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The polymorphisms of genes associated with the profile of fatty acids of sheep

[*Os polimorfismos de genes associados ao perfil de ácidos graxos de ovinos*]

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ABSTRACT

The present study aimed to evaluate the occurrence of polymorphisms in Diacylglycerol acyltransferase (*DGTA-1* and *2*), Fatty acid synthase (*FASN*), Stearoyl-CoA desaturase (*SCD*) genes and the Thioesterase domain of *FASN* (*TE-FASN*) gene that may be related to the lipid profile. In the experiment, a total of 84 sheep from different genetic groups were used. For the evaluation of the polymorphism of the genes, PCR-Single Strand Conformation Polymorphism (SSCP) technique and subsequent sequencing were used. In *DGAT-2* gene, four genotypes were identified with the presence of 6 polymorphisms, with two (c.229T> C; c.255T> C) that resulted into the exchange of phenylalanine by leucine. In *FASN* gene, two genotypes were identified. In *TE-FASN* gene, three genotypes and 17 polymorphisms were identified. *DGAT-1* and *SCD* genes did not reveal the occurrence of polymorphism. There was difference in relation to C14: 0, C18: 0 fatty acids and Δ9-desaturase ^{C18} for *DGAT-2* gene and of C18: 2ω6t for *TE-FASN*. There were differences among the genetic groups for C10: 0, C12: 0, C17: 0, C18: 2ω6t, C18: 3ω3, C20: 2, total of ω3, ω3/ω6 and atherogenicity index. There is occurrence of polymorphism of DGAT-2 and *TE-FASN* genes and these should be further studied in sheep since they revealed influence of the genotypes on the fatty acid profile.

Keywords: diacylglycerol acyltransferase, fatty acid synthase, stearoyl-CoA desaturase

RESUMO

O presente estudo teve o objetivo de avaliar a ocorrência de polimorfismos nos genes Diacilglicerol aciltransferase (DGTA1 e 2), Ácido graxo sintase (FASN), Estearoil-CoA dessaturase (SCD) e o Domínio da tioesterase do gene FASN (TE-FASN), que possam estar relacionados ao perfil lipídico. No experimento, foram utilizados um total de 84 ovinos de diferentes grupos genéticos. Para avaliação do polimorfismo dos genes, foi utilizada a técnica de polimorfismo de conformação de cadeia simples (PCR-SSCP) e posterior sequenciamento. No gene DGAT-2, foram identificados quatro genótipos com a presença de seis polimorfismos, com dois (c.229T>C; c.255T>C) que resultaram na troca da fenilalanina por leucina. No gene FASN, foram identificados dois genótipos. No gene TE-FASN, foram identificados três genótipos e 17 polimorfismos. Os genes DGAT-1 e SCD não revelaram a ocorrência de polimorfismo. Houve diferença em relação aos ácidos graxos C14:0, C18:0 e Δ9-desaturase^{C18} para o gene DGAT-2 e de C18:2ω6t para TE-FASN. Houve diferença entre os grupos genéticos para C10:0, C12:0, C17:0, C18:2ω6t, C18:3ω3, C20:2, total de ω3, ω3/ω6 e índice de aterogenicidade. Há ocorrência de polimorfismo dos genes DGAT-2 e TE-FASN, e estes devem ser mais estudados em ovinos, pois revelaram influência dos genótipos sobre o perfil de ácidos graxos.

Palavras-chave: diacilglicerol aciltransferase, ácido graxo sintase, estearoil-CoA dessaturase

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INTRODUCTION

At present, much attention has been paid to the composition of fatty acids in meats, due to their influence on quality, since intramuscular fat is responsible for the tenderness and juiciness of cooked meat (Wood *et al*., 2003) and human health. The lipid composition, in saturated fatty acids (AGS), affects adipose tissue firmness and oxidative stability of muscles, influencing meat processing (Wood *et al*., 2008). It is known that some AGS, commonly found in meat and dairy products, especially myristic and palmitic acids, raise total cholesterol and the low-density lipoprotein levels and are therefore risk factors for heart disease (Erkkilä *et al*., 2008). Although, the effects of fatty acids on health are well studied and known, the molecular mechanisms that control their synthesis in different species are not yet fully understood.

The process of AG synthesis in meat involves some enzymes, such as Fatty Acid Synthase - *FASN* (Chu *et al*., 2015; Zhang *et al*., 2008), Diacylglycerol Acyltransferase - *DGAT* (Bouwman *et al*., 2012; Zhang *et al*., 2014) and Stearoyl-CoA desaturase - *SCD* (Henriquez-Rodriguez *et al.*, 2015; Barton *et al.*, 2010). Stearoyl-CoA desaturase enzyme (*SCD*) is required in the biosynthesis of monounsaturated fatty acids such as oleic acid (C18: 1ω9) and palmitoleic (C16: 1ω9), which are produced after insertion of unsaturation in the Δ9 position of their precursors, C18: 0 and C16: 0 fatty acids (Guillou *et al*., 2010). While, Diacylglycerol acyltransferase enzymes (*DGAT-1* and *DGAT-2*) catalyze the final step of triacylglycerol (TAG) formation (Cases *et al*., 1998). *DGAT-1* acts by incorporating an oleoyl-CoA in TAG and *DGAT-2* is responsible for the accumulation of lipids (McFie *et al*., 2011).

