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FABP1 and *SLC2A5* expression levels affect feed efficiency-related traits

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ABSTRACT

Improving the efficiency of production to reduce the environmental footprints is pivotal to the sustainability of livestock systems. Despite the advances in cattle feed efficiency (FE) measurement and identification of potential mechanisms involved, much is still unclear regarding the genetic and biological basis of this trait. Nevertheless, lipid and carbohydrate metabolism have been outlined as important in determining efficient and inefficient animals. To address the role of genes partaking in these processes and previously involved with residual feed intake (RFI), we carried out a liver expression profile in Nelore steers (n = 83). Six target genes (*FABP1, FADS2, PPP1R26, RGS2, SLC2A5,* and *UCP2)* were measured by qPCR analysis. A general linear mixed model approach was applied to associate them with dry matter intake (DMI), body weight (BW), metabolic BW (MBW, kg), DMI as a percentage of BW (DMI%BW), and average daily gain (ADG, kg/d). Residual feed intake (RFI), feed conversion ratio (FCR), feed efficiency (FE), Kleiber index (KI), and relative growth rate (RGR) were also evaluated. Our results support that increased expression of *FABP1* gene was associated with enhanced values for RFI and DMI. Likewise, higher expression level of *SLC2A5* was related to higher KI and RGR. There was no phenotypic correlation between RFI and ADG, BW, and MBW. The positive correlations between *FABP1* and *SLC2A5,* and between *FABP1* and *FADS2* gene expression suggest a putative co-regulation affecting feed efficiency phenotypes.

1. Introduction

There is a growing concern over the livestock systems impact on climate change and the use of natural resources. Concomitantly, strategies to improve the efficiency in the resource usage and animal yield have been developed and applied (Rojas-Downing et al., 2017). Regarding cattle production, one of the strategies is to identify animals with increased efficiency of feed conversion for the same production level (Herd and Bishop, 2000; Karisa et al., 2014). This approach would lead to cost reduction, as well as the environmental footprints (Nkrumah et al., 2006). However, many factors affect feed efficiency (FE), including genetic and environment (Arthur et al., 2001).

Accordingly, several approaches have been applied to measure the FE in beef cattle, since there is a genetic and phenotypic variation involved (Herd and Arthur, 2009). Despite the different methods to estimate FE, residual feed intake (RFI) has become a desirable measure as it is independent of other production and growth traits (Herd and Arthur, 2009; Moore et al., 2009). Extensive progress has been made to clarify the biological process behind the biological variation on FE. Gene expression profiling, as well as genome-wide association analysis

Abbreviations: ADG, Average daily gain; Cq, Cycle of quantification; BW, Body weight; DMI, Individual dry matter intake; DMI%BW, DMI as a percentage of BW; *FABP1*, Fatty acid binding protein 1; *FADS2*, Fatty acid desaturase 2; FCR, Feed conversion ratio; FE, Feed efficiency; KI, Kleiber index; MBW, Metabolic body weight; *PPP1R26*, Protein phosphatase 1 regulatory subunit 26; RFI, Residual feed intake; RGR, Relative growth rate; *RGS2*, Regulator of G protein signaling 2; *SLC2A5*, Solute carrier family 2 member 5; *UCP2*, Uncoupling protein 2

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(GWAS), have pointed out genes and metabolic processes acting on FE-related traits (Mukiibi et al., 2018; de Oliveira et al., 2014; Tizioto et al., 2015, 2016; Zhang et al., 2017).

These studies showed different quantitative trait loci (QTLs) and differentially expressed (DE) genes among breeds, and between efficient and inefficient animals. Although genomic regions and genes may not hold across studies, biological processes as lipid, protein, insulin, and carbohydrate metabolism involved with FE are in agreement among them (Diniz et al., 2018; Mukiibi et al., 2018; de Oliveira et al., 2018). Additionally, most of the genome-wide expression profiling studies employed case-control approaches, which does not consider the continuous variation of gene expression across the whole population once divergent groups are selected. By applying a differential expression framework, Tizioto et al. (2015) selected 20 Nelore steers, which are part of the animals evaluated here, genetically divergent for RFI. These authors reported several DE genes in liver partaking in the aforementioned process, including fatty acid binding protein 1 (*FABP1*), fatty acid desaturase 2 (*FADS2*), protein phosphatase 1 regulatory subunit 26 (*PPP1R26*), regulator of G-protein signaling 2 (*RGS2*), solute carrier family 2 member 5 (*SLC2A5*), and uncoupling protein 2 (*UCP2*). Although these genes have also been reported as DE for FE in other studies (Chen et al., 2011, 2012), it still missing to assess their expressions as a continuous variable.

