The Effects of Stress Related Genes on Carcass Traits and Meat Quality in Pigs

H. J. Jin*, B. Y. Park[1], J. C. Park[1], I. H. Hwang[2], S. S. Lee[3], S. H. Yeon, C. D. Kim
C. Y. Cho, Y. K. Kim, K. S. Min[4], S. T. Feng[5], Z. D. Li[6], C. K. Park[7] and C. I. Kim[7]
Animal Genetic Resources Station, National Livestock Research Institute, RDA, Namwon, 590-832, Korea

ABSTRACT : The current study was conducted to investigate the relationship between stress related gene and meat quality in pigs. A total number of 212 three-way cross bred (Landrace-Yorkshire×Duroc) and 38 Duroc were sampled from the Korean pig industry to determine genotype frequency of porcine stress syndrome (PSS) and heat shock protein 70 kDa (HSP70) genes and their relationship with carcass traits and longissimus meat quality. Screen of HSP70 was performed by the single strand conformation polymorphism (SSCP) technique. Based on the analysis of restriction fragment length polymorphism (RFLP) in ryanodine receptor 1 (RYR1) gene, genetic disorder of PSS was related to a mutation at 18,168th (C to T) of exon 17. There was no significant difference in ultimate meat pH and backfat thickness between HSP70 K1-AA type and -BB type in pure Duroc breed. In Landrace-Yorkshire×Duroc (L-YxD) cross bred pig, our results indicated that HSP70 derivate type in Duroc had a limited effect on backfat thickness, but L-YxD type had a noticeable linkage with HSP70 K1-AA and K3-AB. This tendency was also observed in hot carcass weight where HSP70 K1-AA and K3-AB resulted in heavier weight with 86.3 kg compared to HSP70 K1-AB and K3-BB of 74.3 kg. Results imply that stress related HSP70 genotype has a potential association with backfat thickness and carcass weight. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 2 : 280-285)***

Key Words : Pig, PSS, HSP70 Gene, PCR-SSCP and Meat Quality

INTRODUCTION

Extensive research has been performed to reduce the most notorious quality defect of pale, soft and exudative (PSE) pork over last decades (Forrest et al., 1997; Rosenvold and Andersen, 2003; Kim et al., 2005). It has been identified that PSE meat is related to a fast glycolysis during the onset of rigor under which condition protein denaturation occurs (Cassens et al., 1975) which consequently reduce water holding capacity (WHC) and juiciness (Cheah et al., 1998; Gispert et al., 2000).

On the other hand, genetic component has been shown as another significant factor determining ultimate meat quality through its interactions with extrinsic components (Riëtte et al., 1993). The genetic influence on pork quality exists between breeds and within a breed. The variation is caused by a large set of genes known as polygenic effects, and in principle most traits of interest for meat quality have a multifactorial background (McPhee and Trout, 1995; Gispert et al., 2000; Andersson, 2001). Fujii et al. (1991) reported that a point mutation in pig RYR1 gene is associated with PSS, and RYR1 gene is a major gene known to have a direct linkage with pork quality (Rosenvold and Andersen, 2003). The causative mutation for the halothane (HAL) gene is in the gene encoding for a pig RYR1 isoform. The HAL or stress gene effect has been well documented since the 1991 discovery of a DNA test for direct identification of genotypes (Fujii et al., 1991).

In general, homozygous and heterozygous pigs for the HAL gene have higher carcass yield and lean percentage (Simpson and Webb, 1989; McPhee and Trout, 1995; Larzul et al., 1997). However, the positive effect of the HAL gene on performance is counterbalanced by its negative effect on WHC and colour. As indicated by name, PSS carrying pigs are highly susceptible to stress. These animals are genetically more susceptible to stress even during normal shipping and handling. The hypermetabolism is increased levels of lactic acid, CO_2, heat and oxygen consumption (Forrest et al., 1997). Even with a particular care, a limited stress prior to slaughter is sufficient to trigger a higher rate of post mortem glycolysis in both homozygous and heterozygous pigs, being most severe in the homozygous pigs (Mitchel and Heffron, 1982; Rosenvold and Andersen, 2003).

