

INVESTIGATION OF CATHEPSIN B (*CTSB*) and CATHEPSIN L (*CTSL*) POLYMORPHISM FOR CARCASS AND MEAT QUALITY IN SWINE

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ABSTRACT: The objectives of the research were to evaluate genotype frequencies and to investigate polymorphism in the cathepsin B gene (*CTSB*) and cathepsin L gene (*CTSL*) with carcass and meat quality in 288 crossbreeds (large white x landrace x duroc). Pigs whose weights ranged from 78-134 kg were assigned to slaughter. PCR-RFLP protocols were used to identify polymorphisms of both genes. The frequencies of AA, AC and CC genotypes of *CTSB* were 0.340, 0.615, and 0.045, respectively. The frequencies of A and C alleles of *CTSB* were 0.648 and 0.352, respectively. Regarding *CTSL*, the frequencies of CC, CT and TT genotypes were 0.625, 0.354, and 0.021, respectively. The frequencies of C and T alleles of *CTSL* were 0.802 and 0.198, respectively. The significant effects of *CTSB* polymorphism were detected on body length (BL), a scan of loin eye area (U_LAE), loin eye area (LAE), and weight loss (WL) ($P < 0.05$). The pigs carrying the AC genotype had a higher level of BL, U_LEA and LEA than the ones carrying the homozygous genotype (AA or CC). The pigs with the CC genotype of *CTSB* had lower WL (1.86 kg) than the ones carrying the AA or the AC genotype (2.19 and 1.97 kg). The *CTSL* variants represented statistically significant effects on BL trait ($P < 0.05$). The CC genotype (77.92 cm) had higher BL value compared to the CT (76.14 cm) and the TT genotypes (76.92 cm). The *CTSB* and *CTSL* were high polymorphism and their alleles could be as the potential genetic markers for swine selection.

Keywords: *Cathepsin B, Cathepsin L, Swine, Polymorphism*

1. INTRODUCTION

The selection of pig has focused on the increase of carcass and meat quality. The carcass and meat characteristics, especially the back fat thickness and ham weight, are economically important for the pig industry [1]. However, making genetic progress by traditional breeding is challenging because meat and carcass qualities are low to moderately heritable [2], [3]. The meat and carcass quality are of complex nature, influenced by several genes with the high impact of environmental effect [4].

Ultrasound technique has been used to predict carcass and meat traits of live animals for several years. Deza [1] has found that the R^2 model between hams weight and slaughter live weight and ultrasound fat thickness was 0.82. Moreover, the correlation between 10th rib back fat measurement by real-time ultrasound and 10th rib back fat was 0.84 [5]. Newcom [6] has used real-time ultrasound technique to predict intramuscular fat percentage in live swine and found that the product moment correlation and rank correlation coefficients between predict loin intramuscular fat percentage and carcass loin intramuscular fat percentage of Duroc population were 0.60 and 0.56, respectively. Results show that real-time ultrasound image analysis can be used to predict intramuscular fat percentage in live swine. The prediction of meat and carcass quality of measurement carried out in live pigs before slaughter and interesting aspect for producer and for the

industry.

Marker-assisted selection (MAS) is one of the possibilities related to the breeding program. Several studies have identified gene marker associated with the genetic variation process through MAS that has an increased possibility for implementation [7]. The cathepsin genes belong to the enzyme family (*CTSL*, *CTSB*, *CTSD*, *CTSH*, *CTSF* and *CTSZ*), which are a group of genes that produce lysosomal proteinases and are important for the degradation of proteins. Their main role is in the *post-mortem* proteolysis of meat. These genes or their mutations may profoundly affect the qualitative properties of meat [8], [9]. Recent studies have shown that the polymorphism in the cathepsin B (*CTSB*) and cathepsin L (*CTSL*) gene is associated with carcass and meat quality traits in swine. Vera [8] has found a trend of allele T increased fatness and the effect of allele C on lean meat in swine. The significant results of *CTSB* mutation were observed for higher carcass yield and weight of ham [9]. Moreover, Russo [10] also found the marker identified at the *CTSB* loci involved in meat quality traits. *CTSL* marker shows a tendency towards back fat thickness and weight of lean cuts [11], [12]. In the present study, we predicted the carcass and meat quality by real-time ultrasound that might improve the efficiency of measure methods for evaluating meat quality on a routine basis. Thus, the objectives of this research are to investigate the genotype of *CTSB* and *CTSL* polymorphism and confirm the

association between the polymorphisms and carcass and meat quality in Thai crossbred swine. The results of this study might improve the efficiency of selection swine.

