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Weighted single-step genome-wide association study and pathway analyses for feed efficiency traits in Nellore cattle

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Abstract

The aim was to conduct a weighted single-step genome-wide association study to detect genomic regions and putative candidate genes related to residual feed intake, dry matter intake, feed efficiency (FE), feed conversion ratio, residual body weight gain, residual intake and weight gain in Nellore cattle. Several protein-coding genes were identified within the genomic regions that explain more than 0.5% of the additive genetic variance for these traits. These genes were associated with insulin, leptin, glucose, protein and lipid metabolisms; energy balance; heat and oxidative stress; bile secretion; satiety; feed behaviour; salivation; digestion; and nutrient absorption. Enrichment analysis revealed functional pathways (p-value < .05) such as neuropeptide signalling (GO:0007218), negative regulation of canonical Wingless/Int-1 (Wnt) signalling (GO:0090090), bitter taste receptor activity (GO:0033038), neuropeptide hormone activity (GO:0005184), bile secretion (bta04976), taste transduction (bta0742) and glucagon signalling pathway (bta04922). The identification of these genes, pathways and their respective functions should contribute to a better understanding of the genetic and physiological mechanisms regulating Nellore FE-related traits.

KEYWORDS

Bos indicus, GBLUP, residual body weight gain, residual feed intake, weighted single-step

1 | INTRODUCTION

In beef cattle, the most costly component is the feedstuff, which has increased significantly in the last years reducing the beef cattle operation profitability (Boaitey, Goddard, Mohapatra, & Crowley, 2017). Approximately 65%–70% of the metabolizable energy required for beef production is used to meet maintenance requirements (Ferrell & Jenkins, 1985). Although *Bos indicus* cattle have less maintenance requirements per kilogram of metabolized weight than *Bos taurus* (Sainz, Barioni, Paulino, Valadares Filho, & Oltjen, 2006), decreasing the feedstuff costs involves reducing the maintenance requirement (Ferrell & Jenkins, 1985). The livestock has been recognized as one of those responsible for environmental impacts due to manure and gas production (Boaitey et al., 2017). These facts make feed efficiency (FE) an economically relevant trait to improve the profitability and reduce the environmental impact (Boaitey et al., 2017).

To increase the efficiency in converting food into carcass components, the residual feed intake (RFI), residual body weight gain (RG) and, the combination of these two traits, residual intake and body weight gain (RIG) were proposed (Berry & Crowley, 2012). These traits were low genetic correlated with adult weight and carcass composition, different from reported for feed conversion ratio (FCR) and FE (Berry & Crowley, 2012; Koch, Swiger, Chambers, & Gregory, 1963; Olivieri et al., 2016; Santana et al., 2014). Thus, RFI, RG and RIG are preferred measures for dissecting the underlying biology related to FE (Seabury et al., 2017).

Complex genetic background and physiology of the FE -related traits limit the understanding of the mechanisms involved in phenotypic expression and identification of more efficient animals regarding feed utilization (Rolf et al., 2012). Several studies identified biological and genetic mechanisms that could explain the differences in beef cattle FE (Gomes et al., 2013; Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014; Seabury et al., 2017). One of the main mechanisms that lead to variation in FE is the maintenance requirement, which is related to the animal's energy expenditure and the ability to increase the carcass weight (Ferrell & Jenkins, 1985). Although this information is valuable, it is still insufficient to elucidate all mechanisms that affect the phenotypic FE expression (Gomes et al., 2013; Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014; Seabury et al., 2017). The development of electronic technologies that allow the automatic measurement of individual feed intake and new evaluation traits led to easier phenotype collection and evaluation of FE in beef cattle, which is an expensive measurement phenotype (Boaitey et al., 2017). Up to date, there are low number of records collected for FE-related traits in zebu cattle (Olivieri et al., 2016; Santana et al., 2014).

The genomic information can be applied in genome-wide association studies (GWAS), which is a relevant methodology that can be applied under different statistical–computational tools and allow the identification of genes and genomic regions that explain part of the genetic variance for the evaluated traits (Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014). In livestock, several genomic regions with small effects and a large number of quantitative trait loci (QTL) were identified through GWAS (Wang, Misztal, Aguilar, Legarra, & Muir, 2012; Webber, 2011; Yang, Lee, Goddard, & Visscher, 2013). The phenotype expression is a result of complex interactions among genes and multiple regulatory mechanisms. Enrichment analyses can be used to identify the functions of genes and complement the GWAS results. This information elucidates the biological mechanisms and genetic architecture involved in phenotypic expression of FE-related traits, since these traits are of complex nature and controlled by several QTLs with small effect (Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014; Seabury et al., 2017). However, most of the GWAS studies for FE in zebu breeds were performed with experimental populations or low number of herds with small sample size (Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014). Thus, additionally studies with larger sample size under different conditions are necessary to increase the knowledge about the genetic background of FE-related traits in zebu cattle under tropical conditions.

The aim of this study was to conduct a weighted single-step genome-wide association study (WssGWAS) to detect genomic regions and putative candidate genes related to FE-related traits in Nellore cattle. In addition, gene set enrichment analysis was performed to better understand the biological processes and pathways shared by FE trait-associated genes.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The research project was approved by the Committee on Ethics in the Use of Animals (CEUA/PRPI) of the Federal University of Goiás (UFG), according to protocol No 088/18 issued by this institution.

2.2 | General data information

Data from 4,329 animals tested for FE, carried out between 2011 and 2018, and genotypic information from 3,594 animals were considered and provided by the Nellore Brazil Breeding Program, coordinated by the National Association of Breeders and Researchers (ANCP). Animals belonged to 39 farms located in the mid-west, south-east, north-east and north regions of Brazil. The relationship matrix used in the analyses was calculated based on pedigree information from 58,374 animals with 6,309 sires and 37,147 dams through nine generations. The animals that composed the data set had an average inbreeding of 0.071%, and the proportion of inbreeding was 0.41% over the total population, with an average inbreeding of 0.27%. These parameters were estimated using the INBUPGF90 program (Misztal, 2017).

A total of 125 FE tests were performed to assess the FE-related traits. The animals were evaluated in feedlot with an average age of 13.5 ± 3.92 months at the beginning of the tests under similar management and environmental conditions. The tests were conducted using the same protocol (Mendes et al., 2020) in three ranches (HoRa Hofig Ramos, Rancho da Matinha and AgroNova) and two research centres (Embrapa Rice and Beans and Federal University of Uberlandia). Even though the diets offered over the years differed in composition and ingredients, they were formulated based on silage and commercial concentrate, with an average of 64% total digestible nutrients, 13% crude protein and 76% dry matter, and formulated for gains of 1.2 kg/day (Mendes et al., 2020).

During the tests, the average weight of each animal was obtained by periodic weighing, as well as at the beginning and end of the evaluation period. Forage, concentrate and waste samples were collected every week to evaluate chemical composition.

DNA samples were obtained from hair follicles taken from animals' tails and placed in card with adhesive film. The animals were genotyped for SNP markers using CLARIFIDE® Nellore 3.1 low-density panel, containing approximately 29,000 SNP markers. DNA extraction and sample genotyping were performed by Zoetis®, through its protocol.

2.3 | Performance traits

The FE traits were estimated within each contemporary group (GC). The CG was composed by farm, management group, FE test, sex, year and birth season (dry season from April to September and the wet season from October to March). The effects included in the CG were those whose significance value was <0.001 obtained in ANOVA results.

The DMI was measured by collective stalls equipped with automated systems (GrowSafe System® and Intergado®), for a minimum of 70 days preceded by adaptation. The DMI, measured in kg/day, was obtained by calculating the average of all valid daily intake values during the test period. As quality control, daily DMI records within ±3.5 *SD* from the average daily DMI of the contemporary group were considered in the analysis. Additionally, daily DMI obtained on days with a power outage or weighing scale adjustments were excluded from the analysis. The DMI was calculated as the amount of individually consumed feed automatically recorded by the electronic systems (GrowSafe System® and Intergado®) (Mendes et al., 2020).

To estimate RFI and RG, ADG and metabolic body weight ($MW^{0.75}$) were calculated. ADG (kg/day) was estimated by the linear regression coefficient of the weights as a function of the days in test, using the *lm* function of R program (2018) and the following equation:

$$y_i = \alpha + \beta \times DIT_j + \varepsilon_i$$

where y_i is the weight of *i*th animal; α is the intercept of the regression equation which represents the initial weight; β is the linear regression coefficient which represents the ADG; DIT_j is the day in the performance test of *j*th observation; and ε is the residual associated with each observation. It was assumed that the residues were independent and not correlated and residual effects were normally distributed with mean zero. The $MW^{0.75}$ was given from body weight and ADG:

$$MW^{0.75} = \left[\alpha + \beta \times \left(\frac{DIT}{2}\right)\right]^{0.75}$$

where $MW^{0.75}$ is the metabolic weight; α is the intercept of the regression equation which represents the initial weight; and β is the linear regression coefficient which represents the ADG, as described and obtained above in estimating ADG.

