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SHORT COMMUNICATION

Proteomic investigation of liver from beef cattle (*Bos indicus*) divergently ranked on residual feed intake

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Abstract

Proteomics studies can be used to identify proteins that affect feed efficiency traits, related to cost and profitability of meat production. We used a proteomic approach based on two-dimensional electrophoresis (2D-PAGE) in combination with mass spectrometry (ESI-MS) to study liver samples of Nellore bulls divergently ranked according to residual feed intake (RFI). The study showed that 71 protein spots were expressed differentially ($P < 0.05$) among RFI groups and 47 were identified by ESI-MS. In RFI, efficient animals (low RFI) eat less than predictions, based on their weights and growth rate, while inefficient animals (high RFI) that eat more than predicted. Data from 18 animals (9 high vs. 9 low RFI) aged 24–26 months in feedlot finishing were used. Immediately after slaughter, liver samples were collected and protein extracts were separated. The gels of RFI groups were scanned and the images analyzed, whereby we found 279 and 215 liver protein spots in high and low RFI bulls, respectively. The proteins identified were related to the following biological functions: (I) oxygen transport and blood flow; (II) mitochondrial function and energy metabolism; (III) amino acid metabolism, ion transport, and cell survival. The study suggests hemoglobin subunit beta and heat shock protein 71 kDa and as molecular markers to study FE in Nellore cattle. Moreover, proteins such as 3-ketoacyl-CoA thiolase and glutamate dehydrogenase 1 were found in liver from high and low RFI animals, respectively. Such protein expression could be associated with changes in the oxidative capacity of RFI phenotypes.

Keywords Feed efficiency · Energy · Metabolism · Proteome · Zebu cattle

Introduction

Energy expenditure and efficiency of energy conversion into body weight gain are highly important. Animal feed efficiency (FE) accounts for most variation in emission of pollutants, greenhouse gases, and in the use of many natural resources for meat production. Brazil, the United States, Oceania and Europe are the main supplier of the increasing meat demand worldwide. In the international scenario, Brazil is expected to play a major role as a strong net exporter of livestock products [1].

The common approach for measuring FE is residual feed intake (RFI) [2]. Efficient animals (low RFI) eat less than what is predicted based on their weights and growth rate, while inefficient (high RFI) animals that eat more than predicted. This measurement checks the large individual variation in feed intake for a growth rate level and animal size. This variation is wide in cattle [3, 4] and few studies have investigated molecular mechanisms that underlie FE-related traits.

Proteomics approaches can be used to study changes in protein expression of liver and muscle, providing insights into the regulatory mechanism of animals [5]. To this end, two-dimensional gel electrophoresis (2D-PAGE) [6] and electrospray ionization mass spectrometry (ESI-MS) [7] are often applied.

Several proteins that impact FE traits were identified by proteomic approaches using 2D-PAGE in combination with ESI-MS [8, 9]. These studies indicated that many proteins

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are expressed differentially in FE phenotypes. Because liver is a central organ of energy metabolism and proteomic investigation allows a better understanding of its role in energy expenditure, we investigated whether the hepatic protein profile differed in Nellore bulls (*Bos indicus*) with high or low RFI.

Materials and methods

All animal care and use protocols were approved by the *Instituto de Zootecnia* (CEUA/IZ—Protocol 213-15) and were conducted in compliance with ethical research standards established by the University of São Paulo (USP/ESALQ).

Animals and RFI calculation

The experimental feedlot was conducted at *Centro Avançado de Pesquisa Tecnológica dos Agronegócios de Bovinos de Corte—Instituto de Zootecnia (IZ)*, Sertãozinho, State of São Paulo, Brazil. Nellore calves underwent an FE test after weaning to identify their RFI class (low or high RFI). For definition [2], efficient animals (low RFI) eat less than what is predicted based on their weights (metabolic body weight—$BW^{0.75}$) and growth rate (average daily gain—ADG), while inefficient (high RFI) animals that eat more than predicted. RFI was calculated as the equation previous described [10], in which no composition of growth parameter was used in the regression.