Regardless of these results, there is still a gap in knowledge related to the genetic basis of FE and the linear gene expression-phenotype relationship. Furthermore, Mukiibi et al. (2018) suggested that the genetic architecture underlying FE is probably not the same across breeds. To better understand the quantitative gene expression-trait relationship regarding candidate genes that were identified by DE analysis in contrasting phenotypes, herein, we tested the hypothesis that the gene expression continuous variation across the population has an "additive effect" on the phenotype. We tested this hypothesis not only for the phenotype used on the previous DE analysis (RFI) (Tizioto et al., 2015) but also for all the FE-related traits measured in the experimental Nelore population. To this end, we carried out an association study based on qPCR liver expression profile in Nelore cattle samples representing the normal distribution of the phenotypes. Our results support that increased expression of *FABP1* and *SLC2A5* genes were related to higher values of some FE-related traits in Nelore.

2. Material and methods

2.1. Animals, phenotypic traits, and target gene selection

Nelore steers (n = 83) were produced and raised as previously described by Tizioto et al. (2015). The animals, allocated to feedlots with 21 months old, were evaluated for growth and FE-related traits. The animal management, diet, and the experimental trial were detailed elsewhere (de Oliveira et al., 2014). In brief, the feeding was offered *ad libitum* twice daily with a diet formulated to contain 40% dry matter of corn silage and 60% of the concentrate. During the 70-days trial, the dry matter intake was measured daily and non-fasted body weight (BW) every 14 days (de Oliveira et al., 2014).

The following parameters were estimated: individual dry matter intake (DMI, kg/d), body weight (BW, kg), metabolic BW (MBW, kg), DMI as a percentage of BW (DMI%BW), average daily gain (ADG, kg/d), residual feed intake (RFI, kg/d), feed conversion ratio (FCR, kg/kg), feed efficiency (FE, kg/kg), Kleiber index (KI, ADG/MBW), and relative growth rate (RGR%/d) (Bergh et al., 1992; Diniz et al., 2018; de Oliveira et al., 2014).

To select the target genes, we based our study on the previous work carried out by Tizioto et al. (2015). In this work, the authors selected 20 animals out of 83 based on the Best Linear Unbiased Prediction (BLUP) of genetic merit for RFI calculated in the context of 585 Nelore steers, and for which liver samples were available. For that study, the animals were grouped in contrasting classes high (n = 10) or low (n = 10) RFI, according to its genetic merit for RFI, and the hepatic genome-wide expression profile from RNA-sequencing was employed. The RFI was estimated as the residuals from the phenotypic regression of DMI on mid-test $BW^{0.75}$ and ADG. The regression model included the contemporary group, defined as feedlot place, year, animal origin, and pen type (individual or collective), as a fixed effect. Considering this approach, Tizioto et al. (2015) reported 112 annotated genes as DE, from which we selected six targets (*FABP1, FADS2, PPP1R26, RGS2, SLC2A5,* and *UCP2)* based on their biological role and involvement with FE traits. Herein, all the 83 animals, from which liver samples were available, from which Tizioto et al. (2015) selected the divergent animals, were used to assess the association of the target genes not only with RFI but also with other FE-related traits. Additionally, we adopted a continuous gene expression distribution to evaluate the gene expression-trait relationship rather than a case-control design.

2.2. RNA isolation and Real-time quantitative PCR

Liver samples (n = 83) collected at the slaughterhouse were frozen immediately in liquid nitrogen and kept at −80 °C until subsequent analyses. TRIzol® (Life Technologies) was used to extract the total RNA, following the manufacturer's instructions. The concentration and RNA quality (260/280 ratio) were measured by spectrophotometry (NanoDrop® ND-1000, Thermo Fisher Scientific). The RNA integrity was evaluated by electrophoresis on 1% agarose gel, and 24 randomly selected samples were further evaluated on Agilent 2100® Bioanalyzer (Agilent Technologies). After DNase I (Deoxyribonuclease I - Invitrogen®) treatment, the reverse transcription was performed individually with 1 μg of total RNA in a 20 μL reaction volume using SuperScript III (Invitrogen®), as suggested in the manufacturer's protocol.

