The effect of gender on the characteristics of muscle fibers in pork

Vliv pohlaví na charakteristiky svalových vláken ve vepřovém mase

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Abstract

The aim of this study was to evaluate the influence of gender on the proportional representation of different types of muscle fibres and their selected characteristics. The experiment was carried out with the use of 72 crossbred pigs of final TOPIGS hybrid combination with balanced gender ratio (24 boars/24 gilts/24 barrows) at an average live weight of 22.5 kg. The average slaughter weight of the animals was 108.7 kg. In order to determine the quantitative (number) and qualitative (area, diameter, length) characteristics of the muscle fibre, samples were obtained from the loin carcass part, specifically the muscle *musculus longissimus lumborum et thoracis (MLLT)*. These samples were used for the manufacture and evaluation of permanent histological slides. There were negative correlations found between the number of muscle fibres and their size. Gilts had the highest number of muscle fibres (208.55) per measured surface (1 mm^2) and simultaneously the smallest diameter (66.31 μm) of muscle fibres in comparison with boars and barrows. Statistically significant ($P<0.05$) differences in the area and diameter of muscle fibres type IIA were detected between gilts and barrows.

Keywords: gender, loin**,** muscles, pigs, type of muscle fibres

Abstrakt

Cílem práce bylo vyhodnotit vliv pohlaví na zastoupení jednotlivých typů svalových vláken a na jejich vybrané charakteristiky. Do pokusu bylo zařazeno 72 kusů jatečných prasat finální hybridní kombinace TOPIGS, vyrovnaného pohlaví (24 vepříků/24 prasniček/24 kanečků). Při naskladnění měla prasat průměrnou hmotnost 22,5 kg. Průměrná porážková hmotnost sledovaných zvířat byla 108,7 kg. Za účelem sledování kvantitativních (počet) a kvalitativních (plocha, obvod, tloušťka) charakteristik svalových vláken byly odebrány vzorky z jatečné partie pečeně ze svalu *musculus longissimus lumborum et thoracis*. Tyto vzorky byly použity ke zhotovení a vyhodnocení trvalých histologických preparátů. Mezi počtem svalových vláken a jejich velikostí se projevily negativní korelace. Prasničky měly nejvyšší počet

svalových vláken (208,55) na měřenou plochu (1 mm^2) a současně nejmenší průměr svalových vláken (66,31 μm) v porovnání s kanečky a vepříky. Mezi prasničkami a vepříky byly zjištěny statisticky významné rozdíly v ploše a průměru (diametru) svalových vláken typu IIA.

Klíčová slova: pečeně, pohlaví, prasata, svaly, typ svalových vláken

Detailní abstrakt

Vliv pohlaví nebo kastrace se projevuje zejména po dosažení pohlavní dospělosti – do živé hmotnosti prasat 50 – 70 kg je efekt samotný zanedbatelný. Rozdíly mezi kanci a prasničkami v počtu svalových vláken může být způsoben právě hormonální aktivitou. Cílem práce bylo zhodnotit vliv pohlaví na zastoupení jednotlivých svalových vláken a jejich charakteristiky u prasat. Pokus byl realizován se 72 kusy prasat finální kombinace TOPIGS (24 vepříků, 24 prasniček, 24 kanečků). Pokus byl ukončen při průměrné porážkové hmotnosti prasat 108,7 kg. Byly analyzovány vzorky z jatečné partie pečeně (*musculus longissimus lumborum et thoracis* - MLLT). Získané vzorky byly použity k tvorbě trvalých histologických preparátů. Vzorky byly zmrazeny pomocí tekutého dusíku, krájeny pomocí Leica microtome, obarveny, fixovány na sklíčka a zhotoveny obrázky histologických řezů pomocí optického mikroskopu s fotoaparátem. Získané hodnoty byly vyhodnoceny statistickým programem SAS. Kvantitativní (počet) a kvalitativní (plocha, obvod, tloušťka) ukazatele svalových vláken byly sledovány. Mezi počtem svalových vláken a jejich velikostí se projevily negativní korelace. Prasničky měly nejvyšší počet svalových vláken (208,55) na měřenou plochu (1 mm^2) a současně nejmenší průměr svalových vláken (66,31 μm) v porovnání s kanečky a vepříky. Mezi prasničkami a vepříky byly zjištěny statisticky významné rozdíly v ploše a průměru (diametru) svalových vláken typu IIA.