2. MATERIALS AND METHODS

2.1 Animals

Two hundred and eighty-eight cross breeds pig (Large White × Landrace × Duroc Jersey) were purchased from the commercial farm with an initial live weight of 78 to 134 kg and no phenotypic criteria.

2.2 Ultrasound Image Collection

All pigs were weighed body weight (BW), body length (BL) and were scaled of loin eye area (U_LAE), backfat thickness (U_BF) obtained 2 h were scanned before slaughter by real-time ultrasound (Honda electronic Inc., a 3.5 MHz, 10 cm long probe and a 1.5 - 2 MHz, 13 cm long). Images were recorded and then linear and area measurements were taken by means of the between 10th rib. The pigs were slaughtered at a commercial slaughterhouse after electrical stunning.

2.3 Carcass and Meat Data Collection

At the slaughterhouse, within 10 min of *post-mortem*, hot carcass weight (Hot_C, kg), pH0 at 0 h Post – mortem (pH0) and carcass length (CL) were collected. pH at 45 min post – mortem (pH45) was determined using pH meter. After chilling, carcass weight after chilling (Chill_C), pH 22 h (pH22), back fat thickness (BF), loin weight (LW), ham weight (HW), fillet weight (FW) and shoulder weight (SW) were determined.

2.4 Tissue Collection and DNA Extraction

Approximately 20-30 mg of tissue was collected from the carcass of each pig into a tube containing 99% of ethanol. Genomic DNA was isolated from tissue using Puregene (Gentra Inc., Minneapolis, MN) according to the supplied protocol. Briefly, tissues were separately minced were washed twice with 0.9% NaCl sample were centrifuged for 5 min at 3,000 rpm at room temperature. Cell lysis buffer and protein precipitation buffer were added to the pellet. The cell lysate was then centrifuged for 5 min at 10,000 rpm at 4°C. The supernatant was then transferred to 1.5 ml tube, and absolute isopropanol was added. The DNA was precipitated at 10,000 rpm for 5 min at 4°C. The supernatant was discarded, and DNA pellet was washed 2 to 3 times with 75% ethanol. The DNA pellet was air-dried at room temperature and

dissolved in DNA hydration buffer. Deoxyribonucleic acid quality and concentration were determined by UV spectroscopy. The DNA was diluted to 50 ng/μL as a working solution and stored at -20°C before use.

2.5 PCR –Restriction Fragment Length Polymorphism (PCR-RFLP) Analysis

The reactions of PCR were carried out in a total volume of 10 μL containing: 1 μL of diluted DNA template (50 ng/μL); 1X PCR buffer (1 μL of 10XPCR buffer), 4 mM MgCl₂ (0.8 μL of 50 mM MgCl₂), 0.1 mM dNTP (1 μL of 1 mM dNTP), 0.5 μM of each primer (1 μL of 5 μM of each primer), and add 0.1 μL of *Taq* DNA polymerase (Promega, San Diego, CA). PCR amplification was carried out in a PCR thermal cycle (COBETT RESEARCH, Australia 2003, iCycler thermal cycler, BioLad, U.S.A) using the following amplifying program: preheating at 95°C for 5 min followed by 35 cycles at denature 95°C, 30 s; annealing temperature (Table 1), 40 s; and extension 72°C, 30 s. The final extension was carried out at 72°C for 5 minutes and the amplified products were hold at 4°C until needed. At the end of the PCR cycle, amplified products were analyzed by 0.8% agarose gel with 1X loading dye. After electrophoresis at 100 V for 35 min, the gel was stained with GELSTAR™ (Gelstar Inc, NY) for 10 min. DNA fragments were visualized by gel documentation.