Eighteen animals not selected by IZ breeding program criteria were assigned to the feedlot for a finishing period and housed in individual pens. These 18 bulls were classified as low (< 0.5 standard deviations below the mean; more efficient), and high (> 0.5 standard deviations above the mean; less efficient) RFI groups.

Slaughter, tissue collection and sample preparation

Animals were slaughtered with an average BW of 557.65 ± 10.39 kg. Harvesting was carried out in an experimental slaughterhouse and was preceded by fasting for 16 h in accordance with Brazilian government inspection procedures. Immediately after slaughter, a section of liver was collected, placed in a sterilized aluminum foil and frozen in liquid nitrogen for transport. Subsequently, all samples were stored at − 80 °C until processing.

Protein extraction from liver samples

Approximately 250 mg of the pooled of liver tissue was macerated with 250 µL of ultrapure water followed by centrifugation at 13,000 rpm at 4 °C for 40 min each. The protein extracts were transferred to 2 mL vials and subjected to precipitation to obtain protein pellets for which 200 µL of the protein extract was mixed with 800 µL of 80% acetone (1:4 ratio) kept at 4 °C. Subsequently, the tube was then centrifuged (13,000 rpm at 4 °C for 15 min) and the obtained pellets were washed prior to quantify proteins and electrophoretic runs (2D-PAGE). One portion of the protein pellets was re-solubilized in 0.5 M NaOH to quantify total protein by Biuret method. An additional protein fraction was re-solubilized in a specific buffer containing 0.07 M urea, 0.02 M thiourea, 2% CHAPS (m/v), 10% ampholyte (pH range 3–10), and 0.002% bromophenol blue. Moreover, 2.8 mg of 1,4-dithiothreitol was added and this solution was used in electrophoretic separations.

Two-dimensional electrophoresis

Proteins in hepatic tissue were separated by 2D-PAGE. To represent each RFI groups (high vs. low), a pooled of liver samples was created from equal amounts of tissue and, subsequently, this standard was used for protein extraction and electrophoresis procedures. Briefly, approximately 375 µg of protein extracts were loaded into first dimension strips (13 cm) and the protein extracts were separated on pH 3–10 gradient as previously described [11]. After this step, the focusing strips were placed on a polyacrylamide gel with a concentration of 12.5% (w/v) in the second dimension of the electrophoretic process was performed in the molecular weight (MW) range of 14–97 kDa.

Image analysis

The gels from RFI groups were scanned and the images analyzed using the ImageMaster Platinum (v. 7.0) as previously described [11, 12] with minor adaptations. In brief, the equivalent spots (matching) were identified, and automatic and manual editing was performed to remove false spots. Additionally, the number of spots per gel, and the amount of protein in each of these spots were scanned.

In-gel digestion of proteins

Initially, the liver protein spots to be characterized were cut from gel and each one was placed in a 1.5 mL microtube for digestion using trypsin as previously described [12]. Protein spots were washed with dye removal and prepared for tryptic digestion (Rapid Digestion Kit—Trypsin, Promega Corporation) followed by peptide elution. Proteins were characterized by electrospray ionization mass spectrometry (ESI-MS/MS) [12].

Protein identification

Peptides were detected using the ESI-MS/MS mass spectrometer (nanoAcquity UPLCR - nanoAcquity HSS T3, Waters Solutions). The column equilibration, peptides separation gradient, and operation mode were performed as described [12]. The proteins were identified by sharing according to the software ProteinLynx Global Server (PLGS) version 3.0 and by searching the MASCOT v.2.2 database system (Matrix Science Ltd). The NCBI (https://www.ncbi.nlm.nih.gov/protein) database was searched [11].

Statistical analysis

A completely randomized design was adopted in the experimental feedlot. Data were analyzed with the GLM procedure of SAS software (SAS Inst. Inc version 9.3) considering the fixed effects of RFI class and the covariate initial body weight (BW). To compare high versus low RFI classes, the PDIFF option of SAS was applied. The protein spot volume data were imported into ImageMaster Platinum (v. 7.0) software. We study the protein expression considering each set of liver gels from high versus low RFI groups, whereby spots with P-value ≤ 0.05 obtained by ANOVA were considered differentially abundant.

