African Journal of Biotechnology Vol. 9(44), pp. 7568-7572, 1 November, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.704
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Expression of porcine myosin heavy chain 1 gene in Berkshire loins with a high pH^{24} value

Jin Hun Kang[1#], Woo Young Bang[1#], Eun Jung Kwon[1], Yong Hwa Lee[1], Da Hye Park[1], Eun Seok Cho[1], Min Ji Kim[1], Jong-Soon Choi[1,2], Hwa Chun Park[3], Beom Young Park[4] and Chul Wook Kim[1]*

[1]Swine Science and Technology Center, Jinju National University, Jinju 660-758, Korea.
[2]Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 305-764, Korea.
[3]Pig Breeding Company, Namwon 590-831, Korea.
[4]National Institute of Animal Science, RDA, Suwon 441-350, Korea.

Accepted 4 October, 2010

An important factor in evaluating porcine meat quality is the pH at post-mortem 24 h (pH^{24}). In the current work, we conducted a GeneFishing polymerase chain reaction (PCR)–based screen to identify differentially expressed genes in Berkshire loins with high versus low pH^{24}. Total RNAs were extracted from Berkshire loins with high and low pH^{24} and subjected to GeneFishing PCR. Four genes were found to be differentially expressed in the high and low pH^{24} groups. Semi-quantitative reverse transcription (RT)-PCR confirmed that *MYH1* encoding myosin heavy chain 1 was specifically expressed in high pH^{24} groups. These results are very useful in establishing the genetic relationship between the composition of muscle fiber type and pH^{24} in the Berkshire loin.

Key words: GeneFishing™ polymerase chain reaction, myosin heavy chain 1, pork meat quality, post-mortem pH value, pale, soft and exudative.

INTRODUCTION

Porcine meat quality is severely affected by a condition known as PSE, which stands for “pale, soft and exudative,” adjectives that describe the resultant poor quality of the meat. This condition, which is very problematic in the pork industry (Barbut et al., 2008), is developed in the muscle as a result of the accelerated glycolysis that occurs during the early post-mortem stage and is also closely related to the rapid initial post-mortem decline in pH (Bowker et al., 2000).

The mechanism underlying PSE development is thought to be anaerobic glycolysis in muscle tissues (Bowker et al., 2000). Specifically, the circulatory failure caused by exsanguination after slaughter results in lack of the oxygen that is required for aerobic glycolysis, leading to an adenosine triphosphate (ATP) homeostatic imbalance in muscle tissues. To maintain the cellular ATP concentration under anoxic conditions, muscle glycogen is metabolized via anaerobic glycolysis, which is less efficient at generating ATP than aerobic glycolysis. Consequently, levels of glycogen and ATP decrease and the lactic acid, a waste product of the anaerobic glycolysis, accumulates lowering muscle pH (Briskey and Wismer-Pedersen, 1961; Kastenschmidt et al., 1968). In particular, the rapid initial post-mortem decline in pH stimulates the denaturation of muscle proteins, leading to the development of PSE in muscle (Bowker et al., 2000). The pH at post-mortem 24 h (pH^{24}), a common indicator for the evaluation of pork quality, can be used to monitor PSE. It shows high genetic correlations with other meat

*Corresponding author. E-mail: cwkim@jinju.ac.kr. Tel: +82-55-751-3281. Fax: +82-55-759-1893.

Abbreviations: ACP, Annealing control primer; **pH^{24},** pH at post-mortem 24 h; **ATP,** adenosine triphosphate; **DEG,** differentially expressed gene; **MYH1,** myosin heavy chain 1; **PCR,** polymerase chain reaction; **RT-PCR,** reverse transcription-PCR; **PSE,** pale, soft and exudative; **RT,** reverse transcription.

#These authors contributed equally to this work.

quality parameters such as drip loss, cooking loss, water-holding capacity and meat tenderness (Nguyen et al., 2006; Duan et al., 2009). In the current study, we first tried to screen the differentially expressed genes (DEGs) in Berkshire breeds with high versus low pH^{24} to identify the pH^{24}-responsible genes, using annealing control primer (ACP)-based GeneFishing PCR (Kim et al., 2004). From the screen, four porcine genes were discovered to be differentially expressed in high and low pH^{24} lines. The *MYH1* gene encoding myosin heavy chain 1 was confirmed to be highly expressed in Berkshire loins with excellent meat quality and a high pH^{24} value. Collectively, our results provide insight into the genetic relationship between pH^{24} and muscle fiber type composition and suggest that *MYH1* may be a novel pH^{24}-responsible gene.

