GENETIC MARKER EFFECTS FOR QUALITY TRAITS IN COMMERCIAL BEEF FROM MEXICO

Alfaro S[1], Rubio LMS[1]*, Parra M[2], Méndez MD[1], Perez LC[3], Figueroa SF[3], Sánchez EA[4], Torrescano G[4], Ríos RFG[5], Braña VD[6], Sifuentes A[2], Arellano W[2], Macedo, REF[7], Jimenez P[1]

[1] Laboratorio de Ciencia de la Carne, Facultad de Medicina Veterinaria y Zootecnia (FMVZ), Universidad Nacional Autónoma de México, Av. Universidad 3000, Del. Coyoacán, Ciudad Universitaria, México, D.F. 04510, México, [2] Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa, Tamaulipas, México. [3]IICV-Universidad Autónoma de Baja California, Mexicali, México, [4]Centro de Investigación en Alimentos y Desarrollo (CIAD, AC), Hermosillo, Sonora, México, [5]FMVZ-Universidad Autónoma de Sinaloa, Culiacán, México, [6]CENID-Fisiología, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. Ajuchitlán, Querétaro, México. [7]Agricultural Sciences and Veterinary Medicine School, Pontifical Catholic University of Paraná, Paraná, Brasil, * Corresponding author e-mail: msalud@unam.mx.

Abstract - The aim of this study was to determine the genotype, allele frequencies and the effect of single nucleotide polymorphisms (SNPs) in genes CAPN, CAST and TG on the quality traits of Mexican commercial beef cuts. *Longissimus dorsi* samples (n=171) were collected and aged for 14 days before Warner Bratzler shear force (WBSF) and cooking loss (CL) were performed. Markers were analyzed by RT-PCR and RFLP-PCR. Marker effect was evaluated by using a general linear model, additionally allelic substitution effect (*α*) and markers dominance deviation (d) were estimated. CAPN4751 marker showed the highest allele frequencies for favorable alleles (C) and an additive allele effect ($\alpha_{(T> C)}$= -0,368 kg) on WBSF values (P=0.05). Dominant deviation in CAPN4751 was -0,210 kg. Presence of at least one T allele in TG5 marker could improve marbling in beef, since their genotypes showed an additive trend (P=0.07) on CL. Identification and selection of favorable alleles in quality traits of economic importance in Mexican beef may improve its quality and increase its commercial value.

Key Words – Mexican beef, molecular markers, quality improvement.

I. INTRODUCTION

In the last years, the meat industry in Mexico has experienced sustained growth by the increasing demand for beef. However, the national beef production has not achieved self-sufficiency, which leads to the importation of meat products to support the domestic demand [1].

In developed countries the increase in meat production and the improvement in quality is the result of cattle genetic improvement. This improvement is a result of the combination of animal productive testing and the implementation of molecular techniques that use genetic markers for the selection of animals with favorable allelic variants associated with quantitative traits (QTL's). These traits influence meat quality, in particular, tenderness and marbling [2, 3].

Meat tenderness is one of the main traits that affect beef consumer's satisfaction. Beef tenderness has been associated with some SNP markers in CAPN and CAST (an inhibitor of the calpain system) genes [4,5,6]. Furthermore, other genes such as LEP and TG promote intramuscular fat deposition. In those genes, polymorphisms with effect on meat tenderness and marbling have already been identified and validated to be use to predict beef quality [7,8].

In Mexico, there is evidence on the significant associations of CAPN 316 and TG5 markers with WBSF and fat deposition, respectively, in commercial beef [9].

So, the aim of this study was to estimate the allele and genotype frequencies in markers CAPN316, CAPN4751, CAST T1 and TG5 and estimate their association with some beef quality traits.

II. MATERIALS AND METHODS

Longissimus dorsi beef samples collected from Federal Inspection Type 171 (TIF) slaughterhouses located in northern, central and southern in Mexico were analyzed. The samples were aged during 14 days under refrigeration (4 °C) and further subjected to Warner Bratzler shear force (WBSF) and cooking loss as described by AMSA [10].

