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Association analyses of DNA polymorphisms in bovine SREBP-1, LXRα, FADS1 genes with fatty acid composition in Canadian commercial crossbred beef steers

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Abstract Two previously reported DNA polymorphisms of sterol regulatory element binding transcription factor 1 (SREBP1) and liver X receptor alpha (LXRα) and two DNA polymorphisms of fatty acid desaturase 1 (FADS1) were evaluated for associations with fatty acids in brisket adipose tissue of Canadian cross-bred beef steers. The polymorphism of 84 bp insert/deletion in intron 5 of SREBP1 was significantly associated with the concentration of 9c C17:1 (P=0.013). The G>A single nucleotide polymorphism (SNP) in the exon 4 of LXRα gene was associated with the concentration of 9c, 11t C18:2 (P=0.04), sum of conjugated linoleic acids (CLA) (P=0.025) and 11c C20:1(P=0.042). Two DNA polymorphisms in the promoter region of FADS1, deletion/insertion of ->GTG in rs133053720 and SNP A>G in rs42187276, were significantly associated with concentrations of C17:0 iso, C17:0 ai, total branched chain fatty acids (BFA), 12t C18:1, 13t/14t C18:1, 15t C18:1, and 13c C18:1 (P<0.05). Further studies are needed to validate the associations and to delineate the roles of the gene polymorphisms in determining the fatty acid composition in beef tissues.

Keywords fatty acid, beef; sterol regulatory element binding protein 1; liver X receptor alpha; fatty acid desaturase 1; association.

1. Introduction

The fatty acid (FA) composition in beef has emerged as an economically relevant trait to the beef industry due to increasing consumer awareness of the health implications of fat intake associated with red meat consumption. Accumulated evidence suggests the type of dietary fat

(or the fatty acid composition) has a more profound impact on human health than the amount of fat in the diet (Hu et al., 2001; Woodside & Kromhout, 2005). It is well established that the intake of saturated fatty acids (SFA) is positively correlated with atherosclerosis and other cardiovascular diseases whereas polyunsaturated fatty acids (PUFA) and rumenic acid (main natural isomer of conjugated linoleic acids (CLA)) can have health benefits (Wolfram, 2003; Bhattacharya et al., 2006; Richard et al., 2009). Recent studies with animal models showed that one of the *trans* fatty acid isomers also found in beef, vaccenic acid (11t C18:1), may also have a number of potential health benefits (Wang et al., 2008; Bassett et al., 2010). Increasing the concentrations of beneficial fatty acids of beef would, therefore, be a way to add values to the beef products.

Improving the content of beneficial fatty acids in beef has been achieved mainly through dietary manipulation (Gillis et al., 2004; Mir et al., 2004; Dugan et al., 2010). However, even when steers are fed the same diet significant variation in the fatty acid composition has been observed (Dugan et al., 2007), indicating a potential to improve the content of beneficial fatty acids through genetic selection. To date, genetic evaluation and selection has not been applied to fatty acids in cattle due to the complexity and relatively high cost of comprehensive fatty acid and genetic analyses. With refinements of methods, and then reduced expenses, identifications of DNA markers or gene polymorphisms influencing the fatty acid composition are now practical, which could facilitate the genetic improvement of fatty acid profiles through marker-assisted or genomic selection and/or marker-based diet management.

DNA polymorphisms in several bovine genes have been reported to have associations with the fatty acid composition in bovine tissues (Taniguchi et al., 2004; Abe et al., 2004;

Bhuiyan et al., 2009; Matsuhashi et al., 2011; Orrù et al., 2011), indicating that genetic control of fatty acid composition in beef tissues does exist. Hoashi et al. (2007) detected an 84bp deletion in the intron 5 of the *SREBP1* gene (rs133958066) and found that the deletion was associated with higher percent MUFA and lower melting point of intramuscular fat in Japanese Black cattle.

In Liver X receptor alpha (LXRα) gene, Hoashi et al. (2008) reported that a G>A single nucleotide polymorphism in the exon 4 of the *LXRα* gene (rs109428603) that causes an amino acid change from 'V' to 'I' at the position of 133 amino acid of the protein (i.e. V133I) was associated with linoleic acid content (C18:2n-6) in intramuscular fat of Japanese Black cattle.

In humans, studies showed that DNA polymorphisms in fatty acid desaturase 1 gene (FADS1) family were associated with arachidonic (C20:4n-6) , linoleic (C18:2n-6), alpha-linolenic (C18:3n-3) and eicosadienoic (C20:2n-6) acids of human serum (Malerba et al., 2008). Recently, Matsuzaka et al. (2002) reported that the mRNA of *FADS1* was elevated in mice overexpressing *SREBP1*, suggesting the link of *FADS1* with *SREBP1*. In cattle, two DNA polymorphisms for *FADS1*, rs42187276 (A>G), and rs133053720 (->GTG), were previously reported in the National Center for Biotechnology Information (NCBI) SNP database. The two polymorphisms are respectively located in the promoter region 134bp and 276bp upstream of the *FADS1* start codon. These two DNA polymorphisms have not been previously tested for associations with fatty acid composition in cattle. In this study, we evaluated the associations of the DNA polymorphisms of *SREBP1*, *LXRα* and *FADS1* genes with the fatty acid composition of brisket adipose tissue in a population of 225 Canadian

commercial crossbred beef steers.

2. Materials and Methods

2.1. Animals and management

Two hundred and twenty-five Angus and Charolais based crossbred commercial steer calves originating from Deseret Ranches near Lethbridge, Alberta were used in this study. The steers were part of a study that examined the impact of nonionophore antibiotics on feedlot cattle production (Aldai et al., 2008). The animals were cared according to the guidelines set out by the Canadian Council of Animal Care (CCAC, 1993). The animal management, diets, and non-ionophore antibiotics treatments were previously described (Aldai et al., 2008). The effect of non-ionophore antibiotic treatments on the fatty acid composition has been analyzed and reported previously (Aldai et al., 2008).