Thus, many works aimed to understand the activity of these enzymes with the purpose of associating the lipid profile of meat with single nucleotide polymorphisms (SNPs) present in their genes. Some results indicate that the presence of SNPs in *FASN, SCD and FABP4* genes (*Fatty acid binding protein 4*) may influence the composition of fatty acids in ruminants (Maharani *et al*., 2012). In cattle, 5 SNPs were observed in *FASN* gene, identified along different breeding studies and one of them was associated with lipid composition, and can be used as a marker in breeding programs (Bhuiyan *et al*., 2009). In the same way, in goats, a SNP in the exon 3 of *DGAT-2* gene was found, being characterized as a possible molecular marker (Fang *et al*., 2012). However, studies in sheep are still scarce for these genes.

The present study intended to analyze the occurrence of SNPs of *DGAT-1 and 2, FASN*, *SCD* genes and Thioesterase domain of *FASN* gene in different genetic groups of sheep and check its association with lipid composition.

MATERIAL AND METHODS

The experiment used a total of 84 lambs, 14 purebred Santa Inês (SI) animals, 16 ½ SI x ½ Black Dorper (BD) animals; 12 animals ½ SI x ½ White Dorper (WD); 19 animals ½ SI x ½ Texel (TX); 14 animals ½ SI x ½ Lacaune (LC); 19 animals ½ SIx ½ East Friesan animals (EF) from SI dams and six different sires, in a randomized block design. The lambs were fed ration containing 80% concentrate and 20% roughage, based on oat hay, coffee husk, soybean meal, corn, limestone, mineral supplement and Rumensin ® and bypass fat (Megalac) at 5, 3% of the ration until the moment of slaughter. The sheep were slaughtered through a humanitarian method and use of good hygiene practices with a live weight of 43.99 ± 1.21kg (CEUA UFLA Protocol Number. 102/12).

Prior to slaughter, to conduct DNA evaluations approximately 5ml of blood was collected in a tube containing EDTA potassium [50μL 15% EDTA (K3)]. The samples were identified and stored at -80°C and the genomic DNA was extracted according to the protocol of Regitano (2001). The PCR was performed from 50ng of genomic DNA in a final volume of 25μl containing 1 × reaction buffer, 200μM of dNTP, 1.25U of Taq DNA polymerase, 2.5mM of MgCl and 0.5μM of each primer and H_2O. The PCR products obtained for the different primers (Table 1) were subjected to 1.0% agarose gel electrophoresis. Amplification was performed in the thermocycler (MasterCycler - Eppendorf, USA).

Table 1. Sequence of the *primers* and conditions established of annealing temperature of PCR for the amplification of the fragments of *DGAT-1, DGAT-2, FASN, SCD* and *TE-FASN* genes

Gene	pb	Sequence of primers (5'-3')	Annealing	Reference
DGAT 1	381	CCATCCTCTTCCTCAAGCTG GGGAAGTTGAGCTCGTAGCA	59ºC for 30''	Conte *et al*. (2010)
DGAT 2	268	CGAGCCCATTACCATCCC AAATAACCCACAGACACCC	61ºC for 30''	Fang *et al*. (2012)
FASN	228	GACCTTGACACGGCTCAACT GGGCACAGCATGAGGTTTAG	60ºC for 30''	Ciecierska *et al*. (2013)
SCD	377	TGAGGGCTTCCACAACTA GCATCATAAAGGCAGAGT	57ºC for 30''	Zhang *et al*. (2010)
TE –FASN	505	AGAGCCTGGCCACCTACTACATC TGCCACACGCGCCTCCAGA	56ºC for 30''	Oztabak *et al*. (2014)

About 1µl of each PCR product was diluted in 10µl of denaturing buffer (98% formamide, 20mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol), denatured at 95°C for 5min and maintained on ice. The total volume was applied onto 12.5% polyacrylamide gel. Electrophoresis was performed at 10°C in 0.5 × TBE buffer (100mM Tris, 9mM boric acid, 1mM EDTA) for approximately 6h at 300V. Thereafter, the polyacrylamide gels were stained with silver according to Byun *et al.* (2009).

