Universidade Federal de Lavras - UFLA
Departamento de Zootecnia – DZO

ELISA BARBOSA DE CARVALHO

PROTEOMIC PROFILE OF SKELETAL MUSCLE AND LIVER TISSUE OF HIGH AND LOW RESIDUAL FEED INTAKE IDENTIFIED NELLORE CATTLE

Lavras - MG
2016

ELISA BARBOSA DE CARVALHO

PROTEOMIC PROFILE OF SKELETAL MUSCLE AND LIVER TISSUE OF HIGH AND LOW RESIDUAL FEED INTAKE IDENTIFIED NELLORE CATTLE

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para obtenção do título de Mestre.

Prof. Mateus Pies Gionbelli
Orientador

LAVRAS - MG
2016

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Carvalho, Elisa Barbosa de.
Proteomic profile os skeletal muscle and liver tissue of high and low residual feed intake identified Nellore cattle / Elisa Barbosa de Carvalho. – Lavras : UFLA, 2016.
65 p. : il.

Dissertação (mestrado acadêmico)–Universidade Federal de Lavras, 2016.
Orientador(a): Mateus Pies Gionbelli.
Bibliografia.

1. Feed efficiency. 2. 2D electrophoresis. 3. Heat shock protein beta-1. 4. Alpha actin 1. 5. 10-Formyltetrahydrofolate dehydrogenase. I. Universidade Federal de Lavras. II. Título.

ELISA BARBOSA DE CARVALHO

PROTEOMIC PROFILE OF SKELETAL MUSCLE AND LIVER TISSUE OF HIGH AND LOW RESIDUAL FEED INTAKE IDENTIFIED NELLORE CATTLE

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para obtenção do título de Mestre.

APROVADA em 16 de agosto de 2016.

Dr. Marcio de Souza Duarte UFV

Dr. Márcio Machado Ladeira UFLA

Prof. Mateus Pies Gionbelli
Orientador

LAVRAS - MG
2016

Aos meus avós Dita e João Carneiro *(in memorian)*... verdadeiros exemplos de vida.

A toda minha família... meu alicerce!

DEDICO

AGRADECIMENTOS

Aos meus pais, Dumbo e Dionéa, pelo amor incondicional, apoio e dedicação, fazendo com que eu acreditasse ser capaz.

Ao meu tio João e a minha tia Marlena, que me ajudam e incentivam em todos os momentos.

Ao meu irmão Juarez pela amizade, companheirismo e torcida.

À minha irmã e amiga Vanessa pelo apoio, conselhos pessoais e profissionais, sempre me mostrando o melhor caminho.

Ao Rafa que, além de cunhado e companheiro de república, mostrou-se um grande amigo, contribuindo em vários momentos.

À Universidade Federal de Lavras e ao Programa de Pós-Graduação em Zootecnia por minha formação.

Ao Conselho Nacional de desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de mestrado.

Ao meu orientador, Dr. Mateus Pies Gionbelli, pela oportunidade, confiança e ensinamentos transmitidos. Sempre será um exemplo como pesquisador e pessoa.

Ao meu coorientador, Dr. Marcio de Souza Duarte, pela confiança, incentivo e imenso apoio em todos os momentos da realização deste trabalho.

Ao professor Dr. Márcio Machado Ladeira pelos ensinamentos transmitidos e pelas importantes contribuições para este trabalho.

A todos do LABTEC e NUBIOMOL da Universidade Federal de Viçosa, em especial aos técnicos Walmir Silva e Edvaldo Barros e ao colega Rafael Torres, que me acolheram e auxiliaram muito nas análises laboratoriais.

Ao NEPEC pelos ensinamentos e experiências trocadas durante este período e, principalmente, pelas amizades que fiz.

“However bad life may seem, there is always something you can do, and succeed at”

Stephen Hawking

ABSTRACT

Residual feed intake (RFI) has been extensively used to evaluate feed efficiency, however the molecular underlying differences in this trait remains unclear. In order to characterize mechanisms driving differences on energy metabolism of beef cattle skeletal muscle and liver tissue proteome was analyzed. From a group of 120 Nellore young bulls identified for RFI, cattle with the highest (n=9) and lowest (n=9) values were selected for protein abundance studies. Samples were collected immediately after slaughter and subjected to two-dimensional gel electrophoresis (2-DE) followed by mass spectrometry to identify differentially abundant proteins associated with RFI values. Two differentially abundant spots were identified in skeletal muscle of high RFI group (Alpha actin 1 e 14-3-3 protein epsilon), one was identified in muscle of low RFI group (Heat shock protein beta-1) and one was identified in liver tissue of low RFI group (10-formyltetrahydrofolate dehydrogenase) (P<0.05). These data indicates that genetic selection for RFI tends to cause changes in skeletal muscle and liver protein profile, suggesting that differences in low and high RFI cattle may be due skeletal muscle protein turnover and liver energy metabolism.

Key words: Feed efficiency, 2D electrophoresis, Heat shock protein beta-1, Alpha actin 1, 10-Formyltetrahydrofolate dehydrogenase.

