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Short Communication

Effects of CSN3 and LGB gene polymorphisms on production traits in beef cattle

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Abstract

The objective of the present study was to estimate the allele and genotype frequencies of the CSN3/*Hin*fI and LGB/*Hae*III gene polymorphisms in beef cattle belonging to different genetic groups, and to determine the effects of these polymorphisms on growth and carcass traits in these animals, which are submitted to an intensive production model. Genotyping was performed on 79 Nelore, 30 Canchim (5/8 Charolais + 3/8 Zebu) and 275 crossbred cattle originating from the crosses of Simmental (n = 30) and Angus (n = 245) sires with Nelore females. Body weight, weight gain, dressing percentage, *longissimus dorsi* area and backfat thickness were fitted using the GLM procedure, and least square means of the genotypes were compared by the *F* test. The results showed that the CSN3/*Hin*fI and LGB/*Hae*III polymorphisms did not have any effect on growth or carcass traits ($p > 0.05$).

Key words: beef cattle, polymorphisms, candidate gene, growth, carcass.

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"Super-young" steers are animals finished on feedlot immediately after weaning and slaughtered as 13 month-olds at the most, weighing at least 450 kg. These animals should also have backfat thickness above 3.0 mm, which, associated with the animals' youth, will guarantee the quality of meat and its by-products. This system takes advantage of maximum biological efficiency, which can basically be defined as live weight gain over energy intake. It provides an excellent model of intensive cattle rearing.

The κ-casein (CSN3) and β-lactoglobulin (LGB) genes are expressed in milk and are important in the evaluation of the milk production potential and milk fat and protein percentage, indicating a maternal ability in beef cattle and therefore representing candidate genes for growth traits. In addition, these genes can also be associated with other loci that have a direct influence on growth. Polymorphisms of these genes have been associated with milk production and quality and weight gain until weaning in dairy cattle. However, the results of these studies are contradictory. On the other hand, the effects of milk protein polymorphisms on production traits in beef cattle have been little investigated. Moody *et al*. (1996) observed an effect of a κ-casein gene polymorphism on growth traits in Hereford beef cattle.

The objectives of the present study were to estimate the allele and genotype frequencies of CSN3 and LGB gene polymorphisms in beef cattle belonging to different genetic groups and to determine the effects of these polymorphisms on production traits in these animals which are submitted to a "super-young" production model.

For this purpose, blood was collected from 384 bulls belonging to four different genetic groups and differing in frame size, including 79 Nelore, 30 Canchim (5/8 Charolais + 3/8 Zebu) and 275 crossbred cattle originating from crosses of Simmental (n = 30) and Angus (n = 245) sires with Nelore females.

Calves were weaned at 210 days of age using a creep-feeding system. At the beginning of the study, the animals were individually identified, treated against endo- and ectoparasites, divided into groups of five animals each in pen according to breed and size, and fed diets formulated according to the recommendations of the National Research Council (NRC, 1996) for an average daily gain higher than 1.2 kg. After entering the pen, the animals were allowed to adapt for a period of approximately 20 days. The

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animals were slaughtered after an average feedlot period of 110 days, presenting a minimal live weight of 450 kg, and aged slightly over one year.

The animals were weighed at the time of entry in the pen (BW0), at the beginning of feedlot (BW1), and close to the time of slaughter (BWEND). Average daily gain (ADG) was calculated for the interval between the last two weight measurements. Upon the last weight measurement, ribeye area (REA) or *longissimus dorsi* area, and backfat thickness (BT) were assessed by ultrasound, according to the method described by Perkins and modified by Gresham (1998). Dressed percentage (DP) and carcass weight (CW) were measured at slaughtering.

Five milliliters of whole blood were collected by puncture of the left jugular vein in the neck region into vacuum tubes containing 7.5 mg of EDTA. Genomic DNA was extracted from a 300 µL aliquot of whole blood, using the Genomic Prep™ Blood DNA Isolation kit (Amersham Biosciences, Piscataway, NJ, USA). The amount and integrity of the DNA were determined on 0.8% agarose gel.

