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Muscle fibre composition and meat quality in pigs with different nutrition level

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Abstract. A total of 60 crossbred pigs were divided to 3 feeding groups - *ad libitum* (AL), restriction (R1) and strong restriction (R2) and the effect of feed restriction on muscle fibre characteristics of *musculus longissimus lumborum* (MLL) and meat quality traits were evaluated. Muscle fibres were stained and classified as fibre types I, IIA and IIB. For each muscle fibre type, the fibre density, fibre cross-section area (CSA) and fibre proportion were determined. Fibres IIB were divided to small- , medium- and large-sized fibres. From qualitative meat parameters, meat colour, tenderness, water-holding capacity, pH, and electrical conductivity were measured. AL group had significantly lower area percentage of IIB fibres and lower CSA of IIB fibres than restricted groups. R2 group had significantly higher content of large-sized IIB fibres and lower content of medium-sized IIB fibres than AL group. *Ad libitum* fed group had the highest back fat thickness and the lowest lean meat content and tended to have better meat quality traits compared to other groups. The results of this study showed that strong feed restriction had a negative effect on muscle fibre composition, especially amount of large-sized fibres IIB, which are associated with poor meat quality.

1. Introduction

Muscle fibres are the basic structural unit of skeletal muscle. They occupy more than 75 % of muscle volume and therefore, morphology of muscle fibre is a major determinant factor of muscle mass [1]. Muscle fibres are divided into individual types using different classification methods. One of the commonly used is method by Brooke and Kaiser [2], which allow to classify muscle fibres into three types (I, IIA and IIB) according their pH sensitivity of myosin adenosine triphosphatase (ATPase) activity. Individual fibre types differ in their metabolic, structural and contractile properties [3], and these varying characteristics are related to meat quality and carcass traits in various animal species [4, 5, 6]. For instance, high proportion and larger area of glycolytic type IIB fibres in muscle is associated with lighter meat and lower water-holding capacity in pigs [4, 7, 8, 9,10]. Higher proportion of muscle fibre type I is related to darker colour, better tenderness and higher content of intramuscular fat in cattle [11, 12, 13]. There are many factors affecting muscle fibre characteristics, like as breed, gender, age and others [14, 15, 16]. One of the extrinsic factors is a nutrition [14, 17].

The effect of feeding intensity on the proportion of different fibre types is controversial when comparing various muscles and/or species [18]. Feed restriction at an early stage does not change FTC in *longissimus* muscle, but does lead to a dramatic increase in the proportion of type I fibres in the red *rhomboideus* muscle and lower CSAF of all fibres [15]. Bee et al. [17] reported that the FTC in *longissimus* muscle is not changed due to feed restriction in post-weaning and growing-finishing pigs. Solomon et al. [19] observed that feed restriction increase the proportion of red fibres in *longissimus dorsi* muscle of pigs. Lefaucheur [20] reported that feed restriction between 7 and 100 kg of body weight did not change fibre type percentages in pig *longissimus* and *tibialiscranialis* muscles, but increased the CSAF [15].The aim of this study is to evaluate the effect of restriction feeding on muscle fibre composition in *longissimus lumborum* muscle and meat quality traits in pigs.

2. Material and Methods

2.1. Animals and diets

A total of 60 crossbred pigs (30 barrows and 30 gilts) of hybrid combination (Pietrain × Large WhiteSire) × (Landrace × Large WhiteDam) were used in this study realised in an experimental and testing station of pigs of the Czech University of Life Sciences Prague. The animals were housed at the age of 56 days, with an average body weight of 26.7 kg and were divided to 3 feeding groups. The first group was fed *ad libitum* (AL) and the second two groups (R1, 80% of *ad libitum*; R2, 70% of *ad libitum*) were restricted. Experimental group R1 was fed a maximum up to 3.01 kg complete feed mixture (CFM) per day (ME 38.5 MJ/day) in the final fattening phase. Group R2 was fed up to 2.81 kg CFM per day (ME 36.0 MJ/day). AL group received 3.44 kg CFM/day (ME 44.0 MJ/day) at this stage of fattening. Two experimental feed mixtures, for growing and finishing pigs with continuous transition depending on actual weight of pigs were used in this study. Pigs were slaughtered at a small commercial abattoir using electrical stunning. The average slaughter weight and age of the groups are presented in Table 1.

