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BTN1A1 , *FABP3* and *TG* genes polymorphism in East Anatolian red cattle breed and South Anatolian red cattle breed

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The aim of the study was to determine butyrophilin, thyroglobulin and fatty acid binding protein genes in East Anatolian Red cattle breed and South Anatolian Red cattle breed. In the study, unrelated 50 South Anatolian red and 50 East Anatolian red cattle were used. Genomic DNA was isolated from whole blood using standart salt-out protocol. The polymerase chain reaction technique was used for gene amplification. Allele and genotype distribution and Hardy-Weinberg equilibrium was calculated by using PopGene32 software program. For *BTN1A1* gene, A allele frequency was higher in East Anatolian red (EAR) and South Anatolian red (SAR) cattles. In *TG* gene, T allele frequency was higher in SAR breed but this frequency was lower in EAR cattle breed. For *FABP3* gene, G allele frequency was lower in SAR breed but it was higher in EAR breed. The presented results should be confirmed in future investigations, taking into consideration all possible genotype at different loci and using other restriction enzymes for recognizing the variants.

Key words: BTN1A1, TG, FABP3, cattle, polymerase chain reaction-restriction fragment lenght polymorphism (PCR-RFLP).

INTRODUCTİON

Since the improvement of cattle linkage maps, many researchers have managed to identify quantitative trait loci (QTL) affecting economically important traits. At the beginning, investigations focused on identifying QTL affecting milk production traits, however traditional selection ways have been effectual in development milk production in dairies without the DNA marker information (Ashwell et al., 2004).

BTN1A1 is a candidate gene which is very important for milk yield and composition. It is a type 1 transmembrane glycoprotein placed between plasma membrane and surface of fat droplets, synthesed from 526 amino acids and conserved in B30.2 domain in the cytoplasmic tail. It was identified as a main component of the milk fat globule

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Abbreviations: QTL, Quantitative trait loci; **MFGM,** milk fat globule membran; **BTN,** butyrophilins; **EAR,** East Anatolian red; **SAR,** South Anatolian red; **EDTA,** ethylene diamine tetra-acetic acid; **PCR,** polymerase chain reaction; **RFLP,** restriction fragment lenght polymorphism.

membran (MFGM), that is important in secreting and stabilizing milk-fat droplets in milk. It is highly expressed only during the lactation and last period of pregnancy. The butyrophilins (BTN) belong to the immu-noglobulin family of transmembran proteins. BTN groups were identified that are not all conserved between mamalian species (Afrache et al., 2012). *BTN1A1* gene has been found in mammalian species (Jack and Mather, 1990; Tetsuya et al., 1995; Taylor et al., 1996a) cattle, human and mice. The bovine butyrophilin gene located to chromosome 23, consists of 8 exon and 7 intron and 893 bp long gene fragment (Ashwell et al., 1996; Komisarek and Dorynek, 2003). BTN1A1 was mapped to the long arm of chromosome 23 (Jack and Mather, 1993; Frank et al., 1981). This gene expression is regulated at the transcription level by lactogenic hormones (Banghart et al., 1998). QTL candidate attempt to characterize the influence of particular polymorphic variants of this gene on production traits, content of individual milk components in milk or somatic cell count (Komisarek and Dorynek, 2002).

In the thyroid gland, TG is the most abundant protein that is the precursor of the thyroid hormones T3 and T4 (Baas et al., 1986). In dairy cattle, lots of studies have showed that QTL affects milk fat yield and milk fat percentage in bovine chromosome 14 (Khatib et al., 2007). *TG* gene, located at the centromeric end of chromosome 14, contains 37 exons and encodes an 8,7 kb mRNA. The association of a C/T single nucleotide polymorphism (SNP) in the 5' untranslated region of TG with marbling in beef cattle has been reported (Barendse et al., 1997).

Milk lipids are synthesized from fatty acids which bind to specific proteins; fatty acid binding proteins (FABPs). They are family of small cytoplasmic proteins; nine of them have been determined as FABP1-FABP9 (Chmurzynska, 2006). They play an important role in fatty acid uptake, transport and metabolism. FABP3 is present in tissues such as heart muscle, skeletal muscles, lactating mammary gland, liver or adipose tissues (Roy et al., 2003). It was mapped to bovine chromosome 2 where QTLs affects milk fat yield and content. It contains 4 exons. The association of a A/G single nucleotide polymorphism has been determined (Calvo et al., 2004; Khatkar et al., 2004).

