Genetic variations in the leptin gene associated with growth and carcass traits in Nellore cattle

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Genet. Mol. Res. 13 (2): 3002-3012 (2014)
Received June 6, 2013
Accepted October 23, 2013
Published April 16, 2014
DOI http://dx.doi.org/10.4238/2014.April.16.9

ABSTRACT. The aim of the study was to detect polymorphisms in the leptin gene and to determine the association of these polymorphisms with growth and carcass traits in Nellore cattle. The single nucleotide polymorphisms (SNPs) -1457 (AJ571671:g.-1457A>G) and A59V (AF536174.1:g. 321C>T), as well as the microsatellite BM1500 (3.9 kb downstream), were genotyped. The measures of body weight and ultrasound examinations (rib eye area, back, and rump fat thickness) were performed in 3 different periods of animal management. During the ¿rst period, the animals were fed with grass and mineralized salt ad libitum. In the second period, they received grass and concentrate, and in the third, only concentrate. After the slaughter of animals, data were collected for classi¿cation and typi¿cation of carcasses. No signi¿cant association was found between the variables assessed and SNP -1457. Conversely, SNP A59V was associated with rump fat thickness and muscle color post-slaughter. BM1500 was associated with rump fat thickness in the ¿rst period (pre-slaughter), subcutaneous fat thickness

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in the second, weight of the animals in the third, and length of the carcass after slaughter. These results suggest that SNP A59V and the microsatellite BM1500 might be useful for marker-assisted selection in Nellore cattle.

Key words: Molecular markers; *Bos taurus indicus*; Beef cattle; Ultrasonographic traits; Weight

INTRODUCTION

The leptin gene has been widely studied since it was ¿rst reported by Zhang et al. (1994). In bovines, this gene is found in chromosome 4 (4q32) and is 16,735 Kb, with 3 exons and 2 introns (Taniguchi et al., 2002). Its ¿nal product is leptin protein (167 amino acids) of hormonal origin, which has an autocrine action that inhibits insulin-stimulated glucose uptake and reduces lipogenesis in the adipose tissue (Ceddia et al., 1998).

The single nucleotide polymorphisms (SNPs) detected in the leptin gene and its receptors have been associated with carcass traits and serum leptin concentrations in different species, particularly bovines (Nkrumah et al., 2005; Kulig and Kmieć, 2009; Orrù et al., 2011; da Silva et al., 2012). SNP-1457 (AJ571671:g.-1457A>G) in the leptin gene promoter region has been associated with fertility and growth in the Holstein breed (Liefers et al., 2005; Clempson et al., 2011). The polymorphism A59V (AF536174.1:g. 321C>T) in exon 3 of the leptin gene has been associated with weight and average daily body weight gains in beef cattle (Kulig and Kmieć, 2009). Previous studies of the microsatellite BM1500 (located 3.9 kb downstream of the leptin gene) showed its association with carcass fat deposition in beef cattle (Fitzsimmons et al., 1998; Montoya et al., 2009).

Several studies investigated the inÀuence of these polymorphisms on the growth and body composition of taurine bovine. In order to improve productivity and meat quality, further studies are required to gain a better understanding of the genetic variation in the leptin gene associated with growth and carcass quality in Nellore cattle (*Bos taurus indicus*), under different environmental conditions. According to Barbosa et al. (2010), the main problems faced by the Brazilian beef industry are the lack of uniformity in slaughter age, fat cover, and marbling. The polymorphisms of structural genes studied in Nellore cattle might have a relevant impact on the Brazilian beef industry (Tizioto et al., 2012). Therefore, the aim of the present study was to detect polymorphisms in the leptin gene and determine their association with growth and carcass traits in bovines of the Nellore breed.