Primers for the target genes (*FABP1, FADS2, PPP1R26, RGS2, SLC2A5,* and *UCP2)* were designed using Primer3 software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3 plus.cgi/) based on the transcript sequences deposited on Ensembl database (Table 1). The primers were designed across exon-exon junctions or in different exons, and up to 2 °C difference in the melting temperature between primer-pairs. Primer quality control and specificity analyses were carried out by NetPrimer software (http://www.premierbiosoft.com/netprimer/netprimer.html), and NCBI Basic Local Alignment and Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/), respectively. The genes *RPL19* and *YWHAZ* were used as reference genes after evaluation by RefFinder software (http://www.leonxie.com/referencegene.php?type=reference) (Table S1).

Table 1
Gene symbols, accession number, primer sequences, and amplicon size for qPCR gene expression analysis in liver of Nelore steers.

Genes	Accession	Sequence (5′ - 3′)	Amplicon size (bp)
SLC2A5	NM_001101042.2	F: CGATCTACTACTACGCAGACCA R: GTTATCAGCACATTGACAGCAC	103
PPP1R26	XM_002691719.3	F: CTCCATAGACAGCGATGACAG R: CCTCCTGGAATTTCTGATCC	96
RGS2	NM_001075596.1	F: AAGATTGGAAGAGCCGTTTGAG R: GAGAAGGCTTGATGAAGGTTTG	105
FADS2	NM_001083444.1	F: GCTTCATACCACCCTCTTTTCT R: CACAGAAGGGCAGAGGATTG	140
UCP2	NM_001033611.2	F: AGACGAGATACATGAACTCTGC R: GAGAAAGGAGGGCATGAACC	119
FABP1	NM_175817	F: GGTTCAGCAGGAAGGTGATAAT R: CCTTCGTCATGGTACTGGTAA	101

F = forward; R = reverse; bp = base pairs; *FABP1*- fatty acid binding protein 1; *FADS2* - fatty acid desaturase 2; *PPP1R26* - protein phosphatase 1 regulatory subunit 26; *RGS2* - regulator of G protein signaling 2; *SLC2A5* - solute carrier family 2 member 5; *UCP2* - uncoupling protein 2.

The relative expression levels of target genes were carried out in duplicate for every gene and sample. The experimental set-up was based on a sample maximization method proposed by Hellemans et al. (2007). *RPL19* gene was adopted as inter-run calibration. All reactions were carried on Applied Biosystems® 7500 Real-Time PCR system. The PCR reaction was performed in a 10 μL volume containing 1 μL of cDNA diluted 0.2×, 5 μL of SYBR® Green I Master Mix (Thermo Fisher Scientific) 2×, and 5 μM of each primer (Sigma-Aldrich®). Amplification conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C, and 1 min at 60 °C. A dissociation curve to test PCR specificity was carried out by one cycle for 15 s at 95 °C, 1 min at 60 °C, 30 s at 95 °C, and 15 s at 60 °C.

2.3. Data association and functional analyses

Based on the LinRegPCR software, the PCR efficiency was calculated by fitting the slope of the regression line taking into account the fluorescence intensities measured after each cycle for each sample (Ruijter et al., 2009). For this, we used at least three points in the log-linear phase from the amplification curve for each sample. Furthermore, the individual efficiencies (Cq – cycle of quantification) for all samples were adjusted for the theoretical maximum (100%) (Bustin et al., 2009), as previously described in Tizioto et al. (2013). A general linear mixed model approach was applied to consider the individual sample variation on gene expression, allowing adjusting for the cycle of quantification. Thus, the model included the reference gene and contemporary group as a fixed effect, and the animal sample as a random effect, according to the equation:

$$y_{ijk} = \mu + C_i + G_i + A_k + \varepsilon_{ijk} \quad (1)$$

Where:

y_{ijk}: is the Cq for the i^{th} contemporary group, for the j^{th} reference gene, of the k^{th} sample;

μ: is the average of Cq;

C_i: is the fixed effect for the i^{th} contemporary group ($i = 1, 2$);

G_i: is the fixed effect for the j^{th} reference gene ($j =$ gene 1, gene 2);

A_k: is the random effect associated with the k^{th} sample, taking account $A_k \sim$ NID (0, σa^2);

ε_{ijk}: is the random residual effect, with $\varepsilon_{ijk} \sim$ NID (0, σe^2).

The Satterthwaite degrees-of-freedom correction (Satterthwaite, 1946) was applied, as well as the variance for each reference gene was considered.