Results

At the end of experimental feedlot, the difference between RFI means from high and low RFI groups was 2.0 kg DMI per day ($P < 0.01$). Low-RFI animals (more efficient) consumed − 1.16 kg/day whereas high-RFI animals (less efficient) consumed + 1.22 kg/day on average. Moreover, low-RFI showed lower DMI (13.2 ± 07 vs. 15.2 ± 0.6 kg/day) and higher gross FE (ADG/DMI = 0.139 ± 0.01 vs. 0.120 ± 0.01) than high RFI animals ($P < 0.01$).

Total protein and electrophoretic separations

The 2D-PAGE experiments with liver samples were performed with at least three replicates per group. Figures 1 and 2 shows representative gels of hepatic tissue samples from bulls with high or low RFI, respectively. Most protein spots were found in the 20–66 kDa MW range, with most frequent isoelectric point (pIs) near range 5–7.

Imaging study

The image processing of the electrophoretic runs of groups high and low RFI showed a correlation between gels (n = 3)

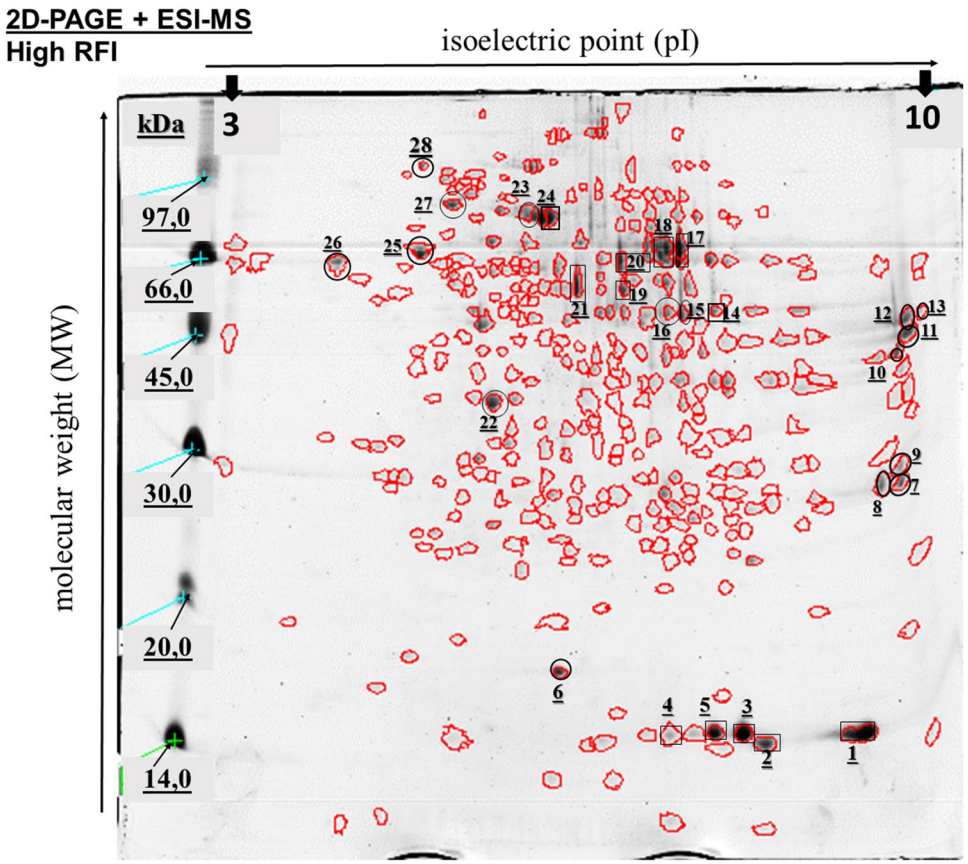

Fig. 1 Representative two-dimensional electrophoresis gel (2D-PAGE) with proteins spots from liver of Nellore bulls classified as high residual feed intake—RFI (less efficient). The numbered circle spots (1–28) where the proteins identified by mass spectrometry (ESI-MS) characterization

