effect of group (castration) (G);
- $(A \times G)_{ij}$ – is the interaction term of A and G;
- e_{ij} – is the random residual error.

Results

Table 1 presents the results of the histopathological evaluation of pectoral and thigh muscles from Rhode Island Red capons and cockerels at different times after caponization. Segmental defragmentation of the fibres, loss of cross striations, accumulation of adipose tissue with muscular atrophy and hyalinization of the fibres were detected in both the capons and the cockerels, as well as in different age groups. Segmental defragmentation of the fibres was observed more often in pectoral muscles than in thigh muscles in both groups. In cockerels and capons, accumulation of adipose tissue with muscular atrophy and loss of cross striations were noted almost entirely in thigh muscles. Seven cases

Table 1. Histological lesions in pectoral and thigh muscles in cockerels and capons in different time of fattening.

Specification n = 6	12 th week		16 th week		20 th week		24 th week		28 th week		Significance/ P-value											
	P		Th		P		Th		P		Th											
	Coc	Cap	Coc	Cap	Coc	Cap	Coc	Cap	Coc	Cap	Coc	Cap	Total P	Total Th								
Segmental defragmentation of the fibres	2	2	3	2	2	3	4	2	4	3	1	1	3	6	0	0	4	3	0	1	13/17	8/6
<i>P-value</i>	0.727		0.500		0.500		0.284		0.500		0.773		0.091		-		0.500		0.500		0.220	0.381
Accumulation of adipose tissue with muscular atrophy	0	0	0	1	0	0	1	5	0	0	1	6	0	1	2	6	0	1	3	6	0/2	7/24*
<i>P-value</i>	-		0.500		-		0.040		-		0.080		0.500		0.303		0.500		0.909		0.246	0.000
Loss of cross striations	0	1	1	2	0	0	3	4	0	0	6	6	0	0	5	5	0	0	2	2	0/1	17/19
<i>P-value</i>	0.500		0.500		-		0.500		-		-		-		0.773		-		0.727		0.500	0.396
Hyperplasia of the smooth muscle in arteries	0	0	1	1	0	0	0	2	1	0	1	1	0	0	1	0	0	0	1	0	1/0	4/4
<i>P-value</i>	-		0.773		-		0.227		0.500		0.773		-		0.500		-		0.500		0.500	0.650
Infiltration of lymphoid cells between fibres	1	2	1	1	1	1	0	1	0	2	0	0	0	2	0	0	1	2	0	0	3/9	1/2
<i>P-value</i>	0.500		0.773		0.773		0.500		0.227		-		0.227		-		0.500		-		0.052	0.500
Atherosclerosis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1/0	1/0
<i>P-value</i>	-		-		-		-		-		0.500		-		-		0.500		-		0.500	0.500
Fibromuscular dysplasia	1	0	0	0	0	1	2	0	0	0	1	1	0	0	0	0	0	0	1	0	1/1	4/1
<i>P-value</i>	0.500		-		0.500		0.227		-		0.773		-		-		-		0.500		0.754	0.177
Focal necrosis of the fibres with infiltration of lymphoid cells	1	2	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	1/2	2/3
<i>P-value</i>	0.500		0.773		-		-		-		0.500		-		-		-		0.227		0.500	0.500
Proliferation of connective tissue around vessels	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1/0	2/3
<i>P-value</i>	-		0.500		-		0.500		-		-		-		-		0.500		0.500		0.500	0.500
Hyalinization of fibres	0	1	2	1	0	0	1	1	1	1	0	1	0	0	2	1	1	2	1	2	2/4	6/6
<i>P-value</i>	0.500		0.500		-		0.773		0.773		0.500		-		0.500		0.500		0.500		0.335	0.626

* Significant at $p \leq 0.05$ - chi-square test - Fisher test for 2x2 tables - Fisher exact test; description: P - pectoral muscles; Th - thigh muscles

of fibromuscular dysplasia (FMD) were noted, mostly in thigh muscle arteries. A larger number of lesions were detected in the thigh muscle than in the pectoral muscle in both groups. Statistical analysis showed significant differences in the accumulation of adipose tissue with muscular atrophy ($p < 0.05$) in thigh muscles between capon and cockerels, with higher values in capons (Fig. 1). No other statistically significant differences were found in the quantity of histopathological changes between the pectoral and thigh muscles of the cockerels and capons.

