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DGAT and LEP gene polymorphisms and their association with carcass characteristics and the lipid profile of meat from Nellore cattle

Polimorfismo dos genes DGAT e LEP e sua associação com características de carcaça e perfil lipídico da carne de bovinos nelore

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ABSTRACT

The objective of this study was to evaluate the association of polymorphisms in the diacylglycerol acyltransferase (*DGAT*) and leptin (*LEP*) genes with the performance, carcass characteristics, meat quality, and lipid profile of Nellore cattle. A total of 100 intact male Nelore cattle were used to analyze the performance, carcass, physicochemical and centesimal composition, and fatty acid profile of beef. To identify the polymorphisms, the PCR–single-strand conformation polymorphism (SSCP) technique was applied to genomic DNA extracted from muscle tissue. The SSCP technique revealed the presence of four band patterns for the *DGAT* gene (AC, AD, AE and BB) with five alleles (A, B, C, D and E). For the *LEP* gene, five band patterns (AA, AB, AC, BB and BC) with three alleles (A, B and C) were observed. For the LEP gene, the AB genotype was associated with higher backfat thickness and ribs weight, while the BB genotype was associated with lower ribs yield; higher hindquarter yield was associated with AC and BB genotypes. Higher contents of C17:0, C18:0 and lower contents of C18:2ω6C, total polyunsaturated fatty acids, total ω6 and ratio of polyunsaturated and saturated fatty acids (PUFA/SFA) were verified for the AC genotype of the LEP gene. The AC and AA genotypes of the LEP gene were associated with higher means of C15:0 and C18:1ω9t. For the DGAT gene, the highest C24:0 content was associated with the AE genotype and

the lowest with the AD and BB genotypes. Polymorphisms in the *DGAT* and *LEP* genes influence carcass parameters and the lipid profile of the meat of Nellore cattle.
Keywords: fatty acids, finishing, PCR-SSCP

RESUMO

Objetivou-se com este estudo avaliar a associação do polimorfismo dos genes da *Diacilglicerol Aciltransferase* (*DGAT)* e Leptina – (L*EP*) em relação ao desempenho, características de carcaça, qualidade de carne e perfil lipídico de bovinos Nelore. Para o estudo, foram utilizados um total de 100 bovinos nelores machos inteiros e avaliado os parâmetros de desempenho, composição da carcaça, composição físico-química, centesimal e perfil lipídico da carne. Para identificação dos polimorfismos foi utilizada a técnica de PCR-SSCP a partir da extração do DNA genômico do tecido muscular. A técnica de *SSCP* revelou a presença de quatro padrões de banda para o gene DGAT (AC, AD, AE e BB) com cinco alelos (A, B, C, D e E) e, para o gene *LEP* foram verificados cinco padrões de bandas (AA, AB, AC, BB e BC) com três alelos (A, B e C). Para o gene LEP, o genótipo AB foi associado a maior espessura de gordura subcutânea e peso do corte ponta de agulha, enquanto o genótipo BB foi associado a menor rendimento do corte ponta de agulha; maior rendimento do corte traseiro foi associado aos genótipos AC e BB. Maiores teores de C17:0, C18:0 e menores de C18: 2ω6C, total de ácidos graxos poli-insaturados, total de ω6 e a relação de ácidos graxos poli-insaturados e saturados (POL/SAT) foram verificados para o genótipo AC do gene LEP. Os genótipos AC e AA do gene LEP foram associados a maiores médias de C15:0 e C18:1ω9t. Para o gene DGAT, os maiores teores de C24:0 foram associados o genótipo AE e o menores aos genótipos AD e BB. A ocorrência de polimorfismo nos genes DGAT e LEP revelaram influência destes sobre parâmetros de carcaça e perfil lipídico da carne de bovinos Nelore.
Palavras-Chave: ácidos graxos, acabamento, PCR-SSCP

INTRODUCTION

Molecular genetics tools have been effecting at identifying polymorphisms at loci associated with the production of enzymes involved in the synthesis of fatty acids and study their possible associations with performance, carcass characteristics, and lipid profile in ruminants. Some polymorphisms affecting these characteristics have been described in the genes encoding the enzymes diacylglycerol acyltransferase (*DGAT*) and leptin (*LEP*) (Grisart et al., 2002; Taniguchi et al., 2002; Casas et al., 2005).