2.2. Animal tissue collection and fatty acid analyses

Steers were slaughtered in a commercial abattoir at 580 ± 34 kg and samples of brisket adipose tissues were collected within 48 h post mortem for each steer, placed in plastic bags, frozen on dry ice and stored at -80ºC. Details on fatty acid analyses have been previously described (Aldai et al., 2008). Briefly, brisket adipose tissue samples were freeze-dried and directly methylated with sodium methoxide. The fatty acid methyl esters (FAME) were analyzed by GC and silver-ion HPLC using the methods outlined by Cruz-Hernandez et al. (2004). However, the *trans*-18:1 isomers were further separated using two complementary GLC temperature programs instead of a preparatory silver-ion TLC separation combined with GLC analyses at 120ºC (Kramer et al., 2008).

The concentrations of fatty acids were expressed as a percentage of total FAME

quantified, and 33 individual and 11 groups of FAME with a concentration of greater than 0.1% were selected and analyzed in this study. In addition, a health index (HI) was also calculated as the inverse of atherogenic index as originally proposed by Ulbricht & Southgate (1991) with a modification as described by Zhang et al. (2008): HI = (total MUFA + total PUFA) / (4 x 14:0 + 16:0).

2.3. Genotyping

DNA was extracted from adipose tissue using the phenol/choloform/isoamyl alcohol method as described by Sambrook and Russel (2001). Genotypes of SREBP1 and LXRα were determined with the PCR method and the PCR restriction enzyme length polymorphism (PCR-RFLP) method, respectively, according to previous studies (Hoashi et al., 2007, 2008). Primers for genotyping were re-designed based on Ensembl bovine sequence NW_001493671.1 for SREBP1 and are listed in Table 1. The amplified DNA fragments were analyzed on a 1.5% agarose gel with 1x SYBR® Safe DNA Gel Stain (Invitrogen, Burlington, ON) in 1×TBE buffer. Genotypes were called based on the size of DNA fragment with the 432 bp assigned to allele 'L' (insertion) and 348bp to allele 'S' (deletion). For the SNP of G/A of LXRα, genotyping was performed using the RFLP PCR primers described in Hoashi et al., 2008 (Table 1). The amplified 1716 bp product was digested using HpyCH4IV (NEB, Pickering, ON). The digestions were performed in a 25 µl reaction volume with approximately 5 µg of PCR products and 1 unit of each restriction enzyme. The reaction was incubated at 37°C overnight. The digested PCR products were run on a 1% agarose gel and then stained with SYBR® Safe DNA Gel Stain. The 'G' allele showed 954bp, 440bp, 259bp and 63bp band sizes (allele 'V'), and the undigested 'A' allele showed a profile of 1017bp,

440bp and 259bp bands (allele 'I'). Several purified PCR products were sequenced to validate the LXRα RFLP genotypes using an ABI Big Dye Terminator v3.1 kit and 3730 capillary sequencer (ABI). The genotyping of the DNA polymorphisms of FADS1 were carried out using an ABI Step-One-Plus^{TM}real-time thermocycler based on allele discrimination using the 5 nuclease assay (Applied Biosystems, Foster City, CA 94404, USA) with a forward primer and a reverse primer and two TaqMan MGB fluorogenic probes targeted at the two alleles (Table 1). The primers and probes were designed according to bovine sequence of the GenBank accession NW_001494536 to genotype one deletion/insertion rs133053720 (allele D for '-' and allele I for 'GTG') and one SNP rs42187276 (allele A for 'A' and allele G for 'G') in FADS1 (Table 1).

2.4. Statistical analysis

Genotypic and allele frequencies were estimated for each of gene DNA polymorphisms based on the genotype counts, and intralocus genotypic proportions of each DNA polymorphism were examined for their conformation to the Hardy-Weinberg equilibrium using a X^2 test. Association analyses between the DNA genotypes and the fatty acid traits were carried out on a single DNA marker association basis using the PROC MIXED procedure in SAS (SAS Institute Inc.). The linear mixed model included fixed effects of antimicrobial (non-ionophore) treatment, SREBP1 rs133958066 genotype, LXRα rs109428603 genotype, FADS1 rs133053720 genotype or rs42187276 genotype, and feedlot pen nested in the treatment as a random factor. Parameters were estimated by the REML method, and the fixed effects were tested with the denominator degrees of freedom computed by the Kenward–Roger method. The additive effect of the DNA polymorphisms was

estimated as the difference between the solutions of means of the two homozygous genotypes divided by two and the dominance effect was estimated by subtracting the average of solutions of homozygous genotypes from the solution of the heterozygous genotype according to Falconer & Mackay (1996).

3. Result

3.1. Genotype and allele frequencies

The DNA polymorphisms of *SREBP1* and *LXRα* were detected in the beef cattle population with counts of genotypes listed in Table 2. The genotype frequencies of *SREBP1* were 0.98 for genotype LL (insertion/insertion), 0.02 for genotype LS (insertion/deletion), and 0 for genotype SS (deletion/deletion). The allele frequency for L was 0.99 while the allele frequency for S was 0.01. The genotype frequencies of LXRα were 0.88 for genotype VV (GG), 0.12 for genotype VI (GA), and 0 for genotype II (AA). The allele frequency were 0.94 for the V allele and 0.06 for the I allele.

The two DNA polymorphisms rs133053720 (->GTG) and rs42187276 (A>G) of *FADS1* were also successfully genotyped and their counts of genotypes were listed in Table 3 and Table 4, respectively. The genotype frequencies of rs133053720 were 0.48 for DD (deletion/deletion), 0.43 for DI (deletion and insertion), and 0.09 for II (insertion/insertion). The allele frequency was 0.70 for the D allele and 0.30 for the I allele. The genotype frequencies of rs42187276 were 0.49 for GG, 0.42 for GA and 0.09 for AA. The allele frequency for G of rs42187276 was 0.70 while the allele frequency for A was 0.30. The x^2 test showed that the genotype frequencies SREBP1, LXRα and FADS1 DNA polymorphisms conformed to the Hardy-Weinberg equilibrium (P>0.05).

3.2. Associations with fatty acid composition

There were only two genotypes detected in the population for each of the SREBP1 and LXRα DNA polymorphisms. As a result, association analyses were performed by contrasting the means of the fatty acid concentration between the two genotypes (Table 2). The 84bp length polymorphism in SREBP1 was found to be associated only with the concentration of 9c C17:1 at $P<0.05$. Steers with the LL genotype had a 23.0% higher concentration of 9c C17:1 than that of steers with the LS genotypes ($P=0.013$). The VV genotype of LXRα gene was associated with the concentration of rumenic acid 9c,11t C18:2 ($P=0.04$), sum CLA content ($P=0.025$) and 11c C20:1($P=0.042$). The steers with the VV genotype had 12.7% higher concentration of 9c,11t C18:2, 9.5% higher concentration of the sum CLA and 13.8% higher concentration of 11c C20:1 as compared to the steers with the VI genotypes.