Table 2 presents the localization of adipose tissue in

pectoral and thigh muscles at different times of fattening. In the pectoral muscles around the vessel area (Fig. 2), the accumulation of adipose tissue was similar in capons and cockerels at 12-20 weeks, but higher values were noted from 24 weeks in capons. At the 28th week, five capons showed low concentrations of adipose tissue in the endomysium in the pectoral muscles compared to one cockerel with low values. In the thigh muscle vessel area, the accumulation of adipose tissue was at a similar moderate to high intensity in capons and cockerels (Fig. 3). From the 16th week, capon thigh muscles showed higher accumulation of adipose tissue

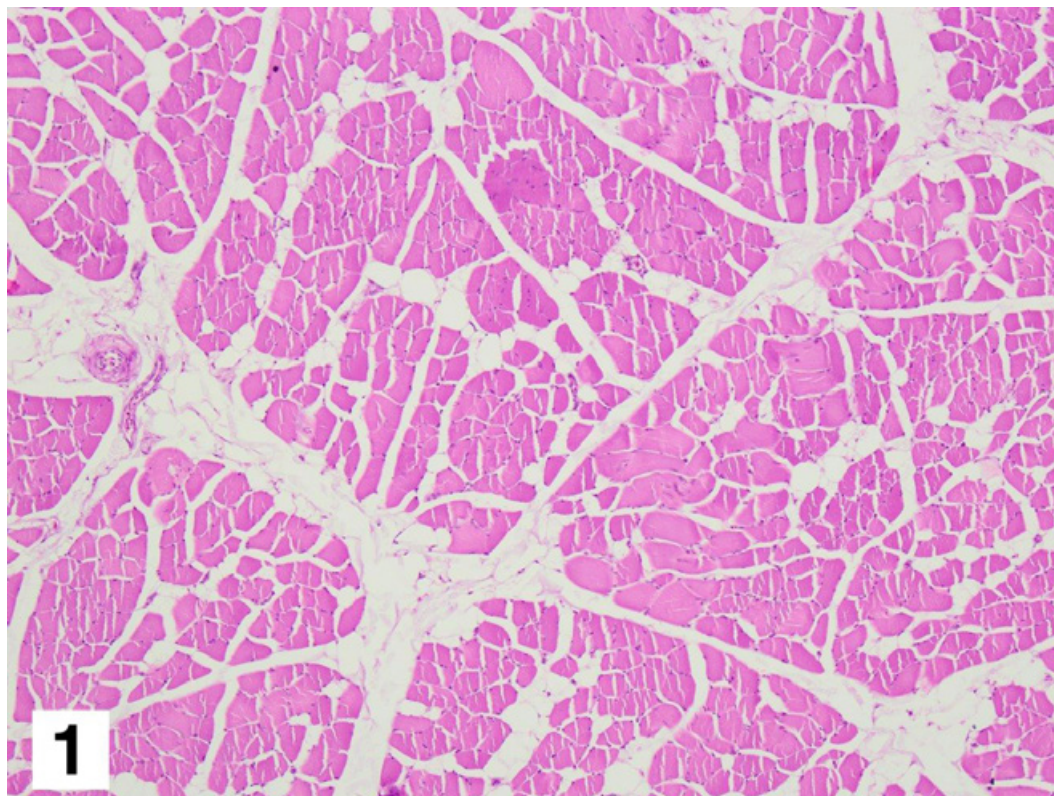


Fig. 1. Thigh muscle sample from a 24-week-old capon shows an accumulation of adipose tissue with muscular atrophy (haematoxylin and eosin staining). x40

Table 2. Accumulation of the adipose tissue in the pectoral and thigh muscles of cockerels and capons after various times of fattening.

