Association between single-nucleotide polymorphisms of fatty acid synthase gene and meat quality traits in Datong Yak (*Bos grunniens*)

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ABSTRACT. Fatty acid synthase (FASN) is a key enzyme in fatty acid anabolism that plays an important role in the fat deposit of eukaryotic cells. Therefore, in this study, we detected 2 novel single-nucleotide polymorphisms (SNPs) in the *FASN* gene in 313 adult individuals of Datong yak using polymerase chain reaction-single strand conformation polymorphism and DNA sequencing techniques. SNP g.5477C>T is located in intron 3 of *FASN*, and 3 genotypes, HH, HG, and GG, were detected in this mutation site. SNP g.16930T>A is located in exon 37 of *FASN*, and 2 genotypes, EE and EF, were detected in this site. Association analysis of these 2 SNPs with meat quality traits showed that in SNP g.5477C>T, yaks with the HH genotype and HG genotype

had significantly higher intramuscular fat content than individuals with the GG genotype (P < 0.01). In SNP g.16930T>A, yaks with the EE genotype also had significantly higher IMF content than individuals with the EF genotype (P < 0.01). The results indicate that *FASN* may be used as a candidate gene affecting intramuscular fat content in Datong yaks.

Key words: Fatty acid synthase gene; Meat quality traits; Polymorphism; Yak

INTRODUCTION

Most fatty acids needed for animal body fat deposition originate from the *de novo* fatty acid synthesis, which requires fatty acid synthase (FASN) to catalyze the reaction between acetyl coenzyme A, malonyl-CoA, and nicotinamide adenine dinucleotide phosphate (Stuart et al., 1991; Chakravarty et al., 2004). *FASN* is thought to be a candidate gene in animal breeding for improving fat deposition and meat quality traits and plays a central role in lipogenesis in mammals (Roy et al., 2005; Zhang et al., 2008). The bovine *FASN* gene is located on chromosome 19 (Roy et al., 2001) and is 18,824 base pairs in length, including 42 exons and 41 introns. Reverse transcription-polymerase chain reaction (PCR) and Western blot analysis have shown that FASN expression in the brain, testis, and adipose tissue is higher than that in the liver and heart (Roy et al., 2005). Genetic variants of *FASN* gene have been reported to be associated with fat percentage in bovine milk (Roy et al., 2006). Five single-nucleotide polymorphisms (SNPs) in 3 different cattle populations have been identified, and association mapping results using these SNPs were associated with variation in fatty acid composition in adipose fat and milk fat (Morris et al., 2007).

Yak (*Bos grunniens*) is a domestic animal that lives on the Qinghai-Tibetan Plateau at altitudes between 2500 and 6000 m. It is the primary source of meat, milk, and hair in this area of Tibet. Yak meat is high in protein and calories, yet low in fat, and is increasing in popularity because of its good taste. Moreover, yak meat is referred to as "green food" because yaks subsist on the alpine grassland without the pollution of modern industry (Guo et al., 2009). With the development of molecular genetics, genetic marker-assisted selection has been widely used in animal breeding (Yan et al., 2010). Thus, biological genetic marker technology is very important in yak breeding.

These findings suggest that the *FASN* gene plays an important role in fatty acid metabolism. Few studies of the *FASN* gene in yak have been reported. Therefore, the objective of this study was to detect potential SNPs in yak *FASN* gene and explore the associations between SNPs and meat quality traits in yak to select animals with better meat quality.

MATERIAL AND METHODS

Animals

During slaughter season (October-November), a total of 313 blood samples of adult (approximately 4 years old) Datong yaks were collected from domestic slaughter houses before slaughter. Several meat quality traits were measured in the *longissimus dorsi* muscle in a sample taken from the last rib. Intramuscular fat content (IMF) was measured using standard

methodology (Honikel, 1998). Muscle pH was measured at 24 h *post mortem* (pH 24 h) using a portable pH meter (Crison Instruments, Barcelona, Spain) (Korkeala et al., 1986). Warner-Bratzler shear force was measured using the Texture Analyser Winopal (Ahnbeck, Germany) with a Warner-Bratzler blade (2.8 mm wide). Water holding capacity was estimated by centrifuging 1 g muscle placed on tissue paper inside a tube for 4 min at 1500 *g*. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. Water holding capacity was calculated as: dried weight/initial weight x 100. Cooked meat percentage was estimated as the percentage of the roasted sample weight (cooled for 30 min, remove the meat samples stored at room temperature after cooling 15 min). Genomic DNA was extracted using a genomic DNA isolation kit (Tiangene, Beijing, China) according to manufacturer instructions.