Sequencing reactions were performed by capillary electrophoresis on ABI3130 apparatus using POP7 polymer and BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Madrid, Spain). The generated spherograms and nucleotide sequences were analyzed by the free software Sequence Scanner Software (Applied Biosystems®) and JustBio's computational tools (www.justbio.com), respectively. Homologous searches and sequence alignment were performed with BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and with ClustalW2 program (http://www.ebi.ac.uk/ Tools/msa/ Clustalw2 /).

For the conduction of the analyses of the fatty acid profile, samples of cover fat of the *longissimus lumborum* muscle of each animal were collected. The extraction of the lipids followed the methodology of Folch *et al*. (1957) and fatty acids were esterified according to Hartman and Lago (1973). Fatty acid analysis was carried out by gas chromatography on a Shimatzu CG 2010 chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA), equipped with flame ionization detector, split injector at the 1:50 ratio and capillary column of Supelco SPTM-2560, 100m X 0.25mm X 0.20µm (Supelco Inc., Bellefonte, PA, USA). The chromatographic conditions were initial column temperature of 140°C/5 min; increased 4°C/min. up to 240°C and maintained for 30min., totaling 60 minutes. The temperature of the injector and detector was 260°C.

The carrier gas used was helium. The fatty acids were identified by comparison to the retention times presented by the chromatographic standard SupelcoTM37 FAME Standard Mix (Supelco Inc., Bellefonte, PA, USA) and expressed in percentage (%) of the total of fatty acids identified and then grouped into: total saturated, monounsaturated and polyunsaturated fatty acids. The activity of Δ9 desaturase C16, C18, elongase and Thioesterase enzymes was estimated according to Malau-Aduli *et al*. (1997) and Kazala *et al*. (1999). The atherogenicity and thrombogenicity indices were obtained according to Ulbricht and Southgate (1991).

Genotypic frequency and allele frequency were performed by direct counting. In the statistical analysis, the SAS statistical package ((Statistical Analysis System, 2011) was used to determine the normality of the data (Shapiro-Wilk). The data were analyzed through the GLM procedure of SAS and the means were compared by the Tukey test ($\alpha = 0.05$) for the effect of the genetic groups and genotypes of each gene.

RESULTS AND DISCUSSION

In the sheep of this study, the occurrence of the band changes in the exon 6 region of *SCD* gene which proved monomorphic by the PCR-Single Strain Conformation Polymorphism (SSCP) technique was not verified. The same behavior also occurred in the region of the exon 8 of the *DGAT-1* gene in the present study (Figure 1).

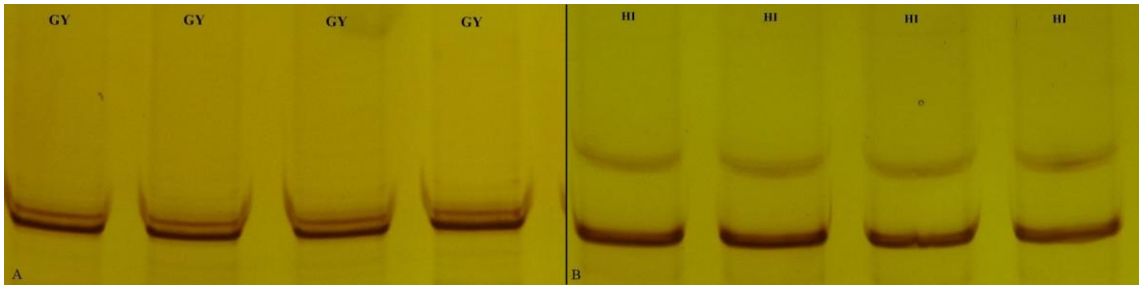

Figure 1. 12.5% polyacrimalyde gel using the PCR-SSCP technique identifying monomorphic genotypes: (A) GY genotypes in *SCD* gene and (B) HI genotypes in *DGAT-1* gene.

Using PCR-RFLP technique in goats, the results found by Ozmen and Kul (2014) did not demonstrate the occurrence of SNPs in the exon 8 region for that *DGAT-1* gene. However, seeking to find polymorphisms for *DGAT-1* gene and with the use of PCR-RFLP technique in sheep of the Assaf breed, Dervishi *et al*. (2015) detected a SNP in the exon 1, responsible for the amino acid alteration in position p. Asp53Glu, two SNPs in the exon 17 and a SNP in the intron 10, which promotes an amino acid alteration in position p. Arg482Cys. Considering the results presented, possible polymorphisms in the exon 8 of those genes, only will be able to be detected with the application of other analytical techniques and/or in other groups of sheep of different genotypes or submitted to different treatments.

Therefore, in relation to the amino acid profile in sheep, it was not possible to infer a relationship of polymorphisms in the studied regions of *SCD* (exon 6) and *DGAT-1* (exon 8) genes and the composition of the fatty acids. According to Sañudo *et al*. (2000), the sheep meat can present different fatty acid profiles according to the breeds, the animals and the diets, by means of genetic selection and alteration in the feeding of the animals, it is possible to obtain meats with better profile of fatty acids and consequently more wholesome.