n = 6	12 th week		16 th week		20 th week		24 th week		28 th week			
	Cockerels		Capons		Cockerels		Capons		Cockerels		Capons	
	P	Th	P	Th	P	Th	P	Th	P	Th	P	Th
Accumulation of adipose tissue around vessels	+	++	+	+++	+	+++	+	+++	+	+++	+	+++
	++	++	++	++	+	+++	++	++	++	+++	++	+++
	+	+++	+	++	++	+	+++	++	+++	+	+++	+++
	+	+++	++	++	+	+++	+	+++	++	+++	++	+++
	+	++	+	++	++	+	+++	++	+++	+	+++	+++
Accumulation of adipose tissue in the perimysium between fascicles		+		++		+++		++		+++		++
		+		+		++		+++		+++		++
		++		++		+	++	+	+++		++	+
		++		+		+++		+++		++		+++
		++		+++		++		+		++		+++
Accumulation of adipose tissue in the endomysium between fibres		+		+		++		+++		++		++
		+		+		+	++	+	+++		+	+
		+		++		++	+	+++		++		++
		++		+		++		+		+		+++
		++		++		+		++		+++		+++
Accumulation of lipid droplets in the sarcoplasm of muscle fibres				+								
				+		+						+
		+		+		+			+			
				+		+						
				+								

Description: +: low; ++: moderate; +++: high concentration of lipids; P: pectoral muscles, Th: thigh muscles. Empty spaces – no lipid accumulation.

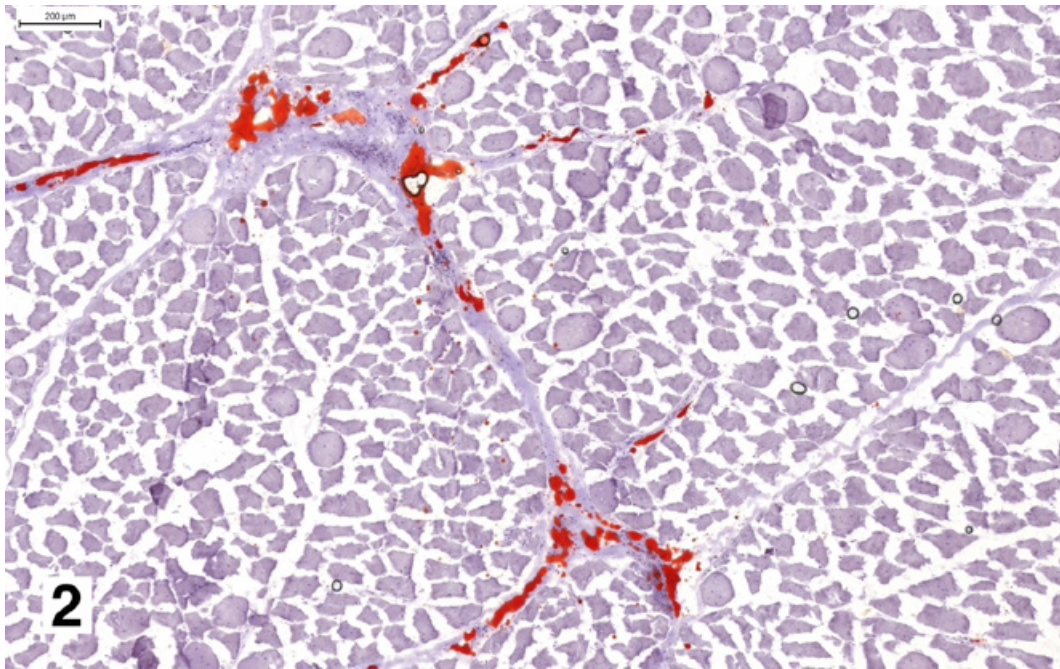


Fig. 2. Pectoral muscle sample from a 20-week-old capon shows an accumulation of adipose tissue around the vessels (++ indicates a moderate concentration; Oil Red O staining)

