

## Association between molecular markers linked to the Leptin gene and weight gain in postpartum beef cows

### Associação entre marcadores moleculares ligados ao gene da Leptina e ganho de peso em vacas de corte no pós-parto

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#### ABSTRACT

Three short tandem repeats (STRs), BMS1074, BM1500, IDVGA-51, and three single nucleotide polymorphisms (SNPs), LEPSau3AI (A/B), LEPSau3AI (+/-) and LEPKpn2I linked to the LEP gene were investigated to verify associations with productive performance in postpartum cows of two beef cattle breeds, Aberdeen Angus (AA, n=98) and Charolais (C, n=83). After polymerase chain reaction, STRs were analyzed by vertical electrophoresis and SNPs in agarose gel after endonucleases cleavage. In AA herd 79% of BMS1074\*151 carriers had a lower average daily weight gain (ADG) when compared with the population mean daily weight gain (103g), while 62% of BMS1074\*151 non-carriers presented a higher ADG ( $P<0.01$ ); AA animals with at least one BMS1074\*151 allele showed a ADG about 159g lower than that of other animals ( $P<0.01$ ). In both herds, carriers of the BM1500\*136 allele presented higher ADG (about 75g day<sup>-1</sup> higher in AA,  $P<0.05$ , and 96g day<sup>-1</sup> in C,  $P<0.10$ ); animals with one BM1500\*136 allele had about a 3-fold higher chance of having a higher ADG than non-carriers, in both populations.

**Key words:** Leptin, STRs, SNPs, beef cattle, weight gain, postpartum.

#### RESUMO

Foram investigadas três repetições curtas em tandem (STRs), BMS1074, BM1500 e IDVGA-51 e três polimorfismos de um único nucleotídeo (SNPs) LEPSau3AI (A/B), LEPSau3AI (+/-) e LEPKpn2I, ligados ao gene da leptina, verificando-se associações com o desempenho produtivo em vacas no pós-parto, em dois rebanhos de gado de corte, Aberdeen Angus (AA, n=98) e Charolês (C, n=83). Após a reação em cadeia da polimerase, os STRs foram

analisados em géis de poliacrilamida e os SNPs em gel de agarose, após a clivagem com endonucleases. Na raça AA, 79% dos portadores do alelo BMS1074\*151 apresentaram ganho médio de peso diário (ADG) menor, quando comparados com a média da população (103g), enquanto 62% dos não-portadores mostraram ADG mais alto ( $P<0,01$ ); os animais AA com pelo menos um alelo BMS1074\*151 possuem ADG cerca de 159g menor que os outros animais ( $P<0,01$ ). Em ambos os rebanhos, portadores do alelo BM1500\*136 apresentaram ADG mais alto (em torno de 75g dia<sup>-1</sup> em AA,  $P<0,05$  e 96g dia<sup>-1</sup> em C,  $P<0,10$ ) e animais com um alelo BM1500\*136 possuem cerca de três vezes mais chance de ter um ADG maior que os não-portadores.

**Palavras-chave:** Leptina, STRs, SNPs, gado de corte, ganho de peso, pós-parto.

#### INTRODUCTION

Leptin is an important regulator of energy metabolism, adiposity and reproduction. It is also involved in the regulation of body weight and can be considered as one of the best biological markers reflecting body fatness in both animals and humans (OPRZADEK et al., 2003; MÜNZBERG et al., 2005).

In ruminants, as in other species, leptin is predominantly secreted by adipocytes and has a primary effect on the hypothalamus, but it is also produced by non adipocyte cells and targets extra hypothalamic tissues. Plasma leptin levels in cattle and sheep increase linearly with increasing body fat

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mass and with increasing energy balance and seem to be an important component of a feedback loop involving key metabolic regulators that include insulin, glucocorticoids and sympathetic nervous system (DELAUVAUD et al., 2002). Leptin concentrations undergo dynamic changes during pregnancy and lactation (BARTHA et al., 2005); the levels decline toward parturition and remain low for about three months after calving despite the increase energy balance which occurs during postpartum (LIEFERS et al., 2005).

The leptin (LEP) gene is highly conserved among mammals, and bovine leptin gene shares over 89% similarities with their human and murine counterparts (JI et al., 1998). The bovine leptin gene has been mapped to chromosome 4 (STONE et al., 1996; POMP et al., 1997), it is composed of three exons, spanning about 18.9kb, equivalent to that of human and mouse gene. The exon-intron organization was perfectly conserved between bovine and human or mouse genes, and the sizes of the first and second introns are about 14 and 1.7kb, respectively (TANIGUCHI et al., 2002).

