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Comparative study of the histochemical properties, collagen content and architecture of the skeletal muscles of wild boar crossbred pigs and commercial hybrid pigs

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ABSTRACT

The histochemical properties, collagen content and architecture of *Musculus longissimus thoracis* (LT), *Musculus pectoralis profundus* (PP) and *Musculus biceps femoris* (BF) were compared in F_1 (half blood) and F_2 (quarter blood) wild boar crossbred pigs and commercial hybrid pigs, and Japanese wild pigs. F_1 pigs showed the lowest growth rate, followed by F_2 pigs. The most rapid growth was shown by the commercial pigs. The percentage weights of LT and PP muscle to body weight were larger in the wild boar crossbred pigs than commercial pigs. The muscles of the crossbred pigs contained type I and IIA myofibers at higher frequency and type IIB at lower frequency than the commercial pigs, except for LT muscle of F_2 pigs. The myofiber diameter in each type of muscle did not differ between pigs except for the smaller type IIA in BF muscle in commercial pigs. The total amount of intramuscular collagen was less in LT muscles than the others. More intramuscular collagen was found in the wild boar crossbred pigs than the commercial pigs in LT and PP muscles. With an increase of collagen content, the perimysial collagen architecture developed but not the endomysial architecture. Traits characteristic of the crossbred pigs seem to be inherited from the wild boar. Our results clarify that cross breeding with wild boar results in pigs with distinctive muscle characteristics in terms of histochemical properties, collagen content and architecture.

1. Introduction

Myofibers are categorized as type I (slow-twitch oxidative), IIA (fast-twitch oxidative glycolytic) or IIB (fast-twitch glycolytic), based on its contractile and metabolic properties (Brooke & Kaiser, 1969; Peter, Barnard, Edgerton, Gillespie, & Stempel, 1972). Type I myofiber repeats a slow contraction for a prolonged period for posture maintenance. Type IIA myofiber repeats a fast contraction in a more powerful motion, and type IIB plays a main role in strong but instantaneous action in rapid locomotion (Hopeler, 1990). The myofiber composition varies in skeletal muscles depending on functional demand (Gotoh, Iwamoto, Nakanishi, Umetsu, & Ono 1999; Suzuki & Tamate, 1988) and environmental factors (Gentry, McGlone, Miller, & Blanton 2004), resulting in variable meat quality (Karlsson, Klont, & Fernadez 1999). In the skeletal muscles of pigs, the typical pattern of myofiber type arrangement has been reported; type I occupies the central position surrounded by type IIA and type IIB distributed outside of the former type (Iwamoto, Kawaida, Ono, & Takahara, 1983; Lefaucheur, Edom, Ecolan, & Butler-Browne, 1995).

Collagen, one of the main components of muscular connective tissue, plays an important role in binding the myofibers into fascicules, and finally skeletal muscle, for preventing disorganization, and propagating contraction power of the myofibers to the bone level during activity (Borg & Caulfield, 1980; Vellema, 1999). Types I and III collagens exist predominantly in skeletal muscle where type I makes up 70–80% of the total collagen and type III makes up 10–20% (Listrat, Picard, & Geay, 1999). Development of collagen architecture during growth, regardless of the total content, makes the

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meat tougher (Fang, Nishimura, & Takahashi, 1999; Light, Champion, Voyle, & Bailey, 1985). Within the animal body, each muscle has distinctive features of collagen content and architecture (Nakamura et al., 2003a). Collagen content is intimately related to meat toughness (Torrescano, Sanshez-Escalante, Gimenez, Roncales, & Beltran, 2003). Collagen fibrils and bundles in endomysia are woven into a fine felt-like wall for encircling individual myofibers, and in the perimysia they usually develop into large bundles (fibers) showing longitudinal, circular or oblique striations around myofiber bundles (Nakamura et al., 2003a; Nakamura et al., 2004a,2004b; Oshima et al., 2007).

The domestic pig, *Sus scrofa domesticus*, was domesticated from the European wild pig, *Sus scrofa scrofa*, and the Asian wild pig, *Sus scrofa vittatus*, long ago and has been continually bred for improved meat production ability to the present day. Through domestication and breed improvement, the domestic pig has attained different characteristics in myofiber composition of skeletal muscle and capillary density around myofibers, compared to the wild type animal (Ashmore, Tompkins, & Doerr 1972; Essen-Gustavsson & Lindholm, 1984; Ruusunen & Puolanne, 2004). Breed and strain differences in myofiber type composition, collagen content and architecture have been reported in domestic pigs (Chang et al., 2003; Iwamoto, Ono, Kawaida, & Takahara 1989; Iwamoto et al., 1983), cattle (Iwamoto, Gotoh, Nishimura, Ono, & Takahara 1999), and chickens (Nakamura et al., 2004a; Oshima et al., 2007).

A darker and tougher meat is often selected for the traditional Japanese method of cooking meat by boiling it with vegetables. Japanese native breed cocks have been crossed with various meat-type hens to produce darker chicken meat (Iwamoto et al., 1997, 1998). Here, Japanese wild boars were crossed with Large White sows to produce a F_1 hybrid strain (half blood) and crossed again to produce a F_2 hybrid strain (quarter blood). The histochemical properties, collagen content and architecture of three muscles were compared between the wild boar crossbred pigs and commercial hybrid pigs to evaluate the crossbreeding effect.