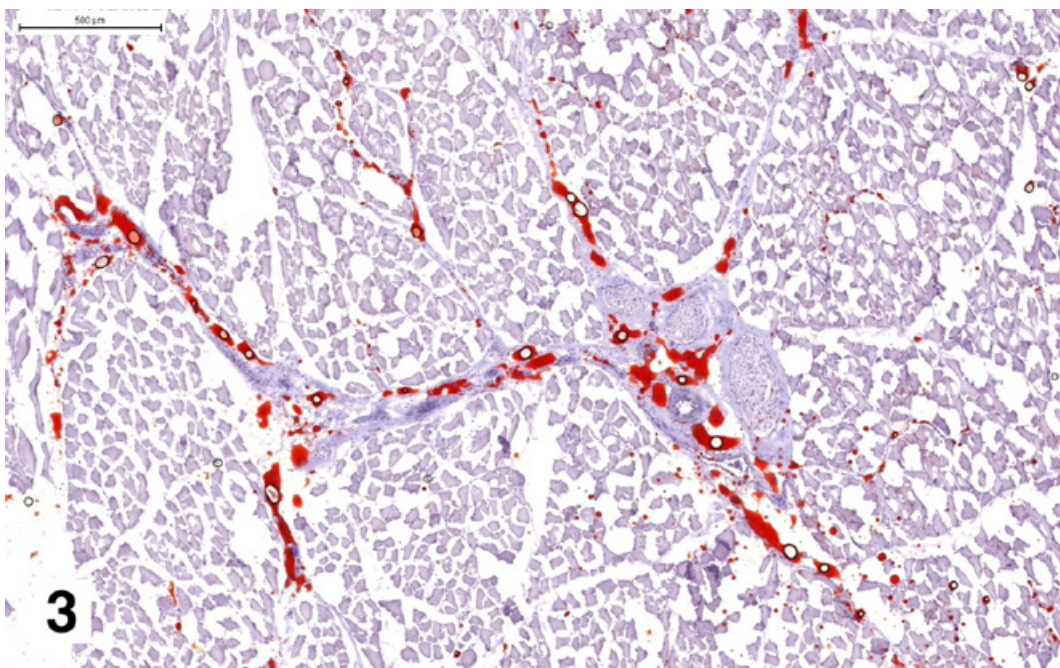


Fig. 3. Thigh muscle sample from a 12-week-old capon shows an accumulation of adipose tissue around the vessels (++ indicates a moderate concentration; Oil Red O staining)

in the perimysium between the fascicles and in the endomysium between the fibres (Fig. 4) compared to cockerels with moderate to high values. Within the sarcoplasm of muscle fibres in thigh muscles, lipid droplets were seen more often in capons than cockerels (12th week; Fig. 5).

Analyses of the pectoral muscle fibre diameters are presented in Table 3. The pectoral fibre diameter in both capons and cockerels continued to increase with age

($p < 0.000$). The influence of caponization on pectoral fibre diameter was examined ($p < 0.000$), and significant differences were noted at the 12th and 16th weeks (interaction, $p = 0.030$; Table 3). When the influence of caponization on giant fibre diameters was evaluated, significant differences were established between age groups, both in capons and cockerels ($p < 0.000$; Table 3). Additionally, the influence of caponization on pectoral giant fibre diameter was examined ($p < 0.000$), and significant

