QUANTIFICATION OF DIFFERENT CONTENTS OF RAPESEED MEAL IN THE RATION ON MUSCLE FIBER CHARACTERISTICS IN PIGS

Brzobohatý L., Stupka R., Čítek J., Okrouhlá M., Šprysl M.

Czech University of Life Sciences Prague, Czech Republic

Abstract

The study deals with the influence of different extracted rapeseed meal (ERM) levels in the diet of pigs on the share of individual muscle fiber types and their characteristics in pork. The experiment included the standard test from 22 to115 kg live weight. The animals were divided into 3 study groups according to the amount of extracted rapeseed meal (EMR) in their diet. All the animals were fed *ad libitum* with the use of complete feeding mixtures (CFM) according to the nutrient standards. The feeding process consisted of 3 phases (22-35, 36-65, 66-115kg) with a continuous transition according to body weight. The first, second and third group of pigs were fed with respect to their body weight phase by CFM containing 3-5-8.1, 5-12-17 and 4-8-14% of ERM.

For monitoring quantitative (number) and qualitative (area, diameter) parameters of the muscle fibers the samples from the *musculus longissimus lumborum et thoracis* (MLLT) were taken. From the final obtained results it is obvious that the animals fed with feed mixture with the highest proportion of ERM had significantly the smallest area (2291.75 mm^2) of slow oxidative muscle fibre type I ($P \leq 0.01$). Furthermore, these animals, compared to others, had significantly ($P \leq 0.001$) a higher proportion of the muscle fibre types I (15.99%) as well as the frequency per 1 mm^2 (31.01pc). The results confirm that different content of the ERM in the pig diet can affect the characteristics of the muscle fibers.

Key Words: Pig, rapeseed, food technology, muscle fibres

Skeletal muscle tissue is very heterogeneous, consisting of a large number of different functional types of fibers (Choi, Kim, 2009). Skeletal muscle is the most important from a quantitative point of view. Skeletal muscle fibers are of tree types: red, white and intermediate (Reece *et al.*, 2009). White muscle fibers are formed by a process of differentiation of red muscle fibers postnatally. A high growth intensity selection results in a higher proportion of the white muscle fibers (Hampl, 2007). Morphological and biochemical characteristics of muscle fiber types represent the major factors affecting energetic metabolism in the skeletal muscles during the life span of the animal, as well as the muscle conversion to meat during the post mortem changes (Choi, Kim, 2009). Muscle fibers can be characterized according to their metabolism, colors, and contractility. According to the different sensitivity of the ATPase activity of myofibrils are after their previous exposure to either high or low pH, determined three different types of muscle fibers: I, IIA and IIB (Klont *et al.*, 1998). The muscle area is determined by the characteristics of muscle fibers, while muscle mass is determined by their number. The number of muscle fibers depends mainly on genetic and environmental factors which influence prenatal myogenesis. Postnatal skeletal muscle growth is realized by increasing the muscle fiber length as well as their circumference (Rehfeldt *et al.*, 2004). The histochemical description of muscle fibers is determined by genetic parameters as well as environmental conditions, such as nutrition, sex, age and physical activity (Bee, 2007; Hampl, 2007).

Various levels of nutrition influence different carcass composition. The main influence on final carcass composition in pigs is the content of nutrients in the ration. In addition to energy content, the level of ration performance depends on the content of crude protein which participates in the meat formation and composition (Stupka *et al.*, 2009). The level of nutrients in the ration can be influenced, for example, by adding rapeseed meal, which affects the quality and meat composition (Payne *et al.*, 1999; Tremona *et al.*, 1999). Different levels of nutrition can influence the quantitative and qualitative composition of meat and fat (Maltin *et al.*, 2003; Bee *et al.*, 2007). Other studies show that animals with a higher number of muscle fibers and the median sizes produce more meat with better quality (Rehfeldt *et al.*, 2004).

Materials and Methods

The test began with 72 Danbred genotype pigs of balanced sex ratio starting at an average live weight (ALW) of 22 kg and slaughtered at an average live weight of 115 kg. The animals were penned in pairs by sex and divided into 3 groups according to the amount of ERM content in the complete feeding mixture (CFM). Nutrition of the monitored animals was carried out according to established nutrient needs (Šimeček, et al. 2000) *ad libitum* in 3 phases with a continuous transition according to body weight (22-35, 36-65, 66-115kg) and according to the particular scheme and nutrient content (Table 1).