DNA was isolated from 40 g of lean meat, free of connective tissue, using the Genelute Mammalian Genomic DNA kit. Analysis of the TG5 marker

was carried out using the PCR-RFLP assay as reported by Barendse et al. [7]. CAPN316 and CAPN4751 genotyping were performed by allelic discrimination assays under the conditions reported by Bonilla et al. [9] and Parra et at. [11]. CAST T1 was determined after Van Eenennaam et al. [2].

Association analysis

The variables were analyzed using a general linear model: $Y_{ij} = \mu + G_i + GG_j + \varepsilon_{ij}$, where: Y = the dependent variables WBSF or CL, G_i = effect of genotype in CAPN316, CAPN4751, CASTD T1 or TG5, GG_J= Genetic group, and ε_{ij}= random error.

Afterward, genotypes with significant effect markers ($p<0.10$) (CAPN4751 y TG5) were recoded with dummy variables: 0 (normal homozygote), 1 (heterozygote) and 2 (favorable homozygote), for the determination, by regression analysis, of the allelic substitution effect (α) and the dominance deviation (d). The allelic substitution (α) estimates the effect of replacing the normal allele by the favorable allele on a characteristic. The dominance deviation is estimated as the difference between the predicted value and the estimated one for the heterozygous genotype in each marker [12]. Analysis was performed using the statistical package MINITAB14.

III. RESULTS AND DISCUSSION

Table 1 shows the allele and genotype frequencies of the markers analyzed.

Table 1. Genotypic and allelic frequencies of CAPN316, CAPN4751, CAST T1 and TG5 markers in Mexican beef

Marker	Genotype	N	Genotypic frequency	Allelic frequency	HW
CAPN316	CC	4	0.03	C= 0.13	NS
	CG	31	0.21	G= 0.87	
	GG	113	0.76		
CAPN4751	CC	23	0.14	C= 0.35	NS
	CT	68	0.41	T= 0.65	
	TT	73	0.45		
CAST T1	AA	88	0.54	A=0.73	NS
	GA	62	0.38	G=0.27	
	GG	14	0.08		
TG5	CC	138	0.86	T= 0.09	**
	CT	14	0.09	C= 0.91	
	TT	8	0.05		

HW (Hardy-Weinberg), ** P<0.05; NS = P>0.05

Allele frequencies were 0.13 for C and 0.87 for G in CAPN316. Van Eenennaam et al. [2] reported a frequency of 0.18 for allele C in U.S. *Brangus*, which was slightly higher than the frequency found in this study. In fact, it is shown a tendency of higher frequency of G allele in commercial bovine breeds, which is related to a lower favorable effect on meat tenderness [13] and it is probably due to the limited genetic selection of the cattle for this meat quality attribute.

Since high frequency of G allele in CAPN316 was estimated in Zebu and crossbred cattle [4], CAPN4751 marker was designed to discriminate differences in tenderness among bovine breeds and their crosses, including those with Zebu influence. In this study a high frequency of heterozygous genotype (CT) (0.41) and frequency of 0.35 for C allele was found in CAPN4751. Pinto et al. [13] reported genotypic and allelic frequencies of 0.18 and 0.32, respectively for C in CAPN4751 in Brazilian Nellore cattle. In Mexican commercial cattle Bonilla et al. [9] found higher frequency (0.49) of the favorable allele C in CAPN4751. This difference in allele frequencies in Mexican cattle could be related to the variability of beef origins across the country and the limited genetic flow directed to quality trait selection in their original populations.

Least squares means, the effect of allelic substitution and dominant deviations for CAPN4751 marker on beef WBSF is shown in Table 2.

The CC genotype showed a significant lower WBSF (P= 0.05) compared to the CT and TT genotypes. The effect of replacing T allele for C allele indicates decrease of 0.368 kg in WBSF of beef aged during 14 days *postmortem*.