Table 1 Sequences of primer and Ta (annealing temperatures) for PCR amplification

Genes	Primer sequence	Ta (°C)
CTSB ^{1/}	Forward:5'GTGGCCGGG TGGTTTAA 3' Reverse:5'TCCTCCTGGT GCTGCTAATTCTGAC 3'	55
CTSL ^{2/}	Forward:5'TCACTGCCG TGAAGAATCAG 3' Reverse:5'GCAGAGCTG TAATGGCAAGA 3'	64

Note: ^{1/} [16], [12]
^{2/} [12], [8]

2.6 Restriction of PCR Products

Polymerase chain reaction (PCR) products from each pig were digested separately with 1 restriction endonucleases, *MspI* for *CTSB* and *TaqI* for *CTSL*. Each digestion reaction contained 2 μL of PCR products, 1 μL of cut smart, and 0.2 μL of the enzyme, add water 4.8 μL in a total volume of 10 μL. Subsequently, each reaction was incubated at 37°C for *MspI* and 65°C for *TaqI* for at least 6 h. Genotyping of *CTSB* deoxyribonucleic acid

fragments were detected on vertical electrophoresis (Mini-Protein III, Bio-Rad Laboratories, Richmond, CA) using a 12% denaturing polyacrylamide gel (Sigma Inc., St. Louis, MO) with 1X polyacrylamide dry 5 μ L. After electrophoresis at 150 V for 120 min, gels were stained with GelStar (Gelstar Inc., Patchogue, NY) for 10 min. Deoxyribonucleic acid fragments were visualized by UV transillumination and photographed with the Syngene gel documentary system (Syngene Inc., Cambridge, IL). For *CTSL*, detected on a horizontal electrophoresis unit (Mini-Protein III, Bio-Rad Laboratories, Richmond, CA) using a 2% agarose gel.

2.7 Statistical Analysis

The means of ultrasound, carcass and meat quality traits were analyzed by PROC MEANS. [13] Associations between the genotypes of the candidate genes (*CTSB* and *CTSL*) on carcass and meat quality were assessed by the procedure MIXED in SAS version 8.02 (SAS Institute Inc. Cary, NC, USA), with a model that included sire as a random effect and the fixed effects sex and genotype of the analyzed DNA markers.

3. RESULTS AND DISCUSSION

3.1 Phenotypic Analysis

The means (Means), standard deviation (Std.), minimum (Min.) and maximum (Max.) of carcass and meat quality traits in the pigs are showed in Table 2. Means of BW and Hot_C weight were 105.62 kg and 78.96 kg, respectively. Data from ultrasound showed that U_LEA and U_BF were 38.30 cm^2 and 17.09 mm, respectively. The previous study of Schwab [2] reported that ultrasound 10th rib loin muscle areas were 41.05 cm^2 and showed larger than this study. Ayuso [14] found the means of the 10th rib by ultrasound were 38.12 mm and carcass measurements were 43.11 mm. Moeller [15] reported loin eye area of swine was 35.2 cm^2 .

Table 2 Means, standard deviations (Std.), minimum (min) and maximum (max) values for carcass and meat quality parameters

Variable	Means	Std.	Min.	Max.
BW, kg	105.62	9.63	80.00	126
BL, cm	73.28	6.41	58.80	90.00
U_LEA, cm^2	38.30	4.61	20.29	50.25
U_BF, mm	17.09	3.45	8.80	25.60
CL, cm	76.64	3.14	69.00	84.00
Hot_C, kg	78.96	8.04	57.10	95.90
Chill_C, kg	77.04	7.94	55.60	93.80
%carcass, %	74.79	4.23	61.69	95.77

