

**EFFECTS OF MICROSATELLITES *CSSM066* AND *ILSTS011*, FLANKING
THE *THYROGLOBULIN* GENE, IN FAT DEPOSITION OF
CANCHIM BEEF CATTLE BREED**

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INTRODUCTION

The total amount and distribution of fat have a strong impact on carcass and meat quality in beef cattle (Wheeler *et al.*, 1994; Lozeman *et al.*, 2001). The use of molecular markers linked to Quantitative Trait Loci (QTL) or Candidate Genes related to fat deposition can be of great value in cattle breeding programs, allowing for Marker Assisted Selection (MAS) together with Traditional Selection. Benefits of MAS are larger for characteristics that have low heritability and that are difficult to measure.

The thyroglobulin is a glycoprotein hormone that is synthesized by the thyroid follicular cell and is the precursor for triiodothyronine (T3) and thyroxin (T4). The thyroidean hormones affect carbohydrate metabolism through several ways, including glucose intestinal absorption and insulin-mediated acquisition of glucose by the cells, influencing adipocytes growth and differentiation (Ailhaud *et al.*, 1992). T3 and T4 hormone levels have been associated with marbling deposition in Wagyu cattle (Mears *et al.*, 2001). Based on the knowledge of thyroglobulin role in metabolism regulation it has been pointed as a candidate gene for fat deposition traits in beef cattle.

The *thyroglobulin* gene was mapped in the centromeric region of bovine chromosome 14 (BTA14), 11.95 cM from the most centromeric marker of that chromosome. The polymorphism that occurs in the 5' leader sequence of the *thyroglobulin* gene has been associated with intramuscular fat deposition in long-fed cattle (Barendse, *et al.* 2001). The same polymorphism was associated to fat tickness in a Brahman population (Casas *et al.*, 2005).

In this study the effects of two microsatellite markers, *CSSM066* and *ILSTS011*, on bovine backfat thickness of Canchim breed were analyzed. These markers are located at 5.12 cM (*CSSM066*) and 25.71 cM (*ILSTS011*) in bovine chromosome 14, flanking the *thyroglobulin* gene (11.95cM).

MATERIAL AND METHODS

Canchim breed (5/8 Charolais + 3/8 Zebu) animals, raised in pasture, were evaluated for backfat thickness at 18 months of age. Measures of fat tickness (mm) were obtained from the image analysis using a Piemedical Scanner 200 Vet ultrasound 200 Vet equipment, with a linear array probe 18 cm long and 3,5 MHz. A total of 264 animals, belonging to two farms, the Embrapa Southeast Cattle Research Center, located at São Carlos in São Paulo state, Brazil, and Ipameri farm, located in Goiás state, Brazil, were evaluated.

DNA was extracted from blood samples using a protein salting out method as described by Regitano (2001). The microsatellites *CSSM066* e *ILSTS011* were amplified by polymerase chain reaction (PCR) and analyzed in a capillary Sequencer ABI 3100 Avant (Applied Biosystems). Genotypes were determined using the softwares GeneScan and Genotyper.

An analysis of variance was performed, using the least squares method, with a statistical model that included the effects of genetic group, combined effect of sex and farm of origin and the effect of month of birth. Marker genotypes were nested within genetic group in order to test for microsatellite effect on backfat thickness. Genotypes with few (≤ 4) observations were excluded from the analysis.

RESULTS AND DISCUSSION

For marker *ILSTS011*, a total of 14 genotypes were found in the evaluated population, while 57 genotypes were obtained for *CSSM066* marker. Variance analysis indicated a significant effect ($p < 0.05$) for *CSSM066* microsatellite marker (Table 1). Fat thickness means observed for each genotype, considered in the *CSSM066* analysis, are presented in table 2. For *ILSTS011*, the analysis of variance indicated an effect only at the level of 10% (Table 1). In both models the trait was significantly affected by the month of birth and farm/sex combined effect.

Table 1. Results from analysis of variance considering the effects of markers *ILSTS011* and *CSSM066*

Source of variation	DF	Mean Square (Model 1)	P	Mean Square (Model 2)	P
Genetic group	1	0.0013	0.9525	0.0927	0.6091
Farm/Sex	2	11.9899	<.0001	7.0694	<.0001
Month of birth	3	2.1978	0.0006	1.6704	0.0035
<i>ILSTS011</i> (GG) (Model 1)	14	0.5679	11.9899	-	-
<i>CSSM066</i> (GG) (Model 2)	13	-	-	0.5828	0.0334

Table 2. Means and standard deviation of fat thickness according to *CSSM066* genotype

Genotype	Genetic Group 1		Genetic Group 2	
	Means (mm)	Standard error (mm)	Means (mm)	Standard error (mm)
178/178	0.7493	0.2453	-	-
178/180	1.5117	0.2514	-	-
178/182	0.9620	0.1899	1.2341	0.3615
178/184	0.6664	0.1736	1.4010	0.3543
178/192	0.8340	0.2999	1.8902	0.6072
180/182	0.8644	0.1687	1.3365	0.2820
180/184	1.4949	0.1898	1.3321	0.2791
180/192	1.0599	0.2282	0.6677	0.3543
182/182	0.8131	0.1861	0.4417	0.3561
182/184	0.6113	0.1470	1.0182	0.4323
182/192	1.0512	0.1855	0.8277	0.2792
184/184	0.9341	0.2012	-	-
184/190	0.5201	0.2685	0.3792	0.4290
184/192	1.0536	0.2136	1.0174	0.3182
184/198	0.8809	0.1901	-	-
192/198	1.1817	0.2668	-	-
198/198	1.3527	0.2726	-	-

In the present work, the choice of markers to be analyzed was done based on linkage to the *thyroglobulin* gene (TG). The results are in accordance with a direct effect of TG when one considers the distance between the reported gene and the markers. In this sense, the closer marker, *CSSM066*, 6.82 cM apart from TG, had a more significant effect in fat deposition than *ILSTS011* marker, located 13.76 cM, presenting, therefore, higher recombination rates with *thyroglobulin* gene.

This work corroborates the results of studies such as the works by Casas *et al.*, (2000) who reported a QTL for fat deposition in the centromeric region of chromosome 14 in multiple populations and Moore *et al.* (2003) detected association at the *CSSM66* locus with the backfat EBV, providing further evidence of a QTL for backfat in this region. Barendse (1999) mapped the *thyroglobulin* gene in this QTL region, suggesting that the causative mutation for this QTL could be on this gene. Direct associations between polymorphisms at this gene and fat deposition traits were described later by Barendse *et al.* (2001) and Casas *et al.* (2005).

CONCLUSION

The results obtained suggest that bovine chromosome 14 centromeric region, where the *thyroglobulin* gene is located, may harbor one or more genes for fat deposition in the studied population of Canchim breed. This information could be used as a start point for studies of marker assisted selection.

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