THE EFFECTS OF DIFFERENT NUTRIENT COMPOSITION IN THE FEED ON THE MUSCLE FIBERS TYPOLOGY IN PIGS

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Abstract

The study deals with the effects of different levels of crude protein in the feed on the muscle fibers typology in pigs. The test included 72 hybrid animals (barrows and gilts) of the Dx(LW_DxL) genotype. The pigs were fattened ad-libitum in three feeding phases. All animals were divided into 3 groups according to the crude protein (CP) level intake. In order to monitor the quantitative (number) and qualitative (area, diameter) parameters of the muscle fibers, there were muscle samples taken from the *musculus longissimus lumborum et thoracis* (MLLT) muscle following the animals‘ slaughter. The study showed that pigs with the highest amount of rapeseed meal level in their diet showed the smallest ($P \leq 0.01$) slow oxidative muscle I type fibers area (2 292 μm^2). Furthermore, the highest ($P \leq 0.001$) percentage of the type I muscle fibers (16 %) and their number/1 mm^2 (31.0) were observed in the same group. The results show that different crude protein levels in the pig diet can affect their muscle fibers typology.

Key Words: Pig, nutrition, rapeseed diet, muscle fibers

Skeletal muscle is a heterogeneous tissue, consisting of a large number of different functional muscle fiber types. Functional fiber types are determined by their characteristics and composition. This affects the speed contractility and endurance. Morphological and biochemical muscle fiber type characteristics are the main factors that affect energy metabolism in skeletal muscle during the life of the animal as well as the muscle conversion to meat during the post-mortem changes (Choi, Kim, 2009).

Traditionally the term „meat“ generally refers to all the muscle originating from the animal. Muscles are divided into smooth, cardiac and the skeletal muscle. From the quantitative point of view, the skeletal muscle is the most important. Skeletal muscle consists of muscle fibers. Muscle fibers of this muscle tissue are of three types, namely red, white and transient. Red fibers, when compared to white, contain more mitochondria and myoglobin (Lefaucheur et al., 2011). White fibers are formed postnatally through the process of red fibers differentiation. Their high proportion in the muscle can serve as a domestication indicator as well as a selection indicator for high growth intensity and lean meat share in the modern pig genotypes (Hampl, 2007).

The smallest structural muscle unit is a myofibril. Several hundred thousand of these form a muscle fiber. Muscle fibers are grouped into muscle fascicles (Katsumata et al., 2008). Muscle area is then determined by the muscle fibers properties, while the muscle mass is based on the number of muscle fibers. The basic functional components of the muscle, such as fat cells, connective tissue, capillary network and nerve fibers have less effect on the muscle area. Number of muscle fibers is influenced primarily by genetic and environmental factors. These are likely to affect the prenatal myogenesis. Postnatal skeletal muscle tissue growth is realized through an increase in the muscle fibers length as well as their perimeter (Rehfeldt et al., 2004).

Muscle fibers can be characterized according to their metabolism, contractility and colour. With regards to the metabolism, there are various types of fibers, varying in different myofibrils sensitivity to an ATP-ase activity following previous exposure to high or low pH and subsequent colouring. Based on these criteria there are three main muscle fiber types: I, IIA and IIB (Klont et al., 1998).

Current research shows that animals with higher number of muscle fibers and higher mean perimeter produce more meat of better quality (Rehfeldt et al., 2004). The muscle fibers histochemistry is determined by genetics and also environmental conditions such as sex, age, nutrition and physical activity (Bee, 2007).

Through different levels of nutrition, and thus different nutrient contents in the diet, the carcass composition can be changed. The level of performance is defined as the energy content and the crude protein content in the diet, which is a function of meat formation and composition (Stupka et al., 2011). By adding extracted rapeseed meal to the regular diet, one can affect the quality and lean meat share in the main meat parts (Stites et al. 1991; Crome et al. 1996; Payne et al. 1999; Tremon et al., 1999).

