PE1.16 Polymorphisms of genes related to carcass and meat traits in Brazilian beef cattle 133.00

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Abstract - Fat deposition is one of the most important factors affecting carcass and beef quality. Our objectives were to study allele frequency and effect on beef traits of polymorphisms of three genes associated with fat metabolism in a population of Brazilian beef cattle. The studied genes were leptin (LEP), thyroglobulin (TG) and diacylglycerol O-acyltransferase 1 (DGAT1). The studied population included Nellore (Bos indicus) and Nellore crossed with Bos taurus cattle. Three hundred animals, including 114 Nellore, 67 Angus x Nellore, 44 Rubia Gallega x Nellore, 41 Canchim, 19 Brangus three-way cross and 15 Braunvieh three-way cross, were genotyped for the polymorphisms: LEP/Kpn2I (C/T in exon 2); TG/PsuI (C/T in 3' UTR) and DGAT1/CfrI (K232A in amino-acid sequence). The phenotype included backfat thickness, intramuscular fat, rib eye area, shear force and myofibrillar fragmentation index. In the association study, data analyses were performed with a linear model that included combined effect of genetic and contemporary group and genotype effect. Least square means of the genotypes were compared using Tukey test and the correction for multiple comparisons was done by the Bonferroni method. LEP/Kpn2I was polymorphic in Bos indicus animals, f(T) = 0.057. The T allele of TG was fixed in Nellore and in Rubia Gallega x Nellore. The DGAT1 was polymorphic in all genetic groups, but the A allele frequency was lower in Nellore animals. In spite of the importance of the studied genes for fat metabolism, it was not possible to find evidences supporting the association between the studied polymorphisms and meat traits. Financial support: Fapesp and CNPq

Key words: polymorphisms, candidate gene, fat deposition, beef cattle

I. INTRODUCTION

Intramuscular and subcutaneous fat depositions have direct influence on beef quality, thus affecting consumers' choice. Subcutaneous fat deposition is necessary for carcass quality and it has been highly associated with carcass yield 1. Marbling, or intramuscular fat deposition, affects the habit of consumers as well as the final shelf price of beef 2. To address these industry issues via selection of superior cattle is an achievement of molecular biology. Today, gene polymorphisms that were associated with traits of economical interest are commercially available 3. Leptin, thyroglobulin and diacylglycerol O-acyltransferase, encoded by LEP, TG and DGAT1 genes, respectively, play important roles in fat metabolism and energy balance. The polymorphisms C/T in exon 2 of LEP 4, C1696T (GenBank M35823) in 5'UTR of TG 5 and GC/AA (positions 10433/10434: GenBank AJ318490) in exon 8 of DGAT1 6 have been included in studies that aim to describe an association between molecular markers and beef or dairy traits. Considering all the above and the relevance of the Nellore breed (B. indicus) and their crosses with B. Taurus in Brazil, the present work aimed to estimate the allele and genotype frequencies of LEP, TG and DGAT1 gene polymorphisms in different beef genetic groups (Nellore and Nellore x B. taurus) and to associate genotypes with backfat thickness, intramuscular fat deposition, rib eye area, shear force and myofibrillar fragmentation index.

II. MATERIALS AND METHODS

Samples of the Nellore breed (B. indicus, n = 114), Angus x Nellore cross (1/2 B. taurus + 1/2 B. indicus, n = 67), Canchim breed (5/8 B. taurus + 3/8 B. indicus, n = 41), Brangus three-way cross (9/16 B. taurus + 7/16 B. indicus, n = 19) and Braunvieh three-way cross (3/4 B. taurus + 1/4 B. indicus, n = 15) were analyzed. These animals, originating from commercial herds, were born in seven farms and bred in the same feedlot sector

(intensive system), in the years 2003, 2005, 2006 and 2007. Additional 44 samples represented the crossing between Rubia Gallega sires (B. taurus) and Nellore dams (Rubia Gallega x Nellore: 1/2 B. taurus + 1/2 B. indicus), which were bred in a semi-intensive cattle-raising system in 2006.

The germplasma composition of each genetic group was previously described by Curi et al. 7. The 300 animals used in the trial, 32 females and 268 males, were bred according to Brazilian legislation for animal well-being and slaughtered at 15, 17 and 19 months of age. After slaughter, the carcasses were identified and chilled for 24 hours. Two 2.54 cm thick samples of Longissimus dorsi muscle were then removed from an area between the 11th and 13th ribs of the left half carcasses. Samples collected between the 12th and 13th ribs provided measurements of the Rib Eye Area (REA), Backfat Thickness (BT) and Shear Force (SF). Samples collected from between the 11th and 12th ribs allowed measurements of the Myofibrillar Fragmentation Index (MFI) and Intramuscular Fat (IF, percentage of total lipids), as well as the extraction of genomic DNA. The REA was measured by the quadrant point method and the BT was determined with the aid of a ruler, both according to the methodology described by the USDA Quality Grade 8.

After these first measurements, carried out in the slaughterhouses, the samples of Longissimus dorsi muscle were de-boned, vacuum wrapped and aged at controlled temperature (between 1 and 2 °C) for 14 days, followed by freezing at -20 °C. The other phenotypes, SF, MFI and IF, were determined in the laboratory following methodologies described by Wheeler et al. 9, Culler et al. 10 and Bligh and Dyer 11, respectively. The extraction of genomic DNA from meat samples (250 mg) was accomplished by the non-phenolic method, utilizing digestion with proteinase K and precipitation with NaCl and alcohol.

