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Effect of feed restriction on muscle fibre characteristics and meat quality traits in pigs

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Abstract. The aim of this study was to evaluate the effect of feed restriction on muscle fibre composition and meat quality traits in pigs. Forty crossbred pigs (Pietrain × Large $\text{White}_{\text{Sire}}$) × (Landrace × Large $\text{White}_{\text{Dam}}$) were divided into two feeding groups: *ad libitum* (AL) and restricted (R1). The effects of feed restriction on muscle fibre characteristics of the *musculus longissimus lumborum et thoracis* (*MLLT*) 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 into small- (diameter < 46 µm), medium- (diameter 46–86 µm) and large-sized (diameter > 86 µm) fibres. The AL group had significantly lower ($P < 0.05$) percentage area of IIB fibres and lower ($P < 0.01$) CSA of IIB fibres than did the R1 group. The R1 group had significantly greater content of large-sized IIB fibres and smaller content of medium-sized IIB fibres than did the AL group ($P < 0.05$). The group fed *ad libitum* had greater backfat thickness and smaller lean meat content and tended to have better meat quality traits compared to the restricted group. The results of this study show that strong feed restriction had a negative effect on muscle fibre composition, especially on the amount of large-sized fibres IIB, which are associated with poor meat quality.

Key words: fibre types, meat quality, nutrition, pig.

INTRODUCTION

Muscle fibres are the basic structural unit of skeletal muscle. They make up more than 75% of muscle volume and, therefore, morphology of muscle fibre is a major determining factor of muscle mass (Lee et al., 2010). Muscle fibres are divided into individual types using various classification methods. One commonly used method is that of Brooke & Kaiser (1970), which classifies muscle fibres into three types (I, IIA, and IIB) according to their pH sensitivity in relation to myosin adenosine triphosphatase (ATPase) activity. Individual fibre types differ in their metabolic, structural, and contractile properties (Choi & Kim, 2009), and these varying characteristics are related to meat quality and carcass traits in various animal species (Lefaucheur, 2010; Kim et al., 2013a; Bogucka & Kapelanski, 2016). Qualitative and quantitative parameters of carcass traits are usually associated with muscle fibre properties, such as total number of fibres, fibre density, cross-section area of fibres (CSA), and fibre type composition in muscle (Joo et al., 2013; Kim et al., 2014). For instance, high proportion and larger area of glycolytic type IIB fibres in muscle are associated with brighter meat and lower water-holding capacity in pigs (Ryu et al., 2006; Lefaucheur, 2010; Kim et al., 2013b). Hypertrophy of fast-twitch oxido-glycolytic fibres is detrimental to meat tenderness, as well (Lefaucheur, 2010). Colour acceptability of fresh meat is positively correlated with CSA and the area and number percentages of type I muscle fibre in study of Nam et al. (2009). Number and area percentage of type I fibres positive correlate with taste of cooked pork at the same study. Kim et al. (2013a) reported that fibre size distribution of type IIB fibres also has an impact on pork meat quality. Pigs with higher percentage of large-sized IIB fibres exhibit tougher, lighter, and more exudative meat than do pigs with higher proportion of small- or normal-sized fibres.

There are many factors affecting muscle fibre characteristics, including breed, gender, age, and others (Rehfeld et al., 2004; Jeong et al., 2012; Joo et al., 2013). One of the extrinsic factors is nutrition (Bee et al., 2007; Jeong et al., 2012). The effect of feeding intensity on the proportion of different fibre types is controversial when comparing various muscles and/or species (Candek-Potokar et al., 1999). According to Harrison et al. (1996), feed restriction at an early stage (7 weeks) does not change fibre type composition in *longissimus* muscle of Large White pigs, but it does lead to a dramatic increase in the proportion of type I fibres in the red *rhomboideus* muscle and lower CSA of all fibres (Joo et al., 2013). Bee et al. (2007) reported that the fibre type composition in *longissimus* muscle is not changed due to feed restriction in post-weaning and growing-finishing barrows of Large White breed. Solomon et al. (1988) observed that feed restriction increased the proportion of red fibres in *longissimus dorsi* muscle of pigs at 55 kg of slaughter weight. Bogucka et al. (2013) observed that the decrease in the intake of protein and energy or protein alone had significant effect only on the percentage and diameter of fast-twitch glycolytic fibres in crossbred pigs (Danish Landrace × Polish Large White) at 119 days of age. They did not observed any significant differences in any of the studied microstructure traits in animals at 168 days of age.

