View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Repositorio Universidad de Zaragoza

The Journal of Animal & Plant Sciences, 27(3): 2017, Page: 743-746
ISSN: 1018-7081

GENETIC POLYMORPHISM IN MEAT FATTY ACIDS IN ARAUCANO CREOLE SHEEPS

J. Quiñones[1], S. Bravo[2], J. H. Calvo[3], and N. Sepúlveda[2*].

[1]Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada. Universidad de La Frontera. Temuco, Chile.
[2]Departamento de Producción Agropecuaria de la Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera. Temuco, Chile.
[3]Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza, Spain.
[*]Corresponding author: nestor.sepulveda@ufrontera.cl

ABSTRACT

Meat is a source of proteins and minerals. However, red meats have high levels of saturated fatty acids (SFA) and a low proportion of monounsaturated fatty acids (MUFA), a combination which has been linked to cancer and cardiovascular diseases. In ruminants, there are several genes that regulate the proportions of MUFA in tissues, but the most important is SCD *(Stearoyl-CoA desaturase).* The polymorphism g.31C >A has been described in the promoter region of the SCD gene, which is associated with changes in the gene expression and MUFA levels in the meat. The aim of this study was to detect the presence of polymorphism g.31C >A in a population of Araucano creole sheep using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Genomic DNA was obtained from 157 Araucano creole sheep. The SCD promoter region was amplified using PCR and the amplicons were digested with restriction enzyme *MnlI*. The allelic frequency was 0.98 for the C allele and 0.02 for the A allele. The *in silico* analysis showed that the A allele could alter the expression of SCD. This is the first report of the presence of polymorphism g.31C >A in Chilean sheep, and its association with SCD expression levels and the proportion of MUFA in the meat will require further investigation.

Key words. Creole sheep, meat, polymorphism, SCD gene.

INTRODUCTION

The consumption of red meats from ruminants is very important for human nutrition because they are a source of proteins and minerals (McNeill and Van Elswyk, 2012). However, the red meats are characterized as having a SFA profile, which has been linked to cancer and cardiovascular diseases (Givens, 2010).

The fatty acid profile of meat is regulated by several genes of lipid metabolism, which encode enzymes responsible for fatty acid synthesis, elongation and desaturation that will be incorporated into cell membranes and various tissues (Gonzales-Calvo *et al.*, 2015; Ling *et al.,* 2015).

In ruminants the SCD gene encodes for *Stearoyl-CoA desaturase*, an enzyme that regulates the proportions of monounsaturated fatty acids (MUFA), considered healthy fatty acids, through the desaturation of palmitic acid (C16:0) to palmitoleic acid (C16:1) and the desaturation of stearic acid (C18:0) to oleic acid (C18:1) (Izadi *et al.,* 2014).

It has been shown in cattle that SCD is polymorphic in encoding regions; it is a SNP (single nucleotide polymorphism) identified as g.878T>C which has been linked to changes in the proportion of conjugated linoleic acid (CLA) and MUFA in meat and milk (Taniguchi *et al.*, 2004, Mele *et al.,* 2007, Inostroza *et al.,* 2012). In goats four polymorphisms have been identified in the SCD gene, three in the intronic region (SCD3 172, SCD3 181, and SCD3 231) and one in exon 2 identified as SCD2, associated with high levels of CLA in meat (Aviles *et al.,* 2016).

In sheep the presence of the g.878T>C SNP has not been described, nor the polymorphisms reported in goats, but a QTL (quantitative trait loci) has been identified in chromosome 22 (the chromosome where the ovine SCD gene is located) related to the amount of CLA in milk from the Churra breed (García-Fernández *et al.,* 2010). In another study, an evaluation of the genetic variability of SCD revealed the absence of variations in the gene coding region. However, four SNPs were identified: two present in intron 2 (g.1473A>G and g.2011T>C); one (g.2893G>A) located in intron 3 and another present in the promoter region (g. 31C>A); the last one is polymorphic in ovine species than those found in the other intronic region (García-Fernández *et al.,* 2009).

