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## THREE-POINT SCALE OF LIPID CONCENTRATION AND LOCALIZATION IN MUSCLE TISSUE OF BIRDS USING OIL RED **O STAINING\***

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#### Abstract

Research on skeletal muscles includes chemical, sensorial, histopathological, microbiological analvsis, and the influence of observed data on meat quality. The aim of this paper was to establish a point scale for analysing the fat concentration in breast and thigh muscles of birds during histological examination. The need for a point scale showing lipid localization arises during the experiment, including the castration of the bird. During necropsy, pectoral and thigh muscles were put into 30% saccharose solution with the addition of sodium azide. Then, frozen samples were cut into 8 µm sections and stained with Oil Red O (Bio-Optica, Milan, Italy) to detect lipids. Four main locations were evaluated: the area around vessels, perimysium between fascicles, endomysium between fascicles, and sarcoplasm of the fibres. Each location was separately evaluated for pectoral and thigh muscles. The percentage of tissue occupied by lipids in different locations was detected using Panoramic Viewer software (3DHISTECH, Budapest, Hungary). The results from the point scale analysis, similar in pectoral and thigh muscles, confirmed data from the chemical analysis. Significant differences were observed in all examined periods in chemical analysis (P<0.05) and were visible in the point scale with a higher number of birds with higher lipid concentration in all examined locations. Our scale analysis of lipid concentration, confirmed by chemical analysis, is an objective tool and can be used separately in muscle tissues in experiments where there is the need for lipid visualization. An established three-point scale can be a tool in poultry muscle tissue evaluation because not only accumulation but also lipid location is crucial in determining the usefulness of meat in culinary processing.

Key words: lipids, localization, concentration, Oil Red O, muscles

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Birds selected for fast growth receive high-energy and high-protein feed for the first day of life. An inadequate proportion of energy and protein in feed led to disturbances in lipid metabolism and caused the accumulation of adipose tissue in the abdomen, subcutaneous tissue, muscles, and visceral organs (Gesek et al., 2010, 2013). During the last days of fattening, fat accumulation is more visible, especially in the liver (Gesek et al., 2010, 2013). Accumulation of adipose tissue can be desirable for culinary reasons, and surgical methods, such as castration/caponization of male birds, can be performed. In other species, such as bulls (Bruns et al., 2004; Killinger et al., 2004), pigs (Bañón et al., 2003), sheep (Kemp et al., 1972), and goats (Johnson et al., 1995), castration enhanced sensorial aspects mainly due to increases in the level of intramuscular fat. Castration, in general, leads to androgen deficiency and changes in lipid metabolism. Caponization in birds can be accomplished chemically or surgically (Calik et al., 2015; Chen et al., 2010; Franco et al., 2016; Quaresma et al., 2017; Rahman et al., 2004; Sirri et al., 2009). Studies analysing the effects of caponization indicated that testes removal changes the lipoprotein profile and increases abdominal fat accumulation. Several papers have described how caponization changes the weight of abdominal fat as well as the triacylglycerol and total content of low and high-density lipoprotein (LDL and HDL) (Chen et al., 2005; Zawacka et al., 2017) and influences the chemical composition of tissues (total lipids, cholesterol, and ash) in both breast and thigh cuts (Sirri et al., 2009). Additionally, studies focused on the chemical composition of intramuscular fat in caponized medium-growing broiler line (Sinanoglou et al., 2011; Symeon et al., 2010) and intramuscular fat in layer line capons (Symeon et al., 2012). Native breeds such as Greenleg Partridge are also used for capon production, and the studies included morphological analysis as the weight of abdominal fat as well as the chemical analysis as the chemical composition of breast and thigh meat (Kwiecień et al., 2015).

All of these studies provide information on only the morphometric (weight) or chemical analysis (level) of fat content in a particular part of a tissue. However, there is no information about the specific localization of lipids in skeletal muscles, and there is no scale to analyse lipid concentration in those locations. Our research aims were to determine if an established three-point scale used in histological muscle samples is an objective tool and whether this is confirmed with another quantitative method, e.g., chemical analysis of muscular tissue.

Thus, the aim of this study was to establish a point scale showing lipid concentrations in pectoral and thigh muscles of birds using Oil Red O staining.