Clarifying the inter-relationships among muscle fibre characteristics and meat quality traits as well as the factors which influence these can be helpful for improving meat quality in pigs. This study's aim is to evaluate the effect of feed restriction on meat quality and muscle fibre composition in *longissimus lumborum et thoracis* muscle in the commercial crossbred pigs used in the Czech Republic. Results of the study could help to suggest the optimal feeding strategy for the chosen hybrid combination of pigs with respect to the required slaughter weight and meat quality.

MATERIALS AND METHODS

Animals and diets

Forty crossbred pigs (20 barrows and 20 gilts) of hybrid combination (Pietrain × Large White$_{\text{Sire}}$) × (Landrace × Large White$_{\text{Dam}}$) were used in this study carried out at a swine experimental and test station belonging to Czech University of Life Sciences Prague. The animals entered the experiment at 60 days of age. Their mean body weight was 26.7 kg, and they were divided into two feeding groups. The first group was fed *ad libitum* (AL, 10 gilts and 10 barrows) and the second group (R1, 10 gilts and 10 barrows) was fed in a restricted manner. The pigs were housed with two animals per pen according to feeding group and gender.

For the R1 group, the following equation was used: MJ per day = 8.6539 + 3.9408x – 0.1435x^2 (where x = age in days, Fig. 1). The equation for *ad libitum* fed group (MJ per day = 6.7786 + 7.4011x –0.3534x^2) shows the actual amount of feed received by these animals. Two experimental feed mixtures for growing and finishing pigs were used in the study.

Their amounts and choice of mixture were adjusted continuously over time depending on the actual weights of the pigs (Table 1). The compositions of the feed mixtures are presented in Table 2.

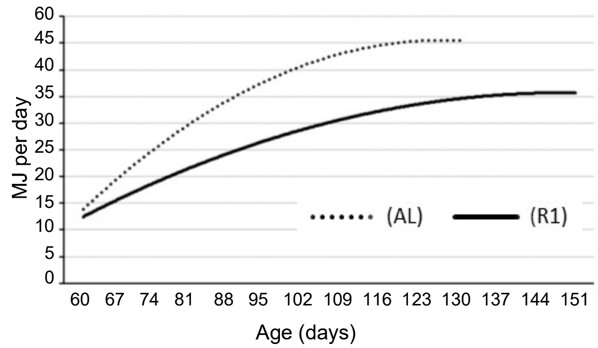

Figure 1. Feeding curves for *ad libitum* (AL) and restricted (RI) groups.

Table 1. The composition of complete feed mixture of two feeding groups during the fattening

Weight of pigs	AL			R1		
	Growing diet (%)	Finishing diet (%)	CFM per day (kg)	Growing diet (%)	Finishing diet (%)	CFM per day (kg)
27	100	0	1.80	100	0	1.25
45	85	15	2.26	85	15	1.80
72	43	57	2.92	43	57	2.57
91	10	90	3.27	10	90	2.72
112	0	100	3.44	0	100	2.81

CFM: complete feed mixture.

Table 2. Ingredient composition of the growing and finishing diets

Item	Diet	
	Growing	Finishing
Ingredient (%)		
Wheat	42.12	42.8
Barley	20.2	20.0
Soybean meal	13.8	2.9
Triticale	9.0	12.5
Rapeseed meal	4.0	7.0
Sunflower meal	3.5	6.0
Oats	2.5	2.5
Animal fat	2.0	1.15
Wheat bran	-	2.3
Calcite	1.15	1.2
Lysine-HCl	0.48	0.45
NaCl	0.45	0.45
Monocalcium phosphate	0.4	0.3
Vitamin–mineral premix	0.3	0.3
Threonine	0.2	0.15
Methionine	0.1	-

Table 2 (continued)

Nutrient composition (g per100 g of dry matter)		
Crude protein	17.48	14.95
Crude fat	3.66	2.91
Crude fibre	4.23	4.96
Lysine	1.12	0.91
Methionine	0.36	0.25
Ca	0.82	0.83
P	0.45	0.50
DE (MJ kg^{-1} of dry matter)	13.89	13.35

In order to assess the growth parameters, the individual animals were weekly weigh and daily feed intake was monitored. The average daily gain, feed conversion ratio and daily feed intake were calculated for each animal in week intervals and the final value was calculated as an average of these values. Pigs in both groups were fattened to an average slaughter weight of 112 kg. They were then slaughtered at a small commercial abattoir using electrical stunning and according to a routine procedure. The average slaughter weight, age, and selected growth parameters are presented in Table 3.