To monitor the quantitative (number) and qualitative (area average) parameters of muscle fibers, *p.m.* samples of muscle tissue from *m. longissimus lumborum et thoracis* (MLLT) at the level of last thoracic vertebra were collected. The 12 obtained muscle samples (size 20x5mm) were frozen with liquid nitrogen. Thin histological slices (7-8 μm thick) were acquired with the help of Leica microtome. To visualize the histological muscle fibers characteristics, ATPase coloring with pH 10.4. preincubation was used. The long-lived histological slides were digitalized with the use of Nikon Eclipse E 200 microscope. Subsequent image processing and measurements were carried out with the use of NIS - Elements AR 3.2 program. The evaluation of obtained data was performed via statistical SAS program (version 9.2), using the MEANS and GLM procedures.

Table 1. Characteristics of the complete feed mixtures (CFM) composition

Item	Group								
	1			2			3		
CFM	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
ALW (kg)	22-35	36-65	66-115	22-35	36-65	66-115	22-35	36-65	66-115
ERM (%)	3	5	8.1	4	8	14	5	12	17
Soybean meal (%)	19.5	12.6	2.5	18.9	10.9	0	18.4	8.8	0
Barley (%)	30	28	22	30	28	22	30	28	22
Wheat (%)	37.9	47.1	63.3	36.6	43.3	55.2	35.2	37.9	51.7
P1-Plus (%)	4	4	4	4	4	4	4	4	3.3
Fat (%)	5.6	3.3	0	6.5	5.8	4.8	7.4	9.3	6
MEp (MJ)	13			12,9			12.8		
LYZ (g)	12.01			10.6			8.6		

Results

Table 2 shows obtained data characterizing the muscle fibers. The smallest area of the muscle fibre type I (2291.75±101.693μm^2) was found in the group of pigs fed with the highest ERM share in the CFM. Concerning the muscle fibers type IIA a IIB belonging to the same group, the obtained values (2533.19±214.271 and 4090.74±99.12 μm^2, respectively) can be characterized as mean. The largest area of muscle fibers type I (3353.57 ± 144.510 mm^2) was found in the group fed with medium share of ERM in the CFM, while the remaining areas of the muscle fibre types show the lowest values.

In the pigs fed with the lowest ERM share in the CFM the area of type I fibers was medium-sized (2666,67±117,900 μm^2), however this group showed the largest areas of type IIA and IIB (2791.90±201.586 and 4350.22±96.511 μm^2, respectively) muscle fibers.

Another measured outcome was the diameter of muscle fibers, which for type I reached the range of 52.89±1.146 to 63.79±1.628 μm, for type IIA the range of 47.79±2.224 to 58.87±2.250 μm and for type IIB the range of 66.46±1.010 to 70.56±0.850 μm.

With regards to the percentage of individual muscle fibers types, the highest percentage of type I fibers (15.99±0.350%) was found in group 3. This group however showed the lowest percentage of type IIB fibers (81.80±0.326%). Group 2 was characterized by the lowest percentage of type I fibers (10.82±0.498%) and the highest percentage of type IIA fibers (7.03±0.493%). In group 1 there was the highest percentage of type IIB fibers (85.93±0.378%) and the lowest percentage of IIA fibers (2.48±0.402%).

Because the diameter of muscle fiber is a function of their frequency per 1mm^2, this indicator was also closely observed. The type I fibers reached the range of 21.01±0.972 to 31.01±0.684 pc, type IIA fibers the range of 4.04±0.830 to 13.99±1.018 pc and type IIB fibers the range of 142.32±2.823 to 162.84±3.460 pc. This finding reflects the fact that there is a negative correlation between the area size and the number of muscle fibers.

Discussion

Larzul et al. (1997) demonstrated that a group of pigs with a lower growth intensity had a greater muscle fibers area. They draw attention especially to the IIB muscle fibers where this phenomenon was particularly noticeable. The effect of growth rate on muscle fiber area is very questionable and many authors specializing in this field express different thoughts and conclusions. Ender (1995) did not observe any significant differences between the groups. The results of this study also did not confirm the above-mentioned phenomenon. The only observed trend was the change in muscle areas (especially in the type IIB muscle fibers). Cerasuolo et al. (2009) reported that there is a negative correlation between the total number of fibers and their average area.