A complete model, including simultaneously the adjusted expression for all the target genes, obtained from eq. 1, was applied to estimate their effect on the studied traits. The genes did not show collinearity in this model. The same degrees-of-freedom correction described was applied. Based on the REG procedure on SAS® software, the analyses were carried out applying the model as follow:

$$y_{ij} = \mu + C_i + b_1(A_{ij} - \overline{A}) + \sum_{k=1}^{6} g_k(G_{ijk} - \overline{G_K}) + \varepsilon_{ijk}$$

Where:

y_{ij}: is the i^{th} trait for the j^{th} animal;

μ: is the overall mean;

C_i: is the fixed effect for the i^{th} contemporary group ($i = 1, 2$);

$b_1(A_{ijk} - \overline{A})$: is the regression coefficient associated with animal's age at slaughter;

A_{ij}: is the animal's age at slaughter; $\overline{A}$ is the mean age at slaughter;

G_{ijk}: is the k^{th} gene expression adjusted by eq. 1, for the i^{th} trait, for the j^{th} animal;

$\overline{G_K}$: is the mean for the k^{th} gene expression adjusted by the eq. 1 ($k = 1, 2, \ldots, 6$);

ε_{ijk}: is the random residual effect, with $\varepsilon_{ijk} \sim$ NID (0, σe^2).

Co-association analysis was performed between traits and genes by applying Pearson's correlation in the corrplot R-package (Wei and Simko, 2017). We calculated both the co-expression values based on our target genes expression (Cq) levels and the predicted co-expressed genes by using GeneMania (Warde-Farley et al., 2010). GeneMania allows us to predict the most closely connected genes based on our target gene list by finding functionally similar genes (Warde-Farley et al., 2010). To gain insights into the pathways in which these genes are partaking, we carried an over-representation analysis using STRING v.11.0 (Szklarczyk et al., 2017).

Table 2
Descriptive statistics for feed efficiency-related traits measured in a sample of Nelore steers.

Traits	N	Mean	SD
ADG (kg/d)	83	1.623	0.25
BW (kg)	83	345.1	32.15
DMI (kg/d)	83	8.806	1.02
DMI%BW (%$BW^{0.75}$)	83	2.561	0.27
FCR	83	5.524	0.81
FE	83	0.185	0.02
KI (kg gain/kg $BW^{0.75}$)	83	0.020	0.002
MBW	83	79.99	5.59
RFI	78	0.0154	0.66
RGR (%/d)	83	0.202	0.028

N - sample size; SD - standard deviation; ADG -average daily gain; BW - body weight; DMI- individual dry matter intake; DMI%BW - DMI as a percentage of BW; FCR - feed conversion ratio; FE - feed efficiency; KI - Kleiber index; MBW - metabolic body weight; RFI - residual feed intake; RGR - relative growth rate.

3. Results

We carried out a qPCR gene expression profiling in Nelore cattle to evaluate their relationship with FE-related traits. Descriptive statistics for the studied traits are presented in Table 2.

Based on the linear regression, we identified significant association among the target gene's expression level, measured as Cq, and the FE-related traits (Table 3). *FABP1* gene's Cq values were negatively associated with RFI and DMI, which decreased 0.2471 kg/d and 0.4001 kg/d, respectively, for each additional Cq unit. As Cq is inversely proportional to gene expression, it means that both traits exhibited a positive association with the gene expression. Thus, increased expression of *FABP1* leads to higher values of RFI and DMI. A similar pattern was observed for *SLC2A5* in relation to KI and RGR traits, where the increment in Cq values were associated with decreasing in the phenotypic measure. Likewise, the gene expression resulted in increased KI and RGR with estimated effects of 0.00006 and 0.00057, respectively. There was no significant association among *FADS2*, *RGS2*, *PPP1R26,* and *UCP2* with the evaluated traits (Table 3).

To identify the gene relationship and its co-association with the FE-related traits, we performed a correlation analysis. Based on that, the genes *FABP1*, *SLC2A5*, and *FADS2* showed a negative correlation with DMI, being the former also negatively correlated with RFI (Fig. 1). Gene co-expression also pointed significant correlations ($p \leq .05$) between *FADS2* and *FABP1*, *FADS2* and *PPP1R26*, and between *RGS2* and *UCP2* (Fig. 1). Furthermore, the co-expression prediction, based on GeneMania, pointed out 20 genes co-expressed with our targets (Fig. 2). The pathway analysis using all co-expressed genes retrieved protein digestion and absorption, fat digestion and absorption, and PPPAR signaling as over-represented KEGG pathways (FDR $\leq .05$).

We also applied correlation analysis to identify the relationship among the FE-related traits (Fig. 1). RFI was strongly and significantly correlated with DMI%BW. Moderate phenotypic correlations were identified between RFI and DMI, FCR, and FE. Strong and significant correlations were also identified for FCR with FE and with KI, KI with RGR, FE with KI, ADG with FCR, KI, and RGR.