Although Turkey is a rich country in total numbers of dairy cows, annual milk production per animal is not encouraging. As a general opinion, the underlying causes of poor milk yield are poor environmental conditions and poor husbandry practices as well as poor genotype (Bakir and Kaygisiz, 2003). However, up to date, there is no published investigation on the presence of potential QTLs in East Anatolian red (EAR) and South Anatolian red (SAR) cattles. SAR cattle are commonly produced in Southern Anatolia. Other than that, varieties of SAR are raised in Syria, Israel and Egypt. Average lactation period of SAR cows is 215 to 280 days. Annual milk production may vary from 1000 to 1500 kg. It has been reported that milk production may be increased up to 5000 kg if the environmental conditions and nutritional factors are optimized. EAR breed cattle are common in Eastern Anatolia. The average lactation period is 210 to 270 days and annual milk production is between 1000 and 1200 kg. The well-known feature of both cattle breed is their adaptation ability to poor conditions (Bakir et al., 2003; Kaymakci et al., 2004). The objective of the present study was to determine the genotype and allele frequency of *BTN1A1*, *FABP3* and *TG* genes which are claimed to be marker genes used for selection of breeding dairy cows in SAR and EAR cattle.

MATERIALS AND METHODS

Our study was conducted on a total of 100 cattle from two different native breeds: 50 cattle from South Anatolian Red cattle breed (SAR) and 50 cattle from East Anatolian Red cattle breed (EAR). From each animal, about 2 mL of blood was collected into vaccum tubes coated with ethylene diamine tetra-acetic acid (EDTA). Genomic DNA was isolated by using salt-out method (Miller et al., 1998). The polymerase chain reaction (PCR) volume of 25 µl contained; 1 U Taq DNA Polymerase (Fermentas Life Sciences, Canada), 2 to 2.5 µl 10X PCR buffer, 1.5 mM $MgCl_2$, 50 to 100 ng genomic DNA, 100mM dNTP (TaKaRa Biotechnology Co.,Ltd.,Japan), and 10 pmol of each primer.

The primer sequences designed for genotyping these candidate genes were: Butyrophilin (BTN1A1), 893 bp fragment, Hae III restriction site, forward 5´TCCCGAGAATGGGTTCTG 3´ and reverse 5´ACTGCCTGAGTTCACCTCA3' (Taylor et al. 1996a), fatty acid binding protein (FABP3), 243 bp fragment, Aci I restriction site, forward 5' GTGAGTTGAGGAAGGGGCTGTG-3' and reverse 5'-TAGGTCTCCACCTCTTGTCCTTCAG-3', thyroglobulin (TG), 242 bp fragment, Bst YI restriction site , forward 5´GGGGATGACTACGAGTATGACTG 3´and reverse 5´GTGAAAATCTTGTGGAGGCTGTA 3´ (Wu et al., 2005).

For BTN1A1, the amplification conditions included; initial denaturation 95°C for 5 min, followed by 33 cycles at 95°C for 30 s, 59°C for 5 min and 72°C for 2 min followed by a final extension at 72°C for 7 min. For FABP3 and TG, the amplification conditions used were, pre-denaturation at 95°C for 10 min, 30 amplification cycles were performed, denaturation at 94°C for 30 s, annealing at 63°C for 30 s and extension at 72°C for 30 s, followed by a final at 72°C for 5 min.

For restriction fragment lenght polymorphism (RFLP) analysis, 10 µl of the PCR products were digested overnight with 10 units of restriction enzyme at 37°C. The digested DNA fragments were seperated by electrophoresis in 2% agarose gel. Genotype and allele frequencies of each polymorphisms were calculated by using the PopGene 32 software program and also the chi-square tests (x^2) was used to check whether the polpulations were in Hardy-Weinberg equilibrium using PopGene32 software (Yeh et al., 2000).