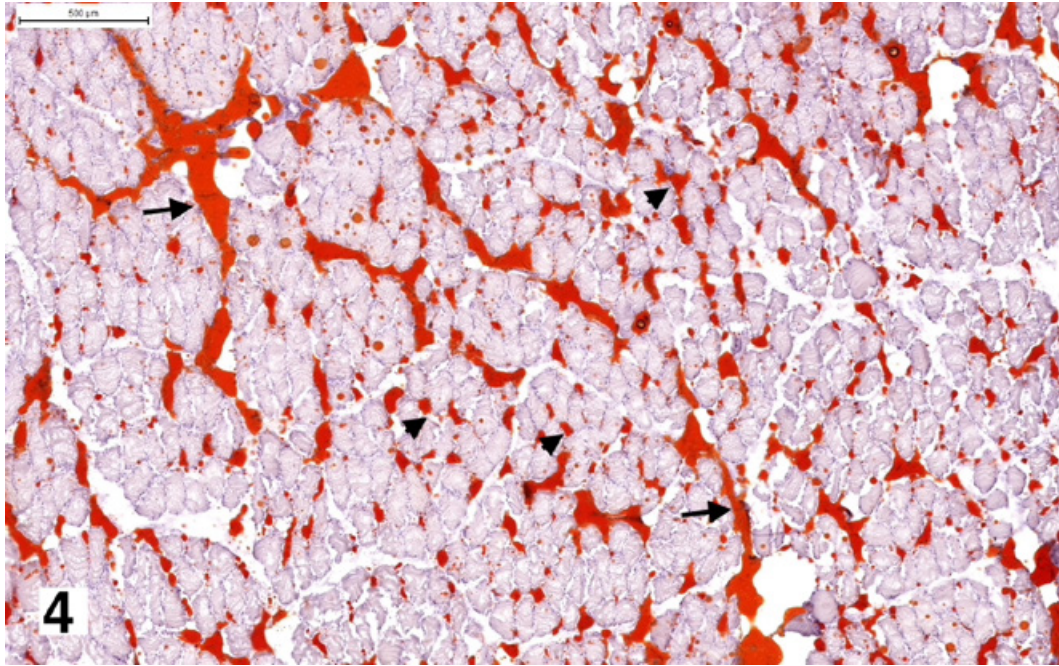


Fig. 4. Thigh muscle sample from a 24-week-old capon shows an accumulation of adipose tissue in the perimysium between fascicles (+++ indicates a high concentration, black arrows), and in the endomysium between fibres (+++, arrowhead; Oil Red O staining)

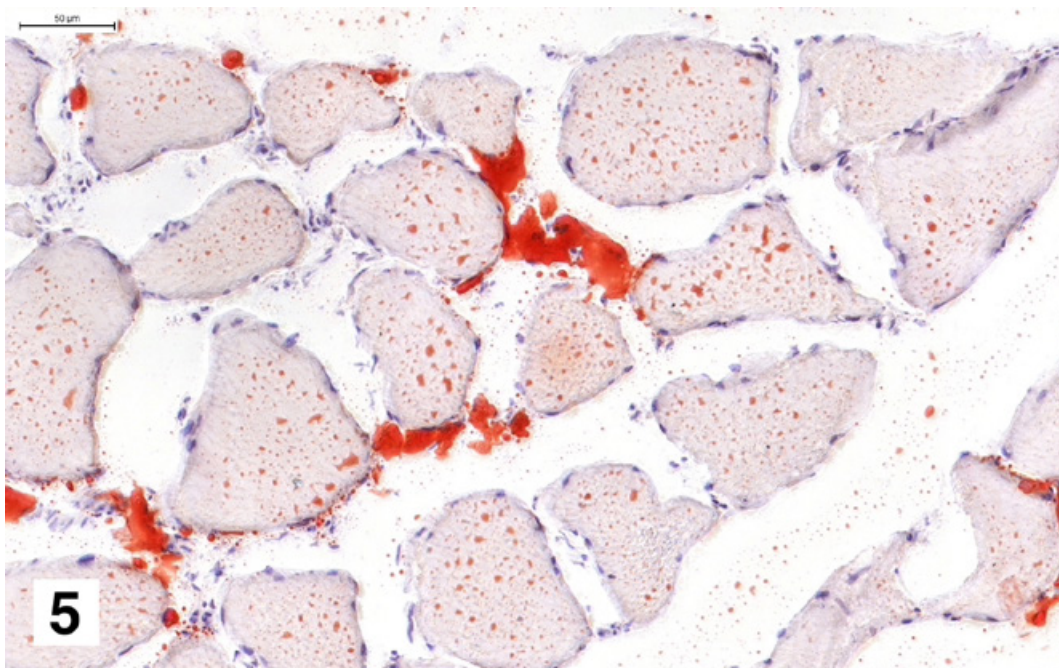


Fig. 5. Thigh muscle sample from a 12-week-old capon shows an accumulation of adipose tissue in the sarcoplasm of the fibres (+ indicates a low concentration; Oil Red O staining)

differences were noted at the 12th and 20th weeks (interaction, $p=0.019$; Table 3).