Our findings concerning the single muscle fiber diameter are consistent with the work of Klont *et al.* (1998). They concluded that the smallest diameter is found in the type I muscle fibers and the largest diameter in the type IIB fibers. IIA fibers show a medium sized diameter. In addition to these results muscle fiber types I and IIA contain more lipids, myoglobin and more blood capillaries than the type IIB fibers.

Regarding the observed percentage of muscle fiber types, our results seem to be in agreement with the work of Bee et al. (2007). Further percentage characteristics of the muscle fibers are reported in the work of Choi and Kim (2009), who calculated the percentage of muscle fibers IIB in MLLT to be between 80-90% and the percentage of type I muscle fibers in MLLT to be between 5-15%.

Finding and validation of new methods for improving meat production quality is very interesting in all sectors of animal production. As shown Dunshea et al. 1993 and Uttar et al. 1993, the addition of rapeseed in the pigs ration bears a positive effect on their growth intensity.

In this context, Merkel (1989) found that the addition of rapeseed increased lean meat share in the carcass as well as growth intensity without showing any negative effects on the quality of porcine meat. The findings showing that the addition of rapeseed in the pigs ration led to a higher lean meat share was also demonstrated in the study of Sites et al. (1991). These authors also found that among other impacts rapeseed increases the yield of ham and neck. Concerning above mentioned conclusions these were also confirmed in the work of Crome et al. (1996), who state that addition of rapeseed into the nutrition has no negative effects on the growth or overall production performance of pigs.

Table 2. Characteristics of the muscle fibers

Parameter	1. group	2. group	3. group
	n = 12	n = 12	n = 12
	L.S.M. ± S.E.	L.S.M. ± S.E.	L.S.M. ± S.E.
Fibre area (μm^2)			
I	2666.67 ± 117.900^{Bc}	3353.57 ± 144.510^{AC}	2291.75 ± 101.693^{aB}
IIA	2791.90 ± 201.586^{B}	1958.31 ± 199.245^{A}	2533.19 ± 214.271
IIB	4350.22 ± 96.511^{B}	3814.83 ± 114.69^{A}	4090.74 ± 99.12
Fibre diameter (μm)			
I	56.17 ± 1.328^{b}	63.79 ± 1.628^{aC}	52.89 ± 1.146^{B}
IIA	58.87 ± 2.250^{B}	47.79 ± 2.224^{A}	54.71 ± 2.392
IIB	70.56 ± 0.850^{B}	66.46 ± 1.010^{A}	68.55 ± 0.873
Fibre type composition (%)			
I	13.53 ± 0.406^{BC}	10.82 ± 0.498^{AC}	15.99 ± 0.350^{AB}
IIA	2.48 ± 0.402^{B}	7.03 ± 0.493^{AC}	3.27 ± 0.347^{B}
IIB	85.93 ± 0.378^{BC}	83.93 ± 0.464^{AC}	81.80 ± 0.326^{BC}
Quantity of fibre type / 1 mm^2			
I	21.91 ± 0.793^{C}	21.01 ± 0.972^{C}	31.01 ± 0.684^{AB}
IIA	4.04 ± 0.830^{Bc}	13.99 ± 1.018^{AC}	6.78 ± 0.716^{aB}
IIB	142.32 ± 2.823^{BC}	162.84 ± 3.460^{A}	158.64 ± 2.435^{A}

Statistical significance: *a,b,c* - $P \leq 0{,}01$; *A,B,C* - $P \leq 0{,}001$

Conclusion

Based on here presented results it could be said that the addition of EMR into the ration shows no negative effects on the muscle fiber characteristics in pigs. Pigs fed with high doses of EMR also showed the highest number of slow oxidative muscle fiber type I per 1 mm^2. This finding confirms that the slow oxidative muscle fibers in this group had the smallest diameter and area. It is also clear that this subject requires further additional research.

References

BEE, G., CALDERINI, M., BIOLLEY, C. GUEX, G., HERZOG, W., LINDEMANN, M. D.: Changes in the histochemical properties and meat quality traits of porcine muscles during the growing-finishing period as affected by feed restriction, slaughter age, or slaughter weight. Journal of Animal Science, 85, 2007, 1030-1045.

CERISUELO, A., BAUCELLS, M. D., GASA, J., COMA, J., CARRIÓN, D., CHAPINAL, N., SALA, R.: Increased sow nutrition during midgestation affects muscle fiber development and meat quality, with no consequences on growth performance. Journal of Animal Science, 87, 2009, 729-739.