Significant additive effects were detected for seven individual and groups of fatty acids including C17:0 iso, C17:0 ai, BFA, 12t C18:1, 13t/14t C18:1, 15t C18:1 and 13c C18:1 at $P<0.05$ for both polymorphisms of FADS1 (Table 3 and 4). These two polymorphisms showed similar association results due to their high linkage disequilibrium (LD) with the value of r^2 equal to 0.97. The DD genotype of rs133053720 or the GG genotype of rs42187276 was significantly associated with higher concentrations of C17:0 iso, C17:0 ai and total BFA in comparison to the II or AA genotypes ($P<0.05$). The DD genotype of rs133053720 or the GG genotype of rs42187276 was also associated with higher concentrations of some MUFA including 12t C18:1, 13t/14t C18:1, 15t C18:1, while lower concentrations of 13c C18:1 ($P<0.05$). No significant dominance effects were detected for the

45 individual and groups of fatty acids examined in the brisket adipose tissue for the two DNA polymorphisms of FADS1 ($P > 0.05$) (Table 3 and Table 4).

4. Discussion

The S allele for the 84bp deletion of SREBP1 has been previously reported with a frequency of 0.37 and 0.51 in different populations of Japanese Black cattle (Hoashi et al., 2007; Matsuhashi et al.,2011) and 0.28 in Korean Hanwoo Cattle (Bhuiyan et al., 2009). In a population of Fleckvieh cattle, a lower frequency of 0.0797 for the S allele was observed (Barton et al., 2010), and a lower frequency of 0.22, 0.00, 0.00, 0.05, and 0.01 for the S allele were also reported in populations of Limousin, Angus, Simmental, Brahman, and Red Chittagong cattle, respectively (Bhuiyan et al.,2009). Similarly, a low frequency of 0.01 was observed in the Canadian crossbred steer population of this study, and as a result, only two genotypes (LL and LS) were detected among the 225 steers. A lower frequency of 0.01 for the allele I of *LXRα* was also observed in the Canadian crossbred steer population in comparison to a frequency of 0.1410 reported in a population of Japanese black cattle (Hoashi et al., 2008). A frequency of 0.016 for the I allele was reported in a population of 31 Canadian Holstein cattle (Narukami et al., 2011), which was similar to what we observed in this study. The difference of the allele frequencies of the two gene DNA polymorphisms may reflect the population divergence of different cattle breeds.

As a member of the basic helix-loop-helix leucine zipper family of transcription factors, SREBP1 gene plays an essential role in adipocyte differentiation, biosynthesis of cholesterol and fatty acids (Shimano et al. 2002). Hoashi et al. (2007) reported that the S allele of the 84

bp insertion/deletion contributed to a 1.3% higher concentration of total MUFA and a 1.6℃ lower melting point in the Japanese black intramuscular fat. Bhuiyan et al. (2009) reported that the Korean Hanwoo cattle with genotype of LL had 5.7% and 6.3% higher stearic acid concentration (C18:0) than the LS genotype and SS genotype ($P<0.05$), respectively, but the linoleic (C18:2n-6) and PUFA contents were 11.06% and 12.20% higher in muscle of animals with the SS genotype compared to the LL genotype ($P<0.05$). In a population of Fleckvieh bulls, Barton et al. (2010) found that the LS genotype was associated with a higher content of 9c C14:1 compared to LL genotype in subcutaneous fat ($P < 0.01$). However, none of the associations described above were observed in this study. Instead, the 84 bp polymorphism was only found to be associated with the concentration of 9*c* C17:1 with steers of the LL genotype having a higher concentration of 9c C17:1 than that of steers with the LS genotypes. In Korean cattle, the LL genotype was associated with a higher concentration of stearic (C18:0) and lower concentrations of linoleic (C18:2n-6) and polyunsaturated fatty acids (PUFA) (Bhuiyan et al., 2009). However, Matsuhashi et al. (2011) reported that the same DNA polymorphism of SREBP1 showed no effects on the fatty acid composition in adipose tissue and longissimus thoracis muscle fat from another population of Japanese Black cattle. The inconsistency of the associations between the DNA polymorphisms in SREBP1 with the fatty acids in different beef cattle populations suggests that the different breeds or populations may have different background gene effects on the fatty acid composition and/or the linkage phase between the DNA marker and the causative DNA polymorphisms are different in different beef cattle populations. Further studies are required to validate and illuminate the effect of SREBP1 gene on the fatty acid composition in beef cattle.

LXRα is a key transcriptional regulator of lipid metabolism, which increases expression of SREBP1 by mediating the induction of SREBP1c via RXR/LXR DNA binding sites in the SREBP1c promoter (Repa et al., 2000; Yoshikawa et al., 2001). The synonymous substitution G>A found in the 4 exon causes an amino acid change from valine to isoleucine at the 133 amino acid position , which is located on a Zinc finger DNA binding domain and is believed to affect the structure and therefore the function of the protein (Hoashi et al. 2008). Hoashi et al. (2008) detected a significant association between the SNP with linoleic acid (C18:2n-6) of intramuscular fat in the Japanese black cattle with the VV homozygote exhibiting a significantly lower percentage of C18:2n-6 than the VI genotype. In the present study, significant associations between the LXRα V133I were observed for 9c,11t C18:2 and also for sum CLA in addition to 11c C20:1. Steers with the VV genotype showed a higher concentration of 9c,11t C18:2, sum CLA and 11c C20:1 than steers with the VI genotype. There was a tendency that the VV genotype also had a higher concentration of 11t C18:1, a precursor for 9c, 11t C18:2, than the VI genotype (P=0.108, Table 2), indicating that associations with 9c,11t C18:2 and sum CLA may due to its association with 11t C18:1. However, validation of the associations in beef cattle populations of a larger sample size and functional analyses of the LXRα polymorphism are necessary in order to elucidate the involvement of LXRα in the production of various fatty acids.