In *DGAT-2* gene by PCR-SSCP technique, the presence of four genotypes (MO, MP, NP and MQ) with 5 alleles (M, O, P, N, Q) was found (Figure 2).

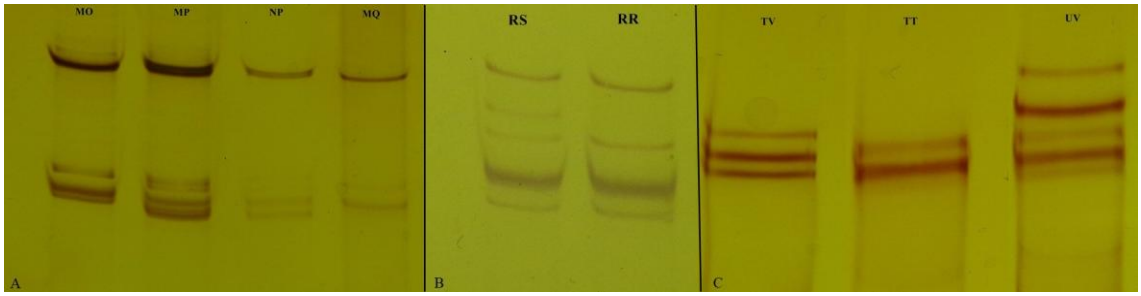

Figure 2. 12.5% polyacrylamide gel using PCR-SSCP technique identifying the genotypes: (A) four genotypes MO, MP, NP, MQ of SNPs in *DGAT-2* gene; (B) two RS and RR genotypes of SNPs on *FASN* gene; (C) three TV, TT and UV genotypes of SNPs in the TE Thioesterase domain of *FASN* gene.

The MO genotype in *DGAT-2* gene showed higher frequency in the Lacaune x Santa Inês (LC) crossing; while the MP occurred in the East Friesian x Santa Inês (EF). Animals. The purebred Santa Inês (SI) animals presented higher frequency of the NP genotype, whereas the MQ genotype was found only in the White Dorper x Santa Inês (WD) animals (Table 2). A higher frequency of the M, R and T alleles was observed in *DGAT-2*, *FASN* and *TE-FASN* genes, respectively. Fang *et al*., (2012) analyzing *DGAT-2* gene in goats found two alleles (A and B) and two genotypes (AA and AB). There was a greater number of individuals with AA genotype than AB, therefore, the A allele was considered a candidate gene for quantitative traits related to fat absorption, triglyceride synthesis and storage in livestock.

Table 2. Frequency of the genotypes and polymorphism alleles (SNP) according to the PCR-Single Strand Conformation Polymorphism technique (SSCP) of Diacylglycerol acyltranferase (*DGAT-2*), Fat Acid Synthase (*FASN*) genes and Thioesterase domain of *FASN* gene (*TE-FASN*) in each genetic group of sheep

Gene	Genotype	Genetic Groups*						Total	Alleles	Frequence
		LC	SI	EF	BD	WD	TX			
		FR(%)	FR(%)	FR(%)	FR(%)	FR(%)	FR(%)	FR(%)	-	-
DGAT-2	MO	78.57	35.71	44.44	62.5	66.67	47.37	55.95	M	0.357
	MP	0.00	0.00	33.33	31.25	16.67	10.53	14.29	N	0.143
	NP	21.43	64.29	22.22	6.25	8.33	42.10	28.57	O	0.280
	MQ	0.00	0.00	0.00	0.00	8.33	0.00	1.19	P	0.214
	-	-	-	-	-	-	-	-	Q	0.006
FASN	RS	21.43	14.29	44.44	12.5	0.00	10.53	15.48	R	0.923
	RR	78.57	85.71	55.55	87.5	100.00	89.47	84.52	S	0.077
TE-FASN	TV	71.43	92.86	88.89	100.00	83.33	73.68	84.53	T	0.493
	TT	14.29	0.00	0.00	0.00	16.67	10.53	7.14	U	0.042
	UV	14.29	7.14	11.11	0.00	0.00	15.79	8.33	V	0.465

*LC - Lacaune x Santa Inês; SI – Purebred Santa Inês; EF - East Friesian x Santa Inês; BD – Black Dorper x Santa Inês; WD - White Dorper x Santa Inês; TX - Texel x Santa Inês; FR = Relative frequence (%).

The data obtained in the sequencing of each genotype of the region of the exon 8 *DGAT-2* gene revealed the presence of six polymorphisms, which represent base change (A/G, C/G and A/T) and base deletion (Figure 3).

