KCNJ11 gene expression is associated to feed consumption and growth traits in Nelore beef cattle

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ARTICLE INFO

ABSTRACT

Feed efficiency is a complex trait influenced by several genes and biological processes. However, there is limited knowledge about the genes and pathways involved in this trait. KCNJ11, related to the insulin secretion pathway, was shown to be a functional candidate gene for beef quality traits in Nelore. Given its role in energy metabolism, we evaluated the effects of KCNJ11 gene expression level on feed efficiency-related, carcass and growth traits in Nelore steers. Skeletal muscle KCNJ11 mRNA levels were positively and significantly associated with dry matter intake (DMI), total digestible nutrients consumption (TDN), average daily gain (ADG), and relative growth rate (RGR) from a general linear mixed model approach. Co-expression analysis, using RNA sequencing data obtained from Longissimus dorsi (LD) muscle of 30 Nelore steers, identified key signalling pathways related to the regulation of energy metabolisms, such as mitogen-activated protein kinases and insulin pathways. Our findings indicate a linear relationship between KCNJ11 gene expression and the phenotypic measures for feed intake, weight gain, and relative growth rate in Nelore steers, but not for residual feed intake or fat deposition traits.

1. Introduction

Feed efficiency has major importance for animal production because it can improve cattle industry profitability (Basarab et al., 2003). However, despite the known moderate heritability (Arthur et al., 2001), including estimates for the Nelore breed (De Oliveira et al., 2014), feed efficiency-related traits have not been prioritized in animal breeding programs probably due to the time and cost to assay these traits (Arthur et al., 2001).

The potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11), is mapped to bovine chromosome 15, within a QTL interval for meat tenderness (Rexroad et al., 2001). This QTL was also described in the Nelore breed (Tizioto et al., 2013b) where it was mapped to 35 Mb on BTA15. In a candidate gene approach, SNPs located in this gene had significant effects on meat tenderness in the Nelore population studied here (Tizioto et al., 2013a). KCNJ11 gene is known to be involved in the insulin secretion pathway, and a study with knockout mice observed reduced glycogen storage, lean phenotype, lower body fat, and muscle weakness related to KCNJ11 loss of function (Alekseev et al., 2010). However, there is no information on the role of KCNJ11 in bovine energy metabolism.

Based on KCNJ11 metabolic function and considering its role on beef tenderness of Nelore cattle (Tizioto et al., 2013a, 2014), we evaluated the effects of KCNJ11 gene mRNA level on feed efficiency-related, growth, and carcass traits in Nelore beef cattle. Taking advantage of other studies being developed by our research group, we also

performed a co-expression network analysis using RNA-seq data generated from 30 samples of LD muscle from Nelore steers (Tizioto et al., 2016a) to identify genes that are co-expressed with *KCNJ11* to understand its role in bovine physiology better.

2. Results and discussion

Feed efficiency is a complex trait in which many physiological processes, genes, and metabolic pathways collectively operate (Karisa et al., 2014; Moore et al., 2009). Among them, *KCNJ11* gene is involved in insulin secretion pathway and activation of genes related to residual feed intake and glucose transport efficiency (Karisa et al., 2014). Also, it has been pointed as a potential positional, physiological, and functional candidate gene for beef quality traits (De Souza et al., 2016; Tizioto et al., 2013a, 2014).

Insulin is one of the main regulators of feed intake in cattle and plays a central role in nutrient metabolism and energy partitioning (Nikkhah, 2014). Increased secretion of insulin in plasma decreased dry matter intake, glucose uptake, and amino acids in sheep (Herd et al., 2004; Oddy, 1993), and low-fat deposition in double musculature cattle (Bernard et al., 2009). Also, Oddy (1993) noted that insulin secretion in response to feed intake occurred more rapidly in sheep lines selected for higher growth when compared to those with lower growth.

As shown in Table 1, four of the evaluated traits in this study were significantly associated ($p \leq 0.05$) with expression levels of *KCNJ11*. We found that lower expression levels, represented in our analysis by higher threshold cycles (Ct) in real-time PCR amplification of *KCNJ11* gene, are related to smaller values of DMI, TDN, ADG and RGR (Table 1).

In contrast to the mice knockout model, were suppression of *KCNJ11* produced a lean phenotype (Alekseev et al., 2010), our results suggest that differences in fat deposition did not accompany reduction in daily gain and relative growth associated to lower *KCNJ11* expression. In our previous work (Tizioto et al., 2013a) lower *KCNJ11* expression values were associated with more tender meat, which would be favourable for the beef industry. However, the present results

indicate an antagonistic relationship among the effects of *KCNJ11* on meat tenderness, feed consumption, and growth traits.

Lower dry matter intake (DMI) is associated with reduced consumption of TDN and consequently affects animal performance (Azevêdo et al., 2010). Sobrinho et al. (2011), evaluating the residual feed intake and its relation to Nelore performance selected for post-weaning weight, observed that DMI, based on metabolic weight, is strongly and positively correlated with ADG and RGR. Thus, as suggested by our results, it is likely that the lower expression of *KCNJ11* leads to a reduction in DMI which, therefore, may affect correlated traits.