The fatty acid desaturase 1 gene (FADS1) has been considered as one of the rate-limiting enzymes to the endogenous formation of long-chain polyunsaturated fatty acids (LC-PUFAs) in humans (Lattka et al., 2010). In mammals FADS1 converts dihomo-γ-linolenic acid (C20:3n-6) to arachidonic acid (C20:4n-6) and eicosatetraenoic acid (C20:4n-3) to

eicosapentaenoic acid (C20:5n-3) with linoleic (C18:2n-6) and linolenic (C18:3n-3) acids as the initial substrate (Schaeffer et al., 2006; Lattka et al., 2010). Linoleic (C18:2n-6) and linolenic (C18:3n-3) acids are essential fatty acids because they cannot be synthesized *de novo* by animals including humans and must be obtained in the diet (Simopoulos, 2010). In humans, Schaeffer et al. (2006) analyzed eighteen SNPs of the FADS1 and FADS2 gene cluster in 727 caucasians and found that polymorphisms or statistically reconstructed haplotypes of FADS1 and FASD2 showed strong associations with the level of the direct precursor of inflammatory eicosanoids, the n-6 fatty acid arachidonic acid (C20:4n-6), and also strong associations with levels of the n-6 fatty acids C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C22:4n-6 and of other n-3 fatty acids C18:3n-3, C20:5n-3 and C22:5n-3 ($P < 1.0\times10^{-13}$). Mathias et al. (2010) assessed associations between the FADS gene cluster polymorphisms and the fasting serum PUFA concentration in humans and a cluster of SNPs in tight linkage disequilibrium in the FADS1 gene were found with strong associations to arachidonic acid (C20:4n-6) ($P = 5.8 \times 10^{-7}$ to 1.7×10^{-8}) and other PUFAs including C22:2n-6, C20:5n-6, C22:4n-6, C20:3n-6. Recently, Knight et al. (2012) analyzed 74 SNP of genes FADS1/2/3, ELOVL2 and SLC26A10 in 1252 Australian sheep and found that no SNPs of FADS1 showed association with levels of long chain omega-3 fatty acids in lamb muscle. The authors suggested that genetic variation in omega-3 levels in lamb muscle might not be directly linked to the omega-3 biosynthesis pathway but may be associated with omega-3 transport mechanisms or regulators that involve the omega-3 biosynthesis pathway (Knight et al., 2012). In this study, we evaluated two closely linked DNA polymorphisms of bovine FADS1 gene with fatty acids in beef cattle steers and significant associations were

found for C17:0 iso, C17:0 ai, and BFA and some *trans*-monounsaturated fatty acid MUFA including 12t C18:1, 13t/14t C18:1 and 15t C18:1 and a *cis*-monounsaturated fatty acid 13c C18:1. However, the DNA polymorphism was not associated with the concentration of any long chain omega-3 fatty acids, which is in agreement with the results reported by Knight et al. (2012) in lamb. These results suggest a different role of bovine FADS1 gene in ruminants than in humans in determining the fatty acid composition. Furthermore, analyses of the FADS1 mutations by TESS (Schug 2008) has identified a possible CAC-binding protein site introduced by the insertion of 'GTG' in rs133053720, and transcription factors binding to this site would enhance the gene expression (Mignotte et al., 1989). Additional analysis of rs42187276 using TESS revealed the G allele has a possible binding site for SP1, which could both activate (Larsson et al., 2010) or repress (Bilsland *et al*,. 2006) the genes expression. However, TFSEARCH (Heinemeyer et al., 1998) suggests that the 'A' allele has a binding site for EGR-2 family which has been reported in cattle to influence stearoyl-CoA desaturase 5 and the lipid biosynthesis of myelin (Lengi & Corl 2012). More studies are needed to confirm the associations and to delineate how the bovine FADS1 gene exerts its effect, directly or indirectly, in affecting the fatty acids in beef cattle.

Fatty acid composition in beef is a complex trait and is affected by multiple factors including the diet composition, rumen microbes, management schemes and genetic factors (Jenkins, 1993; Bauman et al., 2000; De Smet et al., 2004). Although the breed effect contributes significantly as a genetic factor to the difference in fatty acid composition among beef cuts, genetic variations among animals within a beef population also explains a proportion of fatty acid variance and the heritability estimates for fatty acids ranged from low

to moderate (0.02 to 0.43), depending on the type of fatty acids (Malau-Adull et al., 2000; Pitchford et al., 2002). Recently, quantitative trait loci (QTL) that are associated with fatty acid composition in beef cattle have been identified on multiple chromosomes (Alexander et al., 2007; Morris et al., 2010; Gutiérrez -Gil et al., 2010) and SNP markers of a few of other genes including SCD, FASN, FABP4, ACACA have been identified to have associations with fatty acid composition in beef cattle populations (Hoashi et al., 2007; 2008; Zhang et al., 2008; Ohsaki et al., 2009; Li et al., 2010; Barton et al., 2010; Zhang et al., 2010; Li et al., 2012). The identification of these QTL and gene SNPs will not only enhance the understanding of the genetic mechanisms regulating lipid metabolism in ruminants but also provide a means to improve beneficial fatty acids through marker-assisted selection and/or marker-based diet management in beef cattle. Further studies focusing on the evaluation of DNA marker predictability on beneficial fatty acids including multiple DNA makers across multiple beef cattle breed populations as well as the assessment of gene-by-diet interaction will greatly facilitate the implementation of marker assisted selection and/or marker-based diet management in beef cattle.