Table 3. Growth and slaughter parameters of pigs

Traits	Feeding groups		Significance[1]
	AL	R1	
Initial body weight (kg)	26.12 ± 3.02	27.27 ± 4.10	ns
Final live body weight (kg)	112.18 ± 7.15	112.41 ± 10.95	ns
Final age (days)	135.42 ± 2.63	155.65 ± 2.98	**
Average daily gain (g)	1,229.40 ± 92.44	935.60 ± 114.99	***
Feed conversion ratio (kg per kg)	2.27 ± 0.23	2.42 ± 0.25	**
Daily feed intake (kg)	2.78 ± 0.41	2.23 ± 0.10	***

[1] ns: not significant; ** $P < 0.01$; *** $P < 0.001$.

Histochemical analysis

Muscle samples for histochemical analysis were taken within 1 h after slaughter from the central part of the *musculus longissimus lumborum et thoracis* (*MLLT*). The samples were cut into 0.5 × 0.5 × 2.0 cm pieces, immediately frozen in isopentane cooled by liquid nitrogen according to the method of Dubowitz & Brooke (1973), then stored at -80 °C until analysis. Transverse serial muscle sections of 12 µm were cut from the entire blocks in a Leica CM1850 cryostat (Leica Microsystems, 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 & Kaiser (1970). Stained muscle section images were obtained using an optical microscope with a Nikon Eclipse E200 camera (Nikon, Tokyo, Japan) and examined using an image analysis program (NIS - Elements AR 3.2., Nikon Instruments Europe B.V., Amsterdam, Netherlands). Approximately 300 fibres per sample were included into the analysis.

Fibres were classified into fibre types I, IIA, and IIB. For each muscle fibre type, the fibre density, fibre cross-section area (CSA), and fibre proportions (percentage by number and area) were determined. For type IIB fibres, the proportions of small (diameter < 46 μm), medium (diameter 46–86 μm) and large (diameter > 86 μm) fibres were specified.

Carcass and meat quality traits

Lean meat percentage was evaluated by ZP method ('Zwei-Punkt-Messverfahren'), which is widely used in smaller slaughterhouses in the Czech Republic (The details of the method are described in Font-i-Furnols et al., 2016). Backfat thickness was measured 45 min *post mortem* using electronic callipers at the levels of the first *thoracic vertebrae*, the first *lumbar vertebrae* and over the *gluteus medius* muscle without skin. The result value was calculated as the mean of these three measurements. Using a portable pH meter (pH 330i/set, WTW GmbH, Weilheim, Germany), pH (pH_{45}, measured together with temperature – t_{45}) was determined 45 min *p.m.* within carcasses at the 13th and 14th *thoracic vertebrae*. Electrical conductivity (EC_{50}) was evaluated 50 min *p.m.* at the same location (Conductometer EV plus, Czech Technical University in Prague, Czech Republic).

Muscle samples for evaluation of meat quality traits were taken 24 h *p.m.* from *MLLT* of right sides of carcasses. Before sampling, photos of transverse cuts of loins were obtained for evaluating loin area. The pictures were evaluated using an image analysis program (NIS - Elements AR 3.2., Nikon Instruments Europe B.V., Amsterdam, Netherlands). Meat colour (CIE L*, a* and b*) was measured 24 h *p.m.* on the muscle surface after 10 min of blooming using a Minolta CM-700d colorimeter (Konica Minolta, Osaka, Japan). Warner-Bratzler shear force (WBSF; N) values were determined by Instron Universal Texture Analyzer 3342 (Instron, Norwood, MA, USA). Muscle samples of raw and cooked meat (6 × 1 × 1 cm, cooked at 80 °C for 1 h) were cut across muscle fibres. The resulting value for each sample was calculated as an average value of at least 6 measurements. Drip loss was evaluated using a bag method (24 h at 4 °C) according to Honikel (1998). Intramuscular fat content was determined gravimetrically via Soxhlet extraction, using petroleum ether as the solvent.

Statistical analysis

The experimental data were analysed by one-way and two-way analysis of variance (ANOVA) using SAS 9.4 statistical software (SAS Institute, Cary, NC, USA). One-way ANOVA with fixed effect of feeding group was used for the data in Table 3. The effect of feed restriction was equal to the age of animals, therefore only the effect of feeding group was used. For Table 4 and Table 5, two-way ANOVA with fixed effects of feeding group and body weight was used. Results in Tables 4 and 5 are presented as least squares means (LSM ± SEM) for the main effect of feeding group and significance for the effect of feeding group and body weight. No significant interactions between feeding group and body weight were observed, therefore they were not used in the Tables 4 and 5. Differences between the LSM were determined by the Tukey's range test. Pearson correlation coefficients were calculated to evaluate the association between muscle fibre characteristics and carcass and meat quality traits.