A recent study on the Rasa Aragonesa breed showed that the A allele of the g.31C>A polymorphism is significantly associated with the increase in gene expression and the proportion of MUFA in the meat compared to the C allele (González Calvo *et al.,* 2014). Consequently, the g.31C>A SNP is the main genetic marker of the MUFA variation in this breed's meat.

The Araucano creole sheep is a local ecotype from southern Chile, bred by small and medium farmers, highly adaptable to its surroundings and it has shown high genetic variability by DNA-microsatellites, which is why the existence of polymorphisms in genes of productive importance such as SCD is likely (Bravo *et al.,* 2015; Paz *et al.,* 2015).

In this light, the aim of this study was to detect the presence of the g.31C>A SNP in a population of Araucano creole sheep using the PCR-RFLP technique.

MATERIALS AND METHODS

Study animals: This study was conducted at the Sheep Genetic Nucleus of the Universidad de La Frontera, located in the commune of Freire, Region of La Araucanía, Chile (38º50´27´´south, 72º41´45´´west). 157 blood samples were drawn by jugular vein puncture from a population of Araucano creole sheep composed of adult males, adult females, lambs and yearlings from the 2015 season.

Genomic DNA extraction: Genomic DNA was obtained from the blood samples using the ULTRA CLEAN extraction kit (MO BIO Laboratories Inc., USA). Sample integrity was assessed by 1% agarose gel electrophoresis and stained with SYBR® Safe (Thermo Fisher Scientific Inc., USA). The genomic DNA was quantified by spectrophotometry using the Gen5™ software (BioTek Instruments, Inc., USA). Finally, the DNA samples were diluted to 10ng/µl for use.

Detection of polymorphisms in the SCD promoter region using PCR-RFLP: A sequence of 527 bp corresponding to the promoter region of the SCD gene was amplified (Genbank: FJ513370.1). For this, the Taq Polymerase enzyme was used (Biotools B&M Labs S.A., Spain) as well as the primers described by Aali *et al.* (2014): Forward: 5´AAATTCCCTTCGGCCAATGAC´3 and Reverse: 5´TCTCACCTCCTCTTGCAGCA´3.

The temperature conditions for the PCR reaction were as follows: initial temperature of 94°C for 3 min, then 35 cycles of 94°C for 50 s, 58°C for 50 s, 72°C for 50s and then a final period of 72°C for 10 min. The PCR-RFLP was done by incubating the PCR product all night at 37°C with the restriction enzyme *MnL1* (Bioengland Biolabs, USA). The PCR-RFLP patterns were checked using 3% agarose gel and stained with SYBR® Safe (Thermo Fisher Scientific Inc., USA). The g. 31 C/C genotype showed a pattern of 3 restriction fragments: one fragment of 269 bp, one of 94 bp and one of 64 bp. The g. 31 C/A genotype showed a restriction fragment pattern identical to g. 31 C/C, but a fragment of 76 bp was also observed, given that the A allele produces a new recognition site for the restriction enzyme *Mnl1*. The g. 31 A/A genotype revealed a fragment of 269 bp, a second 76 bp, and a fragment of 64 bp. All fragments were identified by NEBcutter (New England Biolabs, USA). Transcription factor recognition sequences were predicted using the AliBaba 2.1 software (BIOBASE, Germany).

RESULTS AND DISCUSSION

Our results show that the A allele of the g.31C>A SNP is present in the Araucano creole sheep as detected using PCR-RFLP.

The Araucano showed a genotypic frequency of 93% for the g. 31 C/C genotype. The g. 31 C/A genotype had a genotypic frequency of 7%. The A/A genotype was not detected in the study population (Figure 1). Likewise, a low frequency of the A allele was observed (0.02). However, this frequency is similar to that described by Aali *et al.* (2014) in Lori-Bakhtiari sheep (0.014). It is interesting that a low frequency of the A allele is present mainly in meat sheep breeds like the Araucano, with a much higher frequency of the A allele being observed in milk breeds (Table 1). We believe that the loss of the A allele over time in the Araucano population could occur due to the selection of heavier animals and therefore a higher fat infiltration, a trait more closely related to the C allele (Costa *et al.,* 2013).