Table 1. Growth and slaughter parameters of pigs

Traits	Feeding groups		
	R1	R2	AL
Initial body weight (kg)	26.70 ± 3.81	27.27 ± 4.10	26.12 ± 3.02
Final live body weight (kg)	118.81^{a} ± 7.21	112.41^{b} ± 10.95	112.18^{b} ± 7.15
Final age (days)	155.33^{A} ± 2.91	155.65^{A} ± 2.98	135.42^{B} ± 2.63

Values in rows with different letters differ significantly at$P < 0.05$ (a,b)/ $P < 0.01$(A,B)

2.2. Histochemical analysis

Muscle samples for histochemical analysis were taken within 1 h after slaughter from the *musculus longissimus lumborum* (MLL). The samples were cut into 0.5 × 0.5 × 2.0 cm pieces, immediately frozen in isopentane cooled by liquid nitrogen and stored at -80°C until analysis. Transverse serial muscle sections of 12 µm were cut from the entire blocks in a cryostat Leica CM1850 (Leica Microsystems GmbH, Nussloch, Germany) at -20°C and mounted onto glass slides. The slides with sections were then incubated for the histochemical demonstration of myosin adenosine triphosphatase using the method of Brooke and Kaiser (1970). Stained muscle sections were obtained by an optical microscope with a camera Nikon Eclipse E200 (Nikon, Tokyo, Japan) and examined with an image analysis program (NIS - Elements AR

3.2., Nikon Instruments Europe B.V., Amsterdam, Netherlands). Approximately 300 fibres per sample were included to the analysis and fibres were classified into fibre types I, IIA and IIB. For each muscle fibre type, the fibre density, fibre cross-section area and fibre proportions were determined. For type IIB fibres, the proportion of small (diameter <46 µm), medium (diameter 46–86 µm) and large (diameter >86 µm) fibres were specified.

2.3. Carcass and meat quality traits

Lean meat percentage (evaluated by ZP method) and back fat thickness were measured 45 min post mortem with the electronic callipers. The value of pH (pH_{45}) and electrical conductivity (EC_{50}) were determined (45 and 50 min*p.m.*, respectively) into carcasses.

Muscle samples for evaluation of meat quality traits were taken 24 h post mortem. Before sampling, the photos of transverse cut of loins were obtained to evaluation of loin area. Meat color (CIE L*, a* and b*) was measured 24 h p.m. on the muscle surface using a colorimeter Minolta CM-700d (Konica Minolta, Osaka, Japan). Values of Warner-Bratzler shear force (WBSF; N) were determined by Instron Universal Texture Analyzer 3342 (Instron Corp., Norwood, USA). Muscle samples of raw and cooked meat (6 × 1 × 1 cm) were cut across muscle fibres and the resulting value was calculated as an average value of at least 6 measurements. Drip loss was evaluated using a bag method (24 h at 4°C). Intramuscular fat content was determined by petroleum ether extraction.

2.4. Statistical analysis

The experimental data were analysed by a one-way analysis of variance (ANOVA) using statistical software SAS 9.4 (SAS Inst. Inc., Cary, NC). The results are presented as means ± standard error of the mean (SEM). Differences between LS means were determined by Tukey's test.