DUNSHEA, F. R., KING, R. G., CAMPBELL, R. G., SAINZ, R. D., KIM, Y. S.: Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. Journal of Animal Science, 71, 1993, 2919-2930.

ENDER, K.: Future demands on meat quality. Proceeding of the 2nd Dummerstorf Muscle Workshop: Muscle Growth and Meat Quality, Rostock, Germany, 1995, 58.

HAMPL, A.: Svalová soustava. In: MARVAN, F.: Morfologie hospodářských zvířat, Praha, 2007, 116-134.

CHOI, Y. M., KIM, B. C.: Muscle fiber characteristic, myofibrilar protein isoforms, and meat quality. Livestock Science, 122, 2009, 105-118.

CROME, P. K., MCKEITH, F. K., CARR, T. R., JONES, D. J., MOWREY, D. H., CANNON, J. E.: Effect of ractopamine on growth performance, carcass composition, and cutting yields of pigs slaughtered at 107 and 125 kilograms. Journal of Animal Science, 74, 1996, 709-716.

KLONT, R. E., BROCKS, L., EIKELENBOOM, G.: Muscle fibre type and meat quality. Meat Science, 49, 1, 1998, 219-229.

LARZUL, C., LEFAUCHER, L., ECOLAN, P., GOGUE, J., TALMAN, A., SELLIER, P., LE ROY, P., MONIN, G.: Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in Large White pigs. Journal of Animal Science, 75, 1997, 3126-3137.

MALTIN, C., BALCERZAC, D., TILLEY, R., DELBAY, M.: Determinants of meat quality tenderness. Proceedings of the Nutrition Society, 62, 2003, 337-347.

MERKEL, R. A.: Is meat quality affected by the use of repartitioning agents? Proceeding Recipient Meat Conference, 41, 1989, 101-112.

PAYNE, H. G., MULLAN, B. P., TREZONA, M., FREY, B.: Variation in pig production and performance. In: CRANWELL P. D.: Manipulating Pig Production VII., 13-26. Aust. Pig Scientist Association, Werribee, Victoria, Austria, 1999, 177 p.

REECE, W. O.: Fyziologie a funkční anatomie domácích zvířat. Grada 2009, 449 s.

REHFELDT, CH., FIEDLER, I., STICKLAND, N. C.: Number and size of muscle fibres in relation to meat production. In: PAS, M. F. W., EVERT, M. E., HAAGSMAN, H. P.: Muscle development of livestock animals. Physiology, genetics and meat quality. Wallington, UK, CABI Publishing 2004, 1-38.

SAS (2010): Release 9.2 of the SAS[â] System for Microsoft[â] Windows[â]. SAS Institute INC., Cary, NC.

STITES, C. R., MCKEITH, F. K., SINGH, S. D., BECHTEL, P. J., MOWREY, D. H., JONES, D. J.: The effect of ractopamine hydrochloride on the carcass cutting yields of finishing swine. Journal of Animal Science, 69, 1991, 3094-3101.

STUPKA, R., ŠPRYSL, M., ČÍTEK, J.: Základy chovu prasat. PowerPrint, Praha, 2009, 182.

ŠIMEČEK, K., ZEMAN, L., HEGER, J.: Potřeba živin a tabulky výživné hodnoty krmiv pro prasata. Česká akademie zemědělských věd, Brno, 2000, 124 s.

NIS (2010): Release 3.2 of the NIS-Elements AR[â] System for Microsoft[â] Windows[â]. Nikon Instruments Europe B.V., Amstelveen, Netherlands.

TREMONA, M., MULLAN, B. P., WILSON, R. H., WILLIAMS, I. H.: Seasonal variation in carcass quality of pigs: Does pattern of nutrition play a role? Recent Advances in Animal Nutrition, Australia, 1999, 12, 6A.

UTTARO, B. E., BALL, R. O., DICK, P., RAE, W., VESSIE, G., JEREMIAH, L. E.: Effect of ractopamine and sex on growth, carcass characteristics, processing yield, and meat quality characteristics of crossbred swine. Journal of Animal Science, 71, 1993, 2439-2949.

This study was supported by an S-grant of the Ministry of Education, Youth and Sports of the Czech Republic and project no. MSM 6046070901