GROWTH HORMONE *ALU*I POLYMORPHISM ANALYSIS IN EIGHT PORTUGUESE BOVINE BREEDS

ANÁLISIS DEL POLIMORFISMO *ALU*I DE LA HORMONA DE CRECIMENTO EN OCHO RAZAS BOVINAS PORTUGUESAS

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ADDITIONAL KEYWORDS

Somatotropin. Polymorphism. Meat production. PCR-RFLP.

PALABRAS CLAVE ADICIONALES

Somatotropina. Polimorfismo. Producción de carne. PCR-RFLP.

SUMMARY

A total of 195 bulls of eight Portuguese beef cattle breeds (Alentejana, Arouquesa, Barrosã, Maronesa, Marinhoa, Mertolenga, Mirandesa and Preta) were genotyped for the GH *Alu*I polymorphism by the polymerase chain reaction and restriction length polymorphism (PCR-RFLP). The genotype and gene frequencies for each breed were determined and shown to be quite variable among the breeds. The overall gene frequencies for L and V were 0.759 and 0.241, respectively. The relation between the bGH-*Alu*I polymorphism and growth performances was ascertained in 168 of the animals analysed. According to our results there is a significant association between the genotypes LL and LV of the bGH and the average live body weight of the animals of the breeds Alentejana, Marinhoa and Preta.

RESUMEN

Un total de 195 bovinos pertenecientes a ocho razas productoras de carne portuguesas (Alentejana, Arouquesa, Barrosã, Maronesa, Marinhoa, Mertolenga, Mirandesa y Preta) fueron genotipados utilizando PCR-RFLP para el polimorfismo CH *Alu*I. Se determinaron el genotipo y las frecuencias génicas para cada raza mostrando una gran variabilidad entre razas. Las frecuencias génicas globales para L y V fueron 0,759 y 0,241 respectivamente. Se estableció en 168 de los animales analizados la relación entre el polimorfismo bGH-*Alu*I y los resultados de crecimiento. De acuerdo con nuestros resultados hay una asociación significativa entre los genotipos LL y LV de bGH y el peso vivo de los animales en las razas Alentejana, Marinhoa y Preta.

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INTRODUCTION

The use of polymorphic specific genes as molecular detectable markers is a promising alternative to the current methods of trait selection, once these genes are proven to be associated with traits of interest in animals.

The bovine growth hormone (bGH) is a 22 KDa single-chain polypeptide hormone produced in the anterior pituitary gland. The encoding gene is approximately 1800 base pairs (bp) and consists of five exons separated by four intervening sequences (Woychick *et al.,* 1982; Gordon *et al.,* 1983). It is well known that it plays an important role in biological processes such as mammary development, lactation, growth and metabolism regulation (reviewed by Etherton, 1998), being therefore a promising candidate gene marker for improving milk and meat production in cattle.

Recently several studies have investigated associations between genetic polymorphisms at the bGH *locus* with production traits, namely to milk protein percentage (Lagziel *et al.,* 1996 and Vukasinovic *et al.,* 1999, and references therein). A *Taq*I RFLP, using a complementary DNA (cDNA) probe for GH, has been associated with the birth-weight of beef cattle (Rocha, 1991). A polymorphism in the fifth exon, responsible for two alternative forms of the hormone, was reported by Lucy (1991). A substitution of a citosine (C) for a guanine (G) at position 2141 (Zang, 1992); [designation from the sequence in work of Gordon (1983)] causes an amino acid change from leucine (L, codon CTG) to a valine (V, codon GTG) at the residue 127. This transversion enables the genotyping at this particular *locus* using the endonuclease *Alu*I since this enzyme does not recognize its target sequence when a G is present instead of a C. The *Alu*I (+/-) polymorphism is believed to be related to plasma levels of GH as suggested by Schlee (1994b). This author observed that genotype LL was usually associated with higher circulating concentrations of GH when compared to genotype LV. Chrenek (1998) reported an association between bGH-*Alu*I polymorphism and meat production traits in Slovak Simmental bulls. This hormone was shown to be polymorphic in many breeds, being the distribution of GH variants (LL, LV, VV) and their frequencies different among each breed. The study of the effects of growth hormone genotypes on growth traits is of great interest in the breeds analysed in this study, as their main purpose is meat production.

The objectives of the present study were: (1) to reveal GH-*Alu*I polymorphism in the eight major indigenous Portuguese cattle breeds and estimate the gene frequencies, (2) to look for an association between growth performances and GH-*Alu*I variants.