Diacylglycerol acyltransferase catalyses the final phase of triglyceride synthesis that occurs in the endoplasmic reticulum membrane, affecting fat metabolism, including its production and percentage in milk (Cases et al., 1998, 2001; Silva et al., 2011). This enzyme is found in several tissues but has greater activity in the liver, adipose tissue, and lactating mammary gland (Fang et al., 2012). Polymorphisms in the *DGAT* gene have been associated with production characteristics, milk composition, subcutaneous fat deposition, and marbling in beef cattle (Casas et al., 2005; Wu et al., 2005; Tantia et al., 2006; Schennink et al., 2007).

Leptin is a hormone encoded by the obesity gene and is expressed in adipocytes, mainly in white adipose tissue, but also in skeletal muscle, mammary epithelium, gastric epithelium, and placenta in smaller proportions (Chilliard et al., 2005). In

cattle, *LEP* gene polymorphisms have been associated with carcass fat deposition (Lien et al., 1997; Pomp et al., 1997; Buchanan et al., 2002), ribeye area (Oprzadek et al., 2003), and growth characteristics and finishing precocity (Yang et al., 2007). Thus, due to its relevance, *LEP* has been studied as a candidate gene in studies related to characteristics of economic interest (Oprzadek et al., 2003; Shin & Chung, 2007; Corva et al., 2009), such as sexual precocity, percentage of milk fat, milk production, weight gain, carcass fat deposition, and meat quality (Lusk, 2007; Kulig & Kmiec, 2009; Lara et al., 2012).

The objective of this study was to evaluate the association of polymorphisms in the *DGAT* and *LEP* genes with the performance, carcass characteristics, meat quality, and lipid profile of the meat of Nellore cattle.

MATERIAL AND METHODS

The experiment was conducted in the feedlot of the Frialto group, located 15 km from the city of Sinop, state of Mato Grosso (MT), Brazil. To perform this study, a population of 100 intact male Nellore animals was used, with an average initial weight of 386.19 ± 4.48 kg and slaughtered at a weight of 527.82 ± 17.96 kg at age 13-36 months. Cattle population numbers were selected from the contemporaneous group of animals, based on environmental conditions and feedlot management practices. The ingredients were supplied as complete feed for 88 days and were offered *ad libitum*. The diets were formulated to meet the requirements of 1.5 kg of daily weight gain, according to the NRC (2000). This study was approved by the Ethics Committee on Animal Use (CEUA) of UFLA under number 040/12.

To evaluate performance, the daily weight gain of the animals was measured, which corresponded to the difference between the initial and final weight divided by days in the feedlot (88 days). After slaughter and cooling of the carcasses for 24 hours at ± 1°C, cuts and linear evaluations of the carcasses were performed, and the following parameters were determined: backfat thickness (BF), ribeye area (Striploin) (REA), cold carcass weight, cold carcass yield, and weights and yields of cuts (forequarter, ribs, and hindquarter).

The REA and BF measurements were taken in the left half carcass through a cut between the 10th and 11th ribs after 24 hours of cooling. To determine the REA, the contour of the *longissimus thoracis* muscle was drawn on tracing paper. This paper with the drawing of the muscle area was scanned in a scanner, and the area was analysed by ImageJ® software. The area was determined in cm^2. The subcutaneous fat area was determined in millimetres (mm) using a digital calliper (DIGIMESS, China).

After slaughter and cooling of the carcass for 24 hours at ± 1°C, samples of the *longissimus thoracis* muscle (Striploin) were collected to determine the physicochemical parameters and proximate composition. The final pH was determined 24 hours after slaughter in the Striploin cut of the left half-carcass using a pH metre with a penetration probe (Hanna Instruments, HI 99163, Romania). For this analysis, a colorimeter (Konica Minolta CM-700, Singapore) was used, operating in the CIEL*a*b* system, with a D65 illuminant, a 10° observer angle, and specular component excluded (SCE) measurement mode, to obtain the lightness (L*), redness (a*), and yellowness (b*) indices. The saturation index (C*) and hue angle (h*) values were determined according to Ramos &

Gomide (2012). To determine the cooking loss (CL), the samples were weighed on a semi-analytical scale (METTLER M P1210, Toledo, Brazil), wrapped in aluminium foil, and cooked on an electric plate at 150°C until reaching 72°C inside of the sample, as described by Amasa (1978). The shear force was determined using a texture analyser with a Warner-Bratzler shear device (Chrystall & Devine, 1991). The proximate composition was determined by A.O.A.C. (1990).