RESUMO

O consumo alimentar residual (CAR) tem sido amplamente utilizado para avaliar a eficiência alimentar, no entanto a base molecular desta característica permanece obscura. A fim de caracterizar os mecanismos responsáveis por diferenças no metabolismo energético de gado de corte, o proteoma do músculo esquelético e tecido hepático foi analisado. A partir de um grupo de 120 machos Nelore avaliados para CAR, os animais com os maiores (n= 9) e menores (n = 9) valores foram selecionados para estudos de abundância de proteína. As amostras foram coletadas imediatamente após o abate e submetidas a eletroforese bidimensional em gel (2-DE) seguida de espectrometria de massas para identificação de proteínas diferencialmente abundantes associadas com os valores de CAR. Dois spots diferencialmente abundantes foram identificados no músculo esquelético do grupo de alto CAR (Alfa actin - 1 e 14-3-3 epsilon), um foi identificado no músculo do grupo de baixo CAR (Heat Shock Protein Beta-1) e um foi identificado no fígado do grupo de baixo CAR (10-Formyltetrahydrofolate dehydrogenase) ($p \leq 0,05$). Estes dados indicam que a seleção genética para CAR tende a causar alterações no perfil de proteínas do músculo esquelético e fígado, sugerindo que as diferenças entre animais com baixo e alto CAR podem ser ocasionadas por turnover proteico do músculo esquelético e metabolismo energético do fígado.

Palavras-chave: Eficiência alimentar, Eletroforese 2-D, Heat shock protein beta-1, Alpha actin 1, 10-Formyltetrahydrofolate dehydrogenase.

SUMMARY

FIRST CHAPTER

1 INTRODUCTION

Feed efficiency is one of the most important parameters related to the efficiency of production. It has a high impact on the production costs and environmental damage. Animals more efficient in processing feed have lower input (feed) costs for a given amount of output (beef). From an economic perspective, the producer could increase profit margins by reducing expenses in the form of lower feed costs **(**WULFHORST et al., 2012).

The residual feed intake (RFI) is a measure used to estimate feed efficiency, defined as the difference between an animal's actual feed intake and its expected feed requirements for maintenance and growth over a specific test period (BASARAB et al., 2003). The physiological basis of the residual feed intake is still not completely understood, and many genes probably controlled it. According to Herd and Arthur (2009), at least five major processes are involved in variation of efficiency: intake, digestion, metabolism, activity, and thermoregulation.

Metabolism of skeletal muscle and liver is an important factor affecting feed efficiency, since these tissues accounts for about two thirds of whole body protein turnover in mammals (FRAYN, 2010). The protein deposition is a complex process and proteins in tissues of the organism suffer constant degradation and renewal (protein turnover), affecting energy requirement for maintenance of the animal (LAWRENCE; FOWLER, 2002).

The liver tissue is highly metabolically active, responsible for a large portion of oxygen consumption in cattle. It is the principal site of gluconeogenesis (CONNOR et al., 2010) and is an important organ in protein metabolism, exerting substantial effects on maintenance requirements (FRAYN, 2010).

Proteomics tools are widely used in the identification of key proteins and the corresponding biological processes by measurements of different protein expression levels in several biological samples (SZABO et al., 2012). Two-dimensional electrophoresis (2DE) is a tool that allows the separation and analysis of proteins extracted from complex samples due to its specific molecular weight and charge (BENDIXEN, 2005).

Proteomic studies have been used in beef cattle researches to explain muscle development and growth, metabolism function beyond the *ante* and *postmortem*, and meat quality. However, there are few studies related to feed efficiency.

Thus, based on this, we hypothesized that animals with different RFI have proteins differentially abundant in skeletal muscle and liver tissues, which may explain changes in metabolism and feed efficiency.

Our objective was to evaluate the consequences of divergent feed efficiency Nellore cattle identified by RFI on protein profile of skeletal muscle and liver tissue.

2 BACKGROUND

2.1 Feed efficiency

The typical definition of efficiency is a measure of output of over input where output and input are measured in the same units. Energetic efficiency has not changed substantially over the years; however, there appears to be variation among cattle (FREETLY, 2015).

Several measures of feed efficiency have been proposed over the years to quantify the capacity of animals to convert the ingested feed into a product and identify those animals with a high production and lowest requirements (ARTHUR; HERD, 2012; BONILHA et al., 2015).

Some indices used for measure efficiency are gross efficiency, feed conversion ratio (FCR), partial efficiency of growth and maintenance efficiency. However, these measures have the undesirable property of correlation with growth rate or other production traits, such as mature body weight (CREWS, 2006; MOORE; MUJIBI; SHERMAN 2009).

The FCR, which is the ratio of some measure of feed intake to some measure of output, have been frequently used to express efficiency in beef production. In the most cases when ratios are used, the numerator and dominator are not in the same units and are not a true measure of efficiency (FREETLY, 2015). Another problem with FCR is no distinction between metabolic efficiency, growth efficiency and larger mature size (KERLEY, 2012). Thus, selection for lower FCR is expected to result in animals with larger mature size and higher intake requirements (CREWS, 2006) due to higher maintenance costs of types of cattle that have high growth rate (FERRELL; JENKINS, 1998).

Some studies of phenotypic and genetic parameters for different measures of feed efficiency showed that FCR have typical positive correlation with feed intake (ARTHUR et al., 2001 a; CROWLEY et al., 2010), is negatively correlated with ADG and have moderate correlation with yearling weight and mature weight (ARTHUR; HERD, 2012).

Feed efficiency is a function of feed intake and growth, and causal differences for variation of these traits may not be the same across populations. To improve feed efficiency is necessary express it in biological terms that allow determination of the underlying biology that is responsible for variation (FREETLY, 2015).

Incorporation of feed efficiency into breeding objectives would increase the genetic potential for animals to have less feed intake while maintaining the same production levels, which has the potential to increase profits for cattle producers and decrease the environmental footprint of beef cattle production.

They are important because address the challenges of increasing feed costs and land pressure (MOORE; MUJIBI; SHERMAN, 2009).