MATERIAL AND METHODS

Animals

One hundred male bovines of the Nellore breed reared in a commercial herd in the São Jorge do Maracay Farm (Municipality of Iguatemi, Mato Grosso do Sul State, Brazil) were used. The growing and ¿nishing phases of the animals were performed in 3 different periods. In the ¿rst period (P1), the animals were fed a low quality pasture (*Brachiaria* sp) with mineralized salt provided *ad libitum* up to 20 months of age. In the second period (P2), they

received the same pasture (Brachiaria sp) until 22 months of age and concentrate (16% crude protein and 3.1 Mcal/kg dry matter). In the third period (P3), the animals were ¿nished in con¿nement (15% crude protein and 2.7 Mcal/kg dry matter) and slaughtered at 24 months of age. The weighing, blood sampling, and ultrasonographic measurements (rib eye area, back, and rump fat thickness) were performed after the animals were fasted for 12 h in each period.

Phenotypic traits

The weight of the animals was adjusted to 600 (W1), 650 (W2), and 700 (W3) kg. The average daily body weight gains between the periods of sampling were registered (ADG 1/2 and ADG 2/3). The carcass measurements were obtained using real-time ultrasound equipped with a linear probe of 17.8 cm and an acoustic coupler. The images of the rib eye area (REA US) and the backfat thickness (BT US) were measured transversally on the Longissimus dorsi muscle in the region between the 12th and 13th ribs. Rump fat thickness (BT P8 US) was also measured in the extremity of the Biceps femoris, above the Gluteus medius.

When the animals reached a body weight of approximately 1480 kg, they were slaughtered in a commercial abattoir in accordance with article No. 110 of the RIISPOA-Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal (Brasil, 1968). After a 24-h freezing period, carcass grade data were collected. The pH, physiological maturity, length, conformation, and fat distribution details were obtained, as described by Gomide et al. (2006). The carcasses were divided into primary retail cuts (fore and hindquarters) between the 5th and 6th ribs. Next, the short ribs were removed to obtain the special hindquarters (Gomide et al., 2006). Measurements of color, texture, REA, BT, and marbling (MAR) were obtained between the 5th and 6th ribs.

Molecular analyses

DNA extraction was performed using an initial volume of 300 μL of blood samples, following previously described methodology (Sambrook et al., 1989). DNA quality and concentrations were determined using spectrophotometry (NanoDrop® ND-2000 UV-Vis). Animals were genotyped for the following markers: 1457 (Liefers et al., 2005), A59V (Haegeman et al., 2000), and BM1500 (Fizsimmons et al., 1998) found in the leptin gene. These markers have been associated with important economical features in bovines.

The polymerase chain reaction ampli¿cations were performed in a thermocycler BIORAD® model MyCycler™ Thermal Cycler. The reactions were carried out in a ¿nal volume of 25 μL, containing 12.5 μL PCR Master MIX (Promega®, USA), 2 μL each primer (10 pmol), and 2 μL genomic DNA (10-20 ng).

The forward (5'GTCGTAGTGGATGCTACTGCCTCTAT3') and reverse (5'TGGCTA AGACTCCCTGCTTCCAAA3') primers were used to amplify a fragment of 293 bp (SNP, 1457), based on the sequences of the bovine leptin gene available in GenBank (AN: AB070368.1). The ampli¿cation protocol consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation (94°C for 40 s), annealing (56°C for 45 s), extension (72°C for 1 min), and a ¿nal extension step at 72°C for 5 min. The amplicons were digested in a ¿nal volume of 12 μL containing 5 μL PCR product, 1X enzyme buffer, and 5 U restriction enzyme AluI.

The forward (5'-GGGAAGGGCAGAAAGATAG-3') and reverse (5'-CCAAGCTCT CCAAGCTCTC-3') primers were used to amplify a fragment of 458 bp (SNP A59V), as previously described by Öztabak et al., 2010. The run protocol consisted of an initial denaturation step (94°C for 2 min), followed by 35 cycles (denaturation at 94°C for 30 s, annealing at 57°C for 1 min, and extension at 72°C for 30 s), and a ¿nal extension step of 72°C for 15 min. The fragments were digested in a ¿nal volume of 15 µL, containing 7 µL PCR product, 1X enzyme buffer, and 2.5 U restriction enzyme HphI (Öztabak et al., 2010). The enzymatic digestions for both SNPs were performed at 37°C for 4 h. The fragments obtained were analyzed using 1% agarose gel.