Primer design and PCR amplification

Five pairs of PCR primers (Table 1) were designed based on the bovine *FASN* gene (GenBank accession No. NC_000176). A 15-μL reaction volume included 1 μL (50 ng/μL) template, 7.5 μL *Taq* PCR MasterMix, 0.2 μL Taq DNA polymerase, 5.7 μL ddH$_2$O, and 0.3 μL of each primer (10 pM/μL), performed in a Tpersonal thermocycler (Biometra, Goettingen, Germany) under the following conditions: 95°C for 5 min (preliminary denaturation), followed by 35 cycles of 94°C for 60 s (denaturation), primer annealing at 58.3° and 57°C (corresponding to 2 primer pairs) for 35 s, and 72°C for 50 s, and a final extension at 72°C for 10 min. PCR products were then electrophoresed on 1.5% agarose gels using 1X TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA Na$_2$), containing 200 ng/mL ethidium bromide to detect the products.

Table 1. Primers used for PCR amplification.

Primers	Sequence of primers	Size (bp)	Annealing temperature (°C)
P1	F: GGTTTGACTTCTGCCTCCT	649	61
	R: CCACTGCTCTCACCTGATG		
P2	F: CCAACCGCCTCTCCTTCTT	355	65
	R: ACTCCCCTCTCTGGATGGC		
P3	F: TCGGCAAAGTGGTCATTCAG	767	62.4
	R: CATTGTACTTGGGCTTGTTGA		
P4	F: GGGCACCTTAGGCTTGG	365	59.3
	R: ACTCAGGGGTCTGGTTATCC		
P5	F: CTGAAGGGCACTAAAGACAAA	256	57
	R: GGAGACCAGACTCGGAAGA		

Genotyping

To screen for polymorphisms in the fragments analyzed, PCR amplifications were subjected to single-strand conformation polymorphism analysis. First, 4 μL PCR products were mixed with 8 μL loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, and 0.025% xylene-cyanol) after denaturing at 98°C for 10 min; the mixture was immediately placed on ice for 10 min. Next, the mixture was loaded onto polyacrylamide gels and electrophoresed at 250 V for 10 min and then at 200 V for 2 h in 1X TBE buffer. The polyacrylamide gel was silver-stained with 0.1% AgNO$_3$ and visualized using 2% NaOH (Lan et al., 2007). According to the PCR-single-strand conformation polymorphism band patterns observed using visual light, individual genotypes were defined (Qu et al., 2005). Amplifica-

tions derived from the 3 allele standards were included on each gel for reference (Byun et al., 2008). Representative PCR products corresponding to different mutation types of homozygous individuals with different genotypes were sequenced. Forward and reverse reactions were both performed to rule out false-positives.

Statistical analysis

Genotypic frequency, allele frequency, Hardy-Weinberg equilibrium, gene homozygosity, gene heterozygosity (HE), effective allele numbers (NE), and polymorphism information content (PIC) were statistically analyzed using the method described by Nei and Roychoudhury (1974) and Nei and Li (1979). Associations between genotypes of SNPs in the *FASN* gene, 5 meat quality traits (water loss, cooked meat percentage, PH_{24}, shear force, and IMF fat content), and genetic effects were analyzed using the general linear model procedure in the SPSS software (Version 18.0; SPSS, Inc., Chicago, IL, USA). The following model was used: $Y_{ijk} = \mu + S_i + N_j + E_{ijk}$, where Y_{ijk} is the trait measured in yak, μ is the population mean, S_i is the fixed effect of gender, N_j is the fixed effect of genotype, and E_{ijk} is random error. Significant differences between least-square means of different genotypes were determined using the Duncan multiple-range test, given that the trait was excluded from the model if its effect was not significant ($P > 0.05$). The values are reported as least square means and standard error. P values of 0.05 were considered to be statistically significant.