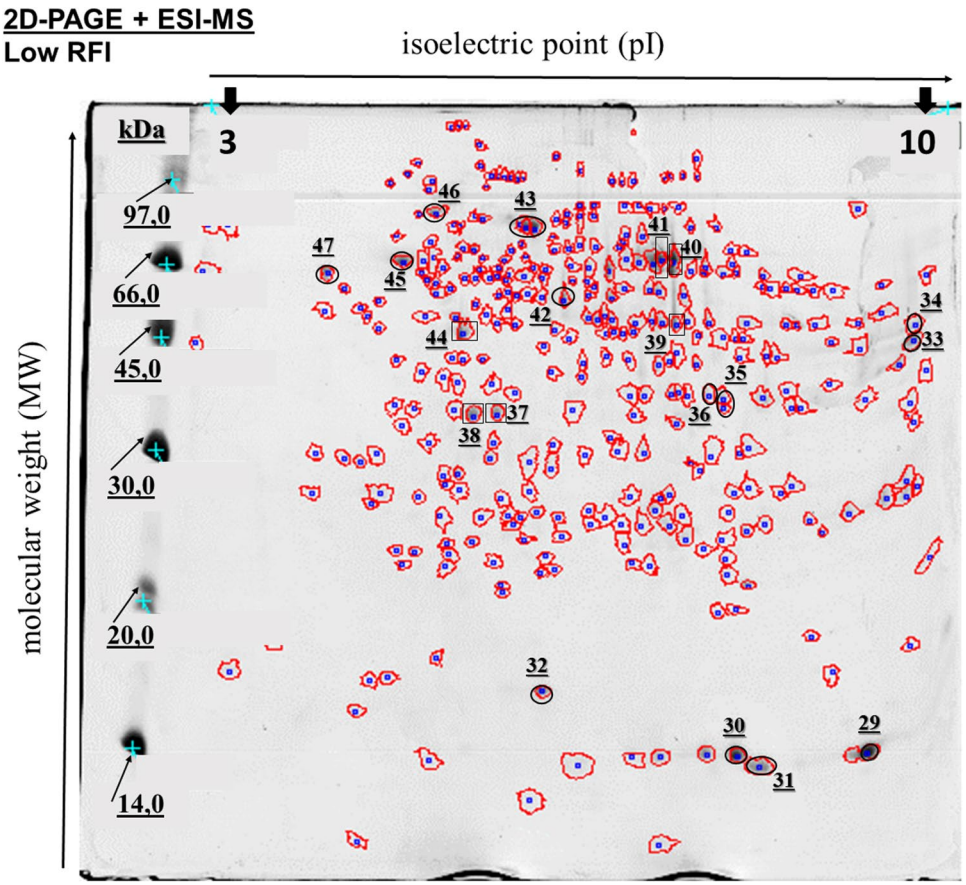

Fig. 2 Representative two-dimensional electrophoresis gel (2D-PAGE) with proteins spots from liver of Nellore bulls classified as low residual feed intake—RFI (more efficient). The numbered circle spots (29–47) where the proteins identified by mass spectrometry (ESI-MS) characterization

of 91 and 93%, respectively, meaning that the protein spots were found in three replicates of these gels. The mean number of protein spots found in the gel replicates of groups were 279 and 215 in high and low RFI bulls, respectively. Significance testing by ANOVA showed that 71 spots were expressed differentially ($P < 0.05$) among RFI groups.

Protein identification

From 71 spots differentially abundant, 47 proteins were identified by mass spectrometry, which identified 28 were in high RFI (Table 1) and 19 proteins in low RFI (Table 2). The protein name, accession number, score, matched peptides, percent of sequence coverage, source and theoretical pI and MW were derived from database.

Discussion

Our hypothesis was that liver protein profile differs between RFI phenotypes. We used a proteomic approach based on 2D-PAGE and ESI-MS to study liver samples of Nellore bulls divergently ranked according to RFI. The study showed that 71 protein spots were expressed differentially ($P < 0.05$) among RFI groups and 47 were identified by ESI-MS. The proteins identified were related to the following biological functions: (I) oxygen transport and blood flow; (II) mitochondrial function and energy metabolism; (III) amino acid metabolism, ion transport, and cell survival. These pathways could help to explain phenotypic differences among RFI groups.