Heat was the first factor known to induce the formation of these stress proteins, which subsequently became known as heat shock proteins (HSP). These proteins are found in

* Corresponding Author: H. J. Jin. Tel: +82-63-620-3536, Fax: +82-63-620-3590, E-mail: genezoo@rda.go.kr
[1] NLRI, RDA, 77 Chuksan gil, Kwonsun-Gu, Suwon, 441-350, Korea.
[2] Department of Animal Resources and Biotechnology, Chonbuk National University, Jeonju, 561-756, Korea.
[3] National Institute of Subtropical Agriculture, RDA, Jeju 690-150, Korea.
[4] Graduate School of Bio. & Information Technology, Hankyong National University, Ansung 456-749, Korea.
[5] Institute of Animal Science, CAAS Beijing 100094, P. R. China.
[6] College of Biological Science, China Agricultural University, Beijing 100094, P. R. China.
[7] College of Animal Resource Science, Kangwon National University, Chunchon 200-701, Korea.
Received March 10, 2005; Accepted September 21, 2005

Table 1. Effects of HSP70 K1 genotype on carcass and meat quality traits of Duroc pure breed

	PCR-SSCP genotypes		
	AA	AB	BB
No. of head	11	14	13
pHu[1]	5.69±0.11[2]	5.87±0.08	5.92±0.08
Backfat thickness (cm)	2.47±0.17	2.32±0.17	2.58±0.12
WB shear force (0.5 in^2/kg)	2.98±0.23	2.83±0.16	2.50±0.16
Cooking loss (%)	28.58±1.05	28.08±0.69	27.12±0.71
Water holding capacity (%)	62.33±1.36	63.04±0.90	64.33±0.93
Meat colour CIE:			
L* (lightness)	51.71±1.03	51.81±0.88	52.39±0.95
a* (redness)	7.44±0.39	7.67±0.33	7.99±0.36
b* (yellowness)	4.49±0.43	4.75±0.37	5.24±0.39

[1] Ultimate (at 24 h post mortem) pH values in the loin. [2] LSM±SE.

several protein families, with 70-kDa (HSP70) and 90-kDa (HSP90) families, being the most abundant (Riëtte et al., 1993). Cells or multi-cell organisms respond to heat or other stresses by inducing or increasing the synthesis of HSP (Lindquist and Craig, 1988; Welch, 1992). In the unstressed cell, HSP70 is diffusely located in both the cytoplasm and the nucleus, but in the heat shocked cell there is a concentration of HSP70 in nucleoli, as well as an increase of the protein in both cytoplasm and nucleus (Welch and Feramisco, 1984). The transient expression of HSP70 in the piglet is likely attributed to periods of increased muscular activity, or the increased metabolic demands (McComb and Spurlock, 1997). Kilgore et al. (1994) suggested that circumstances that fit either an increase or decrease in muscle mass may invoke expression of HSP70. Stress response in organisms has been a subject of extensive scientific investigation.

Riëtte et al. (1993) investigated the pork quality and expression of stress protein HSP70 in swine. There was no difference in the HSP70 express patterns of tissues from HAL-positive and HAL-negative pigs. The pig that showed the strongest expression of HSP did not appear to be related to the meat quality characteristics from stressed pigs. DNA polymorphisms of PSS and HSP70 gene might be due to individual difference of pork quality from preslaughter treatment (Christian and Lundstrom, 1992; Houde et al., 1993; Riëtte et al., 1993; Rosenvold and Andersen, 2003). Therefore, the purpose of this study was to determine the effects of RYR1 and HSP70 genes on carcass characteristics in pigs.

MATERIALS AND METHODS

Animals and DNA sampling

Number of animals and genetic characteristics were described in Tables 1 and 2. *M. longissimus* samples were taken from two hundred and twelve pigs from commercial abattoir (Han Naeng Co. LTD., Ochang, Chungbuk) in Korea, while 38 blood samples were collected from the National Livestock Research Institute (NLRI). Genomic DNA was isolated from both whole blood and muscle samples, and purified according to the method of Sambrook et al. (1989). The isolated DNA was diluted to a final concentration of 50 ng/ml.