RESULTS AND DISCUSSION

As a result of digestion of 893 bp site in *BTN1A1* gene

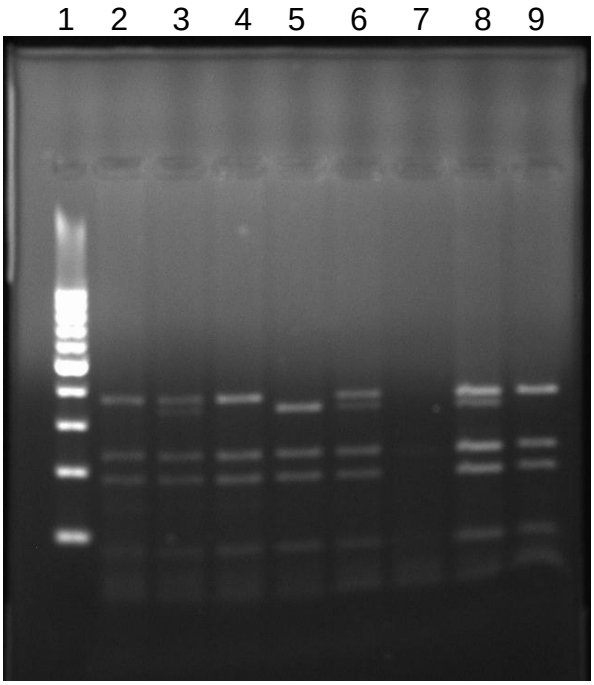

Figure 1. BTN1A1 genotyping by polymerase chain reaction-restriction fragment lenght polymorphism (PCR-RFLP) method. Lane 1, DNA marker (100 bp); lanes 2, 4 and 9, AA genotype; lanes 3, 6 and 8, AB genotype; lane 5, BB genotype.

with HaeIII restriction enzyme AA (371, 231, 185, 83 and 32 bp), BB (338 and 83 bp) and AB (371, 231, 185, 83 and 32 bp), genotypes were determined (Figure 1). Then, the TG 5' flanking region (242 bp) was digested with Bst YI restriction enzyme CC (295, 178 and 75 bp), TT (473, 75 bp), CT (473, 295, 178 and 75 bp) genotypes determined. *FABP3* gene with AciI restriction enzyme (243 bp) was digested. AA (438 bp) , GG (299 and 139 bp), AG (438, 299 and 139 bp) genotypes were determined.

Information on product size, genotype frequencies and allele frequencies for *BTN1A1*, *FABP3*, *TG* genes are listed in Table 1. For *BTN1A1* gene, the obtained results indicate that B allele frequency was lower in EAR and SAR. For *TG* gene, it was found that T allele frequency was higher in SAR breed but C allele frequency was higher in EAR cattle breed. For *FABP3* gene, G allele frequency was lower in SAR breed but it was higher in EAR breed.

The aim of the genetic studies on livestock animals was to identify, map and determine the polymorphisms of genes which are important in metabolic pathways affecting animal growth. The EAR and SAR cattle breeds are the most common breeds in Turkey, and also investigations on the QTL of these animals have been going on (Iraz et al., 2012).

In *BTN1A1* gene, five Hae III restriction sites, the butyrophilins were determined that were the most important proteins in the milk fat globule membrane (Sadr et al., 2008). The studies reported that they were produced in the end of pregnancy. It was also found that the *BTN1A1* gene was affective during the process of lactogenesis (Bhattacharya et al., 2005; Frank et al., 1981; Ogg et al., 2004).

In this study, in the SAR and EAR cattle, the highest frequency was observed for AA genotype and A allele whereas the lowest frequency was found for BB genotype and B allele. Such type of polymorphic informations were also reported in cattle by Sadr et al. (2008), Taylor et al. (1996a) Husaini et al. (1999) and Komisarek and Dorynek (2003). The high frequency of the A allele indicates that this allele might have been supported by selection for dairy production (Komisarek and Dorynek, 2003; Taylor et al., 1996a; Sadr et al., 2008; Husaini et al., 1999). The results determined in this study suggest that the *BTN1A1* gene is polymorphic in this population.

TG is one of the main gene which affect lipid metabolism and mapped to the centromeric region of

Table 1. Loci, product size, genotypes and allele frequencies for polymorphisms in three candidate genes.