## Material and methods

The need of a point scale showing lipid localization arises during experiments performed on Leghorn cockerels (approval of the Local Ethics Committee in Olsztyn) and concerns castration of the birds at 8 weeks of age. Feed composition was the same as presented in our earlier studies (Gesek et al., 2017). In the 20th, 24th, and 28th week, 6 cockerels and 6 capons were slaughtered (at each age), and a necropsy

was performed (36 birds in total). During the necropsy, pectoral (one section 2 cm  $\times$  2 cm  $\times$  2 cm from right *pectoralis major* muscle) and thigh muscles (one section  $2 \text{ cm} \times 2 \text{ cm} \times 2 \text{ cm}$  from right *semimembranosus* muscle) were put into a 30% saccharose solution with the addition of sodium azide (+4°C). During next 4 days, muscle tissues were covered by cryostat embedding medium and frozen (-25°C), then samples were cut into 8 µm sections in cryostat microtome (-25°C). Directly before staining, slides with sections were unfrozen in the first step of Oil Red O staining protocol (Bio-Optica, Milan, Italy) to detect lipids. Each section was imaged using a Panoramic Scanner MIDI 3DHISTECH (3DHISTECH, Budapest, Hungary). Four main locations were evaluated: the area around vessels, perimysium between fascicles, endomysium between fascicles, and sarcoplasm of the fibres. Each location was separately evaluated for the pectoral and thigh muscles and area about 100-300 mm<sup>2</sup> in all examined muscles was evaluated. The images were prepared using Panoramic Viewer software (3DHISTECH, Budapest, Hungary). The percentage of tissue occupied by lipids in the different locations was detected using Panoramic Viewer software (3DHISTECH, Budapest, Hungary), which identifies positive areas (red lipids) based on an automatic colour separation. Evaluation was performed by two pathologists.

The proximate chemical composition of breast and thigh meat was determined. Breast and thigh muscles were chilled separately at 4°C, put through a laboratory mincer with a 2 mm mesh, and homogenized. Analytical samples of meat were collected to determine fat content by standard methods (AOAC, 2005).

#### Statistical analysis

Two-way ANOVA was performed (chemical composition; the effects of age and sex were analysed). The statistical analysis included the determination of characteristics of analysed traits and significant differences in mean values between age groups by Duncan's test. Computations were performed using STATISTICA 10.0 software (StatSoft Inc. 2011).

#### Results

The specific scheme/procedure, described below, was used in all examined tissues, and it was crucial to obtain objective data to compare with data from the chemical analysis. In the vessel area of pectoral muscles, individual vessels did not show lipid accumulation (Figure 1a). When more than 50% of the vessel area in the examined region of the pectoral muscle did not show lipids, the accumulation of lipids around the vessels was established as a 0 grade (Figure 1a). The low lipid content (+) around vessels was established when there were single lipid droplets, and sometimes merged adipose tissue was observed in more than 50% of the examined pectoral muscle (Figure 1b). The moderate lipid content (++) around vessels was established when merged adipose tissue was observed in more than 50% of the examined pectoral muscle (Figure 1c). High lipid content (+++) around vessels was established when a wide area of adipose tissue in more than 50% of the examined tissue was observed, as shown in Figure 1d.



Figure 1. Area around the vessels in a pectoral muscle. No lipid accumulation – 0 grade (Figure 1a); single, lipid droplets, sometimes merged adipose tissue – low lipid content (+) (Figure 1b); merged adipose tissue – moderate lipid content (++) (Figure 1c); wide area of adipose tissue – high lipid content (+++) (Figure 1d). Oil Red O staining



Figure 2. Area in the perimysium between fascicles in a pectoral muscle. No lipid accumulation (arrows) – 0 grade (Figure 2a); single, small and large lipid droplets around fascicles (arrows) – low lipid content (+) (Figure 2b); small and large lipid droplets and merged adipose tissue surrounds fascicles (arrows) – moderate lipid content (++) (Figure 2c). Oil Red O staining

When the perimysium between fascicles in pectoral muscles was examined, similar observations were made. When this location did not show lipid accumulation, as shown in Figure 2a, in more than 50% of identified pectoral muscle, a 0 grade was established. The low lipid content (+) in the perimysium between fascicles was established when single, small and large lipid droplets were observed near fascicles in more than 50% of the examined pectoral muscle (Figure 2b). The moderate lipid content (++) in the perimysium between fascicles was established when small and large lipid droplets as well as merged adipose tissue surrounded fascicles in more than 50% of the examined pectoral muscle (Figure 2c). High lipid content (+++) in the perimysium between fascicles can be observed, as a wide range of adipose tissue surrounding the fascicles, but our studies did not find that concentration.