Finally, the forward (5'-GATGCAGCAGACCAAGTGG-3') and reverse (5'-CCCATTG CTAGAACCCAGG-3') primers were used to amplify the microsatellite BM1500 (Fitzsimmons et al., 1998). The ampli¿cation protocol consisted of an initial denaturation step at 94°C for 3 min, followed by 25 cycles (denaturation at 94°C for 45 s, annealing at 57°C for 30 s, and extension at 72°C for 45 s), and a ¿nal step at 4° for 4 min (Fitzsimmons et al., 1998). The alleles were analyzed by electrophoresis on 7% denaturing polyacrylamide gel stained with silver nitrate.

Statistical analysis

The population analysis of gene frequency, genotype, the Hardy-Weinberg equilibrium test (HWE), observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphic information content (PIC) were calculated using the CERVUS 3.0 software (Kalinowski et al., 2007). The statistical analyses were performed using SAS 9.2 (SAS Institute, 2000). All characteristics studied were analyzed using the Shapiro-Wilk test and Bartlett's test to con¿rm the normality of residuals and the homogeneity of variances, respectively. The features of weight and average weight gain that met the assumptions previously tested (i.e., normality and homogeneity) were submitted to regression analysis for 1457 and BM1500, as well as analysis of variance (ANOVA) for A59V.

The ultrasound measures of carcass traits did not meet the normality and homogeneity assumptions and, consequently, were assessed using the Kruskal-Wallis test or the Chi-Square test. The measurements were dichotomized by their respective medians. When the observed and expected frequencies in a cell of the contingency table were less than or equal to 5 for A59V, Fisher's exact test was used.

RESULTS

Genetic diversity

Table 1 shows the gene and genotypic frequency estimates, H_O and H_E, and PIC for the polymorphisms studied.

Two allelic forms of SNP -1457 were recorded. Two restriction fragments of 201 and 92 bp were detected when the genotype AA was present. The genotype GG was reported by the observation of a fragment of 293 bp. The heterozygotes exhibited 3 different amplicon sizes: 293, 201, and 92 bp. The AG genotype was found in 54% of the population and the G allele was the most frequent (Table 1).

Table 1. Population analysis using molecular markers located in the bovine leptin gene.

Marker	Genotypic frequency	Allele frequency	H_O	H_E	PIC	HWE
-1457	AA = 0.05	A = 0.26	0.41	0.38	0.30	NS
	AG = 0.54	G = 0.74				
	GG = 0.41					
A59V	CC = 0.00	C = 0.15	0.30	0.25	0.22	NS
	CT = 0.30	T = 0.85				
	TT = 0.70					
BM1500	138/138 = 0.02	138 = 0.08	0.20	0.37	0.33	NS
	147/147 = 0.08	147 = 0.15				
	149/149 = 0.70	149 = 0.77				
	138/147 = 0.06					
	138/149 = 0.06					
	147/149 = 0.08					

H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content; HWE = Hardy-Weinberg Equilibrium; NS = no signi¿cative.

Two allelic variants were found for A59V. When the TT genotype was present, fragments of 311 and 147 bp were observed, whereas the CC (fragment of 458 bp) was not detected in the populations studied. The heterozygotes (CT genotype) showed fragments of 458, 311, and 147 bp, but were only found in 30% of the population.

Six different genotypes were obtained using the microsatellite BM1500. The 149/149- and 138/138-bp alleles were the most and least abundant, respectively (Table 1). The allele 149 bp was the most frequent and found in 77% of the populations. According to the classi¿cation reported by Menezes et al. (2006), which considered a polymorphic locus when the frequency of the most common allele was lower than 0.95, all markers in the present study were polymorphic.

The herd studied was not in HWE ($P < 0.05$) for any polymorphism. The PIC described by Botstein et al. (1980) indicates the quality of markers in genetic studies. According to this classi¿cation, a marker with a PIC value higher than 0.5 is considered to be very informative, whereas values between 0.25 and 0.5 are mildly informative, and values lower than 0.25 are not informative. The PIC values found for SNP -1457 and BM1500 (Table 1) indicated that the markers were mildly informative, whereas those for SNP A59V were not informative.

