

Variability of *Leptin* gene promoter in cattle

C. Lisa¹, S. Sartore², L. Di Stasio¹

¹ Dipartimento di Scienze Zootecniche. Università di Torino, Italy

² Dipartimento di Produzioni Animali, Epidemiologia e Ecologia. Università di Torino, Italy

Corresponding author: Claudio Lisa. Dipartimento di Scienze Zootecniche. Università di Torino. Via Leonardo da Vinci 44, 10095 Grugliasco, Italy - Tel. +39 011 6708570 - Fax: +39 011 6708563 - Email: claudio.lisa@unito.it

ABSTRACT: A preliminary analysis on the variability of *Leptin* gene promoter in seven cattle breeds was carried out, focusing the attention on the SNP at nt 1759, which has been suggested to affect some quantitative traits in cattle. In addition, the linkage disequilibrium with the C305T mutation in exon 2 of *Leptin* gene was tested. The results indicate that the *Leptin* gene promoter is polymorphic in all the analysed breeds, with significant between-breed differences. Pairwise comparison of genotypes at the two considered SNPs revealed a significant linkage disequilibrium, with the presence of the haplotypes 1759^C - 305^T and 1759^G - 305^C.

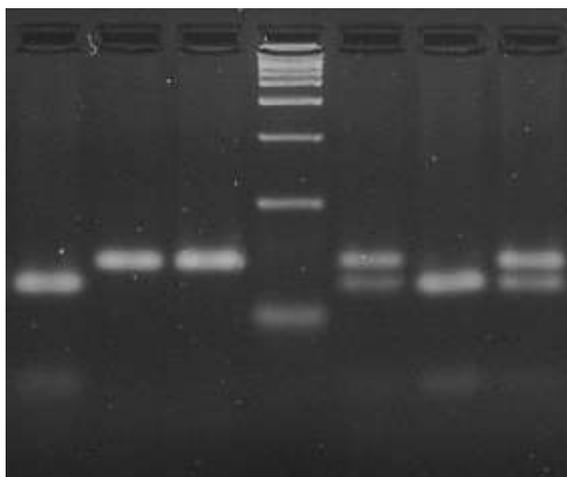
Key words: Leptin, Promoter, SNP, Cattle.

INTRODUCTION – Leptin is a protein hormone with important effects in regulating body weight, metabolism and reproduction. Several mutations in *Leptin* gene have been found in cattle, both in introns and exons, and relationships between some of the mutations and meat production have been reported (Buchanan *et al.*, 2002; Lagonigro *et al.*, 2003; Schenkel *et al.*, 2005). More recently the interest has been focused on the gene promoter, in order to better understand the genetic mechanism underlying the variability of gene expression, for the possible effect on the phenotype. The bovine *Leptin* promoter, which has been sequenced (Taniguchi *et al.*, 2002), showed a high variability, with at least 20 mutations identified up to now in a region of about 1.6 kb (Liefers *et al.*, 2005). This investigation was aimed at studying the variability at position 1759 in the promoter, which has been suggested to affect leptin concentration, growth, feed intake and carcass merit in cattle (Nkrumah *et al.*, 2005).

