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Effects of Muscle Mass and Fiber Number of *Longissimus dorsi* Muscle on Post-mortem Metabolic Rate and Pork Quality

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Abstract The aim of this study is to investigate the effects of the muscle mass and fiber number on post-mortem metabolic rates and pork quality. Carcass traits, muscle fiber characteristics, and type of fiber composition were evaluated using a sample of 200 cross-bred pigs. The muscle mass was divided into two groups according to carcass weight and loin-eye area measurements (heavy or light). In addition, the muscle histological characteristics were divided into two groups according to the muscle fiber density and total number of muscle fibers (high or low). All the carcass traits were significantly different in the muscle mass groups. Increasing weight significantly affected the cross-sectional area (CSA) of all fibers. The low group, which had a low muscle fiber number indicating a larger CSA of fibers, and especially the heavy-low group had the highest CSA levels of fibers. The fiber number percentage and the area percentage were significantly different in the groups categorized by fiber number. The heavy-high group indicated a normal rate of pH decline and the R-value. In addition, pigs with a heavy muscle mass and high muscle fiber number indicated normal drip loss, lightness, and protein denaturation. The present results suggest that increasing the total muscle fiber number has a beneficial effect on increasing the muscle mass without deteriorating the meat quality.

Keywords: muscle mass, fiber number, fiber type composition, pork quality

Introduction

In recent decades, pigs have been selectively bred for the rapid production of lean meat. The rapid production and muscle mass are closely related to two major muscle fiber characteristics. One is the number of muscle fibers and the other is the size of muscle fibers (1). It is generally believed that the muscle growth potential is related to the total number of muscle fibers (2), and that the rate of muscle growth depends on the fiber hypertrophy rate (1, 3). These two muscle fiber characteristics are inversely correlated with each other and both the fiber number and size are positively correlated with the muscle mass (4).

An important question is the extent to which each of the two fiber characteristics contributes to muscle growth and how this affects meat quality. Pigs with more muscle fibers grew faster and had a greater muscle mass (5, 6). The estimated number of fibers has been reported to be related to the muscle mass as well as the growth rate and meat quality (7, 8, 9). A low muscle fiber number correlates with the fibers that exhibit greater hypertrophy. Moreover, animals with large muscle fibers often grow rapidly and are quite muscular (10).

It is well known that changes in the composition of muscle fiber types affect the metabolic properties of a muscle and meat quality (11). Some results suggested that the muscle mass was also related to the composition of fiber types. Heavier pigs tend to have greater amounts of I and IIa myosin heavy chain (12). Candek-Potokar et al. (13) observed that the cross-sectional area of βR and αW fibers increased in heavier pigs. These previous findings suggest that muscle mass and its histological characteristics influence the composition of fiber types and ultimately, meat quality. The purpose of the present study was to investigate the effects of muscle mass and histological characteristics on post-mortem metabolic rates and pork quality.

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Materials and Methods

Animals A total of 200 cross-bred Duroc × (Yorkshire × Landrace) pigs (129 gilts, 71 castrated male pigs) were evaluated. The pigs were slaughtered after 172.7 ± 1.7 days during the winter. The abattoir used a traditional scalding-singeing process. After electrical stunning, the carcasses were exsanguinated for 5 min prior to scalding in 65°C water. After evisceration, the carcass weight was measured and the back fat thickness was also measured at the 11th and last *thoracic vertebrae*. The mean of these two measurements was used to determine the back fat thickness. The loin-eye area was measured at the level of the last rib.

Histochemical analyses Within 45 min after slaughter, muscle samples for histochemical analysis were taken from *longissimus* muscle at the 8th *thoracic vertebrae*. The samples were cut into 0.5 × 0.5 × 1.0 cm pieces, promptly frozen in isopentane cooled by liquid nitrogen and stored at -80°C until needed.