Association analysis

Tables 2 and 3 show the ANOVA and linear regression results for the associations between the markers and weight characteristics in the 3 periods (P1, P2, and P3), as well as the average daily weight gain between periods P1 and P2 and between P2 and P3 (ADG 1/2 and ADG 2/3), respectively.

Table 2. Adjusted means for weight and average daily gain and their association (ANOVA) with SNP-A59V (AF536174.1:g. 321C>T) in Nellore cattle.

	Characteristics				
Polymorphism	W1 (kg)[1]	W2 (kg)[2]	W3 (kg)[3]	ADG1/2 (g)[4]	ADG2/3 (g)[5]
A59V					
TT	397.35 ± 3.11	412.21 ± 3.11	475.37 ± 3.62	0.30 ± 0.02	1.50 ± 0.04
CT	402.77 ± 2.77	414.76 ± 3.40	472.16 ± 3.87	0.24 ± 0.02	1.37 ± 0.05
P	0.43	0.72	0.70	0.45	0.24

[1]□ = 400.06; [2]□ = 413.48; [3]□ = 473.76; [4]□ = 0.27; [5]□ = 1.43. W = weight; ADG = average daily gain. TT and CT = genotypes obtained for the marker A59V.

Table 3. Adjusted means for weight and average daily gain and their association (linear simple regression) with the markers BM1500 and 1457 (AJ571671:g.-1457A>G) in Nellore cattle.

Polymorphism	Characteristics				
	W1 (kg)[1]	W2 (kg)[2]	W3 (kg)[3]	ADG1/2 (g)[4]	ADG2/3 (g)[5]
-1457					
AA	399.82	426.93	498.65	0.55	1.69
AG	393.18	408.72	466.61	0.31	1.37
GG	403.10	414.82	478.33	0.23	1.50
CV(%)	7.56	7.73	7.78	120.88	33.22
P	0.21	0.86	0.70	0.06	0.67
	W1 (kg)[6]	W2 (kg)[7]	W3 (kg)[8]	ADG1/2 (g)[9]	ADG2/3 (g)[10]
BM1500					
138/138	408.01	439.15	491.24	0.63	1.24
147/147	417.60	430.48	491.07	0.26	1.44
149/149	398.58	412.70	476.37	0.28	1.51
138/147	384.00	400.01	456.20	0.32	1.34
138/149	395.38	401.50	456.64	0.12	1.32
147/149	390.88	406.42	460.20	0.31	1.30
CV(%)	7.52	7.60	7.60	122.83	33.09
P	0.12	0.06	0.02	0.50	0.34

[1]□ = 398.7; [2]□ = 416.82; [3]□ = 481.19; [4]□ = 0.36 ; [5]□ = 1.52; [6]□ = 399.07; [7]□ = 415.04; [8]□ = 500.48065-7.96185X, R2 = 0.0483; [9]□ = 0.32; [10]□ = 1.35; W = weight; ADG = average daily gain; CV = coef¿cient of variation.

No signi¿cant association was found between the weight gain of the animals and SNP A59V (Table 2). On the other hand, a signi¿cant association was observed between the microsatellite BM1500 (P < 0.05) and weight in the third period of feeding treatment (Table 3). However, no signi¿cant associations were found between SNP-1457 and the phenotypic traits studied.

Tables 4 and 5 show the means, standard errors, median, and the number of animals used to obtain the ultrasound measurements of carcass traits. No signi¿cant associations (P > 0.05) were found between carcass traits and SNP -1457.

Table 4. Mean (μ), Standard Deviation (SD), median and number of individuals for the analyzed characteristics in Nellore cattle pre-slaughter.