To analyse the fatty acid profile, samples of *longissimus thoracis* (Striploin) muscle tissue were collected from each animal. Lipid extraction followed the method of Folch et al. (1957), and the fatty acids were esterified according to Hartman & Lago (1973). Fatty acid analysis was performed by gas chromatography in a Shimadzu GC-2010 chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with a flame ionization detector, split injector at a 1:50 ratio, and Supelco SPTM-2560 capillary column, 100 m × 0.25 mm × 0.20 µm (Supelco Inc., Bellefonte, PA, USA). The chromatographic conditions were an initial column temperature of 140°C/5 min, an increase of 4°C/min to 240°C, and a hold of 30 minutes, totalling 60 minutes. The injector and the detector temperature were 260°C. The carrier gas used was helium. The fatty acids were identified by comparing the retention times observed against those of the chromatographic standard SupelcoTM37 standard FAME Mix® (Supelco Inc., Bellefonte, PA, USA) and are expressed as a percentage (%) of the total fatty acids identified.

Genomic DNA was extracted from samples of the *longissimus dorsi* muscle (Striploin) muscle following CTAB protocol for DNA extraction (Catonichexadecyl trimethyl ammonium bromide) described by Stefanova et al. (2013). The concentration and purity of the extracted DNA were quantified by reading the absorbance at 260 nm and 280 nm in a NanoDrop ND-1000 UV/Vis Spectrophotometer. The samples were diluted with sterile water to obtain the desired final concentration of 10 ng DNA/µL. Next, the integrity gel was prepared to ensure we only took excellent-quality genomic DNA for experiments.

To perform the DNA analysis, primers were designed for the evaluated genes *DGAT* (exons 16 and 17) (427 bp): forward (5'TCTTCCACGAGGTCAGTGC3') and reverse (5'GGCAAAGCAGTCCAACACC3'); and *LEP* (exon 2) (500 bp): forward (5'CTCTAGGGAAAGGCGGAGTC3') and reverse (5'CAGCCAGAAGCTCAGGTTTC3') using online software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi)). First, the genetic sequences homologous to the selected genes were analysed using the Basic Local Alignment Search Tool software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the sequences available in GenBank (http://www.ncbi.nlm.nih.gov/). Next, Oligo Perfect and Oligo Analyser software were used to design the primer pairs for each gene.

Conventional polymerase chain reaction (PCR) was performed on 50 ng of genomic DNA in a final volume of 25 µl, containing 1× reaction buffer, 200 µM dNTPs, 1.25 U *Taq* DNA polymerase, 2.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 0.5 µM of each primer, and H_2O. The PCRs were performed in a thermocycler (Mastercycler Eppendorf, USA) with the following thermal cycling profile: 5 minutes for initial denaturation

at 95°C, 30 seconds for denaturation at 95°C, 30 seconds for annealing of primers (61°C and 60°C for *DGAT* and *LEP*, respectively), and 1 minute for extension at 72°C. The final extension was 2 minutes at 72°C. The PCR products were visualized by electrophoresis in a 1% agarose gel using TAE buffer and 1× staining with 200 ng/ml ethidium bromide.

The amplified products from the different primers were subjected to electrophoresis in a 1.0% agarose gel in 1× TBE (45 mM Tris-borate, pH 8.0, and 1 mM EDTA) with 0.8 µg/mL ethidium bromide, and the resulting electrophoretic profiles were visualized in the gel and photo documented (Spectroline Ultraviolet Transilluminator).

Gene mutations were analysed using the single-strand conformation polymorphism (PCR-SSCP) comparative method, in which 1 µL of each PCR product was added to 10 µL of denaturing buffer (98% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylenocyanol). After denaturation at 95°C for 5 minutes, the samples were immediately placed on ice and then loaded into a 12.5% acrylamide: bisacrylamide (37.5:1) gel. The samples were subjected to polyacrylamide gel electrophoresis in a horizontal vat (35×15) at 20°C in 0.5× TBE buffer for 6 hours for each gene, at 180 V, 150 mA, and 100 w. The gels were stained according to Byun et al. (2009).