The animals were genotyped for the CSN3 and LGB genes by PCR-RFLP. The A and B alleles of the CSN3 gene (chromosome 6) were identified based on the amplification of a 350-bp fragment located between nucleotide 201 of exon IV and nucleotide 149 of intron IV, using the forward primer 5'-ATCATTTATGGCCATTCCACCAA AG-3' and the reverse primer 5'-GCCCATTTCGCTTTCT CTGTAACAGA-3', followed by digestion with the restriction enzyme *Hinf*I (Medrano and Cordova, 1990a). For the determination of the A and B alleles of the LGB gene (chromosome 11), a 247-bp fragment located between nucleotide 23 of exon IV and nucleotide 158 of intron IV was amplified, which contains a polymorphism at the *Hae*III restriction site, was amplified using the forward primer 5'-TGTGCTGGACAGCGACTACAAAAAG-3' and the reverse primer 5'-GCTCCCGGTATATGACCACCCT CT-3' (Medrano and Cordova, 1990b).

Each PCR was performed in a final volume of 25 µL, with the amplification mixture consisting of 50 ng genomic DNA, 0.20 µM of each primer, 10 mM Tris-HCl pH 8.0, 50 mM KCl, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, and 1 U *Taq* DNA polymerase. DNA was amplified in five steps: 1) initial denaturation of the double strand at 94 °C for 4 min; 2) denaturation at 94 °C for 1 min; 3) annealing of the primers at 56 °C for 45 s; 4) extension at 72 °C for 1 min; and 5) a final extension at 72 °C for 4 min. Steps 2, 3 and 4, corresponding to one cycle, were repeated 35 times.

The amplified fragments were digested in a reaction mixture containing 10 µL of the PCR product and 2.5 and 5 U of the restriction enzymes *Hinf*I and *Hae*III, respectively. The digestion mixtures were incubated for 4 h in a thermocycler at 37 °C. After digestion of the amplified products, the DNA fragments were separated on 3% agarose gel, in a horizontal electrophoresis system. A 100-bp molecular weight standard was applied to each gel, to permit the calculation of the size of the amplified and digested fragments. The DNA fragments were visualized on the agarose gel by staining with ethidium bromide followed by exposure to ultraviolet light. The gels were photographed for later analysis of the data, using a digital photodocumentation system. The genotypes of the individuals were determined for each polymorphism by analyzing the size of the fragments reported as base pairs (bp).

Genotype and allele frequencies were calculated according to Weir (1996) for each polymorphism, based on the genotypes identified on the gels. Differences in the allele frequencies of the polymorphisms within and between genetic groups were determined by the method of Goodman, adapted by Curi and Moraes (1981).

The traits of interest were analyzed by least square variance analysis (significance at 0.05) using the General Linear Model (GLM) procedure of the SAS program (Statistical Analysis System, 1999). The linear model used to fit the quantitative variables included, in addition to the genotype effect, the contemporary group effect that considered the interaction between genetic groups (1,.., 4), feedlots (1,.., 3) and farms of origin (1,.., 6), as follows: $Y_{ijk} = \mu + G_i + GC_j + e_{ijk}$, where Y_{ijk} = production trait, μ = overall mean, G_i = fixed effect of the i^{th} genotype, GC_j = fixed effect of the j^{th} contemporary group, and e_{ijk} = random error.

Genetic groups showing only one genotype or genotypes with very low frequencies (less than 0.10) in the total animal sample were excluded from the analysis. The sire effect was not included in the linear model because the number of genotyped animals which were progenies of the same sires was very small (8.5 on average). As a consequence of the large number of small half-sib families, the possibility of confounding the genotype and sire effects on production traits was small.

Two genetic variants (A and B) of the CSN3/*Hinf*I polymorphism were identified in the genetic groups studied. Genotype AA was characterized by the presence of three restriction fragments of, respectively, 134, 132 and 84 bp (the 134- and 132-bp fragments could not be separated on the gel under the present conditions). Genotype BB showed two fragments of 266 and 84 bp, respectively. Heterozygous individuals (AB) were characterized by the presence of four fragments of 266, 134, 132 and 84 bp, respectively.