Table 3
Estimated effect of target genes' expression, measured as adjusted real-time PCR cycle of quantification (Cq), on feed efficiency-related traits in Nelore.

Traits[a]	*RGS2*	*SLC2A5*	*UCP2*	*FABP1*	*FADS2*	*PPP1R26*
RFI	0.09699 (± 0.09514)	0.001473 (± 0.006028)	−0.01232 (± 0.06309)	−0.2471* (± 0.1146)	0.02909 (± 0.04856)	0.02051 (± 0.05328)
BW	−16.438 (± 47.441)	0.08352 (± 0.3048)	23.049 (± 32.159)	−35.760 (± 57.730)	−0.9858 (± 24.655)	45.295 (± 26.733)
MBW	−0.2704 (± 0.8265)	0.01462 (± 0.05310)	0.3981 (± 0.5602)	−0.6275 (± 10.057)	−0.1799 (± 0.4295)	0.7902 (± 0.4657)
ADG	−0.02203 (± 0.03856)	−0.00446 (± 0.002478)	0.04469 (± 0.02614)	0.004610 (± 0.04693)	0.005056 (± 0.02004)	0.002327 (± 0.02173)
DMI	0.1132 (± 0.1406)	−0.00132 (± 0.009031)	0.07979 (± 0.09528)	−0.4001* (± 0.1710)	0.01109 (± 0.07305)	0.09995 (± 0.07921)
DMI%BW	0.03700 (± 0.03581)	−0.00095 (± 0.03581)	0.005550 (± 0.02427)	−0.08531 (± 0.04357)	0.01275 (± 0.01861)	−0.00578 (± 0.02018)
FCR	0.1133 (± 0.1226)	0.01346 (± 0.007874)	−0.1034 (± 0.08308)	−0.1855 (± 0.1491)	−0.01195 (± 0.06370)	0.05686 (± 0.06907)
FE	−0.00432 (± 0.004219)	−0.00040 (± 0.000271)	0.003182 (± 0.002860)	0.007766 (± 0.005134)	0.000502 (± 0.002193)	−0.00172 (± 0.002377)
KI	−0.00016 (± 0.000400)	−0.00006* (± 0.000026)	0.000459 (± 0.000271)	0.000114 (± 0.000487)	0.000130 (± 0.000208)	−0.00018 (± 0.000226)
RGR	−0.00115 (± 0.004109)	−0.00057* (± 0.000264)	0.003041 (± 0.002785)	0.001992 (± 0.005000)	0.001141 (± 0.002135)	−0.00153 (± 0.002315)

[a] Estimated effect (± standard error, SE); *p ≤ .05; ADG -average daily gain; BW - body weight; DMI- individual dry matter intake; DMI%BW - DMI as a percentage of BW; FCR - feed conversion ratio; FE - feed efficiency; KI - Kleiber index; MBW - metabolic body weight; RFI - residual feed intake; RGR - relative growth rate; *FABP1*- fatty acid binding protein 1; *FADS2* - fatty acid desaturase 2; *PPP1R26* - protein phosphatase 1 regulatory subunit 26; *RGS2* - regulator of G protein signaling 2; *SLC2A5* - solute carrier family 2 member 5; *UCP2* - uncoupling protein 2.

4. Discussion

Feed efficiency in cattle is an economically important trait controlled by many factors, including the genetic background. Therefore, identifying the factors affecting FE and selecting animals with high breeding values for FE can be advantageous under different perspectives, such as land occupation, greenhouse gas emission, and profitability. Many genes have been pointed out acting in several biological processes related to FE. However, previous works have mainly adopted a case-control approach (Alexandre et al., 2015; Tizioto et al., 2015), thus overlooking the continuous variation of the expression profile in complex traits. Using that approach, Tizioto et al. (2015) reported 112 genes as DE, identified by liver RNA-seq, between groups of Nelore steers divergent for RFI. To overcome the limitations of this study, we applied a quantitative approach to associate the continuous gene expression with several FE-related traits. For this, we selected six target genes (*FABP1, FADS2, PPP1R26, RGS2, SLC2A5,* and *UCP2*) previously reported as DE by Tizioto et al. (2015). Then, we quantified their hepatic expression level in 83 Nelore steers, from where the animals used by Tizioto et al. (2015) were selected, representing the continuous variation of the traits, and associated them with ten FE-related traits.