Genotypic frequency and allele frequency were performed by direct counting in relation to the total polymorphisms identified in each gene (Ramalho et al., 2012). The data were analysed using the GLM procedure of the Statistical Analysis System (SAS 9.3, 2011, SAS Inst. Inc., Cary, NC). The age of the animals was considered a blocking variable, and the means were compared by Tukey's test (P=0.05) to evaluate the genotypes of each gene in relation to performance variables, carcass traits, physicochemical parameters, proximate composition, and fatty acid profile.

RESULTS

In this study, the *DGAT* and *LEP* genes were evaluated in Nellore cattle. The PCR products of these genes were subjected to gel electrophoresis, separating the DNA fragments according to size, i.e., DNA fragments of the same length formed a single band in the gel and were put directly into PCR-SSCP analysis.

Four different genotypes of the *DGAT* gene were found: AE (n=12), AD (n=21), BB (n=58), and AC (n=7), with five different alleles (A, B, C, D, and E). Five different genotypes of the *LEP* gene were found: AA (n=12), AB (n=36), BB (n=36), BC (n=10), and AC (n=4), with three different alleles (A, B and C). The frequency of the genotypes and alleles of the *DGAT* and *LEP* genes are shown in Table 1.

Table 1 - Frequency of the genotypes and polymorphism alleles according to the PCR-SSCP of Diacylglycerol acyltranferase (DGAT) and Leptin (LEP)

Genes	Genotype	Frequencies	Allele	Frequencies
LEP	AA	0.1224	A	0.3421
	AB	0.3673	B	0.5658
	AC	0.0408	C	0.0921
	BB	0.3673	-	-

	BC	0.1020	-	-
DGAT	AC	0.0714	A	0.2899
	AD	0.2143	B	0.4203
	AE	0.1224	C	0.0507
	BB	0.5918	D	0.1522
	-	-	E	0.0870

Statistical analysis showed no difference in performance variables or carcass parameters with *DGAT* genotype. However, there was a difference in BF, ribs weight (RW), ribs yield (RY), and hindquarter yield (HY) with *LEP* genotype (Table 2). However, there was no difference in the physicochemical parameters or proximate composition according to the *DGAT* or *LEP* gene.

Table 2 - Statistical analysis of performance, carcass parameters, physicochemical and chemical composition parameters of the meat from Nellore cattle associated with the *DGAT* and *LEP* genes.

Variables	*DGAT*			*LEP*		
	Mean	SEM	P value*	Mean	SEM	P value*
Performance						
Initial weight (kg)	382.72	17.27	0.969	378.40	27.95	0.823
Final Weight (kg)	526.68	27.70	0.873	516.36	35.23	0.191
DWG (kg/day)	1.64	0.16	0.969	1.57	0.21	0.334
Carcass Parameters						
REA (cm^2)	70.56	3.51	0.807	68.31	5.80	0.686
BF (mm)	6.32	1.02	0.770	6.66	1.31	0.047
CCW (kg)	282.43	15.96	0.988	275.03	20.21	0.181
CCY (%)	53.60	0.68	0.241	53.25	0.91	0.580
FW (kg)	111.83	7.46	0.950	107.62	9.43	0.108
RW (kg)	31.96	2.53	0.941	31.04	3.09	0.010
HW (kg)	138.63	6.48	0.881	136.36	8.37	0.432
FY (%)	39.45	0.52	0.583	39.04	0.66	0.095
RY (%)	11.28	0.43	0.542	11.25	0.53	0.044
HY (%)	49.28	0.70	0.976	49.71	0.84	0.002
Physicochemical						
pH final	5.66	0.12	0.971	5.68	0.15	0.219
L* - lightness index	26.79	1.23	0.964	26.70	1.98	0.129
A* - redness index	18.11	1.71	0.993	17.60	2.17	0.082
B* - yellowness index	16.75	1.13	0.642	16.36	1.45	0.104
C* - saturation index (chroma)	24.69	1.97	0.950	24.06	2.50	0.101
H* - color hue angle	42.86	1.30	0.151	43.04	1.73	0.474
Cooking Loss (%)	31.99	6.11	0.510	33.54	8.08	0.930
Shear Force (kgf)	11.29	1.38	0.705	10.51	1.79	0.237
Proximate composition						
Ashes	1.30	0.11	0.669	1.27	0.14	0.764
Moisture	75.30	0.39	0.849	75.32	0.50	0.226
Ether extract	3.02	0.43	0.311	3.05	0.59	0.878
Protein	17.94	0.65	0.070	17.97	0.88	0.565

*F test (α=0.05); *DGAT* = effect of the genotype of the *DGAT* gene; LEP = effect of the genotype of the *LEP* gene; DWG - daily weight gain; REA – ribeye area; BF - backfat thickness; CCW - cold carcass weight, CCY - cold carcass yield; FW - forequarter weight; RW - ribs weight; HW - hindquarter weight; FY - forequarter yield; RY - ribs yield; HY - hindquarter yield. SEM = standard error of the mean.