Design of primers

PSS mutation (Fujii et al., 1991; Houde et al., 1993) was assessed by applying PCR-restriction fragment length polymorphism (PCR-RFLP) primer pairs, which was composed of detection of PSS mutations: PSS-F 5´- GAC ATC ATC CTT CTG GCT TCC -3´ and PSS-R: 5´- ATA GTT GAT GAG GTT TGT CTG C -3´ yield 221 bp normal products. PCR 221 bp sequence lying within an exon 17 (18,475 to 18,695) was amplified from the RYR1 gene (Brenig and Brem, 1992). HSP70 mutations was determined by PCR-single strand conformation polymorphism (PCR-SSCP) primer pairs method where the primer comprised HSP70 K1 (290-512; 223 bp; F 5´- CCC TGA ATG CGC AGA ATA CC -3´, R 5´- TAC GCC TCC GCA GTC TCC TT -3´) and HSP70 K3 (830-1,424; 595 bp; F 5´- ACT TCA TGG AGG AGT TTC G -3´, R 5´- ACT CCA GGT TGG TGG TCT GAA TAA G -3´). HSP70 K1 and K3 were amplified from the HSP70 gene (Dezeure et al., 1993).

Polymorphisms

PCR for both fragments were obtained by the cocktail of a reaction volume of 25 μl using 50 ng of DNA, 10 pM of each primer, 10 μM dNTP, 1×PCR buffer [10 mM Tris-HCL (pH 8.3), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$] and 1 U of Taq DNA polymerse (Bioneer Co., Korea). After denaturation for 5 minutes at 94°C, amplications were carried out for 35 cycles at 94°C×30 s, 56°C×45 s and 72°C×30 s with a final extension step of 5 minutes at 72°C in a GeneAmp PCR System 9700 (Applied Biosystems, CA, USA). A PCR product (221 bp) of exon 17 (18,475 to 18,695) was amplified from the RYR1 gene (Brenig and Berm, 1992). The PSS genotype of PCR products was separately confirmed by PCR-RFLP. For

Table 2. Effects of PSS and HSP70 genotype on carcass and meat quality traits of Landrace-Yorkshire×Duroc pigs

	Genotype (PSS-HSP70 K1-HSP70 K3)					
	Normal-AA-AB	Normal-AB-AB	Normal-AB-BB	Normal-BB-AB	Normal-BB-BB	Carrier-AB-AB
Carcass traits:						
No. of head	11	12	4	3	73	
Backfat thickness (cm)	2.40±0.14[a]	1.98±0.13[b]	2.18±0.22[ab]	2.08±0.25[b]	2.10±0.51[b]	-
Carcass weight (kg)	86.28±1.93[a]	80.58±1.79[b]	74.31±3.22[b]	84.99±3.62[a]	82.99±0.74[a]	-
Loin weight:						
No. of head	20	32	9	5	132	3
Left loin (kg)	3.28±0.15[a]	2.91±0.11[b]	2.98±0.22[a]	3.00±0.29[a]	3.03±0.06[a]	3.32±0.37[a]
No. of head	21	32	10	6	140	3
Right tender loin (g)	442.55±3.18[a]	434.54±2.52[c]	436.54±4.53[bc]	442.91±5.75[a]	440.24±1.21[ab]	439.41±8.27[b]
pH:						
No. of head	14	32	10	6	127	3
pHu[1]	5.49±0.05	5.46±0.03	5.51±0.06	5.55±0.08	5.49±0.02	5.56±0.11
Meat colour CIE:						
No. of head	21	32	10	6	140	3
L* (lightness)	48.49±1.02	48.31±0.64	48.13±1.17	48.48±1.48	48.42±0.33	46.26±2.13
a* (redness)	9.94±0.55	9.82±0.35	10.63±0.63	10.14±0.79	10.24±0.18	8.95±1.15
b* (yellowness)	3.28±3.36[a]	3.04±0.23[b]	3.62±0.41[a]	2.79±0.55[b]	3.49±0.11[a]	1.92±0.74[b]

[1] Ultimate (at 24 h post mortem) pH values in the loin.

[a, b, c] LSM±SE with different superscripts in the same row are significantly different ($p<0.05$).

