SINGLE NUCLEOTIDE POLYMORPHISMS IN MMP-1 AND THE RELATION WITH BEEF FATTY ACID PROFILE

Sara Christensen,[1,2*], Laura B. Pouzo[1,3,4]; María E. Latorre[1,2], Cristina Monteavaro, [5] Enrique Pavan[4] and Peter P. Purslow[2]

[1]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina.

[2]Departamento de Tecnología y Calidad de los Alimentos, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina.

[3]Facultad de Ciencias Exactas, Universidad Nacional de La Plata. La Plata, Buenos Aires, Argentina.

[4]Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Balcarce, Argentina

[5]Laboratorio de Microbiología Clínica y Experimental CICPBA – NACT – SAMP CIVETAN, CCT-CONICET. Facultad de Ciencias Veterinarias – Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina.

*Corresponding author email: christensensarita@gmail.com

Abstract - Matrix metalloproteinase -1 (MMP-1) is an extracellular collagenase that is also known to have cell signaling effects controlling adipogenesis. Previous research indicates that single nucleotide polymorphisms (SNPs) in MMP-1 can affect the fatty acid profile and fat content of beef muscles. The aim of the research is to determine if the SNPs ss77831914 and ss77831924 in MMP-1 have effects on the development of fatty acids in meat in comparison to the effect of diet. Thirty (30) Angus cattle were raised on three diets and the Tetra primers ARMS-PCR was the technique used to identify the single nucleotide polymorphisms. Fatty acid methyl esters were analysed using a CLARUS 500 gas chromatograph. In our study, no correlation were found between the SNPs ss77831914 and ss77831924 and the fatty acid profile but a higher amount of fatty acids was found in homozygotic animals (GG genotype) for the SNP ss77831914 than for heterzygotic (CG) animals. However, this effect was insignificant compared to the effect of diet. We conclude that SNPs in MMP-1 may affect the intramuscular fat content of beef *longissimus thoracis* muscle in the absence of dietary effects.

Key Words: fat content, , meat quality, MMPs (matrix metalloproteinases), SNPs,

I. INTRODUCTION

The control of meat quality and sensory characteristics are important for beef producers to satisfy consumer preferences. Meat quality depends not only on production factors such breed, genotype, age, diet, growth path or slaughter weight, but technological factors are important too [1]. Genotypes have an important relation with meat quality traits and are greatly influence by a variety of environmental factors [2]. The level of intramuscular fat content and fatty acid composition is among the factors determining meat palatability and consumers satisfaction [3]. Muscle lipid characteristics determine meat flavour, lipid oxidation and contribute to beef colour and affect the juiciness and tenderness of meat [1]. The intramuscular fat composition affects two important characteristics, i.e. healthfulness and palatability of meat. Red meat has relatively high concentration of saturated fatty acids and low concentration of beneficial polyunsaturated fatty acids [2].

Numerous single nucleotide polymorphism (SNPs) have been identified from the bovine genome-sequencing project. SNPs within genes cause variations and probably are responsible for variations in the phenotype. Different mutations in different genes have been associated with different carcass traits, which defined the meat quality in bovines.

Dunner & colleagues [2] describe a large panel of candidate genes involved in adipogenesis, lipid metabolism and energy homoeostasis in 15 European breeds. They found that SNPs in matrix metalloprotease -1 (MMP-1) have effects on different lipid traits, specifically on docosahexaenoic acid (22:6 n-3) and conjugated linoleic acid. Meissburger et al., [4] consider that MMP-1 plays a key role in adipogenesis, stimulating tissue remodeling during adipose tissue expansion in obesity. The principal function of MMP-1 is as an extracellular collagenase. Studies have shown that adipocytes and fibroblasts in muscle share common progenitor cells located in the evolving extracellular matrix of muscle fibers Uezumi, et al. [5]. During embryonic muscle growth, adipogenesis and fibrogenesis may be considered as a competitive process Du et al., [6] ; Miao et al., [7] and the balance between the differentiations of mesodermal progenitor cells into fibroblasts or adipocytes is partly under the control of MMPs [8]. Considering this influence of the MMPs in the differentiation of the progenitor cells, SNPs in the MMPs probably cause variation in the function of the enzyme and affect the fatty acid profile and composition.