Two different alleles (A and B) were found for the LGB/*Hae*III polymorphism. Genotype AA was characterized by the presence of two fragments of 148 and 99 bp, respectively. Genotype BB was identified based on the presence of two bands of 99 and 74 bp, respectively, and three restriction fragments of 99, 74 and 74 bp, respectively. Heterozygous individuals (AB) showed three bands of 148, 99 and 74 bp, respectively.

The allele and genotype frequencies of the CSN3/*Hinf*I and LGB/*Hae*III loci obtained for the different genetic groups are shown in Tables 1 and 2, respectively.

Table 1 - Allele frequencies of the CSN3/*Hinf*I and LGB/*Hae*III loci obtained for the four genetic groups and for the sample as a whole.

Locus	Allele	Genetic group				Total
		Nelore	Canchim	1/2 Simmental	1/2 Angus	
CSN3/*Hinf*I	A	$0.892^{A;a}$	$0.767^{A;a}$	$0.800^{A;a}$	$0.782^{A;a}$	0.806
	B	$0.108^{B;a}$	$0.233^{B;a}$	$0.200^{B;a}$	$0.218^{B;a}$	0.194
LGB/*Hae*III	A	$0.160^{B;b}$	$0.550^{A;a}$	$0.400^{A;a}$	$0.226^{B;b}$	0.261
	B	$0.840^{A;a}$	$0.450^{A;b}$	$0.600^{A;b}$	$0.774^{A;a}$	0.739

^{A,B}significantly different allele frequencies within genetic groups ($p < 0.05$).
^{a,b}significantly different allele frequencies between genetic groups ($p > 0.05$).

Table 2 - Genotype frequencies of the CSN3/*Hinf*I and LGB/*Hae*III *loci* obtained for the four genetic groups and for the sample as a whole.

Locus	Genotype	Genetic group				Total
		Nelore	Canchim	1/2 Simmental	1/2 Angus	
CSN3/*Hinf*I	AA	0.810	0.633	0.633	0.588	0.641
	AB	0.177	0.267	0.333	0.388	0.331
	BB	0.013	0.100	0.034	0.024	0.028
LGB/*Hae*III	AA	0.000	0.200	0.133	0.025	0.052
	AB	0.321	0.700	0.533	0.403	0.417
	BB	0.679	0.100	0.334	0.572	0.531

Segregation of the CSN3/*Hinf*I polymorphism was observed in the four genetic groups studied, the frequency of allele A being significantly higher than that of allele B in all groups. No significant differences in allele frequencies were observed between the various genetic groups. Genotypes AA, AB and BB for the CSN3/*Hinf*I polymorphism were detected in all four genetic groups. The homozygous genotype AA predominated over the other two genotypes in all groups, the difference being greater in Nelore animals.

The LGB/*Hae*III polymorphism also showed segregation in the four genetic groups. The frequency of allele B was significantly higher than that of allele A in the Nelore and 1/2 Angus groups, whereas in Canchim and 1/2 Simmental animals the frequencies of alleles A and B were similar. The frequency of allele A was lower in the Nelore and 1/2 Angus animals than in the Canchim and 1/2 Simmental groups. No AA genotype for the LGB/*Hae*III polymorphism was observed in the Nelore group. Genotypes AA, AB and BB showed a heterogeneous distribution in Canchim, 1/2 Simmental and 1/2 Angus animals.

Tables 3 and 4 show the comparison of the least square means and respective standard errors of quantitative growth and carcass traits, respectively, between the genotypes of the CSN3/*Hinf*I and LGB/*Hae*III polymorphisms.

