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ASSOCIATION OF THE ACSL5 GENE G.33185918G>A AND G.33186348C>T MUTATIONS WITH CARCASS AND MEAT QUALITY TRAITS OF CHINESE SIMMENTAL-CROSS STEERS

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ABSTRACT

Acyl coenzyme A long-chain 5 synthetase (ACSL5), a member of the ACSL family, plays a key role in fatty acid beta-oxidation and fat synthesis in mammals. In this study, two SNPs g.33185918G>A and g.33186348C>T in the 5' flanking sequence of the bovine ACSL5 gene were identified by PCR-RFLP. Thus, we aimed to evaluate the association between the two SNPs and meat quality traits, in addition to the carcass quality of Chinese Simmental-cross steers. The two SNPs were genotyped, and 36 carcass and meat quality traits were measured in a population of 354 individuals. Statistical analysis revealed that the two SNPs were significantly associated with carcass and meat quality traits. g.33185918G>A was very significantly ($p<0.01$) associated with the weight of the omasum and the pH of beef. For g.33186348C>T, the weight of the liver, hind hooves, kidney, and testis and the fat coverage rate of the carcass were very significantly associated ($p<0.01$). The present work indicated that polymorphisms in ACSL5 might be important genetic factors affecting meat quality and carcass yield in Chinese Simmental-cross steers. Moreover, such polymorphisms may serve as practical markers for carcass and meat quality traits in future marker-assisted selection (MAS) programs in beef cattle breeding and production.

Keywords: ACSL5, Chinese Simmental cattle, SNPs, Meat quality trait, MAS.

INTRODUCTION

With the rapid development of molecular genetics, it is possible to identify DNA polymorphisms that affect various traits of interest in livestock(Charlier *et al*., 2008; Mullen & Kenny, 2015). Nevertheless, it remains a challenge to identify variation in the carcass and meat quality traits of beef cattle involving meat tenderness, fatty acid contents, meat sensory quality, dressed weight and intramuscular fat levels, which mainly results from different genotypes(Esmailizadeh *et al*., 2011; Bonfatti *et al*., 2013; Poleti *et al*., 2015). A better understanding of the association between genetic variants and meat quality could allow us to adapt bovine meat quality to meet consumer and market demands through marker-assisted selection (MAS) programs(Williams, 2005).

Acyl-coenzyme A synthetases (ACSs) catalyze the fundamental, initial reaction in mammalian fatty acid metabolism: the "activation" of fatty acids (FAs) via the thioesterification to CoA allows FAs to participate in both the anabolic and catabolic pathways of triglycerides(Watkins *et al*., 2007; Ellis *et al*., 2010). Long-chain acyl-coenzyme A synthetases (ACSLs) belong to the ACS family and include 5 isoforms, designated ACSL1, ACSL3, ACSL4, ACSL5 and ACSL6, listed in order of their discovery(Kuwata *et al*., 2014). ACSLs mainly identify saturated and unsaturated FAs with carbon chain lengths of 12-20. Because long-chain fatty acids are the main dietary fatty acids consumed by mammals, ACSLs are more important than other ACS family members. ACSLs are key enzymes in the synthesis of triglycerides, phospholipids and cholesterol esters as well as in the oxidation of fatty acids(Lopes-Marques *et al*., 2013).

ACSL5, a unique ACSL isoform that is the only isoform to localize on mitochondria, also differs from other family members in its tissue distribution(Mashek, Li, *et al*., 2006). Molecular characterization of the bovine ACSL5 gene revealed that this gene is located on chromosome 26:33,184,653-33,234,956 and spans approximately 5 kb, comprising 23 exons and 22 introns and encoding a 683-amino acid protein with a CoA binding site and an AMP binding site. ACSL5 is essential for triglyceride synthesis and the β-oxidation of fatty acids, especially when using exogenous-source long-chain fatty acids as substrates, and it is abundant in the liver and duodenum(Teng *et al*., 2009). However, it is not clear whether ACSL5 is related to carcass or meat quality.