Figure 3. Area in the endomysium between fibres in a pectoral muscle. No lipid accumulation – 0 grade (Figure 3a); single, small lipid droplets around fibres – low lipid content (+) (Figure 3b); small and medium lipid droplets and merged lipids around and between fibres – moderate lipid content (++) (Figure 3c); small, medium, and large lipid droplets and merged adipose tissue around fibres and between the fibres – high lipid content (+++) (Figure 3d). Oil Red O staining

In the endomysium between pectoral muscle fibres, a 0 grade was established when no lipids were observed in more than 50% of the identified tissue (Figure 3a). The low lipid content (+) in the endomysium between fibres was established when single, small lipid droplets around and between fibres were observed in more than 50% of the examined pectoral muscle (Figure 3b). The moderate lipid content (++) in the endomysium between fibres was established when small and medium lipid droplets and sometimes merged lipids around and between fibres were observed in more than 50% of the examined muscle (Figure 3c). High lipid content (+++) in the endomysium between fibres was established when small, medium, and large lipid

droplets as well as merged adipose tissue were observed around fibres and between the fibres in more than 50% of the examined tissue, as shown in Figure 3d.



Figure 4. Sarcoplasm of the muscle fibre in a pectoral muscle. No lipid droplets in the fibre sarcoplasm – 0 grade (Figure 4a); single, small lipid droplets – low lipid content (+). (Figure 4b); presence of small, parallel lipids – moderate lipid content (++) (Figure 4c); small, medium, and parallel lipid droplets – high lipid content (+++) (Figure 4d). Oil Red O staining

Lipids were also observed in the sarcoplasm of the fibres in pectoral muscles. Figure 4a shows a cross-section image of the pectoral fibres with no lipids in the cytoplasm/sarcoplasm. When more than 50% of the examined tissue showed a similar observation, a 0 grade in the muscle fibre sarcoplasm was established. The low lipid content (+) in the muscle fibre sarcoplasm was established when single, small lipids occurred randomly in more than 50% of the examined pectoral muscle (Figure 4b). The presence of small parallel lipids occurring in the muscle fibre sarcoplasm, as shown in Figure 4c, determined the moderate lipid content (++) (in more than 50% of the examined muscle). High lipid content (+++) in the muscle fibre sarcoplasm was established when small, medium and parallel lipid droplets occurred side by side between cytoplasmic myofilaments in more than 50% of the examined tissue, as shown in Figure 4d.

Table 1 shows results of the established three-point scale used in pectoral muscles in Leghorn capons and cockerels, which are partial results of studies concerning histology of the muscles (Gesek et al., 2019). At 20 weeks of age, only the vessel area showed lipid accumulation in cockerels and capons (with one exception, in capons, in the endomysium). In this period, in the vessel area, compared to capons where all birds revealed low lipid concentration, cockerels showed higher value of lipid concentration (one with moderate and five with low lipid concentration). At the same time, the chemical analysis, presented in Table 2, showed significant differences (P < 0.05) between sex groups with higher values in cockerels compared to capons (0.77% to 0.54%, respectively). The opposite finding was revealed at 24 weeks of age, where compared to six cockerels with low lipid content, higher concentration of lipids was noted in capons (two birds with moderate, and four with low lipid concentration). Similarly, in chemical analysis, compared to cockerels (0.50%) capon pectoral muscles showed significantly higher (P<0.05) lipid value (0.70%). Increased tendency was also noted in 28-week-old birds. In three-point-scale capons, the pectoral muscle showed higher lipid concentration around blood vessels, five capons showed moderate and one low concentration in the perimysium between fascicles and in the endomysium between fibres, three capons showed low lipid accumulation, and in the sarcoplasm of muscle fibres, one capon showed low concentration. Lower values were noted in cockerels using the three-point scale, where there were three birds with moderate and three with low concentration around the vessels, and only one cockerel with low concentration in the endomysium. Chemical analysis confirms these observations, and significantly higher lipid content (P<0.05) was observed in capons compared to cockerels (0.88% to 0.56%, respectively).