According to Herd and Arthur (2009), metabolism, feed intake and digestion, and thermoregulation are among the main physiological processes affecting FE. Regarding energy metabolism, the efficiency of nutrient utilization and energy partition towards growth and muscle deposition are improved in animals with negative RFI values (Herd and Arthur, 2009). Thus, the lipid and carbohydrate metabolism have been reinforced as pivotal in more efficient animals (Karisa et al., 2014; Mukiibi et al., 2018; Tizioto et al., 2015). The genes *FADS2* and *FABP1*, are involved, respectively, with unsaturated fatty acid (FA) synthesis (Graugnard et al., 2009), FA uptake and transport (Ballester et al., 2017). Also, the *SLC2A5* gene codes a transporter acting in fructose absorption (Barone et al., 2009). Biological functions related to mitochondrial energy production (Schrauwen and Hesselink, 2002), protein synthesis modulation (Nguyen et al., 2009), and cell proliferation regulation by phosphatase activity (Hendrickx et al., 2009) have been described for the genes *UCP2*, *RGS2*, and *PPP1R26*, respectively.

Among the over-represented pathways, PPAR signaling has a pivotal role by modulating lipogenic gene expression and lipid synthesis (Graugnard et al., 2009). Acting in this pathway, we identified the lipogenic genes *FABP1* and *FADS2*, which were already reported as candidates for FE determination in Nelore steers. The positive relationship identified between *FABP1* and *FADS2,* considering both the correlation based on qPCR expression data and on prediction analysis, reinforces the joint action of these genes towards the energy metabolism by the interaction with the *UCP2* gene. Besides a role in resting energy expenditure, the *UCP2* gene has also been associated with insulin secretion (Schrauwen and Hesselink, 2002), which has an effective role in protein synthesis, lipolysis, and fatty acid biosynthesis (Graugnard et al., 2009; Karisa et al., 2014).

Our association model pointed not only a direct and linear relationship between *FABP1* and RFI but also its increased expression drives to enhanced RFI and DMI trait values. *FABP1* gene act as a metabolic sensor modulating the lipid homeostasis (Newberry et al., 2006). Furthermore, its co-expression with *FADS2*, which partakes in the FA beta-oxidation pathway, may promote increased fatty oxidation, leading to increased circulating free FA levels. Fatty acid oxidation acts as a modulator of feed intake (Scharrer, 1999) and thus, may affect growth performance through the consequent reduction of available nutrients (Azevêdo et al., 2010). Hepatic *FABP1* expression has been reported as up-regulated in inefficient Nelore animals (Tizioto et al., 2015). On the other hand, efficient animals were reported to have a down-regulation in lipid synthesis, being the energy input likely derived towards muscle deposition (Mukiibi et al., 2018), which is consistent with the observed down regulation on *FABP1* expression in this group of animals herein.

The involvement of the *SLC2A5* gene in the metabolism of carbohydrates (Barone et al., 2009) supports its association with FE-related traits, as identified here. Our approach showed that increased *SLC2A5* expression was associated with enhanced RGR and KI indices. However, although animals with higher weight gains exhibit the best efficiency for these indices (Sobrinho et al., 2011), it may also increase energy maintenance requirements (Manuel et al., 2019). The expression of nutrient transporter is essential for proper nutrient uptake and animal metabolism. The increased expression of the *SLC2A5* has been associated with increased adipocyte differentiation and fat deposition (Du and Heaney, 2012). The negative relationship identified between *SLC2A5* and FE is likely due to the fact that lipogenesis is energetically more expensive than muscle accretion (Herd and Arthur, 2009; Mukiibi et al., 2018).

Different biological mechanisms tightly regulate lipid and carbohydrate metabolism, as well as the efficiency of energy utilization (Ballester et al., 2017; Herd et al., 2004; Newberry et al., 2006). Similarly, several environmental and genetic factors underlie feed intake behavior, which has a major role in feed efficiency. Unlike the approach adopted by Tizioto et al., (2015), we showed that only the genes *FABP1* and *SLC2A5* were associated with FE-related traits in a continuous variation context. Considering that, *FABP1* and *SLC2A5* genes have a potential role in the previously discussed biological functions, and are putative candidates for FE traits.