2.2 Residual feed intake

Residual feed intake (RFI) is a more direct measure of metabolic efficiency than FCR, and is now broadly considered to be the best available measure of feed efficiency to use in the context of genetic selection (KERLEY, 2012).

The concept of RFI was first defined by Koch et al. (1963) as the difference obtained when the actual feed intake of an animal is adjusted for growth and maintenance requirements. Basarab et al. (2003) defined RFI as the difference between an animal's actual feed intake and its expected feed requirements for maintenance and growth over a specific test period.

The RFI is calculated by linear regression of individual dry matter intake (DMI) observed based on the average metabolic bodyweight (MBW) and average daily gain (ADG). This regression generates the average coefficients of the group ($\beta 0$, $\beta 1$ and $\beta 2$) and the residue (ε). The most used equation was proposed by Koch et al. (1963):

$$DMI = \beta 0 + \beta 1\ ADG + \beta 2\ MBW + \varepsilon$$

The DMI predicted by the equation indicates the amount of feed that would be necessary to keep this metabolic weight and gain rate. The residue obtained is RFI, which is the difference between the observed and expected intake. Thus, more efficient animal has a negative RFI (observed intake is less than predicted) and the less efficient animal has a positive RFI (observed intake higher than predicted).

Residual feed intake is phenotypically independent of body weight, average daily gain and other production traits that are included in the equation of estimation of DMI (BASARAB et al., 2003; KERLEY, 2012).

While FCR is expressed as a ratio, RFI is a linear index. The use of ratio traits for genetic selection presents problems relating to prediction of the change in the component traits in future generations. However, a linear index places a predetermined amount of selection pressure on the traits and results in a predictable amount of genetic changes. For this reason, RFI is preferred as the feed efficiency trait for genetic selection in beef cattle (ARTHUR; HERD, 2012).

Residual feed intake allows inclusion of any requirement, or "energy sinks" that differentiate cattle in different industry segments and stages of production, while still describing individual animal differences (CREWS, 2006).

The RFI is a characteristic that has genetic variability between animals within a population and a moderate-high heritability, ranging from 0.34 to 0.60 (LANCASTER et al., 2009), allowing its use in selection and breeding programs.

Results from a population of cattle divergently selected for RFI have confirmed the expectation that progeny of cattle selected for low RFI consume less feed for the same level of growth as progeny of cattle selected for high RFI (ARTHUR; HERD, 2012).

The opportunity to improve production efficiency through exploitation of genetic variation in RFI is dependent not only of the genetic variation in young cattle, but also on the magnitude of the genetic correlations with other key production traits, such growth and feed intake during finishing, carcass and meat quality traits at slaughter (HERD; ARCHER; ARTHUR, 2003).

Arthur and Herd (2012) present a review with estimates genetic correlations of traits measured in animals tested for RFI (Table 1).

The genetic correlation coefficients shows that RFI is moderate to highly correlated with FCR, positively correlated with feed intake, have close to zero

genetic correlations with ADG and MW, low to moderate negative correlation with rib eye area and low to moderate positive correlation with rib fat depth in carcass.

The RFI is individual measure and accuracy measurements of individual feed intake, growth and feed efficiency in animals require a test over a period. Therefore, RFI should be determined for at least 70 days (CASTILHOS et al., 2011). Animals should be fed individually in stalls or in groups, using electronic devices that measure individual intake. The cost of the individual feeding of animals is high, which may limit the use of this parameter. However the use of electronic equipment of individual intake measurement may allow a greater number of animals by testing, enabling the use of residual feed intake in selection programs (CHIZZOTTI et al., 2011).

The independence of RFI from production traits has led some authors to suggest that RFI may represent inherent variation in basic metabolic processes (HERD; ARCHER; ARTHUR, 2003).

Table 1 Genetic correlations (±standard error) between feed efficiency traits and other economically important traits (adapted from studies where over 600 animals were used)

	Source[a]								
	1	2	3	4	5	6	7	8	9
Number of observations	1180	792	1481	2284	740	1304	1463	2102	1141
	Genetic correlations with RFI (kg/d) [b]								
Feed intake (kg)	0.69 ± 0.03	0.79 ± 0.04	0.43 ± 0.15	0.81∗	0.78 ± 0.06	0.41 ± 0.28	0.59 ± 0.12	0.59 ± 0.13	0.66 ± 0.12
Average daily gain (kg)	−0.04 ± 0.08	−0.10 ± 0.13	0.09 ± 0.20	0.01	0.25 ± 0.16	−0.14 ± 0.32	0.18 ± 0.21	0.01 ± 0.13	0.15 ± 0.25
Metabolic body weight	−0.06 ± 0.06		−0.20 ± 0.16	−0.17	0.16 ± 0.13	0.01 ± 0.28	0.15 ± 0.18	0.17 ± 0.09	
Feed conversion ratio	0.66 ± 0.05	0.85 ± 0.05	0.41 ± 0.32	0.69∗	0.64 ± 0.10			0.48 ± 0.10	
Carcass rib eye area			0.27 ± 0.22		−0.42 ± 0.33		−0.42 ± 0.18		
Carcass rib fat depth			0.48 ± 0.12		−0.30 ± 0.27		0.49 ± 0.19		

1, Arthur et al., 2001a; 2, Arthur et al., 2001b; 3, Robinson and Oddy, 2004; 4, Schenkel et al., 2004; 5, Hoque et al., 2006a, 2006b; 6, Okanishi et al., 2008; 7, Barwick et al., 2009 and Wolcott et al., 2009; 8 Crowley et al., 2010, 2011; 9, Rolfe et al., 2011.

b In the study by Okanishi et al. (2008), feed intake was converted to total digestible nutrients (TDN) hence FCR was expressed as TDN (kg/d):gain (kg/d) and RFI as kg TDN/d. In the studies by Crowley et al. (2010, 2011), RFI was expressed as MJ/d.