Rehfeldt et al. (2012) found that low levels of protein in the diet lead to lower lean meat share and higher fat content in pigs. They also indicate, that low protein content in the feed leads to a lower skeletal muscle mass and a lower total number of muscle myofibrils. These authors also reported that different protein levels in the diet do not change each type of muscle fiber proportion, capillary density, creatine kinase activity, as well as the size and number of subcutaneous fat cells. Lafaucheur et al. (2011) reported, that effective nutrition in pigs leads to a greater muscle volume as a result of enlargement and a higher type II fibers proportion. Katsumata et al. (2008) concluded, that a reduced lysine intake increases oxidative muscle fibers proportion and thus the muscle oxidative capacity. Increased oxidative muscle fibers proportion leads, among other things, to drip loss reduction (Lafaucheur et al., 2011).

The differences in the body development, growth and carcass quality are a common problem and frequently represent hidden costs in the total economy of pig production (Payne et al., 1999; Tremon et al., 1999). Different levels of nutrition can therefore affect both the quantitative and qualitative meat and fat composition. In this context

Maltin et al. (2003) found an interaction between the growth rate of lean meat, its tenderness and nutrition intensity. Feeding regimes can affect meat quality characteristics, especially its water holding capacity as well as juiciness (Bee et al., 2007).

The aim of this work was to determine the effect of different nutrient composition in the feed and how does this affect the muscle fiber typology in pigs.

Material and Methods

The total number of 72 hybrid pigs of the Dx (LW_DxL) genotype and balanced sex (barrows, gilts) were included in the test. The test ran from 22 to 115 kg of live weight (LW). The fattening of pigs ran ad-libitum in three stages according to reached LW and the content of rapeseed meal (REM) in the complete feeding mixtures (CFM).

The first stage included the weight interval of 22-35,9 kg and the CFM contained 3-5-8,1% of REM, translating into 187.9-169.1-142.8 g of CP. The second stage included the weight interval of 36-65,9 kg and the CFM contained 4-8-14% of REM, meaning 187,9-170-146,9 g of CP. The third stage covered the weight interval of 66-115 kg, with the CFM containing 5-12-17% of REM, meaning 188,2-171,2-153,7 g of CP. The composition of feed mixtures, including their nutrient composition, is documented in Table 1.

Following the slaughter, 36 samples of muscle tissue were taken from the *Musculus longissimus lumborum et thoracis* (MLLT) in order to perform the monitoring of quantitative (number) and qualitative (area, diameter) parameters of the muscle fibers. Muscle samples of size 20x5x5 mm were frozen using liquid nitrogen and 2-methylbutane. The cutting of thin histological sections of the loin was carried out with the use of Leica CM 1850 microtome. The optimum section thickness was chosen to be 12 microns. In order to evaluate the histological characteristics of the muscle fibers, the ATPase coloring with preincubation at pH 10.4 (Brooke and Kaiser, 1970) was used. The resulting permanent slides were digitalized with the use of Nikon Eclipse E 200 microscope. Customized image processing and measurements were carried out using the NIS - Elements AR 3.2. The data evaluation was performed via statistical program SAS version 9.2, using the MEANS and GLM procedures.

Table 1. The CFM composition and nutrient contents with respect to the feeding phases in pigs

Component	1. group			2. group			3. group		
Phase									
CFM/LW(kg)	A1 22-35	A2 36-65	A3 66-115	A1 66-115	A2 36-65	A3 66-115	A1 22-35	A2 36-65	A3 66-115
REM (%)	3	5	8.1	4	8	14	5	12	17
Soybean (%)	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
Premix (%)	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
Analysed nutrient composition									
MEp (MJ)	13	12.9	12.8	13	12.9	12.8	13	12.9	12.8
CP (g)	187.9	169.13	142.82	187.94	170	146.93	188.23	171.18	153.692
Crude fiber (g)	42.66	41.57	39.45	43.23	43.65	43.82	44.02	46.43	46.73
LYZ (g)	12.01	10.6	8.6	12	10.61	8.7	12.01	10.64	8.61
MET (g)	3.3	3.1	2.84	3.32	3.17	2.99	3.34	3.27	3.07
MET+CYS (g)	6.64	6.26	5.78	6.68	6.4	6.08	6.72	6.58	6.31
THRE (g)	7.81	7.08	6.06	7.84	7.19	6.33	7.88	7.34	6.47
TRY (g)	2.3	2.01	1.63	2.28	2.04	1.67	2.28	2.01	1.76
Ca (g)	7.5	7.21	6.86	7.67	7.7	7.67	7.85	8.41	7.14
P (g)	6.17	6.14	6.12	6.22	6.31	6.42	6.29	6.56	6.3
Na (g)	1.9	1.91	1.93	1.9	1.9	1.87	1.9	1.9	1.6