FE, measured in kg ADG/kg DMI, was obtained as the ratio between ADG and DMI. FCR, measured in kg DMI/kg ADG, was obtained by the inverse ratio (DMI/ADG). RFI (kg of DM/day) was estimated, within each CG, by the residual of the DMI regression as a function of ADG and $MW^{0.75}$, using the R program (2018) and the equation (Koch et al., 1963):

$$y_i = \beta_o + \beta_1 ADG + \beta_2 MW^{0.75} + \varepsilon\ (RFI)$$

where y is individual dry matter intake of *i*th animal; β_o is the intercept; β_1 and β_2 are the linear regression coefficient of ADG and $MW^{0.75}$, respectively; and ε is the residual error, that is RFI. It was assumed that the residues were independent and not correlated and residual effects were normally distributed with mean zero (Sen & Sen, 2014). Regression analysis was performed, and no effect of backfat thickness on RFI was observed; thus, the RFI was not adjusted for fat thickness.

The RG (Berry & Crowley, 2012; Koch et al., 1963) (kg of ADG/day) was obtained as the difference between the observed ADG and the estimated ADG based on DMI and $MW^{0.75}$. The estimated average daily gain (ADGe) was obtained using the *lm* function on the R program (2018), within CG and by:

$$ADG_{ei} = \beta_o + \beta_1 DMI + \beta_2 MW^{0.75} + \varepsilon\ (RG)$$

where β_o is the intercept, β_1 and β_2 are the regression coefficients of *DMI*and $MW^{0.75}$, respectively; and ε is the residual error, that is RG. It was assumed that the residues were independent and not correlated and residual effects were normally distributed with mean zero (Sen & Sen, 2014).

Residual intake and body weight gain was calculated as RG-RFI, after standardizing both traits to a variance of 1, allowing their combination into single value (Berry & Crowley, 2012). Both traits, RFI and RG, are linear functions of their component traits: DMI, ADG and $MW^{0.75}$. The number of records and descriptive statistics for the evaluated traits are summarized in Table 1.

2.4 | Statistical and quality control analyses

Records within ±3.5 *SD* from the CG mean were considered in the analysis. Additionally, all CG should have at least four animals in order to proceed with the analysis. In the quality control for genomic data, SNPs with minor allele frequency (MAF), call rate and *p*-value for Hardy–Weinberg equilibrium test less than 0.02, 0.95 and 0.15,

Trait	*N*	Mean	*SD*	*N*° CG	σ_a^{2a}	σ_e^{2a}	$h^2 \pm SE^a$
RFI	4,080	0.00	0.70	125	0.09	0.042	0.17 ± 0.04
DMI	4,097	7.97	1.75	126	0.21	0.68	0.23 ± 0.04
FE	2,242	0.09	0.03	93	0.00030	0.00404	0.07 ± 0.03
FCR	2,235	12.18	4.43	125	0.80	8.14	0.09 ± 0.03
RG	2,056	0.00	0.20	93	0.03	0.16	0.17 ± 0.05
RIG	2,033	0.02	0.74	93	0.11	0.43	0.20 ± 0.05

Abbreviations: DMI, dry matter intake; FCR, feed conversion ratio; FE, feed efficiency; RFI, residual feed intake; RG, residual body weight gain; RIG, residual intake and body weight gain.

[a]The variance components were estimated by single-trait analyses in a single-step genomic approach (Brunes et al., 2020).

TABLE 1 Number of observations (*N*), phenotypic mean, standard deviation (*SD*), number of contemporary groups (*N*° CG), additive genetic variance (σ_a^2), residual variance (σ_e^2) and heritability ($h^2 \pm SE$) for feed efficiency-related traits in Nellore cattle

TABLE 2 Descriptive analysis of genetic distance in Nellore cattle

Statistics	Mean	Median	Minimum	Maximum	1° quartile	3° quartile
Whole population	0.0865	0.0835	0.0000	0.1816	0.0526	0.1030
Between folds of random validation	0.0912	0.0971	0.0000	0.1710	0.0421	0.0991
Training and validation population for age approach	0.1161	0.1158	0.0002	0.1816	0.0635	0.1174
Training and validation population for EBV accuracy	0.1095	0.1087	0.0001	0.1796	0.0603	0.1125

respectively, were excluded. Only SNPs in autosome chromosomes and with known position according to UMD 3.1 bovine genome were considered. Samples with call rates below to 0.95 were excluded from the analysis. This process was performed with R program (2018), using scripts developed for this purpose, resulting in a data set with 19,602 SNPs and 3,467 animals.

To evaluate the existence of population substructure, a principal component analysis (PCA) was performed using information from SNPs and genomic relationship matrix of individuals (VanRaden, 2008) (Figure A1). The proportion of variance explained by the two first principal components was 33.95%. The PC1 and PC2 did not group the animals into clear-cut clusters, implying that genetic admixture probably existed for the evaluated population. The animals' dispersion in the PCA plot indicated the absence of subgroups among the evaluated animals, since there is no formation of major components.

The genetic distance between individuals was calculated based on their genotypes using the method of Jukes–Cantor (Jukes & Cantor, 1969) and R program (2018). The genetic distances value was, on average, 0.08 (0.0–0.1816), indicating that the data are not dispersed or subgrouped in whole population (Table 2). Among training and validation populations, the genetic distances showed the same pattern, with low values and no dispersion, pointing out that considering the animals' genetic structure constitutes a unique population.

2.5 | Weighted single-step genome-wide association studies

The model to perform the WssGWAS included the direct additive genetic and residual effects as random effects, and the CG was included as a fixed effect and animal's age as covariable (linear effect). The variance components necessary to perform the WssGWAS analysis were estimated by single-trait analyses (Brunes et al., 2020), through the restricted maximum-likelihood method, with REMLF90 program (Misztal, 2017) and using single-step genomic approach (Aguilar et al., 2010). The variance components and heritability estimates obtained by Brunes et al. (2020) are summarized in Table 1.

The effects and variances of SNPs were estimated by the WssGWAS proposed by Wang et al. (2012), using the BLUPF90 adapted for genomic analyses (Misztal, 2017). The WssGWAS uses matrix H^{-1} (Aguilar et al., 2010) that combines pedigree and genomic information:

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

where G^{-1} is the inverse of genomic relationship matrix; A^{-1} is the inverse of additive relationship matrix; and A_{22} is the inverse pedigree relationship matrix for genotyped animals. The genomic matrix (G) was created as follows (VanRaden, 2008):

$$G = \frac{ZDZ'}{\sum_{i=1}^{M} 2p_i(1-p_i)}$$

where Z is an incidence matrix adjusted for allele frequencies; D is a diagonal matrix of weights for SNP variances; M is the number of markers; and p_i represents the MAF of the ith SNP. The SNP effects and weights for WssGWAS were calculated iteratively as follows (Wang et al., 2012):

1. Set $t = 1$, $\boldsymbol{D}_{(t)} = \boldsymbol{I}$; $G_{(t)} = \lambda \boldsymbol{Z}\boldsymbol{D}_{(t)}\boldsymbol{Z}'$

$$\lambda = \frac{1}{\sum_{i=1}^{M} 2p_i(1-p_i)}$$

2. Estimate GEBV for all animals using ssGBLUP approach;
3. Compute SNP effects as $\hat{u}_{(t)} = \lambda D_{(t)} Z' G_{(t)}^{-1} \hat{a}_g$, where $\hat{u}_{(t)}$ was a vector of the SNP effect estimation and $\hat{a}_g$ is the GEBV of animals that were also genotyped;
4. Calculate SNP weights for the next iteration using $d_{i\,(t=1)} = \hat{u}^2_{i\,(t)} 2p_i(1-p_i)$ where i is the ith SNP;
5. The SNP weights were normalized to keep the total genetic variance constant:

$$D_{(t+1)} = \frac{tr\,(D_{(t)})}{tr\,(D_{(t+1)})} D_{(t+1)}$$

6. Calculate $G_{(t+1)}$

$$G_{(t+1)} = \frac{ZD_{(t+1)}Z'}{\sum_{i=1}^{M} 2p_i(1-p_i)}$$

7. $t = t + 1$;

Exit or loop to step 2 or 3.

This procedure was run for four iterations. At each iteration, the weights for SNPs were updated (steps 4 and 5) and used to construct the G matrices (step 6) and update the GEBV (step 2) and, consequently, the estimated SNP effects (step 3). The results were presented for windows with 10 adjacent SNPs (±1 Mb). The window size was defined after analyses performed with R. R Core Team (2018), in which the average and mode haplotype block were obtained in studied population. In addition, the window size was based on the linkage disequilibrium of zebu genome (Espigolan et al., 2013). Windows based on the number of the SNPs instead of physical size were chosen in order to avoid biases due to uneven distributed SNPs in the genotype panel. The percentage of genetic variance explained by the ith window was calculated as follows:

$$\frac{Var(a_i)}{\sigma_a^2} = \times 100 = \frac{Var\left(\sum_{j=1}^{10} Z_j \hat{u}_j\right)}{\sigma_a^2} \times 100$$

where a_i is the genetic value of the ith SNP window that consists of a region of 10 adjacent SNPs; σ_a^2 is the total additive genetic variance; Z_j is the vector of gene content of the jth SNP for all individuals; and $\hat{u}_j$ is the effect of the ith SNP with the ith window. Manhattan plots based on the proportion of additive genetic variance explained by the windows were generated using *qqman* package of R software (2018).