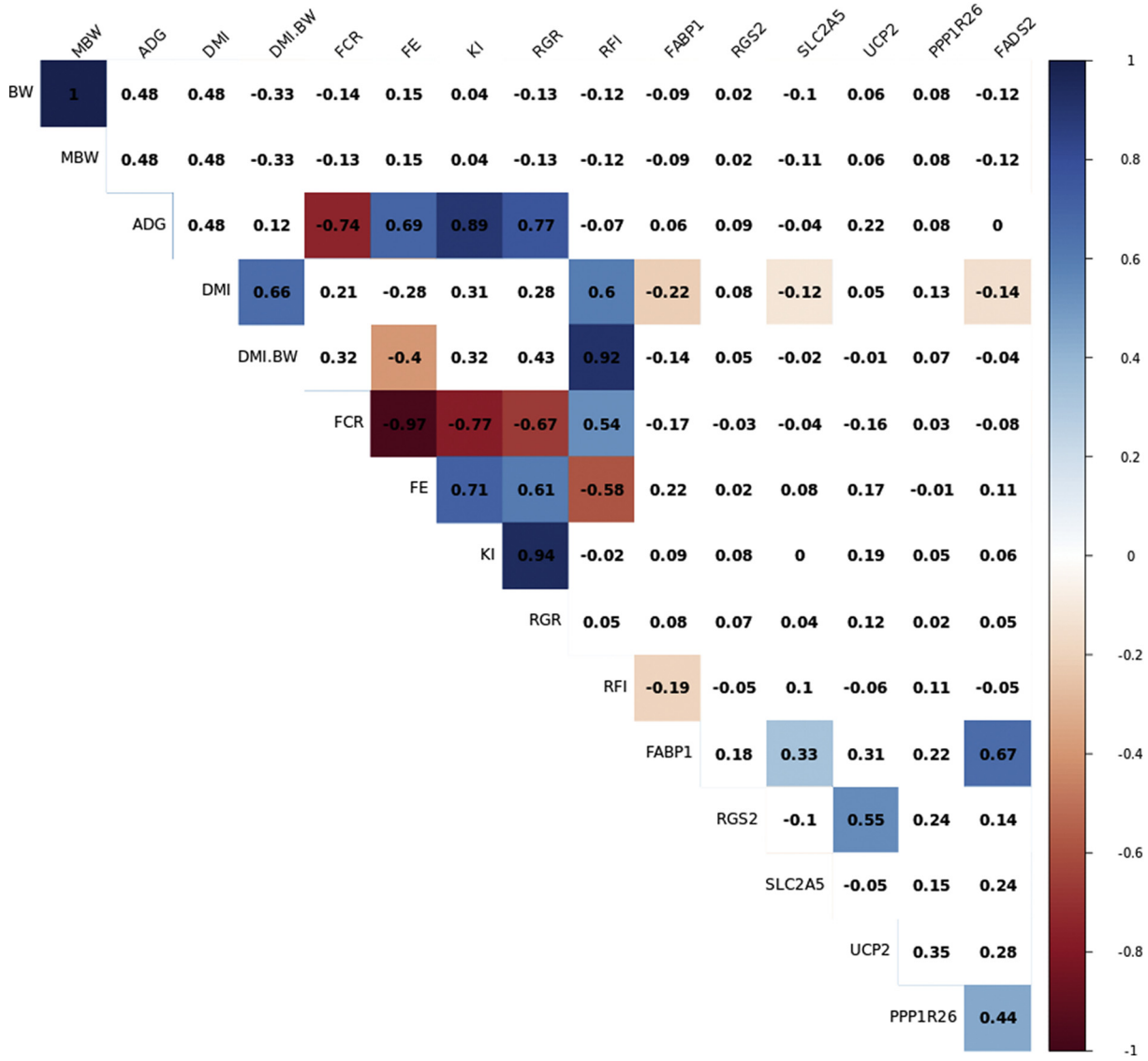

Fig. 1. Pairwise Pearson's correlation coefficients between feed efficiency-related traits and between the qPCR cycle of quantification in Nelore steers liver.
The matrix is color-coded ($p \leq .05$) based on their Pearson's correlation values according to the color legend. Blue and red colors represent, respectively, a positive or negative correlation. White cells are not significant. ADG -average daily gain; BW - body weight; DMI- individual dry matter intake; DMI%BW - DMI as a percentage of BW; FCR - feed conversion ratio; FE - feed efficiency; KI - Kleiber index; MBW - metabolic body weight; RFI - residual feed intake; RGR - relative growth rate; *FABP1*- fatty acid binding protein 1; *FADS2* - fatty acid desaturase 2; *PPP1R26* - protein phosphatase 1 regulatory subunit 26; *RGS2* - regulator of G protein signaling 2; *SLC2A5* - solute carrier family 2 member 5; *UCP2* - uncoupling protein 2.

5. Conclusion

Our results reinforce the role of *FABP1* and *SLC2A5* expression in the quantitative variation of feed efficiency-related traits. We suggested that the increased *FABP1* expression is unfavorable for RFI and DMI traits. Although *SLC2A5* gene expression may be favorable to KI and RGR indices, it may increase the energy requirements and compromise animal efficiency. The positive co-expression between some of the target genes suggests a putative co-regulation on the feed efficiency traits studied here.

