The Relationship between Muscle Fiber Characteristics and Meat Quality of *Longissimus dorsi* Muscle of Pigs

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Abstract

This experiment was designed to investigate the histochemical parameters of muscle fiber, and to estimate the correlation of muscle characteristics to meat quality traits in 63 crossbred pigs (Landrace x Large White x Duroc). Samples of the *longissimus dorsi* muscle were taken to evaluate the meat quality traits and histochemical characteristics using a combined NADH dehydrogenase and myofibrillar acid stabile ATPase reaction staining. Three muscle fiber types were detected, I, IIA, and IIB types. The results showed that type I, IIA, and IIB muscle fiber diameters were 40.12, 45.69, and 61.49 µm, respectively. For the percentage of muscle, the frequencies of type I and IIA were quite similar (19.93 and 15.98, respectively). Whereas, the IIB type presented the greatest proportion of muscle fibers (63.43). Fiber type was mainly related to and meat quality traits among various muscle fiber characteristics. The pH_{24} was negatively correlated to those of T_{24}, a^*, and b^* (-0.38, -0.36, -0.37, respectively). L^* value also negatively correlated with pH_1 (r = -0.35). For pH_{24} and T_1 showed positively correlated to WBS (r = 0.28 and 0.30, respectively), whereas T_{24} and L^* value were negatively correlated with WBS (r = -0.26 and -0.30, respectively). Moreover diameter of type I and type I muscle fiber is positively correlated with WBS. The results suggested that pH values which reflect the early post-mortem metabolic rate are reliable parameters for prediction ultimate pork quality. The results imply that accelerated metabolic rate and poor meat quality could be partially explained by the decline of pH in muscle during early post-mortem.

Keywords: muscle fiber, meat quality, crossbred pigs, histochemical parameters

Introduction

Mechanisms controlling meat quality development are often associated with altered post-mortem muscle metabolism. Specifically, changes in the extent or rate of glycolysis can create unfavorable muscle pH. A high rate of pH decline and low ultimate pH result in muscle protein denaturation and diminished quality parameters (Hammelman et al., 2003). One of the main factors determining muscle biochemical pathways is skeletal muscle fiber.

Skeletal muscle fibers can be histochemically classified into different fiber types based on differences in the pH dependence of the myosin adenosine triphosphatase (m-ATPase) activity. At alkaline pH, slow-contracting, type I fibers, have a low ATPase activity, whereas fast-contracting, type II fiber, have a high ATPase activity. Type II fibers can be further subdivided into type II A and type II B fibers on the basis of their differences in m-ATPase under different acidic conditions (Burke and Edgerton, 1970). Type I fibers prefer the aerobic usage of glucose and fat as energy sources. They are surrounded by many capillaries which facilitate oxygen diffusion, because oxygen is needed to utilize energy for contraction. Type IIB fibers are glycolytic, and therefore contain more glycogen and need less oxygen than type I fibers for energy production. Whereas type IIA is intermediate between type I and type IIB.

During the post-mortem transformation of muscle to meat, changes in the relative contribution of various proteins are related to pork quality traits (Warner et al., 1997). Several studies have shown that the metabolic properties of muscle are related to variation in glycogen content and ultimate pH of meat. This may explain part of variation in some meat quality characteristics, such as water holding capacity, color and tenderness (Bendall and Swatland, 1988). Several studies, mostly in logissimus muscle, have reported difference in muscle fiber characteristics. For the practical application of this knowledge to improve and control meat quality, more information of the fiber type characteristics on post-mortem metabolic rate is necessary. Therefore the aim of this study was to investigate the histochemical parameters of muscle fiber, and to estimate the correlation of muscle characteristics to meat quality traits.

Materials and Methods

Animals

The experiment was carried out on 63 crossbred (castrated males and females) pigs (Landrace x Large White x Duroc. Both maintenance and feeding were similar for all animals. The animals were fattened and then slaughtered at a live body weight of about 100 kg. Pigs were slaughter by electrical stunning. The abattoir used a traditional scalding singeing process.

Histochemical Analysis

Muscle samples from *m. longissimus thoracic* at the location of $13^{th}/14^{th}$ rib were taken 24 h post mortem. Samples were cut into cube-shaped 1 x 3 x 1 cm^3 pieces (parallel to the muscle fibers), immediately frozen in liquid nitrogen, and stored at -80^oC pending analysis

Serial transverse muscle sections (3 μm) were obtained from each sample with a cryostat microtome (CM 1850, Leica, Germany) at -20 $^{\circ}C$ and mounted on glass slides.