2.6 | Search for candidate genes and functional enrichment analysis

To determine possible QTLs, genomic regions that explained more than 0.5% of the additive genetic variance were selected. These analyses were based on WssGWAS for all FE-related traits: RFI, DMI, FE, FCR, RG and RIG. The threshold of 0.5% was chosen based on the previous reports (Medeiros de Oliveira Silva et al., 2017; Stafuzza et al., 2019), visual inspection of Manhattan plots, small proportion of explained variance of polygenic traits and expected contribution of SNP windows (Sollero, Junqueira, Gomes, Caetano, & Cardoso, 2017).

For identification and positioning of the selected segments in the bovine genome, a survey was made in the database available using the *B. taurus* UMD 3.1 genome assembly and Ensembl BioMart tool with Genes 94 database (Haider et al., 2009). The gene content of genomic regions selecting a 500-Kb window around each region (upstream and downstream) was identified. Previous studies also suggested that a similar distance could be used in the GWAS approach to capture the genomic regions affecting quantitative trait in Nellore cattle (Stafuzza et al., 2019), since the average linkage disequilibrium (r^2) is .34 in genomic regions within 500 kb length size (Espigolan et al., 2013).

Classification of genes for biological function, metabolic pathway and gene set enrichment analyses, considering $p < .05$ threshold for significance in Fisher's exact test, was performed with ENSEMBL database and Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 toll (da Huang, Sherman, Lempicki, & Lempick, 2009; Huang, Sherman, & Lempicki, 2009), from annotated genes in the Ensembl and to seek for significant clusters.

3 | RESULTS

A total of 14, 15, 21, 22, 26 and 27 genomic regions that explained more than 0.5% of additive genetic variance and

harboured genes with known functions associated with RFI, DMI, FE, FCR, RG and RIG, respectively, were identified (Tables 3–8 and Figures 1 and 2).

Manhattan plots (Figures 1 and 2) displayed the genomic regions that explained more than 0.5% of the additive genetic variance for FE-related traits. There were several genomic regions found on BTA 2, 3, 6, 7, 9, 11, 14, 16, 19, 20, 21, 24 and 29 explaining more than 0.5% of additive genetic variance for more than one evaluated trait (Tables 3–8).

A large number of genomic regions explaining more than 0.5% of the additive genetic variance were identified for the studied traits. The genes found in the regions that accounted for more than 0.5% of additive genetic variance and enrichment pathways in each functional category ($p < .05$) for FE-related traits are shown in Table 9. The functional enrichment analysis revealed 21 biological processes, six molecular functions, five cellular components and five KEGG pathways. It highlighted the following terms related to FE: neuropeptide signalling pathway (GO:0007218), negative regulation of canonical Wnt signalling pathway (GO:0090090), detection of chemical stimulus involved in sensory perception of bitter taste (GO:0001580), bitter taste receptor activity (GO:0033038), neuropeptide hormone activity (GO:0005184), bile secretion (bta04976), taste transduction (bta0742) and glucagon signalling pathway (bta04922).

4 | DISCUSSION

Several genomic regions with small effect for FE-related traits were also reported in previous studies with zebu cattle (de Oliveira et al., 2014; Olivieri et al., 2016; Santana et al., 2014). Thus, some small-effect genomic markers contribute to differences in these traits, which may be related to their polygenic architecture (Serão et al., 2013). Several genomic regions explaining more than 0.5% of the additive genetic variance for at least two traits were identified. Genes that could be related to FE-related traits in Nellore cattle, according to their functions, were highlighted below.

Hormones such as insulin, leptin and glucose affect energy metabolism and, consequently, the FE, since higher supply and utilization of energy result in divergent animals in terms of FE (Richardson & Herd, 2004). As an example, RFI is related to the basal energy needs and differences in growth efficiency. Thus, this trait works as an indicator of metabolic efficiency and energy expenditure, which supports the large number of RFI-associated genes that act on energy, insulin and glucose metabolism (Richardson & Herd, 2004). The same concept may be extrapolated to the other FE -related traits, whereas genes related to processes that constantly demand and expenditure energy were observed associated with all evaluated traits.

TABLE 3 Genomic regions of 10 adjacent SNPs that explain more than 0.5% of the additive genetic variance (Var) for residual feed intake (RFI) in Nellore cattle

BTA	Start position (bp)	End position (bp)	Var (%)	Genes
2	20,023,792	21,555,517	1.12830	NFE2L2, HOXD3, HOXD4, HOXD9, HOXD10, ATF2, CHN1
2	120,775,372	122,330,054	1.11169	PDE6D, COPS7B, ALPI, ECEL1, CHRND, CHRNG, EIF4E2, PHC2, A3GALT2, ZNF362, TRIM62, AZIN2, AK2, YARS, RBBP4, ZBTB8A, TSSK3, MARCKSL1, HDAC1, LCK, MTMR9, PTP4A2, SPOCD1, PEF1, TINAGL1
3	54,028,274	54,062,811	0.50873	LRRC8D, LRRC8C, LRRC8B, GBP6, GBP5
5	70,280,639	71,120,972	0.51857	NUAK1, POLR3B, RFX4, RIC8B, BTBD11, PRDM4, ASCL4, RTCB
6	73,547,830	74,233,023	0.57135	PPAT, PAICS, HOPX, REST, POLR2B, IGFBP7
11	83,875,552	84,970,005	0.63952	TRIB2
11	29,736,720	30,551,963	0.50447	CALM2, EPCAM, MSH2, MSH6, FBXO11, FOXN2
16	75,847,620	76,091,078	0.52570	IRF6, HSD11B1, CAMK1G
19	42,988,287	43,755,425	1.99706	EIF1, JUP, NT5C3B, KLHL10, ACLY, TTC25, CNP, DNAJC7, NKIRAS2, DHX58, KAT2A, HSPB9, RAB5C, STAT5B, STAT5A, STAT3, ATP6V0A1, NAGLU, HSD17B1, COASY, MLX, TUBG1, TUBG2, EZH1, RAMP2, VPS25, CNTD1, PSME3, AOC2, AOC3, SAO, G6PC, AARSD1, RND2, BRCA1, ARL4D, DHX8, ETV4, MEOX1
20	7,103,987	7,736,726	1.50032	GFM2, HEXB, ENC1, UTP15, ANKRA2, CALM
20	65,636,880	67,125,349	0.65687	MTRR, ADCY2, NSUN2, MED10
21	21,333,104	22,991,710	0.67895	ACAN, HAPLN3, MFGE8, RLBP1, FANCI, POLG, TICRR, ANPEP, AP3S2, ZNF710
24	52,165,674	54,752,773	0.53601	POLI, RAB27B, TCF4

Abbreviations: bp, base pair, BTA, *Bos taurus* autosomes.

Several genes related to insulin metabolism were identified, such as *OSM* (Komori, Tanaka, Senba, Miyajima, & Morikawa, 2014), *NOD2* (Rodriguez-Nunez et al., 2017), *IQGAP2* (Brisac et al., 2016) and *AOC3* (Carpene, Iffiu-Soltesz, Bour, Prevot, & Valet, 2007) genes. The action of insulin-related genes results in differences in the mechanisms of hunger and satiety due to energy homeostasis and growth (Kelly et al., 2011; Nascimento et al., 2015), and in the total energy extracted from food, which may cause variation in weight gain, despite there was no difference in feed intake (Rodriguez-Nunez et al., 2017). Low-RFI animals have a higher sensation of satiety due to insulin signalling (Kelly et al., 2011), and serum concentrations of this hormone can be used as indicators of efficient feed utilization in Nellore cattle (Nascimento et al., 2015).

Some leptin-related genes were identified, such as *LIF* (Beretta, Dhillon, Kalra, & Kalra, 2002), *IAPP* (Muff, Born, & Fischer, 1995) and *STAT3* (Weber et al., 2016) genes. The last one gene was reported associated with RFI in Angus cattle (Weber et al., 2016). Neurons of the area postrema are co-activated by *IAPP* and glucagon-like peptide-1, regulating feeding, digestive functions, satiety and gastric emptying (Muff et al., 1995), and FE indirectly. Leptin influences the action of the alpha-melanocyte-stimulating hormone (α-MSH), which is responsible for satiety, acting on the animals' feeding behaviour, appetite and emitting signals that stop the seeking for food by the animal. Leptin emits signals through the central nervous system to elicit changes in feeding behaviour, energy balance and nutritional status (Zieba, Amstalden, & Williams, 2005).

Genes related to energy and glucose metabolism, one of the main sources of energy for cattle, were identified, such as *FBXO32* (Cleveland & Evenhuis, 2010), *MAF1* (Cherry et al., 2012) and *AK2* (Burkart, Shi, Chouinard, & Corvera, 2011) genes. The *FBX032* gene was reported related to leanness and fatness traits and enhanced growth efficiency in cattle (A. Wang et al., 2013). Under conditions of nutrient limitation, *MAF1* is associated with reduced fitness, stress sensitivity, altered respiratory metabolism and decreased sporulation efficiency (Cherry et al., 2012). This pattern may be due to increased O_2 consumption by mitochondrial complex 2 and establish faster phosphorylation homeostasis, reduced caloric intake and increased energy expenditure, being inefficient metabolically to transform calories into biomass (Bonhoure et al., 2015). These findings explain reduced intake, lower body fat thickness and blood glucose and insulin concentrations in low-RFI animals. These mechanisms can be ceasing their intake in less time, because they achieve satiety first (Kerley, 2010).