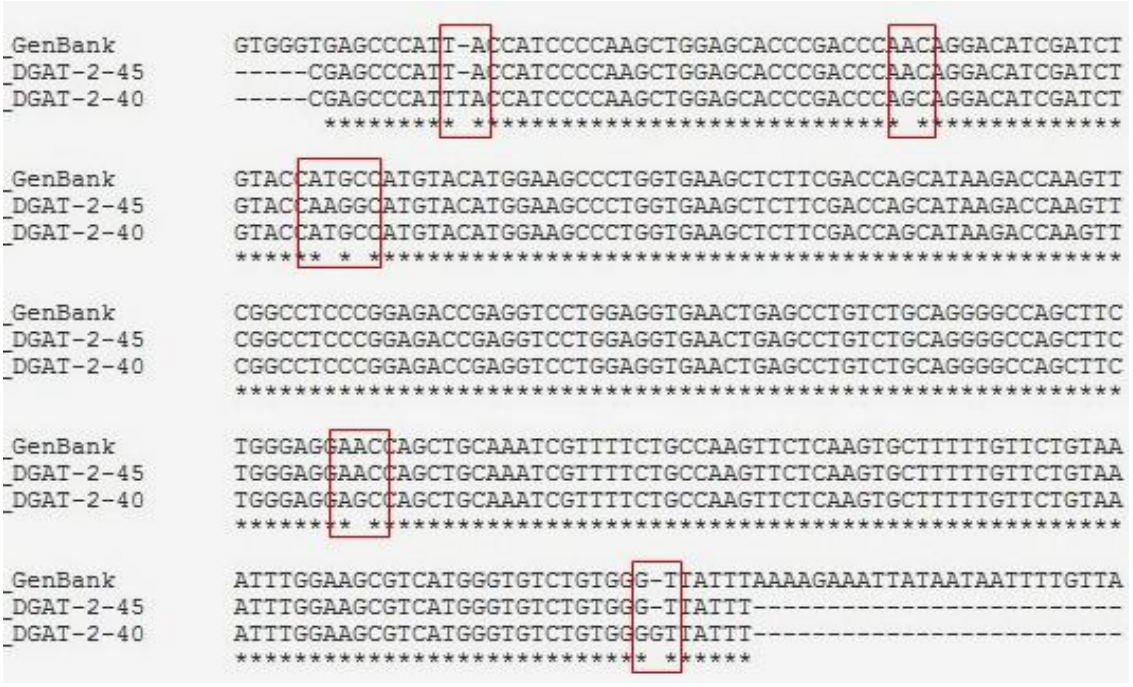

Figure 3. Partial alignment using tools from the JustBio site (2016) of two samples of *DGAT-2* gene and of the *DGAT-2* gene sequence deposited in GenBank, accession number XM_012154874.1. The similarities are indicated by an asterisk (*) and the differences by the absence of the same.

In two SNPs of *DGAT-2* gene, the polymorphisms (c.229T>C; c.255T>C), resulted into the change of phenylalanine for leucine (Figure 4).

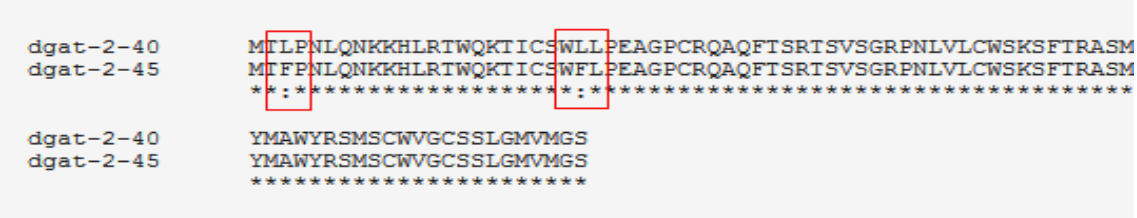

Figure 4. Partial alignment using tools from the Clustal omega site, from three different protein samples of *DGAT-2* gene. The similarities are indicated by an asterisk (*) and the differences by the absence of the same.

Fang *et al*. (2012) also reported the presence of SNPs for *DGAT-2* gene, with the presence of 7 polymorphisms in the exon 3 in the study with different goat breeds, using PCR-SSCP technique, one of which is characterized by the base change A/G) which resulted in the exchange of lysine by arginine, however, for the region of the exon 8 of this gene, the authors did not find the presence of SNPs.

In the present study, among the genetic groups evaluated, it was possible to observe two genotypes (RS and RR) and two alleles (R and S) in the exon 6 region of the *FASN* gene. The frequencies of the genotypes observed were of 15.48% and 84.52%, respectively, for the RS and RR genotypes (Table 2). Among the crosses, it is found that the EF animals presented higher frequency for the RS genotype; while for the RR genotype, its greatest expression occurred in the Texel x Santa Inês (TX) group. The allele frequencies observed for the *FASN* gene were of 0.923 and 0.077for R and S, respectively.