Oxygen transport and blood flow

The protein *hemoglobin subunit beta* was found only in high RFI animals (Fig. 1, spot 5 and 2). We demonstrated previously that efficient Nellore cattle (low RFI) showed lower hemoglobin concentration in blood samples [3]. Lower hemoglobin and hematocrit in more efficient animals (low RFI) is consistent with the economic design theory of FE (e.g. less spare physiological capacity relates to more feed efficient animals) as discussed by Hudson [13]. This pioneer work [3] is corroborated in the present study, now with molecular data, highlighting its importance. The biological functions of this protein such as heme/iron binding, oxygen binding and oxygen transporter may help explain phenotypic differences in FE. When it passes through pulmonary blood capillaries, the hemoglobin inside red blood cells binds to oxygen, forming oxyhemoglobin [14]. Thus, differences in liver proteome may be associated with oxygen-carrying

Table 1 Proteins identified by mass spectrometry in gels from liver tissue of Nellore bulls (*B. indicus*) with high residual feed intake—RFI (less efficient)

Spot	Protein	Access number	Score	pI/MM	pI/MM theoretical	Cover (%)	Molecular function
Oxygen transport and blood flow							
1	Hemoglobin subunit alpha	HBA_BOVIN	53198.95	9.30/14.59	8.07/15.18	91.55	Heme binding; iron ion binding; oxygen binding; oxygen transporter activity
2	Hemoglobin subunit beta	HBB_BOVIN	6008.626	8.34/14.31	7.02/15.95	79.31	
5	Hemoglobin subunit beta	HBB_BOVIN	10714.92	7.85/14.62	7.02/15.95	93.79	
Antioxidant mechanisms							
4	D-Dopachrome decarboxylase	DOPD_BOVIN	7122.275	7.87/14.12	6.59/12.87	85.59	D-Dopachrome decarboxylase activity
6	Superoxide dismutase [Cu–Zn]	SODC_BOVIN	359.006	6.28/16.91	5.85/15.68	23.68	Chaperone binding; copper ion binding; protein homodimerization activity; protein phosphatase 2B binding; superoxide dismutase activity; ubiquitin-protein transferase activity; zinc ion binding
7	Glutathione S-transferase	A0A140T854_BOVIN	5555.339	9.70/27.41	8.66/25.42	31.53	Glutathione transferase activity
9	Glutathione S-transferase A2	GSTA2_BOVIN	5287.668	9.67/28.74	8.66/25.71	45.74	
Mitochondrial function and energy metabolism							
3	Fatty acid-binding protein, liver	G3MXK2_BOVIN	456.7763	8.10/14.59	9.61/15.28	36.3	Fatty acid binding; transporter activity
8	GTP:AMP phosphotransferase AK3, mitochondrial	KAD3_BOVIN	1145.186	9.51/27.41	9.02/25.67	45.81	Adenylate kinase activity; ATP binding; GTP binding; nucleoside triphosphate adenylate kinase activity
10	Glyceraldehyde-3-phosphate dehydrogenase	G3P_BOVIN	12946.94	9.65/42.41	8.51/35.86	51.95	Glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity; NAD binding; NADP binding
11	Fructose-bisphosphate aldolase	A5PK73_BOVIN	10630.34	9.75/46.37	8.69/39.52	59.62	ATPase binding; cytoskeletal protein binding; fructose-1-phosphate aldolase activity; fructose binding; fructose-bisphosphate aldolase activity; identical protein binding
12	Acetyl-CoA acetyltransferase, mitochondrial	THIL_BOVIN	1465.572	9.75/48.70	8.98/44.88	46.92	Acetyl-CoA C-acetyltransferase activity; carbon–carbon lyase activity; ligase activity, forming carbon–carbon bonds; metal ion binding
16	3-Ketoacyl-CoA thiolase, mitochondrial	THIM_BOVIN	1283.978	7.37/50.35	8.06/42.13	58.19	Cellular response to hypoxia; fatty acid beta-oxidation; negative regulation of mitochondrial membrane permeability involved in apoptotic process
18	UDP-glucose 6-dehydrogenase	UGDH_BOVIN	5019.493	7.30/68.42	7.51/55.13	65.79	NAD binding; UDP-glucose 6-dehydrogenase activity
20	Aldehyde dehydrogenas, mitochondrial	ALDH2_BOVIN	7573.718	6.88/64.37	7.54/ 56.65	53.85	Aldehyde dehydrogenase (NAD) activity; NAD binding