MATERIALS AND METHODS

Animals and meat samples

Three hundred and twenty three Berkshire breeds were bred under same condition (Da-San-Jong-Don Co. Ltd., Namwon, Korea) and then slaughtered in 10 batches when their body weight reached 110 kg. Subsequently, the samples were used for meat quality evaluation, GeneFishing™ PCR and semi-quantitative RT-PCR.

Meat quality evaluation

The analysis was carried out according to AOAC (Association of Official Analytical Chemists) method. The contents of protein, lipid, water and collagen in Berkshire loins were measured by FOSS (Foodscan Near-Infrared Spectrophotometer), and the meat quality parameters such as pH^{24} value, water-holding capacity, drip loss and cooking loss were determined by previously described methods (Prevolnik et al., 2009; Tan et al., 2009).

First-strand cDNA synthesis

Total RNAs extracted from frozen samples were used for the synthesis of first-strand cDNAs by reverse transcriptase. Reverse transcription was performed for 1.5 h at 42°C in a final reaction volume of 20 µl containing 3 µg of the purified total RNA, 4 µl of 5' reaction buffer (Promega, Madison, WI, USA), 5 µl of dNTPs (2 mM each), 2 µl of 10 µM dT-ACP1 (5'-CGTGAATGCTGCGA CTACGATIIIIIT(18)-3'), 0.5 µl of RNasin® RNase Inhibitor (40 U/µl; Promega) and 1 µl of Moloney murine leukemia virus reverse transcriptase (200 U/µl; Promega). First-strand cDNAs were diluted by the addition of 80 µl of ultra-purified water for the GeneFishing™ PCR and stored at -20°C until use.

ACP (annealing control primer)-based GeneFishing™ PCR

DEGs were screened by ACP-based PCR method using the GeneFishingTM DEG kits (Seegene, Seoul, South Korea) (Kim et al., 2004). Briefly, second-strand cDNA was synthesized at 50°C during one cycle of first-stage PCR in a final reaction volume of 20 µl containing 3 - 5 µl (about 50 ng) of diluted first-strand cDNA, 1 µl of dT-ACP2 (10 µM), 1 µl of 10 µM arbitrary ACP and 10 µl of 2 × Master Mix (Seegene, Seoul, South Korea). The PCR protocol for second-strand synthesis was one cycle at 94°C for 1 min, followed by 50°C for 3 min and 72°C for 1 min. After second-strand DNA synthesis was completed, the second-stage PCR amplification protocol was 40 cycles of 94°C for 40 s, followed by 65°C for 40 s, 72°C for 40 s and 5 min final extension at 72°C. The amplified PCR products were separated in 2% agarose gel and stained with ethidium bromide.

Cloning and sequencing

The differentially expressed bands were extracted from the gel by using the GENCLEAN® II Kit (Q-BIO gene, Carlsbad, CA, USA), and directly cloned into a TOPO TA cloning vector (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cloned plasmids were sequenced with ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Semi-quantitative RT-PCR

The differential expression of DEGs was confirmed by RT-PCR using *MYH1*-specific primers. The first-strand cDNA was normalized by the pig *GAPDH* gene. The normalized cDNA was used as a template. The PCR reaction was conducted in a final reaction volume of 20 µl containing 2 - 4 µl (about 50 ng) of diluted first-strand cDNA, 1 µl of each MYH1-specific primer (10 µM) (forward, 5'-AGCAGGAGCTGATTGAGACC-3'; reverse, 5'-TTCAGTCTGA AGCTGGGACA-3') and 10 µl of 2× Master Mix (Seegene, Seoul, South Korea). The PCR amplification protocol was an initial 3 min denaturation at 94°C, followed by 20 - 25 cycles of 94°C for 40 s, 60°C for 40 s, 72°C for 40 s and a 5 min final extension at 72°C. The amplified PCR products were separated in 2% agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

