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MICROSTRUCTURE OF THE MASSETER MUSCLE (*MUSCULUS MASSETER*) IN CATTLE

MIKROSTRUKTURA MIĘŚNIA ŻWACZA (*MUSCULUS MASSETER*) U BYDŁA

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Streszczenie. Celem pracy jest ocena mikrostruktury mięśnia żwacza (*musculus masseter*) bydła rzeźnego w zależności od płci i wieku. Materiał doświadczalny stanowiło 40 sztuk bydła (7 krów powyżej 5. roku życia, 12 jałówek i 21 buhajów w wieku do 24 miesięcy). Po uboju pobierano próbki mięśnia i zamrażano w ciekłym azocie. Zamrożone próbki ścinano w kriostacie na 10 µm skrawki. Następnie skrawki umieszczano na szkiełku podstawowym i poddawano barwieniu na aktywność NADH-TR w celu wyróżnienia włókien mięśniowych o metabolizmie oksydatywnym (βR) i glikolitycznym (αW). Stwierdzono istotny wpływ zarówno wieku, jak i płci bydła na średnicę włókien mięśniowych. Większe średnice obu typów włókien stwierdzono u zwierząt starszych. U samic, zarówno włókna βR, jak i αW charakteryzowały się większą średnicą niż u samców. W badanym mięśniu stwierdzono większy procentowy udział włókien oksydatywnych w stosunku do glikolitycznych. Nie obserwowano wpływu wieku badanych zwierząt na zawartość poszczególnych typów włókien mięśniowych, proporcje włókien u młodego i dorosłego bydła rzeźnego kształtowały się na podobnym poziomie. Wykazano natomiast wpływ płci zwierząt na udziały procentowe włókien βR i αW. Istotnie większy udział włókien oksydatywnych w mięśniu żwaczu stwierdzono u samców. Odwrotną relację stwierdzono w przypadku włókien glikolitycznych, których więcej obserwowano w mięśniu samic.

Key words: masseter muscle, muscle fibres, slaughter cattle.
Słowa kluczowe: bydło rzeźne, mięsień żwacz, włókna mięśniowe.

INTRODUCTION

Beef is one of the most nutritionally valuable types of meat. This is determined by the content of highly available essential amino acids, as well as low energy value and low content of fat, which is very important in view of current dietary trends. Beef is also a source of CLA, many B vitamins and minerals (mainly iron, zinc, copper and selenium) (Łaska and Stawska 2012, Zymon 2012).

Meat quality is largely influenced by the diet, growth potential, breed, sex, body weight and age of slaughtered cattle (Kłosowski et al. 1992, Młynek et al. 2006, Enderr 2008). They result not only from somatic maturity, which determines muscle size, but also from muscle

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microstructure and metabolism (Warren et al. 2008). The muscle structure, which develops during the animal's lifetime, is associated with meat tenderness, water holding capacity, colour and acidity, which are of essential importance for both consumers and processors (Wegner et al. 2000, Młynek et al. 2006).

The masseter muscle (*musculus masseter*) is a very strong muscle with a multipennate arrangement of fibres, which elevates and retracts the mandible. In herbivores, it is one of the masticatory muscles, which also allows lateral movements of the mandible (Akajewski 1997, Janowicz 1999, Krysiak et al. 2001). Together with other muscles it forms part of beef cheeks, which are considered a delicacy.

The aim of the study was to examine the microstructure of the masseter muscle in slaughter cattle by determining the percentage of oxidative and glycolytic fibres as well as their thickness, depending on sex and age.

MATERIAL AND METHODS

The experimental material used in this study consisted of 40 cattle: 12 heifers, 21 bulls and 7 cows. At slaughter, the estimated age of the animals was less than 24 months for heifers and bulls, and more than 5 years for cows.

Animals were slaughtered in a meat plant located in the Pomorskie province, Poland. Immediately after slaughter (within 45 minutes), samples of the masseter muscle were collected, chilled and transported to the laboratory of the Department of Animal Biochemistry and Biotechnology of the University of Technology and Life Sciences in Bydgoszcz. The collected muscle samples were frozen in liquid nitrogen (–196ºC) and stored until analysis. Frozen samples were cut into 10 µm sections on a cryostat (Thermo Scientific, Waltham, USA) at –25ºC. Next, the sections were mounted on a glass slide. Oxidative and glycolytic fibres were distinguished using staining according to Ziegan (1979). The microscopic images were saved on a computer disk using a Delta Optical Evolution 300 microscope equipped with a *ToupCam*^{TM} digital camera. Multiscan v. 18.03 image analysis software (Computer Scanning Systems II, Warsaw, Poland) was used to measure muscle fibre diameters and percentages of oxidative (βR) and glycolytic fibres (αW) in a 1.5 mm^2 area.