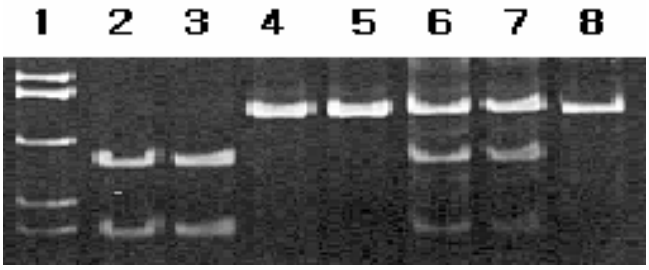

Figure 1. The PCR-RFLP analysis of RYR1 gene. Lane 1, standard markers pUC18/*Hae*III; Lanes 2 and 3, DNA from a C/C pig; Lanes 4 and 5, DNA from a T/T pig; Lanes 6 and 7, DNA from a C/T pig; Lane 8, PCR products that amplified from 18,475 to 18,695.

RFLP analysis 10 μl of the 221 bp PSS fragment was digested with 5 units of *HhaI* at 37°C for 2 h. The digested DNA fragment was then separated by electrophoresis on 12% polyacrylamide gel in 1×TBE. The gel was stained with ethidium bromide (2 μg/ml) and visualized under UV light. The HSP70 genotypes of PCR products were separately confirmed by PCR-SSCP. For SSCP analysis, 8 μl of PCR products were mixed with 8 μl of loading buffer (0.05% xylene cyanole FF, 0.05% bromophenol blue, 2 mM EDTA and 95% deionized formamide), denatured at 95°C for 5 min and snap-chilled on ice for 5 min. Sixteen microliters of denatured mixture were then separated by electrophoresis on 30% MDE Gel Solution (Cambrex Bio Science Rockland, Inc., ME, USA) gel (1.0 mm) with 0.7×TBE. The gel was stained with ethidium bromide and visualized under UV light.

Meat quality measurements

The carcass weight (kg) and the tenth backfat thickness (cm) were measured on the hanging carcasses. Longissimus thoracis ultimate pH was evaluated at 24 h post mortem. The day after slaughter, longissimus muscles (from the last thoracic vertebrae to the last lumbar vertebrae) were taken from left side of each carcass, and prepared for the measurements of objective meat colour, Warner-Bratzler (WB) shear force and cooking loss. WB-shear force was measured on steaks (2.54 cm thick) cooked in a pre-heated water bath for 60 min until the core temperature had reached 70°C and then cooled in running water (ca. 18°C) for 30 to reach a core temperature below 30°C. Eight cores of 1.27 cm diameter were made for each sample, and peak force was determined using a V-shaped shear blade with a cross-head speed of 400 mm/min (Wheeler et al., 2001). Cooking loss was determined by calculating the weight difference in steaks before and after cooking, expressed as percentage of initial weight. Objective meat colour was determined by a Minolta Chromameter (CR300, Minolta, Japan) on freshly cut surface after a 30 min blooming at 4°C.

Statistical analysis

The effects of PSS and HSP70 genotypes on objective meat qualities were evaluated by analysis of variance using GLM procedure of SAS/STAT. The model included HSP70 K1 genotype effect for pure Duroc Breed and PSS-HSP70 K1-HSP70 K3 for three-way cross bred. Differences in least squares means were analysed by Fisher's least significant difference test (SAS, 1990) with a comparison error rate of 0.05.

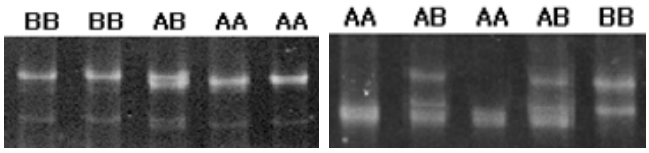

Figure 2. PCR-SSCP polymorphisms detection of HSP70 segments for (A) HSP70 K1 (223 bp, 290-512) and (B) HSP70 K3 (595 bp, 830-1,424).

RESULTS AND DISCUSSION

Genotyping

As shown in Figure 1, PSS genotyping with *HhaI* RFLP showed that *HhaI* (C/C) PCR products exhibited two fragments of 145 bp and 76 bp (normal pig). *HhaI* (T/T) PCR products exhibited one fragment of 221 bp (mutant pig). *HhaI* (C/T) PCR product exhibited three fragments of 221 bp, 145 bp and 76 bp (carrier pig). Three other genotype alleles were observed after *HhaI* digestion of the 221 bp PCR-amplified fragment. The normal C/C allele of PSS digested and produced fragments of 145 and 76 bp. The mutant T/T allele of PSS did not cut but produced a 221 bp fragment. The PCR-SSCP polymorphisms of pig HSP70 segments for K1 (223 bp, 290-512) and K3 (595 bp, 830-1,424) were detected and the mutant band consisted of AA, AB and BB (Figure 2). The three genotypes (AA, AB and BB) alleles were obtained at 10°C, 30% MDE gel using PCR amplification of HSP70 K1 290-512 (2a) and HSP70 K3 830-1,424 (2b) fragments.