	20th week				24th week				28th week			
n=6	cocl	kerels	ca	pons	cocl	cerels	cap	oons	cock	erels	caj	oons
	Р	Th	Р	Th	Р	Th	Р	Th	Р	Th	Р	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		+		+		+		+				+
				+				+				++
				+								

Table 1. Accumulation of adipose tissue in the pectoral and thigh muscles of cockerels and capons after various times of fattening

Description: + low; ++ moderate; +++ high concentration of lipids; P – pectoral muscles, Th – thigh muscles (Gesek et al., 2019).

_	Age			Analysis of variance (significance)			
Item	(weeks)	Cockerels	Ccapons	Sex	Age	Sex × A	
Crude fat pectoral muscle	20	0.77*±0.01 a	0.54±0.01 a	0.000	0.000	0.000	
	24	0.50±0.01 b	0.70*±0.01 b				
	28	0.56±0.02 c	$0.88*{\pm}0.02$ c				
Crude fat thigh muscle	20	1.63±0.03 a	3.98*±0.03 a	0.000	0.000	0.064	
	24	3.65±0.01 b	5.07*±0.02 b				
	28	5.07±0.02 c	6.37*±0.03 c				

Table 2. Crude fat content (%) in chemical analysis (mean ± standard error)

a-c - mean values in columns with different letters differ significantly, P<0.05.

\*Means in rows differ significantly.

The same procedure to evaluate lipid concentration was used in thigh muscles. Microscopic analysis of thigh muscle samples was performed for the same locations. In the vessel area, a 0 grade was established when more than 50% of the identified vessel area in the examined part of the thigh muscle did not show lipids, but our experiments did not find that concentration. The low lipid content (+) around vessels was established when single small and large lipid droplets, and sometimes merged adipose tissue, were observed in more than 50% of the examined pectoral muscle (Figure 5a). The moderate lipid content (++) around the vessels was established when merged adipose tissue was observed in more than 50% of the examined tissue (Figure 5b). High lipid content (+++) around the vessels, as shown in Figure 5c, was set when a wide area of adipose tissue was visible in more than 50% of the examined tissue.

Figure 6a shows no lipids in the perimysium between fascicles in thigh muscles. When this location did not show lipids in more than 50% of diagnosed tissue, a 0 grade was established. The low lipid content (+) in the perimysium between fascicles was established when single, small and large droplets of lipid were observed focally around fascicles in more than 50% of the examined thigh muscle (Figure 6b). The moderate lipid content (++) in the perimysium between fascicles was established when small and large lipid droplets and merged adipose tissue surrounded fascicles in more than 50% of the examined muscle (Figure 6c). High lipid content (+++) in the perimysium between fascicles is observed when a wide range of merged adipose tissue surrounds the fascicles in more than 50% of examined muscle, as shown in Figure 6d.

In the endomysium between fibres of the thigh muscles, a 0 grade was established when no lipids were observed in more than 50% of the identified tissue (Figure 7a). The low lipid content (+) in the endomysium between fibres was established when single, small and medium lipid droplets were observed around and between fibres in more than 50% of the examined muscle (Figure 7b). The moderate lipid content (++) in the endomysium between single around and between fibres in more than 50% of the examined muscle (Figure 7b). The moderate lipid content (++) in the endomysium between fibres was established when small and medium lipid

droplets, and sometimes merged lipids, were observed around and between fibres in more than 50% of the examined muscle (Figure 7c). High lipid content (+++) in the endomysium between fibres was established when small, medium, and large lipid droplets as well as merged adipose tissue surrounded fibres in fascicles in more than 50% of the examined tissue, as shown in Figure 7d.