Statistical analysis revealed significant differences in lignoceric acid (C24:0) with *DGAT* genotype and in the pentadecanoic (C15:0), margaric (C17:0), stearic (C18:0), elaidic (C18:1ω9t), and linoleic (C18:2ω6c) fatty acids; PUFA; total ω6 fatty acids (∑ω6); and the PUFA/SFA ratio with *LEP* genotype (Table 3).

Table 3 - Statistical analysis of the lipid profile of meat from Nellore cattle associated the *DGAT* and *LEP* genes.

Variables	*DGAT*			*LEP*		
	Mean	SEM	P value*	Mean	SEM	P value*
Fatty acids (%)						
C10:0	0.11	0.03	0.139	0.09	0.02	0.371
C12:0	0.10	0.02	0.577	0.10	0.02	0.351
C14:0	2.46	0.19	0.550	2.53	0.25	0.188
C14:1	0.42	0.06	0.580	0.40	0.07	0.618
C15:0	0.34	0.03	0.336	0.36	0.04	0.037
C16:0	24.50	0.38	0.911	24.55	0.49	0.335
C16:1	2.97	0.14	0.168	2.93	0.18	0.210
C17:0	0.91	0.06	0.223	0.95	0.08	0.015
C17:1	0.69	0.05	0.470	0.67	0.07	0.364
C18:0	15.32	1.17	0.590	16.15	1.48	0.038
C18:1ω9t	1.67	0.23	0.210	1.79	0.29	0.010
C18:1ω9c	39.17	1.21	0.650	39.23	1.62	0.688
C18:2ω6t	0.11	0.01	0.150	0.12	0.02	0.065
C18:2ω6c	8.96	1.77	0.099	8.13	2.32	0.044
C20:0	0.15	0.01	0.324	0.15	0.01	0.939
C18:3ω6	0.03	0.01	0.091	0.03	0.01	0.841
C20:1	0.29	0.05	0.875	0.28	0.06	0.327
C18:3ω3	0.52	0.05	0.264	0.47	0.07	0.242
C20:2	0.16	0.04	0.316	0.16	0.05	0.405
C22:0	0.10	0.02	0.523	0.08	0.03	0.484
C20:3ω6	0.16	0.03	0.532	0.14	0.03	0.125
C22:1ω9	0.01	0.04	0.234	0.01	0.05	0.311
C20:4ω6	0.64	0.11	0.154	0.53	0.15	0.367
C24:0	0.02	0.02	0.022	0.02	0.02	0.793
C20:5ω3	0.15	0.04	0.436	0.12	0.05	0.340
Sum						
SFA	44.05	1.59	0.559	44.98	2.03	0.080
MUFA	45.20	1.34	0.525	45.31	1.79	0.674
PUFA	10.73	1.86	0.118	9.69	2.43	0.045
∑ω3	0.66	0.07	0.201	0.59	0.10	0.128
∑ω6	9.90	1.81	0.118	8.94	2.38	0.046

Ratios						
∑ω6/∑ω3	15.00	3.22	0.332	14.99	4.30	0.676
PUFA/SFA	0.25	0.05	0.152	0.23	0.07	0.046

*F test (α=0.05); *DGAT* = genotype effect of the *DGAT* gene; *LEP* = genotype effect of the *LEP* gene; SFA = sum of total saturated fatty acids; MUFA = sum of total monounsaturated fatty acids; PUFA = sum of total polyunsaturated fatty acids; ∑ω3 = sum of total omega-3 fatty acids; ∑ω6 = sum of the total omega-6 fatty acids; SEM = standard error of the mean.

The animals with the highest BF and RW had the *LEP* AB genotype, while the lowest values were found in the AA(BF) and BB(RW) genotypes. RY only differed under the BB genotype, which had lower means. The highest mean HY was found with the AC and BB genotypes, and the AB genotype had the lowest mean, Table 4.