Duroc pure breed meat quality

No significant differences were observed in carcass and objective meat quality characteristics among the three PSS-free HSP70 K1 types (AA, AB and BB) in Duroc pure breed (Table 1). There was, however, a consistent tendency of the genotypes to have linear effects on meat quality traits. For example, the least square means showed that ultimate pH, WHC and meat colour values increased with the expression of type B (i.e. from AA to AB to BB). In contrast, WB-shear force and cooking loss displayed a reverse trend (i.e. decreasing from AA to AB to BB) (Table 1). The ultimate pH value recorded in the current study for type BB (5.92) was relatively higher than those reported for normal pig longissimus muscle in other studies (De Smet et al., 1996; Depreux et al., 2002). It is generally known that ultimate pH in pig muscle is a reflection of many confounded factors including feeding regime, transit stress and slaughtering process and that has a significant effect on meat quality (Hwang et al., 2004), but the higher pH for the currently used animal group was not clearly identified. However, results of PSS and HSP70 genotypes showed no significant difference for ultimate pH (Table 2). Similarly, Klont et al. (1993) did not find any significant difference for ultimate pH between carriers and normal genotypes. The range in WB-shear force values in our investigations (2.50-2.98 0.5 in^2/kg) were lower than those observed by Moelich et al. (2003) in PSS genotype group (3.0-3.2 kg/1.27 cm).

Backfat thickness and carcass weight

Our results indicate that HSP70 genotype from Duroc had a limited effect on meat quality characteristics but L-Y×D crossbred had a noticeable linkage with HSP70 K1-AA and K3-AB. This effect was observed in the hot carcass weight where significantly ($p<0.05$) higher weight was recorded in HSP70 K1-AA and K3-AB (86.3 kg) compared to HSP70 K1-AB and K3-BB (74.3 kg) (Table 2). Consequently, backfat thickness was also higher in HSP70 K1-AA and K3-AB (2.40 cm) at $p<0.05$ than other groups, although significant terms were not found, indicating a relationship between backfat thickness and carcass weight. Therefore, the data imply that heavier carcass weight had higher backfat thickness although in some cases this is variable. This trend also suggests that stress-related HSP70 genotype could be associated with backfat thickness and carcass weight. In spite of the limited number of pigs for some genetic groups, the trend corroborates observations by Gispert et al. (2000) that PSS-carrier yielded heavier carcass weight and thicker backfat. Other studies have, however, showed little but non-significant effects of stress-related HSP70 genotype on backfat thickness (McPhee and Trout, 1995; Nanni Costa et al., 2002).

Loin weight

Left loin and right tender loin weights were significantly ($p<0.05$) influenced by stress-related genotype (Table 2). The left loin weight for the PSS-Carrier pigs with genotype HSP70 K1-AB and K3-AB (3.32 kg) was significantly higher than that for the PSS-Normal genotype HSP70 K1-BB and K3-AB (2.91 kg). The PSS-Normal genotype HSP70 K1-BB and K3-AB had the highest yield of tenderloin (442.9 g) while the lowest tenderloin yield was produced by the PSS-Normal genotype HSP70 K1-AB and K3-AB (434.5 g). Leach et al. (1996) reported significantly ($p<0.01$) lower Least Mean Squares for wholesale loin cut weights for heterozygous (normal carrier) pig than for the homozygous normal.