In addition, several studies on livestock involving other isoforms of ACSLs have suggested that there is a notable relationship between ACSLs and both carcass and meat quality (Hoashi *et al*., 2008; Rusc *et al*., 2011; Fang *et al*., 2014). ACSL5 is the only isoform that has been found to localize to the mitochondria in mammals; therefore, ACSL5 was hypothesized to be involved in fatty acid oxidation, which may be essential

for meat quality and carcass quality(Mashek, *et al*., 2006). In the current study, PCR-RFLP would be used to distinguish the genotypes of 354 cattle, then the association between genotypes and 36 traits of meat and carcass quality would be analyzed by statistical software. We aimed to identify SNPs in bovine ACSL5 gene to be used for MAS, which could be a fast, convenient, cost-effective andpractical way to identify the advantaged genotypes and may play a significant role in cattle breeding.

MATERIALS AND METHODS

Ethics statement: Animal experiments were strictly performed in accordance with the guidelines for the care and use of laboratory animals of the animal care and use committee of Jilin University (Permit number: SYXK(J) 2008-0010/0011). All of the examined meat quality and standardized carcass parameters were measured by the Chinese Academy of Agricultural Sciences Meat Laboratory.

Experimental Materials: A total of 354 Chinese Simmental steers (28 months old) from the Inner Mongolian Baolongshan cattle farm were used in this study. These steers were randomly selected from the offspring of a Simmental population with more than 2,000 cows and 25 bulls. Blood samples (10 ml each) were collected from the jugular vein, then blended with an anticoagulant (acid citrate dextrose, ACD), and stored at -40ºC. DNA extraction kit (Tiangen, Beijing, China) was used to extract DNA from 1 ml of whole blood, in accordance with the manufacturer's protocol.

Traits analyzed: The carcasses were stored at between 0ºC and 4ºC for 24 h. All of the meat traits and carcass traits were subsequently determined based on the GB/T17238-1998 cutting standard criterion for fresh and beef in China (China Standard Publishing House).

The live backfat thickness, bone weight and rib eye area (by ultrasound) were investigated before slaughter. Thirteen carcass traits that were used to determine the yield grades for the carcasses were measured at the slaughter plant. Six meat quality traits that were employed to determine quality grades were measured at the Chinese Academy of Agricultural Sciences Meat Laboratory.

The following 36 meat and carcass traits were measured as described by Tian *et al*., (2013): the weight of the carcass, bone, green hide, head, front hooves, hind hooves, furs, rumen, reticulum, abomasum, omasum, heart, liver, kidney, lung and weasand, pizzle, testes, gonad fat, spleen, and oxtail; carcass yield; kidney fat; degree of marbling; meat color score, fat color score; fatty acid composition content; carcass length; carcass depth; hind leg circumference; hind leg width; thigh meat thickness; thorax depth; thickness of the loin; hind leg length; thickness of fat coverage; pH of the fresh carcass; and pH of beef. The number of records and the means for the analyzed traits are shown in Table 1.

Primers and PCR amplification: Primers were designed based on the published sequence of the bovine ACSL5 gene (GenBank accession number: AC_000183.1) using the program Oligo 6. The primer sequences were ACSL5-11F: 5'-ggcaccctcatcctaacctac-3', ACSL5-11R: 5'-acaccttgaaagcgtcttgag-3', ACSL5-31F: 5'-ctgatgagactactatgcgtcc-3', and ACSL5-31R: 5'-aacaacagtgaaaaggcagac-3'. PCR was performed in a total volume of 25 µl using the following mixture: 100 ng of bovine genomic DNA, 200 µmol/L of each dNTP, 10 pmol/L of each primer, 2.5 µl of buffer (10× concentrate: 200 mmol/L Tris-HCl, pH 8.4, 500 mmol/L KCl), 1.5 mmol/L $MgCl_2$, and 1.0 units of Taq DNA polymerase. The amplification protocol involved an initial denaturation step at 94ºC for 5 min, followed by 35 cycles of denaturation (94ºC for 40 s), annealing (56ºC for 45 s), and extension (72ºC for 1 min), with a final extension step at 72ºC for 5 min.

SNP detection and genotyping: Two polymorphisms of the ACSL5 gene were identified through sequencing and confirmed via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using restriction enzymes (HpyCH4IV and BsmAI, New England Biolabs, MA, USA). Digestion of the amplicons of the two SNPs was performed in a final volume of 18 µl, containing 8 µl PCR product, 1.8 µl enzyme buffer, and 0.4 µl of restriction enzyme. The enzyme-digested products of the ACSL5 gene segments were visualized in a 1.5% agarose gel.