The histochemical differentiation of the three main fiber types, slow twitch oxidative (STO), fast twitch oxidative (FTO), and fast twitch glycolytic (FTG), was obtained by the use of a combind NADH dehydrogenase and myofibrillar acid stabile ATPase reaction (Szentkuti and Eggers, 1985). All histochemical samples were examined by an image analysis system. The operational system consisted of an optical microscope equipped with a CCD color camera (SSC-C370P, Sony, Japan).

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All portions of the analyzed sections were free from tissue disruption and freeze damage. At least 300 myofibers per sample on the two series of photographs in each tissue taken at the same location on the different specimens were counted for the determination of fiber type percentage. Muscle fiber diameter (μm) was calculated using Scan Image scientific imaging software. From each section, 10 muscle fibers /type (I, IIA, and IIB) were measured and then averaged for the determination of muscle fiber diameter. For the calculation of the total fiber number, the fiber density per mm^2 was multiplied with the loin-eye area (cm^2) determined at the level of the $13^{th}/14^{th}$ rib.

Meat Quality Traits

Following 24 h of chilling, *Longissimus dorsi* (LD) was taken to evaluate the meat quality traits. For each of the 63 loins, every effort was made to maintain consistency in using the same anatomical location for each procedure.

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Drip loss was determined by suspending slices of *Longissimus dorsi* muscle (2.5 cm) inside polythene bags and held at 1^{o}C for 24 h. Drip loss was expressed as a percentage of the initial sample weight.

The color of the meat was measured at 24 h post mortem at room temperature (20^{o}C) using a Minolta Chroma Meter CR 300 (Minolta co., Ltd., Japan) after blooming for 30 min at 4^{o}C. The average of triplicate measurements was recorded and the results were expressed as CIE L*, a*, and b* light reflectance coordinates.

Warner-Bratzler shear force (WBS) was determined using an Instron Universal Testing Machine Model 1011 (Instron Corporation, USA) equipped with a Warner-Bratzler shear device. Six cubes (1 x 3 x 1 cm^{3}) were removed from each cooked steaks parallel to the longitudinal orientation of muscle fibers. Samples were sheared perpendicular to the long axis of the core.

Loin eye area (LEA) of *Longissimus dorsi* muscle was traced on a acetate film between the 13th and 14th ribs and determined by planimeter.

At 24 h post mortem, pH was measured at the loin around 13th rib (*Longissimus thoracis*), using Knick Model 651-2 pH meter (Knick, Berlin, Germany). Temperature was measured at 24 h post mortem in the same muscle as pH measurement using Mettler Toledo (SevenGo^{TM}pH meter SG2, Germany)

Statistical Analysis

Descriptive statistics were performed using the MEANS procedure of the SAS system for window to calculate mean values and standard deviations for all variables. Pearson correlation coefficients were evaluated to describe the relationship between meat quality traits and muscle fiber characteristics.

Results and Discussion

Post-Mortem Metabolic Rate and Meat Quality Traits

The mean values, standard deviations of the post-mortem parameters and meat quality traits are given in Table 1. The results indicate that there are differences in post-mortem metabolic rate and meat quality. The data showed no marked problems with deficiencies as documented by pH_1, which were within the normal ranges. In the case of accelerated

Table 1 Post mortem parameters and meat quality traits of *Longissimus dorsi* muscle.

Parameter	Mean±SD	Min	Max
45 min Post mortem			
Temperature(T_1)	36.47±3.01	28.10	41.30
pH_1	6.34±0.31	5.6	7.60
24 h Post mortem			
Temperature (T_{24})	1.77±0.91	0	4.00
pH_{24}	5.70±0.24	5.11	6.28
Lightness (L*)	49.73±3.49	41.63	57.03
Redness (a*)	5.51±1.47	2.65	8.83
Yellowness (b*)	1.41±1.06	-0.73	3.73
Drip loss (DL); %	0.002±0.002	0	0.008
Loin eye are (LEA); c m^{2}	50.89±8.67	25.90	78.20
Warner-Bratzler shear- force (WBS); N	6.15±1.74	3.09	10.81

glycolysis, the metabolism in muscle contributes to a fast pH decline. This glycolytic pathway is related to the occurrence of the PSE condition (Candek-Potokar et al., 1999).