The relative growth rate is not a frequently used trait in animal breeding. Moreover, it is correlated with performance measures such as weight, growth, feed conversion, and daily gain (Sobrinho et al., 2011). Although selection for RGR may improve feed efficiency during the growth and termination phases, animals with higher growth rates tend to have higher nutritional maintenance requirements, which is usually not desired in the production system (Sobrinho et al., 2011).

Regarding the co-expression analysis, we identified 593 genes significantly co-expressed with *KCNJ11* in LD muscle whose pairwise correlations ranged from 0.50 to 0.80, (Fig. 1, Table S1). These genes are involved in seven KEGG database pathways (Table S2), including mitogen-activated protein kinases (MAPK) and insulin pathways. Both pathways have been previously related to feed efficiency in beef cattle (De Oliveira et al., 2014; Karisa et al., 2014; Serão et al., 2013).

The genes co-expressed with *KCNJ11* participate in signalling pathways that act in the regulation of energy metabolism, cell proliferation, and differentiation. Eighteen co-expressed genes were included in MAPK signalling pathway (Table S2) that regulate cell functions such as differentiation, proliferation, and cell death (Pearson et al., 2001). Protein kinases activate most of the genes (*e.g. FGF11*, *SRF*, *FGFR4*) identified in this pathway. Chen et al. (2011) found that genes related to these processes were up-regulated in Angus cattle with low-RFI. Serão et al. (2013) identified 11 genes harboring SNPs associated with feed efficiency that are involved in the MAPK pathway. From the genes found by these authors, four (*CACNA1S*, *FGF11*, *MAP2K2*, *MAPKAPK2*) were common to this study. Do et al. (2014) suggested that the effect of MAPK on feed intake may be mediated by a variety of pathways that act through tyrosine kinase receptor such as insulin.

The insulin signalling pathway is important for feed efficiency as evidenced in various species as cattle (Karisa et al., 2014) and swine (Do et al., 2014; Kim et al., 2014). This pathway was found enriched with eleven genes co-expressed with *KCNJ11*. These genes are related to biological processes such as regulating protein synthesis, lipid synthesis, glucose uptake, and storage (Kim et al., 2014).

3. Conclusions

Experimental data on *KCNJ11* function has pointed out its putative paper in main metabolic pathways for feed efficiency-related traits. Although the genetic architecture of feed efficiency is multifatorial, our findings support the role of the *KCNJ11* gene on feed intake, weight gain, and relative growth rate in Nelore steers, without considerably affecting common measures of feed efficiency such as RFI and FE, nor the fat deposition. Co-expression of *KCNJ11* in bovine muscle reinforces its role in the regulation of energy metabolism, cell proliferation, and differentiation.

4. Material and methods

The population consisted of 460 steers, half-sib families produced by artificial insemination with 32 sires representative of the main lineages of Nelore cattle in Brazil (Tizioto et al., 2013a, 2014). The animals were allocated into feedlots at EMBRAPA Pecuária Sudeste, São Carlos - SP, and evaluated for growth and feed efficiency traits. The

Table 1
Effect of real-time PCR cycle threshold (Ct) of *KCNJ11* gene in feed efficiency-related, carcass and growth traits in Nelore steers.

Traits	Estimated effect $\pm$ SE	p-Value
IFBW (kg)	-1.2911 ± 1.78	0.4723
FFBW (kg)	-3.8665 ± 2.10	0.0706
MBW (kg)	-0.4258 ± 0.31	0.1748
ADG (kg/d)	-0.02984 ± 0.01	0.0419*
DMI (kg/d)	-0.1065 ± 0.05	0.0478*
DMI/BW	-0.01319 ± 0.00	0.1481
FCR (kg/kg)	0.1581 ± 0.09	0.1182
FE (kg/kg)	-0.00178 ± 0.00	0.2174
KI	-0.00026 ± 0.00	0.0959
TDN (kg/day)	-0.09145 ± 0.04	0.0417*
ME (kg/kg)	-0.00795 ± 0.00	0.1004
PEG (kg/kg)	-0.00033 ± 0.00	0.8456
RGR (%/d)	-0.00274 ± 0.00	0.0461*
WGE (kg/Mcal)	-0.00279 ± 0.00	0.2286
RFI	-0.02905 ± 0.03	0.4079
BFT (mm)	-0.07936 ± 0.09	0.4233
REA (cm^2)	0.1638 ± 0.35	0.6437
IMF (%)	-0.03145 ± 0.05	0.0542

Initial fasting body weight (IFBW); Final fasting body weight (FFBW); Metabolic body weight (MBW); Dry matter intake (DMI); Dry matter intake per body weight (DMI/BW); Average daily gain (ADG, kg/d); Feed conversion ratio (FCR); Feed efficiency (FE); Kleiber index (KI); Total digestible nutrients consumption (TDN); Maintenance efficiency (ME); Partial efficiency of growth (PEG); Relative growth rate (RGR); Weight Gain Efficiency (WGE); Residual feed intake (RFI); Backfat thickness (BFT); Ribeye muscle area (REA); Intramuscular fat (IMF).

* significant p -value.