Statistical analysis: The allelic and genotypic frequencies of the polymorphism information content were calculated. The Chi-squared test was used to determine the Hardy-Weinberg equilibrium of the mutations. A significance test was performed to analyze the values of the genotype frequencies calculated from the examined Simmental-cross steers. Then, we employed two-way ANOVA in SPSS 13.0 to determine the association between the ACSL5 gene polymorphisms and carcass trait data following the fixed model

$Y_{ijkl} = u + fys_j + m_k + e_{ijkl}$.

Where Y_{ijkl} is the observed value of the l^{th} individual from Simmental breed i of genotype k in the j^{th} farm-year-season; u is the least square means of the observed values; fys_j is the effective value of the j^{th} farm-year-season; mk is the effective value of genotype k; and e_{ijkl} is the random residual effect corresponding to the observed value.

RESULTS

PCR amplification: The genomic DNA of the 354 Simmental steers was amplified using primers targeting the ACSL5 gene. Two amplified fragments of 441 bp and 348 bpwere completely identical tothe target fragment, and showed good specificity (Figure c), which could be directly used in PCR-RFLP analysis.

RFLP analysis and sequencing of different genotypes: The amplified products of the ACSL5 gene were analyzed via PCR-RFLP. Two polymorphisms were detected in the PCR products through DNA sequencing (by Shenggong, Shanghai, China), as shown in Figure d. Two loci, a G/A substitution at the -1,107 base pairs position and a C/T substitution at the -677 base pairs position, both in the ACSL5 gene flanking sequence were detected, which might be associated with the efficiency of the promoter. For G/A site, digestion of the 441 bp band PCR fragment of ACSL5 with HpyCH4IV showed two bands of 239 and 202 bp lengths for genotype GG individual (homozygous); GA (heterozygous) showed 441 bp, 239 bp, and 202 bp bands; AA (homozygous) showed 441bp bands (Figure d). For T/C site, digestion of the 348 bp band PCR fragment of ACSL5 with BsmAI showed two bans of 259 and 89 bp lengths for genotype CC individual (homozygous); GA (heterozygous) showed 348 bp, 259 bp, and 89 bp bands; TT (homozygous) showed 348 bp bands (Figure d).

Genetic diversity of the ACSL gene in the Chinese Simmental steer population: The genotype frequency and allele frequency calculated for the two polymorphisms of the ACSL5 gene detected in this study are shown in Table 2. For g.33185918G>A, allele G exhibited a frequency of 0.944, whereas for g.33186348C>T, allele C presented a frequency of 0.697 in the Simmental steer group.

The values of genetic diversity and characteristic, including PIC and χ^2 values are shown in Table 3. The PIC ranged from 0.17-0.46, indicating that SNPs in bovine ACSL5 gene of this investigated populations was moderate polymorphism. Chi squared (χ^2) tests showed that the two SNPs of the ACSL5 gene did not fit with Hardy-Weinberg equilibrium in the studied populations ($P<0.05$; Table 2)

Association analysis of ACSL5 polymorphisms with carcass and meat quality traits: It was revealed through statistical analysis that the two identified polymorphisms, g.33185918G>A and g.33186348C>T, both showed a significant association with meat quality and carcass traits.