The polymorphisms of the genes LEP, TG and DGAT1 were genotyped with the PCR – RFLP method as previously reported. Alleles C and T of the LEP gene were identified by the amplification of a 94 bp fragment, part of exon 2, followed by digestion with Kpn2I as per Choudhary et al. 12. Alleles C and T of the polymorphism C1696T (GenBank M35823), mapped to 5' UTR region of TG gene, were identified by the amplification of a 548 bp fragment, followed by restriction with PsuI as per Thaller et al. 13. Alleles A and K of the polymorphism GC/AA (positions 10433/10434: GenBank AJ318490) of DGAT1, were identified by the amplification of a 411 bp fragment, part of exon 8, followed by digestion with CfrI as per Lacorte et al. 14.

The allele and genotype frequencies were calculated for each of the polymorphisms and differences in allele frequencies within and among genetic groups were calculated using contingency tables 15. For the association studies, the traits of interest were analyzed utilizing General Linear Model (GLM) of the program Statistical Analysis System 16 and the least square means of the genotypes were compared by the Tukey test. The Bonferroni correction was applied to analyses involving multiple comparisons.

The linear model for adjustment of quantitative variables included the genotype and the contemporary group effects as follows: $Yijk = \mu + Gi + CGj + eijk$, where Yijk = trait of interest, μ = general mean, Gi = fixed effect of ith genotype (i = 1, ..., 3), CGj = fixed effect of jth contemporary group (j = 1, ..., 13), and eijk = random error. The definition of contemporaries groups included variations of genetic group, sex, age of slaughter, feedlot year and farm of origin.

Groups that presented a single genotype and genotypes whose frequencies were less than or equal to 0.10 in the total sample of studied animals were excluded from analyses. The sire effect was not included in the linear model since the number of genotyped offspring of individual sires was very small.

III. RESULTS AND DISCUSSION

We have identified in these study animals both alleles, C and T, for the polymorphism LEP/Kpn2I, as previously identified by Buchanan et al. 4. The alleles of TG/PsuI, C and T, present on our study were originally described by Barendse 5. We were also able to report the alleles A and K of the polymorphism DGAT1/CfrI as previously identified by Winter et al. 6. Allele and genotype frequencies for all genetic groups are presented in tables: 1 for LEP/Kpn2I; 2 for TG/PsuI and 3 for DGAT1/CfrI. The LEP/Kpn2I was polymorphic in Bos indicus animals (Nellore). The T allele

frequency of 0.057 is similar than the previously 0.043 reported in a smaller sample size 17. The T allele of TG/PsuI polymorphism was fixed in Nellore and in Rubia Gallega x Nellore. The DGAT1/CfrI was polymorphic in all genetic groups, but the A allele frequency was lower in Nellore animals. Hence, for Bos indicus of the Nellore breed, the three polymorphisms were not ideal for association studies with meat traits, do to low or zero frequency of one of the alleles.

These results have confirmed a previous report 17 in a greater sample size and so the use of these three markers is not encouraged for most Brazilian herds. Tables 4, 5 and 6 show comparisons between least square means and standard errors of the quantitative traits, determined for the genotypes of LEP/Kpn2I, TG/PsuI and DGAT1/CfrI, in that order. These attempt to associate the polymorphisms LEP/Kpn2I, TG/PsuI and DGAT1/CfrI with Backfat Thickness (BT) and intramuscular fat (IF) was not successful.

Despite the lack of appropriated allele distribution in Nellore, it should have been possible to detect LEP/Kpn2I and DGAT1/CfrI associations with BT and IF in the crossbred genetic groups (Bos taurus x Bos indicus). The allele distribution of TG/PsuI was inappropriate in Nellore and other genetic groups (Angus x Nellore and Rubia Galega x Nellore). Thus, the association study was limited by the number of individuals in each genotype. According to Hocquette et al. 18, association studies between markers and fat deposition presented mostly conflicting results 13, 19, 20, 21, 22, 23, 24, 25, 26. Van Eenennaam et al. 3 confirmed this tendency for validation studies that worked with commercial gene markers.

Therefore, the failure of our association attempt was not entirely unexpected. Probably, the conflict in results may be explained in light of the complexity of fat deposition physiology, which involves a great number of genes. In complex traits such as this, each gene has a small effect and detecting the association between marker and trait becomes a challenge. The lack of connection between allele forms of the LEP, TG and DGAT1 genes and growth related traits (such as REA) and tenderness (SF and MFI) was expected since, in principle, leptin, thyroglobulin and diacylglycerol O-acyltransferase 1 are not involved in the physiology of these characteristics.

IV. CONCLUSION

The polymorphisms LEP/Kpn2I, TG/PsuI and DGAT1/CfrI showed impropriated allele distribution in Bos indicus, providing difficulty for studies that aim to associate the markers with beef traits in exclusively zebu animals. In spite of the importance of the studied genes in fat metabolism, it was not possible to find evidences supporting association between the LEP/Kpn2I, TG/PsuI and DGAT1/CfrI polymorphisms and backfat thickness, intramuscular fat deposition, rib eye area, shear force and myofibrillar fragmentation index in the sample of used animals. These results and supporting literature draw attention for the little applied potential of these polymorphisms in the marker assisted selection of beef cattle of different genetic backgrounds.

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