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Table 1
Summary of DNA polymorphisms, primers, and probes of SREBP1, LRXa and FADS1 for genotyping

Gene symbol	Reference gene sequences	DNA variant position	Genotyping Method	Primer pairs and Probes	Allele assignment
SREBP1	NW_001493671.1	Intron 4 -/84bp: rs133958066	PCR	F:CCACAACGCCATCGAGAAACGCTAC R:GGCCTTCCCTGACCNCCCAACTTAG	L (432 bp) S (348 bp)
LRXα	NW_001493360	Exon 4 G>A: rs109428603	PCR-RFLP	F:CTAGGACAGCGTGGCGTATGAGAGC R:ATCTTGGAAAAACAGGTGGGTCTT	V (G) I (A)
FADS1	NW_001494536	Promoter -/GTG: rs133053720	Real-time PCR	F:CGCCGGCCCAGCTT R:GGCGACCCCAGCAAGAT Probe1:CTCCTGCCACCACGAC-VIC Probe2: CCCTCCTGCCACGAC-FAM	D (-) I (GTG)
FADS1	NW_001494536	Promoter A>G: rs42187276	Real-time PCR	F:GGTGCGCGTCCGGAT R:CGCCAACACCCCTCAGT Probe1: CCGCTCACGCACCT-VIC Probe2: CGCTCGCGCACCT-FAM	A (A) G (G)

Table 2. Least-square means and estimated effects of DNA polymorphisms SREBP1 rs133958066 and LXRα rs109428603 on fatty acids in commercial crossbred beef steers

Trait[1]	SREBP1 rs133958066 genotype[2]		Genotype effect[3]	P-value	LXRα rs109428603 genotype[2]		Genotype effect[3]	P-value
	LL (220)	LS(5)			VV (197)	VI(26)		
C14:0	3.54±0.046	3.974±0.296	-0.434±0.298	0.147	3.53±0.052	3.694±0.131	-0.164±0.137	0.234
C15:0	0.621±0.008	0.587±0.05	0.034±0.05	0.503	0.615±0.009	0.656±0.022	-0.041±0.023	0.072
C16:0	25.57±0.124	26.647±0.824	-1.077±0.832	0.197	25.527±0.137	25.982±0.366	-0.455±0.388	0.242
C17:0	1.404±0.017	1.277±0.106	0.127±0.107	0.236	1.392±0.02	1.453±0.047	-0.061±0.049	0.216
C18:0	8.916±0.103	8.933±0.683	-0.017±0.69	0.98	8.923±0.105	8.77±0.288	0.153±0.307	0.619
SFA	40.291±0.205	41.674±1.348	-1.383±1.362	0.311	40.232±0.219	40.766±0.588	-0.534±0.625	0.394
C15:0 iso	0.117±0.002	0.139±0.013	-0.022±0.013	0.083	0.117±0.002	0.113±0.005	0.004±0.006	0.49
C15:0 ai	0.156±0.003	0.186±0.018	-0.031±0.018	0.091	0.156±0.003	0.156±0.008	-0.001±0.008	0.934
C16:0 iso	0.166±0.003	0.187±0.017	-0.021±0.017	0.225	0.166±0.003	0.169±0.008	-0.003±0.008	0.71
C17:0 iso	0.287±0.003	0.301±0.017	-0.014±0.017	0.414	0.289±0.003	0.282±0.007	0.007±0.008	0.374
C17:0 ai	0.594±0.005	0.635±0.031	-0.04±0.031	0.199	0.596±0.005	0.591±0.013	0.005±0.014	0.729
C18:0 iso	0.136±0.002	0.144±0.011	-0.008±0.011	0.443	0.137±0.002	0.135±0.005	0.002±0.005	0.73
BFA	1.484±0.016	1.627±0.095	-0.142±0.095	0.137	1.489±0.015	1.473±0.041	0.017±0.043	0.7
SFA+BFA	41.776±0.216	43.288±1.39	-1.512±1.403	0.282	41.719±0.228	42.249±0.608	-0.53±0.644	0.411
9c C14:1	1.485±0.042	1.683±0.229	-0.197±0.23	0.392	1.492±0.044	1.455±0.102	0.037±0.105	0.726
7c C16:1	0.159±0.002	0.176±0.011	-0.016±0.011	0.144	0.16±0.002	0.153±0.005	0.008±0.005	0.132
9c C16:1	5.605±0.097	5.43±0.501	0.176±0.502	0.727	5.573±0.102	5.827±0.226	-0.254±0.23	0.271
12c C16:1	0.366±0.011	0.401±0.058	-0.035±0.059	0.545	0.367±0.012	0.355±0.026	0.012±0.027	0.639
9c C17:1	1.488±0.017	1.21±0.11	0.278±0.111	**0.013***	1.474±0.018	1.538±0.048	-0.063±0.051	0.22
9c C18:1	40.121±0.235	39.067±1.296	1.054±1.302	0.419	40.207±0.253	39.45±0.585	0.758±0.603	0.211
11c C18:1	2.472±0.026	2.292±0.168	0.18±0.17	0.292	2.481±0.029	2.429±0.074	0.052±0.077	0.504
12c C18:1	0.138±0.002	0.15±0.015	-0.011±0.016	0.468	0.14±0.003	0.139±0.007	0.001±0.007	0.93
13c C18:1	0.747±0.017	0.743±0.095	0.004±0.096	0.965	0.751±0.018	0.701±0.041	0.05±0.043	0.243
9c C20:1	0.114±0.001	0.112±0.008	0.002±0.008	0.792	0.116±0.001	0.109±0.003	0.006±0.004	0.091

