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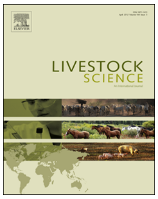

Short communication

Single nucleotide polymorphisms in genes linked to ion transport and regulation of appetite and their associations with weight gain, feed efficiency and intake of Nellore cattle

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ABSTRACT

The aim of this study was to evaluate the effects of associations and allele substitution of DNA single nucleotide polymorphism (SNP) on genes linked to appetite control (*NPY* and *PDE3B*) and ion transport (*ITPR1* and *TRPM3*) of average daily gain (ADG), feed conversion ratio (FCR), residual feed intake (RFI), residual BW gain (RWG), residual intake and body weight gain (RIG) and dry matter intake (DMI) of Nellore (*Bos indicus*) cattle. We evaluated the DMI, body weight and ADG of two hundred fifty-one animals kept in feedlots. The data were used to calculate the FCR, RFI, RWG and RIG. The associations of these variables with four SNPs (one in each gene cited above) were analyzes and the effects of allele substitution were estimated using PROC MIXED of SAS 9.3. The SNPs showed no association in genes *ITPR1*, *TRPM3* and *NPY*, but we observed a significant association ($P \leq 0.05$) and effect of allele substitution in one SNP in gene *PDE3B* with DMI, RFI and RIG. The SNPs evaluated are polymorphic in Nellore cattle and the gene *PDE3B* should be further studied because it seems to have effect on feed intake and efficiency.

1. Introduction

Advances in genetics and molecular biology in the last decades have enabled studies on single nucleotide polymorphism (SNP) in candidate genes. The use of SNPs in candidate genes can be directly related to some change in this gene, which is associated with a phenotype. Feed intake and efficiency are important for beef cattle, mainly because feed accounts for a large share of production costs (Sherman et al., 2009). Additionally, these phenotypes are expensive to obtain on a large scale.

Several metabolic factors can contribute to feed efficiency and hundreds of genes control its physiological variation (Barendse et al., 2007; Sherman et al., 2009). Neuropeptide Y (*NPY*) is a compound of the hypothalamic-pituitary axis that participates in the processes that control animal appetite regulating feed intake (Campfield et al., 1996). *PDE3B* is an enzyme related to insulin and leptin

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secretion, which affects feed intake (Cong et al., 2007). *ITPR1* reduces calcium release from the endoplasmic reticulum, an essential ion transport process (Huang et al., 2012). *TRPM3* is important in cellular calcium signaling and homeostasis. The protein encoded by this gene decreases calcium intake, reducing stored calcium (Clapham et al., 2005).

This study investigated the effects of allele substitution and association of SNP-type molecular markers on genes related to appetite control (*NPY* and *PDE3B*) and ion transport (*ITPR1* and *TRPM3*) on feed efficiency traits, feed intake and performance of Nellore cattle.

2. Material and methods

2.1. Animals, facilities, and management

We evaluated 251 males of the Nellore breed (103 steers and 148 young bulls) in five different experiments between 2007 and 2011 (in three experiments were steers and young bulls). The trials were carried out at the Universidade Federal do Paraná (2009, $n=47$, Santana et al. (2013)) and at Faculdade de Zootecnia e Engenharia de Alimentos of Universidade de São Paulo (2007–2011, $n=204$, Gomes et al. (2013)). The animals were evaluated from 70 to 90 days in feedlots with individual pens (155) and Calan Gates (96). The average age was 21 ± 2 months and the average weight was 380 ± 19 kg at the beginning of the experiment.

Every 21 days, the animals were weighed to obtain body weight (BW), and the average daily gain (ADG) was estimated using the linear regression of body weights by the weighing days. During the experiment, we measured the dry matter intake (DMI) daily subtracting the feed provided by leftovers, both adjusted for DM. Feed efficiency traits were calculated from the data on DMI, ADG and BW. We used feed conversion ratio (FCR), residual feed intake (RFI) and residual body weight gain (RWG) proposed by Koch et al. (1963) and residual intake and body weight gain (RIG) proposed by Berry and Crowley (2012) as measurements of feed efficiency.