Polymorphisms linked to the leptin gene could influence gene regulation and affect the weight gain metabolism. In Holstein-Friesian dairy cows some mutations are associated with milk yield, feed intake and plasma leptin concentrations during pregnancy but not during lactation (LIEFERS et al., 2005).

This paper was aimed at analyzing the effect of six polymorphisms, BMS1074, BM1500 and IDVGA-51 STRs (short tandem repeats) and LEPSau3AI (A/B), LEPSau3AI (+/-), and LEPKpn2I SNPs (single nucleotide polymorphisms) linked to LEP gene on the average daily weight gain and reproductive performance in postpartum cows of two beef cattle herds, Aberdeen Angus and Charolais. The two SNPs and the BM1500 STR are mapped within the LEP gene, which is at 83cM from the beginning of BTA 4 chromosome: LEPSau3AI is at the intron 2, LEPKpn2I is at the exon 2 and BM1500 is at 2.6Kb downstream the stop codon. The BMS1074 and IDVGA51 STRs are at 75cM and 84cM, respectively, from the beginning of BTA 4 chromosome (KAPPES et al., 1997).

## MATERIALS AND METHODS

Blood samples were obtained from 181, Aberdeen Angus (AA, n=98) and Charolais (C, n=83), multiparous cows, in the postpartum period. The animals came from a previous study which compared the efficiency of different hormonal treatments associated with 96-hour calf removal in relation to

complete weaning of animals fed with different forages (TERRA, 2001). The animals were adult cows (ages varying from 4 to 6 years), with mean body condition at delivery of 3.0 (in a classification range from 1 - very thin - to 5 - obese; LOWMAN et al., 1976). Fifty to 70 days postpartum, the cows previously classified at delivery, were ranked according to their body condition and submitted to hormonal treatment. All animals were weighed twice (at delivery and at weaning - performed 7 days after start of treatment) and sorted in two groups on the basis of forage availability: group one was managed on native pasture with 960kg of dry matter per hectare (DM ha<sup>-1</sup>) and a stocking rate of 0.96 animal unit per hectare (au ha<sup>-1</sup>; au=400kg live weight) at delivery, and 400kg DM ha<sup>-1</sup> at weaning; Group 2 was also managed on native pasture but with 600kg DM ha<sup>-1</sup> and a stocking rate of 1.44au ha<sup>-1</sup> at delivery and 240kg DM ha<sup>-1</sup> at weaning. The dry matter of the pasture was estimated by the double sample method (WILM et al., 1944). Some animals gained while others lost weight in both groups (Table 1) suggesting that factors other than forage availability (which was above the maintenance diet, estimated based on dry matter only) must have influenced daily weight gain.

All cows that showed oestrous between days 7 and 17 from the beginning of the treatment were artificially inseminated; they were then bred with a cow: bull ratio of 100:12 up to day 67; clinical and ultrasonic diagnosis of pregnancy were performed at day 60 from the beginning of experiment to calculate the proportion of cows that conceived to the first oestrous after treatment and at day 127 to estimate the final pregnancy rate.

Blood was obtained from the jugular vein using ACD (acid-citrate-dextrose) as anticoagulant (ALMEIDA et al., 2003). Genomic DNA was extracted from peripheral blood leukocytes by the method of MILLER et al. (1988). Short tandem repeats were PCR-amplified as indicated in STEIGLEDER et al. (2004) and SNPs according to POMP et al. (1997) and BUCHANAN et al. (2002). The STR amplification products were analyzed by vertical electrophoresis in non-denaturing polyacrylamide gel (LAHIRI et al., 1997) and those of SNPs in agarose gel after *Sau3AI* or *Kpn2I* endonucleases cleavage.

Variance analyses (one-way ANOVA) were performed to compare productive parameters [average daily weight gain (ADG) and pregnancy according to the diagnosis at days 60 (P1) and 127 (P2)] in the different genotype classes according to the model:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where,  $Y_{ij}$  is the ADG, P1 or P2 phenotype of the  $j$ th individual;  $\mu$  is the effect of the population

Table 1 - Distribution of animals analyzed in this study according to nutrition condition and information about average daily weight gain (ADG) in grams

Breed	Conditions (n)		Total
	1	2	
A. Angus (n)	53	45	98
WG (%)	64	58	
ADG (range)	-267 to 544	-297 to 575	-297 to 575
ADG (Mean±sd)	112 ± 177	104 ± 217	103 ± 198
Charolais (n)	40	43	83
WG (%)	88	84	
ADG (range)	-278 to 833	-219 to 575	-278 to 833
ADG (Mean ± sd)	244 ± 258	207 ± 188	232 ± 225

Conditions: 1= 960 kg of dry matter per hectare (DM ha<sup>-1</sup>) and stocking rate (SR) of 0.96 animal unit per hectare (au ha<sup>-1</sup>); 2 = 600 kg of DM ha<sup>-1</sup> and SR of 1.44 au ha<sup>-1</sup> (au=400kg live weight); n= investigated number; WG (%)= percentual of animals gaining weight; sd = standard deviation.

mean;  $A_i$  is the effect of the  $i$ th genotype class; and  $e_{ij}$  is the random error component. The Tukey post hoc test was employed to identify the significant group, when a significant association was detected. Descriptive statistics was carried out beforehand to verify the normality of the distribution of productive parameters.