Table 2. Least squares means, allelic substitution effect (α) and dominance deviation (d) of CAPN4751 on WBSF (kg/cm^2) of Mexican beef

Genotype	n	WBSF (Kg/cm^2) $\bar{X}\pm$ S.E.	α T>C	d	P value
TT	70	$6.48^{b}\pm0.25$	-0.368±0.21	-0.21	0.05
CT	65	$6.38^{b}\pm0.29$			
CC	23	$5.32^{a}\pm0.41$			

X = average; S.E. = standard error. Values with different superscript letters are significantly different (P<0.10).

CAPN4751 showed important dominant deviation effect; however, it was not higher than α value. White et al. [5] reported CAPN4751 dominance deviations on WBSF in Brahman, *Bos taurus* and *Bos taurus-Bos indicus* beef aged during 14 days, with values of -0.40±0.15, -0.27±0.10 and -0.44±0.10kg, respectively. The replacement of T allele for C allele decreased WBSF by 4 kg. A decrease in WBSF by the replacement of T allele was also found in this study. Pinto et al. [13] also found association of CAPN4751 with lower WBSF in Nellore meat aged during 14 days and reported an additive effect of the favorable allele C of -0.27±0.09 kg and a dominant deviation of -0.28±0.11 ($P<0.05$). This trend suggests that the presence of CAPN4751 marker is significantly associated with the decrease of WBSF in beef; and explains tenderness variation in meat of different bovine subspecies.

Table 3 shows the effect of TG5 on the beef cooking loss. The TT genotype had higher cooking loss (32.97±2.66%) than CC and CT genotypes (27.15± 0.83% and 29.43 ± 2.16%, respectively). Since T allele is associated with the increase of intramuscular fat (IMF) deposition in the *Longissimuss dorsi* muscle, higher cooking loss might be due to the dripping of melted IMF along with meat juices during the cooking process [14].

The α allelic substitution effect shown in Table 3 indicates that animals showing at least one copy of the favorable T allele will have a CL increase of 2.73% due to the higher IMF deposition.

Table 3. Least squares means, allelic substitution effect (α) and dominance deviation (d) of TG5 on the cooking loss (%) of Mexican beef.

Genotype	n	CL (%) $\bar{X}$±S.E.	α C>T	d	P value
CC	145	27.15^{b}± 0.83	2.73±1.17	0.40	0.07
CT	14	29.43^{b} ± 2.16			
TT	8	32.97^{a} ± 2.66			

CL= cooking loss. X = average; S.E. = standard error.
Values with different superscripts letters are significantly different ($P<0.10$).

Bonilla et al. [9] analyzed the frequencies of this single nucleotide polymorphism (SNP) in Mexican commercial cattle populations and determined the association of this marker with meat marbling. Those authors found that T allele significantly increased the IMF by 4.4 to 6.6 %.

In a previous study reported by Casas et al. [15] evaluating *Wagyu* breed, TT homozygotes (*Wagyu)* animals had higher degree of marbling than those with TC and CC genotypes, suggesting that the functional variation of this allele is predominant in the *Wagyu* breed and occurs in lower frequencies in the other bovine breeds.

IV. CONCLUSION

CAPN4751 and TG5 markers have a significant association to WBSF and CL, respectively. The use of CAPN4751 for the selection of favorable genes in Mexican commercial cattle might be an efficient tool to the improvement of tenderness in beef produced in Mexico. Relationship showed by TG5 with intramuscular fat, measured indirectly through the CL, showed that those animals with at least one T allele may have higher marbling. The application of genetic marker selection to the identification and the selection of alleles that improve meat tenderness and marbling in combination with breeding programs could lead to an increase in the commercial value of the Mexican beef.

ACKNOWLEDGEMENTS

Authors thank SAGARPA-CONACYT for financial support through project number 109127.