The serial transverse muscle sections (10 μm) were obtained from each sample with a cryostat (CM1850, Leica, Germany) at -20°C and mounted on glass slides. Unfixed sections were preincubated at room temperature for 5 min in a buffer consisting of 100 mM potassium chloride in 100 mM sodium acetate, adjusted to pH 4.7 with acetic acid (14). The sections were stained for their myofibrillar ATPase reactivity according to the methods of Brooke and Kaiser (15). The muscle fibers were identified

by dark, light, and intermediate staining as being type I, IIa, or IIb, respectively. About 600 fibers per each sample were evaluated. All the histochemical samples were examined using an image analysis system (Image-Pro Plus, Media Cybernetics, L.P., USA).

Fiber number percentage refers to the ratio of counted fiber numbers of each fiber type to the total counted fiber number. The relative area of fibers denotes the ratio of the total cross-sectional area (CSA) of each fiber type to the total measured fiber area. The average CSA of type-identified fibers were also measured. The density of fibers was calculated using the mean number of fibers per mm^2. With the fiber density and the loin-eye area measures, the total number of fibers was calculated.

Meat quality parameters Samples were taken within 45 min after slaughter from the *longissimus* muscle at the 8th *thoracic vertebrae* and immediately frozen in liquid nitrogen to determine the post-mortem metabolic rate. Early post-mortem muscle pH and temperature were determined on carcasses after muscle samples were removed. After 24 hr of chilling, the M. *longissimus dorsi* was examained to evaluate the meat quality attributes. For each of the 200 loins, maximum effort was made to maintain consistency using the same anatomical location for each procedure.

In order to determine the post-mortem metabolic rate, the R-values were measured using the procedure reported by Calkins et al. (16). R248, R250, and R258 were defined as the ratios of A248/A260, A250/A260, and A258/A250, respectively, and the absorbances were measured using a calibrated spectrophotometer (Model Du-64, Beckman Co., U.S.A.).

The post-mortem protein denaturation was evaluated by measuring the solubility of the sarcoplasmic and total (sarcoplasmic + myofibrillar) proteins according to the methods reported by Joo et al. (17). Myofibrillar protein solubility was obtained by measuring the difference between total and sarcoplasmic protein solubility levels.

The level of drip loss was determined by suspending the muscle samples normalized for surface area in an inflated plastic bag (2°C) for 48 hr (18). The color of meat was measured after exposing it to surface air for 30 mins at 2°C. A Minolta chromameter (CR-300, Minolta Camera Co., Osaka, Japan) was used for the measurements and the results were expressed as C.I.E. (Commission International de l'Eclairage) L*a*b*.

Statistical analysis Cluster analysis was performed to classify the muscle mass and the muscle histological property using the FASTCLUS procedure of the SAS program. The observations were allocated to different groups according to the smallest Euclidean distance from the initial seeds of the cluster. The muscle mass was classified into two clusters (heavy or light group) using the carcass weight and loin-eye area, and the numerical abundance of muscle fibers was classified into two clusters (high or low group) using the density of muscle fibers and the total number of muscle fibers.

A General Linear Model was used to evaluate the significant differences ($P < 0.05$) among the muscle mass and fiber number clusters. The results were presented as least square means (LSM) for the four groups together with the standard errors of these LSMs.

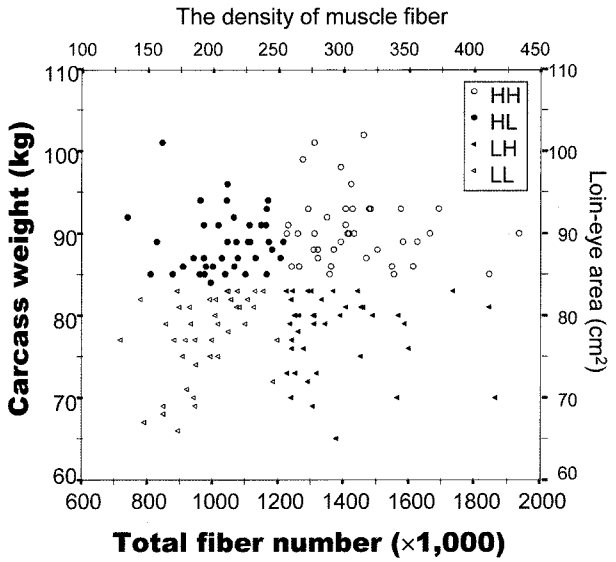

Fig. 1. A scattered plot of carcass weight (kg) vs. total fiber number (×1,000) of longissimus muscle in pigs. The muscle mass was divided into heavy (H) or light (L) groups according to the carcass weight and the loin-eye area measurements. In addition, the numerical abundance of muscle fibers was divided into high (H) or low (L) groups according to the density of muscle fibers and the total number of muscle fibers.