It is known that in cattle a greater SCD expression influences the increase in oleic acid levels (C18:1) and MUFA in fatty tissue as well as the total lipid levels in meat (Da Costa *et al.,* 2013).

The *in silico* analysis using the AliBaba 2.1 software established that the C allele of the SNP has an affinity for transcription factors Sp1, WT1, NF-1 and AP-2 α, whereas the sequence of the A allele has an affinity for Sp1 and CCAAT/enhancer-binding protein (C/EBP alpha) (Figure 2).

The mechanisms by which the A allele acts as a regulator are unknown. However, this could be explained by the *in silico* analysis of the SCD sequence, which indicates that the C allele is found in a region of high affinity to nuclear proteins such as WT1 and AP-2 α. Both transcription factors may be involved in lipid metabolism proliferation and regulation processes (Bakhtiarizadeh *et al.,* 2014)

The A allele causes the loss of affinity sites for WT1, AP-2 α, having exclusive affinity with C/EBP alpha, a transcription factor that serves as a co-activator, binding to the promoter regions of several lipid metabolism genes, including SCD, and is associated with lipid deposition in the adipocyte proliferation and growth processes (Reardon *el al.,* 2010; Hirwa *et al.,* 2011). Nevertheless, there are reports in cattle that consider C/EBP as not directly related to the regulation of SCD (Ohsaki *et al.,* 2007).

García–Fernández *et al.* (2009), however, report that the g.31C>A SNP is the most important polymorphism in the SCD gene in sheep given its

polymorphic nature in various breeds and because of its position in the promoter region attached to gene expression; therefore, it is necessary to delve more deeply into this study for a better understanding of the factors related to healthier fatty acid synthesis in animals of productive interest.

Table 1. Comparison of frequencies of the A allele of the g.31C>A SNP in the promoter region of the SCD gene present in meat and milk sheep breeds.

Breed	Specialization	Frequency of the A allele
Lacaune	milk	0.45
Churra	milk	0.37
Castellana	milk	0.15
Assaf	milk	0.4
Ojalada	meat	0.2
Lori bakhtiari	meat	0.014
Ossimi	meat	0,05
Araucana	meat	0.02
Berrichon Du Cher	meat	0

Table adapted from the results shown by: García Fernández *et al*., 2009; Aali *et al*., 2014

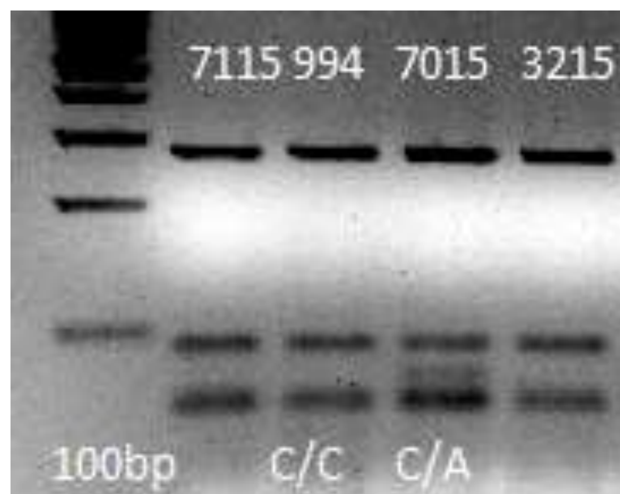

Fig 1. Genotypes present in the Araucano creole from the polymorphism g.31C>A. PCR-RFLP performed with *MnL1* (Bioengland Biolabs, USA), visualized in 3% agarose gel, stained with SYBR® Safe (Thermo Fisher Scientific Inc, USA). The genotypes C/C and C/A can be observed compared to the 100 bp marker.