Excellent meat quality in Berkshire loins with a high pH^{24} value

Berkshire breeds are known to have excellent meat quality (Suzuki et al., 2003), and hence their loins were used to identify the DEGs in the loins with high vs. low pH^{24} values. To evaluate meat quality, 323 Berkshire breeds were raised under the same conditions (Da-San-Jong-Don Co. Ltd., Namwon, Korea) and then slaughtered in 10 batches when their body weight reached 110 kg. Subsequently, the sample loins were divided into high (pH >5.88) and low (pH <5.88) pH^{24} groups and then evaluated for meat quality using standard methods (AOAC: Association of Official Analytical Chemists; Prevolnik et al., 2009; Tan et al., 2009). Mean values for meat quality parameters (pH^{24}, water-holding capacity, drip loss and cooking loss) for representative loins with high and low pH^{24} are shown in Table 1. Noticeably, most meat with high pH^{24} value exhibited a higher water-holding capacity and lower drip and cooking losses than did the meats with low pH^{24} value. These data demonstrate that the Berkshire loins with a high pH^{24} are meats with excellent quality compared to those of the low pH^{24} group and suggest that the high pH^{24} value may be used as a meat quality parameter for identifying excellent Berkshire loins.

Table 1. Meat quality traits of Berkshire loins

Sample group[a]/number		pH^{24} value[b]	Water-holding capacity (%)	Drip loss (%)	Cooking loss (%)
High group	H1	6.36	56.22	1.39	21.31
	H2	6.31	55.75	1.87	25.75
	H3	6.23	58.69	1.37	22.49
	H4	6.06	56.25	3.86	22.40
	H5	6.23	61.04	1.91	21.28
	H6	6.24	61.72	1.95	29.09
	H7	6.30	63.22	1.28	28.03
	H8	6.30	61.95	1.61	24.00
	H9	6.46	59.96	1.78	24.87
	H10	6.35	60.96	1.84	24.90
Average[c] (n = 10)		6.28	59.58	1.89	24.41
Low group	L1	5.65	56.31	8.79	28.70
	L2	5.61	55.20	8.78	30.61
	L3	5.57	57.07	8.81	30.77
	L4	5.53	55.72	6.92	30.02
	L5	5.48	55.20	8.35	29.48
	L6	5.63	56.24	6.55	29.30
	L7	5.63	56.36	6.16	24.89
	L8	5.46	55.11	14.38	30.90
	L9	5.63	60.93	8.22	27.15
	L10	5.64	56.81	7.54	31.91
Average[d] (n = 10)		5.58	56.49	8.45	29.37
Total average[e] (n = 323)		5.88	58.05	4.33	28.20

[a]Berkshire loins were divided into high (pH >6.0) and low (pH < 5.7) pH^{24} groups; [b]pH^{24} indicates the pH value, measured at post-mortem 24 h; [c,d]Averages represent mean values for meat quality parameters of high (n = 10) and low (n = 10) pH^{24} groups, respectively; [e]Total average represents mean value for meat quality parameters of 323 Berkshire breeds slaughtered.

Screening of differentially expressed genes (DEGs) in Berkshire loins with high vs. low pH^{24}

Next, to identify the DEGs, the total RNAs extracted from loins with high and low pH^{24} were used for GeneFishing PCR (Kim et al., 2004) with a combination of six ACPs. As shown in Figure 1, of the six ACPs, four showed differentially expressed DNA bands, in which two bands increased and two bands decreased in intensity in the high pH^{24} loin when compared with the low pH^{24} loin. The sizes of the bands varied from 200 to 700 bp (Figure 1). Subsequently, the four differentially expressed DNA bands were purified from the agarose gels and cloned into TOPO TA cloning vectors for sequencing analysis. The National Center for Biotechnology (NCBI) Basic Local Alignment Search Tool (BLAST) searches revealed that the four DEGs were matched with known genes: ACP1 with *MYH1* (myosin heavy chain 1; Acc. No. NM 00110495.1), ACP2 with *SMPX* (small muscle protein, X-linked; Acc. No. NM_001078687), ACP3 with *MYH2b* (myosin heavy chain type 2b; Acc. No. NM001123141.1) and ACP4 with *HSP40* (heat shock protein 40; Acc. No. AK237314.1).