Introduction

The effect of gender on the differences in muscle fibre characteristics was evaluated in studies published by Klont et al. (1998), Ozawa et al. (2000), Wojtysiak et al. (2004) and Čítek et al. (2012). The effect of gender, or castration, applies especially after reaching sexual maturity – until the pigs reach at least 50 – 70 kg of life weight the effect itself is negligible (Hovorka, 1983). The differences in the number of muscle fibres between boars and gilts can be caused by hormonal activity (Rehfeldt et al., 2004). Wojtysiak et al. (2004) evaluated the effect of gender on the composition and size of muscle fibres in *m. longissimus thoracis* in pigs. The gender had no effect on the proportion of muscle fibre types, but it did influence their diameter. Boars showed a higher diameter of muscle fibre type IIA (fast-twitch, oxido-glycolytic) and IIB (fast-twitch, glycolytic) than gilts. Conversely, Larzul et al. (1997) found higher area of muscle fibre type IIB in gilts than boars. Also, Bee (2004) detected higher area of muscle fibre in *m. semitendinosus* in gilts. In addition, there were significant differences between gilts and barrows concerning the percentage of IIA and IIB fibres proportion in *m. rectus femoris*. There were no significant differences found between the values for *m. longissimus* and *m. semitendinosus*. Ryu et al. (2008) didn't find any statistically significant differences between muscle fibre characteristics in relation to gender. On the other hand, their study reported significant differences between the

muscle fibers proportion among different breeds.The objective of this study was to evaluate the muscle fibre characteristics in relation to gender.

Material and Methods

Animals

The experiment was carried out at the pig breeding test station at Ploskov near Lány. A total of 72 pigs of the TOPIGS hybrid combination with balanced gender ratio (24 barrows/ 24 gilts/ 24 boars) at an average age of 69 days and at an average live weight of 22.5 kg were included in the experiment. The placing and housing of the pigs was carried out in pairs, with both genders being separated.

Diet

The pigs were fed with a complete feed mixture (CFM) containing three main components (wheat, barley and soybean meal) and premix. The diet was mixed separately for each box. The animals were slaughtered at an average live weight of about 108.7 kg.

Sampling and freezing

The quantitative (number) and qualitative (area, diameter, length) characteristics of the muscle fibres were evaluated in the carcass part of loin (musculus longissimus lumborum et thoracis - MLLT). The size of muscle samples was 0.5 x 0.5 x 2 cm. The obtained samples were used in order to produce permanent histological slides. The slides were marked for subsequent identification and frozen with the use of liquid nitrogen. Subsequently the slides were stored in a deep freezer at a temperature of – 80 °C.

Cutting, dying and fixation of the histological slides

Thin histological slides (12 μm) were acquired using Leica microtome at a temperature of – 20°C. Following this, the samples were dyed in accordance with the method of Brooke and Keiser (1970) and fixated on slides with the use of the Pertex mounting medium.