Results and Discussion

Table 2 shows the calculated data characterizing the typology of muscle fibers. As it is evident from the table, the differentiation of individual muscle fiber types with regard to realized nutrition was significant ($P \leq 0.001$). The first group, fed with the lowest CP level, showed the lowest proportion of the muscle fiber type IIA (2.5 %) and the highest proportion of the type IIB fibers (85.9 %). The second group is characterized by the lowest proportion of type I fibers (10.8 %) and the highest proportion of type IIA fibers (7.0 %). The highest proportion of type I muscle fibers (16.0 %) was found in the third group, where the lowest proportion of type IIB fibers (81.8 %) was also demonstrated. The detected proportions of different muscle fibers types are in accordance with the study published by Bee *et al.* (2007). The work of Choi, Kim (2009) puts the muscle fibers percentage of type IIB and type I in the MLLT muscle to be 80-90 % and 5-15 %, respectively.

The lowest number of muscle fibers type IIA per 1 mm^2 (4.0) and IIB (142.3) was shown in group 1 (the group with the lowest level of crude protein and amino acids). The highest number of muscle fibers type IIA (14) and IIB per 1 mm^2 (162.8) was found in group 2. It can be stated, that the findings correspond to the relationship between the size area and the muscle fibers number. There is a negative correlation between these two values, as it was demonstrated by Cerisuelo *et al.*(2009). The muscle mass and muscle growth do not only depend on the number of muscle fibers, but also on the degree of the muscle fiber cells volume increase (Larzul et al. 1997; Rehfeldt et al., 2004, Ryu et al., 2008; Cerisuelo et al., 2009).

The first group also showed the largest average area of muscle fiber type IIA (2791.9 μm2) as well as type IIB (4350.2 μm2). The second group then reached the lowest average muscle fiber area of type IIA (1958.3 μm2) and IIB (3814.8 μm2). Animals in the third group had the lowest average fibers area of type I (2291.8 μm2).

Concerning the muscle fibers diameter, the obtained results are in accordance with Klont *et al.* (1998). These authors also found that the muscle fibers type I had the smallest diameter. The IIB muscle fibers had the largest diameter, while the type IIA fibers showed only average size. In addition to that, muscle fibers type I and IIA contain more lipids, myoglobin and more capillaries in the fiber when compared with the type IIB. Larzul et al. (1997) reported that pigs with a lower growth rate reach a larger muscle fibers area. In their work they especially point to the type IIB muscle fibers, where this phenomenon was most evident. The effect of growth intensity on the muscle fibers area is highly debatable and many authors express different views. For example, concerning the growth intensity and muscle area, the study published by Ender (1995) did not find any significant differences between the muscle fibers types.

The results of our study show that muscle fibers diameter corresponds to the average area. The first test group achieved the greatest muscle fiber type IIB diameter (70.6 mm). The largest muscle fiber type I diameter (63.8 mm) was achieved in pigs from the second group. The smallest muscle fiber type I diameter (52.9 mm) was found in the last test group.

Table 2. Muscle fiber characteristics with respect to different treatment

Parameter	1. group (n=12)	2. group (n=12)	3. group (n=12)
	L.S.M. ± S.E.		
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
Diameter (μm)			
I	56.17 ± 1.328^{Bc}	63.79 ± 1.628^{AC}	52.89 ± 1.146^{aB}
IIA	58.87 ± 2.250^{B}	47.79 ± 2.224^{Ac}	54.71 ± 2.392^{b}
IIB	70.56 ± 0.850^{B}	66.46 ± 1.010^{A}	68.55 ± 0.873
Muscle fibers proportion (%)			
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}
Number of fibers/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}

a, b, c = $P \leq 0{,}01$; A, B, C = $P \leq 0{,}001$

Conclusion

Based on the obtained results it could be said that higher CP level in the CFM (when supplied by REM) influenced the muscle fibers typology in pigs, namely the number of muscle fibers per 1 mm^2, muscle fibers proportion and average area of type I and IIB fibers. The higher CP content increased the number of type I muscle fibers per 1 mm^2, muscle fibers proportion of type I and decreased the IIB fibers proportion. In addition to that, a higher CP content decreased the diameters of muscle fibers type I.

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