Discussion

The main aim of this study was to establish whether Rhode Island Red cockerels can be used in the production of capon meat. Histopathological and lipid local-

ization analyses were used to provide the necessary data. Only high-quality meat without extensive pathological lesions can reach the market. The present study revealed morphological changes in capons and cockerels in both pectoral and thigh muscles during the entire experiment, but the number of those changes was relatively low. Accumulation of adipose tissue with muscular atrophy show differences between groups. This lesion is a consequence of castration, where

Table 3. Effect of caponization and age on diameters of fibres and diameters of giant fibres in *Pectoralis major* muscles (mean).

Specifi- cation	#Means for subgroups \pm SD (n=6)			Age (wk)					Group (capons/cockerels)		P-value		
	Age (wk)	capons	cockerels	12	16	20	24	28	Cap	Coc	Age	Group	AxG
Diameter of fibres (μ m)	12	30.32 ^a \pm 0.44	*33.27 ^a \pm 0.50										
	16	35.40 ^b \pm 0.46	*36.6 ^b \pm 0.30										
	20	38.06 ^c \pm 0.48	38.41 ^c \pm 0.36	33.27	36.06	38.23	42.07	44.00	38.30	39.30	0.000	0.001	0.030
	24	41.97 ^d \pm 0.42	42.18 ^d \pm 0.46										
	28	43.74 ^e \pm 0.53	44.28 ^e \pm 0.57										
Diameter of giant fibres (μ m)	12	51.97 ^a \pm 0.86	*58.26 ^a \pm 0.87										
	16	65.7 ^b \pm 0.92	66.16 ^b \pm 0.96										
	20	66.66 ^b \pm 1.37	*70.73 ^c \pm 0.97	55.6	65.93	66.40	74.76	76.93	66.41	68.37	0.000	0.001	0.019
	24	74.32 ^c \pm 0.56	75.22 ^d \pm 1.17										
	28	75.44 ^c \pm 1.06	78.43 ^e \pm 1.09										

#Description:

* or ^{a-d} means for subgroups values followed by * or different letters differ significantly ;* on the left side of the value denotes means in rows, letters on the right side of the value denote means in columns (p< 0.05)

adipose tissue accumulates within muscle structures. The present study showed a higher concentration of this lesion in thigh muscles in Rhode Island Red capons (24 capons) than that observed in Leghorn and Green-legged Partridge capons (18 and 15 capons, respectively) (Gesek et al. 2017, 2019b), and we can assume that Rhode Island Red capons are more predisposed to desirable adipose tissue accumulation. Pathological changes that can influence meat quality are necrosis of muscle fibres and inflammation. Necrosis of the fibres with degeneration and infiltration of T lymphocytes is a problem in heavy-size male broilers (Mazzoni et al. 2015). Mazzoni et al. (2015) observed this lesion in the *pectoralis major* muscles and assumed that it can influence meat quality. In the present study, only a few cases of focal necrosis of the fibres with infiltration of lymphoid cells were diagnosed, and this number (3 within pectoral muscles and 5 within thigh muscles) in both capons and cockerels is similar to that diagnosed in Green-legged Partridges (2 within pectoral muscles and 3 within thigh muscles) but half as low as that in Leghorn birds (10 within pectoral muscles and 11 within thigh muscles) (Gesek et al. 2017, 2019b). Minimal necrotic foci can induce inflammatory reactions and result in the infiltration of inflammatory cells (MacRae et al. 2006, Kuttappan et al. 2013). Our study revealed a higher number of infiltrations within pectoral muscles with a higher number in capons. However, the total number of lesions was low and was equal to the number diagnosed in Green-legged Partridges but was half as low as that in Leghorn birds. After histological analysis, we can assume that Rhode Island Red cockerel and capon meat was healthier, with only a few pathological lesions compared to meat with extensive

necrosis in meat-type broilers (McRae et al. 2006, Mazzoni et al. 2015). Histology has also confirmed that Rhode Island Red capons have fewer undesirable changes than Leghorn and Green-legged Partridge capons (Gesek et al. 2017, 2019b).