Relationships between Post-Mortem Parameters and Muscle Fiber Traits

The correlation coefficients between post-mortem parameters and meat quality traits are presented in Table 2.The pH_{24} was negatively correlated to those of T_{24}, a^*, and b^* (-0.38, -0.36, -0.37, respectively). L^* value also negatively correlated with pH_1 (r = -0.35). For pH_{24} and T_1 showed positively correlated to WBS (r = 0.28 and 0.30, respectively), whereas T_{24} and L^* value were negatively correlated with WBS (r = -0.26 and -0.30, respectively). These results indicated that pork with a lower in pH_1 and pH_{24} becomes lighter in color as reported by Allison et al. (2003). The rate of pH decline and ultimate pH are the two main determinants of water holding capacity (WHC) and meat color. Brewer et al. (2001) reported that as pH approached the isoelectric points of muscle protein, free water increases and scatters more light. Thus, decreasing pH_1 is related to increasing drip loss and lightness. Moreover previous studies (Fernandez and Tornberg, 1991; Karlsson et al., 1993) have been shown that pH_{24} is dependent on the muscle glycogen content at slaughter, which also affects meat tenderness. According to Henckel et al. (1997), the glycogen content in longissimus muscle is negatively correlated with pH_{24}, and shear force and muscle glycogen were inversely correlated.

Muscle Fiber Characteristics and Meat Quality Traits

The variation in muscle fiber type is also related to heterogeneity in glycogen depletion between different fiber types. The results form this study showed that type I, IIA, and IIB muscle fiber percentage were 19.93, 15.98, and 63.43, respectively. The frequencies of type I and IIA were quite similar. Whereas, type IIB represented the greatest proportion of fibers. At birth, all fibers are oxidative, and intense conversion towards glycolytic fibers. Muscle fiber type percentage are influenced by environmental factor, genetics, nutrition and exercise (Gentry et al., 2004). In the domestic pig longissimus muscle, 10% of fibers are type I, 7% type IIA, and 83% type IIB as determined by classical histochemical techniques (Lefaucheur and Etienne, 1991).

The fiber diameter and fiber type composition of the slow twitch oxidative (STO), fast twitch oxidative (FTO) and fast twitch glycolytic (FTG) types showed values and variation in normal range when compared to earlier results (Maltin, et al., 1997). The data show that the fiber sizes of oxidative types (STO and FTO) were 40.12 and 45.69 μm, respectively, whereas the glycolytic type

Table 2 Correlation coefficients between post-mortem parameters and meat quality traits of *Longissimus dorsi* muscle.

	pH_1	pH_{24}	T_1	T_{24}	$L^{1/}$	$a^{1/}$	$b^{1/}$	DL	LEA
pH_1									
pH_{24}	0.00								
T_1	- 0.05	-0.03							
T_{24}	0.11	-0.38**	0.29*						
$L^{1/}$	- 0.35**	-0.13	-0.09	0.07					
$a^{1/}$	- 0.18	-0.36**	0.11	0.08	0.26*				
$b^{1/}$	- 0.16	-0.37**	0.13	0.18	0.67**	0.78**			
DL	0.04	0.14	-0.01	-0.12	0.10	-0.22	0.01		
LEA	0.07	0.26*	0.30*	-0.26*	-0.25*	0.11	-0.09	-0.06	
WBS	0.00	0.28*	0.30*	-0.26*	-0.32**	0.00	-0.19	-0.02	0.27*

[1/] L = Lightness, a = redness, b = yellowness
Level of significance: * P<0.05, ** P<0.01, *** P<0.001

Table 3 Muscle fiber characteristics of the *Longissimus dorsi* muscle.

Charactersitic	Mean	SD	Min	Max
Muscle fiber type (%)				
Type I	19.93	5.30	10.81	34.73
Type IIA	15.98	8.69	23.49	75.89
Type IIB	63.43	4.60	7.14	30.27
Diameter of muscle fiber (μm)				
Type I	40.12	7.27	28.26	59.47
Type IIA	45.69	8.41	33.56	75.41
Type IIB	61.49	8.97	44.53	94.77
Number of muscle fiber (cell/cm)	1,288,229.17	317,250.52	637,772.00	1,910,923.00

(FTG) was higher (61.49 μm).The FTG fiber occur at a higher frequency than the STO fiber, with FTG fibers occurring at a higher frequency than FTO fibers. These results were consistent with those Karlsson et al. (1993), supporting the contention that longissimus muscle of the pig can be regarded as a 'white' muscle. Actually, pig muscles are white and dark in color depending on the histochemical composition. Dark muscle primarily contains small, oxidative, slow contracting and small, oxidative, fast contracting fibers. Each fiber type has different physiological capacity, and they therefore use different source of energy. Type I fiber, slow twitch oxidative (STO), prefer the aerobic usage of glucose and fat as energy sources. They are surrounded by many capillaries which facilitate oxygen diffusion, because oxygen is needed to utilize energy for contraction. Type IIB fibers, fast twitch glycolytic (FTG), are glycolytic, and therefore contain more glycogen and need less oxygen than type I fibers for energy production. Muscle fibers affect post-mortem changes in muscle due to the differences in their glycolytic or oxidative capacity (Klont et al, 1998).