Only genotypes AA and AB were considered in the comparative analysis between CSN3/*Hinf*I polymorphism genotypes. The results showed no significant effects of genotypes on growth: BW0 ($p \leq 0.5024$), BW1 ($p \leq 0.2473$), BWEND ($p \leq 0.7566$) and ADG ($p \leq 0.2609$) and carcass traits: CW ($p \leq 0.1144$), DP ($p \leq 0.5531$), REA ($p \leq 0.8847$) and BT ($p \leq 0.1154$).

The comparative analysis of the LGB/*Hae*III polymorphism genotypes only considered genotypes AB and BB. No significant effects of these genotypes on the quantitative traits analyzed were observed: BW0 ($p \leq 0.1564$), BW1 ($p \leq 0.5293$), BWEND ($p \leq 0.8638$), ADG ($p \leq 0.9273$), CW ($p \leq 0.4956$), DP ($p \leq 0.3351$), REA ($p \leq 0.1551$) and BT ($p \leq 0.1651$).

The CSN3/*Hinf*I polymorphism identified by Medrano and Cordova (1990a) results in the substitutions of the amino acid alanine (allele A) with aspartic acid (allele B) at position 148 in the protein sequence. Thus, this polymorphism has the potential to directly or indirectly affect production traits.

The present results showed high frequencies of allele A, similar to those reported in the literature. Kemenes *et al*. (1999) and Tambasco *et al*. (2000) observed frequencies of 0.91 and 0.94, respectively, in Nelore animals. Regitano *et al*. (1999) found a frequency of 0.67 for the A allele in animals of the Canchim breed. In addition, these and other studies have shown higher frequencies of allele A compared to allele B in both taurine (Ron *et al*., 1994; Moody *et al*., 1996; Vasconcellos *et al*., 2003) and Zebu breeds (Mitra *et al*., 1998; Kemenes *et al*., 1999; Tambasco *et al*., 2000), regardless of whether dairy or beef cattle were studied. This finding indicates that differences in the selection criteria for milk or meat production in these breeds do not favor different alleles of this polymorphism. However, allele B has been found to predominate in the taurine dairy

Table 3 - Least square means and standard errors of the growth traits for the genotypes of the CSN3/*Hinf*I and LGB/*Hae*III loci. The number of animals compared in each genotype is given in parentheses.

Locus	Genotype	Growth trait			
		BW0 (kg)	BW1 (kg)	BWEND (kg)	ADG (kg)
CSN3/*Hinf*I	AA	274.25 ± 2.18 (130)	316.89 ± 1.78 (246)	472.27 ± 2.41 (225)	1.48 ± 0.01 (225)
	AB	276.89 ± 2.97 (87)	313.35 ± 2.46 (127)	470.91 ± 3.49 (112)	1.45 ± 0.02 (112)
LGB/*Hae*III	BB	276.97 ± 2.23 (109)	314.33 ± 1.89 (205)	471.79 ± 2.59 (183)	1.48 ± 0.01(183)
	AB	272.04 ± 2.48 (100)	316.11 ± 2.15 (160)	471.10 ± 2.91 (145)	1.47 ± 0.02 (145)

BW0 = body weight at the time of entry in the pen; BW1 = body weight at the beginning of feedlot; BWEND = body weight at the time of slaughter; ADG = average daily gain.
No significant differences were observed between genotypes ($p > 0.05$).

Table 4 - Least square means and standard errors of the carcass traits for the genotypes of the CSN3/*Hinf*I and LGB/*Hae*III loci. The number of animals compared in each genotype is given in parentheses.

Locus	Genotype	Carcass trait			
		CW (kg)	DP (%)	REA (cm^2)	BT (cm)
CSN3/*Hinf*I	AA	262.07 ± 1.48 (244)	55.61 ± 0.11 (223)	71.82 ± 0.47 (242)	5.07 ± 0.10 (242)
	AB	257.84 ± 2.03 (126)	55.50 ± 0.16 (111)	71.70 ± 0.64 (127)	4.80 ± 0.14 (126)
LGB/*Hae*III	BB	259.75 ± 1.56 (204)	55.63 ± 0.11 (182)	71.42 ± 0.51 (203)	4.80 ± 0.10 (202)
	AB	261.34 ± 1.78 (158)	55.49 ± 0.13 (143)	72.51 ± 0.58 (158)	5.00 ± 0.12 (158)