The results were subjected to two-way analysis of variance using STATISTICA AXAP v. 10.0 MR1. Arithmetic mean ($\bar{x}$) and standard deviation (SD) were calculated. Significant differences between the groups were determined with Tukey's HSD test for unequal numbers.

RESULTS AND DISCUSSION

As a result of histological analyses, the percentage of muscle fibres and their diameter in the masseter muscle were determined in cattle. Photographic documentation (Figs. 1, 2 and 3) is provided at the end of this article to show microscopic images of the muscle analysed in bulls, cows and heifers.

The diameters of oxidative (βR) and glycolytic fibres (αW) are given in Tables 1, 2 and 3 to show differences between the studied groups of cattle.

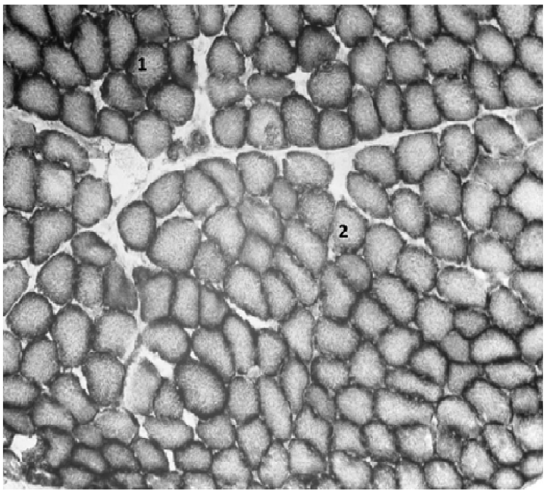

Fig. 1. The cross-section *musculus masseter* of heifer (1 – fiber βR, 2 – fiber αW). 100x
Rys. 1. Przekrój poprzeczny mięśnia *musculus masseter* jałówki (1 – włókno βR, 2 – włókno αW). Pow. 100x

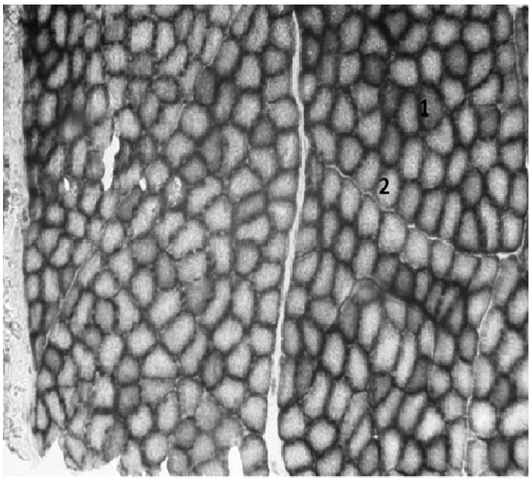

Fig. 2. The cross-section *musculus masseter* of bull (1 – fiber βR, 2 – fiber αW). 100x
Rys. 2. Przekrój poprzeczny mięśnia *musculus masseter* buhaja (1 – włókno βR, 2 – włókno αW). Pow. 100x

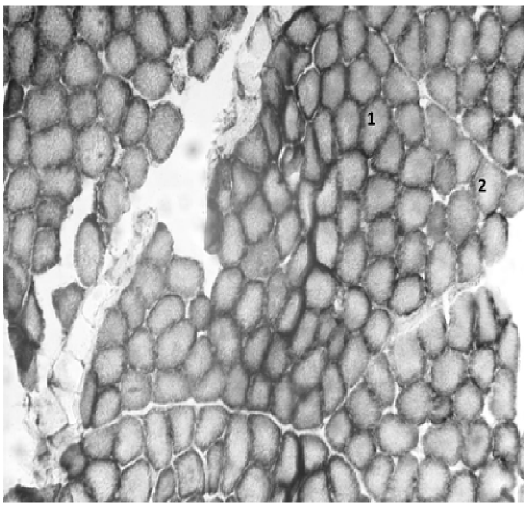

Fig. 3. The cross-section *musculus masseter* of cow (1 – fiber βR, 2 – fiber αW). 100x
Rys. 3. Przekrój poprzeczny mięśnia *musculus masseter* krowy (1 – włókno βR, 2 – włókno αW). Pow. 100x