The animals with the AA and AC genotypes of the *LEP* gene showed higher levels of pentadecanoic (C15:0) and elaidic (C18:1ω9t) fatty acids, while the animals with the AB and BB genotypes had lower values of margaric (C17:0), stearic (C18:0), and elaidic (C18:1ω9t) fatty acids. The animals with the AC genotype showed higher values of margaric (C17:0) and stearic (C18:0) fatty acids and lower values of conjugated linoleic acid (C18:2ω6c), PUFA, total ω6 fatty acids, and the ratio of PUFA/SFA. The BB and BC genotypes of the *LEP* gene had the highest values of conjugated linoleic acid (C18:2ω6c), PUFA, total ω6 fatty acids, and PUFA/SFA. In the *DGAT* gene, the animals with the highest lignoceric fatty acid values (24:0) had the AE genotype, while the lowest values were found in the AD and BB genotypes (Table 4).

Table 4 - Breakdown of the parameters measured in the carcasses and the lipid profile of the meat (Striploin) of Nellore cattle according to the genotypes of the *DGAT* and *LEP* genes.

Gene		Genotype				
	Parameters	AA	AB	AC	BB	BC
	Backfat thickness (mm)	5.41b	7.54a	6.90ab	5.90ab	6.33ab
	Ribs weight (kg)	31.38ab	35.07a	29.75ab	29.16b	30.19ab
	Ribs yield (%)	11.26a	11.74a	11.16a	10.75b	11.37a
	Hindquarter yield (%)	49.53ab	48.47b	50.67a	50.12a	49.56ab
	C15:0	0.39a	0.33ab	0.42a	0.32b	0.33ab
LEP	C17:0	1.00ab	0.89b	1.08a	0.88b	0.91ab
	C18:0	15.60ab	15.32b	19.55a	14.81b	15.46ab
	C18:1ω9t	2.06a	1.52b	2.18a	1.53b	1.63ab
	C18:2ω6c	7.31ab	8.89ab	4.65b	9.66a	10.15a
	PUFA	8.89ab	10.56ab	5.75b	11.45a	11.81a
	∑ω6	8.14ab	9.75ab	5.22b	10.59a	11.00a
	PUFA/SFA	0.20ab	0.25ab	0.12b	0.27a	0.29a
Gene		Genotype				
DGAT	Fatty acid	AC	AD	AE	BB	
	C24:0	0.03ab	0.01b	0.05a	0.01b	

*Tukey's test (α=0.05); ∑ω6 = sum of the total omega-6 fatty acids; PUFA/SFA = ratio sum of total polyunsaturated fatty acids/total Saturated Fatty acids.

DISCUSSION

The different genotypes of the *DGAT* gene showed no relationship with the carcass parameters of Nellore cattle. In fact, no associations between *DGAT* gene polymorphisms and any of the performance and carcass characteristics analysed in cattle have been reported (Casas et al., 2005; Fortes et al., 2009; Ardicli et al., 2019). The physicochemical parameters and proximate composition related to meat quality were also not associated with the polymorphisms in the *LEP* or *DGAT* gene. The same trend was observed by Casas et al. (2005) and Fortes et al. (2009). This corroborates the results found in the present study, suggesting that the lack of association of the genes with carcass composition parameters and meat quality characteristics in *Bos indicus* cattle may be due to the genetic composition of the breed or to the polygenic effects associated with the expression of each trait (Ardicli et al., 2017).

For *LEP*, the AB genotype showed higher BF and RW, while the BB genotype had lower RY. The AC and BB genotypes had higher HY. In the study by Nkrumah et al. (2004), evaluating polymorphisms in the *LEP* gene and their association with fat content in bovine carcasses using the PCR–restriction fragment length polymorphism (RFLP) technique, the authors observed that polymorphisms in the *LEP* gene showed associations with carcass fat content and carcass and lean meat yield, where the animals with the TT genotype had higher carcass fat content while the CT animals showed greater BF. However, TT animals had lower lean meat yield and yield grades than CT and CC animals. Ardicli et al. (2017) investigated the effects of *LEP* gene polymorphisms on the fattening performance parameters and carcass traits of 81 Simmental cattle in Turkey and found *LEP* SNPs that had a significant effect on hot carcass weight, chilled carcass weight, and carcass yield ($P<0.05$). The animals with the TT genotype had higher hot carcass weight and chilled carcass weight than animals with the CC or CT genotype.