In the present study, we used the same three-point scale to analyse lipid localization and accumulation, similar to Leghorns and Green-legged Partridges (Gesek et al. 2017, 2019b). The established scale shows the level of fat accumulation at specific locations within muscle structures (Gesek et al. 2019a). When the fat concentration within the muscle structure is high, lipids enhance the quality and improve the flavour, texture and tenderness of the meat, which make it a unique product (Calik et al. 2015, Amorim et al. 2016).

In pectoral muscles, the highest lipid concentration was noted in an area around the vessels, and differences between capons and cockerels were visible from the 24th week, with higher values in capons. Differences were also established within the endomysium between fibres (28th week) with higher lipid content in capons (five capons with low lipid content compared to one cockerel with low lipid content). When other breeds of capons were compared, 28-week-old Rhode Island Red capons showed the highest lipid content (in vessel areas), with a high to moderate lipid content, whereas Leghorn capons showed a low to moderate lipid content (Gesek et al. 2019b), similar to Green-legged Partridges (Gesek et al. 2017). In the endomysium, similar to our experiment, a higher lipid content was observed in Leghorn capons (Gesek et al. 2019b), but no differences were noted in Green-legged Partridges between capons and cockerels (Gesek et al. 2017). Other studies used a chemical analysis of the pectoral muscles. Kwiecień

et al. (2015) reported data similar to our observations, with a higher fat composition in Green-legged Partridge capon pectoral muscles. In Amarela Portuguesa and native Pedres Portuguesa capons, the same higher values were noted in pectoral muscles (2.23 and 1.97%, respectively) compared to cockerels (Amorim et al. 2016). Additionally, Beijing-You capons had significantly more intramuscular fat than the controls (Cui et al. 2017). In pectoral muscles, a three-point scale determined that dual-purpose breed Rhode Island Red capons are a better source of high-lipid, high-quality meat compared to laying-type Leghorn capons and native breed Green-legged Partridge capons. Compared to other capon breeds, Rhode Island Red capons showed higher levels of fat in pectoral muscles from the 16th week of age.

In thigh muscles at 12 weeks of age, similar moderate to high lipid concentrations were observed around the vessels in capons and cockerels. From the 16th week of age, higher concentrations (three birds with high and three with moderate concentrations in cockerels, compared to four birds with high and two with moderate concentrations in capons) were observed in capons. From this age, in the vessel area in capons, the concentration of lipids is high. Similar results were observed for the thigh muscle perimysium between fascicles where, from 16 week of age, the accumulation of lipids was higher in capons. From this age, all examined capons showed moderate to high lipid content, whereas cockerels had moderate content. The highest values within this area were noted at the 20th and 24th weeks. Differences between capons and cockerels in the endomysium between fibres were visible from the 12th week of age. At 12 weeks of age, six capons showed low to moderate lipid concentrations, but three cockerels showed low and one moderate lipid concentrations. From this age and in this location, capons showed higher values with moderate to high lipid content. In the sarcoplasm of the thigh muscle fibres, fat accumulation was more intense in capons (five capons with low lipid concentration compared to one cockerel with low lipid concentration) only at 12 weeks of age. Interestingly, only at the 16th week did birds show lipid content, with two capons and cockerels with low lipid concentrations. Using the three-point scale, we observed a difference between data from Rhode Island Red, Leghorn and Green-legged Partridge capons and cockerels. In the present experiment, in the vessel area, from the 20th week, capons showed enterally high lipid content, whereas in Leghorn capons, only at 28 weeks of age was high lipid content observed (Gesek et al. 2019b). Green-legged Partridge capons had lower lipid content and, in this area, 4 capons had a high lipid content only at the 24th week (Gesek et al. 2017). In the