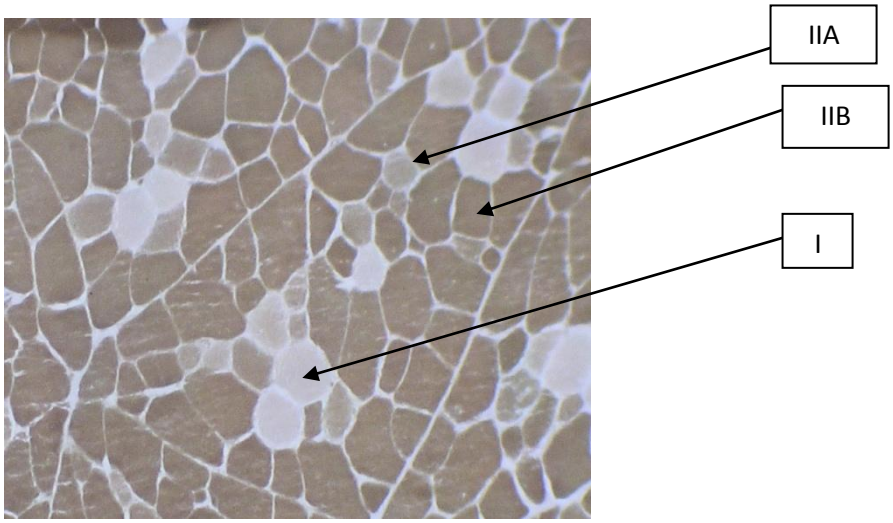

Fig. 1: Classification muscle fibers following preincubation at pH 10.7; type I muscle fibre (slow-twitch, oxidative); type IIA (fast-twitch, oxido-glycolytic); type IIB (fast-twitch, glycolytic).

Obr. 1: Klasifikace svalového vlákna po preinkubaci při pH 10,7, I typ svalového vlákna (pomalé, oxidativní), typ IIA (rychlé, oxido-glykotické), typ IIB (rychlé, glykotické).

Evaluation using a microscope

The images of histological sections were obtained using an optical microscope with camera (CAMEDIA-5060, OLYMPUS) and subsequently evaluated with the use of the image analysis program NIS – Elements 3.2.(2010; Nikon Instruments Europe B.V., Amstelveen, Netherland; Fig. 1).

The following parameters were obtained:

- the number of fibre types I, IIA and IIB per 1 mm^2 area,
- the proportion of fibres (%) of the type I, IIA and IIB per 1 mm^2 area,
- the average area of the cut of muscle fibre types I, IIA, IIB, and the sum (μm^2),
- the average diameter of the muscle fibre types I, IIA, IIB and total (mm),
- the length of the muscle fibre types I, IIA, IIB and total (mm),
- the circularity of muscle fibres (value 1 = ring, value 0 = line),
- the colour intensity of the muscle fibres.

Statistical analyses

The results of the experiment were evaluated with the use of the statistical program SAS ® Propriety Software Release 9.2 (2008) using analysis of variance (ANOVA). The differences between the individual traits were tested by the GLM procedure. The model included the fixed effect of gender (boars, gilts and barrows).

Results and discussion

Table 1 shows the representation of the different types of muscle fibres in relation to gender. The highest number of muscle fibres per measured area was observed in gilts (208.55). Statistically insignificant difference was recorded between boars (202.39) and barrows (202.78). Higher proportion of muscle fibres type IIB was found in gilts (84.99 %) when comparing with barrows (82.98 %) and boars (83.80%). Gilts showed a lower proportion of type I muscle fibres (11.70 %) and type IIA fibres (3.58 %) contrary to boars and barrows. According to the measured values gilts had a higher proportion of muscle fibres type IIB and lower proportion of muscle fibres type I and IIA as compared to boars and barrows. Contrary to the results of this study, Larzul et al. (2009) indicated higher proportion of muscle fibres type IIB (74.3 vs. 73.9 %) in barrows when compared to gilts. They also found a higher proportion of fibres type IIA in gilts when compared to barrows (6.6 vs. 6.2 %), however the differences were not statistically significant. Bee (2004) determined significant differences between gilts and barrows in the proportion of muscle fibres type IIA and type IIB in the muscle *m. rectus femoris*. In accordance with the results of this study, Bee determined higher proportion of fast glycolytic fibres (type IIB) in gilts when compared to boars (64.15 vs. 58.65 %, P = 0.01) and higher proportion of fast

oxidative-glycolytic fibres in barrows than gilts (34.03 vs. 29.32 %, P <0.01). He found no significant differences between the characteristics of the muscle fiber types (in the muscles *m. longissimus* and *m. semitendinosus)* in relation to gender. Similarly, Brocks et al. (1998) reported higher proportion of muscle fibres type IIB and lower proportion of muscle fibres type I in gilts when compared to boars. In comparison with the findings of this study, Brocks et al. found higher proportion of muscle fibres type IIA in gilts. According to Rehfeldt et al. (1999), concerning the number of muscle fibres in the muscle *m. longissimus* in pigs there are no differences between the genders.