CW = carcass weight; DP = dressing percentage; REA = ribeye area; BT = backfat thickness.
No significant differences were observed between genotypes ($p > 0.05$).

breeds Jersey and Brown Swiss (Van Eenennaan and Medrano, 1991). Literature data have demonstrated higher frequencies of the allele A in Zebu breeds compared to taurine breeds. These findings are in accordance to those of the present study, which showed lower frequencies (although non-significant) of allele A in the genetic groups Canchim, 1/2 Simmental and 1/2 Angus as compared to Nelore.

The results obtained in the present study differ from those reported by Moody *et al*. (1996) in Hereford beef cattle. These authors observed a negative effect of substitution of allele A with allele B of the κ-casein polymorphism on EPD (Expected Progeny Difference) for birth weight, weaning weight and maternal milk production, indicating that the A allele is favorable to these traits. However, our results agree with those obtained by Regitano *et al*. (1999) and Tambasco *et al*. (2003), who did not observe any influence of this polymorphism on growth traits in Canchim beef cattle and F1 animals of Canchim x Nelore, Simmental x Nelore and Angus x Nelore crosses, respectively. According to Pomp *et al*. (1994), these contradictions can be explained by differences in the linkage equilibrium between markers and quantitative traits loci in the various populations studied, or by different epistatic interactions between the genetic bases of these populations and quantitative trait loci. In addition, epigenetic factors resulting from different experimental conditions may also play a role.

The A and B alleles of the LGB/*Hae*III polymorphism were identified by Medrano and Cordova (1990b). This polymorphism is located in a coding region of the LGB gene (exon IV), leading to changes in the amino acid sequence of the protein and therefore being able to directly or indirectly affect production traits.

In the present study, a low frequency of allele A was observed in the Nelore group compared to the results reported by Rosa (1997), Kemenes *et al*. (1999) and Tambasco *et al*. (2000), who found frequencies of 0.41, 0.40 and 0.24, respectively. However, Faria *et al*. (2000) reported the same frequency (0.16) as obtained in the present study. This variation in the results might be explained by different selection criteria applied to the herds, or by differences in the allele frequencies between base populations. Regitano *et al*. (1999, 2000) and Vasconcellos *et al*. (2003) observed frequencies of 0.40, 0.46 and 0.17 for allele A in Canchim, Simmental and Angus breeds, respectively. In other studies, the frequency of the A allele of the β-lactoglobulin polymorphism was 0.43 and 0.39 in the dairy taurine breeds Holstein and Brown Swiss, respectively (Van Eenennaan and Medrano, 1991), and 0.37 in the Zebu GirGir breed (Kemenes *et al*., 1999). The allele frequencies of this polymorphism are heterogeneous among breeds and seem to be independent of origin (Zebu or taurine) and type of production (meat or milk).

Although a number of studies have shown a significant effect of the A allele of the LGB/*Hae*III polymorphism on milk production (Geldermann *et al*., 1985; Cowan *et al*., 1992; Bovenhuis and Weller, 1994), and of the B allele on

percent milk fat in dairy cattle (Bovenhuis and Weller, 1994), Moody *et al*. (1996) did not detect any significant effect of this polymorphism on the growth traits of Hereford beef cattle, despite significant allele differences between the selected and the control populations. Regitano *et al.* (1999) did not observe allele variations between different generations of a Canchim herd and suggested that this polymorphism is not associated with phenotypic traits subjected to selection in this herd. Similarly, Tambasco *et al*. (2003) did not observe an isolated influence of the LGB/*Hae*III polymorphism on the growth traits of crossbred Canchim x Nelore, Simmental x Nelore and Angus x Nelore cattle. However, these authors found a significant interaction (epistasis) between this marker and a polymorphism of the growth hormone gene influencing weight gain. The same authors called attention to the importance of epistasis studies and of the influence of the β-lactoglobulin polymorphism on weight gain.

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