Traits[a]	μ	SD	Median		Marker/Genotype										
					1457			A59V		BM1500[1,2]					
					AA	AG	AG	CT	TT	138/138	138/147	138/149	147/147	147/149	149/149
REA US 1	59.46	0.52	60.08	<	26	19	5	16	34	2	3	39	3	0	3
				≥	27	22	0	11	38	0	5	32	2	6	4
REA US 2	62.09	0.50	62.22	<	26	20	4	11	39	2	3	37	3	1	4
				≥	27	27	1	16	33	0	5	34	2	5	3
REA US 3	68.99	0.60	68.55	<	28	17	4	16	33	2	6	33	2	3	3
				≥	25	24	1	11	39	0	2	38	3	3	4
BT US 1	2.29	0.05	2.3	<	9	11	1	5	16	1	0	17	2	1	0
				≥	44	30	4	22	56	1	8	54	3	5	7
BT US 2[1]	2.58	0.04	2.3	<	2	3	0	1	4	1	0	2	1	1	0
				≥	51	38	5	26	68	1	8	69	4	5	7
BT US 3	3.01	0.05	3.0	<	15	9	0	5	16	1	1	18	0	3	1
				≥	38	32	5	22	53	1	7	53	5	3	6
BT P8 US 1[2]	2.79	0.06	3.0	<	26	19	3	9	39	2	0	36	3	4	3
				≥	27	22	2	18	33	0	8	35	2	2	4
BT P8 US 2	2.97	0.06	3.0	<	15	14	1	3	27	2	1	19	3	3	2
				≥	38	27	4	24	45	0	7	52	2	3	5
BT P8 US 3	3.42	0.06	3.3	<	24	22	4	12	38	1	4	36	2	5	2
				≥	29	19	1	15	34	1	4	35	3	1	5

[a]REA US 1, 2, 3 = sonographic measurements of the rib eye area in the three periods; BT US 1, 2, 3 = sonographic measurements of the backfat thickness in the three periods; BT P8 US 1, 2, 3 = sonographic measurements of the rump fat thickness in the three periods. [1]Signi¿cative association between BT US 2 and BM1500; [2]signi¿cative association between BT P8 US 1 and BM1500.

Table 5. Mean (μ), Standard Deviation (SD), median and number of individuals for the characteristics analyzed in Nellore cattle post-slaughter.

Traits[a]	μ	SD	Median		Marker/Genotype										
					-1457			A59V[1,3,4]		BM1500[2]					
					AA	AG	GG	CT	TT	138/138	138/147	138/149	147/147	147/149	149/149
Physiological maturity	13.62	0.04	13.0	<	1	3	0	1	3	0	1	2	0	0	1
				≥	52	38	5	26	69	2	7	69	5	6	6
Colour	4.32	0.05	4.0	<	4	7	0	2	9	1	2	5	0	1	2
				≥	49	34	5	25	63	1	6	66	5	5	5
Texture	3.88	0.05	4.0	<	21	15	1	13	24	1	4	27	2	2	1
				≥	32	26	4	14	48	1	4	44	3	4	6
REA	28.89	0.37	28.85	<	30	22	2	16	380	0	3	41	4	2	4
				≥	23	19	3	11	34	2	5	30	1	4	3
MAR	4.81	0.25	4.0	<	19	15	3	10	27	0	5	26	1	3	2
				≥	34	26	2	17	45	2	3	45	4	3	5
BT[1]	3.23	0.05	3.17	<	26	21	3	12	38	1	4	38	3	1	3
				≥	27	20	2	15	34	1	4	33	2	5	4
Carcass lenght[2]	1.32	0.00	1.33	<	31	26	2	16	43	0	7	38	3	6	5
				≥	22	15	3	11	29	2	1	33	2	0	2
pH	5.81	0.00	5.82	<	27	21	2	14	36	1	4	37	3	1	4
				≥	26	20	3	13	36	1	4	3	2	5	3
Conformation	6.26	0.12	6.0	<	21	15	2	7	31	1	4	23	3	3	4
				≥	32	26	3	20	41	1	4	48	2	3	3
Fat distribution	1.60	0.03	1.5	<	10	9	2	3	18	0	4	15	1	0	1
				≥	43	32	3	24	54	2	4	56	4	6	6
Muscle colour (a*)[3]	23.96	0.14	23.88	<	27	22	1	18	32	0	4	38	3	2	3
				≥	26	19	4	9	40	2	4	33	2	4	4
Muscle colour (b*)[4]	11.32	0.10	11.27	<	28	19	3	19	31	0	5	36	2	3	4
				≥	25	22	2	8	41	2	3	35	3	3	3
Muscle colour (a*)	11.03	0.29	10.35	<	24	25	1	15	35	0	2	39	2	4	3
				≥	29	16	4	12	37	2	6	32	3	2	4
Muscle colour (b*)	13.99	0.23	14.23	<	24	23	3	13	37	1	1	40	2	3	3
				≥	29	18	2	14	35	1	7	31	3	3	4