2. Materials and methods

2.1. Animals and muscle samples

A Japanese wild boar crossbred F_1 strain was produced by crossing Large White sows and the F_2 strain by crossing a F_1 boar with Large White sows at the Livestock Station, Fukuoka Agricultural Research Center. Commercial hybrid pigs were produced by crossing a Duroc boar with (Large White ♂ × Landrace ♀) sows at the Experimental Farm, Saga University. The male piglets of F_1, F_2 and commercial hybrid strains, were castrated and reared together with the female piglets in the pen house of each farm. Standard maintenance diets were fed for growth to 100 kg live weight. Two wild pigs were caught using a trap in the orange orchard of the Saga University Farm in December 2003.

After slaughter, the carcasses were chilled for several hours. Then *Musculus longissimus thoracis*, *Musculus pectoralis profundus* and *Musculus gluteobiceps* were extracted and weighed after removal of peripheral adipose tissue and tendons. Two tissue samples of about 1 cm^3, with parallel myofiber striation, were cut out from the core portion of *M. longissimus thoracis* (LT) at level of the 11th thoracic vertebra, the core portion of *M. pectoralis profundus* (PP), and the core portion of the cranial part of *Musculus biceps femoris* (BF) in the *gluteobiceps* muscle. One of the samples was frozen with dry ice-acetone mixture and stored at −50 °C until histochemical preparation. The other sample was fixed in 4% glutaraldehyde/4% paraformaldehyde in 0.02 M phosphate buffer solution (pH 7.4) for several days at 4 °C. Additional samples (5 g or more) were taken off the epimysium, frozen with dry ice and stored at −50 °C until used for measuring the total amount of collagen.

2.2. Histochemical methods

Frozen cross sections 8 μm thick were stained by the histochemical reactions for myosin adenosine triphosphatase (ATPase) activities (Padykula & Herman, 1955), after acid (pH 4.3) or alkaline (pH 10.5) pretreatment (Brooke & Kaiser, 1969), and reduced nicotinamide adenine dinucleotide dehydrogenase (NADH-DH) activity (Okamoto, Ueda, Maeda, Mizutani, & Sugiyama, 1976). Using microscopic photographs (250×) of the specimen, a total of 400–600 myofibers in each sample were categorized into types I, IIA and IIB (Brooke & Kaiser, 1969). The number of myofibers in each type was counted for the calculation of percentage distribution. The diameters of 100 myofibers in each type were measured as the maximum width of the short axis perpendicular to the long axis (Oshima et al., 2007).

2.3. Determination of total collagen content

A sample solution was prepared according to the method of Hill (1966). After isopropanol, an oxidant solution (7% w/v chloramines T, 1 volume, and acetate/citrate buffer, pH 6.0, 3 vol.) and Ehrlich's reagent were added to each sample, the sample was then incubated at 60 °C for 25 min, cooled to 20 °C, and diluted with isopropanol. Within 4 h of sample preparation, spectrophotometric determination of the hydroxyproline at 560 nm was carried out (Bergman & Loxley, 1963). Because skeletal muscle collagen contains 13.3% hydroxyproline, total collagen content was calculated by multiplying the hydroxyproline content by 7.25 (Cross, Carpenter, & Smith, 1973; Goll, Hoerstra, & Bray 1963). To measure collagen content, three replicates were used for each muscle sample.

2.4. Scanning electron microscopy

The fixed material was sliced transversely with a razor and macerated in 2 N NaOH for 5 days, using slightly modified methods of Ohtani, Ushiki, Taguchi, & Kikuta, 1988. The macerated specimens were rinsed in distilled water for 3 days at 25 °C, treated with 1% tannic acid for 2 h, and postfixed with 1% osmium tetraoxide solution for 2 h. After dehydration in a graded series of ethanol, the specimens were placed in *t*-butyl alcohol and freeze-dried (TIS-U-DRY, FIS Systems, New York, USA) (Inoue & Osatake, 1988). The specimens were mounted on aluminum holders and

Table 1
Age, body weight and muscle weights in F_1, F_2 general and wild pigs

Breed	F_1	F_2	Commercial	Wild pig
No of animals	9	8	4	2
Age (days)	229 ± 1[a]	196 ± 6[b]	159 ± 3[c]	–
Body weight (kg)	92 ± 2[b]	105 ± 2[a]	103 ± 2[a]	38 ± 1
M. longissimus thoracis				
Absolute weight (kg)	1.75 ± 0.02[b]	2.06 ± 0.04[a]	1.77 ± 0.07[b]	0.59 ± 0.07
% of body weight	1.91 ± 0.03[a]	1.96 ± 0.04[a]	1.72 ± 0.05[b]	1.57 ± 0.24
M. pectoralis profundus				
Absolute weight (kg)	0.47 ± 0.02[b]	0.56 ± 0.03[a]	0.45 ± 0.02[b]	0.21 ± 0.02
% of body weight	0.51 ± 0.02[a]	0.53 ± 0.02[a]	0.43 ± 0.01[b]	0.56 ± 0.07
M. gluteobiceps				
Absolute weight (kg)	1.16 ± 0.04[b]	1.32 ± 0.05[a]	1.22 ± 0.03[b]	0.48 ± 0.03
% of body weight	1.26 ± 0.04[a]	1.26 ± 0.04[a]	1.19 ± 0.02[a]	1.29 ± 0.01

Means ± standard errors.
F_1: Wild boar (♂) × Large White (♀), F_2: (Wild boar (♂) × Large White (♀)) (♂) × Large White (♀), Commercial: Duroc (♂) × (Large White (♂) × Landrace (♀)) (♀) and Wild pig: female yearling.
[a,b,c]Means with the same letter do not differ significantly between pig types.