RESULTS AND DISCUSSION

We evaluated the effect of restricted feeding at uniform slaughter weight on the characteristics of muscle fibres and qualitative parameters of meat. The average slaughter weight of all animals (n = 40) was 112.3 kg, which corresponds to the average slaughter weight of pigs slaughtered in the Czech Republic. As expected, we observed variations in growth parameters in these groups (Table 3). The AL group had significantly higher average daily gain and daily feed intake compared to the R1 group, and therefore the final age of the R1 group was almost 3 weeks greater. The feed conversion ratio was lower in the AL group, so we presume that these animals were able to utilize the maximum of their genetic potential in achieving growth performance.

The values obtained for muscle fibre parameters in relation to feed restriction are shown in Table 4. Within the evaluated groups, there were significant differences in mean CSA and CSA of IIB fibres. The higher CSA of fibres (μm^2) was found in the R1 group ($P < 0.01$). These results correspond to the values for fibre density, which values were higher in the AL group. This is probably related to the difference in lean meat content between these groups (Table 5). 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. (2007) also had evaluated the effect of feed restriction on the characteristics of muscle fibres, but they observed no differences in CSA of fibres between the groups of Swiss Large White barrows at the same body weight. Surprisingly, in their study barrows of the restricted group were not leaner than were barrows of the *ad libitum* group at 100 kg of body weight.

Table 4. Effect of feed restriction on muscle fibre characteristics of *longissimus lumborum et thoracis* muscle

Traits	Feeding groups		Significance[1]	
	AL	R1	FG	BW
Fibre density (number per mm^2)				
Type I	38.46 ± 2.75	32.27 ± 2.68	ns	*
Type IIA	32.33 ± 2.83	29.51 ± 2.76	ns	ns
Type IIB	192.25 ± 8.09	183.94 ± 8.59	ns	**
Cross-sectional area (μm^2)				
Mean	2,826.9 ± 98.3	3,063.3 ± 111.4	**	**
Type I	2,209.1 ± 89.5	2,375.2 ± 101.3	ns	*
Type IIA	1,699.1 ± 131.6	1,666.6 ± 198.8	ns	ns
Type IIB	3,174.8 ± 124.6	3,477.8 ± 161.2	**	**
Fibre number composition (%)				
Type I	14.70 ± 0.78	13.05 ± 0.76	ns	ns
Type IIA	12.00 ± 0.66	11.87 ± 0.64	ns	ns
Type IIB	73.30 ± 1.03	75.09 ± 1.00	ns	ns
Fibre area composition (%)				
Type I	11.20 ± 0.70	9.42 ± 0.69	ns	ns
Type IIA	7.43 ± 0.51	6.39 ± 0.50	ns	ns
Type IIB	81.37 ± 0.95	84.19 ± 0.92	*	ns
Proportion of fibre size IIB (%)				
Small	16.85 ± 2.03	18.04 ± 1.98	ns	ns
Medium	72.34 ± 3.24	60.70 ± 3.16	*	ns
Large	10.81 ± 3.57	21.27 ± 3.48	*	*

FG – feeding group; BW – body weight; [1] ns: not significant; * $P < 0.05$; ** $P < 0.01$.

There were no significant differences in fibre number composition between the groups. In both groups, the largest number was of IIB fibres (74.20%), followed by fibres of type I (13.89%) and of type IIA (11.94%).

The restricted group, which was significantly leaner, also had a higher percentage area of type IIB fibres (84.19%) than did 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 (Lefaucheur, 2010; Joo et al., 2013), and diameters of type II fibres increase faster than diameters of type I (Oksbjerg et al., 1994). The positive relationship between high muscularity and proportional representation of type IIB fibres is reported in many studies (Wimmers et al., 2008; Lefaucheur, 2010; Joo et al., 2013; Kim et al., 2013b). Brzobohaty et al. (2015) also observed higher proportion of IIB fibres in restricted group of crossbred pigs in comparison to *ad libitum* fed group, which had lower lean meat content.