3. Results and Discussion

The values obtained for muscle fibre parameters in relation to feed restriction are shown in table 2. Table 3 shows the effect of feed restriction on carcass and meat quality traits. To assess the effect of feed restriction on muscle fibre parameters, we compared groups R2 with AL and groups R1 with R2. Within the monitored groups, there were significant differences in mean CSA and CSA of fibres IIB. All feeding groups had the largest area of type IIB fibres. Highest values were in the group R1 (3642.7 µm2), then in R2 (3481.9 µm2) and AL (3177.3 µm2). The smallest CSA had fibres IIA in all groups. The largest area in the restricted R1 group could be expected due to the higher slaughter weight of this group. In general, postnatal growth of skeletal muscle mass is mainly due to muscle fibre hypertrophy than fibre hyperplasia [16]. However, larger muscle fibres were also observed in group R2 compared to AL, which had the same slaughter weight. This is probably related to the different lean meat content of these groups. The highest lean meat content was in R2 group, on the contrary, the lowest content was in AL group. It corresponded to results found for backfat thickness, which was significantly highest in AL group. The same trend was observed in intramuscular fat content. AL group had the highest content of IMF, but that was without significant differences among the groups.

Table 2. The effect of feed restriction on muscle fibre characteristics of the *longissimus* muscle of pigs

Traits	Feeding groups		
	R1	R2	AL
Fibre density (number/mm^2)			
Type I	28.93^b ± 2.71	32.21^{ab} ± 2.57	38.52^a ± 2.64
Type IIA	28.73 ± 3.16	29.48 ± 3.00	32.37 ± 3.08
Type IIB	175.39 ± 10.29	183.72 ± 9.76	192.48 ± 10.01
Cross-sectional area (μm^2)			
Mean	3222.4^A ± 163.7	3071.3^A ± 128.5	2819.4^B ± 121.3
Type I	2466.2^A ± 133.6	2347.1^{AB} ± 105.1	2221.7^B ± 96.6
Type IIA	1718.6 ± 199.5	1629.4 ± 207.8	1670.5 ± 180.4
Type IIB	3642.7^A ± 89.8	3481.9^B ± 68.3	3177.3^C ± 131.2
Fibre area composition (%)			
Type I	9.16^b ± 0.68	9.43^{ab} ± 0.64	11.19^a ± 0.66
Type IIA	6.81 ± 0.56	6.39 ± 0.53	7.43 ± 0.55
Type IIB	84.03^a ± 0.95	84.18^a ± 0.90	81.38^b ± 0.92
Proportion of fibre size IIB (%)			
Small	13.75 ± 2.10	18.02 ± 2.00	16.86 ± 2.04
Medium	67.89^{ab} ± 3.40	60.65^b ± 3.23	72.39^a ± 3.31
Large	18.36^{ab} ± 3.83	21.34^a ± 3.64	10.74^b ± 3.73

Values in rows with different letters differ significantly at $P < 0.05$ (a, b)/ $P < 0.01$ (A, B)

The group fed *ad libitum* had a significantly lower percentage of lean meat and a higher backfat thickness, which is reflected in the loin area and thus in the cross-section area of muscle fibres. Bee et al. [17] also monitored the effect of feed restriction on the characteristics of muscle fibres, but they did not observe any differences in CSA of fibres between the groups at the same body weight.

Both restricted groups, which were significantly leaner, also had a higher area percentage of type IIB fibres than the AL group, which was almost three weeks younger. The age of animals has a significant effect on the composition of muscle fibres. In general, postnatal transitions of fibres proceed from the oxidative to the glycolytic type of fibres [4, 15], and diameter of type II fibres increase faster than diameter of type I [21]. The positive relationship between high muscularity and proportional representation of type IIB fibres is reported in many studies [22, 4, 15,9]. Groups R2 and AL differed not only in composition of fibre IIB but also in proportion of fibre size of IIB. The AL group had significantly less represented fibres IIB with a large area (> 86 μm), in favour of medium-sized fibres IIB (46-86 μm). Higher proportions of large IIB fibres in restricted groups could be associated with higher lean meat content of these groups again. Kim, Jeong et al. [5] in their study also observed higher muscularity in groups with higher content of large-sized fibres IIB. Similarly Ryu et al. [7] reported that pigs with higher muscle mass had a higher CSA and lower fibre density compared to pigs with lower muscle mass.