∗Significantly different from zero ($P < 0.05$).

Source: Adapted from Arthur and Herd (2012).

2.3 Efficiency of energy utilization

The efficiency of energy utilization in farm animals means energy transference of from feed to animal products or work (KLEIBER, 1960). Feed efficiency is defined as the gain in body weight from the intake of a given amount of feed or its inverse (KOCH et al., 1963).

Metabolizable energy (ME) is the original gross energy of feed subtracting the energy lost in the form of faeces, urine and ruminal fermentation gases. This represents the portion of energy that is effectively available to animal metabolism and is drawn on to produce heat, work or growth. The ME supplied to the animal is partitioned in retained energy (RE) as body tissues and total heat loss. The recovery of ME from the diet for tissues laid is about 40% or less. The components of the heat loss are biologically important and considerable scientific effort has been expended in attempting a logical partition of the contribution of different metabolic processes (LAWRENCE; FOWLER, 2002).

The ME for maintenance (MEm) is defined as the rate of heat production (HP) in an animal kept in a thermoneutral environment when the rate of ME intake in feed exactly balances the rate of heat loss (LAWRENCE; FOWLER, 2002). Maintenance energy requirement was defined as the amount of dietary energy intake that resulted in no net loss or gain of energy from body tissues (NRC, 1996).

The most efficient animals converts ME to RE more efficiently by expending less energy for maintenance. While HP increases exponentially with metabolizable energy intake (MEI), RE increases at a lower incremental rate (FERRELL; OLTJEN, 2008). This occurs because as MEI increases, the energy utilized for feed intake, digestion, absorption, and metabolism of nutrients increases. In addition, the metabolic activity of the visceral organs increases with MEI, resulting in a greater HP (CHIZZOTTI et al., 2008).

Maintenance requirements are the major component of the feed energy required for beef production, accounting for 30 to 50% of ME required by growing-finishing cattle, and it estimative can differ among types of animals evaluated within a study for more than 50%. These differences may have a substantial impact on the efficiency of beef production (FERRELL; JENKINS, 1988).

Considerable genetic variation exists for MEm, which is a result of individual differences in body composition and vital organ mass. Animals with high production have higher metabolic rate and active organs increase in response to increased metabolism (DICOSTANZO et al., 1990).

Potential for growth is a function of energy consumed above maintenance energy requirement. The magnitude of variation in energy requirements for growth is higher than variation in nutrient requirements, that is, amino acid requirements for protein accretion do not vary by the same magnitude as energy intake varies among animals with different efficiency phenotypes (KERLEY, 2012).

The partial efficiency of ME use to gain (kg) is highly dependent on the composition of the tissue deposited. When 1 g of protein is deposited, there is an associated deposit of 4 to 5 g of water and when 1 g of fat is deposited, less than 1 g of water is deposited (MARCONDES et al., 2013).

2.4 Physiology and metabolism of animals differing in feed efficiency

According to Herd and Arthur (2009), at least five major processes are involved in variation of efficiency: intake, digestion (associated energy costs), metabolism (anabolism and catabolism associated with variation in body composition), activity, and thermoregulation.

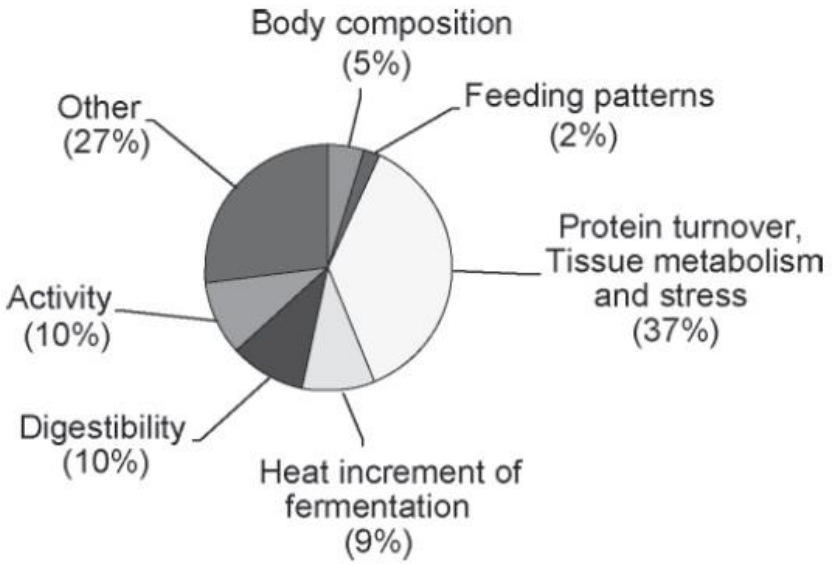

Figure 1 Contributions of biological mechanisms to variation in residual feed intake as determined from experiments on divergently selected cattle.
Source: Herd and Arthur (2009).

Variation in feed intake per se is associated with variation in maintenance requirements of ruminants. As feed intake increases, the amount of energy expended to digest the feed increases, in part because of an increase in size of the digestive organs and increase in energy expended within the tissues themselves. Those animals that eat less for the same performance could have less energy expended as heat increment of feeding (HIF) (HERD; ARTHUR, 2009).

The differences in energy retained in the body accounted for only 5% of the difference in feed intake, with the remainder (95%) due to heat production (HERD; ARTHUR, 2009).