Table 1 (continued)

Spot	Protein	Access number	Score	pI/MM	pI/MM theoretical	Cover (%)	Molecular function
Amino acid metabolism and ion transport							
13	Aspartate aminotransferase, mitochondrial	AATM_BOVIN	4335.197	9.88/50.98	9.19/47.51	52.09	L-Aspartate:2-oxoglutarate aminotransferase activity; L-phenylalanine:2-oxoglutarate aminotransferase activity; pyridoxal phosphate binding; RNA binding
14	Argininosuccinate synthase	ASSY_BOVIN	3222.709	7.84/50.56	7.15/46.41	41.99	Amino acid binding; argininosuccinate synthase activity; ATP binding; identical protein binding; RNA binding
15	Betaine--homocysteine S-methyltransferase 1	BHMT1_BOVIN	633.6555	7.49/50.35	6.40/44.87	39.31	Betaine-homocysteine *S*-methyltransferase activity; zinc ion binding
17	Catalase	CATA_BOVIN	15829.51	7.49/68.42	6.79/59.91	65.46	Aminoacylase activity; catalase activity; enzyme binding; heme binding; metal ion binding; NADP binding; protein homodimerization activity; receptor binding
19	Alpha-enolase	ENOA_BOVIN	12498.39	6.92/57.05	6.37/47.32	70.51	Magnesium ion binding; hosphopyruvate hydratase activity
21	Glycine amidinotransferase, mitochondrial	GATM_BOVIN	943.5532	6.45/58.49	8.00/48.35	44.21	Amidinotransferase activity; glycine amidinotransferase activity
22	Regucalcin	RGN_BOVIN	10182.5	5.61/35.72	5.54/33.30	74.92	Calcium ion binding; enzyme regulator activity; luconolactonase activity; zinc ion binding
26	Protein disulfide-isomerase	PDIA1_BOVIN	8035.609	4.03/64.64	4.80/57.26	61.57	Procollagen-proline 4-dioxygenase activity; protein disulfide isomerase activity; protein heterodimerization activity
Cell survival							
23	Actin, cytoplasmic 1	ACTB_BOVIN	132.2245	6.20/80.33	5.29/41.3	24.53	ATP binding
24	Heat shock 70 kDa protein 1B	HS71B_BOVIN	2836.001	6.20/80.33	5.67/ 70.22	52.57	
28	78 kDa glucose-regulated protein	GRP78_BOVIN	9023.517	4.89/102.60	5.07/72.40	53.89	
25	60 kDa heat shock protein, mitochondrial	CH60_BOVIN	8032.277	4.87/68.42	5.60/61.10	46.95	ATP binding; hydrolase activity; protein binding involved in protein folding; unfolded protein binding
27	Heat shock cognate 71 kDa protein	HSP7C_BOVIN	8248.237	5.18/85.31	5.37/71.24	57.08	ATPase activity, coupled; ATP binding; G-protein coupled receptor binding; RNA binding; ubiquitin protein ligase binding; unfolded protein binding

Table 2 Proteins identified by mass spectrometry in gels from liver tissue of Nellore bulls (*B. indicus*) with low residual feed intake—RFI (more efficient)