Table 3. The effect of feed restriction on carcass and meat quality traits of pigs

Traits	Feeding groups		
	R1	R2	AL
Lean meat (%)	$59.71^{A} \pm 0.42$	$60.58^{A} \pm 0.39$	$57.28^{B} \pm 0.40$
Backfat thickness (mm)	$20.37^{B} \pm 0.82$	$17.71^{C} \pm 0.79$	$23.66^{A} \pm 0.80$
Loin area (mm^2)	$5253^{A} \pm 126.4$	$5000^{AB} \pm 119.6$	$4739^{B} \pm 119.6$
Intramuscular fat content (%)	2.17 ± 0.17	2.16 ± 0.16	2.34 ± 0.17
$pH_{45\ min}$	6.29 ± 0.06	6.29 ± 0.06	6.34 ± 0.06
$EC_{50\ min}$	3.58 ± 0.08	3.61 ± 0.08	3.42 ± 0.08
Lightness (L*)	53.40 ± 0.76	52.92 ± 0.72	51.48 ± 0.74
Redness (a*)	$0.72^{A} \pm 0.24$	$0.94^{A} \pm 0.23$	$0.10^{B} \pm 0.24$
Yellowness (b*)	$10.73^{A} \pm 0.30$	$10.68^{A} \pm 0.28$	$9.15^{B} \pm 0.29$
WBSF of raw meat (N)	$43.45^{B} \pm 1.95$	$49.26^{A} \pm 2.05$	$38.78^{B} \pm 1.86$
WBSF of cooked meat (N)	34.78 ± 1.72	38.89 ± 1.72	35.98 ± 1.28
Drip loss (%)	4.92 ± 0.44	4.69 ± 0.42	4.27 ± 0.43

Values in rows with different letters differ significantly at $P < 0.01$ (A, B)
WBSF – Warner-Bratzler shear force (N); EC - electrical conductivity

Electrical conductivity, pH, colour and water-holding capacity are parameters that can be used to classify abnormalities of meat quality. In these parameters, there were no differences between groups, and we did not observed any meat abnormalities in pigs used in our study. Significant differences were observed in redness (a*) and yellowness (b*), where the values were lower in AL group. Variations in meat colour are related to a different myoglobin content. Red muscle fibres (I and IIA) have a higher myoglobin content and therefore darker colour [23]. However, during meat storage and blooming, myoglobin is oxidized into metmyoglobin, which could result in a decrease in redness and increase in yellowness of meat. Faster colour change during storage is in the muscles with the predominance of red oxidative fibres due to faster oxygen consumption [15].

We also observed differences in WBSF of raw meat. The R2 group had a significantly higher shear force compared to R1 and AL groups. The AL group had a significantly smaller CSA of fibre and the lower content of IIB fibres. This corresponds to the claim that the higher fibre CSA, especially IIB fibres, increases instrumental toughness of meat [24,15, 5]. The R1 group had larger muscle fibres compared to R2, what was related to the slaughter weight of these animals, but this did not reflect the composition of large-sized IIB fibres, which could cause higher meat toughness. There were no significant differences in WBSF of cooked meat.

4. Conclusion

Feed restriction has a significant effect on lean meat content, which is accompanied by a different muscle fibre size. It can be observed in animals slaughtered at the same weight or the same final age as well. Different age at the same slaughter weight is reflected in the amount of fast glycolytic fibres (IIB) in muscle and their proportional composition. These results show that strong feed restriction negatively affects the composition of muscle fibres in *longissimus* muscle of pigs. Meat quality traits tend to be better in *ad libitum* fed group, however, the lean meat content is lower in this group.

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