Table 1. Diameter of muscle fibres (μm) in masseter muscle in the animal groups under study
Tabela 1. Średnica włókien mięśniowych (μm) w mięśniu żwaczu w badanych grupach zwierząt

Group Grupa		Diameter of fibres Średnica włókien	
		oxidative [βR] oksydatywne [βR]	glycolytic [αW] glikolityczne [αW]
Heifers – Jałówki [n = 12]	$\bar{x}$	44.11^{ABx}	40.58^{Ay}
	SD	2.95	2.80
Bulls – Buhajki [n = 21]	$\bar{x}$	41.63^{Bx}	38.41^{Ay}
	SD	3.45	3.50
Cows – Krowy [n = 7]	$\bar{x}$	50.52^{Ax}	48.86^{ABy}
	SD	7.34	8.37
On average Średnio	$\bar{x}$	43.93^{x}	40.89^{y}
	SD	5.25	5.83

A, B – statistically significant differences for p < 0.01 between groups of animals; x, y – statistically significant differences for p < 0.05 between the types of muscle fibers (βR and αW).
A, B – różnice statystycznie istotne dla p < 0,01 między badanymi grupami zwierząt; x, y – różnice statystycznie istotne dla p < 0,05 między typami włókien mięśniowych (βR i αW).

Table 2. Diameter of muscle fibres (μm) in masseter muscle depending on sex
Tabela 2. Średnica włókien mięśniowych (μm) w mięśniu żwaczu w zależności od płci

Group Grupa		Diameter of fibres Średnica włókien	
		oxidative [βR] oksydatywne [βR]	glycolytic [αW] glikolityczne [αW]
Females – Samice [n = 19]	$\bar{x}$	46.47^{Ax}	43.63^{Ay}
	SD	5.78	6.71
Males – Samce [n = 21]	$\bar{x}$	41.63^{Bx}	38.41^{By}
	SD	3.45	3.50
On average Średnio	$\bar{x}$	43.93^{x}	40.89^{y}
	SD	5.25	5.83

A, B – statistically significant differences for p < 0.01 between females and males; x, y – statistically significant differences for p < 0.05 between the types of muscle fibers (βR and αW).
A, B – różnice statystycznie istotne dla p < 0,01 między samicami i samcami; x, y – różnice statystycznie istotne dla p < 0,05 między typami włókien mięśniowych (βR i αW).

Table 3. Diameter of muscle fibres (μm) in masseter muscle depending on age
Tabela 3. Średnica włókien mięśniowych (μm) w mięśniu żwaczu w zależności od wieku

Group Grupa		Diameter of fibres Średnica włókien	
		oxidative [βR] oksydatywne [βR]	glycolytic [αW] glikolityczne [αW]
Young cattle for slaughter Młode bydło rzeźne [n = 33]	$\bar{x}$	42.53^{Ax}	38.20^{Ay}
	SD	3.45	3.39
Grown cattle slaughter Dorosłe bydło rzeźne [n = 7]	$\bar{x}$	$50{,}52^{Bx}$	48.86^{By}
	SD	7.34	8.37
On average Średnio	$\bar{x}$	43.93^{x}	40.89^{y}
	SD	5.25	5.83

A, B – statistically significant differences for p < 0.01 between young and grown cattle slaughter; x, y – statistically significant differences for p < 0.05 between the types of muscle fibers (βR and αW).
A, B – różnice statystycznie istotne dla p < 0,01 między młodym i dorosłym bydłem rzeźnym; x, y – różnice statystycznie istotne dla p < 0,05 między typami włókien mięśniowych (βR i αW).

As the data of Table 1 suggest, significantly greater diameters of both fibre types were found in cows (50.52 μm for βR and 48.86 μm for αW) than in heifers and bulls. Myofibre hypertrophy that occurs with age is consistent with the literature data (Kłosowska 1984, Wegner et al. 2000). The muscle fibre diameter in the masseter muscle averaged 43.93 μm for red fibres and 40.89 μm for white fibres; these values were slightly lower than those obtained for the same bovine muscle by Suzuki (1971). Based on the data presented in Table 1, it was also found that in each studied group, the oxidative fibres had a significantly higher diameter compared to glycolytic fibres ($p < 0.05$).

