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Histological characteristics of musculus longissimus dorsi and their correlation with restriction fragment length polymorphism of the myogenin gene in Jinghua × Pietrain F_2 crossbred pigs

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ABSTRACT

PCR-RFLP, periodic acid Schiff (PAS) and myosin heavy chain (MHC) immunohistochemistry were employed to survey the relationships between the genotypes of the myogenin gene (*myog*) and the histological characteristics of the muscle and other postmortem traits in the Jinghua × Pietrain (JP) F_2 crossbred pigs. The muscle fibers were classified into three groups according to their PAS-reactivity: PAS(−), PAS(+∼++), and PAS(+++) and accounted for approximately 16%, 74% and 10% of the total muscle, respectively. MHC immunohistochemistry was used to categorize muscle fibers into either slow or fast fibers; the proportions of slow and fast fibers were 6% and 94%, respectively. Two different cleavage patterns and three genotypes (AA, AB and BB) were identified and their frequencies were 0.4087, 0.4986 and 0.0928, respectively, for the three genotypes and 0.6580 and 0.3420, respectively, for cleavage patterns A and B. Different genotypes were variably associated with cross-sectional area ($p = 0.074$), water-holding capacity ($p = 0.002$), pH ($p < 0.001$) and carcass temperature ($p < 0.001$) of the loin muscle. Different genotypes showed marked correlation with the *L* value ($p < 0.001$), *a* value ($p = 0.002$), and *b* ($p < 0.001$) of the Minolta meat color index. The genotypes were also significantly related to the cross-sectional area ($p < 0.001$), diameter ($p < 0.001$), aspect ratio ($p < 0.001$) and the density ($p < 0.001$) of muscle fibers from the longissimus dorsi muscle. The results also revealed that the genotypes showed no significant association with the circularity ($p = 0.132$) or the percentage of intramuscular connective tissue ($p = 0.193$).

1. Introduction

The *myog* gene is a member of *myod* gene family (Hasty et al., 1993; Olson et al., 1990). The proteins (including myogenin) encoded by these genes control embryonic muscle development (Olson et al., 1990) and regulate the expression of muscle-specific gene by binding to particular microRNA during myogenesis (Rao, Kumar, Farkhondeh, Baskerville, & Lodish, 2006). Myogenin is a muscle-specific transcription factor that can induce myogenesis in a variety of cell types in tissue culture. Mice with a targeted mutation at the myogenin gene showed a severe reduction of all skeletal muscles (Hasty et al., 1993; Nabeshima et al., 1993).

The porcine *myog* gene was physically mapped to chromosome 9q2.1-q2.6 (Ernst, Mendez, Robic, & Rothschild, 1998; Soumillion, Erkens, Lenstra, Rettenberger, & te Pas, 1997; te Pas et al., 1999). PCR-RFLP analysis using *Nla*IV revealed that the *myog* gene existed as at least two different restricted nuclease polymorphic fragments (Mendez, Ernst, & Rothschild, 1997). Southern blot analysis of 105 unrelated pigs revealed that there were three polymorphic *Msp*I sites (Soumillion et al., 1997; te Pas et al., 1999).

In meat-producing animals such as pigs and cattle, the number of myofiber was found to be related to growth capacity and meat quality (Henckel, Oksbjerg, Erlandsen, Barton-gade, & Bejerholm, 1997), properties which were taken into account in breeding programs. The number of muscle fibers present at birth appears to determine the maximal lean meat growth capacity and meat quality of the adult animal, and seems to be dependent on the number of embryonic myocytes (Hanset, Michaux, Dessy-doize, & Buronboy, 1982). The number of embryonic myocytes is however, genetically determined by the *myog* gene (Olson et al., 1990). The genetic variation in this gene is probably responsible for the differences in myoblast and myofiber numbers.

The heritability of meat quality and histological characteristics varies with swine species. Meat quality can be evaluated by several important traits, including appearance, color, taste, fat content, texture, tenderness and its histochemical characteristics (Henckel et al., 1997). It has become evident that many histological characteristics of muscle such as density, diameter, cross-sectional area of the fibers, the percentage of red fibers and glycogen content are

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directly associated with meat quality. Meat with 30% or more glycogen-depleted fibers has a tendency to appear dry, firm and dark (Karlsson, Klont, & Fernandez, 1999). Muscle samples with a high initial pH and a large proportion of PAS-positive fibers are ultimately likely to produce meat of good quality. A high density of small fibers also enhances the quality of the meat. The proportion of intramuscular fat and connective tissue are also important factors which influence the taste of pork.

An investigation into the histological characteristics of the meat, postmortem traits, and the polymorphism of *myog* gene was undertaken to evaluate the influence of different *myog* genotypes on the muscle of JP F_2 crossbred pigs. Up to now, there have been few reports concerning the relationship between the polymorphism of the *myog* gene and the histological characteristics of muscle. The divergence in meat quality and muscle traits between Jinhua and Pietrain was described in a report by Shen and Xu (1984). In order to combine the merit of the Jinhua pig which is a Chinese breed known for the excellent quality of the meat and the Pietrain which is famous for its high percentage of lean meat, a crossbred (JP pig) is commonly used.