[a]REA = rib eye area; MAR = Marbling; BT = backfat thickness. [1]signi¿cative association between BT and A59V; [2]signi¿cative association between BT and BM1500; [3]signi¿cative association between muscle color (a*) and the A59V; [4]signi¿cative association between muscle color (b*) and the A59V.

Signi¿cant associations ($P < 0.05$) were found between SNP A59V and 3 characteristics (post-slaughter): backfat thickness, and muscle color in terms of redness (a*) and yellowness (b*) (Table 5). Signi¿cant associations ($P < 0.05$) were found between the microsatellite BM1500 (Tables 4 and 5) and the following features: rump fat thickness in the ¿rst period and fat thickness in the second period (pre-slaughter); and carcass length (post-slaughter; Figure 1).

Figure 1 shows the 95% con¿dence intervals of the trait estimates according to the genotypes. On the basis of these estimates, it was possible to observe a great overlap of con¿dence intervals for the characteristic BT US 2. Overlapping of con¿dence intervals was not observed between genotypes 138/138 and 147/147 bp and between 149/149 and 147/149 bp.

With respect to the trait BT P8 US 1, the con¿dence interval of the 138/138-bp genotype was signi¿cant in relation to the 147/147-, 149/149-, and 147/149-bp genotypes. The 147/147-bp genotype also showed signi¿cant differences in con¿dence intervals in relation to the 138/149- and 147/149-bp genotypes. There was a remarkable overlap of con¿dence intervals for the carcass length trait, except between the 138/138-, 147/147-, and 138/149-bp genotypes.