TABLE 4 Genomic regions of 10 adjacent SNPs that explain more than 0.5% of the additive genetic variance (Var) for dry matter intake (DMI) in Nellore cattle

BTA	Start position (bp)	End position (bp)	Var (%)	Genes
2	20,929,001	21,696,086	0.83911	HOXD3, HOXD4, HOXD9, HOXD10, HOXD11, ATF2, CHRNA1
2	120,775,372	122,330,054	0.78975	ECEL1, CHRND, CHRNG, PHC2, RBBP4, ZBTB8A, MARCKSL1, HDAC1, LCK, KHDRBS1, SPOCD1, HCRTR1
5	33,430,648	34,965,958	0.59848	AMIGO2, SCAF11, ARID2
10	6,617,470	7,427,251	0.57428	COL4A3BP, POLK, IQGAP2, F2RL2, F2R
10	3,004,311	3,691,643	0.52988	TRIM36
12	18,440,125	19,212,646	0.65670	MED4, RB1, LPAR6, RCBTB2, CYSLTR2, FNDC3A, MLNR, PHF11, KPNA3
14	22,297,785	22,983,665	0.56690	NPBWR1, OPRK1
14	24,049,812	24,229,059	0.50996	ATP6V1H, RGS20, TCEA1, SOX17
14	21,452,744	21,976,451	0.50976	SPIDR, H3F3C, PRKDC, SNAI2
19	43,001,952	43,948,803	0.96340	JUP, ACLY, CNP, DNAJC7, HCRT, STAT5B, STAT5A, STAT3, ATP6V0A1, NAGLU, HSD17B1, COASY, MLX, TUBG1, TUBG2, EZH1,VPS25, BECN1, PSME3, AOC2, AOC3, SAO, BRCA1, DHX8, ETV4, SOST, DUSP3, PPY, PYY
20	7,103,987	7,736,726	0.85015	HEXB, ANKRA2
21	21,333,104	22,991,710	0.53186	ABHD2, FANCI, RHCG, TICRR, AP3S2, ZNF710, CIB1, VPS33B, PRC1, BLM, CRTC3, ZSCAN2, NMB, AP3B2
21	9,033,285	9,741,507	0.52817	
23	22,300,959	23,477,473	0.56001	CENPQ, RHAG, TFAP2B
29	23,411,243	24,642,061	0.69345	PKHD1, HTATIP2, DBX1, NAV2

Abbreviations: bp, base pair, BTA, *Bos taurus* autosomes.

TABLE 5 Genomic regions of 10 adjacent SNPs that explain more than 0.5% of the additive genetic variance (Var) for feed efficiency (FE) in Nellore cattle

BTA	Start position (bp)	End position (bp)	Var (%)	Genes
6	73,547,830	74,233,023	1.18301	PPAT, PAICS, HOPX, REST
7	24,982,024	25,784,499	1.29587	
9	33,173,529	34,265,873	1.05822	NEPN, VGLL2, RFX6
9	12,744,212	13,502,951	0.79536	EEF1A1,
9	34,495,344	35,276,579	0.52239	TSPYL4, FRK
10	6,018,257	7,329,188	0.51571	DRD1
11	84,227,146	84,997,734	1.61074	TRIB2
14	16,387,114	17,752,395	0.77942	TRIB1, ZNF572, RNF139
14	19,649,604	20,725,667	0.63480	
14	18,460,103	19,605,085	0.58282	FBXO32, WDYHV1, ATAD2, ZHX1, DERL1, ZHX2
14	21,224,382	21,735,604	0.55173	H3F3C, PRKDC, UBE2V2, SNAI2
14	15,551,978	16,285,123	0.52712	
16	77,099,277	77,825,967	0.92552	CD34, CD46, ASPM
16	75,847,620	76,091,078	0.84157	IRF6
16	74,134,974	74,161,201	0.59412	NEK2, RCOR3
17	46,858,681	47,425,590	0.83153	PIWIL1
20	7,351,732	8,078,272	0.61095	ANKRA2
20	5,767,456	6,495,026	0.59919	CPEB4, MSX2
24	56,386,139	56,436,773	0.96898	
24	54,964,769	55,980,406	0.74121	TCF4
24	51,961,637	54,724,737	0.53527	POLI

Abbreviations: bp, base pair; BTA, *Bos taurus* autosomes.

The association of oxidative stress-related genes and FE occurs because oxidative stress can decrease energetic efficiency as oxidation products that must be degraded by processes such as the ATP-dependent ubiquitin system that needs energy (Bottje & Kong, 2013). This association is due to physiological responses to stress, which include increased metabolic rate and energy expenditure, as well as increased catabolic processes (increased lipolysis and protein degradation) (Iqbal et al., 2005). Thus, higher tolerance for oxidative stress may lead to lower energy expenditure and greater tissue accretion, which may partially explain differences in FE (Arthur & Herd, 2008). Seen in these terms, *HSF1* (Ebrahimi et al., 2015), *MSH6* and *MSH2* (Lindholm-Perry et al., 2017) genes related to stress response were identified. Indeed, Lindholm-Perry et al. (2017) observed a difference in transcript abundance of *MSH2* among beef cattle with low gain–high intake phenotype.

Zinc is a structural element in protein and is essential for several biochemical and cellular pathways, characterized by coordination and stabilization of one or more zinc ions in several ionic exchange process, participates in DNA and RNA synthesis, cell division and activation, and is indispensable for immune response (Klug & Rhodes, 1987). In addition, zinc finger proteins were reported associated with DMI, FE and ADG in Nellore cattle (Olivieri et al., 2016; Santana et al., 2014). Indeed, genes such as *ZHX1* and *ZHX2* are zinc finger member family and were related to ADGe in cattle (Serão et al., 2013).

The association of protein metabolism and FE can be attributed to the energy expenditure from the turnover of body proteins, which can reach 30% of the maintenance energy (Carvalho et al., 2019; Richardson & Herd, 2004). High-RFI animals presented higher levels of protein catabolism or more efficient mechanism of protein utilization, identified by the highest concentration of total plasma protein, blood urea and aspartate aminotransferase (Richardson & Herd, 2004). As a result, higher protein turnover nutrient use efficiency results in different energy expenditures. In this way, *POLR2K*, *PDE6D*, *HDDC3* and *POLR2B* genes that play roles in purine metabolism pathways and the *PAICS* and *PPAT* genes that play a role in purine biosynthesis were found (Cheung et al., 2019; Liu et al., 2008).

Similar to other physiological mechanisms, such as insulin, stress and protein metabolism, the key point of the association between FE and lipid metabolism is energy expenditure. In general, to deposit fat, cattle need more energy than to deposit protein; thus, protein synthesis is energetically more efficient than fat synthesis. As a result,

TABLE 6 Genomic regions of 10 adjacent SNPs that explain more than 0.5% of the additive genetic variance (Var) for feed conversion ratio (FCR) in Nellore cattle

BTA	Start position (bp)	End position (bp)	Var (%)	Genes
3	54,028,274	54,062,811	0.60141	LRRC8D, LRRC8C, GBP6, GBP5
5	87,926,197	88,878,999	0.50680	C2CD5, SPX, IAPP, SLCO1A2, SLCO1B3
7	24,982,024	25,784,499	0.97067	
9	12,744,212	13,502,951	1.20275	EEF1A1
9	33,173,529	34,265,873	0.73280	PLN
9	15,959,442	16,564,251	0.58648	
11	83,875,552	84,970,005	1.01124	TRIB2
14	16,387,114	17,752,395	2.28115	RNF139, DERL1
14	19,649,604	20,725,667	2.26805	
14	21,224,382	21,735,604	2.21004	PRKDC
14	21,967,712	22,317,344	1.80052	SNTG1
14	15,551,978	16,285,123	1.60483	TRIB1
14	18,460,103	19,605,085	1.59205	
14	24,115,422	24,406,302	1.31728	
14	23,510,902	23,929,089	1.25423	OPRK1, SOX17
14	24,437,778	24,590,812	1.09788	
14	24,607,527	24,892,678	1.07645	
14	22,392,760	23,017,421	1.06328	
14	24,909,247	25,307,116	1.02923	PENK
20	5,767,456	6,495,026	0.63336	GFM2
24	56,386,139	56,436,773	0.71319	
24	54,964,769	55,980,406	0.50680	RAB27B

Abbreviations: bp, base pair; BTA, *Bos taurus* autosomes.

variations in weight gain and body composition influence the efficiency of nutrient utilization (Arthur & Herd, 2008), often reflecting on fat thickness in the carcass (Basarab et al., 2003).

The reduced lipid synthesis and fat accumulation in high gain–low intake animals may be an indication of energy prioritization away from lipid deposition and towards lean growth or maintaining better health or function of organs (Mukiibi et al., 2018). In this sense, more efficient animals have lower levels of triacylglycerol, indicating increased mobilization of this lipid to be used as an energy source and to supply the requirement for lean meat deposition that is higher in these animals (Duarte, 2018). This biological mechanism is also related to insulin response (Richardson & Herd, 2004), which supports the relationship between insulin genes and FE, as previously stated. Indeed, several lipid-related genes were identified, such as *SDCBP* (Santos, 2018), *ACLY* (Ji, Osorio, Drackley, & Loor, 2012), *OLR1* (Vinsky, Islam, Chen, & Li, 2013), *CRTC3* (Raza et al., 2019), *PRKDC* (Horodyska, Hamill, Varley, Reyer, & Wimmers, 2017), *HNRNPA3* (Wang et al., 2018), *HTATIP2* (Liao et al., 2014) and *NFE2L2* (Wu, Cui, & Klaasen, 2011) genes. The *OLR1* and *CRTC3* genes were reported associated with body weight, rib eye area and fat thickness in Nellore (Fonseca et al., 2015), and fat deposition in Qinchuan cattle (Raza et al., 2019), respectively. The *SDCBP* and *NFE2L2* genes were reported associated with RFI (Santos, 2018) and FE (Lima, 2019) in Nellore cattle, respectively.