According to Chu *et al*. (2015), fatty acid synthase enzyme (*FASN*) presents multiple functions related to fatty acid biosynthesis, its gene being an important candidate as a molecular marker, since its expression can interfere with the lipid deposition and consequently in the meat quality of the animals.

In Polish Holstein-Friesian cattle, *FASN* gene was analyzed by RFLP technique, and three AA, AG and GG genotypes were identified. The AA individuals were characterized by higher milk yield and protein yields (Ciecierska *et al*., 2013). In *Datong yak* cattle, studies showed a significant ratio between the polymorphisms in *FASN* gene and intramuscular fat content (IMF), confirming that genotyping of this gene may be useful to select animals with higher lipid content in the muscle tissue and thus improve meat flavor (Chu *et al.*, 2015).

After several alignments with sequences deposited in the NCBI database, it was not possible to detect the possible polymorphisms in the sequences generated for *FASN* gene in this study. The results showed that the size of the amplification fragment (228 bp) was consistent with the target and had a good specificity, which could be directly analyzed by PCR-SSCP technique. In spite of not being possible to make considerations about the occurrence of polymorphism in the present study for the sheep *FASN* gene in cattle, Dongyep *et al*. evaluating the presence of SNPs for a total of 10 exons (1, 19, 21, 23, 24, 28, 34, 37, 39 and 42) only found the occurrence of polymorphisms for five sites (exon 23, 24, 34, 37 and 39), demonstrating that distinct behavior may occur in relation to the region of the gene evaluated.

The Thioesterase domain of the *FASN* gene (*TE-FASN*) was analyzed in the region of the exon 6 by the PCR-SSCP technique and revealed three original band patterns (TV, TT and UV) for the animals of this study (Figure 2).

The animals of the cross between Black Dorper and Santa Inês (BD) presented only frequency for the TV genotype; while for the cross between White Dorper and Santa Inês (WD), a higher frequency was observed for the TT genotype and the Texel x Santa Inês (TX) animals presented higher frequency for the UV genotype. Among the genetic groups evaluated, the TV genotype was the one with the highest prevalence in the group of animals studied. The allele frequencies observed for *TE-FASN* were 0.493, 0.042 and

0.465 for T, U and V respectively (Table 2), T allele being the most frequent.

The *TE-FASN* domain genotyping revealed 17 polymorphisms, with two base changes and 15 base deletions (Figure 5).

Figure 5. Partial alignment using tools from the JustBio site (2016) of two samples of *TE-FASN* gene and the *TE-FASN* gene sequence deposited in GenBank, accession number *JN570752*.2. The similarities are indicated by an asterisk (*) and the differences by the absence of the same.

One of the first investigations of polymorphisms in *TE-FASN* gene was performed in Angus cattle with the use of RFLP technique. The polymorphism found by Zhang *et al*. (2008) in that region (g.17924A> G) was associated with the composition of fatty acids in the *Longissimus dorsi* muscle. According to these authors, this mutation would be related to the change in the function of Thioesterase enzyme, so that the release of the fatty acid chain occurs when they reached 18 carbons. Thus, reducing the proportions of C14:0 and C16:0 in the adipose tissue and generating difference in the composition of fatty acids between the individuals.

For the lipid composition, there was effect of the genetic groups on the values of fatty acids C10:0, C12:0, C17:0, C18:1ω9c, C18:2ω6t, C18:3ω3, C20:2, Total of ω3/Ω6 and atherogenicity index. Influence was observed in relation to the polymorphism presented in *DGAT-2* gene for fatty acids C14:1, C18:0 and index of Δ9-desaturase ^{C18} and of the genotypes of *TE-FASN* gene for C18:2ω6t (Table 3). According to Faria *et al*. (2005), the lipid profile is related to increased genetic predisposition for fat deposition and these variations may be related to the activity of the Stearoyl-CoA desaturase enzyme (*SCD*). However, in this study the presence of SNPs for *SCD* gene was not found, indicating that the effects on the fatty acid profile may also be related to its expression and/or variability in the different species.

The sheep of the LC genetic group presented higher contents of C10:0 and C12:0 fatty acids and C17:0, C18:3ω3 fatty acids; total ω3 and ω3/ω6 ratio were observed in greater quantity in TX sheep (Table 4). Influence of breeds and genetic groups on the lipid profile is found in the literature. Juárez *et al.* (2009) who described differences in the levels of C12:0, C14:0 and C18:0 fatty acids, with higher values for the Churra Lebrijana breed (mutton breed) than for Grazalema Merino (dairy breed). Landim *et al.* (2011) studying the profile of fatty acids in sheep, found higher percentage of C17:1 in the muscle of Texel × Santa Inês in relation to the Santa Inês and Ille de France x Santa Inês animals, considering this difference due to the greater earliness of the Texel breed.