RESULTS

Genotype distribution and genetic diversity

The PCR products obtained at the most suitable temperature were detected, and the amplification results were satisfactory (Figure 1A and B). The 2 target fragments of the gene were denatured; polymorphisms were identified by polyacrylamide gel electrophoresis (Figure 2A and B). For convenience, the 2 mutations were referred to as sequence variant 1 (SV1) and sequence variant 2 (SV2).
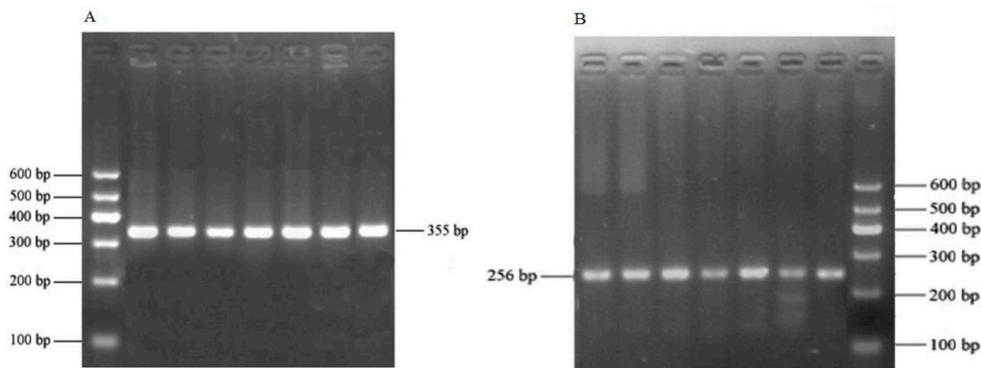

Figure 1. Detection of PCR products of SV1 (A) and SV2 (B). **A.** *Lane M* = marker I (600; 500; 400; 300; 200; 100 bp); *lanes 1-7* = PCR products of SV1 (355 bp). **B.** *Lane M* = marker I (600; 500; 400; 300; 200; 100 bp); *lanes 1-7* = PCR products of SV2 (256 bp).
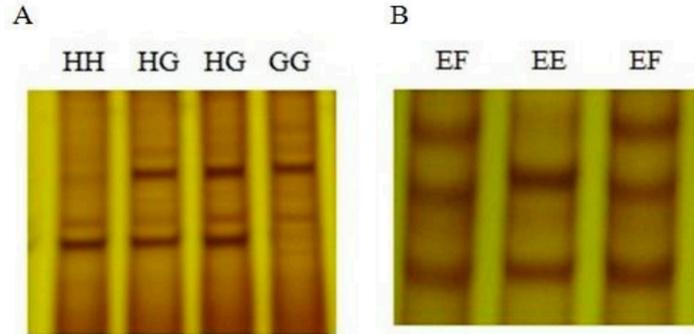
Figure 2. Electrophoresis pattern of PCR-SSCP of SV1 (A) and SV2 (B). **A.** Three genotypes HH, HG, and GG in SV1 locus. **B.** Three genotypes EE and EF in SV2 locus.

At the SV1 locus, 3 different genotypes (HH, HG, and GG) were identified in the Datong yak population. The frequencies of the 2 alleles were near and the heterozygote genotype (HG) showed a high prevalence with a frequency of 0.645. The genotypic frequencies of the SV1 locus in the Datong yak population were not in Hardy-Weinberg disequilibrium (P < 0.05) (Table 2). At the SV2 locus, only 2 genotypes (EE, EF) were identified. The frequency of allele E was dominant in the Datong yak population and EE genotype was more frequent than the EF genotype. The genotypic frequencies of the SV2 locus in Datong yak population agreed with Hardy-Weinberg disequilibrium (P > 0.05) (Table 2).

Table 2. Genotypic frequencies at *FASN* gene for the SNPs in Datong yak.

Locus	Genotype frequencies			Allele frequencies		χ^2
	HH	HG	GG	H	G	
SV1	0.232	0.610	0.158	0.537	0.463	15.88
SV2	EE	EF	FF	E	F	3.16
	0.801	0.199	0	0.900	0.100	

$\chi^2 0.05 = 5.991$, $\chi^2 0.01 = 9.21$. The value of $\chi^2 > 5.991$ means P < 0.05, on the contrary, P > 0.05.

The allelic and genotypic frequencies, genetic diversity parameters (H_O, H_E, N_E, and PIC) of the 2 SNPs are shown in Table 3. According to the genetic diversity classification of PIC (PIC value < 0.25, low polymorphism; 0.25 < PIC value < 0.5, intermediate polymorphism; and PIC value > 0.5, high polymorphism), the SV1 locus possessed intermediate polymorphism and the SV2 locus possessed low polymorphism.