High level of MYH1 gene expression in Berkshire loins with high pH^{24}

Noticeably, of the four DEGs, ACP1 and ACP3 encoding myosin heavy chain isoforms, showed significant increases in band intensity in the loin with high pH^{24} (Figure 1). A few recent studies have suggested that the myosin heavy chain composition of muscles has an important effect on meat quality in pigs (Chang et al., 2003; Hu et al., 2008; Wimmers et al., 2008). In particular, the ACP1 encoding myosin heavy chain 1 (MYH1) isoform is reported to be a critical factor for muscle growth and meat quality (Chang et al., 2003; Hu et al., 2008), suggesting that porcine *MYH1* may be closely associated with the high pH^{24} levels found in Berkshire loins of excellent quality. To prove the hypothesis, we sought to determine whether *MYH1* is highly expressed in the high pH^{24} group (H1 to H10) with excellent meat quality. For this, total RNAs extracted from high (H1 to H10) and low (L1 to L10) pH^{24} groups in Table 1 were used for semi-quantitative RT-PCR with *MYH1*-specific primers. As shown in Figure 2, the *MYH1* gene was expressed more significantly in the high pH^{24} group than in the low pH^{24}

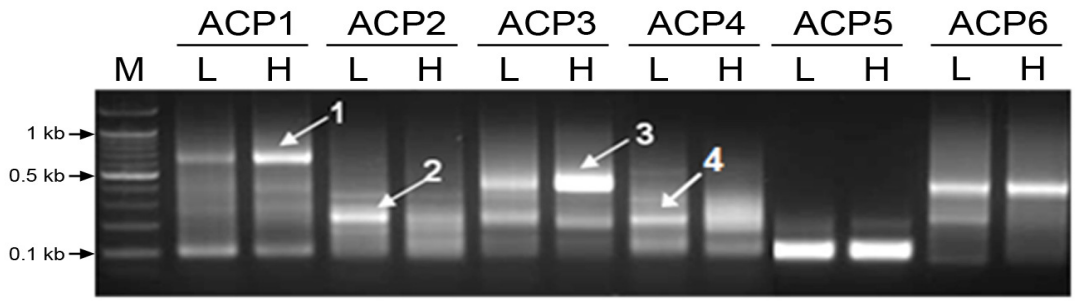

Figure 1. GeneFishing PCR results for identifying differentially expressed genes in Berkshire loins with high vs. low pH^{24} using six arbitrary annealing primers (ACP1 to ACP6). Total RNAs were extracted from Berkshire loins with high and low pH^{24} using TRI-Reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions and were then subjected to ACP-based PCR using the GeneFishingTM DEG kits (Seegene, Seoul, South Korea) (Kim et al., 2004). Lane M, L and H indicate 100-bp size marker, low pH^{24} and high pH^{24}, respectively. The differentially expressed PCR products are indicated as arrows (numbers 1 to 4).