Weight loss, kg	2.43	0.42	0.24	3.81
LAE, cm^2	40.20	5.14	39.00	56.00
BF, mm	31.98	8.19	14.00	51
pH ₀	6.40	0.16	5.98	6.79
pH ₄₅	6.22	0.15	5.82	6.66
pH ₂₂	6.00	0.21	5.32	6.78
FW, kg	0.80	0.13	0.54	1.32
LW, kg	4.94	0.83	3.06	7.49
HW, kg	9.87	1.02	7.25	12.08
SW, kg	7.04	0.81	5.32	9.58

3.2 PCR-RFLP Analysis and Genotype Frequency

The expression of *CTSB* and *CTSL* genes was analyzed by PCR-RFLP. The length of the PCR products was 139 bp (*CTSB*) and 380 bp (*CTBL*) (Fig.1). The frequencies of three investigated genotypes in the *CTSB* (AA, AC and CC) and *CTSL* (CC, CT and TT) gene are presented in Table 3. Of 177 investigated pigs, 0.61 were heterozygous AC genotype, 0.34 were homozygous AA genotype, and 0.05 were homozygous CC genotype in *CTSB*. The frequencies of A and C alleles of *CTSB* were 0.65 and 0.35, respectively. Russo [16] showed that alleles frequency A was higher than alleles C. Regarding *CTSL*, the frequencies of CC, CT and TT genotypes were 0.625, 0.354, and 0.021, respectively. The frequencies of C alleles (0.80) were higher than T alleles (0.20) (Table 3). The high frequency of CC genotype of *CTSL* is in accordance with previous reports [8]. However, Fontanesi [12] reported the highest frequency of TC genotype, which is not in accordance with the results of this study.

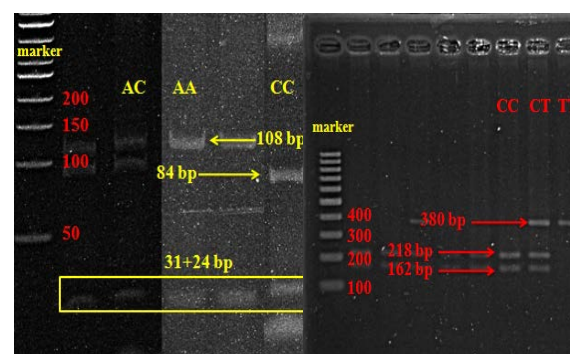


Fig.1 The genotype of *CTSB* gene: AA (108, 31+24 bp), AC (108, 84, 31+24 bp) and CC (84, 31+24); *CTSL* gene: CC(218, 162 bp), CT(380, 218, 162 bp) and TT (380 bp) digested with *MspI* and *TaqI*, respectively

Table 3 Genotype and allele frequency of *CTSB* and *CTSL* in crossbreed swine

Genes	Genotype	Total	Allele	Total
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	frequency				Frequency		
	AA	AC	CC		A	C	
<i>CTSB</i>	0.34	0.61	0.05	1	0.65	0.35	1
N	98	177	13	288			
	CC	CT	TT		C	T	
<i>CTSL</i>	0.63	0.35	0.02	1	0.80	0.20	1
N	180	102	6	288			

3.3 Association of *CTSB* and *CTSL* Polymorphism on Carcass and Meat Quality

The significant effects of *CTSB* polymorphism were detected on body length (BL), ultrasound scan of loin eye area (U_LAE), weight loss (WL) and loin eye area (LAE) ($P < 0.05$, $P < 0.01$). The pigs carrying the AC genotype had a higher level of BL, U_LEA and LEA than the ones carrying the homozygous genotype (AA or CC). The pigs with the CC genotype of *CTSB* had lower WL (1.86 %) than the ones carrying the AA or the AC genotype (2.19 % and 1.97 %). The *CTSL* variants represented statistically significant effects on BL trait ($P < 0.05$). The CC genotype (77.92 cm) had higher BL value compared to the CT (76.14 cm) and the TT genotypes (76.92 cm). The *CTSB* and *CTSL* were high polymorphism and their alleles could be potential genetic markers for swine selection (Table 4). Previous studies have shown that the significant associations were observed between *CTSB* and back-fat thickness [10]. In addition, Piorkowska [9] found that AC genotype characterized higher carcass yield and loin than in AA pig.