Spot	Protein	Access number	Score	pI/MM	pI/MM theoretical	Cover (%)	Molecular function
Oxygen transport and blood flow							
29	Hemoglobin subunit alpha	HBA_BOVIN	37032.49	9.30/14.59	8.07/15.18	84.51	Heme binding; iron ion binding; oxygen binding; oxygen transporter activity
Mitochondrial function and energy metabolism							
30	Fatty acid-binding protein, liver	G3MXK2_BOVIN	2349.144	8.10/14.59	9.61/15.28	70.37	Chromatin binding; fatty acid binding; transporter activity
31	Fatty acid-binding protein, liver	FABPL_BOVIN	3033.83	8.34/14.31	7.78/14.22	66.93	
33	Malate dehydrogenase_ mitochondrial	MDHM_BOVIN	1033.267	9.75/46.37	8.82/35.66	44.38	L-Malate dehydrogenase activity; protein homodimerization activity
36	Dihydrodiol dehydrogenase-3	DDBX_BOVIN	6581.2	7.71/36.57	7.65/36.78	57.59	Oxidoreductase activity
38	Glyceraldehyde-3-phosphate dehydrogenase	G3P_BOVIN	597.6974	7.71/36.57	8.51/35.86	24.32	Disordered domain specific binding; glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity; microtubule binding; NAD binding; NADP binding; peptidyl-cysteine S-nitrosylase activity
41	Glutamate dehydrogenase 1, mitochondrial	DHE3_BOVIN	4610.172	7.27/68.28	8.03/61.63	50.54	Glutamate dehydrogenase (NAD+) activity
Amino acid metabolism and ion transport							
32	Superoxide dismutase [Cu–Zn]	SODC_BOVIN	3095.422	6.28/16.91	5.85/15.68	36.18	Chaperone binding; copper ion binding; protein homodimerization activity; protein phosphatase 2B binding; superoxide dismutase activity; ubiquitin-protein transferase activity; zinc ion binding
34	Fructose-bisphosphate aldolase	A5PK73_BOVIN	6224.233	9.75/48.37	8.69/39.52	57.97	Cytoskeletal protein binding; fructose-bisphosphate aldolase activity
35	Glycine *N*-acyltransferase	GLYAT_BOVIN	7275.037	7.84/36.57	7.11/33.90	52.54	Glycine *N*-acyltransferase activity; glycine *N*-benzoyltransferase activity
37	Regucalcin	RGN_BOVIN	6546.422	5.47/34.34	5.54/33.30	80.27	Calcium ion binding; enzyme regulator activity; gluconolactonase activity; zinc ion binding
39	Argininosuccinate synthase	ASSY_BOVIN	5334.355	7.40/48.45	7.15/46.41	53.64	Amino acid binding; argininosuccinate synthase activity; ATP binding; identical protein binding; RNA binding
40	Catalase	CATA_BOVIN	17686.75	7.39/67.00	6.79/59.91	70.97	Aminoacylase activity; catalase activity; enzyme binding; heme binding; metal ion binding; NADP binding; protein homodimerization activity; receptor binding
42	Adenosylhomocysteinase	SAHH_BOVIN	7955.73	6.45/58.49	5.88/47.63	54.86	Adenosylhomocysteinase activity; NAD binding
43	Serum albumin	ALBU_BOVIN	9051.104	6.20/80.33	5.82/69.29	77.59	DNA binding; drug binding; fatty acid binding; metal ion binding; pyridoxal phosphate binding; toxic substance binding
44	Actin, cytoplasmic 1	ACTB_BOVIN	11634.91	5.51/47.50	5.29/41.3	71.47	ATP binding
45	Protein disulfide-isomerase	PDIA1_BOVIN	6133.078	4.87/68.42	4.80/57.26	55.69	Procollagen-proline 4-dioxygenase activity; protein disulfide isomerase activity; protein heterodimerization activity
46	78 kDa glucose-regulated protein	GRP78_BOVIN	8370.471	5.18/85.31	5.07/72.40	52.82	ATP binding
47	Calreticulin	CALR_BOVIN	2004.88	4.03/64.64	4.31/48.03	25.18	Calcium ion binding; carbohydrate binding; unfolded protein binding

capacity because the number of red blood cells influences the oxygenation capacity of tissues. Therefore, we suggest that hemoglobin is associated to FE and should be measured in blood or hepatic tissue. This is the first research to find consistent results regarding hemoglobin in Nellore cattle.

Mitochondrial function and energy metabolism

Although some studies have investigated FE traits at the transcription level [15], only a few studies have been performed at the protein level from beef cattle [9] and pigs [16]. We found several proteins related to mitochondrial function and energy metabolism in liver samples that could help explain the differences in FE. Proteins, such as *3-ketoacyl-CoA thiolase* and *glutamate dehydrogenase 1*, were found in high and low RFI groups, respectively. Such protein expression could be beneficial to change fatty acid β-oxidation and oxidative capacity in liver of high versus low RFI animals. Similarly, in skeletal muscle of pigs, proteins involved in mitochondrial energy metabolism and glucose metabolism were also expressed differentially between the high- and low-FE phenotypes [16].