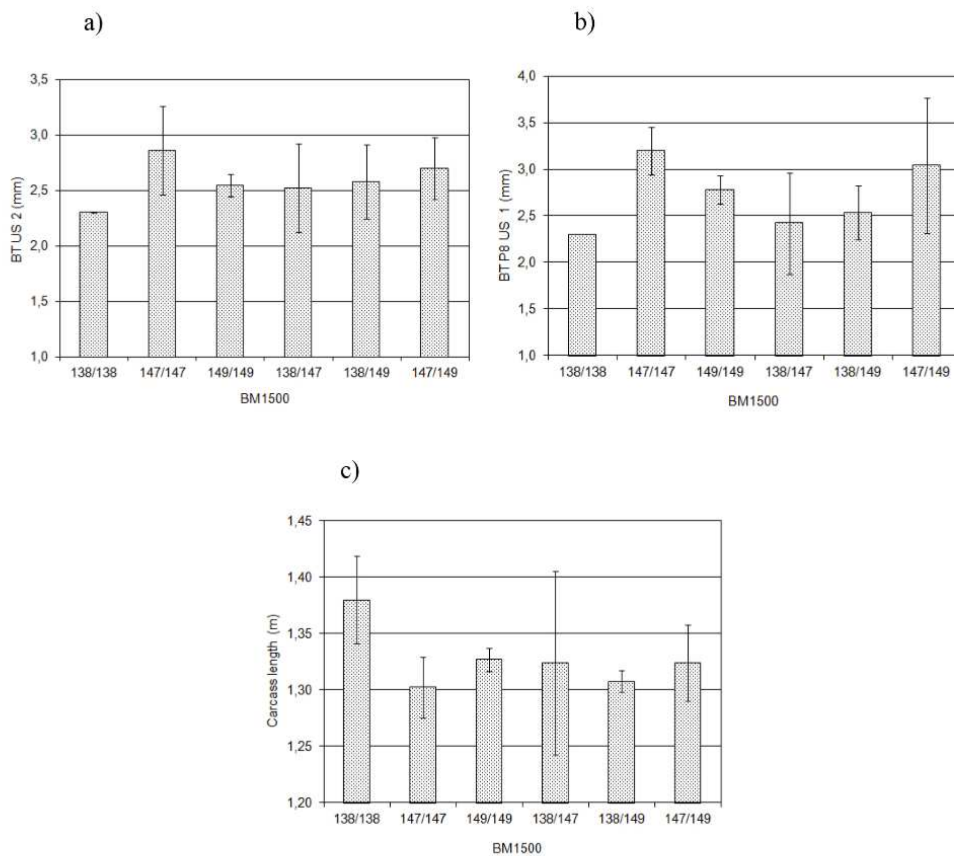

Figure 1. Signi¿cant association between the microsatellite BM1500 and phenotypic traits in Nellore cattle. a. BT US = backfat thickness; b. BT P8 US = Rump fat thickness; c. Carcass length.

DISCUSSION

Genetic diversity

da Silva et al. (2012) studied SNP -1457 in Nellore bulls and reported frequencies of approximately 0.77 for the A allele and 0.23 for the G allele. In the present study, the highest frequency (0.74) was observed for the G allele (Table 1). Giblin et al. (2010) also reported the highest frequency for the G allele (0.52) in Holstein-Friesian cattle.

For the marker A59V, da Silva et al. (2012) found a higher frequency for the C allele (0.99), whereas in the present study the highest frequency was found for the T allele (0.85; Table 1). Öztabak et al. (2010) studied SNP A59V in 120 cattle (Bos taurus taurus) of 3 beef breeds from Turkey (South Anatolian Red, East Anatolian Red, and Turkish Grey) and found a higher frequency for the T allele. Fitzsimmons et al. (1998) studied the microsatellite BM1500 in Angus, Charolais, Hereford, and Simmental cattle breeds and observed a higher genotypic

frequency (0.47) for the 138-bp allele. This differs from the ¿ndings of the present study in which the highest allele frequency detected was for 149 bp.

When comparing the results found in the present study with those obtained by previous studies, the discrepancies in relation to allelic and genotypic frequencies observed for the markers analyzed are notable. Therefore, further genetic diversity studies are needed in cattle populations, particularly the Nellore breed.

The allele and genotype frequencies are expected to vary between breeds and even between different populations of the same breed. Considering not only the genotype/environment interaction, but also the existence of epistatic interactions, pleiotropy, genetic linkage, or segregation of other genes that compose the genome is important to display different combinations of the markers described (Carvalho et al., 2012).

The markers used for the polymorphic sites exhibited reduced levels of observed heterozygosity, revealing a loss of genetic variability. The fact that the herd was not in HWE for any of the polymorphisms was possibly due to arti¿cial selection that occurred in the environment of the animals, thus controlling gene frequencies and genotype of each generation to improve herd productivity. Genetic variability might also decrease due to possible losses and ¿xation of alleles, which might occur as a result of the selection method used.

Association analysis

The polymorphisms 1457 and A59V did not directly inÀuence the weight of the animals of the Nellore breed. However, SNP A59 signi¿cantly affected the body weight of 210-day-old Limousin breed and the average daily gains between 3- and 210-day-old breed (Kulig and Kmieć, 2009). SNP -1457 was associated with the ¿rst luteal activity post-partum and weight during lactation in the Holstein-Friesian breed (Liefers et al., 2005).

Montoya et al. (2009) found a signi¿cant association between the microsatellite BM1500 and body weight at 18 and 24 months of age in cattle of the Velásquez breed. The animals studied might have expressed compensatory gain weight due to the change in their diet (e.g., pasture for grazing plus supplementation).

