International Journal of GEOMATE, Sept., 2019 Vol.17, Issue 61, pp. 91 - 96
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2019.61.4792
Special Issue on Science, Engineering & Environment

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

*Doungnapa Promket[1], Kajorngiat Nabundit[2], and Khanitta Ruangwittayanusorn[3]

[1,3]Faculty of Technology, Mahasarakham University, Thailand; [2]Graduate student of Animal science, Faculty of Technology, Mahasarakham University, Thailand

*Corresponding Author, Received: 27 Nov. 2018, Revised: 03 Jan. 2019, Accepted: 25 Jan. 2019

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), 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 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]. The assessment of meat quality is defined by the characteristics of sensory indexes, such as tenderness, juiciness, flavor, pH, color, shear force, intramuscular fat, moisture content, protein content, drip loss and sensory analysis. 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. Recent developments in real-time ultrasound technology have allowed accurate prediction of intramuscular fat percentage in the live animal [2]. 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 10^{th} rib back fat measurement by real-time ultrasound and 10^{th} 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 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 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 cathepsinB (*CTSB*) and cathepsinL (*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 was 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 genotype of *CTSB* and *CTSL* polymorphism and confirm the association between the polymorphisms and carcass and meat quality in Thai crossbred swine. The results from 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 any 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), back fat 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 10^{th} rib. The pigs were slaughtered at commercial slaughter house 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 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. 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 $MgCl_2$ (0.8 μL of 50 mM $MgCl_2$), 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 RECHEARCH, 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. 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 PCR cycle, amplified products were analyzed by 0.8% agarose gel with 1X loading dye. After electrophoresis at 100 V for 35 min, 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 (^{0}C)
CTSB[1/]	Forward:5´GTGGCCGGGT GGTTTTA 3´ Reverse:5´TCCTCCTGGTG CTGCTAATTCTGAC 3´	55
CTSL [2/]	Forward:5´TCACTGCCGT GAAGAATCAG 3´ Reverse:5´GCAGAGCTGT AATGGCAAGA 3´	64

Note: [1/] [17], [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, *Msp*I *for CTSB* and *Taq*I 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 μL. 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 10^{th} rib loin muscle areas were 41.05 cm^2 and showed larger than this study. Ayuso [14] found the means of 10^{th} 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. Moreover, the mean and the standard deviation of rib eye muscle area at the 10th-11th and the last ribs were 31.45±6.58 cm2, and 32.69±7.28 cm2, respectively [16].

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_LAE, 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 PCR products were 139 bp *(CTSB)* and 380 bp (*CTBL)* (Fig.1 and Fig.2). 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 [17] 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 results of this study.

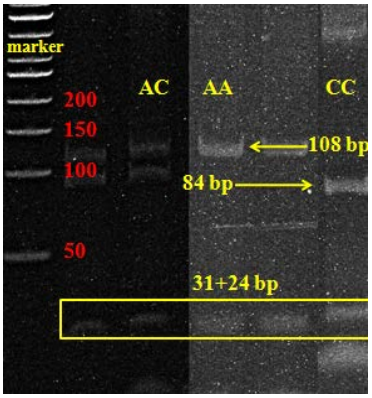

Fig.1 The genotype of *CTSB* gene: AA (108, 31+24 bp), AC (108, 84, 31+24 bp) and CC (84, 31+24) digested with *Msp*I

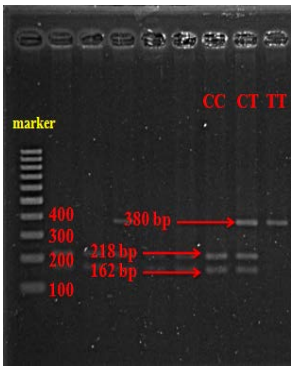

Fig.2 The genotype of *CTSL* gene: CC(218, 162 bp), CT(380, 218, 162 bp) and TT (380 bp) digested with *Taq*I

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

Genes	Genotype frequency			Total	Allele Frequency		Total
	AA	AC	CC		A	C	
CTSB	0.34	0.61	0.05	1	0.65	0.35	1
N	98	177	13	288			
	CC	CT	TT		C	T	
CTSL	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), an 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			*P-value*
		AA	AC	CC	
CTSB	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	*P-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

Table 4 continued

	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
CTSL	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. CONCLUSIONS

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 × Landrace × Duroc Jersey). The means of Hot_C and Chill_C were 78.78 and 76.71 kg, respectively. LEA was 40.20 cm^2. For the *CTSB* polymorphism was significant with BL, U_LEA, WL and LEA. The pigs carrying the AC genotype had 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

We would like to thank Mahasalakham University development fund 2019, Mahasakham, Thailand, for financial support and Betagro Company Limited, Thailand, for tissue supply and the data, which were the main information of this research.