The aim of this research is to determine whether the SNP ss77831914 and the ss77831924 in MMP-1 have effects on the development of fatty acids in beef meat, especially in relation with the synthesis of long-chain polyunsaturated fatty acids (LCPUFAs) derived from n-6 fatty acid linoleic acid (LA, 18:2n-6) and n-3 alpha-linolenic acid (ALA, 18:3n-3) and of conjugate linoleic acid (CLA) especially isomer cis-9,trans-11 (CLA9c,11t).

II. MATERIALS AND METHODS

Animals and diets - Thirty Angus cattle from the same herd were grown on a grazing system with free access to pasture mainly composed of Festuca, white clover and red clover. Target live weight for slaughter was circa 300 kg. Animals were randomly assigned to three dietary treatments defined by duration of background on grazing and time of finishing on a high concentrate diet:

- Group 1 (n=10) diet 1: Animals were finished for 49 days on a concentrate diet, without grass. The final average weight reached was 352 kg (±17.4); 16 month of age.
- Group 2 (n=10) diet 2: Animals were finished for 98 days on a concentrate diet, without grass. The final average weight reached was 299 kg (±14.2); 18 month of age.
- Group 3 (n=10) diet 3: Animals were background by 49 days on grazing system and finished by 49 days on a concentrate diet. The final average weight reached was 293 kg (±14.5); 18 month of age.

The concentrate finishing diet was based on corn grain, 70%DM.

Sample collection - The *Sternomandibularis* muscle was used to provide samples for SNP detection. Samples of this muscle were collected within 1 hour post-mortem, refrigerated for 3 hours at 4 ºC and then frozen at -18°C. For the fatty acids profile, *Longissimus Thoracis* muscle was removed between 9th and–12th ribs twenty-four hour post mortem. The longissimus muscle samples were placed in a cold room (4 ºC) for 24 hours. From each muscle a 2.54-cm-thick steaks were removed. The samples were lyophilized with equipment FreeZone 12 (7960032, LABCONCO, Missouri, USA), pulverized through of a MOULINEX grinder and stored at -25ºC until used for fatty acid analysis.

SNPs in candidate genes - DNA extraction was performed with a DNA-Puriprep-T kit (BioHighway, Argentina) and the yield and purity were measured with a NanoDrop spectrophotometer. The SNPs are identified with the tetra primer ARMS-PCR technique. Two different types of thermocycling were used, according to the polymorphism. For ss77831914 the procedure was incubation for 2 min at 95°C, followed by 35 cycles of 1 min denaturation at 95°C, 1 min annealing at 60°C and 1 min extension at 72°C, and an additional 10 min extension at 72°C at the end of the 35 cycles. On the other hand, for ss77831924 the incubation was for 2 min at 95°C, followed by 35 cycles of 1 min denaturation at 95°C, 1 min annealing at 65°C and 1 min extension at 72°C, and an additional 10 min extension at 72°C at the end of the 35 cycles. A 10µl aliquot of each PCR product was mixed with loading buffer and subjected on horizontal agarose gel (2%) electrophoresis. The gel was stained with SYBR Safe DNA and visualized by UV light.