Locus	Breed	n	Product size (bp)	Genotype frequency			Allele frequency		Chi square[1]
BTN1A1 (Hae III)									
				AA	AB	BB	A	B	51.02378^{XXX}
	SAR	50	893	26	0	24	0.52	0.48	27.03446^{XXX}
	EAR	40		26	4	10	0.71	0.29	
TG (Bst YI)									
				TT	CT	CC	T	C	
	SAR	50	242	28	2	20	0.58	0.42	43.11022^{XXX}
	EAR	50		6	12	32	0.25	0.75	6.02907^{XXX}
FABP3 (Aci I)									
				AA	AG	GG	A	G	
	SAR	50	243	45	0	5	0.90	0.10	55.05618^{XXX}
	EAR	50		4	30	16	0.38	0.62	3.04372^{XXX}

[1]Hardy-Weinberg equilibrium; SAR, South Anatolian Red; EAR, East Anatolian Red; $^{XXX}P \leq 0.001$.

bovine chromosome 14 (Moore et al., 2003). Many studies reported that a QTL for fat yield and percentage in milk of dairy cattle was mapped to a similar region on bovine chromosome 14 (Coppieter et al., 1998; Heyen et al., 1999; Riquet et al., 1999). For that reasons, the previous studies focused on this region to find out the relation between the genes located on chromosome 14 and milk production traits. Khatkar et al. (2004) reported that several QTL affected milk fat and fat percentage on bovine chromosome 14 in the region of TG. Khatip et al. (2007) investigated the association between TG and milk production traits and they did not find any significant association. Moore et al. (2003) also investigated the correlation between the backfat thickness which is one of the major quantative traits that affects carcass quality. They reported that there was no significant association between TG and backfat and also they supposed that other alleles or genes in the region of TG were related with fat metabolism (Moore et al., 2003).

In the present study, the EAR cattle breed, the T allele frequency and TT genotype frequency were determined to be lower than the frequencies of C allele and CC genotypes, similar to the results determined by Moore et al. (2003), Khatib et al. (2007) and Casas et al. (2005). High T allele and TT genotype frequency were found in SAR cattle breed. We suggest that the low frequency of these genotypes in EAR cattle which support the previous studies are not enough to verify the association between the TG genes and production traits in cattles.

The genes encoding fatty acid binding proteins are candidate genes for milk. They are intracellular proteins included in fatty acid transport from the plasma membrane to the beta oxidation, triacylglycerol or phospholipid synthesis (Veerkamp and Maatman, 1995; Veerkamp et al., 1993). Genetic variation at the FABP3 loci was observed by Kulig et al. (2010).They reported that the FABP3, single nucleotide polymorphism (SNP) affected fat and protein content in the milk of Jersey cows with A as a constructive allele for developing these traits. They determined that the AA genotyped cows had higher protein content and milk fat content than the AG and GG genotyped cows. They also suggested that chromosomal location of FABP3 might be considered as candidate gene for milk production traits (Kulig et al., 2010).

In another study, Cho et al. (2007) investigated the association between subcutaneous fat thickness in beef cattle and polymorphisms in FABP3 gene; they determined the low A allele frequency and low AA genotypes frequency. In order to determine the relation between SNP in the FABP3 gene and carcass weight and backfat thickness, Cho et al. (2007) reported that FABP3 was not associated with carcass weight and backfat thickness.

The findings of SAR cattle breed in this study are similar to the report of Kulig et al. (2010), which found higher A allele frequency and AA genotype frequency in SAR but in EAR cattle breed, we found higher G allele frequency and GG genotype frequency which are similar to the results of Wu et al. (2005) that indicated the association between FABP3 gene and subcutaneous fat thickness. The presented results suggest that the FABP3 genotypes might be used to increase fat and protein content in milk and AA genotype cows should be preferred in selection for improving the traits.

As a conclusion, it might be suggested that AA genotype frequency for BTN1A1 Hae III polymorphism which is effective on milk production traits of the two breeds, was found equal to each other. This may be advantageous for improving the milk production traits of both Turkish cattle breeds. The A allele frequency and AA genotype frequency for FABP3 Aci I polymorphism showed a relationship in which the economically important traits were found to be higher in SAR cattle breed. We recommend that further studies be conducted on genotypes and allele frequencies of BTN1A1, FABP3 and TG genes in native Turkish cattle breeds using linkage analysis.

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REFERENCES

Afrache H, Gouret P, Ainouche S, Pontarotti P, Olive D (2012). The butyrophilin (BTN) gene family: from milk fat to the regulation of the immune response. Immunogenetics 64(11):781-794.

Ashwell MS, Ogg SL, Mather IH (1996). The bovine butyrophilin Gene maps to chromsome 23. Anim. Genet. 27: 235-242.