In this context, to investigate the association between mitochondrial function and cattle FE, a study evaluated the differential expression of *peroxisome proliferator-activated receptor α* (PGC1α), *mitochondrial transcription factor A* (TFAM), and *uncoupling proteins* (UCP2 and UCP3), and showed differences in the mRNA expression levels of the UCP2 gene in hepatic tissue of Nellore divergently ranked by RFI [17]. The paradigm in mitochondrial metabolism is that uncoupling (by UCP or other proteins) may represent a cellular inefficiency but also reduces oxidative stress by attenuating mitochondrial ROS production [18]. We found proteins such as *aspartate aminotransferase, aldehyde dehydrogenase and glycine amidinotransferase* (Fig. 1) in high RFI animals which could impact on these processes.

However, additional studies measuring specific genes, protein amount or enzyme activity are needed. Changes of very small magnitude in either mitochondrial function or enzyme activities could greatly alter energy metabolism and cause the changes in feed efficiency observed in vivo. Most of our biochemical or proteomic studies are unable to detect the magnitude of the changes in RFI observed in beef cattle. These small energy expenditure changes are of enormous economic significance.

Amino acid metabolism, ion transport and cell survival

Another important protein, heat shock protein (HSP71), was identified in liver from high RFI animals (Fig. 1, spot 24). Heat shock proteins (HSPs) are induced in response to heat stress and are classified into subfamilies based on their molecular weight (kDa), such as HSP90, HSP70, HSP40, and HSP27. These proteins can bind to and stabilize other proteins, which contribute to cell survival by interfering with cellular signal transduction pathways regulating apoptotic cell death [19].

Most studies have investigated HSPs in muscle and their association with meat tenderness in Nellore [20] and other breeds [21]. Based on 2D-PAGE and ESI-MS, this is the first study to show that HSP71 was found only in liver of inefficient animals (high RFI). Additionally, we study the hepatic tissue due to its role in energy metabolism. In this context, proteomic investigation of liver allows a better understanding of its role in energy expenditure and FE. These results suggest that discrepancy in FE of Nellore cattle as RFI may be due to differences energy expenditure of hepatic tissue (protein turnover), which was indicated by the presence of HSP71 in liver of inefficient phenotype (high RFI).

Moreover, in FE studies where animals were ranked for RFI, different compositions of body weight growth was reported. More efficient animals (low RFI) have leaner carcasses [22] and less internal fat [23]. Thus, changes in metabolism and intake may not only be related to changes in energy expenditure, but also to changes in body tissue composition. Although we consider liver as the key organ to study FE, investigation of protein expression in muscle tissue will be considered for future studies.

We found some important proteins related to amino acid metabolism and ion transport among high and low RFI animals. Peptides such as *alpha-enolase* (ENO1) and *protein disulfide-isomerase* (PDI) were found in the high and low RFI groups, respectively. Such protein expression could be responsible and/or contribute partially to differences in cellular energy expenditure and ion pumping (e.g. magnesium ion binding). PDI (Fig. 2, spot 45), for example, belongs to a large, functionally diverse family of proteins that catalyze thiol/disulfide exchange reactions, including disulfide formation, reduction and isomerization [24]. On the other hand, ENO1 (Fig. 1, spot 19) is a multifunctional glycolytic enzyme involved in cellular stress. This protein is responsible for conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid in the glycolytic pathway [25].

Conclusion

This study showed several proteins expressed differentially among Nellore bulls with high or low RFI. The proteomic investigation of liver suggests *hemoglobin subunit beta* as a molecular marker to study FE. Additionally, HSP71 was found only in inefficient animals (high RFI). We also found proteins related to mitochondrial function and energy metabolism. Protein *3-ketoacyl-CoA thiolase* and *glutamate dehydrogenase 1* were found upregulated in the high and low

RFI groups, respectively. This information could be useful for beef cattle breeding programs and FE studies.

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