2. Materials and methods

2.1. Animals and treatments

Three hundred and forty-five barrows and gilts (Jinhua × Pietrain crossbred F_2) were employed to study the relationships among meat histological characteristics, postmortem traits and the polymorphism of *myog* gene. When pigs had achieved the appropriate slaughter weight, they were electrically stunned (225–380 V, 0.5 A, for 5–6 s) and exsanguinated. The blood samples were collected and the carcasses were eviscerated according to standard commercial procedure. Hot carcass weights were recorded. At 45 min postmortem, carcass length (from the posterior edge of the symphysis pubis to the anterior edge of the first rib) was recorded. A 2 cm × 1 cm × 1 cm clip of longissimus muscle (LM) section was excised on the center region at the level between the first rib and the second rib from last in left side after carcass data was collected. The tissue clips were prefixed in 10% neutral buffer formalin (10% formalin, 29 mM $NaH_2PO_4 \times H_2O$, 46 mM Na_2HPO_4) for 18–24 h. Furthermore, a temperature sensor was inserted into the longissimus dorsi muscle to measure the initial carcass temperature. After that, an incision was made into the longissimus dorsi (LM) and initial muscle pH was recorded with a portable pH meter (Knick, Berlin, Germany). At 24 h postmortem, Minolta color index (*L*, *a* and *b* values) was detected on the cross-section of the loin muscle at the 10th rib with a Minolta chroma meter CR-210 (Minolta Corp., Osaka, Japan). Water-holding capacity (WHC) was computed according to the Carver Press method. The cross-section of loin muscle at the 10/11th rib of carcass was outlined on cellophane paper. The outline was scanned (600 dpi) and the area of the cross-section of loin muscle was analyzed by Image J 1.37 (NIH shareware, available at http://rsb.info.nih.gov/ij/).

2.2. Histological examination

The muscle samples of JP F_2 crossbred pigs were fixed in 10% neutral formalin for another 18 h. The tissue samples were embedded in paraffin blocks and sectioned to 8 μm thickness. The sections were stained with haematoxylin (Shanghai Chemical Co. Ltd., Shanghai, China) and eosin (Shanghai Chemical Co. Ltd., Shanghai, China). The slides were examined by an Olympus microscope and photographed. The resulting photos were analyzed with Image J software (NIH shareware; National Institutes of Health, Bethesda, MA, USA). The diameters of muscle fiber were calculated by the size of the equivalent circle, i.e. diameter $= 2 \times \sqrt{\frac{\text{area}}{\pi}}$. The value of circularity was calculated by the following formula: circularity $= \frac{4 \times \pi \times \text{area}}{\text{perimeter}^2}$. A value of 1.0 indicates that the cross-section of a fiber is a perfect circle. As the value approaches 0.0, it indicates that the cross-section of the fiber is an elongated ellipse or polygon. The aspect ratio of fiber was calculated by dividing the length of the major axis by the length of the minor axis of the cross-section. The fiber density was calculated by dividing the fiber number by the area of interest. The percentage of intramuscular connective tissue (IMCT) was estimated by dividing the area of IMCT (estimated by the total area minus the total area of fibers) by the total area of interest.

2.3. Preparing the Schiff reagents

One gram of basic fuchsin (Shanghai Chemical Co. Ltd., Shanghai, China) was dissolved in 200 mL of boiled distilled water. When the temperature went down to 50 °C, 1 g of sodium pyrosulfite ($Na_2S_2O_5$) and 20 mL of 1 M HCl was mixed into the solution. The reagent was stood for 1–2 days at room temperature in the dark. After that, 2 g of activated charcoal was mixed in the mixture was then filtered with chromatographic filter paper and was stored at 4 °C for later use.

2.4. Periodic acid Schiff reaction

The sections were dewaxed and rehydrated, oxidized in 0.5% periodic acid solution for 5 min, and rinsed in distilled water. The slides were then placed in Schiff reagent for 15 min and washed in tap water for 5 min. Subsequently, they were counterstained with Mayer's haematoxylin and dehydrated, mounted with a synthetic medium. The slides were examined by an Olympus microscope and photographed. The photos were analyzed by Image J software. The PAS-reactive of fibers was classified into three groups with the following cutoff points: PAS(−) fiber is stainless in PAS, the value for its grayscale is greater than 176 (i.e. optical density or OD < 0.16); PAS(+++) fiber is stained deep red in PAS, the value for its grayscale is less than 128 (i.e. OD > 0.30); PAS(+∼++) fiber is stained pink, the value for its grayscale ranges from 128 to176 (i.e. $0.16 \leqslant \text{OD} \leqslant 0.30$).

2.5. Immunohistochemistry

Mouse anti-myosin heavy chain antibody NCL-MHCs (Novocastra Laboratories Ltd., Newcastle, Tyne, UK) and NCL-MHCf (Novocastra Laboratories Ltd., Newcastle, Tyne, UK) were employed as the primary antibodies. After deparaffination and rehydration, endogenous peroxidase activity was blocked with 3% H_2O_2. Antigen retrieval was performed by immersing the sections into EDTA antigen retrieval solution (1.0 mM EDTA, pH 8.0) for 30 min at 98 °C in a water bath, and allowed to cool naturally. The sections were immersed into cool PBS (7.7 mM Na_2HPO_4, 2.7 mM $HaHPO_4$, 154 mM NaCl; pH 7.2) for 10 min and incubated with 0.1% trypsin (Biobasic Inc., Markham, ON, Canada) in PBS for 5 min at 37 °C. The sections were then incubated with 10% normal goat serum (Dingguo Biotechnology Inc., Beijing, China) for 30 min at room temperature to block nospecific binding. Subsequently, they were incubated with primary antibody at 4 °C overnight at a dilution of 1:60. Substitution of PBS for the primary antibody served as a negative control. This was followed by incubation with biotin-conjugated rabbit anti-mouse IgG reagent (Dingguo Biotechnology Inc., Beijing, China) for 60 min at 37 °C. After that, the sections were incubated with horseradish peroxidase-conjugated streptin (Dingguo Biotechnology Inc., Beijing, China) for 40 min at 37 °C. Between each step, sections were washed three times in PBS. The reaction was visualized with 3,3′-diaminobenzidine tetrahydro-