Mechanisms associated with feed digestives processes affect the intake capacity, absorption process and also the utilization of nutrients by animals (Arthur & Herd, 2008; Richardson & Herd, 2004). Salivation is associated with ruminal motility and function, food passage rate and digestive disorders (Carter & Grovum, 1990). In agreement, *EPCAM* (Mignon-Grasteau et al., 2015), *ATP6V0A1* (Kern et al., 2016) and *RFX6* (Freeman et al., 2010) genes that act on digestive processes and salivation were identified. The *ATP6V0A1* and *RFX6* genes were reported associated with FE divergent beef steers (Kern et al., 2016) and with DMI in Nellore cattle (Olivieri et al., 2016), respectively.

Regarding pathway identified by gene set enrichment analyses, some of them and the main genes within metabolic and/or functional pathways related to FE traits or with functions that may be associated with the phenotypic expression of the evaluated traits were discussed below.

TABLE 7 Genomic regions of 10 adjacent SNPs that explain more than 0.5% of the additive genetic variance (Var) for residual body weight gain (RG) in Nellore cattle

BTA	Start position (bp)	End position (bp)	Var (%)	Genes
7	24,982,024	25,784,499	0.73172	
9	12,744,212	13,502,951	0.64508	EEF1A1, CD109
9	15,953,490	16,452,859	0.62922	
11	84,227,146	84,997,734	1.65889	TRIB2
14	16,387,114	17,752,395	1.74571	NSMCE2, ZNF572, MTSS1, RNF139
14	21,224,382	21,735,604	1.39310	SPIDR, H3F3C, PRKDC, UBE2V2, SNAI2
14	18,460,103	19,605,085	1.38702	FBXO32, WDYHV1, ATAD2, ZHX1, DERL1, ZHX2
14	19,649,604	20,725,667	1.37614	HAS2
14	22,297,785	22,983,665	1.31933	ST18
14	21,778,139	22,251,785	1.16037	
14	15,551,978	16,285,123	0.95066	TRIB1
14	24,008,839	24,225,369	0.83847	
14	23,510,902	23,929,089	0.82056	RB1CC1, OPRK1, RGS20, TCEA1, POLR2K, SOX17
14	24,237,304	24,553,162	0.75930	
14	25,528,516	26,385,476	0.70828	UBXN2B
14	2,194,228	2,342,883	0.68498	VPS28, CPSF1, SCRT1, HSF1, BOP1, SCX, MAF1, SHARPIN, CYC1, PUF60, TIGD5, EEF1D, NAPRT, MAFA, SLURP1
14	24,909,247	25,307,116	0.68013	TGS1, LYN, PLAG1, PENK
14	24,582,124	24,828,922	0.62384	
16	47,341,761	49,343,163	0.51830	PHF13, NOL9, ESPN, HES2, CHD5, NPHP4, CSRP1
17	70,443,042	71,146,543	0.60670	CHEK2, HSCB, XBP1, ZNRF3, RHBDD3, NF2, HORMAD2, LIF, OSM, SEC14L2
18	17,497,121	18,822,874	1.11781	C18H16orf78, ZNF423, BRD7, NKD1, SNX20, NOD2, CYLD
18	16,307,788	17,399,669	1.09141	LONP2, SIAH1, N4BP1
24	56,386,139	56,436,773	0.84133	
24	54,964,769	55,980,406	0.76039	TCF4
24	51,961,637	54,724,737	0.63574	POLI, C24H18orf54
29	13,219,091	14,408,041	0.79633	

Abbreviations: bp: base pair; BTA, *Bos taurus* autosomes.

Several genes were identified as related to neuropeptide signalling pathway (GO:0007218) and neuropeptide hormone activity (GO:0005184). The neuropeptide signalling pathway (GO:0007218) was associated with growth and feed utilization traits of Japanese Black cattle (Okada et al., 2018), suggesting that this pathway may affect FE. Among the genes harboured in these pathways, we highlighted the *PYY*, *HCRT*, *HCRTR*, *NMB*, *MLNR*, *NPBWR1*, *PPY*, *SPX*, *OPRK1* and *PENK* genes, which are related to feeding behaviour, satiety and amount of food consumed (Arora, 2006; Hoggard, Bashir, Cruickshank, Miller, & Speakman, 2007; Martín-García et al., 2011; McGregor, Wu, Barber, Ramanathan, & Siegel, 2011; Reid et al., 2017; Reyes-Alcaraz et al., 2016; Sakuraia, 2013; Takahashi, Rikimaru, Komatsu, Uemoto, & Suzuki, 2014; Tyree, Borniger, & Lecea, 2018; Xu et al., 2013).

The *PYY* gene acts as feeding promotion and feeding suppression by the hypothalamus, send signals to the central nervous system and regulate functions of the gastrointestinal tract, appetite regulation and feed intake (Arora, 2006). The *NMB* gene action is closely associated with FE since it represents a mediator between the gut and the brain and serves as a satiation signal to terminate meals and indicate energy balance, reflecting the nutritional status and regulating feed intake over a longer term (Hoggard et al., 2007). The *SPX* gene was implicated in the regulation of appetite regulation, feed intake, satiety factor, leptin signalling and related metabolic processes (Reyes-Alcaraz et al., 2016). This gene was acting by inhibiting feed intake via a drop in feed-seeking behaviour with an increase in feed rejection activity (Wong et al., 2013). The actions of these genes modulate the animal's nutritional

TABLE 8 Genomic regions of 10 adjacent SNPs that explain more than 0.5% of the additive genetic variance (Var) for residual intake and body weight gain (RIG) in Nellore cattle

BTA	Start position (bp)	End position (bp)	Var (%)	Genes
1	95,979,250	96,791,940	0.71167	ECT2, TNIK, SLC2A2
1	129,646,287	130,413,011	0.50496	KPNA6
2	20,929,001	21,696,086	1.02488	ATF2, CHRNA1
2	120,775,372	122,330,054	0.85774	PDE6D, ECEL1, CHRND, CHRNG, PHC2, AK2, RBBP4, ZBTB8A, HDAC1, LCK, KHDRBS1, SPOCD1, HCRTR1, TINAGL1
2	19,851,161	20,896,601	0.59712	NFE2L2, HNRNPA3, HOXD3, HOXD4, HOXD9, HOXD10, HOXD11
5	99,077,991	100,222,618	0.69229	TAS2R42, TAS2R46, T2R65A, T2R12, BOTA-T2R10B, TAS2R10, T2R10C, YBX3, STYK1, KLRJ1, KLRD1, GABARAPL1, OLR1, CLEC7A, CLEC1A, CLEC1B, CLEC12B
5	33,732,100	34,992,983	0.57163	SCAF11, ARID2
6	73,547,830	74,233,023	0.61928	PPAT, PAICS, HOPX, REST, POLR2B, IGFBP7
9	33,173,529	34,265,873	1.02901	ROS1, KPNA5, RSPH4A
11	29,736,720	30,551,963	0.51941	MSH2, MSH6, LHCGR
14	19,649,604	20,725,667	1.72493	
14	21,224,382	21,735,604	1.43592	HAS2, SPIDR, H3F3C, PRKDC, UBE2V2, SNAI2
14	21,976,451	22,392,760	1.37106	
14	16,387,114	17,752,395	1.09489	ZNF572, MTSS1, TATDN1, RNF139, ANXA13
14	24,543,370	24,643,266	0.93121	
14	24,864,286	25,147,967	0.89330	LYN
14	15,551,978	16,285,123	0.88898	
14	23,929,089	24,222,338	0.87523	POLR2K, SOX17
14	18,460,103	19,605,085	0.87461	FBXO32, ATAD2, ZHX1, DERL1, ZHX2
14	24,225,369	24,539,053	0.80941	
14	23,252,097	23,893,220	0.69756	RB1CC1, NPBWR1, OPRK1, TCEA1
19	5,974,265	6,709,868	0.69818	
19	42,988,287	43,755,425	0.53954	NT5C3B, ACLY, CNP, DNAJC7, HCRT, STAT5B, STAT5A, STAT3, ATP6V0A1, HSD17B1, COASY, TUBG1, TUBG2, EZH1, VPS25, BECN1, PSME3, AOC2, AOC3, SAO, BRCA1, DHX8
20	7,077,978	7,661,649	0.93475	
20	66,782,391	67,959,003	0.72484	MED10
21	21,333,104	22,991,710	0.64695	ACAN, HAPLN3, MFGE8, FANCI, TICRR, AP3S2, CIB1, VPS33B, PRC1, HDDC3, MAN2A2, FES, BLM, CRTC3, ZSCAN2, NMB, PDE8A, AP3B2
30	86,838,544	87,524,773	0.58080	

Abbeviations: bp, base pair, BTA, *Bos taurus* autosomes.

status, feed behaviour and intake and thus may lead to obtaining divergent animals for FE.