Steers with high RFI (inefficient) consumed more ME and had higher maintenance and HIF costs, but retained no more energy as live weight gain (RE) compared to steers with low RFI (BASARAB et al., 2003).

According to Basarab et al. (2003), low RFI steers consumed 6.4% less feed than medium RFI steers and 10.4% less feed than steers with high RFI. The

feed:gain (FG) ratios was lower in low and medium RFI steers (9.4% and 4.2%) when compared high RFI steers.

Low RFI was associated with lower feed intake, similar growth rate and greater lean meat content compared with high RFI (VINCENT et al., 2015). In high genetic potential of RFI− animal, the energy intake is primarily used for protein deposition in detriment to fat accretion, which contributes to reduce metabolic cost of tissue growth. (FAURE et al., 2013).

Any variation in gain composition and composition of the body can influence the apparent efficiency of nutrient utilization (HERD; ARTHUR, 2009). Steers with low RFI (efficient) have a slightly slower rate of empty body fat deposition than steers with high RFI. This relationship between RFI and body composition manifested a trend of toward in 2.2% increase in protein gain by low RFI steers as compared to high RFI steers (BASARAB et al., 2003).

There is more variation in the efficiency of lean gain than fat gain, due to greater variation in protein turnover than in fat. Moreover, protein turnover varies largely between organs than fat turnover (HERD; ARTHUR, 2009). Metabolism, protein turnover, and stress are the least understood of the seven proposed processes contributing to variation in RFI (GRUBBS et al., 2013).

Protein synthesis per gram of tissue is higher in liver than in most other tissues (25% of whole body protein synthesis). Muscle protein synthesis is slower. However, the mass of protein in muscle represents 50% of the total body protein, thus its contribution to whole body protein synthesis is substantial (KRAFT et al., 2012). The turnover of body proteins is about 30% of the energy spent on maintenance. Concluding that the metabolism of muscle and hepatic tissues plays an important role in the feed efficiency of an animal.

Mitochondria are responsible for 90% of ATP production in the body. Consequently, modification of mitochondrial functionality has the potential to influence dietary energy utilization and oxidative stress. Mitochondrial oxidative

stress can be caused by electron leakage that leads to production of ROS (reactive oxygen species). Excessive ROS divert dietary energy from growth towards cellular repair and/or autophagy mechanisms and may play a role in the phenotypic expression of feed efficiency (GRUBBS et al., 2013).

A study of Grubbs et al. (2014), provided evidence that genetic selection for RFI impacts the protein profile of mitochondria from the liver and *Longissimus* muscle in growing pigs. Identification of proteins related to ATP production and cellular rescue indicate changes in metabolism that impact efficiency.

2.5 Energy metabolism and protein turnover of skeletal muscle in cattle

According to Jing et al. (2015), the energy metabolism and growth of skeletal muscle may be the two key factors responsible for low RFI and therefore increased efficiency of pigs. They found reduction in energy metabolism of muscle, particularly mitochondrial metabolism, and/or increase of skeletal muscle growth in more efficient animals.

The balance between the amount of muscle protein synthesized and the amount of muscle protein degraded determines muscle size. There are three scenarios that can lead muscle hypertrophy: increased protein synthesis and decreased protein degradation; decreased protein synthesis and decreased protein degradation (most efficient method to increase muscle growth rate); and increased protein synthesis and increased protein degradation (least efficient method to increase muscle growth rate), the protein turnover (KOOHMARAIE et al., 2002).

Muscle consists of three protein fractions, myofibrillar (salt-soluble), connective tissue (acid soluble), and sarcoplasmic (water-soluble) proteins (KOOHMARAIE et al., 2002). Myofibrillar proteins, that form the myofibril or contractile structure in skeletal muscle, constitute 55 to 60% of total protein in

muscle tissue by weight. For this reason, they present a special challenge to metabolic turnover of muscle proteins (GOLL et al., 2008).

Intracellular turnover of proteins requires proteolytic enzymes to degrade the proteins. There are four classes of proteolytic enzymes that are present in amounts sufficient to catalyze intracellular protein turnover: the lysosomal system, caspase system, calpain system and proteasome. However, only two have a major role in metabolic turnover of myofibrillar proteins, the calpain system and the proteasome (GOLL et al., 2008).

The poor efficiency attributed to muscle protein turnover is due to the energy expended in the processes. This is a very energetically expensive cellular process, in terms of ATP required to operate the proteasome system and the loss of energy expended in synthesizing the proteins. Therefore, it may represent a major contributing process in the phenotypic expression of poor feed efficiency in animals (CRUZEN et al., 2013).

Although variations in maintenance and F:G are sometimes more highly associated with weight and metabolic activity of the visceral organs (such as the gut and liver) than with body proteins or composition of gain, the energy requirement was positively related to the rate of myofibrillar protein turnover. Reduced rates of protein degradation allow an increase in lean body mass without a proportionate increase in maintenance energy needs (CASTRO BULLE et al., 2007).

The calpain (calcium activated protease) is an ATP independent pathway responsible for some protein turnover and is endogenously inhibited by calpastatin (GRUBBS et al., 2013). Cruzen et al. (2013) found a combination of reduced calpain activities and increased calpastatin activity in the low RFI pigs, which indicates the potential for decreased capacity for protein degradation in the muscles of these animals.

2.6 Energy metabolism and protein turnover of liver in cattle

Tissues of the splanchnic bed include the gastrointestinal tract, liver, spleen, pancreas and mesenteric fat depots. These organs, together with the associated connective tissue and blood vessels, comprise approximately 15 to 20% of the total body mass in ruminants. Estimates of the total oxygen consumption attributed to the tissues of the whole splanchnic bed in ruminants range from 35 to 60% and approximately 20% for the gastro-intestinal tract alone (HERD; ARTHUR, 2009).