Fatty acid profile - Fatty acid methyl esters from lyophilized *Longissimus dorsi* muscle samples were obtained by direct trans-methylation according to the method of Park and Goins [13]. Fatty acid methyl esters were analysed using a CLARUS 500 (PerkinElmer, Shelton U.S.A) gas chromatograph equipped with a MO61-LF-517 (SLO-SYN) automatic sampler. Separations were accomplished using a 100-m CP7420 (Varian, USA) capillary column (0.25-mm i.d. and 0.28 µm film thickness). The column oven temperature was held at 150ºC for 1 min, increased from 150ºC to 174ºC at 2ºC per min, from 174 ºC to 178ºC at 0.2ºC per min, from 178ºC to 225 at 2 ºC per min, and then held at 225ºC for 6.50 min. The injector and detector were maintained at 250°C and 275ºC respectively. The total run time was 63 min. Sample injection volume was 1 µl with nitrogen as the carrier gas at a flow rate of 1 mL per min. Identifications of sample fatty acids were made by comparing the relative retention times of standard fatty acid methyl-esters (Matreya, Pleasant Gap, PA). The FA were quantified by incorporating an internal standard, methyl Tricosanoic acid (C23:0) into each sample during methylation and expressed as a percentage of total fatty acid. Fatty acids with concentrations lower than 0.5 % was considered minor. Total lipids from *Longissimus dorsis* were extracted by the Bligh and Dyer procedure [14] and expressed as mg/100g of wet tissue.

III. RESULTS AND DISCUSSION

SNPs in MMP-1 The allele frequency and genotypic frequency for the two SNPs analyzed were different. For ss77831914, the allele frequencies are: f(C): p=0.25 and f(G): q=0.75 whereas the genotypic frequencies are f(CC): 0.5; f(CG): 0.5; f(GG):0.0. In the case of ss77831924, the allele frequencies are: f(C): p= 0,467 and f(T): q=0,533 whereas the genotypic frequencies are f(CC): 0,033; f(CT): 0,867; f(TT):0.1.

Fatty acid content and profile The total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) did not present differences between animals with or without the SNP ss77831914 or ss77831924. Individual FA analysis did not show significant differences between animals, which may partly be due to the relatively small number of animals studied. As shown in figure 1, there were significant variations in the total amount of fatty acids in the longissimus muscle between animals of different diets, with fat content being lowest on diet 1.

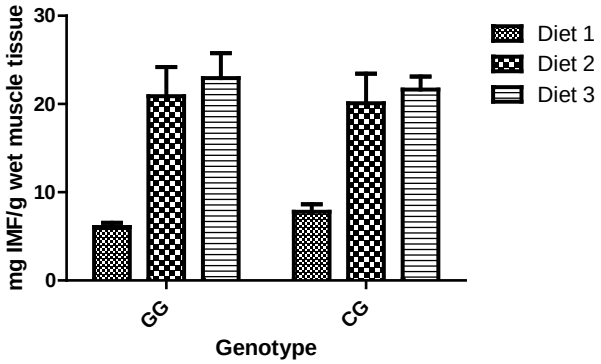

Figure 1. Fat content (mg/100 of wet muscle tissue) in animals with the GG genotype (left) and CG genotype (right) of SNP ss77831914, divided by diet group.

It is interesting to note that animals on diet 1, with a short finishing diet and a younger age at slaughter time, had higher liveweights and were mainly heterozygote C/G(ss77831914) and C/T (ss77831924). The fatty acid profile was not analysed in relation to the SNP ss77831924 as the allele frequency in this SNP was very small.

Table 1 shows the amount of fatty acids, the fat content and the percentage of moisture in longissimus muscles of all diet groups of animals separated by animals that are heterocygote (CG) versus homozygote (GG) for the ss77831914 SNP. Higher amounts of fatty acids/100mg of fat and higher fat contents are observed in homocygotes animals(C allele). However, as shown in figure 1, this effect is insignificant compared with the effect of diet.

Table 1. Miligrams of fatty acids, fat content and moisture according the samples are heterogygote or homocygote allele G.

	ss77831914-Heterocygte C/G	ss77831914-Homocygote allele G
mg FA/100g tissue (w.b.)	2179 ± 814 (235)	3250 ± 1732 (462)
Fat content (w.b.)	13,68 ± 7,80 (2,25)	19,61 ± 8,87 (2,37)
Moisture (%)	74,0 ± 0,9 (0,3)	72,8 ± 1,38 (0,37)

*All the results are average of mg/ g wet tissue (w.b.), standard deviation and numbers in parentheses represent SE.