Table 3. Population genetic indices at the 2 loci of the *FASN* gene in Datong yak.

Locus	H_O	H_E	N_E	PIC
SV1	0.502	0.498	1.992	0.375
SV2	0.810	0.190	1.234	0.190

H_O = gene homozygosity; H_E = gene heterozygosity; N_E = effective allele numbers; PIC = polymorphism information content. PIC > 0.5 means high diversity, 0.25 < PIC < 0.5 means moderate diversity, PIC < 0.25 means low diversity.

Sequence variants identified in the *FASN* gene

Comparison between sequences of the bovine *FASN* gene and the pooled yak DNA samples revealed 2 SNPs, g.5477C>T and g.16930T>A in intron 3 and exon 37, respectively (Figure 3A and B). The SNP g.16930T>A was a synonymous mutation.
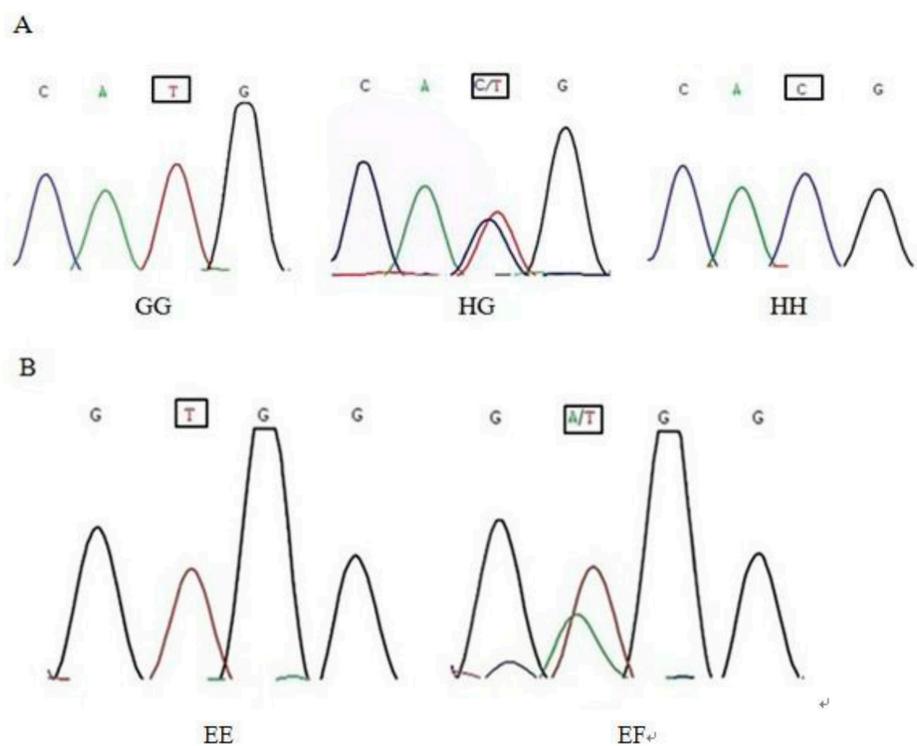

Figure 3. Sequences of SV1 (A) and SV2 (B). **A.** Sequences of 3 genotypes HH, HG, and GG in SV1 locus. **B.** Sequences of 2 genotypes EE and EF in SV2 locus.

Association analysis

The association analysis of the 2 mutations in the *FASN* gene of the Datong yak and meat quality traits are shown in Table 4. For the SV1 locus, the animals with genotype HH and HG showed significantly higher IMF content (P < 0.01) than those with genotype GG, implying that the H is associated with fat content. For the SV2 locus, significant differences in IMF content (P < 0.01) were observed between cattle with genotype EE and those with EF. The animals with genotype EE appeared to be superior in fat content compared to those with genotype EF. No significant differences were observed in PH$_{24}$, water loss, cooked meat percentage, and shear force for these 2 mutations. The remaining records of meat quality traits showed no significant association (P > 0.05).

Table 4. Association between genotypes of the 2 SNPs of the *FASN* gene and meat quality traits in Datong yak.