Ethics statement

All experimental procedures involving steers in this study were approved by the Institutional Animal Care and Use Committee Guidelines (IACUC) from Brazilian Agricultural Research Corporation (EMBRAPA) and sanctioned by the President Dr. Rui Machado.

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Availability of data and materials

All relevant data are within the paper and its Supporting Information files.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

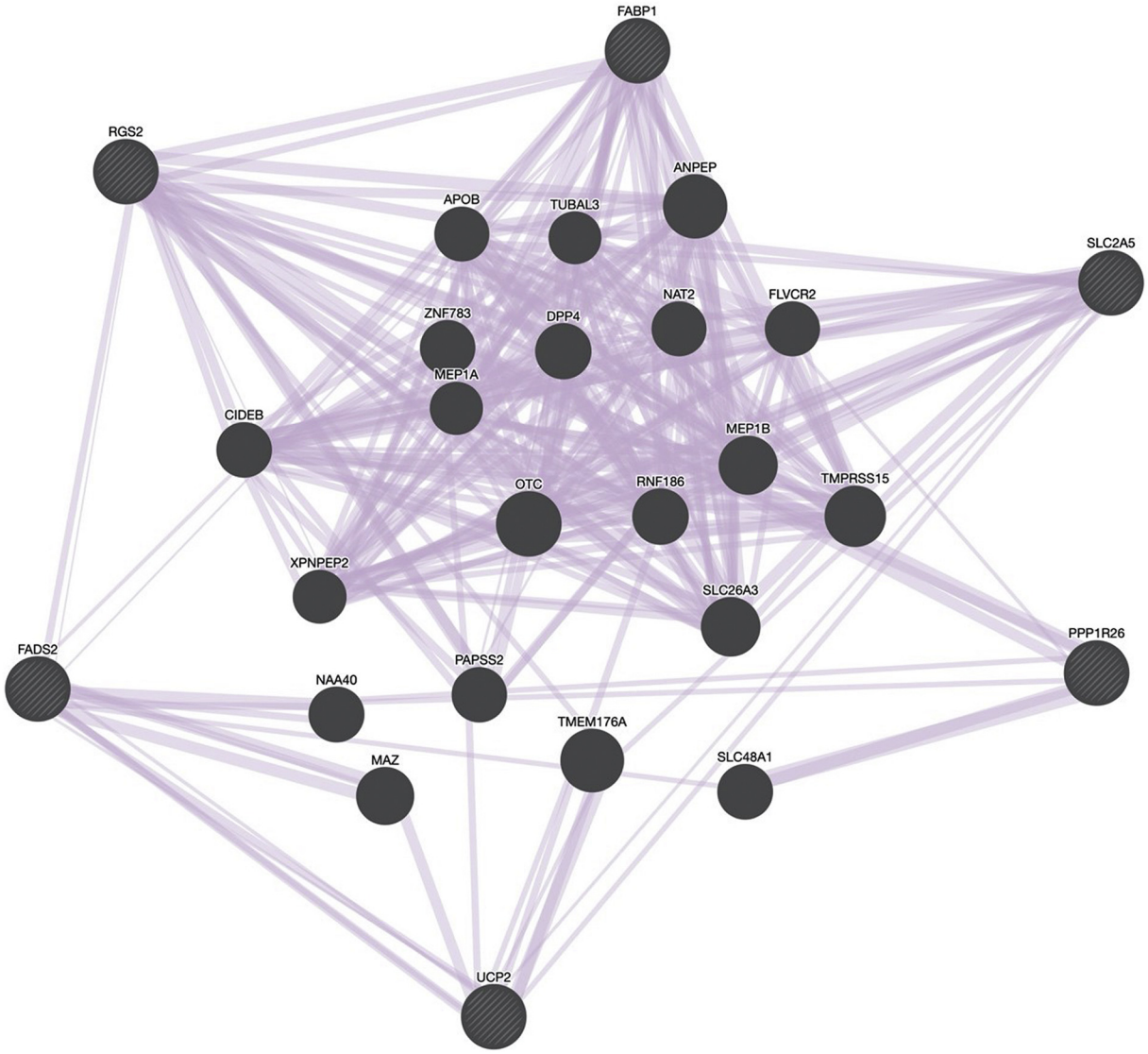

Fig. 2. Co-expression network prediction of selected hepatic genes in Nelore steers from GeneMania. The edges are based on co-expression prediction, and target genes (nodes) are indicated with stripes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aggene.2019.100100.

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