11c C20:1	0.274±0.006	0.269±0.036	0.006±0.036	0.878	0.28±0.008	0.246±0.016	0.034±0.017	**0.042***
6t/7t/8t C18:1	0.11±0.003	0.108±0.016	0.001±0.016	0.931	0.111±0.003	0.107±0.007	0.004±0.007	0.581
9t C18:1	0.181±0.001	0.181±0.009	-0.001±0.009	0.955	0.183±0.002	0.174±0.004	0.009±0.005	0.06
10t C18:1	0.82±0.042	0.66±0.227	0.161±0.228	0.481	0.812±0.047	0.915±0.104	-0.103±0.106	0.335
11t C18:1	0.538±0.011	0.619±0.072	-0.081±0.073	0.268	0.544±0.011	0.491±0.031	0.053±0.033	0.108
12t C18:1	0.121±0.002	0.112±0.012	0.009±0.012	0.467	0.122±0.002	0.12±0.005	0.002±0.006	0.759
13t/14t C18:1	0.369±0.005	0.342±0.035	0.027±0.035	0.44	0.373±0.006	0.366±0.016	0.007±0.017	0.669
15t C18:1	0.099±0.002	0.09±0.011	0.008±0.011	0.465	0.099±0.002	0.099±0.005	0±0.005	0.943
Sum *trans*18:1	2.293±0.045	2.155±0.271	0.138±0.273	0.615	2.298±0.053	2.325±0.124	-0.027±0.128	0.833
MUFA	55.419±0.212	53.826±1.354	1.594±1.367	0.245	55.473±0.224	54.992±0.593	0.481±0.627	0.444
9c,13t/8t,12c C18:2	0.197±0.002	0.174±0.014	0.023±0.014	0.1	0.197±0.003	0.197±0.006	0±0.007	0.994
9c,15c C18:2	0.241±0.004	0.227±0.023	0.014±0.024	0.562	0.24±0.004	0.239±0.01	0.001±0.011	0.891
9c,11t C18:2	0.35±0.007	0.407±0.043	-0.058±0.043	0.181	0.354±0.007	0.314±0.019	0.04±0.02	**0.04***
sumCLA	0.588±0.008	0.66±0.05	-0.072±0.05	0.154	0.595±0.009	0.543±0.022	0.051±0.023	**0.025***
C18:2n-6	1.263±0.017	1.278±0.094	-0.015±0.094	0.873	1.262±0.018	1.254±0.043	0.008±0.044	0.849
C18:3n-3	0.161±0.002	0.168±0.015	-0.007±0.015	0.648	0.161±0.002	0.158±0.006	0.003±0.007	0.667
PUFA	2.805±0.024	2.889±0.149	-0.084±0.15	0.577	2.809±0.025	2.749±0.066	0.06±0.07	0.391
n-3	0.187±0.002	0.198±0.016	-0.011±0.016	0.485	0.186±0.003	0.185±0.007	0.001±0.007	0.883
n-6	1.463±0.018	1.499±0.101	-0.036±0.101	0.723	1.462±0.02	1.456±0.046	0.006±0.047	0.903
n-6/n-3	7.987±0.086	7.688±0.555	0.299±0.561	0.594	7.996±0.087	7.998±0.239	-0.002±0.254	0.993
Health Index	1.489±0.016	1.335±0.104	0.154±0.105	0.143	1.494±0.018	1.431±0.046	0.063±0.049	0.195

[1] The concentrations of fatty acids were expressed as a percentage of total FAME quantified. SFA: sum of saturated fatty acids. SFA+BFA: sum of saturated and branched chain fatty acids. Sum *trans*18:1: sum of trans-18:1. MUFA: sum of all cis and all trans mono-unsaturated fatty acids. sumCLA: sum of conjugated linoleic acids. PUFA: sum of polyunsaturated fatty acids. n-6/n-3: ratio between n-6 and n-3 PUFA. Health Index: (total MUFA + total PUFA) / (4 x 14:0 + 16:0). Values of standard errors for the least square means and estimated genotype effects that were less than 0.0001 were rounded down to zero.

[2] Least square means ± standard error for genotypes LL (insertion/insertion) and LS (insertion/deletion) of *SREBP1* or VV (GG) and VI (GA) *of LXRα*. The counts of the genotype LL and LS of SREBP1 or VV and VI of LXRα or are in parentheses. Intralocus genotypic frequencies conformed to Hardy-Weinberg equilibrium proportions at

P>0.05. [3] Estimated as the difference between the means of the two genotype genotypes. * $P<0.05$.

Table 3

Least-square means and estimated effects of FADS1 DNA polymorphism rs13305370 [-/GTG] on fatty acids in commercial crossbred beef steers

Trait[1]	FADS1 rs133053720 genotype[2]						
	DD(108)	DI(95)	II(20)	Additive effect[3]	P-value	Dominance effect[4]	P-value
C14:0	3.543±0.066	3.569±0.07	3.527±0.148	0.008±0.08	0.923	0.034±0.106	0.75
C15:0	0.624±0.011	0.618±0.011	0.63±0.025	-0.003±0.013	0.809	-0.009±0.018	0.629
C16:0	25.676±0.178	25.542±0.191	25.474±0.414	0.101±0.226	0.654	-0.033±0.296	0.912
C17:0	1.411±0.024	1.39±0.026	1.433±0.053	-0.011±0.029	0.704	-0.032±0.038	0.396
C18:0	8.996±0.141	8.843±0.152	8.516±0.329	0.24±0.179	0.182	0.087±0.235	0.713
SFA	40.491±0.286	40.2±0.308	39.832±0.666	0.329±0.363	0.365	0.038±0.476	0.936
C15:0 iso	0.118±0.003	0.116±0.003	0.108±0.006	0.005±0.003	0.151	0.003±0.004	0.454
C15:0 ai	0.158±0.004	0.154±0.004	0.144±0.009	0.007±0.005	0.149	0.003±0.006	0.642
C16:0 iso	0.166±0.004	0.167±0.004	0.157±0.009	0.004±0.005	0.33	0.005±0.006	0.439
C17:0 iso	0.29±0.004	0.287±0.004	0.27±0.008	0.01±0.004	**0.026***	0.007±0.006	0.218
C17:0 ai	0.601±0.007	0.593±0.007	0.56±0.015	0.021±0.008	**0.013***	0.012±0.011	0.271
C18:0 iso	0.137±0.002	0.137±0.002	0.126±0.005	0.005±0.003	0.069	0.005±0.004	0.161
BFA	1.5±0.021	1.483±0.022	1.394±0.047	0.053±0.025	**0.037***	0.036±0.033	0.278
SFA+BFA	41.991±0.295	41.683±0.317	41.225±0.686	0.383±0.374	0.307	0.075±0.49	0.879
9c C14:1	1.455±0.054	1.492±0.057	1.651±0.113	-0.098±0.06	0.105	-0.062±0.08	0.439
7c C16:1	0.16±0.003	0.159±0.003	0.156±0.006	0.002±0.003	0.46	0±0.004	0.925
9c C16:1	5.487±0.122	5.712±0.129	5.719±0.252	-0.116±0.132	0.382	0.11±0.176	0.533
12c C16:1	0.357±0.014	0.367±0.015	0.416±0.029	-0.03±0.015	0.05	-0.02±0.02	0.32
9c C17:1	1.478±0.024	1.487±0.026	1.533±0.056	-0.028±0.031	0.367	-0.019±0.04	0.645
9c C18:1	40.098±0.307	40.065±0.326	40.304±0.659	-0.103±0.351	0.77	-0.136±0.464	0.77
11c C18:1	2.434±0.036	2.52±0.038	2.452±0.083	-0.009±0.045	0.841	0.077±0.059	0.196
12c C18:1	0.141±0.003	0.14±0.004	0.13±0.008	0.006±0.004	0.183	0.005±0.006	0.377
13c C18:1	0.734±0.021	0.74±0.023	0.847±0.046	-0.057±0.024	**0.022***	-0.051±0.032	0.12
9c C20:1	0.116±0.002	0.114±0.002	0.108±0.004	0.004±0.002	0.053	0.001±0.003	0.633