MATERIAL AND METHODS – The bovine *Leptin* promoter polymorphism was analysed in beef breeds (Blonde d'Aquitaine, BA: 58; Belgian Blue, BB: 17; Piemontese, PI: 39) and dairy breeds (Italian Friesian, IF: 41; Aosta Red Pied, ARP: 41; Aosta Black Pied, ABP: 20), as well as in 45 samples of African Zebu cattle (ZE). For European breeds, individuals as unrelated as possible were sampled; for Zebu cattle, samples were collected from different herds, in order to prevent the sampling of closely related animals. DNA was extracted from blood using ISOCODE™STIX (Schleicher and Schuell, Germany). As the C1759G mutation in the promoter does not alter any restriction enzyme recognition site, we set up a PCR-RFLP method designing the right primer with a mismatch (C for G at position 1763 of the published sequence, GenBank Accession No. AB070368), which creates a recognition site for *HinfI* in the G allele. The primer sequences were: forward ⁵GCTTAAAGATTCCTGATCCTAC³ and reverse ⁵GTGTTTCTGATCACACAGATT³. The PCR reaction (25 µl) contained 1.5 mM MgCl₂ and the amplification was carried out with an annealing temperature of 55°C. The amplicons were restricted with *HinfI* enzyme and the digested fragments were electrophoresed on 3% agarose gel, stained with ethidium bromide and visualised under UV light. In addition, the samples were analyzed for C305T polymorphism in exon 2 of *Leptin* gene (GenBank Accession No. AJ236854) according to Buchanan *et al.* (2002), in order to test for linkage disequilibrium with the 1759 SNP in the promoter. The allele frequencies at 1759 SNP in each breed, the deviations from Hardy-Weinberg equilibrium, the genotypic differentiation for each population pair, as well as the linkage disequilibrium between the two analysed SNPs were examined using the *Genepop* software (Raymond and Rousset, 1995).

RESULTS AND CONCLUSIONS – The PCR-RFLP method set up for the 1759 SNP genotyping gave good results: the amplification originated products of the expected size (95 bp, extending from nt 1687 to nt 1781); the *G* allele, presenting the restriction sequence, yielded two fragments of 73 and 22 bp, while the *C* allele remained undigested (Fig.1).

Fig 1. Electrophoretic pattern of *Leptin* promoter polymorphism. From the left: GG, CC, CC, size marker (2000, 1500, 1000, 750, 500, 300,150, 50), CG, GG, CG.



The polymorphism was observed in all the analysed breeds (Table 1), with the general predominance of the *G* allele (0.56 [I 0.88), except for Blonde d'Aquitaine breed. A lower variability had been reported by Nkrumah *et al.* (2005) in a hybrid experimental population and in six different lines of a commercial population, where the *G* allele had frequencies ranging from 0.32 to 0.59.

Table 1. Allele frequencies at nt 1759 of *Leptin* gene promoter.

Breed	n.	allele	
		C	G
Aosta Black Pied	20	0.25	0.75
Aosta Red Pied	41	0.35	0.65
Belgian Blue	17	0.12	0.88
Blonde d'Aquitaine	58	0.59	0.41
Italian Friesian	41	0.44	0.56
Piemontese	39	0.18	0.82
Zebu	45	0.42	0.58

The genotypic distributions were in agreement with the Hardy-Weinberg equilibrium in all the populations. The analysis of the genotypic differentiation for each population pair showed significant differences in 12 cases out of 21 (Table 2).

Table 2. Genotypic differentiation for breed pairs: significant P values.

	ABP	ARP	BB	BA	IF	PI	ZE
ABP	-						
ARP	n	-					
BB	ns	0.017	-				
BA	0.000	0.001	0.000	-			
IF	ns	ns	0.002	0.048	-		
PI	ns	0.026	ns	0.000	0.000	-	
ZE	ns	ns	0.002	0.022	ns	0.002	-

Italian Friesian, Aosta Red Pied and Zebu were all significantly different from beef breeds and not from dairy breeds. Blonde d'Aquitaine was the only one that significantly differed from all the other breeds. As the positions 1759 in the promoter and 305 in exon 2 of *Leptin* gene are quite close, we considered it important to verify the existence of linkage disequilibrium between the two SNPs, because it could lead to a misinterpretation of the results when studying the relationships with production traits. The pairwise comparison of genotypes at the two SNPs revealed a highly significant linkage disequilibrium ($P = 0.000$), with the presence of the haplotypes 1759^C-305^T and 1759^G-305^C. This finding is consistent with results previously reported; for example, Nkrumah *et al.* (2005) observed a positive effect of 1759^G allele on average daily gain and Di Stasio *et al.* (2007) a positive effect of 305^C allele on the same trait. The existence of such a tight linkage suggests that the effect of the allele at one SNP may be confounded with that of the linked allele. Investigations on *Leptin* gene expression are in progress, in order to discriminate the effects of the two SNPs.

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