The *HCRT* and *PPY* genes have shown a change in circulation within minutes to hours after feeding (Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004; Reid et al., 2017; Tyree et al., 2018). The action of these genes is mediated via leptin and insulin, leading to sensation satiety and acting in feed intake, energy homeostasis and balance (Graaf et al., 2004; Reid et al., 2017; Tyree et al., 2018).

The *OPRK1* gene mediates stress responses, cortisol response, salivation regulation and opioid receptor activity (Xu et al., 2013). Also as part of the opioid system (de Silva, 2018), the *PENK* gene is involved in behaviour responses and has a role in the feeding behaviour of mice (Martín-García et al., 2011). Changing opioid levels alters feed behaviour, intake and efficiency through action on the central nervous system and is related to different physiological functions (Glass, Billington, & Levine, 2000).

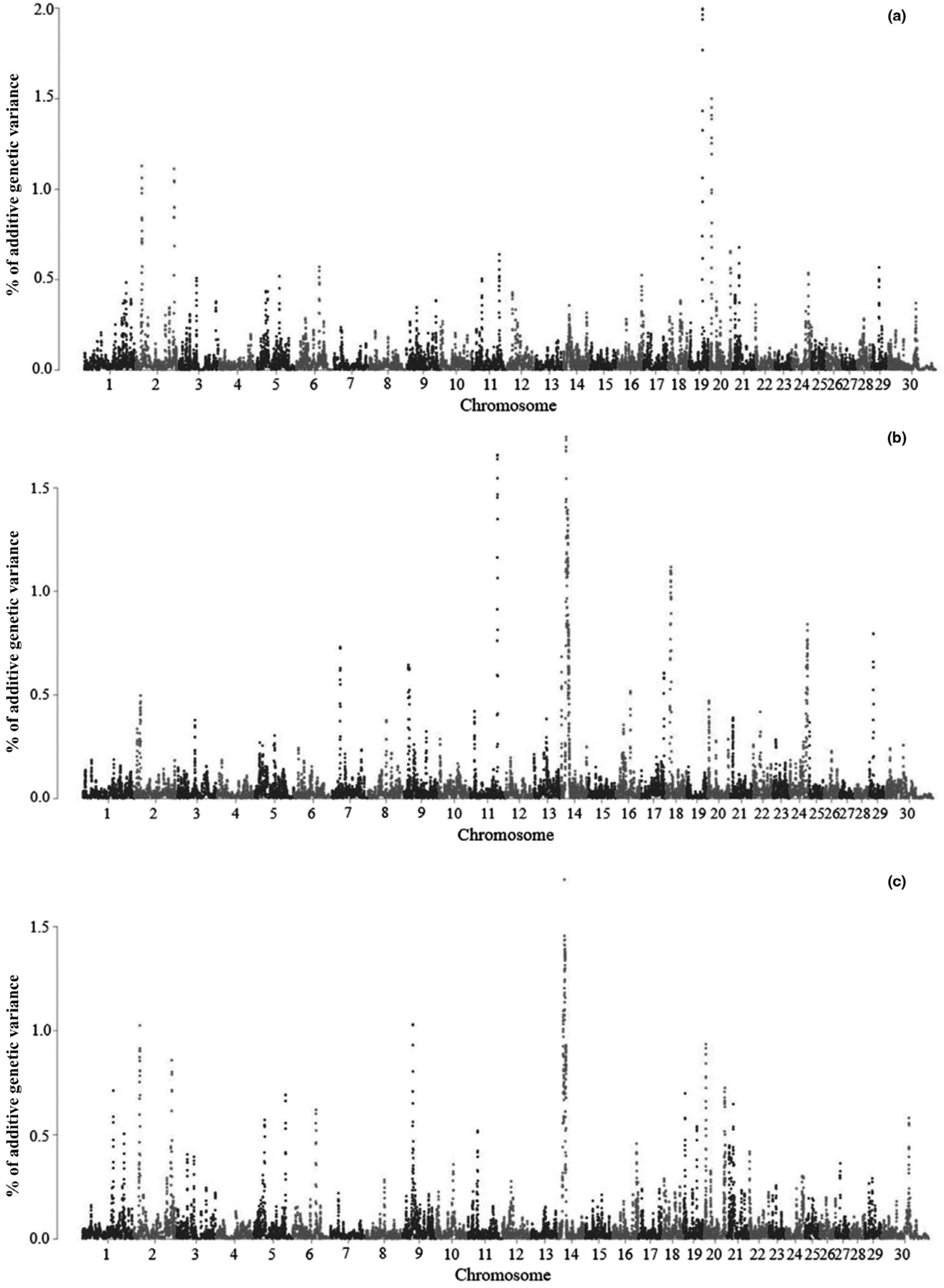

FIGURE 1 Proportion of additive genetic variance explained by windows of 10 adjacent SNPs for residual feed intake (a), residual body weight gain (b) and residual intake and body weight gain (c) in Nellore cattle

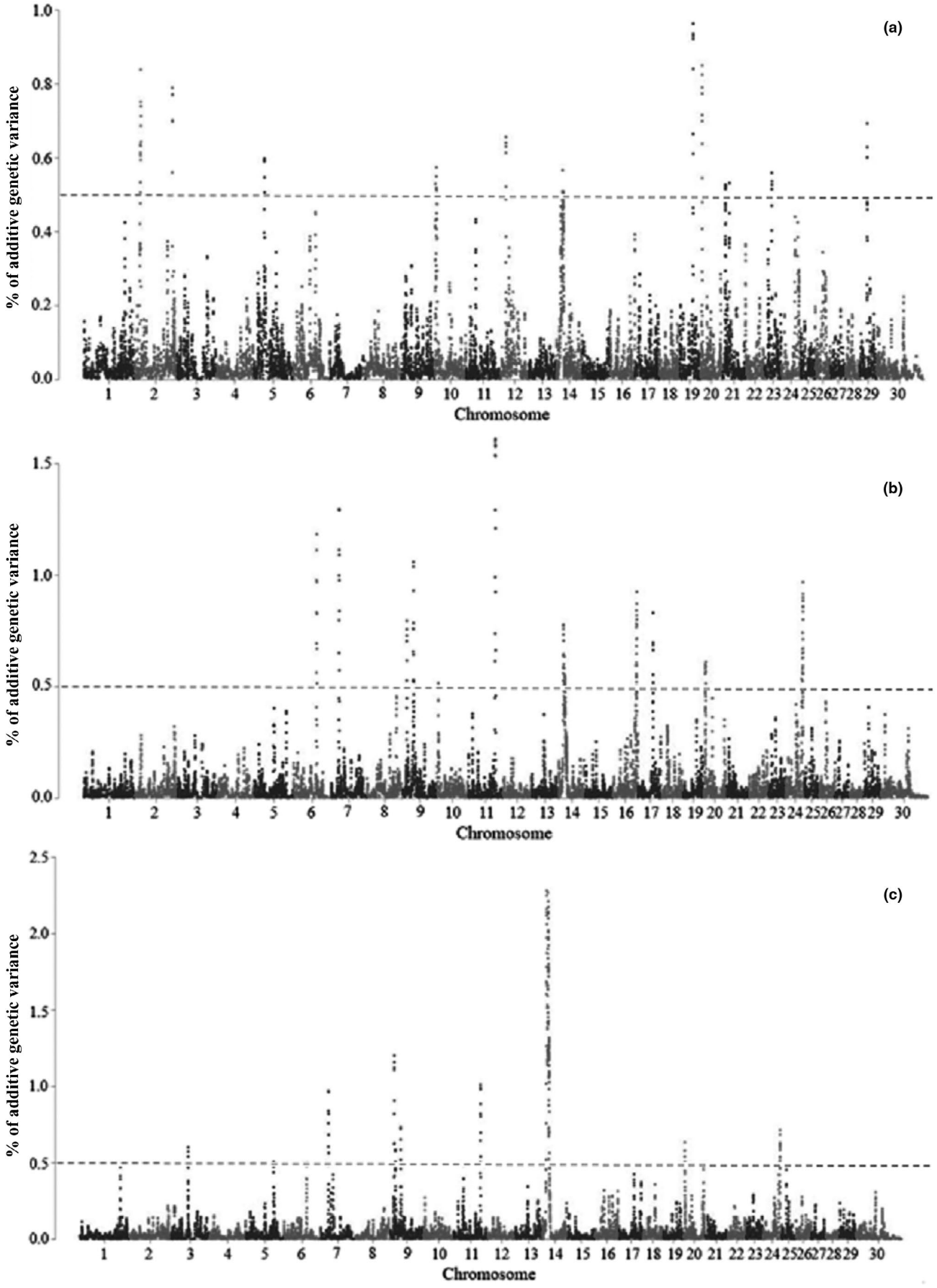

FIGURE 2 Proportion of additive genetic variance explained by windows of 10 adjacent SNPs for dry matter intake (a), feed efficiency (b) and feed conversion ratio (c) in Nellore cattle

TABLE 9 KEGG pathways and Gene Ontology terms revealed by DAVID analyses for feed efficiency-related traits in Nellore cattle