The liver has a central role in metabolism and its anatomical position, receiving the blood from the intestinal tract that contains the products of digestion and absorption, reflects it (FRAYN, 2010).

Liver tissue is highly metabolically active, responsible for a substantial portion of the total oxygen consumption in cattle and have substantial effects on basal maintenance requirements (CONNOR et al., 2010). The portal drained viscera consumed 25.4% and liver consumed 20.5% of whole-body oxygen uptake in steers (HERD; ARTHUR, 2009).

Oxygen consumption by the portal drained viscera has been reported to be directly associated with feed intake in beef cattle. Given the strong correlation between feed intake and RFI, it is possible that decreases in oxygen consumption of these tissues is associated with feed efficiency (HERD; ARTHUR, 2009).

It is an important organ in protein metabolism for a number of reasons: because it is the first organ through which amino acids pass after absorption from the intestine; because some important links between amino acid and carbohydrate metabolism occur there; and because it is the organ where urea synthesis takes place (FRAYN, 2010).

Liver tissue is the principal site of gluconeogenesis. It is estimated that hepatic gluconeogenesis provides 90% of the glucose requirement of ruminants (CONNOR et al., 2010).

Liver from low RFI animals may have a greater metabolic capacity than those animals selected for high RFI (GRUBBS et al., 2013). This can be associated with reducing in ATP production, an event increasing energy requirements and heat generation. Moreover, reduction in hepatic cell division and growth may contribute to a reduction in liver size and spare energy required for body maintenance (FONSECA et al., 2015).

Liver is a logical tissue for transcript profiling to identify key regulatory pathways affecting nutrient use efficiency of cattle (CONNOR et al., 2010).

2.7 Feed efficiency markers in beef cattle

The main inhibition of adoption of selection strategies based on RFI is the difficulty and expense of measuring individual animal BW and feed intake. This makes the development of predictive genetic markers an attractive alternative to direct measurement on large numbers of animals. Moreover, the trait is moderately heritable and DNA or other predictive markers could be used in selection programs (MOORE; MUJIBI; SHERMAN 2009).

Considerable genetic variation has been demonstrated within populations and across different breeds tested for RFI. This indicates that selection for RFI is possible and benefits of reduced feed intake can be passed on between generations (MOORE; MUJIBI; SHERMAN 2009).

Most economically important traits in beef cattle are controlled by genetic as well as no genetic factors, and it is expected that feed efficiency traits are no exception (ARTHUR; HERD, 2012). A combination of genetic markers, when

examined jointly, can explain a large proportion of the genetic variation (MOORE; MUJIBI; SHERMAN 2009).

The distinction between genetically selected RFI lines and phenotypic RFI differences is important when interpreting the molecular and cellular differences between high and low RFI animals. Genetic selection for RFI may provide a good model for understanding molecular differences in RFI when compared to single phenotypic comparisons (GRUBBS et al., 2014).

Address the mechanisms by which a genetic variable gives rise to the phenotypic differences observed is important to understand the biological processes associated with RFI. The candidate gene approach is well suited to identify markers that explain a larger proportion of variation in RFI whose effects can be reproducible across populations (KARISA et al., 2013).

The biological networks could also be used to identify additional genes and metabolites involved in the biological processes and that may have an effect on RFI. (KARISA; MOORE; PLASTOW, 2014).

2.8 Proteomics in feed efficiency of beef cattle

Proteomics is the scientific area that characterize proteins present in a cell or tissue type during a time. The aim of proteomics is to obtain information about cellular protein coding, and thereby reveal the function of genes, and ultimately to explain how heredity and environment interact to control cellular functions, and form the physiological traits of living organisms (BENDIXEN, 2005).

While the genes remain constant during the lifetime of the animal, the expression of the genes to mRNA and proteins is very dynamic and regulated by a large number of factors. The proteins expressed from the genome may thus be viewed as the mirror image of the gene activity (HOLLUNG et al., 2007). It is a complementary technology to expression profiling on the level of the

transcriptome for monitoring gene expression at the protein level (MALTIN; PLASTOW, 2004).

Proteins are frequently the functional molecules and, therefore, the most likely to reflect differences in gene expression. Genes may be present, they may be mutated, but they are not necessarily transcribed. Some messengers are transcribed but not translated, and the number of mRNA copies does not necessarily reflect the number of functional protein molecules. For this reason, the final definition of the phenotype depends on the proteome and proteomics is an alternative technology to complement and enhance the effectiveness of cDNA expression profiling technologies (MALTIN; PLASTOW, 2004).

The functional genomics/transcriptomic and proteomic approaches provide the opportunity to investigate global changes in known or unknown gene/gene product expression in muscle and to associate them with known phenotypic characteristics (MALTIN; PLASTOW, 2004).