MATERIAL AND METHODS

Animals

A total of 195 bulls of the following indigenous breeds were included in the present report: Alentejana (AL, n=22), Arouquesa (AR, n=24), Barrosã (BA, n=23), Marinhoa (MO, n=32), Maronesa (MA, n=24), Mertolenga (ME, n=22), Mirandesa (MI, n=21) and Preta (PR, n=27). Animals born between

April 1996 and January 1997 came from various herds in Portugal and were purchased through Associations of Breeders. Rearing was made at the feedlot of the Estação Zootécnica Nacional (Santarém, Portugal), being each breed physically isolated from each other. Initial average age (IAW) and initial weights were, respectively:

237.5 ± 11.3 d and 248.4 ± 8.8 kg for AL; 247.3 ± 10.6 d and 221.9 ± 8.2 kg for AR; 217.0 ± 10.2 d and 178.7 ± 7.9 kg for BA; 173.6 ± 10.2 d and 190.6 ±7.9 kg for MO; 247.3 ± 9.9 d and 207.2 ± 7.7 kg for MA; 246.5 ± 11.8 d and 195.7 ± 9.2 kg for ME; 261.8 ± 11.9 d and 277.2 ± 9.2 kg for MI and 294.6 ± 10.3 d and 217.0 ± 8.0 kg for PR.

Animals were all fed with the same feeding ration (maize silage and concentrate). The control of body weights was made at the arrival of each animal and subsequently each 21 days. Among the eight races, four are currently considered to be small breeds (Arouquesa, Barrosã, Maronesa and Mertolenga) reaching a mature weight of 700 kilos and four are considered to be heavy breeds (Alentejana, Marinhoa, Mirandesa, Preta) reaching largest mature weights of 1000 kg. A first group of the animals was slaughtered when reaching approximately 50 p.100 of the expected mature weight (P2). A second group was slaughtered at 70 p.100 of the expected mature weight (P3) and finally a third group was weighted until the mature weigh (P4).

Genotyping of bulls

DNA was extracted from peripheral blood leukocytes using DNA Isolation kit from Puregene.

Twenty-five µl polymerase chain reactions (PCR) were carried out in a Biometra UNO II 48 thermalcycler, using PCR beads Ready-To-Go (Amersham Pharmacia Biotec) with 50 ng of bovine genomic DNA and 16 pmol of each primer. The primers GH5F (5´-GCTGCTCCTGAGGGCCCTTC-3´) and GH5R (5´CATGACCCTCAGGTACGTCTCCG-3´) flanked a 211 base pair (bp) fragment, consisting of 49 bp of the fourth intron and 162 bp from the fifth exon according to the published sequence by Gordon *et al.* (1983). After a first denaturation step at 95º for 5 min, the samples were amplified for 30 cycles: denaturation 95º x 30 s; primer annealing 62º x 30 s; primer extension 72º x 30 s; followed of a 5 min final extension step at 72º. Amplification products (8.5 µl) were digested at 37º for at least 14 hours with 5 Units of *Alu*I [AG|CT] (Gibco BRL, Life Technologies) and separated on a 3,5 p.100 agarose gel containing 0.1 µg/ml EtBr.

Statistical analysis

Allele frequencies were calculated by allele counting.

Of the 195 animals, 27 were excluded of the statistical approach due to missing values or unreliable data. The data collected regarding the weights was analysed with the SAS procedure (SAS system for Windows 6.12, 1996 SAS Institute INC.) with mixed procedure according to the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma\,(x_{ijk} - \bar{x}) + \\ + \gamma\,(x_{ijk} - \bar{x})^2 + Animal_{ijk} + \varepsilon_{ijk}$$

where:

Y_{ijk}: phenotypic value of the weight of the animal *k* of the *i* breed with a genotype *j*
μ: overall mean
α_i: fixed effect of the breed
β_j: fixed effect of the genotype (j = LL, LV, VV)
$\gamma\ (x_{ijk} - \bar{x})$: linear effect of covariate age
$\gamma\ (x_{ijk} - \bar{x})^2$: quadratic effect of covariate age
$Animal_{ijk}$: random effect
ε_{ijk}: random error

This model was firstly adjusted for the heavy breeds and then for the small breeds considering 3 distinct periods of growth. The first period (P2) refers to the period before the first slaughter when the animals had reached approximately 50 p.100 of the mature weight (which is for small breeds 350 kg and for the heavy breeds 500 kg). The second period (P3) corresponds to the average weights since the initial weight until 70 p.100 of the mature weight (which is for small breeds 500 kg and for the heavy breeds 700 kg). The third period (P4) refers to the period until the third slaughter (> 500 kg for small breeds and > 700 kg for heavy breeds). Afterwards the model was adjusted for each breed individually without considering the breed effect. Multiple comparison tests were performed when a significant effect was observed ($p<0.05$).