The WD animals revealed higher means of C20:2 and C18:1ω9c (Table 4). That result may be related to the earliness of the WD group. The animals in this study were slaughtered at younger ages, so the lipid profile is represented by the phospholipids of the cell membranes with highest amounts of monounsaturated fatty acids (Wood *et al*., 2008). As the animals reach maturity, the deposition of lipids in the form of triglycerides tends to increase and provide modification of the fatty acid profile, since the

amount of phospholipids remains relatively constant. And that can be verified in the study by Madruga *et al.* (2006) by evaluating the lipid profile of lambs of the Santa Inês and ½ Santa Inês x ½ Dorper breeds slaughtered late (mean weight of 90kg), where lower proportions of monounsaturated fatty acids and higher proportions of polyunsaturated fatty acids for the animals of the cross with Dorper were found.

Table 3. Means of fatty acids, lipid composition and significance (P value) according to the genetic group and gene polymorphism

Fatty acids (AG)	General Mean	Standard Deviation	Value of P*			
			Genetic Group	Genes		
				DGAT-2	FASN	TE-FASN
C8:0	0.003	0.006	0.354	0.753	0.690	0.228
C10:0	0.220	0.112	0.020	0.711	0.493	0.764
C11:0	0.053	0.031	0.207	0.812	0.843	0.464
C12:0	0.093	0.027	0.039	0.248	0.976	0.468
C13:0	0.086	0.043	0.776	0.794	0.289	0.174
C14:0	2.747	0.621	0.130	0.631	0.607	0.261
C14:1	0.196	0.095	0.174	0.042	0.508	0.382
C15:0	1.608	0.548	0.571	0.698	0.540	0.471
C15:1	0.029	0.040	0.152	0.649	0.052	0.569
C16:0	23.274	3.076	0.066	0.555	0.399	0.874
C16:1	3.334	0.813	0.237	0.380	0.604	0.185
C17:0	3.432	0.856	0.001	0.834	0.370	0.967
C17:1	3.023	1.261	0.793	0.391	0.211	0.846
C18:0	9.198	2,834	0.208	0.031	0.407	0.660
C18:1ω9t	6.143	3.336	0.120	0.546	0.180	0.071
C18:1ω9c	42.738	5.707	0.049	0.486	0.224	0.597
C18:2ω6t	0.165	0.069	0.029	0.591	0.634	0.027
C18:2ω6c	3.124	0.893	0.105	0.162	0.464	0.906
C20:0	0.045	0.031	0.081	0.465	0.425	0.417
C18:3ω6	0.116	0.100	0.721	0.733	0.168	0.648
C18:3ω3	0.184	0.094	0.014	0.168	0.730	0.597
C21:0	0.046	0.056	0.907	0.922	0.163	0.792
C20:2	0.020	0.021	0.028	0.716	0.508	0.382
C20:3ω6	0.017	0.011	0.064	0.394	0.402	0.359
C20:4ω6	0.102	0.040	0.354	0.505	0.512	0.336
Summation						
∑ Saturated (SAT)	40.876	4.627	0.094	0.550	0.387	0.877
∑ Monounsaturated (MON)	55.463	4.857	0.098	0.474	0.334	0.915
∑ Polyunsaturated (POL)	3.731	0.989	0.098	0.193	0.580	0.855
∑ω3	0.184	0.094	0.014	0.117	0.730	0.597
∑ω6	3.525	0.921	0.110	0.206	0.536	0.810
ΣPOL/ΣSAT	0.092	0.027	0.098	0.291	0.800	0.789
Ratio						
Σω6/Σω3	24.054	15.139	0.104	0.302	0.158	0.383
Σω3/Σω6	0.051	0.018	0.017	0.223	0.219	0.085
Index						
Δ9-desaturase^{C16}	12.705	3.488	0.289	0.608	0.345	0.401
Δ9-desaturase^{C18}	84.203	4.534	0.155	0.048	0.400	0.805
Elongase$^{C16-C18}$	68.578	3.436	0.094	0.345	0.574	0.455
Thioesterase^{C16-14}	89.525	1.305	0.625	0.924	0.810	0.079
Atherogenicity	0.767	0.076	0.004	0.066	0.831	0.452
Thrombogenicity	1.188	0.249	0.176	0.566	0.335	0.890

*Tukey's test (α=0.05).