Term	*N*	*p*-value	Genes	FDR
Biological process				
GO:0007218 ~ neuropeptide signalling pathway	10	.00076	*HCRTR1*, *HCRT*, *PPY*, *PENK*, *ECEL1*, *CYSLTR2*, *OPRK1*, *NPBWR1*, *NMB*, *PYY*	1.27
GO:0071498 ~ cellular response to fluid shear stress	4	.00313	*MTSS1*, *XBP1*, *HAS2*, *NFE2L2*	5.16
GO:0008285 ~ negative regulation of cell proliferation	17	.00444	*NF2*, *BECN1*, *REST*, *RB1*, *STAT3*, *OSM*, *LIF*, *MSX2*, *HSF1*, *IRF6*, *BRD7*, *TFAP2B*, *RNF139*, *DIS3L2*, *C24H18ORF54*, *CIB1*, *F2R*	7.23
GO:0048863 ~ stem cell differentiation	5	.00797	*EPCAM*, *LIF*, *MSX2*, *HOXD4*, *ETV4*	12.64
GO:0071479 ~ cellular response to ionizing radiation	5	.01077	*BLM*, *SPIDR*, *SNAI2*, *EEF1D*, *ECT2*	16.71
GO:0038083 ~ peptidyl-tyrosine autophosphorylation	6	.01149	*FRK*, *STYK1*, *LYN*, *LCK*, *FES*, *ROS1*	17.73
GO:0009308 ~ amine metabolic process	3	.01159	*SAO*, *AOC2*, *AOC3*	17.87
GO:0035137 ~ hindlimb morphogenesis	3	.01159	*HOXD9*, *TFAP2B*, *HOXD10*	17.87
GO:0090090 ~ negative regulation of canonical Wnt signalling pathway	9	.01162	*CYLD*, *NPHP4*, *RGS20*, *NKD1*, *SOST*, *HDAC1*, *SOX17*, *SNAI2*, *ZNRF3*	17.92
GO:0006357 ~ regulation of transcription from RNA polymerase II promoter	17	.01303	*ANKRA2*, *RFX4*, *RFX6*, *RB1*, *STAT3*, *ATF2*, *MED4*, *MLX*, *BRD7*, *HOPX*, *ZNF710*, *TCEA1*, *NFE2L2*, *MAFA*, *ASCL4*, *DBX1*, *ETV4*	19.87
GO:0043123 ~ positive regulation of I-kappa B kinase/NF-kappa B signalling	11	.01447	*TNFSF10*, *NOD2*, *GOLT1B*, *SHARPIN*, *PLEKHG5*, *TRIM13*, *EEF1D*, *ECT2*, *TRIM62*, *TRAF5*, *F2R*	21.82
GO:0031648 ~ protein destabilization	5	.02032	*DERL1*, *XBP1*, *RNF139*, *PRKDC*, *SOX17*	29.30
GO:0070493 ~ thrombin receptor signalling pathway	3	.02321	*F2RL2*, *IQGAP2*, *F2R*	32.75
GO:2001214 ~ positive regulation of vasculogenesis	3	.02321	*RAMP2*, *CD34*, *TMEM100*	32.75
GO:0019233 ~ sensory perception of pain	5	.02523	*PENK*, *IAPP*, *OPRK1*, *NIPSNAP1*, *HOXD1*	35.06
GO:0045647 ~ negative regulation of erythrocyte differentiation	3	.03024	*STAT5A*, *STAT5B*, *GAS2L1*	40.46
GO:0001580 ~ detection of chemical stimulus involved in sensory perception of bitter taste	4	.03626	*T2R10C*, *T2R12*, *BOTA-T2R10B*, *TAS2R10*	46.41
GO:0006351 ~ transcription, DNA-templated	31	.03694	*EZH1*, *HINT1*, *STAT5A*, *STAT5B*, *REST*, *MAF1*, *SEC14L2*, *MSX2*, *HSF1*, *XBP1*, *TCEA1*, *SCX*, *RBBP4*, *PHF11*, *SNAI2*, *SPOCD1*, *ZSCAN2*, *BRCA1*, *MED10*, *STAT3*, *HOXD9*, *HDAC1*, *IRF6*, *HOXD3*, *HES2*, *HOPX*, *NFE2L2*, *PUF60*, *ZNF572*, *ZBTB8A*, *VPS25*	47.05
GO:0008284 ~ positive regulation of cell proliferation	16	.03942	*PRC1*, *MARCKSL1*, *PKHD1*, *STAT5A*, *STAT5B*, *ST8SIA1*, *OSM*, *EPCAM*, *LIF*, *HDAC1*, *TFAP2B*, *SDCBP*, *HAS2*, *SCX*, *CIB1*, *F2R*	49.30
GO:0042149 ~ cellular response to glucose starvation	4	.04585	*MTMR3*, *XBP1*, *BECN1*, *NFE2L2*	54.74
GO:0051930 ~ regulation of sensory perception of pain	3	.04639	*SPX*, *TMEM100*, *F2R*	55.17

(Continues)

TABLE 9 (Continued)

Term	*N*	*p*-value	Genes	FDR
Molecular function				
GO:0030246 ~ carbohydrate binding	10	.00489	*CLEC1A*, *MAN2A2*, *OLR1*, *CD34*, *KLRJ1*, *CLEC12B*, *ACAN*, *CLEC7A*, *KLRD1*, *CLEC1B*	6.83
GO:0033038 ~ bitter taste receptor activity	4	.00743	*T2R10C*, *T2R12*, *BOTA-T2R10B*, *TAS2R10*	10.20
GO:0008131 ~ primary amine oxidase activity	3	.01143	*SAO*, *AOC2*, *AOC3*	15.28
GO:0003824 ~ catalytic activity	8	.01697	*PHKB*, *HINT1*, *SYN3*, *CDADC1*, *ISOC1*, *AZIN2*, *PGGT1B*, *AASDH*	21.87
GO:0008565 ~ protein transporter activity	7	.01820	*RAMP2*, *AP1B1*, *AP3S2*, *KPNA6*, *KPNA5*, *IPO9*, *KPNA3*	23.27
GO:0005184 ~ neuropeptide hormone activity	4	.04018	*PPY*, *PENK*, *SPX*, *PYY*	44.66
Cellular component				
GO:0000228 ~ nuclear chromosome	4	.00981	*MSH6*, *MSH2*, *SPIDR*, *THOC5*	12.66
GO:0031234 ~ extrinsic component of cytoplasmic side of plasma membrane	7	.01306	*FRK*, *CYLD*, *STYK1*, *KCNAB2*, *LYN*, *LCK*, *FES*	16.52
GO:0005654 ~ nucleoplasm	62	.01768	*HTATIP2*, *ZMAT5*, *PRC1*, *TIMM17A*, *STAT5A*, *CNP*, *MAF1*, *HOXD11*, *SENP6*, *ACOT7*, *HSF1*, *FANCI*, *RSPH4A*, *COL4A3BP*, *DNAJC7*, *PHC2*, *CIB1*, *KHDRBS1*, *POLK*, *TNIK*, *ZHX1*, *ZHX2*, *PARP10*, *TATDN1*, *MTRR*, *RECQL*,	
GO:0005654 ~ nucleoplasm	62	.01768	*NAV2*, *HOXD3*, *KPNA6*, *KPNA5*, *PSME3*, *KPNA3*, *CRTC3*, *COASY*, *HSD17B1*, *TICRR*, *GSDMD*, *PRKDC*, *BOP1*, *ARID2*, *SCRIB*, *ATF2*, *HNRNPA3*, *MTMR3*, *SCAF11*, *ETNK1*, *SPIDR*, *DHX8*, *MSH6*, *ZBTB48*, *OLR1*, *CENPQ*, *ATAD2*, *ACLY*, *SF3A1*, *STAT3*, *DUSP3*, *ATP6V0A1*, *ANXA13*, *FBXO32*, *CLEC7A*, *PUF60*	21.72
GO:0009986 ~ cell surface	20	.02864	*RAMP2*, *CLSTN2*, *CD109*, *IQGAP2*, *LY6K*, *FURIN*, *EPCAM*, *NOD2*, *LYNX1*, *LY6D*, *CD46*, *CCR10*, *CLEC9A*, *KCNN2*, *CHRNA1*, *GHSR*, *ANO6*, *ROS1*, *AOC3*, *F2R*	32.89
GO:0030123 ~ AP−3 adaptor complex	3	.04704	*AP3B2*, *AP3S2*, *VPS33B*	48.39
KEGG pathway				
bta04742:Taste transduction	7	.00098	*T2R10C*, *TAS2R46*, *TAS2R42*, *T2R12*, *TAS1R1*, *BOTA-T2R10B*, *TAS2R10*	1.25
bta04976:Bile secretion	8	.00413	*SLCO1B3*, *NCEH1*, *ADCY2*, *SLCO1A2*, *ADCY7*, *HMGCR*, *CYP7A1*, *KCNN2*	5.15
bta04922:Glucagon signalling pathway	9	.00759	*LDHB*, *G6PC*, *ADCY2*, *CALM*, *PHKB*, *SLC2A2*, *GYS2*, *CALM2*, *ATF2*	9.27
bta00230:Purine metabolism	12	.01689	*PDE6D*, *ADCY2*, *ADCY7*, *NT5C3B*, *POLR2K*, *AK2*, *HDDC3*, *PDE8A*, *PAICS*, *POLR3B*, *PPAT*, *POLR2B*	19.55
bta04950:Maturity onset diabetes of the young	4	.04192	*IAPP*, *RFX6*, *SLC2A2*, *MAFA*	42.14

Abbreviations: FDR, false discovery rate; *N*, number of genes; *p*-values, significance level at 5%.