Table 2 shows muscle fibre diameters in the masseter muscle depending on sex of the studied animals. Both oxidative and glycolytic fibres have a greater diameter in females than in males ($p < 0.01$). The present study failed to confirm the results of Młynek (2009), who obtained slightly smaller muscle fibre sizes in heifers compared to bulls.

Table 3 presents the results concerning fibre thickness in the masseter muscle depending on the age of animals. The diameter of βR and αW fibres was highly significantly lower in young compared to adult slaughter cattle (42.53 vs 50.52 μm for βR, 38.20 μm vs 48.86 μm for αW fibres). The results obtained are consistent with the findings of Młynek et al. (2006), who performed histological analysis of LD muscle in crossbred bulls (Black-and-White × Limousin and Black-and-White × Charolaise) and showed the age of cattle to have a significant effect on muscle microstructure by increasing myocyte thickness.

Tables 4, 5 and 6 present the percentage of the two fibre types in the masseter of the animal groups studied. The masseter muscle contained a higher percentage of oxidative compared to glycolytic fibres. These results agree with the findings of Tuxen and Kirkeby (1990), who reported that the masseter of cows is composed mainly of type I fibres, although type II fibres can also be observed in some animals. The mainly oxidative metabolism of the masseter fibres was also reported by Picard et al. (1996). The proportion of fibres with oxidative metabolism in the masseter muscle ranged from 67.30% in heifers to 71.56% in bulls. No statistically significant differences were found in βR fibre percentage between the studied groups of cattle. Oxidative fibres were most abundant in the muscle of heifers (32.70%) and least abundant in the muscle of bulls (29.44%; $p < 0.05$). What is more, there were significantly more oxidative than glycolytic fibres in each group of slaughter cattle. In the study by Młynek (2009), in which three muscles (*longissimus lumborum, semimembranosus* and *biceps brachii*) from Black-and-White (BW) bulls and heifers and from BW crosses with other beef breeds were subjected to microscopic examination, it was found that all the three muscles had a higher proportion of glycolytic fibres. It can therefore be concluded that the masseter, which is a continuously working muscle that powers the jaw movements during mastication, should be characterized by a greater proportion of oxidative fibres, because these fibres are tough and resistant to fatigue.

A significantly higher proportion of oxidative fibres in the masseter muscle was found in males compared to females – 71.56 vs 68.19% (Table 5). An inverse relation occurred for white fibres, which were more abundant in the muscle of females ($p < 0.05$). The effect of sex of the slaughtered animals on microstructure and quality traits of meat is also stressed by Młynek (2009), although the available literature contains no information concerning the effect of sex of animals on the microstructure of the masseter muscle.

Table 4. Proportion of muscle fibres (%) in masseter muscle in the animal groups under study
Tabela 4. Udział włókien mięśniowych (%) w mięśniu żwaczu w badanych grupach zwierząt

Group Grupa		Proportion of fibres Udział włókien	
		oxidative [βR] oksydatywne [βR]	glycolytic [αW] glikolityczne [αW]
Heifers – Jałówki	$\bar{x}$	67.30^{x}	32.70^{ay}
[n = 12]	SD	3.29	3.50
Bulls – Buhajki	$\bar{x}$	71.56^{x}	29.44^{by}
[n = 21]	SD	3.58	3.34
Cows – Krowy	$\bar{x}$	69.71^{x}	30.29^{aby}
[n = 7]	SD	1.98	1.98
On average	$\bar{x}$	69.43^{x}	30.57^{y}
Średnio	SD	3.48	3.44

a, b – statistically significant differences for $p < 0.05$ between groups of animals; x, y – statistically significant differences for $p < 0.05$ between the types of muscle fibers (βR and αW).
a, b – różnice statystycznie istotne dla $p < 0{,}05$ między badanymi grupami zwierząt; x, y – różnice statystycznie istotne dla $p < 0{,}05$ między typami włókien mięśniowych (βR i αW).