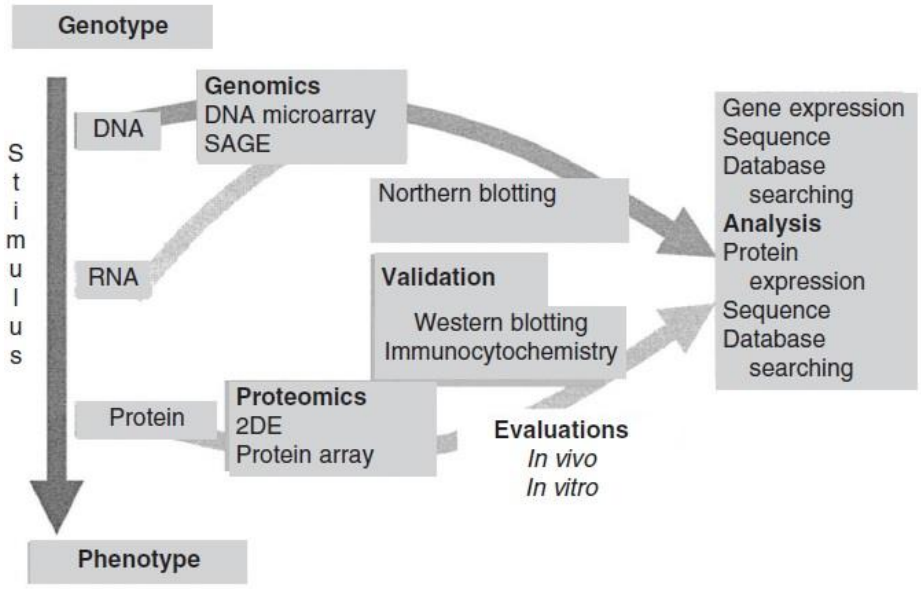

Figure 2 A schematic illustration of the way in which the integration of the '-omic' technologies provide a network of experimental tools for application at various levels to provide data of physiological relevance.
Source: Maltin and Plastow (2004).

There are two approaches to proteome characterization. The mapping proteomics is similar to genome sequencing projects and aims to characterize and make comprehensive databases of ''cellular proteomics''. Comparative proteomics aims to characterize the biological mechanisms that link phenotypes and genotypes (MULLEN et al., 2006). The technique is based in quantitative analyses of relative abundance of proteins between complex tissue samples (BENDIXEN, 2005).

Two-dimensional electrophoresis (2DE) based proteomics is a multi-step procedure that has remained useful in comparative proteomics (BENDIXEN, 2005).

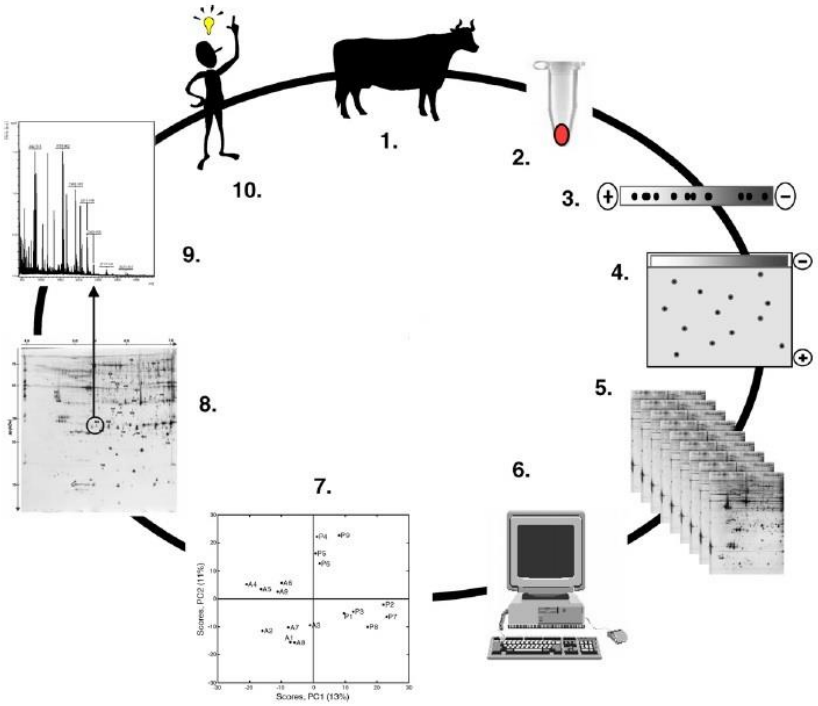

Figure 3 Schematic drawing of steps in the work-Xows in proteome analysis using 2-DE and mass spectrometry. (1) Animal or sample chosen for analysis, (2) sample extraction, (3) isoelectric focusing (IEF), (4) SDS-PAGE, 2-dimensional electrophoresis, (5) alignments and comparisons of 2-DE images, (6) data analysis, (7) data interpretation and selection of significantly changed proteins, (8) extraction of significantly changed protein spots, (9) identification of protein spots by MALDI-TOF MS, (10) interpretation of the results.
Source: Adapted from Hollung et al. (2007).

The 2DE involves two separation parameters, isoelectric point (by isoelectric focusing - IEF) and molecular weight (by SDS-PAGE), which improves fractionation resolution of complex mixtures and allows separation of multiple proteins. Spot patterns are formed, each spot theoretically represents an individual protein and the spot intensity indicates how much of that protein is present. (MULLEN et al., 2006).

Within the last years, mass spectrometry has been developed from methods that allow analyses of small volatile molecules, to a wide range of

applications, which includes characterization of proteins and peptides (BENDIXEN, 2005).

The discovery of matrix-assisted laser desorption ionization (MALDI) was major step towards applying MS for protein identification. In MALDI technology, laser energy is used to convert matrix-embedded peptides into gas phase. When MALDI is combined with a time-of-flight (TOF)-based mass analyzer, the technique becomes robust, low in cost, have low-sample consumption and high-speed. It is ideally suited for protein identification using peptide mass fingerprinting (PMF), which is currently the most widely used method for analysis of 2DE separated proteins and peptides (BENDIXEN, 2005).

During the last decade, high-throughput technologies such as transcriptomics and proteomics have been used for large-scale genome expression analysis. These technologies may allow the identification of genes, proteins, or biological pathways that were not described so far to be responsive to the divergent selection for RFI (VINCENT et al., 2015).