FCR was calculated by the direct ratio of DMI/ADG and RIG is the sum of the RFI (multiplied by -1) and RWG. RFI and RWG were obtained from the residue (ε) of the regression equation that considers the estimated dry matter intake (DMI) and average daily gain (ADG) as the dependent variable for RFI and RWG, respectively, as the models below:

$$\mathrm{DMI} = \beta_0 + \beta_1(\mathrm{ADG}) + \beta_2(\mathrm{BW})^{0.75} + \beta_3(\mathrm{SC}) + \beta_4(\mathrm{CG}) + \varepsilon$$

$$\mathrm{ADG} = \beta_0 + \beta_1(\mathrm{DMI}) + \beta_2(\mathrm{BW})^{0.75} + \beta_3(\mathrm{SC}) + \beta_4(\mathrm{CG}) + \varepsilon$$

where: *SC* is the sex condition (young bulls and steers) and CG = is the contemporaneous group.

2.2. DNA extraction and genotyping

For genomic DNA extraction and sampling genotype, we collected blood from animals by a jugular puncture in vacuum tubes containing K2 EDTA as anticoagulant. The genomic DNA was extracted using NaCl precipitation protocol described in (Olerup et al., 1993).

Table 1
Summary of SNP information.

Gene	BTA	Gene ID	Location	dbSNP reference	Substitution
PDE3B	15	533323	Intron	rs29009964	T/A
NPY	4	504216	Intron	rs110418893	A/G
ITPR1	22	317697	Intron	rs29020542	C/T
TRPM3	8	540699	Intron	rs29024915	C/T

The genotypes for the polymorphism analysis was determined in real-time PCR using ABI Prism® 7500 Sequence Detection System (Applied Biosystems®, Foster City, CA, USA) and the detection system TaqManTM by fluorescence was used to read the probes.

We analyzed the variation of four SNPs alleles (Table 1) in different genes selected from the international genomic database National Center for Biotechnology Information (NCBI) according to Barendse et al. (2007). The SNPs in genes *NPY* (rs110418893) and *PDE3B* (rs29009964) are related to appetite control and genes *ITPR1* (rs29020542) and *TRPM3* (rs29024915) are linked to ion transport and associated with feed efficiency in beef cattle.

2.3. Statistical analysis

We carried out association analyses using the MIXED procedure of the SAS 9.3 statistical package, considering a mixed linear model as follows:

$$Y_{ijk} = \mu + \mathrm{CG}_i + \mathrm{SC}_j + \alpha_1(\mathrm{age}) + \alpha_2(\mathrm{genotype}) + S_{ik} + \varepsilon_{ijk}$$

where: Y_{ijk} = variable phenotypic value; μ = general mean of the trait; CG_i = contemporaneous group as fixed effect (considering the animals of the same test as CG); SC_j = sex condition as fixed effect (steers and young bulls); α_1 = regression coefficient of the age as co-variable; α_2 = regression coefficient associated to the favorable number of alleles; S_{ik} = random sire effect; ε_{ijk} = random error of residue.

The same models were used to calculate the allele substitution effect, however, the genotype was used as continuous fixed effect based on favorable alleles (0–2) in the genotype. The dominance effect was estimated as the heterozygote deviation from the mean of the two homozygotes (Falconer et al., 1996). The probability of Hardy-Weinberg equilibrium (HWE) associated with the observed genotype frequencies was tested in the Chi-square test.

3. Results and discussion

The *P*-value for HWE test and the allele and genotype frequencies for each of the four SNPs are shown in Table 2.

Table 3 shows the allele substitution effect, association and dominance effects of the SNPs with DMI, ADG, FCR, RFI, RWG and RIG.

The four SNPs evaluated were polymorphic in Nellore cattle, differently from SNPs found in taurine cattle (*Bos taurus*) deposited in genomic datasets of *Bos indicus* cattle, which may show SNPs monomorphic to population (Gomes et al., 2013). In our study, the SNPs had all alleles,

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