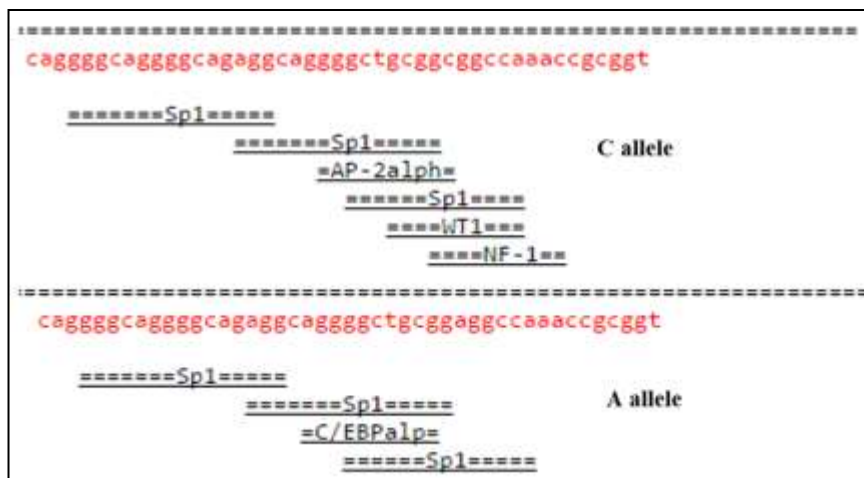

Fig 2. *In silico* analysis of transcription factor binding using AliBaba 2.1. Effect of the C allele and A allele of the polymorphism g.31A>C in the promoter region of the SCD gene of the Araucano creole sheep. The A allele eliminates the WT1 and NF-1 recognition sites and replaces the recognition site of the factor AP-2 alpha for that of the C/EBP alpha protein.

Conclusions: This preliminary study is the first report of the presence of the g.31C>A SNP in Chilean creole sheep, this new molecular marker present in the SCD gene could be used in improvement programs to increase the proportions of MUFA in sheep meat. We agree that there are still few studies related to the effect of the g.31C>A SNP, which is why the continuation of this study will focus on increasing the sample population and studying the effect of the presence of the A allele on the expression of the SCD gene and the composition of fatty acids in sheep tissue.

Acknowledgements: The authors wish to thank to Laboratory of Animal Production Technology and Health Department, CITA Aragón, Zaragoza, Spain for his collaboration. We also appreciate partial financing of DIUFRO: DI16-1001 and the Experimental Farm Maquehue, Universidad de La Frontera, Temuco, Chile.

REFERENCES

Aali, M., M. Shahrbabak, H. Shahrbabak and M. Sadeghi (2014). Identifying novel SNPs and allelic sequences of the stearoyl-Coa desaturase gene (SCD) in fat-tailed and thin-tailed sheep breeds. Biochem. Genet. 52(3-4): 153-158.

Ahlberg, C., L. Schiermiester, C. Calkins and M. Spangler (2014). Genome wide association study of cholesterol and poly-and monounsaturated fatty acids, protein, and mineral content of beef from crossbred cattle. Meat Sci. 98(4): 804-814.

Avilés, C., A. Horcada, O. Polvillo, A. Membrillo, G. Anaya, A. Molina and B. Panea (2016). Association study between variability in the SCD gene and the fatty acid profile in perirenal and intramuscular fat deposits from Spanish goat populations. Small Rumin. Res. 136(1): 127-131.

Bakhtiarizadeh, M., M. Moradi-Shahrbabak and E. Ebrahimie (2014). Transcriptional regulatory network analysis of the over-expressed genes in adipose tissue. Genes. Genom. 36(1): 105-117.

Bravo, S., G. Larama, M. Ortíz and N. Sepúlveda (2015). Genetic differentiation between 'Araucana' creole and 'Hampshire Down' sheeps in Chile. Chil. J. Agric. Res. 75(1): 131-136.

Costa, A. S., M. P. Silva, C. P. Alfaia, M. V. Pires, C. M. Fontes, R. J. Bessa and J. A. Prates (2013). Genetic background and diet impact beef fatty acid composition and stearoyl-CoA desaturase mRNA expression. Lipids. 48(4): 369-381.