11c C20:1	0.279±0.009	0.276±0.009	0.251±0.018	0.014±0.01	0.142	0.011±0.013	0.398
6t/7t/8t C18:1	0.111±0.004	0.11±0.004	0.11±0.008	0.001±0.004	0.877	0±0.006	0.939
9t C18:1	0.183±0.002	0.18±0.002	0.18±0.005	0.002±0.003	0.52	-0.001±0.003	0.743
10t C18:1	0.818±0.057	0.821±0.06	0.889±0.116	-0.036±0.061	0.561	-0.033±0.081	0.687
11t C18:1	0.551±0.015	0.526±0.016	0.5±0.035	0.026±0.019	0.187	0.001±0.025	0.98
12t C18:1	0.125±0.003	0.121±0.003	0.11±0.006	0.007±0.003	**0.028***	0.003±0.004	0.443
13t/14t C18:1	0.382±0.008	0.368±0.008	0.334±0.018	0.024±0.01	**0.018***	0.01±0.013	0.452
15t C18:1	0.102±0.003	0.098±0.003	0.089±0.006	0.007±0.003	**0.03***	0.002±0.004	0.598
Sum *trans*18:1	2.33±0.063	2.281±0.067	2.254±0.139	0.038±0.074	0.613	-0.011±0.098	0.909
MUFA	55.211±0.288	55.514±0.31	55.966±0.672	-0.377±0.366	0.304	-0.075±0.482	0.877
9c,13t/8t,12c C18:2	0.199±0.003	0.197±0.004	0.188±0.007	0.006±0.004	0.139	0.003±0.005	0.498
9c,15c C18:2	0.242±0.005	0.237±0.006	0.254±0.012	-0.006±0.006	0.332	-0.011±0.008	0.199
9c,11t C18:2	0.343±0.01	0.352±0.01	0.357±0.021	-0.007±0.011	0.524	0.002±0.015	0.876
sumCLA	0.581±0.011	0.591±0.012	0.605±0.024	-0.012±0.013	0.344	-0.002±0.017	0.926
C18:2n-6	1.262±0.022	1.259±0.024	1.262±0.047	0±0.025	0.999	-0.003±0.033	0.925
C18:3n-3	0.16±0.003	0.162±0.003	0.154±0.007	0.003±0.004	0.47	0.005±0.005	0.34
PUFA	2.798±0.033	2.803±0.035	2.812±0.075	-0.007±0.04	0.865	-0.002±0.053	0.973
n-3	0.186±0.003	0.188±0.004	0.178±0.008	0.004±0.004	0.357	0.005±0.006	0.349
n-6	1.462±0.024	1.46±0.025	1.459±0.051	0.001±0.027	0.961	0±0.036	0.989
n-6/n-3	7.994±0.116	7.946±0.125	8.228±0.27	-0.117±0.147	0.429	-0.166±0.193	0.392
Health Index	1.481±0.023	1.486±0.024	1.507±0.052	-0.013±0.028	0.644	-0.008±0.037	0.824

[1] The concentrations of fatty acids were expressed as a percentage of total FAME quantified. SFA: sum of saturated fatty acids. SFA+BFA: sum of saturated and branched chain fatty acids. Sum *trans*18:1: sum of trans-18:1. MUFA: sum of all cis and all trans mono-unsaturated fatty acids. sumCLA: sum of conjugated linoleic acids. PUFA: sum of polyunsaturated fatty acids. n-6/n-3: ratio between n-6 and n-3 PUFA. Health Index: (total MUFA + total PUFA) / (4 x 14:0 + 16:0). Values of standard errors for the least square means and estimated genotype effects that were less than 0.0001 were rounded down to zero.

[2] Least square means ± standard error for genotypes DD (deletion/deletion), DI (deletion/insertion) and II (deletion/deletion). The counts of the genotype DD, DI and II are in parentheses. Intralocus genotypic frequencies conformed to Hardy-Weinberg equilibrium proportions at P>0.05 [3] Estimated as the difference between the means of the

two homozygous genotyped divided by two.

[4] Estimated by subtracting the average of solutions for homozygous genotypes from that for heterozygous genotype. * $P<0.05$.

Table 4

Least-square means and estimated effects of FADS1 rs42187276 [A/G] on fatty acids in commercial crossbred beef steers