To compare the significant group detected by ANOVA with the other genotypes, average daily weight gain was also dichotomized into classes consisting of animals heavier or lighter than the mean ADG population values (see Table 1). For the analysis considering both populations simultaneously, the mean value was corrected by the herd weight; this analysis was made only when the positive association was detected in both populations. The genotype frequencies were compared between heavier and lighter animals by the chi-square test. To estimate the effect of the genetic markers on ADG, the odds ratio (OR) with 95% confidence interval was obtained by univariate logistic regression analysis. All the statistic analyses were performed using the SPSS® for Windows™ software (SPSS Inc), version 10.0.5 (1999).

## RESULTS

Allele frequencies for the two breeds are presented in table 2. In relation to the STRs, nine *BM1074* and five *IDVGA-51* alleles were observed in both populations; six *BM1500* alleles were detected in AA and four in C; *BMS1074\*157*, *BM1500\*144* and *IDVGA-51\*175* alleles were the most frequent in AA while *BMS1074\*147*, *BM1500\*142* and *IGVGA-51\*175* were the most common in C. Some alleles (*BMS1074\*159*, *BM1500\*138*, *BM1500\*146*,

*BM1500\*148* and *IGVGA-51\*183*) occurred only in AA, while *BMS1074\*141*, *BM1500\*150* and *IGVGA-51\*173* were exclusively detected in Charolais. Concerning to SNPs, *LEPSau3A1\*A* and *LEPSau3A1\**-alleles were the most frequent in both populations; *LEPKpn21\*C* were more common in Charolais, while *LEPKpn21\*T* were more frequent in Aberdeen.

No association was observed between genetic markers and pregnancy rates, but positive association with average weight gain was detected in two markers: *BM1500* and *BM1074* STRs (Table 3). Carriers of the *BM1500\*136* allele presented a higher ADG as compared to the other animals (about 75g day<sup>-1</sup> higher in Angus,  $P < 0.05$  and 96g day<sup>-1</sup> in Charolais,  $P < 0.10$ ). The analysis considering the pooled population values showed an important association ( $P < 0.01$ ). When the frequencies of *BM1500\*136* carriers and non-carriers were compared between animals heavier or lighter than the mean ADG population value (232g for C and 103g for AA; Table 4) the association results were confirmed: carriers of *BM1500\*136* had 72% and 60% higher ADG when compared with the mean population in AA and C breeds, respectively. The odds ratio test indicated that carriers of the *BM1500\*136* allele had about a 3-fold higher chance of having a higher ADG as opposed to the other animals ( $P < 0.01$ ).

In relation to *BMS1074* significant association was verified for AA cows only (Table 3): carriers of at least one *BMS1074\*151* allele showed less ADG (159g) than the other animals ( $P < 0.01$ ). The comparison of *BMS1074\*151* carriers and non-carriers frequencies between animals heavier or lighter than the mean ADG population value (103g; Table 4) indicated that 79% of *BMS1074\*151* carriers had ADG

Table 2 – Molecular markers allele frequencies in Aberdeen Angus and Charolais cows

STRs	Alleles									
Breeds (n)										
BMS1074	*141	*143	*145	*147	*149	*151	*153	*155	*157	*159
A. Angus (98)	0.00	0.02	0.01	0.10	0.17	0.10	0.15	0.16	0.19	0.10
Charolais (83)	0.08	0.01	0.06	0.25	0.03	0.04	0.14	0.30	0.09	0.00
BM1500	*136	*138	*142	*144	*146	*148	*150			
A. Angus (98)	0.25	0.12	0.13	0.31	0.16	0.03	0.00			
Charolais (83)	0.28	0.00	0.46	0.19	0.00	0.00	0.07			
IDVGA-51	*173	*175	*177	*179	*181	*183				
A. Angus (98)	0.00	0.40	0.10	0.09	0.16	0.25				
Charolais (83)	0.06	0.47	0.32	0.12	0.03	0.00				
LEP <i>Sau3A1</i> (A/B)	*A	*B								
A. Angus (98)	0.87	0.13								
Charolais (83)	0.67	0.33								
LEP <i>Sau3A1</i> (+/-)	* -	* +								
A. Angus (98)	0.97	0.03								
Charolais (83)	0.92	0.08								
LEP <i>Kpn2I</i>	*C	*T								
A. Angus (98)	0.42	0.58								
Charolais (83)	0.63	0.37								

n= sample size. Genotype frequencies are available on request.