perimysium between fascicles in thigh muscles, differences were visible between the birds in this study and Leghorn and Green-legged Partridge birds (Gesek et al. 2017, 2019b). From 20 weeks of age, all Rhode Island capons had high lipid content in this area; in Green-legged Partridge capons, a moderate level was observed, whereas in Leghorn capons, not all birds showed a moderate level of fat (Gesek et al. 2017, 2019b). Similar observations were made in the endomysium between fibres, where the concentration of fat in Rhode Island Red capons from 20 weeks of age was moderate to high, whereas other breeds of capons had only low levels (Gesek et al. 2017, 2019b). Contrasting findings were noted in the sarcoplasm of muscle fibres, where Rhode Island Red capons had lower lipid content compared to Leghorns and Green-legged Partridges (Gesek et al. 2017, 2019b). Our experiment revealed low lipid content in all capons at only the 12th week, whereas in Green-legged Partridges, these levels were observed at 16 and 28 weeks of age, and Leghorn capons showed similar levels throughout all examined periods (Gesek et al. 2017, 2019b). In general, the thigh muscles of Rhode Island Red capons exhibited higher and more intense levels of fat accumulation compared to Leghorns and Green-legged Partridge capons. Earlier experiments on the chemical composition of the muscles confirmed these observations where, in medium-growing capons, significantly higher fat content in thigh muscles was noted compared to control cockerels (Symeon et al. 2010, Sinanoglou et al. 2011). Kwiecień et al. (2015) reported a three times higher lipid content in the thigh muscles of Green-legged Partridge capons than in cockerels. Calik et al. (2017) performed chemical analysis of Rhode Island Red cockerels and capons and noted double the fat content in the leg muscles of capons, which influences the aroma, juiciness, tenderness and flavour of the meat. However, sometimes in native breeds, such as Amarela Portuguesa and native Pedres Portuguesa, differences are not visible, and there was no difference in intramuscular fat between capons and roosters in leg muscles (5.91 and 5.38%, respectively) (Amorim et al. 2016).

Another important determinant affecting the quality of meat is muscle fibre diameter. Lower levels of testosterone can change the diameter of muscle fibres at different times of rearing (Lin and Hsu 2002, Cui et al. 2017). For instance, the authors of an earlier paper detected that smaller diameters of pectoral muscle fibres in 28-week-old Taiwan country chicken capons were associated with lower plasma concentrations of testosterone than those in intact birds (Lin and Hsu 2002). In the present study, these differences were observed at the 12th and 16th week with smaller fibres in capons. Older birds did not show significant differences

between the examined groups. Similarly to our data, smaller pectoral muscle fibre diameters were noted in Beijing-You capons at 17 weeks of age, along with a higher fibre density (Cui et al. 2017). Contrasting data were found in Green-legged Partridge birds. Calik et al. (2015) observed smaller fibre diameters in 24-week-old capons. Gesek et al. (2017) found significant differences in muscle fibres occurring at 20, 24 and 28 weeks of fattening (older capons) in Green-legged Partridge capons. Similarly, in Leghorn capons, differences were noted at the 28th week (Gesek et al. 2019b). Our study also observed that the diameters in both groups increased over time. Studies in Leghorn capons (Gesek et al. 2019b) showed similar results, but in Green-legged Partridges, the diameters of the 24-week-old and 28-week-old capons and cockerels were not increased, indicating that the growth of the fibres was arrested (Gesek et al. 2017).

Significantly larger giant fibres were observed in cockerels only at the 12th and 16th weeks. The diameters of the giant fibres increased throughout the experiment, and differences were found between both groups in all examined periods. Therefore, pectoral giant fibres are similar to other muscle fibres and continue to increase in diameter. Other studies including giant fibre diameters showed significantly larger giant fibres in Leghorn cockerels, similar to our study, at the 16th week (Gesek et al. 2019b). In Green-legged Partridges, cockerels also showed a higher diameter but in older birds at 24 and 28 weeks (Gesek et al. 2017). Smaller fibres of pectoral muscles and smaller giant fibres in capons together with a higher fat content influence meat quality through the tenderness and sensory qualities of the meat (Lin and Hsu 2002).

Rhode Island Red capons have shown a higher accumulation of fat in the pectoral and thigh muscles compared to cockerels and, in earlier periods, the diameters of the fibres in the pectoral muscle are smaller, which enhances meat quality. According to the accumulation and localization of fat in the skeletal muscles, 20 week, is best time to receive high-quality meat from capons.

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