Ashwell MS, Heyen DW , Sonstegard TS , Van Tassell CP , Da Y , VanRaden PM , Ron M , Weller JI , Lewin HA (2004). Detection of quantative trait loci affecting milk production. J. Dairy. Sci. 87(2):468-475.

Baas F, Van Ommen GJ, Bikker H, Arnberg AC, de Vijlder JJ (1986). The human thyroglobulin gene is over 300 kb long and contains introns of up to 64 kb. Nucleic Acids Res. 14(13):5171-5186.

Bakir G, Kaygisiz G (2003). *Guney Anadolu kirmizisi sigirlarinda dunden bugune yapilan islah calismalari.* Gap 3. tarım kongresi, Sanliurfa, Turkey.

Banghart LR, Chamberlain CW, Velarde J, Korobko IV, Ogg SL, Jack LJ, Vakharia VN, Mather IH (1998). Butyrophilin is expressed in mammary epithelial cells from a single-sized messenger RNA as a type I membrane glycoprotein. J. Biol. Chem. 273:4171-4179.

Barendse W (1997). Assessing Lipid Metabolism. Patent Publication WO9923248, Patent US 6383751. Available at: http://ep.espacenet.com.

Bhattacharya TK, Sheikh FD, Sukla S (2005). Differences of ovine butyrophilin gene (exon 8) from its bovine and bubaline counter part. Small Rumin. Res. 3037-3042.

Calvo JH, Marcos S, Jurado JJ, Serrano M (2004). Association of the heart fatty acid-binding protein (FABP3) gene with milk traits in Manchega breed sheep. Anim. Genet. 35: 347-349.

Casas E, White SN, Riley DG, Smith TP, Brenneman RA, Olson TA, Johnson DD, Coleman SW, Bennett GL, Chase CC (2005). Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in *Bos indicus* cattle. J. Anim. Sci. 83:13-19.

Chmurzynska A (2006). The multigene family of fatty acid binding proteins (FABPs): Function, structure and polymorphism. J. Appl. Genet. 47: 39-48.

Cho S, Sung Park T, Du-Hak Y, Hyun Sub C, Namgoong S, Byung LP, Hye Won L,Chang SH, Eun MK, Il-Cheong C, Heebal K, Hyoung DS (2007). Identification of genetic polymorphisms in FABP3 and FABP4 and putative association with back fat thickness in Korean native cattle. BMB reports 29-34.

Coppieter WJ, Riquet J.-J, Arranz B, Berzi N, Cambisano B, Grisart L, Kari F, Marcq L, Morea C, Nezer P, Simon P, Vanmanshove D,Wagenaar MG (1998). A QTL with major effect on milk yield and composition maps to bovine Chromosome 14. Mamm. Genome 9:540-544.

Frank WW, Heid HW, Grund C, Winter S, Freudenstein C, Schmid E, Jarasch ED, Keenan TW (1981). Antibodies to the major insoluble milk fat globule membrane-associated protein: specific location in apical regions of lactating epithelial cells. J. Cell. Biol. 89:485-494.

Heyen DW, Weller JI, Ron M, Band M, Beever JE, Feldmesser E, Da Y, Wiggans GR, Vanraden PM, Lewin HA (1999). A genome scan for QTL influencing milk production and health traits in dairy cattle. Physiol. Genomics 1:65-175.

Husaini Y, Wilkins RJ, Davey HW (1999). Identification of five point mutation including an AluI RFLP in the bovine butyrophilin gene. Anim. Genet. 30:400-401.

Iraz AA, Ahmet M, Kemal OO (2012). A determination of growth hormone receptor gene polymorphisms in East Anatolian Red cattle, South Anatolian Red cattle, and Turkish Grey cattle. Turkish J. Vet. Anim. Sci. 36(1):27-33.

Jack LJW, Mather IH (1990). Coloning and analysis of cDNA encoding bivine butyrophilin, an apical glycoprotein expressed in mammary tissue and secreted in association with the milk fat globule membrane during lactation. J. Biol. Chem. 256:2532-2538.

Jack LJW, Mather IH (1993). A Review of the Molecular and Cellular Biology of Butyrophilin, the Major Protein of Bovine Milk Fat Globule Membrane. J. Dairy. Sci. 76(12):3832-3850.