Table 1: Representation of different types of muscle fibres in relation to gender

Tabulka 1: Zastoupení jednotlivých typů svalových vláken ve vztahu k pohlaví

Sex	Boar	Gilts	Barrows
	n = 24	n = 24	n = 24
	L.S.M. ±S.D.	L.S.M. ±S.D.	L.S.M. ±S.D.
Quantity of fibre type (1mm^2)			
type I	24.76 ±9.634	23.88 ±6.647	26.70 ±12.613
type IIA	7.57 ±5.837	9.16 ±6.954	7.79 ±7.772
type IIB	170.20 ±49.641	176.99 ±43.643	168.30 ±29.915
SUMA	202.39 ±54.683	208.55 ±51.991	202.78 ±34.517
Proportion of fibre type (%)			
type I	12.26 ±3.462	11.70 ±2.86	12.94 ±4.520
type IIA	4.04 ±3.302	3.58 ±2.942	4.09 ±4.511
type IIB	83.80 ±4.573	84.99 ±3.707	82.98 ±6.021

Table 2 shows the characteristics of the different types of muscle fibres in relation to gender. The largest average area of all types of muscle fibres was observed in boars (3885 μm^2) and the smallest area was recorded in gilts (3771 μm^2). Concerning the diameter of all types of muscle fibres, gilts showed the lowest values (66.31 μm) while the highest diameter was recorded in barrows (67.76 μm). Concerning the muscle fibres type I, barrows reached the highest values of both the area (3173 μm^2) and diameter (62.12 μm) of muscle fibres. Gilts on the other hand showed the lowest values (2994 μm^2 and 60.67 μm). The table also shows that the area and diameter of muscle fibres type IIA was significantly influenced by the effect of gender. The highest recorded value of area (2553 μm^2) and diameter (56.10 μm) of the muscle fibres was found in barrows, while the lowest values were found in gilts. The largest area of muscle fibres type IIB was observed in boars (4062 μm^2). The biggest diameter was recorded in barrows (69.16 μm). In accordance with these results (Tables 1 and 2), Larzul et al. (1997) also informed about the negative correlation between the number of different types of muscle fibres and their size. According to our measurements, the diameter of the muscle fibres increases in the following order: IIA, I and IIB. However Klont et al. (1998) concluded that the diameter values displayed by the muscle fibers type IIA puts them between the types I and IIB. They also stated that the muscle fibres type I and IIA contained more fat and myoglobine and were surrounded by denser capillary network than IIB fibres. This finding is confirmed by the works of Essen-Gustavsson et al. (1992). Conversely, Henckel et al. (1997) stated that the content of intramuscular fat was in positive correlation with the frequency of fibres type IIB.

Table 2: Characteristics of different types of muscle fibres in relation to gender