Results and Discussion

Effect of muscle mass and fiber number on carcass traits The muscle mass was classified into two clusters (heavy or light group) using the carcass weight and loin-eye area. The muscle fiber number was also classified into two clusters (high or low group) using the density of muscle fibers and the total number of muscle fibers (Fig. 1).

All the carcass traits were significantly different in the pig groups categorized by muscle mass (Table 1). The back fat thickness and loin-eye area were also affected by fiber number groups. The carcass weight was significantly higher in the heavy group; however, the fiber number traits had no significant effect on the carcass weight. The loin-eye area was significantly affected by both muscle mass and fiber number traits. The heavy group had larger loin-eye areas than the light group, while the increase in fiber number (high group), indicated an increase in the loin-eye area. Increases in both weight and fiber number resulted in larger loin-eye area.

The back fat thickness was the highest in the heavy-low group, indicating heavy muscle mass and a low muscle fiber number. During the post-natal growth, the increase in the skeletal muscle mass was mainly due to increased muscle fiber size (1). The growth rate of the individual muscle fibers was lower when there were a high number of fibers (1). In this study, the heavy-low group indicated rapid growth rates in individual muscle fiber. This suggests that the pigs with a low fiber number have greater levels of fat deposition. Animals showing a larger muscle mass with a low fiber number might have attained their target

Table 1. Effects of muscle mass and fiber number on pig carcass traits

Muscle mass (MM)	Heavy		Light		Level of significance	
Fiber number (FN)	High	Low	High	Low	MM	FN
Number of animals	53	52	20	75		
Carcass weight (kg)	88.26^{a} (0.67)[1]	88.29^{a} (0.67)	76.70^{b} (1.09)	76.71^{b} (0.56)	***	NS
Back fat thickness (mm)	19.81^{b} (0.78)	23.44^{a} (0.79)	18.50^{b} (1.27)	19.69^{b} (0.65)	**	**
Loin-eye area (cm^2)	52.30^{a} (0.58)	47.60^{b} (0.59)	44.44^{c} (0.95)	41.48^{d} (0.49)	***	***

[1]Standard error of least square means.
Levels of significance: NS = not significant; **P<0.01; ***P<0.001.
[a,b,c,d]Least square means with different superscripts in the same row significantly differ ($P < 0.05$).

body weight at an earlier stage during the fattening process.

Histological characteristics of *longissimus dorsi* muscle Heavier pigs exhibited a lower fiber density than the lighter ones, while the total number of muscle fibers was significantly higher in the heavy group (Table 2). Heavier pigs exhibited larger fibers than the lighter ones. The number of muscle fibers also significantly affected the cross-sectional area (CSA) of the fibers. The low group which had a low muscle fiber number indicated a larger CSA of fibers, and especially the heavy-low group had the highest CSA levels of fibers. Increasing the loin-eye area was generally accompanied by an increase in the CSA of the muscle fibers. (In this study, the muscle fiber number was negatively related to loin-eye area.) The mean fiber number and the CSA were mainly related to those of type IIb fibers as a result of the high percentage of type IIb fibers in the *longissimus* muscle.

An increase in weight was reported to accompany an enlargement of the muscle fibers (19). Furthermore, the CSA of fibers were found to increase in the low group which had low muscle fiber numbers. The observed fiber hypertrophy is consistent with the larger loin-eye area in the heavy-low group.

A similar result was observed between the CSA of the fibers, and the loin-eye area can be explained by the simultaneous effect of weight on both traits, as suggested in other reports (13, 20). This indicates that the total number and length of the muscle fibers also determine the composition of the loin-eye area (21).