The glucagon signalling pathway (bta04922) is involved in energy homeostasis and led to suppression of feed intake and behaviour, besides being acting in the sign of satiety (Inokuchi, Oomura, & Nishimura, 2007). Glucagon is secreted when the animal feeds on a protein-rich diet and promotes protein synthesis (Hentze, Carlsson, Kondo, Nassel, & Rewitz, 2015). This mechanism may be important for growth, lean mass and FE. The glucagon signalling pathway was identified as a significant overrepresented pathway for intramuscular fat content and fatty acid composition in *Longissimus dorsi* muscle of Simmental and Yunling cattle (Zhang et al., 2018).

Among the genes harboured in this pathway, the *SLC2A2*, *G6PC* (Foote, Keel, Zarek, & Lindholm-Perry, 2017) and *PHKB* (Nadeau et al., 2012) genes are related to glucose, insulin and energy metabolism. The *SLC2A2* gene was reported associated with weight gain under the same feeding conditions due to more efficiency at small intestinal starch digestion in beef steers (Foote et al., 2017). The *G6PC* gene catalyses the final steps of gluconeogenesis and glycogenolysis, and was expressed in the fasting or in increased glucose demand in cattle, being related to satiety (Foote et al., 2017). The glycolysis, glycogenesis and glycogenolysis metabolic pathways are related to the energy supply in the body, being activated to generate ATP (Rui, 2014), which may be related to the use of energy from food for conversion to body weight. The *PHKB* gene was associated with the carbohydrate metabolic process, glycogenolysis regulation, generation of precursor metabolites and energy. *PHKB* action in the energy metabolism occurs because this gene catalyses the Ca^{2+}-dependent phosphorylation of glycogen phosphorylase in skeletal muscle and stimulates the breakdown of glycogen to ensure a continuous energy supply (Nadeau et al., 2012). This gene was previously suggested as a candidate gene for FE-related traits in Nellore cattle (Oliveira et al., 2014).

The negative regulation of canonical Wnt signalling pathway (GO:0090090), also known as Wnt/β-catenin signalling, was associated with carcass traits in Nellore cattle (Silva-Vignato et al., 2017). Studies showing that this pathway plays important role in skeletal muscle homeostasis (von Maltzahn, Chang, Bentzinger, & Rudnicki, 2012) and the adipocyte differentiation (Li, Luo, Liu, Yang, & Yang, 2008). Among the genes in this pathway, the *ZNRF3* gene that encodes a zinc finger protein and inhibits adipogenesis stimulated lipolysis and affected energy expenditure (Chen & Wang, 2018) that may influence the animal's response to weight gain as a function of feed intake. The *SOX17* gene plays a role in regulating insulin secretion in response to fasting and feeding, and controls genes that regulate insulin secretion, as *GLP1R* and *GLUT2* (Jonatan et al., 2014).

The GO term detection of chemical stimulus involved in sensory perception of bitter taste (GO:0001580), bitter taste receptor activity (GO:0033038) and taste transduction (bta04742) harbours genes that are proteins co-expressed in distinct subpopulations of taste bud cells of the human gustatory system (Valente et al., 2018). The bitter taste receptors affect the release of anorexigenic gut hormones and inhibit gastric contractility that, in turn, may regulate appetite; perception affects feed intake and influences production traits, as body weight (Avau et al., 2015; Ribani et al., 2017). To our knowledge, none of the genes in this pathway were previously reported as candidate genes for FE in cattle; nevertheless, taste perception affects feed intake and production traits, as body weight (Ribani et al., 2017), and appears to function in FE phenotype.

The bile secretion pathway (bta04976) is related to diet and acts in solubilization of fats and subsequently increases absorption (Reshetnyak, 2013), affecting the feed intake and increased weight gain (Parsaie, Shariatmadari, Zamiri, & Khajeh, 2007). Abo-Ismail et al. (2014) identified it as a potential pathway to contributing to variation in FE traits in cross-bred beef cattle. Among the genes harboured in this pathway, the *CYP7A1* (Alexandre, 2015) and *HMGCR* (Mukiibi et al., 2019) genes play a role in cholesterol and lipid metabolism. The *CYP7A1* action was reported differentially expressed for RFI in Nellore cattle (Alexandre, 2015), which may be due to enhancing the absorption of lipids and lipid-soluble nutrients and follows the aim of improving the supply with metabolizable energy (Wooton-Kee et al., 2010). The *HMGCR* was identified associated with ADG and DMI in Angus (Mukiibi et al., 2019). The *KCNN2* gene plays a role in activity in calcium/potassium channels (Shakkottai et al., 2001) and feeding motivation (Kommadath, 2012). These physiological processes (calcium/potassium channels) are energetically expensive, *KCNN2* being a particularly intriguing candidate gene for FE as reported by Olivieri et al. (2016) in a GWAS with FE in Nellore cattle.

Some of the genomic regions, genes and pathways identified in this study were not reported in public databases related to FE in cattle (Alexandre, 2015; Gomes et al., 2013; Lima, 2019; Lindholm-Perry et al., 2017; Mujibi et al., 2011; de Oliveira et al., 2014; Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014; Santos, 2018; Seabury et al., 2017; Serão et al., 2013; de Silva, 2018). Probably, the FE -related traits in Nellore cattle are regulated by different biological mechanisms than other cattle subspecies; and several physiological mechanisms may be behind FE control in beef cattle. The maintenance requirement is one of the key points in the FE regulation, and zebu cattle have lower maintenance requirement than taurine, reaching up to 20% lower, which normally leads to lower DMI (CSIRO, 1999; Sainz et al., 2006). This difference is related to less fast heat production; less basal metabolism; the smaller size of their organs; more pronounced peripheral fat deposit, to the detriment of the interposed fat deposit; and the more efficient use of energy for maintenance in zebu cattle than taurine cattle (Paulino,

Fontes, Jorge, Pereira, & Gomes Júnior, 1999). These different physiological responses may justify the identification of genomic regions associated with FE in Nellore that was not reported in taurine cattle. Also, the variation in SNP allele frequencies, linkage disequilibrium of markers between *B. taurus* and *B. indicus* and genetic constitution of the population could result in identifying different markers associated with evaluated traits (de Oliveira et al., 2014; Gomes et al., 2013; Mujibi et al., 2011; Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014). In addition, the different methods and larger sample size in this study than commonly observed in studies with Nellore cattle may have resulted in variants that had not yet been identified (de Oliveira et al., 2014; Olivieri et al., 2016; Rolf et al., 2012; Santana et al., 2014).

A large number of genomic regions associated with FE-related traits were identified in this study. These results support the premise that these traits are highly polygenic and have their expression controlled by many QTL with small individual effects on FE traits. Pathways involved, mainly, insulin, leptin, glucose, protein and lipid metabolism; energy balance; heat and oxidative stress; zinc finger system; bile secretion; satiety; feed behaviour; salivation; digestion; and absorption of nutrients, were identified and are associated with FE. Understanding of enriched molecular processes, pathways and genes associated with FE-related traits will help shed some light on the underlying DNA variants and candidate genes that are associated with a phenotypic expression of these traits. Therefore, the results obtained in this study would support a better understanding of the genetic and physiological mechanisms that determine FE and would contribute to increase the reliability of genomic evaluation for FE-related traits in indicine cattle.

5 | CONCLUSION

The genetic architecture for FE-related traits shows a polygenic model of inheritance with several genomic regions with small effects, harbouring possible candidate genes for FE-related traits.

The candidate genes identified are involved in insulin, leptin, glucose, protein and lipid metabolism; energy balance; heat and oxidative stress; zinc finger system; bile secretion; satiety; feed behaviour; salivation; digestion; and absorption of nutrients and were highlighted as candidates for FE-related traits in cattle. The identification of these genes and their respective functions should contribute to a better understanding of the genetic and physiological mechanisms regulating Nellore FE-related traits. These results would support the selection for these traits, as developing genomic models incorporating and pondering causal variations or by allowing the associated SNPs to be assigned with higher weights in genomic selection. This information can serve as a basis for fine-mapping studies, aiming to identify causal mutations for these traits or incorporating functional SNPs in the development of new SNP chip panels. The associated genotypes that were identified can potentially be used in animal breeding programmes to select for FE within cattle production systems in a tropical environment.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or legal restrictions, because it belongs to a commercial breeding programme.

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APPENDIX 1

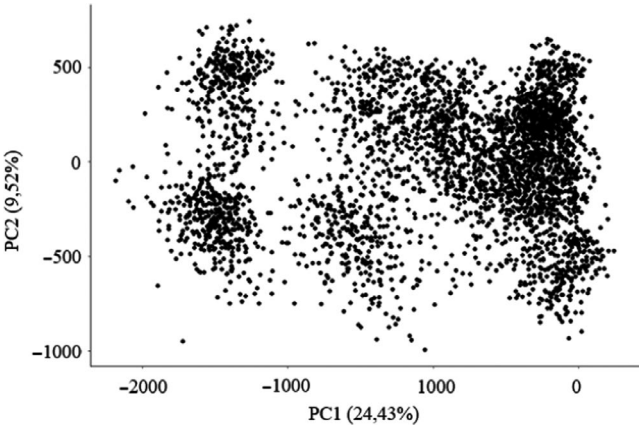

FIGURE A1 Principal component analyses of genomic relationship among Nellore cattle evaluated for related feed efficiency traits