Trait[1]	FADS1 rs42187276 genotype[2]			Additive effect[3]	P-value	Dominance effect[4]	P-value
	GG(110)	AG(95)	AA(20)				
C14:0	3.546±0.065	3.575±0.071	3.45±0.144	0.048±0.078	0.539	0.077±0.104	0.46
C15:0	0.623±0.011	0.619±0.012	0.616±0.024	0.003±0.013	0.794	-0.001±0.017	0.94
C16:0	25.666±0.175	25.583±0.191	25.252±0.402	0.207±0.22	0.347	0.124±0.29	0.67
C17:0	1.41±0.024	1.388±0.026	1.42±0.052	-0.005±0.028	0.852	-0.027±0.037	0.464
C18:0	9.012±0.14	8.816±0.152	8.656±0.321	0.178±0.175	0.31	-0.018±0.232	0.938
SFA	40.499±0.282	40.216±0.307	39.647±0.646	0.426±0.353	0.229	0.143±0.467	0.76
C15:0 iso	0.119±0.003	0.116±0.003	0.108±0.006	0.006±0.003	0.09	0.003±0.004	0.566
C15:0 ai	0.159±0.004	0.154±0.004	0.143±0.009	0.008±0.005	0.097	0.003±0.006	0.675
C16:0 iso	0.168±0.004	0.165±0.004	0.158±0.008	0.005±0.004	0.281	0.003±0.006	0.658
C17:0 iso	0.291±0.004	0.286±0.004	0.271±0.008	0.01±0.004	**0.019***	0.005±0.006	0.38
C17:0 ai	0.603±0.006	0.591±0.007	0.563±0.015	0.02±0.008	**0.015***	0.008±0.011	0.467
C18:0 iso	0.137±0.002	0.136±0.002	0.129±0.005	0.004±0.003	0.157	0.003±0.004	0.501
BFA	1.506±0.021	1.477±0.022	1.4±0.046	0.053±0.025	**0.031***	0.024±0.033	0.471
SFA+BFA	42.005±0.292	41.694±0.318	41.044±0.67	0.481±0.366	0.19	0.169±0.485	0.728
9c C14:1	1.451±0.054	1.508±0.058	1.6±0.111	-0.074±0.059	0.206	-0.017±0.078	0.827
7c C16:1	0.161±0.002	0.158±0.003	0.155±0.005	0.003±0.003	0.304	-0.001±0.004	0.879
9c C16:1	5.493±0.122	5.73±0.129	5.62±0.245	-0.064±0.129	0.62	0.173±0.172	0.314
12c C16:1	0.356±0.014	0.371±0.015	0.404±0.028	-0.024±0.015	0.103	-0.009±0.02	0.646
9c C17:1	1.478±0.024	1.486±0.026	1.518±0.055	-0.02±0.03	0.507	-0.011±0.04	0.776
9c C18:1	40.062±0.304	40.042±0.325	40.677±0.639	-0.307±0.34	0.367	-0.327±0.454	0.471
11c C18:1	2.435±0.036	2.524±0.039	2.449±0.081	-0.007±0.044	0.871	0.082±0.058	0.16
12c C18:1	0.141±0.003	0.139±0.004	0.129±0.008	0.006±0.004	0.132	0.004±0.006	0.466

13c C18:1	0.732±0.022	0.747±0.023	0.833±0.045	-0.051±0.024	**0.036***	-0.035±0.032	0.275
9c C20:1	0.116±0.002	0.113±0.002	0.11±0.004	0.003±0.002	0.122	0±0.003	0.918
11c C20:1	0.278±0.009	0.277±0.009	0.261±0.018	0.008±0.009	0.381	0.007±0.013	0.558
6t/7t/8t C18:1	0.111±0.004	0.109±0.004	0.109±0.008	0.001±0.004	0.747	-0.001±0.006	0.831
9t C18:1	0.184±0.002	0.18±0.002	0.18±0.005	0.002±0.003	0.526	-0.002±0.003	0.487
10t C18:1	0.823±0.056	0.808±0.06	0.873±0.114	-0.025±0.06	0.674	-0.039±0.08	0.621
11t C18:1	0.556±0.015	0.522±0.017	0.503±0.035	0.026±0.019	0.167	-0.007±0.025	0.772
12t C18:1	0.125±0.003	0.119±0.003	0.111±0.006	0.007±0.003	**0.028***	0.001±0.004	0.764
13t/14t C18:1	0.384±0.008	0.364±0.009	0.337±0.018	0.024±0.01	**0.017***	0.004±0.013	0.77
15t C18:1	0.103±0.002	0.097±0.003	0.09±0.006	0.007±0.003	**0.027***	0±0.004	0.915
Sum trans18:1	2.342±0.063	2.253±0.068	2.251±0.135	0.045±0.072	0.531	-0.043±0.096	0.654
MUFA	55.186±0.286	55.515±0.311	56.156±0.653	-0.485±0.356	0.175	-0.156±0.472	0.742
9c,13t/8t,12c C18:2	0.199±0.003	0.196±0.004	0.187±0.007	0.006±0.004	0.086	0.003±0.005	0.576
9c,15c C18:2	0.241±0.005	0.238±0.006	0.254±0.011	-0.007±0.006	0.271	-0.009±0.008	0.248
9c,11t C18:2	0.345±0.009	0.351±0.01	0.36±0.021	-0.007±0.011	0.516	-0.002±0.015	0.888
sumCLA	0.585±0.011	0.588±0.012	0.606±0.024	-0.01±0.013	0.43	-0.007±0.017	0.669
C18:2n-6	1.268±0.022	1.251±0.024	1.257±0.046	0.005±0.025	0.827	-0.012±0.033	0.727
C18:3n-3	0.161±0.003	0.161±0.003	0.153±0.007	0.004±0.004	0.343	0.004±0.005	0.43
PUFA	2.81±0.032	2.789±0.035	2.803±0.073	0.004±0.04	0.929	-0.017±0.053	0.749
n-3	0.187±0.003	0.186±0.004	0.177±0.008	0.005±0.004	0.227	0.004±0.006	0.452
n-6	1.468±0.024	1.451±0.025	1.453±0.05	0.008±0.027	0.773	-0.009±0.035	0.802
n-6/n-3	7.977±0.115	7.947±0.125	8.237±0.263	-0.13±0.144	0.368	-0.16±0.19	0.401
Health Index	1.479±0.023	1.483±0.025	1.542±0.051	-0.032±0.028	0.249	-0.028±0.037	0.449

[1] The concentrations of fatty acids were expressed as a percentage of total FAME quantified. SFA: sum of saturated fatty acids. SFA+BFA: sum of saturated and branched chain fatty acids. Sum *trans*18:1: sum of trans-18:1. MUFA: sum of all cis and all trans mono-unsaturated fatty acids. sumCLA: sum of conjugated linoleic acids. PUFA: sum of polyunsaturated fatty acids. n-6/n-3: ratio between n-6 and n-3 PUFA. Health Index: (total MUFA + total PUFA) / (4 x 14:0 + 16:0). Values of standard errors for the least square means and estimated SNP effects that were less than 0.0001 were rounded down to zero.

[2] Least square means ± standard error for genotypes GG, AG and AA. The counts of the genotype GG, AG and AA are in parentheses. [3] Estimated as the difference between the means of the two homozygous genotyped divided by two.

[4] Estimated by subtracting the average of solutions for homozygous genotypes from that for heterozygous genotype. * $P<0.05$,

Highlights:

1. The 84 bp insert/deletion in intron 5 of SREBP1 was associated with 9c-17:1.
2. A SNP of LXRα showed associations with 9c, 11t-18:2, sum of CLA and 11c-20:1.
3. DNA polymorphisms in FADS1 were associated with iso17:0, ai17, BFA and some MUFA.