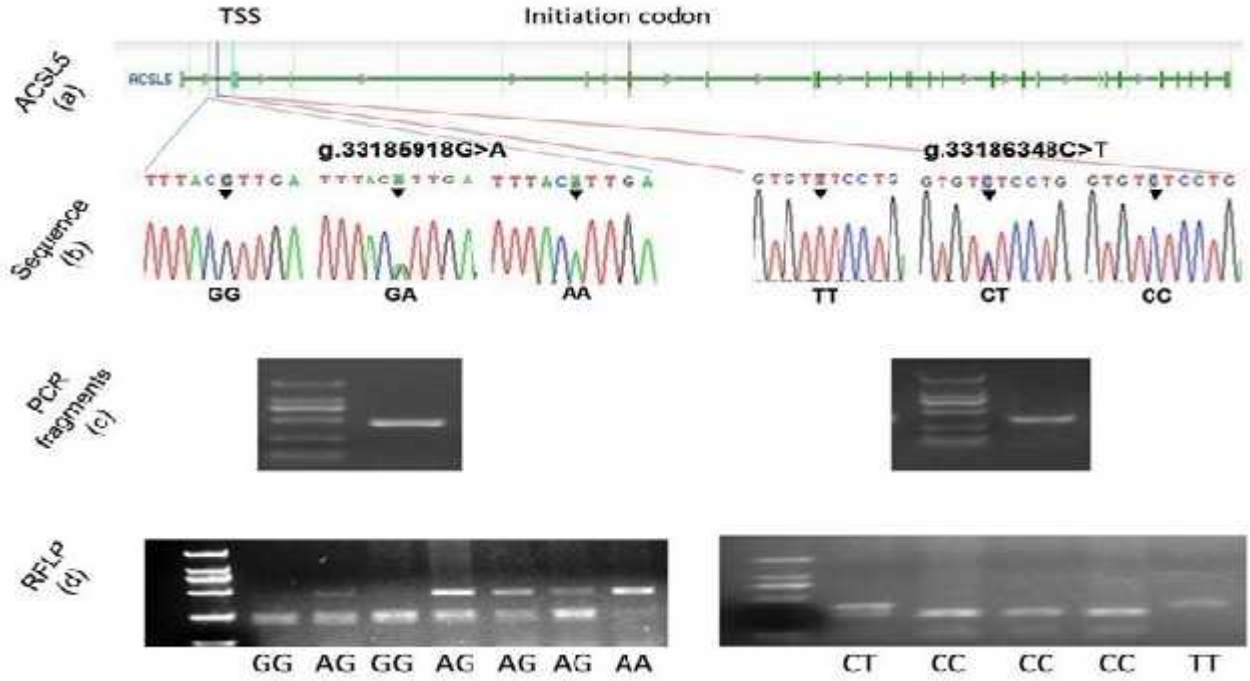

Figure 1.Sequencing and PCR-RFLP analysis of bovine ACSL5 gene.

(a) Two SNPs located in bovine genome were showed(TSS, transcriptional start site). (b) Sequencing results, there was a G/A substitution at position -1,107 bp and a C/T substitution at position -677 bp of ACSL5 gene 5' flanking sequence. (c) Two amplified 441 bp and 348 bp products showed good specificity. (d) 354 Chinese Simmental steers were genotyped using RFLP with restriction enzymes HpyCH4IV and BsmAI.

For g.33185918G>A, the G-bearing genotypes (GA heterozygotes or G allele homozygotes) were associated with a greater weight of the rumen, omasum and testes and a higher fat color score (7.48±0.91 kg, 3.94±0.59 kg, 0.69±0.13 kg, and 2.97±0.74, respectively) than the AA genotype, which was related to a higher pH of beef (5.91±0.78 kg) ($P<0.05$, Table 3). Moreover, the weight of the omasum and the pH of beef were very significantly influenced by genotype variation ($P<0.01$).

For g.33186348C>T, the T-bearing genotypes (TC heterozygotes or T allele homozygotes) were associated with a greater weight of the liver (6.33±1.24kg), whereas the CC genotype was related to a greater weight of the hind hooves, a greater length of the hind leg, a higher genital fat content, a greater carcass length, a lower weight of the kidney, heart and testes, and a lower fat coverage rate of the carcass and loin fat thickness (3.39±1.03 kg, 79.87±3.98 cm, 0.91±0.37, 139.50±7.79 cm, 1.17±0.19 kg, 1.84±0.36 kg, 0.65±0.13 kg, 50.38±20.46%, and 6.89±0.87 cm, respectively) ($P<0.05$, Table 3). Moreover, the weight of the liver, hind hooves, kidney, and testes and the fat coverage rate of the carcass were very significantly influenced by genotype variation ($P<0.01$).

Table 1. Number of records, mean and standard deviations for traits included in the association analysis (N=354).