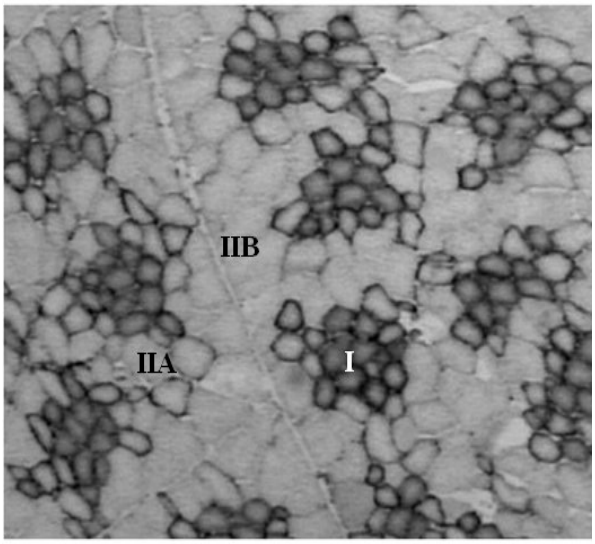

Figure 1 Serial sections of *longissimus dorsi* muscle, staining for a combined NADH dehydrogenase and myofibrillar acid stabile ATPase reaction Magnification of 40X was used. Abbreviations: I, type I fiber (slow-twitch, oxidative); IIA, type IIA fiber (fast-twitch, oxidative); IIB, type IIB fiber (fast-twitch, glycolytic).

Relationships between Myofiber Characteristics and Muscle Fiber Traits

To evaluate the histochemical characteristics of pig longissimus muscle, this study used a combind NADH dehydrogenase and myofibrillar acid stabile ATPase reaction as shown in Figure 1. The relationship between myofiber histological traits and meat quality traits were limited, and only a few significant correlations were found (Table 4). For muscle fiber size parameters, there was a positively correlation with WBS (r = 0.28). However, drip loss and lightness were not association with muscle fiber size parameters, as suggest by Candek-Potokar et al. (1999). Also, no relationship between pH and muscle fiber size was observed.

Table 4 Correlation coefficients between muscle fiber type composition, muscle fiber number and meat quality traits of the *Longissimus dorsi* muscle.

	Fiber number percentage			Fiber number percentage			Number of muscle fiber
	Type I	Type IIA	Type IIB	Type I	Type IIA	Type IIB	
45 min Post mortem							
$T_{45\ min}$	-0.17	-0.15	0.07	-0.15	-0.12	0.22	-0.04
$pH_{45\ min}$	0.21	0.18	-0.02	-0.02	-0.14	-0.02	0.27
24 h Post mortem							
T_{24h}	0.01	0.00	0.03	0.16	0.22	0.16	0.12
pH_{24h}	0.03	0.07	-0.15	-0.39**	-0.45**	-0.31**	-0.08
Lightness	-0.03	-0.01	-0.05	0.10	0.11	0.18	-0.10
Redness	0.14	0.10	-0.20	0.16	0.07	0.09	0.09
Yellowness	0.08	0.02	-0.13	0.14	0.11	0.17	0.04
Drip loss	0.07	-0.08	0.08	0.05	-0.10	0.04	-0.11
Loin eye area	0.07	-0.09	0.01	0.16	0.03	-0.07	0.55***
Warner-Bratzler Shear force	0.28*	0.10	-0.20	0.29*	0.20	0.13	0.12

Level of significance: * P<0.05, ** P<0.01, *** P<0.001

In addition, the number of muscle fibers was positively correlated with loin eye area (LEA). This observation probably resulted from the prenatal myogenesis. Generally, the number of muscle fibers is determined before birth. Muscle fibers grow in size towards a plateau, whereas fiber number remains constant after initial increase shortly after birth (Rehfeldt et al., 2000). In understand the effect of fiber type composition, this study analyzed the relationships between histochemical characteristics and meat quality traits as shown in table 4. The results showed that type I fiber was positively correlated to WBS.

Conclusions

This study shows that pH value reflect the early post-mortem metabolic rate are reliable parameters for prediction ultimate pork quality. These results imply that accelerated metabolic rate and poor meat quality are partially explained by the decline of pH in muscle during early post-mortem.

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