Table 5. Proportion of muscle fibres (%) in masseter muscle depending on sex
Tabela 5. Udział włókien mięśniowych (%) w mięśniu żwaczu w zależności od płci

Group Grupa		Proportion of fibres Udział włókien	
		oxidative [βR] oksydatywne [βR]	glycolytic [αW] glikolityczne [αW]
Females – Samice	$\bar{x}$	68.19^{ax}	31.81^{ay}
[n = 19]	SD	3.01	3.20
Males – Samce	$\bar{x}$	71.56^{bx}	29.44^{by}
[n = 21]	SD	3.58	3.34
On average	$\bar{x}$	69.43^{x}	30.57^{y}
Średnio	SD	3.48	3.44

a, b – statistically significant differences for $p < 0.05$ between females and males; x, y – statistically significant differences for $p < 0.05$ between the types of muscle fibers (βR and αW).
a, b – różnice statystycznie istotne dla $p < 0{,}05$ między samicami i samcami; x, y – różnice statystycznie istotne dla $p < 0{,}05$ między typami włókien mięśniowych (βR i αW).

Table 6. Proportion of muscle fibres (%) in masseter muscle depending on age
Tabela 6. Udział włókien mięśniowych (%) w mięśniu żwaczu w zależności od wieku

Group Grupa		Proportion of fibres Udział włókien	
		oxidative [βR] oksydatywne [βR]	glycolytic [αW] glikolityczne [αW]
Young cattle for slaughter Młode bydło rzeźne	$\bar{x}$	69.37^{x}	30.63^{y}
[n = 33]	SD	3.75	3.70
Grown cattle slaughter Dorosłe bydło rzeźne	$\bar{x}$	69.71^{x}	30.29^{y}
[n = 7]	SD	1.98	1.98
On average	$\bar{x}$	69.43^{x}	30.57^{y}
Średnio	SD	3.48	3.44

x, y – statistically significant differences for $p < 0.05$ between the types of muscle fibers (βR and αW).
x, y – różnice statystycznie istotne dla $p < 0{,}05$ między typami włókien mięśniowych (βR i αW).

There were no differences between young and adult slaughter cattle in the percentages of different fibre types, which were at a similar level (Table 6). A significant effect of the age of slaughtered animals on microstructural characteristics of muscles has been reported by many authors (Wegner et al. 2000, Warren et al. 2008, Młynek et al. 2012). Młynek and Guliński (2007) observed that in older cattle muscle fibres have a greater proportion and show increased oxidative activity. A study by Kłosowski et al. (1992) showed that percentages of type I and IIB fibres increased, and those of type IIA fibres decreased with age in *longissimus dorsi* muscle of calves.

CONCLUSIONS

It is concluded that both the age and sex of cattle had a significant effect on muscle fibre diameter. Greater diameters of both fibre types (βR and αW) were observed in older animals. In females, both oxidative and glycolytic fibres had a greater diameter than in males. The masseter muscle had a greater percentage of oxidative compared to glycolytic fibres. The age of the studied animals had no effect on the content of different muscle fibre types and the proportions of fibres were at a similar level in young and adult slaughter cattle. The sex of cattle had an effect on percentages of βR and αW fibres. A significantly higher proportion of oxidative fibres in the masseter muscle was noted in males compared to females. An inverse relationship was found for white fibres, which were more abundant in the muscle of females.

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Abstract. The aim of the study was to examine the microstructure of the masseter muscle (*musculus masseter*) in slaughter cattle depending on sex and age. The experiment used 40 cattle (7 cows more than 5 years old, 12 heifers and 21 bulls less than 24 months old). Following slaughter, muscle samples were collected and frozen in liquid nitrogen. Frozen samples were to cut into 10 μm sections on a cryostat. Next, the sections were placed on a glass slide and stained for NADH-TR activity to identify oxidative (βR) and glycolytic (αW) muscle fibres. Age and sex of cattle had a significant effect on the diameter of muscle fibres. Older animals had a greater diameter of both fibre types. In females βR and αW fibres had a greater diameter than in males. The masseter muscle contained a greater percentage of oxidative compared to glycolytic fibres. The age of the studied animals had no effect on the content of different muscle fibre types and the fibre proportions in young and adult slaughter cattle were at a similar level. Sex of animals was found to have an effect on the percentage of βR and αW fibres. A significantly greater proportion of oxidative fibres in the masseter muscle was found in males. An inverse relation occurred for glycolytic fibres, which were more abundant in the muscles of females.