Figure 5. Area around the vessels in a thigh muscle. Single, small, large lipid droplets and merged adipose tissue – low lipid content (+) (Figure 5a); merged adipose tissue – moderate lipid content (++) (Figure 5b); wide area of adipose tissue – high lipid content (+++) (Figure 5c). Oil Red O staining



Figure 6. Area in the perimysium between fascicles in a thigh muscle. No lipid accumulation – 0 grade (Figure 6a); single, small and large droplets of lipid around fascicles – low lipid content (+) (Figure 6b); small and large lipid droplets and merged adipose tissue surrounds fascicles – moderate lipid content (++) (Figure 6c); wide range of merged adipose tissue surrounds the fascicles – high lipid content (+++) (Figure 6d). Oil Red O staining



Figure 7. Area in endomysium between fibres in a thigh muscle. No lipid content -0 grade (Figure 7a); single, small and medium lipid droplets around fibres - low lipid content (+) (Figure 7b); small, medium lipid droplets and merged lipids around fibres - moderate lipid content (++) (Figure 7c); small, medium, and large lipid droplets and merged adipose tissue surrounds fibres - high lipid content (+++) (Figure 7d). Oil Red O staining



Figure 8. Sarcoplasm of the muscle fibre in a thigh muscle. No lipid droplets in the fibre sarcoplasm – 0 grade (Figure 8a); single, small, and random droplets – low lipid content (+) (Figure 8b); presence of small, parallel lipids – moderate lipid content (++) (Figure 8c); small, medium, and parallel lipid droplets – high lipid content (+++) (Figure 8d). Oil Red O staining

Figure 8a shows a cross-sectional image of thigh muscle fibres with no lipids in the cytoplasm/sarcoplasm. When more than 50% of the examined tissue showed a similar observation, a 0 grade in muscle fibre sarcoplasm was established. The low lipid content (+) in muscle fibre sarcoplasm was established when single, small, and random lipids were observed in more than 50% of the examined fibres (Figure 8b). The presence of small, parallel lipids occurring in muscle fibre sarcoplasm, as shown in Figure 8c, determined the moderate lipid content (++) (in more than 50% of examined muscle). High lipid content (+++) in muscle fibre sarcoplasm was established when small, medium, and parallel lipid droplets, occurring side by side, were between cytoplasmic myofilaments in more than 50% of the examined tissue, as shown in Figure 8d.

The results of the three-point scale in thigh muscles are presented in Table 1 and are partial results of studies concerning histology of the muscles (Gesek et al., 2019). Compared to pectoral muscles, in general, thigh muscles showed more lipid content. At the 20th week of age, differences between cockerels and capons were noted. At that time, around vessels, cockerels showed high lipid content in two males and moderate content in four males, whereas in capons, higher values were noted for three capons with high, and three with moderate lipid content. Similar to other locations, in the perimysium between fascicles, there were two cases of moderate and two with low lipid content in cockerels and in capons, where one capon showed high lipid content, two moderate, and one low. In the endomysium, three capons showed moderate, with only one cockerel that showed moderate lipid content. Additionally, in fibre sarcoplasm, more capons (five birds) showed low lipid content than cockerels (three birds). The higher lipid content pointed out during histological examination was compared with chemical analysis, and at the same time (in 20-week-old birds), significant differences were noted (P<0.05), with higher values in capons compared to cockerels (3.98% to 1.63%, respectively). At 24 weeks, significantly higher fat content was also noted in capons (5.07% to 3.65%; P<0.05), and similar observation was made using the point scale. Although, in vessel area, lipid content was equal, in other locations, more capons showed lipid presence (in the perimysium and endomysium). Significantly higher differences were observed in capons compared to cockerels in 28-week-old birds in both chemical and point-scale analysis. In chemical analysis, capons showed 6.37% of fat in thigh muscles with 5.07% in cockerel thigh muscles (P < 0.05). When the point scale was used, higher lipid concentration was observed in capons around vessels and in the perimysium. Within the endomysium and fibre sarcoplasm, higher number of capons showed lipid presence and with higher concentration.