Trait	Mean	Standard Deviation
Hair weight(kg)	491.69	61.91
carcass weight(kg)	257.02	38.97
dresssing percentage(%)	52.14	2.41
net weight of bone(kg)	20.01	3.02
head weight(kg)	23.47	2.42
front hooves weight(kg)	5.93	0.69
hind hooves weight(kg)	3.40	0.99
hide weight(kg)	41.77	6.12
stomach weight(no omasum)(kg)	7.46	0.92
omasum weight(kg)	3.92	0.67
heart weight(kg)	1.83	0.35
liver weight(kg)	5.98	1.09
weight of lung and trachea(kg)	3.15	0.49
kidney weight(kg)	1.18	0.21
kidney fat weight(kg)	4.92	2.81
pizzle weight(kg)	0.43	0.08
testis weight(kg)	0.67	0.15
genital fat weight(kg)	0.87	0.35
spleen weight(kg)	0.85	0.19
tail weight(kg)	1.36	0.25
pH of carcass	6.21	0.51
pH of chilled meat	5.54	0.34
carcass length(cm)	139.37	7.90
carcass depth(cm)	64.34	3.26
carcass brisket depth(cm)	65.13	3.62
hind legs circumference(cm)	49.00	3.78
hind leg width(cm)	44.56	2.71
hind leg length(cm)	79.86	3.98
thigh meat thichness(cm)	17.92	1.70
thickness of loin(cm)	6.90	0.91
backfat thickness(cm)	1.01	0.62
fat coverage rate(%)	50.51	20.98
marbling score	5.34	0.71
rib eye area(cm^2)	79.55	12.91
beef color	5.65	1.07
fat color score	2.64	0.95

Table 2.Genotypic frequencies and genetic diversity of ACSL gene in Simmental cattle.

SNPs	Heads	Allele frequency		Genotype frequency			χ^2	P	PIC
g.33185918G>A	354	G(0.94)	A(0.06)	AA(0.01)	GA(0.09)	GG(0.90)	11.28	0	0.46
g.33186348C>T	318	C(0.70)	T(0.30)	TT(0.07)	CT(0.47)	CC(0.46)	5.06	0.02	0.17

SNPs, single nucleotide polymorphisms; PIC, polymorphism information contents.

Table 3. Association of ACSL5 gene SNPs with carcass and meat quality traits in Chinese Simmental-cross steers.

Trait	Genotypes (g.33185918G>A)						Genotypes (g.33186348C>T)					
	AA(n=4)		GA(n=32)		GG(n=318)		CC(n=149)		CT(n=145)		TT(n=24)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HHW(kg)	3.19	1.23	3.32	0.86	3.41	1.01	3.39^{A}	1.03	3.18^{B}	0.84	3.22^{AB}	0.89
STW(kg)	6.50^{b}	1.37	7.34^{a}	0.9	7.48^{a}	0.91	7.46	0.99	7.38	0.9	7.47	0.88
OW(kg)	3.08^{B}	1.11	3.94^{A}	0.59	3.92^{A}	0.66	3.87	0.71	3.87	0.61	3.84	0.64
HTW(kg)	1.73	0.48	1.84	0.36	1.83	0.35	1.84^{b}	0.36	1.85^{b}	0.32	1.97^{a}	0.39
LW(kg)	6.28	0.8	5.87	1.17	5.98	1.09	5.92^{B}	1.08	6.17^{A}	1	6.33^{A}	1.24
KW(kg)	1.11	0.16	1.15	0.17	1.19	0.21	1.17^{B}	0.19	1.20^{AB}	0.19	1.28^{A}	0.33
PW(kg)	0.35^{B}	0.06	0.43^{AB}	0.08	0.43^{A}	0.08	0.43	0.08	0.43	0.08	0.43	0.08
TW(kg)	0.51^{b}	0.2	0.69^{a}	0.13	0.68^{a}	0.15	0.65^{B}	0.13	0.69^{A}	0.16	0.70^{AB}	0.16
GFW(kg)	0.67	0.46	0.92	0.41	0.87	0.35	0.91^{a}	0.37	0.83^{b}	0.35	0.84^{ab}	0.34
CMpH	5.91^{A}	0.78	5.60^{AB}	0.19	5.53^{B}	0.34	5.55	0.34	5.49	0.32	5.47	0.32
CL(cm)	133.5	8.89	139.48	6.89	139.43	7.98	139.50^{a}	7.79	138.29^{ab}	7.92	136.08^{b}	9.49
HLL(cm)	78.5	6.12	79.42	3.47	79.92	4	79.87^{a}	3.98	79.18^{b}	3.86	79.27^{ab}	4.35
LT(cm)	6.55	1.2	6.85	0.98	6.91	0.9	6.89^{b}	0.87	7.08^{a}	0.91	6.92^{ab}	1.03
FCR(%)	48	19.73	50.69	22.6	50.53	20.89	50.38^{B}	20.46	54.15^{A}	19.55	55.63	19.23^{AB}
FCS	2.50^{ab}	1.29	2.97^{a}	0.74	2.60^{b}	0.96	2.65	0.93	2.53	0.98	2.5	0.78