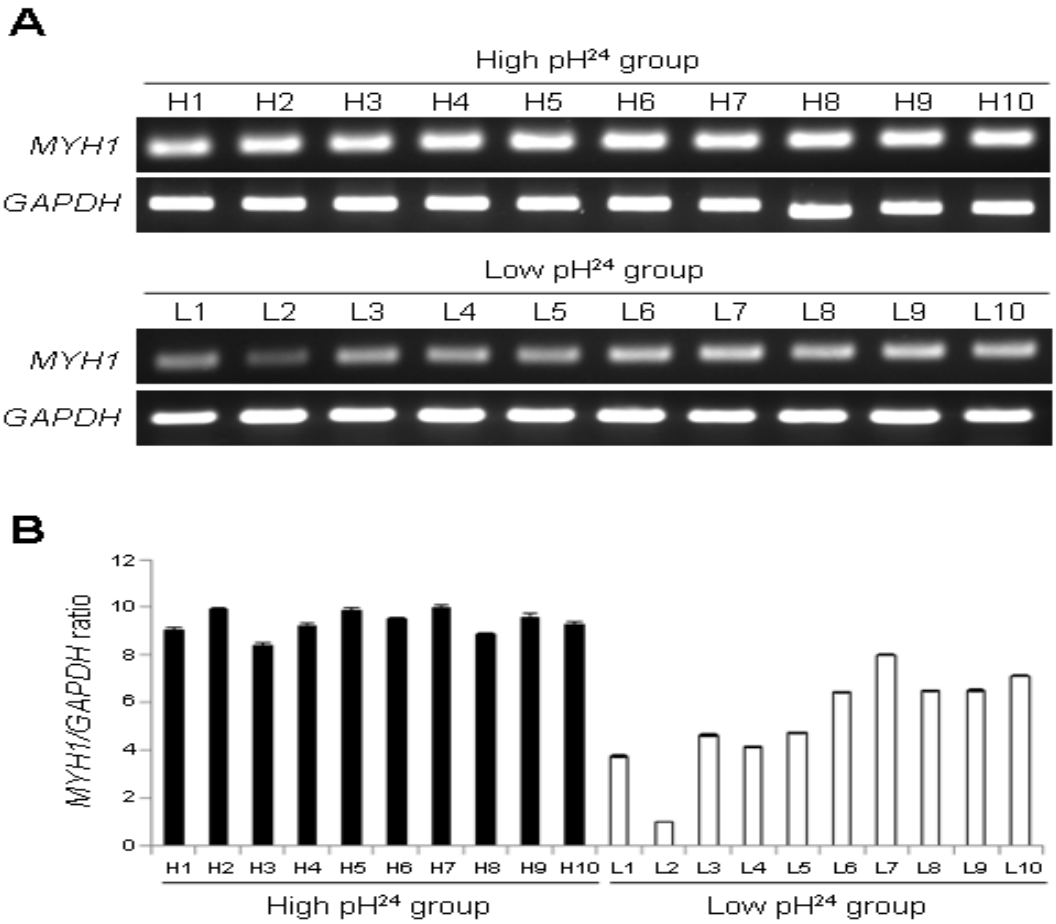

Figure 2. Confirmation of the significant expression of *MYH1* gene in Berkshire loins with a high pH^{24} value. (A) Semi-quantitative RT-PCR analysis of *MYH1* transcript level in Berkshire loins with high versus low pH^{24} values. For each reverse transcription reaction, total RNAs were extracted from each high pH^{24} (H1 to H10) and low pH^{24} (L1 to L10) groups; they were then used for first-strand cDNA synthesis and finally subjected to PCR using *MYH1*-specific primers (forward, 5'-AGCAGGAGCTGATTGAGACC-3'; reverse, 5'-TTCAGTCTGAAGCTGGGACA-3'). The *GAPDH* level was used as an internal control to normalize the amount of cDNA template. The RT-PCR analysis was carried out at least three times, with similar results. (B) Measurement of the *MYH1*/*GAPDH* ratio. The relative intensities of the *MYH1* and *GAPDH* bands in (A) were measured with a densitometer.

group. Collectively, our data demonstrate that porcine *MYH1* is more highly expressed in the high pH^{24} group, suggesting that it may be a novel pH^{24}-responsible gene. Porcine skeletal muscles are heterogeneous tissues consisting of different types of fibers. Traditionally, they contain three types of muscle fibers: type I (red; slow-twitch), type IIB (white; fast-twitch) and type IIA (red; fast-twitch) (Bowker et al., 2000; Eggert et al., 2002). Type I fibers are generally oxidative, whereas type IIB fibers are glycolytic, meaning that the type I fiber is much less susceptible to accelerated post-mortem glycolysis and PSE development than type IIB (Bowker et al., 2000; Eggert et al., 2002). Type IIA fiber is intermediate between type I and IIB fibers with respect to the post-mortem metabolism (Hamalainen and Pette, 1993). Muscle fiber type composition has been reported to have important effects on post-mortem metabolism during the conversion of muscle to meat (Eggert et al., 2002; Lefaucheur et al., 2004). The muscle fiber type composition may also be closely related to the pH^{24} value.

In this study, the *MYH1* gene, which is reported to be a main component for the low-twitch high-oxidative type I fiber (Termin et al., 1989; Gil et al., 2001), was highly expressed in Berkshire groups associated with high pH^{24}. This finding suggests that muscles of the high pH^{24} groups with excellent meat quality may commonly have a high level of type I fibers composed of myosin heavy chain 1 isoforms. Taken together, our results provide a starting point for the understanding of the genetic relationship between pH and muscle fiber type composition after slaughter. Furthermore, the association between high pH^{24} and *MYH1* expression may have important industrial applications for recognizing and promoting excellent quality in Berkshire loins.