Da Costa, A., V. Pires, C. M. Fontes and J. A. Prates (2013). Expression of genes controlling fat deposition in two genetically diverse beef cattle breeds fed high or low silage diets. BMC Vet. Res. 9(1): 118.

García-Fernández, M., B. Gutiérrez-Gil, E. García-Gámez and J. Arranz (2009). Genetic variability of the Stearoyl-CoA desaturase gene in sheep. Mol. Cell. Probes. 23(2): 107-111.

García-Fernández, M., B. Gutiérrez-Gil, E. García-Gámez, J. P. Sánchez and J. Arranz (2010). Detection of quantitative trait loci affecting the milk fatty acid profile on sheep chromosome 22: role of the stearoyl-CoA desaturase gene in Spanish Churra sheep. J. Dairy Sci. 93(1): 348-357.

Givens, D. (2010). Milk and meat in our diet: good or bad for health? Animal. 4(12): 1941-1952.

González-Calvo, L., L. Iguácel, A. Bolado, E. Dervishi, M. Serrano, G. Ripoll, and J. Calvo (2014). A functional variant in the promoter region of ovine stearoyl-CoA desaturasegene (SCD) affects gene expression and fatty acid profile in muscle. Abstracts of the 34th International Society for Animal Genetics (ISAG) Conference: Xi'an, China, p. 93-93.

González-Calvo, L., M. Joy, M. Blanco, E. Dervishi, F. Molino, P. Sarto and J. Calvo (2015). Effect of vitamin E supplementation or alfalfa grazing on fatty acid composition and expression of genes related to lipid metabolism in lambs. J. Anim. Sci. 93(6): 3044-3054.

Hirwa, C., P. Wallace, X. Shen, Q. Nie, G. Yang and X. Zhang (2011). Genes related to economically important traits in beef cattle. Asian. J. Anim. Sci. 5: 34-45.

Inostroza, K., G. Larama, and N. Sepúlveda (2012). Fatty Acid Composition (MUFA and CLA) in Bovine Muscle Tissue Related with the Presence of the g.878TC SCD Gene Polymorphism. Int. J. Morphol. 30(3): 934-936.

Izadi, M., A. Naserian, M. Nasiri and R. Heravi (2014). An Evolutionary Relationship between Stearoyl-CoA Desaturase (SCD) Protein Sequences Involved in Fatty Acid Metabolism. Rep. Biochem. Mol. Biol. 3(1): 1-6.

Ling, Y., K. Wang, J. Yin, L. Zhu, X. Zhang, C. Han and J. Ding (2015). Molecular analyses for genetic polymorphisms of the LPL gene and their associations with intramuscular fat content in goats, The J. Anim. Plant Sci. 25(5): 1238-1244.

McNeill, S. and M. Van Elswyk (2012). Red meat in global nutrition. Meat Sci. 92(3): 166-173.

Mele, M., G. Conte, B. Castiglioni, S. Chessa, N. Macciotta, A. Serra and P. Secchiari (2007). Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy. Sci. 90(9): 4458-4465.

Ohsaki, H., T. Sawa, S. Sasazaki, K. Kano, M. Taniguchi, F. Mukai and H. Mannen (2007). Stearoyl-CoA desaturase mRNA expression during bovine adipocyte differentiation in primary culture derived from Japanese Black and Holstein cattle. Comp. Biochem. Physiol. Part A: Molecular & Integrative Physiology. 148(3): 629-634.

Paz, E., J. Quiñones, S. Bravo, H.H. Montaldo and N. Sepúlveda (2015). Genotyping of BMPR1B, BMP15 and GDF9 genes in Chilean sheep breeds and association with prolificacy. Anim. Genet. 46(1): 98-99.

Reardon, W., A. Mullen, T. Sweeney and R. Hamill (2010). Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine M. longissimus and M. semimembranosus. Meat. Sci. 86(2): 270-275.

Taniguchi, M., T. Utsugi, K. Oyama, H. Mannen, M. Kobayashi, Y. Tanabe and S. Tsuji (2004). Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. Mamm. Genome. 15(2): 142-148.