Significance of difference from each other: the traits means with different capital letters in the same column show very significant difference (P<0.01); the trait means with different small letters in the same column show significant difference (P<0.05); SD, standard deviations of mean; HHW, hind hooves weight; STW, stomach weight (no omasum); OW, omasum weight; HTW, heart weight; LW, liver weight; KW, kidney weight; PW, pizzle weight; TW, testis weight; GFW, genital fat weight; CMpH, pH of chilled meat; CL, carcass length; HLL, hind leg length; LT, thickness of loin; FCR, fat coverage rate; FCS, fat color score.

DISCUSSION

Although the meat quality traits that are important for beef production are influenced by the environment, e.g., management conditions, nutritional status and handling pre-slaughter, the genetically controlled variation (heritability) of the meat quality traits important for production, and product quality is relatively high, between 0.15 and 0.35(Wheeler *et al*., 2004). This suggests that an appreciable proportion of the variation is under genetic control, and hence could be improved by selection. By choosing to breed from the beef cattle with the most favorable alleles at important genes, the rate of beef cattle improvement could be significantly increased. Importantly, these gene-based methods have the potential to facilitate the improvement of meat quality traits that are difficult to select for by the traditional phenotype based methods(Toldrá, 2008).

Intramuscular fat, seen as marbling fat in meat, is important for eating quality, as itaffects flavor, juiciness, and possibly toughness(De la Fuente *et al*., 2009).The varying fatty acid compositions of adipose tissue and muscle have profound effects on meat quality(Pannier *et al*., 2010; Cesar *et al*., 2014). Fatty acid composition determines the firmness/oiliness of adipose tissue and the oxidative stability of muscle, which in turn affects flavour and muscle colour(Wood *et al*., 2008).

Long-chain acyl-CoA synthetases (ACSLs) and fatty acid transport proteins (FATPs) activate fatty acids (FAs) to acyl-CoAs prior to their downstream metabolism(Catala-Rabasa *et al*., 2011). Of numerous ACSL and FATP isoforms, ACSL5 is expressed predominantly in tissues with high rates of triacylglycerol (TAG) synthesis, suggesting it may have an anabolic role in lipid metabolism.

Thus, we inferred that the ACSL5 gene might be associated with carcass and meat quality traits of beef cattle. Therefore, To elucidate the specific roles of ACSL5 in fatty acid metabolism of beef cattle, we identified two novelSNPs, g.33185918G>A and g.33186348C>Tupstream of ACSL5 exon. The genotype frequencies of the 2 SNPs were analyzed with a Hardy-Weinberg equilibrium test. The reason for the observed disequilibrium may be the use of artificial insemination and the high intensity of unnatural selection in recent

years. Then totally 36 meat quality and carcass traits for the 354 Simmental population were measured. The association between the two SNPs and 36 important economic traits of beef cattle were analyzed. Statistical analysis revealed that SNP1(g.33185918G>A)was extremely significantly associated with carcass and meat quality, and particularly with the weight of the omasum and the pH of beef. Furthermore, steers with the AA genotype showed the highest pH of the meat. The pH of meat may partially result from the fatty acid composition and content and may be associated with meat tenderness, which is considered to be the most important quality trait in beef(Liu *et al*., 2015). SNP2(g.33186348C>T) was significantly associated with the weight of the liver and the fat coverage rate of the carcass. Steers carrying the CC genotype showed the lowest fat coverage rate of the carcass, indicating a higher lean rate of the carcass, which is essential for beef quality and is desirable for cattle husbandry and market regulation. We also detected a greater liver weight corresponding to the T-bearing genotype, but whether this genotype is responsible for high expression of ACSL5 in liver is not yet clear.

Our results revealed that the two SNPs identified in the 5' flanking sequence of the ACSL5 gene, g.33185918G>A and g.33186348C>T, were completely linked in the studied population and were significantly related to the fat catabolism and deposition capacity. Furthermore, we demonstrated that the two genotypes were associated with a good fattening ability, meat quality and carcass quality in the Simmental population. Therefore, using the two SNPs for MAS in a larger population as well as investigating whether the ACSL5 gene plays a significant role in commercial traits will be necessary in future studies.

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