≤103 g, while 62% of non-carrier animals presented ADG >103 (P<0.01), but the odds ratio test was not significant.

## DISCUSSION

Two positive associations were detected, involving BM1500 and BMS1074 STRs which are mapped respectively at 3.6 kb and 8cM downstream the LEP gene. The BM1500 markers is in the 5'UTR region and BMS1074 STR could be in an enhancer region, defined as sequences which activate transcription and contains repetitive units of short sequences and can be located thousands base pairs upstream or downstream the gene they control. Therefore, both markers could play a role on DNA transcription.

The association between *BMS1074\*151* carriers and ADG was observed in Aberdeen Angus but not in Charolais animals, probably due to some of the following reasons: the low frequency of this allele in Charolais animals may be masking the results; other genes involved in weight gain metabolism may be hiding the effect of this STR in Charolais, due to different genetic background of the two breeds; the alleles involved in the association could be in linkage disequilibrium in Aberdeen Angus but not in Charolais animals.

The association between *BM1500\*136* carriers and ADG seems to be more interesting since it occurred in both populations. Previous data indicated positive association between BM1500 STR and

Table 3 - Significant association results of ANOVA analyses

Population	<i>BMS1074*151</i>		P value	<i>BM1500*136</i>		P value
	Carriers	Non Carriers		CarriersNon	Carriers	
Aberdeen Angus						
ADG mean ( $\pm$ sd)	-24.7 (179.8)	134.2 (191.0)	<0.01	151.7 (162.9)	76.5 (216.7)	<0.05
N	19	79		46	48	
Charolais						
ADG mean ( $\pm$ sd)	155.1 (240.1)	246.1 (224.3)	>0.25	272.7 (248.0)	176.7 (194.8)	<0.10
N	7	73		47	35	
AA and C						
ADG mean ( $\pm$ sd)		NP		206.8 (209.2)	123.5 (207.1)	<0.01
N				93	83	

ADG = average daily weight gain; sd = standard deviation; NP = pooled analysis not performed, because significant association was detected only in AA animals.

phenotypic characteristics of beef carcass (FITZSIMMONS et al., 1998).

BUCHANAN et al. (2002) verified a correlation between BM1500 STR and LEP*Kpn2I* SNP (the same exon 2 C/T mutation here investigated) alleles and the bovine carcass fat composition and suggested that the C/T change may be the causative mutation, the effect of BM1500 STR resulting from linkage disequilibrium. However, in this paper no association was verified between LEP*Kpn2I* SNP polymorphism and the weight gain.

## CONCLUSIONS

The association studies performed between six molecular markers linked to the LEP gene and the average daily weight gain (ADG) and reproductive

performance in postpartum cows of two beef cattle breeds, Aberdeen Angus (n=98) and Charolais (n=83) indicated two positive associations and suggested that AA animals that carry the *BMS1074\*151* allele had bad performance in relation to the other animals, gaining about 159g day<sup>-1</sup> less. Otherwise, Charolais and Aberdeen Angus cows with *BM1500\*136* allele had three times higher chance to gain weight than for other animals.

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Table 4 - Comparison of BMS1074\*151 and BM1500\*136 carriers and non-carriers between animals heavier or lighter than the mean ADG population value

	<i>BMS1074</i>		P value	<i>BM1500</i>		P value
	<i>BMS1074*151</i>			<i>BM1500*136</i>		
	Carriers	NonCarriers		Carriers	NonCarriers	
	N (%)			N (%)		
Aberdeen Angus						
Weight gain > 103.38g <sup>a</sup>	4 (21)	49 (62)	<0.01	33 (72)	20 (42)	<0.01
Weight gain ≤ 103.38g <sup>a</sup>	15 (79)	30 (38)		13 (28)	28 (58)	
Charolais						
Weight gain > 232.12g <sup>a</sup>	3 (43)	36 (49)	>0.99	28 (60)	11 (31)	<0.01
Weight gain ≤ 232.12g <sup>a</sup>	4 (57)	37 (51)		19 (40)	24 (69)	
Charolais and A. Angus						
Weight gain > 162.41g <sup>b</sup>		NP		61 (66)	31 (37)	<0.01
Weight gain ≤ 162.41g <sup>b</sup>				32 (34)	52 (63)	

<sup>a</sup>mean ADG population values; <sup>b</sup>pooled populations mean ADG value, corrected by herd weight; NP = pooled analysis not performed, because significant association was detected only in AA animals.

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