Locus	Genotype	Water loss (%)	Cooked meat percentage (%)	pH_{24}	Shear force kg/cm²	IMF content (%)
SV1	HH	28.12 ± 0.38	66.49 ± 0.43	5. 37 ± 0.06	4.85 ± 0.62	1.99 ± 0.64[A]
	HG	27.96 ± 0.55	66.52 ± 0.49	5.37 ± 0.04	4.83 ± 0.86	1.97 ± 0.54[A]
	GG	27.95 ± 0.48	66.57 ± 0.26	5.34 ± 0.06	4.83 ± 0.67	1.88 ± 0.52[B]
SV2	EE	28.12 ± 0.73	66.52 ± 0.16	5.37 ± 0.08	4.84 ± 0.67	1.96 ± 0.62[A]
	EF	28.19 ± 0.52	66.38 ± 0.17	5.36 ± 0.06	4.85 ± 0.91	1.88 ± 0.63[B]

[A,B]Means with different superscript letters are highly significantly different ($P < 0.01$). pH_{24} means pH value measured at 24 h after slaughtered.

DISCUSSION

As a central multifunctional enzyme responsible for *de novo* fatty acid biosynthesis, FASN is an important candidate gene affecting fat deposit and meat quality traits in animals. Numerous studies have been reported the effect of the *FASN* gene on fat acid in beef and milk. Morris et al. (2007) reported 5 SNPs in the *FASN* gene related to adipose fat and milk fat in 3 bovine breeds. Abe et al. (2009) and Matsuhashi et al. (2011) both reported SNPs in the *FASN* gene in Japanese Black beef; mutations were associated with fatty acid content of C18:0, C18:1, C14:0, C14:1, C16:0, and C16:1. Hou et al. (2011) reported the mutation g.16009 A/G in exon 34 of the *FASN* gene; this SNP had a significant effect on short-chain fatty acid content. Matsumoto et al. (2012) compared coding sequences of the *FASN* gene among 8 Holstein cattle. Thirteen SNPs were identified in Holstein cattle, and T1950A and W1955R affected milk fat content and C14 index. However, there have been no studies examining SNPs in the *FASN* gene in yak.

Meat quality is influenced by heredity, age, circumstances, and other factors. Heredity factors mainly refer to breed, while circumstance factors mainly refer to feeding conditions such as nutrition and temperature. To exclude the influence of different circumstance factors and age, we selected yaks of 1 breed (Datong yak) living in the same meadow of the same age of 4 years old as experimental animals. The quality of the beef was evaluated in the longissimus thoracis muscle because it is regarded as the most palatable cut (Matsuhashi et al., 2011).

In this study, 2 SNPs in the *FASN* gene were found in the Datong yak population, and affected meat quality traits. The first mutation was located in intron 3, which was not in Hardy-Weinberg equilibrium. The second mutation was located in exon 37, which was in Hardy-Weinberg equilibrium. Allele H in intron 3 and allele E in exon 37 were dominant, and individuals with genotype HH and HG in the first mutation and of genotype EE in the second mutation performed better regarding IMF content than those with genotypes GG and EF. A previous study showed that higher IMF content indicates better tenderness and thus beef flavor. Yak meat has lower IMF content compared with cattle meat. Therefore, increasing the IMF content of yak meat is an effective method for improving meat flavor. Allele H in intron 3 and allele E in exon 37 both had a positive effect on IMF content in yak meat. In this study, no individuals with genotype FF were found in 313 yaks. Datong yak is a cultivated yak breed in China, and after nearly 30 years of artificial selections and cultivation, the yaks with better performance have already been selected for use as breeders. This mating mechanism disrupted the Hardy-Weinberg equilibrium of natural mating mechanism. As a result, alleles associated with high performances were maintained, while alleles associated with low performances were reduced.

The results of this study as well as those of previous studies (Oh et al., 2012; Souza et al., 2012) suggest that *FASN* is an effective candidate gene that can be used as a DNA marker for improving IMF content through marker-assisted selection in yaks.

CONCLUSIONS

This study is the first to investigate the association between polymorphisms in the *FASN* gene and meat traits in Datong yak. Statistical results showed a significant relationship between the *FASN* polymorphisms and IMF content in Datong yak. In conclusion, the study indicated that genotyping of the *FASN* gene may be useful for selecting yaks with higher IMF content and thus improving the beef flavor.

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