#### Discussion

The main idea of this study was to establish a three-point scale for lipid concentration in specific locations, in parts of muscular tissue stained with Oil Red O. Within the pectoral muscle, chemical analysis confirmed our assumption. The threepoint scale showing specific localization and concentration of the lipids showed the same differences in fat level as chemical analysis. Previous analysis of pectoral muscles showed various interesting data, with higher fat values in capons. Sirri et al. (2009) compared partial and complete caponization on chicken meat quality and found higher total lipid content in the breast muscle of capons compared with cocks. Capons, in Sinanoglou et al.'s (2011) study, had significantly higher content of total intramuscular fat in the Pectoralis major muscle compared with intact medium-growing broilers. Greenleg Partridge capons also displayed a tendency to have increased fat composition in pectoral muscles (Kwiecień et al., 2015). In the Castella Negra native Spanish chicken, Miguel et al. (2008) showed that compared with cocks, there is a higher fat content in capon pectoral muscle; consequently, the capon meat was more juicy and less fibrous. Symeon et al. (2012), in laying-type capons, found a similar tendency to the authors mentioned and with our data. In their studies, compared to cockerels, capon breast muscle showed significantly higher fat content. The previously mentioned studies only used a chemical analysis of lipid content. Thus, our three-point-scale analysis of lipid concentration, confirmed by chemical analysis, is an objective tool, which can be used to set the lipid level in tissue samples of pectoral muscle.

Similar to pectoral muscles, in thigh muscles, the point scale also confirmed data from chemical analysis. Significant differences observed in chemical analysis were visible in the point scale, as there were a higher number of birds with higher lipid concentration in all locations examined. Many previous studies concerning caponization showed higher lipid content of fat in thigh muscles of capons in chemical examination of tissue compared to intact birds. Sirri et al. (2009) examined partial and complete caponization in chickens and found that compared with cocks, lipid content was higher in the thigh muscle of capons. Similar observations were made by Symeon et al. (2010). Compared with intact cockerels in medium-growing broilers, the authors reported a higher fat percentage in thigh muscles. In Greenleg Partridge capons, the fat composition was three times higher in thigh muscles, similar to the cockerels (Kwiecień et al., 2015). In Lohmann Silver laying-type capons, Symeon et al. (2012) found fat content twice as high in thigh muscles. In our Leghorn capon, fat accumulation was higher compared to cockerels, but not twice as high. Our study finished at 28 weeks, and Symeon et al.'s (2012) study lasted until 34 weeks of age, and perhaps the longer fattening period increased fat content.

An established three-point scale can be used in the analysis of lipid concentration in capon production, in experiments, including chemical analysis of the fat content in part of the muscle tissue combined with chemical and sensorial analysis as well as in other research concerning a bird's muscles. For example, An et al. (2010) showed that the perimysium and endomysium muscle membranes can change meat/breast tenderness in broilers and White Leghorns. Similar observations by Liu et al. (1996) suggest that the total amount of collagen and structures of the perimysium are major factors involved in determining the toughness of chicken meat. Thus, analysis of lipid concentrations in this area, together with thickness of the perimysium and endomysium, can expand the understanding of meat quality, and in those cases, an established three-point scale of lipid concentration allows for the determination, of which lipid location enhances the culinary parameters of the meat.

Pectoral and thigh muscles in both cockerels and capons showed increased age tendency of fat accumulation. The results of fat accumulation obtained in our threepoint scale are equal to the chemical analysis of the muscular part of the tissue. Established three-point scale analysis of lipid concentration, confirmed by chemical analysis, is an objective tool and can be used separately in muscle tissues in experiments where there is the need for lipid visualization. Red Oil O staining is an easy, fast and reliable method, which can be used in small laboratories.