Tabulka 2: Ukazatelé jednotlivých typů svalových vláken ve vztahu k pohlaví

Sex	Boar	Gilts	Barrows	Significance
	n = 24	n = 24	n = 24	
	L.S.M. ± S.E.	L.S.M. ± S.E.	L.S.M. ± S.E.	
Muscle fibre type I				
Fibre area (μm^2)	3154 ± 93.5	2995 ±149.2	3173 ±95.2	ns
Fibre diameter (μm)	62.01 ±0.990	60.67 ±1.580	62.12 ±1.008	ns
Length (μm)	75.28 ±1.376	76.42 ±2.195	75.07 ±1.401	ns
Circularity	0.83^a ±0.006	0.78^b ±0.010	0.83^a ±0.006	*P*<0.05
Intensity	170.46^b ±1.162	183.46^a ±1.853	173.30^b ±1.183	*P*<0.05
Muscle fibre type IIA				
Fibre area (μm^2)	2229^{ab} ±130.2	2016^b ±212.5	2554^a ±142.1	*P*<0.05
Fibre diameter (μm)	51.98^{ab} ±1.482	49.57^b ±2.420	56.10^a ±1.618	*P*<0.05
Length (μm)	64.10 ±2.080	62.67 ±3.396	68.21 ±2.270	ns
Circularity	0.78 ±0.012	0.74 ±0.020	0.78 ±0.013	ns
Intensity	132.96 ±1.915	136.08 ±3.127	132.55 ±2.090	ns
Muscle fibre type IIB				
Fibre area (μm^2)	4063 ±62.2	3944 ±97.1	4017 ±66.6	ns
Fibre diameter (μm)	68.48 ±0.588	67.66 ±0.917	69.16 ±0.630	ns
Length (μm)	85.26 ±0.794	85.67 ±1.240	85.87 ±0.850	ns
Circularity	0.77^a ±0.003	0.76^b ±0.004	0.77^{ab} ±0.003	*P*<0.05
Intensity	107.91^b ±0.439	113.99^a ± 0.685	102.68^c ± 0.470	*P*<0.05
Muscle fibre type SUMA				
Fibre area (μm^2)	3885 ±54.8	3772 ±86.0	3852 ± 58.4	ns
Fibre diameter (μm)	67.11 ±0.518	66.31 ± 0.813	67.76 ± 0.552	ns
Length (μm)	83.29 ±0.702	83.92 ± 1.101	83.80 ± 0.748	ns
Circularity	0.78^a ±0.002	0.76^b ± 0.004	0.77^a ± 0.003	*P*<0.05
Intensity	116.33^b ±0.710	122.72^a ± 1.114	113.06^c ± 0.756	*P*<0.05

Rehfeldt et al. (2004) states, that animals with higher number of muscle fibres and medium size of these fibres produced overall more meat with better quality. Maltin et al. (1997) stated that the diameter of fibres type IIA significantly influenced the meat tenderness.

According to Miller et al. (1993) the diameter of muscle fibres is smaller if it is found in a castrated male. In uncastrated male pigs this diameter is larger. Solomon et al. (1990) indicated in their work that boars had a larger area of muscle fibres than the gilts and barrows. Barrows had the smallest area of fibres. Based on our results it is clear that gilts show a smaller area of all types of muscle fibres in comparison with boars and barrows. Similarly, Bee (2004) found smaller area in selected muscle samples in gilts when compared to barrows. Contrary to our results, Larzul et al. (1997) found a larger area especially in fibres of type IIB in gilts when compared to

barrows (3940 vs. 3666 μm^2, P <0.001). Bee (2004) also observed a larger area of fibres in gilts, but only in the muscle *m. semitendinosus*. In accordance with our findings, Brocks et al. (1998) observed a greater area of muscle fibres of type I and IIB in barrows than in gilts.

Conclusions

As it is evident from the results of our observation the gender of pigs influences the representation of different fibre types and their characteristics. Negative correlation was found between the number of muscle fibres and their size. The muscle fibres type IIB show the highest proportion in the muscle m. *longissimus lumborum et thoracis*. Higher proportion of muscle fibres type IIB was determined in gilts when compared to barrows and boars. Simultaneously, the largest area and diameter were also determined for this muscle fibre type. The gilts had the highest number of muscle fibres per 1 mm^2 and the smallest diameter of muscle fibres. Therefore it can be concluded that the meat produced by gilts will be more tender than meat obtained from the barrows and boars. There were statistically significant differences found in the area and diameter of muscle fibres type IIA between the gilts and barrows.

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References

Bee, G. (2004) Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. Journal of Animal Science. 82. 826-836.

Brocks, L., Hulsegge, B., Merkus, G. (1998) Histochemical charateristics in relation to meat quality properties in the *longissimus lumborum* of fast and lean growing lines of Large White pigs. Meat Science. 50 (4). 411-420.