ACKNOWLEDGEMENTS

This work was supported by grants from the Priority Research Centers Program (Code #2009-0093813) of the Ministry of Education, Science and Technology, and the BioGreen 21 Program (Code #20080401034059) of Rural Development Administration of Korea.

REFERENCES

Barbut S, Sosnicki AA, Lonergan SM, Knapp T, Ciobanu DC, Gatcliffe LJ, Huff-Lonergan E, Wilson EW (2008). Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Sci. 79: 46-63.

Bowker BC, Grant AL, Forrest JC, Gerrard DE (2000). Muscle metabolism and PSE pork. J. Anim. Sci. 79: 1-8.

Briskey EJ, Wismer-Pedersen J (1961). Biochemistry of pork muscle structure. I. Rate of anaerobic glycolysis and temperature change versus the apparent structure of muscle tissue. J. Food Sci. 26: 297-305.

Chang KC, Da Costa N, Blackley R, Southwood O, Evans G, Plastow G, Wood JD, Richardson RI (2003). Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. Meat Sci. 64: 93-103.

Duan YY, Ma JW, Yuan F, Huang LB, Yang KX, Xie JP, Wu GZ, Huang LS (2009). Genome-wide identification of quantitative trait loci for pork temperature, pH decline, and glycolytic potential in a large-scale White Duroc x Chinese Erhualian resource population. J. Anim. Sci. 87: 9-16.

Eggert JM, Depreux FFS, Schinckel AP, Grant AL, Gerrard DE (2002). Myosin heavy chain isoforms account for variation in pork quality. Meat Sci. 61: 117-126.

Gil M, Serra X, Gispert M, Angels Oliver M, Sanudo C, Panea B, Olleta JL, Campo M, Olivan M, Osoro K (2001). The effect of breed-production systems on the myosin heavy chain 1, the biochemical characteristics and the colour variables of longissimus thoracis from seven spanish beef cattle breeds. Meat Sci. 58: 181-188.

Hamalainen N, Pette D (1993). The histochemical profiles of fast fiber types IIB, IID, and IIA in skeletal muscles of mouse, rat, and rabbit. J. Histochem. Cytochem. 41: 733-743.

Hu HM, Wang JY, Zhu RS, Guo JF, Wu Y (2008). Effect of myosin heavy chain composition of muscles on meat quality in Laiwu pigs and Duroc. Sci. China Ser C: Life Sci. 51: 127-132.

Kastenschmidt LL, Hoekstra WG, Briskey EJ (1968). Glycolytic intermediates and co-factors in fast and slow-glycolyzing muscles of the pig. J. Food Sci. 33: 151-158.

Kim YJ, Kwak CI, Gu YY, Hwang IT, Chun JY (2004). Annealing control primer system for identification of differentially expressed genes on agarose gels. Biotechniques, 36: 424-426.

Lefaucheur L, Milan D, Ecolan P, Le Callennec C (2004). Myosin heavy chain composition of different skeletal muscles in Large White and Meishan pigs. J. Anim. Sci. 82: 1931-1941.

Nguyen NH, McPhee CP, Trout GR (2006). Responses in carcass lean pH at 24 hours post-mortem in lines of Large White pigs selected for growth rate on a fixed ration over a set time. Livest. Prod. Sci. 100: 84-90.

Prevolnik M, Andek-Potokar M, Novi M, Korjanc D (2009). An attempt to predict pork drip loss from pH and colour measurements or near infrared spectra using artificial neural networks. Meat Sci. 83: 405-411.

Suzuki K, Shibata T, Kadowaki H, Abe H, Toyoshima T (2003). Meat quality comparison of Berkshire, Duroc and crossbred pigs sired by Berkshire and Duroc. Meat Sci. 64: 35-42.

Tan B, Yin Y, Liu Z, Li X, Xu H, Kong X, Huang R, Tang W, Shinzato I, Smith SB (2009). Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs. Amino Acids, 37: 169-175.

Termin A, Staron RS, Pette D (1989). Myosin heavy chain isoforms in histochemically defined fiber types of rat muscle. Histochem. Cell Biol. 92: 453-457.

Wimmers K, Ngu NT, Jennen DGJ, Tesfaye D, Murani E, Schellander K, Ponsuksili S (2008). Relationship between myosin heavy chain isoform expression and muscling in several diverse pig breeds. J. Anim. Sci. 86: 795-803.