The characteristics of the animals and their rates of growth and maturation determine the weight and age of the animal for slaughter (Santos et al., 2002). In addition to the genotype, these rates are inÀuenced by the production environment since the phenotypes of the individuals are the result of the interaction between the genotype and environment (Corrêa et al., 2007). The same genotype in different production systems can generate differentiated products and animals with very distinct weights and slaughter ages. The rate at which an animal approaches its adult and slaughter weight is very sensitive to environmental changes, particularly nutrition (Santos et al., 2002).

The animals should have reached physiological maturity in the third management period. The environment could have had a signi¿cant effect on the genotype because the animals were under controlled feeding conditions in the third period.

da Silva et al. (2012) also found no signi¿cant association between polymorphism 1457 and carcass traits in Nellore cattle. This lack of association, both in the study of da Silva et al. (2012) and in the present study, suggested that the marker does not have the potential to be used in genetic improvement programs for the Nellore breed.

In the association analyses, the polymorphism A59V signi¿cantly affected the BT and muscle color in terms of redness (a*) and yellowness (b*; Table 5). Among the factors consid-

ered signi¿cant in the statistical model, the BT measured on the Longissimus dorsi muscle was an effective indicator of carcass ¿nish in the present study and was inversely correlated with the percentage of retail cuts (Silva et al., 2004). The combined inÀuence of these genotypes on environmental conditions and other genes (associated with carcass traits) might determine the factors related to meat quality.

Under normal conditions of storage, color is the main attraction of food. According to Weglarz (2010), meat color is induced mainly by the presence of pigments (myoglobin). However, the color also depends on the composition and structure of the muscle tissue. Therefore, the color of fresh meat is an important quality parameter that determines the consumer response and their decision to buy the product. An intense red color might indicate a higher concentration of myoglobin and is also associated with the age of the animal. Although the values of muscle color (redness) were higher for the TT than the CT genotype (Table 5), the coloration values observed in the present study were within the normal range for redness in bovine meat. The differences observed in relation to the genotypes are not perceptible by the consumer.

Fitzsimmons et al. (1998) reported that the alleles of 138 and 147 bp obtained for the marker BM1500 were associated with high and low levels of fat deposition, respectively, in beef cattle from Angus, Charolais, Simmental, and Hereford breeds. In the present study, signi¿cant associations ($P < 0.05$) between 3 characteristics and the marker BM1500 (Figure 1) indicated that this might be a good marker for assessing the quality of bovine meat.

Associations with BT, BT P8 US, and carcass length might be related to the fact that the alleles for the marker BM1500 are involved in the reduction of the leptin function, leading to increased fat accumulation. Leptin is a regulator of body energy balance, and an organism tends to decrease the amount of hormones when low levels of stored fat are detected. Therefore, the stimulation of lipolysis in adipose tissue does not occur, thereby decreasing energy expenditure in the body, increasing the amount of fat and consequently, body weight (Barsh et al., 2000).

It is also important to consider that the environment inÀuences the expression of genes of animals in studies, with the nutritional factor proving decisive in the appearance and conformation of the animals (Montoya et al., 2009). However, the leptin gene has a profound effect on the metabolism, which indicates a strong possibility that polymorphisms in this gene are associated with other economically important traits (pleiotropic effect).

On the basis of the signi¿cant associations between alleles for the markers A59V and BM1500 and the productive traits analyzed in the present study, it can be concluded that these markers are effective for association studies with production traits in Nellore cattle. Therefore, the markers might be used to aid in the selection of animals with greater potential for weight gain and other productive traits. Despite the results of the present study, further studies are needed to explain the biological mechanism and the relationship of these polymorphisms with phenotypes in Nellore cattle.

ACKNOWLEDGMENTS

The authors thank Agropecuária Jorge Ferreira (São Jorge do Maracay Farm), Frigorí¿co Minerva S/A, Empresa Brasileira de Pesquisa Agropecuária (Embrapa Gado de Corte), as well as the Universidade Federal da Grande Dourados (UFGD) and the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT-MS), for ¿nancial support.

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