Furthermore, differences were observed in fibre size proportion of type IIB fibres. The AL group had significantly less representation of IIB fibres with a large area (> 86 μm; $P < 0.05$) in favour of medium-sized fibres IIB (46–86 μm; $P < 0.05$). Again, higher proportions of large IIB fibres in the restricted group could be associated with greater lean meat content of this group. Kim et al. (2013a) also had observed more muscularity in groups with greater content of large-sized IIB fibres. This corresponds to the statistically significant correlation coefficients that we found between lean meat content and representation of large- ($r = 0.28$) and medium-sized ($r = -0.33$) IIB fibres ($P < 0.05$). Similarly, Ryu et al. (2005) had reported that pigs with greater muscle mass had higher CSA and lower fibre density in type IIB fibres compared to pigs with lower muscle mass. Lee et al. (2016) observed that crossbred pigs with large CSA of fibres had larger loin area than pigs with middle or small CSA. They reported very high correlation coefficient between loin area and CSA of all fibres ($r = 0.92$).

Table 5 shows the effects of feed restriction on carcass and meat quality traits. Significantly greater lean meat content and lower backfat thickness ($P < 0.001$) were found in group R1. Greater backfat thickness in the AL group is probably related to the higher *post mortem* temperature (t_{45}) of meat ($P < 0.01$) measured in this group, where the thick fat layer slows the chilling of carcasses.

Electrical conductivity, pH, and water-holding capacity are parameters that can be used to classify abnormalities of meat quality. There were no differences between groups in these parameters, and we observed no meat abnormalities in the pigs used in our study.

Significant differences were observed in redness (a*) and yellowness (b*), where these values were lower in the AL group ($P < 0.01$). Variations in meat colour are related to differences in myoglobin content. Red muscle fibres (I and IIA) have greater myoglobin content and therefore darker colour (Listrat et al., 2016). During meat storage and blooming, however, myoglobin is oxidized into metmyoglobin, and that could result in a decrease in meat's redness. Faster colour change occurs during storage in muscles where red oxidative fibres predominate due to their faster oxygen consumption (Joo et al., 2013).

Table 5. Effect of feed restriction on carcass and meat quality traits of pigs

Traits	Feeding groups		Significance[1]	
	AL	R1	FG	BW
Lean meat (%)	57.27 ± 0.33	60.59 ± 0.32	***	***
Backfat thickness (mm)	23.69 ± 0.61	17.68 ± 0.59	***	***
Loin area (mm^2)	4,737 ± 112.8	5,002 ± 112.8	ns	**
Intramuscular fat content (%)	2.35 ± 0.17	2.16 ± 0.16	ns	ns
$pH_{45\ min}$	6.34 ± 0.06	6.29 ± 0.06	ns	ns
$t_{45\ min}$	39.53 ± 0.17	38.73 ± 0.17	**	**
$EC_{50\ min}$	3.42 ± 0.09	3.61 ± 0.09	ns	ns
Lightness (L*)	51.49 ± 0.66	52.91 ± 0.64	ns	ns
Redness (a*)	0.10 ± 0.25	0.94 ± 0.23	**	ns
Yellowness (b*)	9.16 ± 0.29	10.68 ± 0.28	***	ns
WBSF of raw meat (N)	38.77 ± 2.02	49.26 ± 1.96	***	ns
WBSF of cooked meat (N)	35.98 ± 1.57	38.88 ± 1.53	ns	ns
Drip loss (%)	4.27 ± 0.42	4.69 ± 0.41	ns	ns

WBSF – Warner-Bratzler shear force; EC – electrical conductivity; FG – feeding group; BW – body weight; [1] ns: not significant; ** $P < 0.01$; *** $P < 0.001$.

Although the R1 group had significantly higher WBSF of raw meat in comparison with the AL group ($P < 0.001$), no significant differences were observed in WBSF of cooked meat. The AL group had a significantly smaller CSA and lower content of IIB fibres. This corresponds to claims that higher fibre CSA, and especially of IIB fibres, increases instrumental toughness of meat (Karlsson et al., 1993; Joo et al., 2013; Kim et al., 2013a).

CONCLUSIONS

Feed restriction has a significant effect on lean meat content, which is accompanied by a different muscle fibre size. Age difference at the uniform slaughter weight, which was caused by feed restriction is reflected in the amount of fast-oxidative glycolytic fibres (IIB) in muscle and their proportion in overall fibre composition. The results of this study suggest that a greater amount in muscle of large IIB fibres, which are associated with poor meat quality, is closely related to lean meat content. Although meat quality traits tend to be better in the group fed *ad libitum*, the lean meat content is lower in this group. These results show that strongly restricting feed consumption negatively affects the composition of muscle fibres in *longissimus* muscle of pigs.

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