Brooke, M. H., Kaiser, K. K. (1970) Three myosin adenosine triphosphatase system: the nature of their pH liability and sulphydryl dependence. J. Histochem. Cyotochem. 18. 670–672.

Čítek J., Stupka R., Šprysl M., Okrouhlá M., Brzobohatý L. (2012) The influence of slaughter weight and sex on the muscle fibers formation in pigs. Research in pig breeding. 6 (1).

Essén-Gustavsson, B., Karlstrom, K., Lundstrom, K. (1992) Muscle fibre characteristics and metabolic response at slaughter in pigs of different halothane genotypes and their relation to meat quality. Meat Science. 31 (1). 1–11.

Henckel, P., Oksbjerg, N., Erlandsen, E.,Barton-Gade, P., Bejerholm, C. (1997) Histo-and biochemical characteristics of the *Longissimus Dorsi* muscle in

pigs and their relationships to performance and meat quality. Meat Science. 47 (3-4). 311-321.

Hovorka, F. (1983) *Biologické aspekty užitkovosti prasat.* Vysoká škola zemědělská, Praha. 148 s.

Klont, R. E., Brocks, L., Eikelenboom, G. (1998) Muscle fibre type and meat quality. Meat Science. 49. 219-229.

Larzul, C., Lefaucher, L., Ecolan, P., Gogue, J., Talmant, A., Sellier, P., Le Roy, P., Monin, G. (1997) Phenotypic and genetic parameters for longissimus muscle fibre characteristics in relation to growth, carcass and meat quality traits in Large White pigs. Journal of Animal Science. 75 (12). 3126-3137.

Maltin, C.A., Warkup, C.C., Matthews, K.R., Grant, C.M., Porter A.D, Delday, M.I. (1997) Pig muscle fibre characteristics as a source of variation in eating quality. Meat Sci. 47-237.

Miller, A.E., MacDougall, J.D., Tarnopolsky, M.A, Sale, D.G. (1993) Gender differences in strength and muscle fiber characteristics. Eur. J. Appl. Physiol. 66, 254-262.

NIS Release 3.2. of the NIS-Elements AR® System for Microsoft® Windows®. Nikon Instruments Europe B.V., Amstelveen, Netherland . 2010.

Ozawa, S., Mitsuhashi, T., Mitsumoto, M., Matsumoto, S., Itoh, N., Itagaki, K., Kohno, Y., Dohgo, T. (2000) The characteristics of muscle fiber types of longissimus thoracis muscle and their influences on the quantity and quality of meat from Japanese Black steers. Meat Science. 54 (1). 65–70.

Rehfeldt, C., Stickland, N. C., Fiedler, I., Wegner, J. (1999) Environmental and genetic factors as sources of variation in skeletal muscle fibre number. Basic and Applied Myology. 9 (5). 235-253.

Rehfeldt, Ch., Fiedler, I., Stickland, N. C. (2004) Number and size of muscle fibres in relation to meat production. Muscle development of livestock animals. 2-38.

Ryu, Y. C., Choi, Y. M., Lee, S. H., Shin, H. G., Choe, J. H., Kim, J. M., Hong, K. C., Kim, B. C. (2008) Comparing the histochemical characteristics and meat quality trans of different pig breeds. Meat Science. 80. 363–369.

SAS® Propriety Software Release 9.2., of the SAS® system for Microsoft® Windows®. SAS Institute Inc., Cary, NC., 2008.

Solomon, M. B., Campbell, R. G., Steele, N. C. (1990) Effect of sex and exogenous porcine somatotropin on longissimus muscle fiber characteristics of growing pigs. Journal of Animal Science. 68 (4). 1176 - 1181.

Wojtysiak, D., Paściak, P., Migdał, W., Połtowicz, K. (2004) Histochemical profile of two parts of longissimus dorsi muscle in relation to sex of fatteners. Animal Science Papers and Reports. 22 (4). 587-593.