RESULTS AND DISCUSSION

In homozygous animals either a unique band (211 bp, VV variants) or two bands (159 and 52 bp, LL variants) patterns were observed. Heterozygous animals gave a three-band (211, 159 and 52 bp) pattern (**figure 1**).

Considering the 195 bulls analysed, the overall genotype frequencies were 0.600 for LL, 0.318 for LV and 0.082 for VV. Gene frequencies of alleles L and V were 0.759 and 0.241, respectively. Significant differences were observed among breeds (**table I**). Genotype VV for example, was absent in the analysed populations of Marinhoa, Mertolenga, Mirandesa and Preta bre-

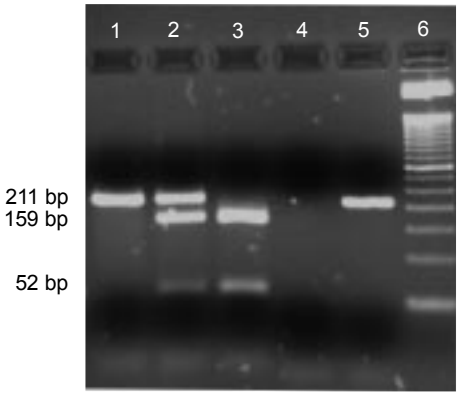

Figure 1. *Analysis of* Alu*I polymorphism at the bGH gene: Electrophoretic patterns of the three genotypes separated on a 3.5 p.100 agarose gel. Lane 1: genotype VV; lane 2: genotype LV; lane 3: genotype LL; lane 4: negative control (PCR reaction without template DNA); lane 5: undigested PCR product; lane 6: 50 bp DNA Ladder (Gibco BRL, Life Technologies).* (Análisis del polimorfismo *Alu*I en el gen bGH: Patrón electroforético de los tres genotipos separados en gel de 3.5 p.100 agarosis. Pozo 1: genotipo VV; pozo 2: genotipo LV; pozo 3: genotipo LL; pozo 4: control negativo (reacción PCR sin DNA); pozo 5: producto PCR indigerido; pozo 6: marcador 50 bp DNA Ladder (Gibco BRL, Life Technologies)).

Table I. *Genotype and gene frequencies of the* Alu*I polymorphism in the bGH gene in the populations analysed.* (Frecuencias genotípicas y genéticas del polimorfismo *Alu*I en el gen bGH en las poblaciones analizadas).

Breed	Genotype frequencies			Allele frequencies	
	LL	LV	VV	L	V
AL(n=22)*	0.773	0.182	0.045	0.8640	0.1360
AR (n= 24)	0.500	0.417	0.083	0.7085	0.2915
BA (n= 23)	0.348	0.478	0.174	0.5870	0.4130
MO (n= 32)*	0.750	0.250	0.000	0.8750	0.1250
MA (n= 24)	0.167	0.458	0.375	0.3960	0.6040
ME (n= 22)	0.910	0.090	0.000	0.9550	0.0450
MI (n= 21)*	0.857	0.143	0.000	0.9285	0.0715
PR (n= 27)*	0.519	0.481	0.000	0.7595	0.2405

*Heavy breeds L= leucine V= valine

eds but has a frequency of 0.375 in the Maronesa breed. The frequency of allele V in this breed (0.604) is significantly higher than that observed in different other breeds. Some of the reported gene frequencies of GH variant V were: 0.32 in Bavarian Simmental bulls by Schlee *et al.* (1994a), 0.20, 0.10, 0.29 in German Black and White, Bavarian and Tyrolean Brown and Simmental respectively, by Schlee *et al.* (1994b), 0.44 in Slovak Simmental bulls by Chrenek *et al.* (1998), 0.08 in Holstein cows by Lucy *et al.* (1991), 0.09 in Holstein bulls and cows and 0.29, 0.24 in Ayrshire bulls and Jersey bulls, respectively, by Sabour *et al*. (1997).

Interestingly, the so-called small breeds have a higher frequency of the V allele than the heavy ones, with the exception of the Mertolenga breed which showed a low V allelic frequency. This fact is in agreement with the previous observations of Lucy *et al.* (1993) who reported that the dairy breeds with the largest mature size (Holstein and Brown Swiss) had the highest frequency of L whereas smaller breeds (Ayrshire and Jersey) had the highest frequency of V.