Table 2. Effects of muscle mass and fiber number on histological characteristics of pig *longissimus dorsi* muscle

Muscle mass (MM)	Heavy		Light		Level of significance	
Fiber number (FN)	High	Low	High	Low	MM	FN
Fiber number per mm^2						
Mean	280^{b} (4.5)[1]	225^{d} (4.6)	321^{a} (7.4)	256^{c} (3.8)	***	***
Type I	22^{a} (1.3)	18^{b} (1.3)	24^{a} (2.1)	20^{ab} (1.1)	NS	*
Type IIa	29^{b} (1.4)	30^{ab} (1.4)	34^{a} (2.2)	31^{ab} (1.6)	*	NS
Type IIb	229^{b} (4.2)	178^{d} (4.3)	262^{a} (6.8)	204^{c} (3.5)	***	***
Total fiber number (×1,000)						
Mean	1450^{a} (19.6)	1069^{b} (19.8)	1417^{a} (31.9)	1058^{b} (16.5)	NS	***
Type I	118^{a} (5.7)	86^{bc} (5.8)	106^{ab} (9.2)	84^{c} (4.8)	NS	***
Type IIa	148^{a} (5.9)	140^{ab} (5.9)	151^{a} (9.6)	129^{b} (4.9)	NS	*
Type IIb	1194^{a} (19.2)	844^{b} (19.3)	1160^{a} (30.9)	843^{b} (16.1)	NS	***
Cross-sectional area of muscle fiber (μm^2)						
Mean	3591^{c} (73.8)	4520^{a} (74.6)	3151^{d} (120.2)	3981^{b} (62.1)	***	***
Type I	2669^{b} (79.7)	2951^{a} (80.4)	2274^{c} (128.5)	2790^{ab} (66.8)	**	***
Type IIa	2268^{b} (68.3)	2565^{a} (68.9)	1963^{c} (110.1)	2301^{b} (57.2)	***	***
Type IIb	3917^{c} (89.5)	5024^{a} (90.4)	3403^{d} (144.4)	4377^{b} (75.0)	***	***

[1]Standard error of least square means.
Levels of significance: NS=not significant; *P<0.05; **P<0.01; ***P<0.001.
[a,b,c,d]Least square means with different superscripts in the same row significantly differ ($P < 0.05$).

Muscle fiber composition of *longissimus dorsi* muscle The fiber number percentage and area percentage were not significantly affected by muscle mass (carcass weight and loin-eye area) at slaughter (Table 3). Among fiber types, the fiber number percentage of type IIb fibers increased in the high group, whereas that of IIa fibers increased in the low group.

In pigs, a correlation between the white fiber percentage and the PSE (pale, soft, exudative) meat condition has been reported (22). Ruusunen and Puolanne (23) reported that the percentage of the fiber types, either in number or area, greatly affected the redness of the meat. The more

Table 3. Effects of muscle mass and fiber number on fiber type composition of pig *longissimus dorsi* muscle

Muscle mass (MM)	Heavy		Light		Level of significance	
Fiber number (FN)	High	Low	High	Low	MM	FN
Fiber number percentage						
Type I	7.79 (0.46)[1]	8.00 (0.47)	7.52 (0.75)	7.90 (0.39)	NS	NS
Type IIa	10.17^{c} (0.53)	13.31^{a} (0.53)	10.78^{bc} (0.85)	12.33^{ab} (0.44)	NS	***
Type IIb	82.10^{a} (0.64)	78.72^{c} (0.65)	81.69^{ab} (1.04)	79.77^{bc} (0.54)	NS	***
Fiber area percentage						
Type I	5.70 (0.31)	5.23 (0.32)	5.33 (0.51)	5.47 (0.26)	NS	NS
Type IIa	6.23^{b} (0.34)	7.53^{a} (0.34)	6.66^{ab} (0.52)	7.10^{a} (0.28)	NS	*
Type IIb	88.07 (0.46)	87.24 (0.46)	88.01 (0.74)	87.43 (0.39)	NS	NS

[1]Standard error of least square means.
Levels of significance: NS=not significant; *P<0.05; ***P<0.001.
[a,b,c]Least square means with different superscripts in the same row significantly differ ($P < 0.05$).

there are type I and the less there are type IIb fibers, the redder the meat color was. Intensive selection for lean muscle growth in pigs may have caused a large genetic change in the fiber type composition, which resulted in a higher proportion of glycolytic fibers and an increase in the mean fiber diameter (24).