The present study found an association between the *LEP* gene polymorphisms of Nellore cattle and their yield and fat deposition traits. Similar results were found by Carvalho et al. (2012), who evaluated allele and genotype frequencies of polymorphisms in the *LEP* gene (exon 2) in 201 crossbred cattle and observed that they had a direct association with backfat thickness.

Regarding the lipid profile and its association with the *LEP* gene, two genotypes showed the opposite behaviour: The AC animals showed higher values of SFA (C 15:0, C17:0 and C18:0) and monounsaturated (C18:1ω9t) fatty acids and lower values of PUFA, ω6 (C18:2ω6c and ∑ω6), and PUFA/SFA ratio than the BB genotype. Kawaguichi et al. (2020) found an association of the CC genotype with higher values of C16:0 and SFA and lower values of C16:1, C18:1ω9, and MUFA. Conversely, the animals with the CT genotype showed higher values of C16:1, C18:1ω9, and MUFA and lower values of C16:0 and SFA (exon 2). Papaleo Mazzucco et al. (2016) observed that the CC genotype showed higher SFA and lower C18:1ω9 and C22:5ω3 fatty acids, while the CT genotype showed higher C22:5ω3 fatty acids and lower C18:1ω9 and MUFA. Conversely,

the TT genotype showed higher C18:1ω9 and monounsaturated fatty acids and lower C22:5ω3 and SFA.

The present study showed that animals with the AC, BB, and BC genotypes may provide meat with a better fatty acid profile for consumption. Animals with the AC genotype had a high amount of stearic acid (C18:0), which represents 43% of the SFA in meat and has a neutral function or even leads to lower cholesterol levels, since the animal metabolically transforms it into oleic acid (C18:1ω9). Likewise, the animals with the BB and BC genotypes had high levels of PUFA, the majority of which plays an important role in decreasing blood cholesterol (Spector, 1999). Similarly, these same animals showed higher amounts of omega-6 fatty acids, which are essential fatty acids and precursors of a set of eicosanoid substances, including thromboxanes, prostaglandins (which have hypotensive effects), prostacyclins (which inhibit platelet aggregation and increase high-density lipoprotein), and leukotrienes (Wynder et al., 1997; Din et al., 2004; Barbosa et al., 2007).

For the *DGAT* gene, an effect of genotype was only observed on the value of lignoceric acid (24:0). Urtnowski et al. (2011) evaluated the association between polymorphisms in the *DGAT* gene and characteristics related to meat production and quality in 156 Holstein cattle and found a relationship between *DGAT* exon 8 and lipid composition, with genotypes AA and GA showing higher values of conjugated linoleic acid (C18:2ω6t) and lower values of lauric acid (C12:0), while the animals with the GG genotype showed the opposite behaviour, higher lauric acid and lower conjugated linoleic acid. Tabaran et al. (2015) evaluated the occurrence of polymorphisms of this gene in 550 Holstein cattle and buffaloes in Romania and their effects on the fat percentage and fatty acid profile in milk, finding that *DGAT* gene polymorphisms resulted in increased production of C16:0 fatty acids and saturated/unsaturated fatty acid ratios and a reduction in the C14:0 and C18:0 values. However, there was no association of the *DGAT* gene polymorphisms with the other parameters of the lipid profile in the present study. This may be due to the genetic profile of the animals. The authors who found associations with *DGAT* gene polymorphisms studied dairy animals, and this effect may be less expressive in zebu beef cattle such as Nellore.

In spite of the positive results in this study, we recognize one limitation **which** was the small sample size and more studies should be carried out to confirm these results and to determine possible associations between these markers and other phenotypic traits in larger populations of Nelore Cattle.

CONCLUSIONS

The *LEP* gene polymorphisms affected carcass parameters and composition in relation to backfat thickness, ribs weight and yield, and hindquarter yield.

DGAT and *LEP* gene polymorphisms influence beef lipid composition differently, with *LEP* polymorphisms having a greater effect on the fatty acid profile than *DGAT* polymorphisms.

The performance, physicochemical and centesimal composition of the meat from nelore cattle, was not associated with polymorphisms of the *LEP* or *DGAT* genes.

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