CTSL is a gene that produces lysosomal proteinases and is important for the degradation of proteins. Its main role is in the post-mortem proteolysis of meat [8]. Fontanesi [12] have claimed that *CTSL* marker showed a tendency towards association with back fat thickness and weight of lean cuts. On the other hand, Vera [8], no statistically significant differences were detected in qualitative traits. However, we identified a trend of allele T on increased fatness and the effect of allele C on lean meat.

Table 4 Association of *CTSB* and *CTSL* on carcass and meat quality in crossbreed swine

Genes	traits	Genotypes			<i>P</i> -value
		AA	AC	CC	
<i>CTSB</i>	BW	102.54	101.93	100.90	0.83
	BL	75.38	77.81	77.79	*
	U_LEA	36.52	39.43	36.56	**
	U_BF	16.34	15.68	15.71	0.23
	CL	76.16	76.93	75.71	0.17

Hot_C	78.72	79.05	77.71	0.88
Chill_C	76.52	77.07	75.84	0.85
%Carcass	73.38	74.21	73.85	0.23
Weight loss	2.19	1.97	1.86	*
LEA	39.79	41.32	38.70	**
BF	31.55	30.78	30.78	0.68
pH0	6.42	6.49	6.49	0.09
pH45	6.25	6.33	6.31	0.15
pH22	6.01	6.03	6.10	0.37
FW	0.75	0.83	0.77	0.37
LW	4.92	5.13	4.80	0.13
HW	9.88	9.86	10.01	0.88
SW	7.12	7.16	7.20	0.96
	CC	CT	TT	<i>P</i> -value
BW	103.30	103.75	98.29	0.73
BL	77.92	76.14	76.92	*
U_LEA	37.61	37.32	36.94	0.57
U_BF	15.74	15.92	14.97	0.68
CL	76.12	76.44	78.99	0.45
Hot_C	78.64	78.33	75.69	0.71
Chill_C	76.65	76.28	73.17	0.71
% Carcass	74.19	73.51	74.57	0.18
<i>CTSL</i> Weight loss	1.99	2.05	2.52	0.50
LEA	40.35	40.16	39.43	0.75
BF	30.13	31.96	29.83	0.06
pH0	6.47	6.47	6.50	0.96
pH45	6.31	6.27	6.36	0.26
pH22	6.07	6.05	6.26	0.58
FW	0.80	0.76	0.78	0.41
LW	4.92	4.98	5.00	0.61
HW	10.00	9.85	9.73	0.28
SW	7.18	7.12	6.60	0.60

Note: * $P < 0.05$; ** $P < 0.01$

4. CONCLUSION

CTSB and *CTSL* were analyzed using the PCR-RFLP technique for detection of genotypes. Three alleles were found on *CTSB* gene (AA, AC and CC) and *CTSL* gene (CC, CT and TT) with frequency ranging from 0.20 to 0.80. The results obtained from the present study indicated that the investigated *CTSB* and *CTSL* gene markers were associated with carcass and meat quality traits in Thai cross breeds pig (Large White \times Landrace \times Duroc Jersey). The means of Hot_C and Chill_C were 78.78 and 76.71 kg, respectively. LEA was 40.20 cm². For the *CTSB* polymorphism was significant with BL, U_LEA, WL and LEA. The pigs carrying the AC genotype had a higher level of BL, U_LEA and LEA than homozygous genotype (AA or CC). CC genotype of *CTSB* had lower WL (1.86 kg)

than AA or the AC genotype (2.19 and 1.97 kg). *CTSL* was related to BL traits. The CC genotype (77.92 cm) had higher BL than CT (76.14 cm) and TT genotypes (76.92 cm). Therefore, this polymorphism of *CTSB* and *CTSL* could be of interest in marker-assisted selection programs.

5. ACKNOWLEDGMENTS

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