IV. CONCLUSION

Whereas Dunner and colleagues [2] found a positive correlation between six SNPs in the MMP-1 in 15 European Bos Taurus breeds and the amount of fatty acids in muscle, the current research presents a slightly different picture.
Dunner and colleagues found that possession of the C allele of the SNP ss77831914 alters the fatty acid profile, as does the C allele in SNP ss77831924 also. Dunner et al. [2] reported an association between the snip ss77831914 and the amount of CLA. For SNP ss77831924 in 3´UTR that affects docosahexaenoic acid (22:6 n-3), the C allele is associated with an increase in the amount of this beneficial n-3 in muscle by 14%. In our analysis, the allele frequency of the allele C was not the highest and this allele could not be associated with variations any fatty acid. This study also shows that in

a group of animals sufficiently large to show a strong effect of diet on fatty acid composition and fat content, the contribution of these SNPs to variation in fatty acid composition is negligible in comparison to the effect of diet.

ACKNOWLEDGEMENTS

The support of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) to SC is gratefully acknowledged. This work was also supported by Fondo para la Investigación Científica y Tecnológica (FONCyT) grant no. PICT 2013-3292.

REFERENCES

1. Bernard C., Cassar-Malek I., Le Cunff M., Dubroeucq H., Renand G.& Hocquette J.F. (2007) New indicators of beef sensory quality revealed by expression of specific genes. Journal of Agricultural and Food Chemistry 55, 5229–37.
2. Dunner, S., Sevane, N., Garcia, D., O´Cortés, A., Valentini, A., Williams, J.L., Mangin, B., Cañón, J., Leveziel, H., and the GeMQual Consortium. (2013). Association of genes involved in carcass and meat quality traits in 15 European bovine breeds. Livestock Science 154: 34-44.
3. Lee S.H., Park E.W., Cho Y.M. et al. (2007). Identification of differentially expressed genes related to intramuscular fat development in the early and late fattening stages of Hanwoo steers. Journal of Biochemistry and Molecular Biology 40, 757–64.
4. Meissburger B., Stachorski L., R€oder E., Rudofsky G. & Wolfrum C. (2011) Tissue inhibitor of matrix metalloproteinase 1 (TIMP1) controls adipogenesis in obesity in mice and in humans. Diabetologia 54, 1468–79.
5. Uezumi, A., Ito, T., Morikawa, D., Shimizu, N., Yoneda, T., Segawa, M., et al., (2011). Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. Journal of cell science 124(Pt21):3654-64.
6. Du, M., Huang, Y., Das, A. K., Yang, Q., Duarte, M. S., Dodson, M. V., & Zhu, M. J. (2010). Meat Science and Muscle Biology Symposium: Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. Journal of Animal Science, 91, 1419–1427.
7. Miao, Z. G., Zhang, L. P., Fu, X., Yang, Q. Y., Zhu, M. J., Dodson, M. V., & Du, M. (2015). Invited review: Mesenchymal progenitor cells in intramuscular connective tissue development animal. Animal, 10(01), 75–81 in press.
8. Christensen, S., Purslow, P. (2016). The role of matrix metalloproteinases in muscle and adipose tissue development and meat quality: A review. Meat Science 119 138–146.
9. Dunner, S., Sevane, N., Garcia, D., Leveziel, H., Williams, J.L., Mangin, B., Valentini, A., GemQual Consortium (2013). Genes involved in muscle lipid composition in 15 European *Bos Taurus* breeds. Animal Genetics, doi: 10.1111/age.12044.
10. Barendse, W.J. (2002). DNA markers for meat tenderness. Patent application WO02064820.
11. Buchanan, F.C., Fitzsimmons, C.J., Van Kessel, A.G., Thue, T.D., Winkelman-Sim, D.C., Schmutz, S.M., 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet. Sel. Evol. 34, 105–116.