Meat colour

The CIE lab values (L*, a* and b*) for the genotypes shown in Table 1 revealed no significant difference ($p>0.05$) for the three genotypes. Various studies have shown that higher L* value are as a result of PSE meat (Lawrie, 1991). Meat from HSP70 KI-BB genotype had a higher mean reflectance value (L* = 52.39) indicating pale

meat compared with both AA (L* = 51.7) and AB (L* = 51.8) genotypes. Studies done by Bartone et al. (1988) revealed that colour changes in meat are associated with abnormal pH values and water holding. This is consistent with these study where ultimate pH and water holding capacity was also similar in trend with L* values. However, contrast patterns were observed in PSS carrier genotype (Table 2) which had a lower L* value (46.3) than PSS normal genotype (48.4). In addition, the b* value was significantly ($p<0.05$) lower in PSS carrier HSP70 K1-AB and K3-AB (1.92) than in PSS Normal (ranging from 2.79 to 3.62). However, there is need to increase the sample size of the PSS carrier to determine accurately the CIE values for comparison purposes. The results obtained in this study, therefore, indicate that there are significant effects on meat quality due to meat colour as described by Brewer and McKeith (1999). The colour of meat is an excellent indicator of pork quality and pale 'very light pink' is regarded as inferior quality indicating the presence of PSE condition. Taken together, we have investigated the effect of stress-related gene of PSS and HSP70 gene on carcass traits and meat quality. These genes were detected in the genotype through the PCR-RFLP and –SSCP from each amplified DNA fragments. In particular, the genotypes of PSS and HSP70 gene have a potential association with backfat thickness and carcass weight. These results can apply to the pig breeding by utilizing polymorphisms detection.

ACKNOWLEDGEMENTS

The authors wish to thank the Rural Development Administration of the Republic of Korea for its financial support.

REFERENCES

Andersson, L. 2001. Genetic dissection of phenotypic diversity in farm animals. Nat. Rev. Genet. 2:130-138.

Barton-Gade, P. A., H. R. Cross, J. M. Jones and R. J. Winger. 1988. Factors affecting sensory properties of meat. In (Ed. H. R. Cross and A. J. Overby). Meat Science, milk science and technology. Elsevier Science Press, Amsterdam. Vol. B3, pp. 141-172.

Brenig, B. and G. Brem. 1992. Genomic organization and analysis of the 5' end of the porcine ryanodine receptor gene (ryr1). FEBS Lett. 298:277-279.

Brewer, M. S. and F. K. McKeith. 1999. Consumer-rated quality characteristics as related to purchase intent of fresh pork. J. Food Sci. 64(1):171-174.

Cassens, R. G., D. N. Marple and G. Eikelenboom. 1975. Animal physiology and meat quality. Adv. Food Res. 21:71-155.

Cheah, K. S., A. M. Cheah and A. Just. 1998. Identification and characterization of pigs prone to producing 'RSE' (reddish-pink, soft and exudative) meat in normal pigs. Meat Sci. 48:249-255.

Christian, L. L. and K. Lundstrom. 1992. Diseases of Swine 7th, Iowa State. Univ. Press. 62:763-771.

De Smet, S. M., H. Pauwels, S. De Bie, D. I. Demeyer, J. Callewier and W. Eeckhout. 1996. Effect of halothane genotype, breed, feed withdrawal, and lairage on pork quality of belgian slaughter pigs. J. Anim. Sci. 74(8):1854-1863.

Depreux, F. F. S., A. L. Grant and D. E. Gerrard. 2002. Influence of halothane genotype and body-weight on myosin heavy chain composition in pig muscle as related to meat quality. Lives. Prod. Sci. 73:265-273.

Dezeure, F., M. Vaiman and P. Chardon. 1993. Characterization of a polymorphic heat shock protein 70 gene in swine outside the SLA major histocompatibility complex. Biochim. Biophys. Acta. 1174(1):17-26.

Forrest, J. C., E. B. Sheiss, M. P. Morgan and D. E. Gerrard. 1997. Pork quality measurement tools-Now and in the future. Pork Quality Summit 73-91.

Fujii, J., K. Otsu, F. Zorzato, S. De Leon, V. K. Khanna, J. E. Weiler, P. J. O'Brien and D. H. Maclennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Sci. 253:448-451.

Gispert, M., L. Faucitano, M. A. Oliver, M. D. Guardia, C. Coll, K. Siggens, K. Harvey and A. Diestre. 2000. A survey of pre-slaughter conditions, halothane gene frequency, and carcass and meat quality in five spanish pig commercial abattoirs. Meat Sci. 55:97-106.