REFERENCES

1. FAS/USDA. 2010. Livestock and Poultry: World Markets and Trade. United States Department of Agriculture, Foreign Agriculture Service. October. 31 p.
2. Van Eenennaam, A.L., Li, J., Thallman, R.M., Quas, R.L., Dikeman, M.E., Gill, C.A., Franke, D.E., & Thomas, M.G. (2007). Validation of commercial DNA tests for quantitative beef quality traits. Journal of Animal Science 85: 891-900.
3. Goddard, M.E. (2009). How can we best use DNA data in the solution of cattle? Proceedings of the Beef Improvement Federation 41st Annual Research Simposium. p. 81-88.
4. Casas, E., White, S.N, Wheeler, T.L, Shackelford, S.D, Koohmaraie, M, Riley, D.G, Chase, Jr.C., Johnson, D.D, y Smith, T.P.L. (2006). Effects of *calpastatin* and μ-*calpain* markers in beef cattle on tenderness traits. Journal of Animal Science 84:520–525.

5. White, S. N., Casas, E., Wheeler T. L., Shackelford S. D., Koohmaraie M., Riley D. G., Chase, Jr C., Johnson D. D., Keele J. W., y Smith T P L. (2005). A new SNP in *CAPN1* is associated with tenderness in cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. Journal of Animal Science 83: 2001-2008.
6. Page B T, Casas E, Quaas R L, Thallman R M, Wheeler T L, Shackelford S D, Koohmaraie M, White, S.N, Bennett, G.L, Keele, J.W, Dikeman, M.E & Smith, T.P.L. (2004). Association of markers in the bovine *CAPN1* gene with meat tenderness in large crossbred populations that sample influential industry sires. Journal of Animal Science 82: 3474–3481.
7. Barendse, W., Bunch, R., Thomas, M., Armitage, S., Baud, S., Donaldson, N. (2004). The TG5 thyroglobulin gene test for a marbling quantitative trait loci evaluated in feedlot cattle. Austalian Journal of Experimental Agriculture 44: 669-674.
8. Kononoff, P.J., Deobald, H.M., Stewart, E.L., Laycock, A.D., Marquess, F.L.S., (2005). The effect of a leptin single nucleotide polymorphism on quality grade, yield grade and carcass weight of beef cattle. Journal of Animal Science 83: 927-932.
9. Bonilla, C.A., Rubio, M.S., Sifuentes, A.M., Parra-Bracamonte, G.M., Arellano, V.W., Méndez, M.R.D., Berruecos, J.M., Ortíz, R. (2010). Association of CAPN1 316, CAPN1 4751 and TG5 markers with Mexican bovine meat quality traits. Genetics and Molecular Research 9 (4): 2395-2405.
10. AMSA (1995). Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. Chicago, IL: American Meat Science Association & National Livestock and Meat Board.
11. Parra-Bracamonte, G.M., Sifuentes-Rincón, A.M., Martínez-Gonzalez, J.C., Cienfuegos-Rivas, E., Tewolde, A. (2007). Polimorfismo en el gen de la μ-Calpaína en ganado Brahman de registro de México. Archivos Latinoamericanos de Producción Animal 15 (1): 3474-3481.
12. Lynch M. and Walsh B. (1998). Genetics and Analysis of Quantitative Traits. Sinauer Associates Inc. 980p.
13. Pinto, L.F.B., Ferraz, J.B.S., Meirelles, F.V., Eler, J.P., Rezende, F.M., Carvalho, M.E., Almeida, H.B., Silva, R.C.G. (2010). Association of SNPs on CAPN1 and CAST genes with tenderness in Nelore cattle. Genetics and Molecular Research 9 (3): 1431-1442.
14. Delgado E. J., Rubio M. S., Iturbe, F. A., Méndez R. D., Cassís L., Rosiles R (2005). Composition and quality of Mexican and imported retail beef in Mexico. Meat Science 69: 465-471.
15. Casas E., White S.N., Shackelford S.D., Wheeler T.L., Koohmaraie M., Bennett G.L. y Smith T.P.L. (2007). Assessing association of single nucleotide polymorphisms at the thyroglobulin gene with carcass cattle. J Anim Sci 85: 2807-2814.