Kaymakci M, Kocak C (2004). *Hayvan Turleri ve Irklari. Tarimsal Uygulamalar Hayvansal Uretim Uygulamalari*. Izmir: Ege University. Agriculture Faculty.

Khatib H, Zaitoun I, Chang YM, Maltecca C, Boettcher P (2007). Evaluation of association between polymorphism within the thyroglobulin gene and milk production traits in dairy cattle. J. Anim. Breed. Genet. 124(1):26-28.

Khatkar MS, Thomson PC, Tammen I, Raadsma HW (2004). Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet. Sel. Evol. 36: 163-190.

Komisarek J, Dorynek Z (2002). Genetic aspects of twinning in cattle. J. Appl. Genet. 43:55-68.

Komisarek J, Dorynek Z (2003). Polymorphism of BTN and GHR genes and its impact on bulls' breeding value for milk production traits. J. Anim. Feed Sci. 12(4):681-688.

Kulig H, Kowalewska-Łuczak I, Kmieć M, Wojdak-Maksymiec K (2010). ANXA9, SLC27A3, FABP3 and FABP4 single nucleotide polymorphisms in relation to milk production traits in Jersey cows. Czech J. Anim. Sci. 55:463-467.

Miller LM, Paszek AA, Schook LB, Louis CF (1998). Denser marker coverage provides greater evidence for carcass trait QTL on SSCP. Anim. Genet. 29: 60-74.

Moore SS, Li C, Basarab J, Snelling WM, Kneeland J, Murdoch B, Hansen C, Benkel B (2003). Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of *Bos taurus*. J. Anim. Sci. 81: 1919-1925.

Ogg SL, Weldon AK, Dobbie L, Smith AJ, Mather IH (2004). Expression of butyrophilin (Btn1a1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. PANS 27: 10084-10089.

Riquet J, Coppieters W, Cambisano N, Arranz J.-J, Berzi P, Davis SK, Grisart B, Farnir F, Karim L, Mni M, Simon P, Tayor JF, Vanmanshoven P, Wagenaar D, Womack JE, Georges M (1999). Fine-mapping of quantitative trait loci by identity by descent in outbred population: Application to milk production in dairy cattle. Proc. Natl. Acad. Sci. 96: 9252-9257.

Roy R, Calvo JH, Hayes H, Rodellar C, Eggen A (2003). Fine mapping of the bovine heart fatty acid-binding protein gene (*FABP3*) to BTA2q45 by fluorescence in situ hybridization and radiation hybrid mapping. Anim. Genet. 34:466-467.

Sadr SMT, Beigi N, Kh. Alami-Saeid J, Fayazi HR, Mohammadi M (2008). DNA polymorphism of butyrophilin gene by PCR-RFLP technique. Afr. J. Biotechnol. 7(14):2527-2529.

Taylor M, Everest M, Smith C (1996a). Restriction fragment length polymorphism in amplification products of the bovine butyrophilin gene: assignment of bovine butyrophilin to bovine chromosome 23. Anim. Genet. 27:183-185.

Tetsuya I, Naohito A, Akihiro N, Takahiro A, Ryo N, Tsukasa M (1995). Carboxy-terminal cytoplasmic domain of mouse butyrophilin specifically associates with a 150-kDa protein of mammary epithelial cells and milk fat globule membrane. Biochimica. Biophysica. Acta. 1245(3):285-292.

Veerkamp JH, Maatman RGHJ (1995). Cytoplasmic fatty acid binding proteins: their structure and genes. Progress. Lipid Res. 34:17-52.

Veerkamp JH, Van Kuppevelt THMSM, Maatman RGHJ, Prinsen CFM (1993). Structural and functional aspects of cytosolic fatty acid binding proteins. Prostaglandins, Leukotrienes and Essential Fatty Acids. 49:887-906.

Wu X-L, MacNeil MD, De S, Xiao Q-J, Michal JJ, Gaskins CT, Reeves JJ, Busboom JR, Wright RWJ, Jiang Z (2005). Evaluation of candidate gene effects for beef backfat via Bayesian model selection. Genet. 125:103-113.

Yeh F, Yang RC, Boyle T (2000). Popgene (v. 1.32), Microsoft Windows-based freeware for Population Genetic Analysis. Retrieved from http://www.ualberta. ca/~fyeh/Pop32.exe.