.

6. REFERENCES

[1] Daza A., Mateos A., Lopez C. C., Rey A., Ovejero I., and Lopez-Bote C. J, Effect of feeding system on the growth and carcass characteristics of Iberian pigs, and the use of ultrasound to estimate yields of joints. Journal of Meat Science, Vol. 72, 2006, pp. 1–8.

[2] Schwab, C. R., Baas T. J., and Stalder K. J, Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. Journal of Animal Science, Vol. 88, 2010, pp. 69-79.

[3] Miar Y., Plastow G. S., Moore S. S., Manafiazar G., Charagu P., Kemp R. A., Van Haandel B., Huisman A. E., Zhang C. Y., McKay R. M., Bruce H. L., and Wang Z, Genetic and phenotypic parameters for carcass and meat quality traits in commercial crossbred pigs. Journal of Animal Science, Vol. 92, 2014, pp. 2869-2884.

[4] Zhang, Q., Boichard D., Hoeschele I., Ernst C., Eggen A., Murkve B., Pfister-Genskow M., Witte L.A., Grignola F.E., Uimari P., Thaller G., and Bishop M.D Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. Journal of Genetics, Vol. 149, 1998, pp. 1959–1973.

[5] Ragland K. D., Christian L. L.,and Baas T. J, Evaluation of real-time ultrasound and carcass characteristics for assessing carcass composition in swine. Journal of Zootec, Vol. 41, 2004, pp. 2463-2466.

[6] Newcom D. W., Baas T. J., and Lampe J. F, Prediction of intramuscular fat percentage in live swine using real-time ultrasound. Journal of Animal Science, Vol. 80, 2014, pp. 3046–3052.

[7] Dekkers, J. C. M, Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. Journal of Animal Science, Vol. 82, 2004, pp. 313-328.

[8] Vera, D., Roman S., Michal S., Jaroslav C., Monika O., Eva K., and Lubos B, The effect of gene CTSL on the quantitative and qualitative production traits of pork meat. Journal of Maso international brno, Vol. 1, 2011, pp. 47-50.

[9] Piorkowska, K., Ropka-Molik K., Eckert R., Tyra M., and Zukowski K, Analysis of polymorphisms of cathepsin B and cystatin B impact on economically important traits in pigs raised in Poland. Journal of Livestock Science, Vol. 146, 2012, pp. 99-104.

[10] Russo V., Fontanesi L., Davoli R., Nanni Costa L., Cagnazzo M., Buttazzoni L., Virgili R., and Yerle M, Investigation of candidate genes for meat quality in dry-cured ham production: the porcine cathepsin B (CTSB) and cystatin B (CSTB) genes. Journal of Animal Genetics, Vol. 33, 2002, pp. 123-131.

[11] Fontanesi, L., Speroni C., Buttazzoni L., Scotti E., Nannicosta L., Davoli R., and Russo V, Association between cathepsin L (CTSL) and cathepsin S (CTSS) polymorphisms and meat

production and carcass traits in Italian Large White pigs. Journal of Meat Science, Vol. 85, 2010, pp. 331-338.

[12] Fontanesi, L., Speroni C., Buttazzoni L., Scotti E., Dallolio S., Davoli R., and Russo V, Association between polymorphisms in cathepsin and cystatin genes with meat production and carcass traits in Italian Duroc pigs: confirmation of the effects of a cathepsin L (CTSL) gene marker. Journal of Molecular Biology, Vol. 39, 2012, pp. 109-115.

[13] SAS.User's Guide: Statistics, V.6.12, 1998, SAS Institute Inc., Cary. NC.

[14] Ayuso D., Gonzalez A., Hernandez F., Corral J. M., and Izquierdo M, Prediction of carcass composition, ham and foreleg weights, and lean meat yields of Iberian pigs using ultrasound measurements in live animals. Journal of Animal Science, Vol. 91, 2014, pp. 1884-1892.

[15] Moeller S. J., Evolution and use of ultrasonic technology in the swine industry. Journal of Animal Science, Vol. 80(E. Suppl. 2), 2002, pp. E19–E27.

[16] Guo, Y., Zhang L., Wang L., and Liu W, Genome-wide association study for rib eye muscle area in a Large White×Minzhu F2 pig resource population. Journal of Integrative Agriculture, Vol.14, 2015, pp. 2095-3119.

[17] Russo, V., Fontanesi L., Scotti E., Beretti F., Davoli R., Nannicosta L., Virgili R., and Buttazzoni L., Single nucleotide polymorphisms in several porcine cathepsin genes are associated with growth, carcass, and production traits in Italian Large White pigs. Journal of Animal Science, Vol. 86, 2008, pp.300-331.