Houde, A., S. A. Pommier and R. Roy. 1993. Detection of the ryanodine receptor mutation associated with malignant hyperthermia in purebred swine populations. J. Anim. Sci. 71(6):1414-1418.

Hwang, I. H., B. Y. Park, S. H. Cho, J. H. Kim, Y. S. Choi and J. M. Lee. 2004. Identification of muscle proteins related to objective meat quality in korean native black pig. Asian-Aust. J. Anim. Sci. 17(11):1599-1607.

Kilgore, J. L., B. F. Timson, D. K. Saunders, R. R. Kraemer, R. D. Klemm and C. R. Ross. 1994. Stress protein induction in skeletal muscle: comparison of laboratory models to naturally occurring hypertrophy. J. Appl. Physiol. 76(2):598.

Kim, Y. S., S. W. Kim, M. A. Weaver and C. Y. Lee. 2005. Increasing the pig market weight: World trend, expected consequences and practical considerations. Asian-Aust. J. Anim. Sci. 18(4):590-600.

Klont, R. E., E. Lambooy and J. G. van Logtestijn. 1993. Effect of preslaughter anesthesia on muscle metabolism and meat quality of pigs of different halothane genotypes. J. Anim. Sci. 71:1477-1485.

Larzul, C., P. Le Roy, R. Gueblez, A. Talmant, J. Gogue, P. Sellier and G. Monin. 1997. Effect of halothane genotype (NN, Nn, nn) on growth, carcass and meat quality traits of pigs slaughtered at 95 kg or 125 kg live weight. J. Anim. Breed. Gene. 114:309-320.

Lawrie, R. A. 1991. Meat Science (5th ed.). Pergamon Press. Oxford.

Leach, L. M., M. Ellis, D. S. Sutton, F. K. McKeith and E. R. Wilson. 1996. The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. J. Anim. Sci. 74:934-943.

Lindquist, S. and E. A. Craig. 1988. The heat shock proteins. Annu.

Rev. Genet. 22:63-77.

McComb, M. A. and M. E. Spurlock. 1997. Expression of stress proteins in porcine tissues: developmental changes and effect of immunological challenge. J. Anim. Sci. 75:195-201.

McPhee, C. P. and G. R. Trout. 1995. The effects of selection for lean growth and the halothane allele on carcass and meat quality of pigs transported long and short distances to slaughter. Lives. Prod. Sci. 42:55-62.

Mitchel, G. and J. J. Heffron. 1982. Porcine stress syndromes. Adv. Food Res. 28:167-230.

Moelich, E. I., L. C. Hoffman and P. J. Conradie. 2003. Sensory and functional meat quality characteristics of pork derived from three halothane genotypes. Meat Sci. 63:333-338.

Nanni Costa, L., D. P. Lo Fiego, S. Dall'Olio, R. Davoli and V. Russo. 2002. Combined effects of pre-slaughter treatments and lairage time on carcass and meat quality in pigs of different halothane genotype. Meat Sci. 61:41-47.

Riëtte, L. J. M., van Laack, C. Faustman and J. G. Sebranek. 1993. Pork Quality and the Expression of Stress Protein Hsp 70 in Swine. J. Anim. Sci. 71:2958-2964.

Rosenvold, K. and H. J. Andersen. 2003. Factors of significance for pork quality - a review. Meat Sci. 64:219-237.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning: a laboratory manual (2nd Ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

SAS. 1999. SAS/STAT User's Guide, Version 8, SAS Institute Inc., Cary, NC, USA.

Simpson, S. P. and A. J. Webb. 1989. Growth and carcass performance of British Landrace pigs heterozygous at the halothane locus, Anim. Prod. 49:503-509.

Welch, W. W. and J. R. Feramisco. 1984. Nuclear and nucleolar localization of the 72,000-dalton heat shock protein in heat shocked mammalian cells. J. Biol. Chem. 259: 4501-4513.

Welch, W. J. 1992. Mammalian stress response: cell physiology, structure/function of stress proteins and implications for medicine and disease. Physiol. Rev. 72:1063-1081.

Wheeler, T. L., L. V. Cundiff, S. D. Shackelford and M. Koohmaraie. 2001. Characterization of biological types of cattle (Cycle V): carcass traits and longissimus palatability. J. Anim. Sci. 79(5):1209-1222.