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#### References

- An J.Y., Zheng J.X., Li J.Y., Zeng D., Qu L.J., Xu G.Y., Yang N. (2010). Effect of myofiber characteristics and thickness of perimysium and endomysium on meat tenderness of chickens. Poultry Sci., 89: 1750–1754.
- AOAC (2005). Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists. Gaithersburg, Maryland, USA.
- Bañón S., Gil M.D., Garrido M.D. (2003). The effects of castration on the eating quality of drycured ham. Meat Sci., 65: 1031–1037.
- Bruns K.W., Pitchard R.H., Boggs D.L. (2004). The relationships among body composition and intramuscular fat content in steers. J. Anim. Sci., 82: 1315–1322.
- Calik J., Połtowicz K., Świątkiewicz S., Krawczyk J., Nowak J. (2015). Effect of caponization on meat quality of Greenleg Partridge cockerels. Ann. Anim. Sci., 15: 541–553.
- Chen K.L., Chi W.T., Chi o u P.W.S. (2005). Caponization and testosterone implantation effects on blood lipid and lipoprotein profile in male chickens. Poultry Sci., 84: 547–552.
- Chen T.T., Huang C.C., Lee T.Y., Lin K.J., Chang C.C., Chen K.L. (2010). Effect of caponization and exogenous androgen implantation on muscle characteristics of male chicken. Poultry Sci., 89: 558–563.
- Franco D., Pateiro M., Rois D., Vazquez J.A., Lorenzo J.M. (2016). Effects of caponization on growth performance, carcass and meat quality of Mos breed capons reared in free-range production system Ann. Anim. Sci., 16: 909–929.
- G e s e k M., S z a r e k J., S z w e d a M., B a b i ń s k a I. (2010). Comparative pathomorphological pattern of the liver in broiler chickens of two breeding lines. J. Comp. Pathol., 143: 343.
- Gesek M., Szarek J., Otrocka-Domagała I., Babińska I., Paździor K., Szweda M., Andrzejewska A., Szynaka B. (2013). Morphological pattern of the livers of different lines of broiler chickens during rearing. Vet. Med.-Czech, 58: 16–24.
- 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. Poultry Sci., 96: 1759–1766.
- Gesek M., Murawska D., Otrocka-Domagała I., Michalska K., Zawacka M. (2019). Effects of caponization and age on the histology, lipid localization, and fiber diameter in muscles from Leghorn cockerels. Poultry Sci., 98: 1354–1362.
- Johnson D.D., McGowan C.H., Nurse G., Anous M.R. (1995). Breed type and sex effects on carcass traits, composition and tenderness of young goats. Small Rumin. Res., 17: 57–63.
- K emp J.D., Shelley J.M., Ely D.G., Moody W.G. (1972). Effects of castration and slaughter weight on fatness, cooking losses and palatability of lamb. J. Anim. Sci., 34: 560–562.

- Killinger K.M., Calkins C.R., Umberger W.J., Feuz D.M., Eskridge K.M. (2004). Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level. J. Anim. Sci., 82: 3294–3301.
- Kwiecień M., Kasperek K., Grela E., Jeżewska-Witkowska G. (2015). Effect of caponization on the production performance, slaughter yield and fatty acid profile of muscles of Greenleg Partridge cocks. J. Food Sci. Technol., 52: 7227–7235.
- Liu A., Nishimura T., Takahashi K. (1996). Relationship between structural properties of intramuscular connective tissue and toughness of various chicken skeletal muscles. Meat Sci., 43: 43–49.
- Miguel J.A., Ciria J., Asenjo B., Calvo J.L. (2008). Effect of caponisation on growth and on carcass and meat characteristics in Castellana Negra native Spanish chickens. Animal, 2: 305–311.
- Quaresma M.A.G., Antunes I.C., Ribeiro M.F., Prazeres S., Bessa R.J.B., da Costa P.M. (2017). Immunocastration as an alternative to caponization: evaluation of its effect on body and bone development and on meat color and composition. Poultry Sci., 96: 3608–3615.
- Rahman M.M., Islam M.A., Ali M.Y., Khondaker M.E.A., Hossain M.M. (2004). Effect of caponization on body weight, hematological traits and blood cholesterol concentration of Nara chickens. Int. J. Poult. Sci., 3: 284–286.
- Sinanoglou V.J., Mantis F., Miniadis-Meimaroglou S., Symeon G.K., Bizelis I.A. (2011). Effects of caponisation on lipid and fatty acid composition of intramuscular and abdominal fat of medium-growth broilers. Brit. Poultry Sci., 52: 310–317.
- Sirri F., Bianchi M., Petracci M., Meluzzi A. (2009). Influence of partial and complete caponization on chicken meat quality. Poultry Sci., 88: 1466–1473.
- Symeon G.K., Mantis F., Bizelis I., Kominakis A., Rogdakis E. (2010). Effects of caponization on growth performance, carcass composition, and meat quality of medium growth broilers. Poultry Sci., 89: 1481–1489.
- Symeon G.K., Mantis F., Bizelis I., Kominakis A., Rogdakis E. (2012). Effects of caponization on growth performance, carcass composition and meat quality of males of a layer type. Animal, 6: 2023–2030.
- Z a w a c k a M., M u r a w s k a D., G e s e k M. (2017). The effect of age and castration on the growth rate, blood lipid profile, liver histology and feed conversion in Green-legged Partridge cockerels and capons. Animal, 11: 1017–1026.

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