Table 4. Means of the lipid composition of sheep by reason of the genetic groups

Variables	Genetic Group						Average	EPM	Value of P **
	BD	EF	LC	SI	TX	WD			
C10:0	0.245ab	0.187ab	0.304a	0.161b	0.206ab	0.208ab	0.220	0.036	0.020
C12:0	0.103ab	0.081ab	0.110a	0.081b	0.090ab	0.092ab	0.093	0.009	0.039
C17:0	3.705ab	3.143b	3.098b	3.21b	4.141a	3.14b	3.432	0.237	0.001
C18:1ω9c	41.215ab	42.171ab	42.019ab	46.172ab	40.565b	45.121a	42.738	1.802	0.049
C18:2ω6t	0.176ab	0.158ab	0.143ab	0.206a	0.126b	0.182ab	0.165	0.022	0.029
C18:3ω3	0.188ab	0.222ab	0.161ab	0.170ab	0.245a	0.123b	0.184	0.029	0.014
C20:2	0.020ab	0.019ab	0.009b	0.021ab	0.018ab	0.038a	0.020	0.007	0.028
∑ω3	0.188ab	0.222ab	0.161ab	0.170ab	0.245a	0.124b	0.184	0.029	0.014
∑ω3/∑ω6	0.055ab	0.056ab	0.046ab	0.046ab	0.063a	0.040b	0.051	0.006	0.017
Atherogenicity	0.756ab	0.759ab	0.822a	0.758ab	0.717b	0.798a	0.767	0.023	0.004

*LC - Lacaune x Santa Inês; SI - Santa Inês puro; EF - East Friesian x Santa Inês; BD – Black Dorper x Santa Inês; WD - White Dorper x Santa Inês; TX - Texel x Santa Inês; SEM =standard error of the mean; ** Tukey's test (α=0.05).

The SI animals presented higher contents of C18:2ω6t (Table 4), which comes to corroborate with the results found by Cruz *et al.* (2011) with Santa Inês sheep, in which they report a higher concentration of this fatty acid.

The highest atherogenicity indices were found for LC and WD animals and the lowest ones for TX sheep (Table 4). These results are related to the ones which present a higher amount of C12:0 found in the LC genetic group, which is a fatty acid with an atherogenic potential as well as C14:0 and C16:0 (Menezes Junior *et al.*, 2014), and lower percentages of the C18:3ω3 group for the WD group, which is a fatty acid of the ω3 series and is related to the metabolic synthesis that will produce the prostaglandins that own antithrombogenic and antiatherogenic effect (Perini *et al.*, 2010). While for the TX genetic group, the result was a higher percentage of C18:3ω3 that showed almost 2 times the amount found for the LC and WD groups.

For *DGAT-2* gene, the MO genotype revealed higher means for C14:1 fatty acids and Δ9-desaturase^{C18} index; for stearic acid (C18:0), an inverse behavior occurred with higher average values for NP and lower for MP, showing influence on the composition of these fatty acids in sheep (Table 5). In pigs, Yin *et al.* (2012) found a variation (A/G) by the PCR-RFLP method in the 3 'UTR region of *DGAT-2* gene and this was related to the modification of the back-fat thickness between the sixth and seventh ribs. Thus, the occurrence of SNPs in the different regions of this gene can promote alteration in lipid composition and deposition.

For the gene of the *TE-FANS* region, higher average values of C18:2ω6t were found for the TV genotype, these being similar to TT and superior to UV.

In this study, the influence of the genetic group and the genotype of the *TE-FASN* gene were found in relation to the values of C18:2ω6t (Table 2, 3). Where the genetic groups (TX and LC) that presented higher frequencies of the TT and UV genotype tended to present, although statistically similar, lower values of this fatty acid.

Table 5. Means of the lipid composition of sheep because of the polymorphism of *DGAT-2* and *TE* genes.

Variables	Genotypes of DGAT- 2 gene				EPM	Value of P *
	MO	MP	NP	MQ		
C14:1	0.215a	0.212ab	0.153b	0.140c	0.028	0.042
C18:0	8.470d	9.441bc	10.428b	11.478a	0.822	0.031
Δ9-desaturase^{C18}	85.317a	83.671ab	82.390b	80.325c	1.327	0.048
Genotypes of TE-FASN gene						
	TV	TT	UV			
C18:2ω6t	0.173a	0.146ab	0.096b		0.027	0.027

* Tukey test (α=0.05); SEM = standard error of the mean.

Studies on cattle of the Korean Hanwoo breed, in *FASN* gene in the exon 39 in the *TE* domain region, g.17924 polymorphism (A> G) in the GG genotype was identified and this was associated with higher concentrations of C18:1ω9 and lower of C16:0 (Bhuiyan *et al*., 2009). This same polymorphism was also associated by Maharani *et al.* (2012) to low levels of myristic fatty acid (C14:0). This demonstrates the importance of conducting further studies on this region of this gene.

CONCLUSION

The results indicate that PCR-SSCP is an efficient technique to detect polymorphisms in the genes involved in lipid metabolism. Different SNPs were found in *DGAT-2* and in *TE* domain of *FASN* gene, which could influence the synthesis of certain fatty acids and alter the lipid profile of the meat.

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