In our study, significant effects of the GH genotype were observed in the AL, MO and PR breeds (**table II**) and considering the four heavy breeds together. The results suggest that the genotype LV is positively associated with higher weights at latter stages of growth (P3 or P4). However, these results should be interpreted with precaution due to the small sample size. It was not possible to study the effect of genotype VV for the heavy breeds. With respect to the small breeds no relationship between GH genotypes and average live weights were apparent either considering each race by itself or grouping the four breeds. Genotype LV of Bava-

Table II. *Means and standard errors of live weights (kg) per breed, genotype and number of animals in each of the intervals (P2, P3 and P4) considered.* (Media y error estándar de los pesos vivos medios (kg) para cada raza, genotipo y número de animales en cada intervalo de tiempo (P1, P2 y P3) considerado).

Breed	P2			P3			P4			Significance
	LL	LV	VV	LL	LV	VV	LL	LV	VV	
AL	(16) 389.8 ±11.20	(4) 409.5 ±22.50	(1) 416.0 ±45.20	(11) 507.8 ±9.05	(4) 527.6 ±15.10	(1) 557.7 ±30.30	(7) 594.6 ±8.41	(1) 648.3 ±22.20	(0) ---	P4* 1: 2
MO	(14) 362.1 ±10.3	(7) 371.2 ±14.65	(0) ---	(11) 478.6 ±11.06	(6) 514.7 ±14.96	(0) ---	(3) 537.1 ±22.83	(3) 578.9 ±22.82	(0) ---	P3** 1: 2
MI	(18) 379.2 ±10.09	(3) 367.3 ±24.75	(0) ---	(16) 521.8 ±11.42	(2) 489.0 ±32.35	(0) ---	(8) 574.1 ±8.83	(0) ---	(0) ---	NS
PR	(11) 356.8 ±20.45	(12) 391.7 ±19.61	(0) ---	(8) 467.0 ±21.17	(8) 540.2 ±21.19	(0) ---	(5) 577.9 ±21.78	(2) 533.6 ±24.60	(0) ---	P3* 1: 2
AL, MO, MI and PR	(59) 372.0 ±6.45	(26) 386.4 ±10.07	(1) 401.6 ±50.38	(46) 497.1 ±6.95	(20) 528.8 ±10.66	(1) 552.6 ±47.53	(23) 573.5 ±8.52	(6) 574.8 ±15.05	(0) ---	P3* 1: 2
AR	(10) 296.5 ±13.59	(9) 281.0 ±14.32	(2) 257.6 ±30.40	(7) 397.8 ±6.16	(8) 383.6 ±16.05	(2) 384.3 ±32.20	(3) 465.8 ±20.23	(5) 484.8 ±15.72	(0) ---	NS
BA	(8) 271.2 ±14.65	(11) 257.5 ±12.49	(4) 284.4 ±20.73	(4) 382.4 ±22.18	(8) 373.2 ±15.67	(2) 416.6 ±31.39	(2) 438.6 ±22.15	(4) 410.1 ±15.6	(1) 490.4 ±31.32	NS
MA	(4) 266.4 ±22.95	(7) 278.3 ±17.35	(7) 283.4 ±17.35	(3) 379.0 ±21.24	(6) 376.0 ±15.02	(5) 376.8 ±16.43	(2) 433.7 ±21.36	(2) 492.0 ±15.11	(3) 482.8 ±12.33	NS
ME	(18) 287.2 ±6.09	(2) 261.6 ±18.43	(0) ---	(15) 391.7 ±7.41	(1) 383.9 ±29.28	(0) ---	(7) 488.6 ±6.63	(0) ---	(0) ---	NS
AR, BA, MA and ME	(40) 283.6 ±6.37	(29) 271.6 ±7.18	(13) 282.8 ±11.00	(29) 388.4 ±7.81	(23) 381.1 ±8.30	(9) 392.8 ±13.34	(14) 458.9 ±10.00	(11) 468.4 ±10.25	(4) 497.6 ±17.74	NS

rian Simmental bulls was associated with significantly higher carcass-gain than homozygotes, either LL or VV, by Schlee *et al.* (1994a) whereas genotype VV was associated with better breeding values regarding the classification score. Chrenek (1998) reported that genotype VV was associated with lower body weight and lower ADG in Slovak Simmental bulls in comparison to the bulls with the genotypes LL or LV.

In the population we have studied, the frequency of *Alu*I (-) allele, corresponding to the V variant, was low or null. The amount of data available was not sufficient to provide an unequivocal statistical evidence of a quantitative effect of the bGH genotypes on the weights. A larger sample including a higher number of animals having the V allele is necessary. The present study is the first report on GH genotyping of Portuguese bovine breeds and has to be considered as a preliminary study. A larger number of observations are needed to establish or deny the existence of an association between GH genotypes and quantitative traits in those breeds.

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