Karlsson et al. (25) indicated that pigs with the highest lean percentage had the highest proportion of type IIb fibers. Thus, the proportion of type I and type IIa fibers decreased. However, this result was found only in the heavy-high group which had heavier muscle mass and higher muscle fiber number. There was no significant difference in the heavy-low group with lower muscle fiber numbers.

Meat quality traits A significant effect of interaction was observed for pH_{45min} ($P < 0.1$), R-value ($P < 0.01$) and protein solubility ($P < 0.05$). The light-high group indicated the fastest rate of pH decline and the highest R-value (Table 4). These results indicated that pigs, which had light muscle mass and high muscle fiber numbers, tended to have faster post-mortem metabolic rates. Post-mortem protein denaturation is caused by increased muscle glycolysis (26, 27). In this study, the light-high group, which indicated fast metabolic rates, had the highest protein denaturation rate.

The simultaneous increase in metabolic rate and protein denaturation has no effect on drip loss or meat color. A lower water holding capacity has sometimes been reported with increasing fiber size (20), whereas, no significant detrimental effect of the increasing CSA levels on the fresh meat quality traits was observed. However, this was not the case in this study.

Lengerken et al. (28) suggested there is a range of optimum muscle fiber numbers, which guarantees both a high meat percentage and good meat quality at a moderate fiber size. In these experiments, increasing muscle mass and fiber numbers resulted in a larger loin-eye area (Table 1), intermediate fiber size (Table 2) and higher percentage of type IIb fibers (Table 3). However, no difference was observed in post-mortem metabolic rate and meat quality traits (Table 4).

Table 4. Effects of muscle mass and fiber number on meat quality traits of pig *longissimus dorsi* muscle

Muscle mass (MM)	Heavy		Light		Level of significance	
Fiber number (FN)	High	Low	High	Low	MM	FN
pH_{45min}	6.03^{a} (0.04)[1]	6.03^{a} (0.04)	5.90^{b} (0.06)	6.04^{a} (0.03)	NS	†
pH_{24hr}	5.56 (0.02)	5.58 (0.02)	5.61 (0.03)	5.58 (0.01)	NS	NS
R-value	1.02^{a} (0.02)	1.01^{a} (0.03)	1.16^{b} (0.04)	0.97^{a} (0.02)	†	***
Drip loss (%)	4.83 (0.34)	4.65 (0.35)	4.80 (0.56)	4.50 (0.29)	NS	NS
Lightness (L*)	47.52 (0.46)	46.83 (0.47)	47.09 (0.75)	47.01 (0.39)	NS	NS
Redness (a*)	6.96 (0.17)	6.95 (0.17)	6.65 (0.27)	6.85 (0.14)	NS	NS
Yellowness (b*)	3.97 (0.14)	3.97 (0.14)	3.68 (0.23)	3.91 (0.12)	NS	NS
Protein solubility (mg/g)						
Total protein	211.90^{a} (2.81)	210.18^{a} (2.78)	194.88^{b} (4.72)	210.51^{a} (2.28)	*	*
Sarcoplasmic protein	80.75^{a} (1.10)	79.30^{ab} (1.09)	75.33^{b} (1.84)	78.71^{ab} (0.89)	*	NS
Myofibrillar protein	131.15^{a} (2.20)	130.88^{a} (2.18)	119.55^{b} (3.70)	131.80^{a} (1.79)	*	*

[1]Standard error of least square means.
Levels of significance: NS=not significant; †$P<0.1$; *$P<0.05$; ***$P<0.001$.
[a,b]Least square means with different superscripts in the same row significantly differ ($P < 0.05$).

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