REFERENCES

ALEXANDRE, P. A. et al. Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. **BMC Genomics**, v. 16, p. 1-13, 2015.

ARTHUR, P. F.; HERD, R. M. Genetic Improvement of Feed Efficiency. In: HILL, R. A. **Feed efficiency in the beef industry**. New York: John Wiley and Sons Inc., 2012. p. 93-104.

ARTHUR, P. F. et al. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency and other postweaning traits in Angus cattle. **Journal of Animal Science**, v. 79, p. 2805–2811, 2001 a.

ARTHUR, P.F.; RENAND, G.; KRAUSS, D. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. **Livestock Production Science**, v. 68, p.131–139, 2001 b.

BARWICK, S.A. et al. Genetics of steer daily and residual feed intake in two tropical beef genotypes, and relationships among intake, body composition, growth and other post-weaning measures. **Animal Production Science**, v. 49, p. 351–366, 2009.

BASARAB, J. A. et al. Residual feed intake and body composition in young growing cattle. **Canadian Journal of Animal Science**, v. 83, p 189–204, 2003.

BENDIXEN, E. The use of proteomics in meat science. **Meat Science**, v. 71, p. 138-149, 2005.

BONILHA, S. F. M. et al. Feed efficiency, blood parameters, and ingestive behavior of young Nellore males and females. **Tropical Animal Health and Production**, v. 47, p. 1381–1389, 2015.

CASTILHOS, A. M. et al. Test post-weaning duration for performance, feed intake and feed efficiency in Nellore cattle. **Revista Brasileira de Zootecnia**, v.40, n.2, p. 301-307, 2011.

CASTRO BULLE, F. C. P. et al. Growth, carcass quality, and protein and energy metabolism in beef cattle with different growth potentials and residual feed intakes. **Journal of Animal Science**, v. 85, p. 928–936, 2007.

CHEN, Y. et al. Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. **Animal Genetics**, v. 42, p. 475–490, 2011.

CHIZZOTTI, M. L. et al., 2011. Consumo alimentar residual e seu impacto sobre a qualidade da carne bovina. In: I SIMBOV MT – SIMPÓSIO MATOGROSSENSE DE BOVINOCULTURA DE CORTE, 1, 2011, Cuiabá. **Proceedings**... Cuiabá: UFMT, 2011, p. 284-305.

CHIZZOTTI, M. L.; TEDESCHI, L. O.; VALADARES FILHO, S. C. A meta-analysis of energy and protein requirements for maintenance and growth of Nellore cattle. **Journal of Animal Science**, v. 86, p. 1588–1597, 2008.

CONNOR, E. E. et al. Enhanced mitochondrial complex gene function and reduced liver size may mediate improved feed efficiency of beef cattle during compensatory growth**. Functional & Integrative Genomics**, v. 10, p. 39–51, 2010.

CREWS, D. H. JR. The genetics of feed efficiency in beef cattle. In: BIF 38th Annual Research Symposium and Annual Meeting, 38, 2006. **Proceedings**… Choctaw, 2006, p. 22–31.

CROWLEY, J.J. et al. Genetic associations between feed efficiency measured in performance-test station and performance of growing cattle in commercial beef herds. **Journal of Animal Science**, v.89, p. 3382–3393, 2011.

CROWLEY, J. J. et al. Phenotypic and genetic parameters for different measures of feed efficiency in different breeds of Irish performance-tested beef bulls. **Journal of Animal Science,** v**.** 88, p. 885–894, 2010.

CRUZEN, S. M. et al. Evidence of decreased muscle protein turnover in gilts selected for low residual feed intake. **Journal of Animal Science**, v. 91, p. 4007–4016, 2013.

DICOSTANZO, A. et al. Within-herd variation in energy utilization for maintenance and gain in beef cows. **Journal of Animal Science**, v. 68, p. 2156-2165, 1990.

FAURE, J. et al. Consequences of divergent selection for residual feed intake in pigs on muscle energy metabolism and meat quality. **Meat Science**, v. 93, p. 37–45, 2013.

FERRELL, C. L.; JENKINS, T. G. Influence of biological types on energy requirements. **Beef Research Program Progress Report**, v. 3, p. 86-90, 1988.

FERRELL, C. L.; JENKINS, T. G. Body composition and energy utilization by steers of diverse genotypes fed a high-concentrate diet during the finishing period: II. Angus, Boran, Brahman, Hereford, and Tuli sires. **Journal of Animal Science**, v 76, p. 647–657, 1998.

FERRELL, C. L.; OLTJEN, J. W. ASAS centennial paper: net energy systems for beef cattle concepts, application, and future models. **Journal of Animal Science**, v. 86, p. 2779-2794, 2008.

FONSECA, L. F. S. et al. Expression of genes related to mitochondrial function in Nellore cattle divergently ranked on residual feed intake. **Molecular Biology Rep**, v. 42, p. 559–565, 2015.

FRAYN, K. N. **Metabolic regulation**: a human perspective. Oxford: Wiley-Blackwell, 2010.

FREETLY, H.C. Feed efficiency in beef cattle: how far have we come, how far can we go? In: INTERNATIONAL SYMPOSIUM OF BEEF CATTLE PRODUCTION, 4, 2015, Lavras. **Proceedings…** Lavras: UFLA, 2015, p. 107-123.

GOLL, D. E. et al. Myofibrillar protein turnover: The proteasome and the calpains. **Journal of Animal Science**. v. 86, p. 19–3, 2008.

GRUBBS, J. K. et al. Liver and skeletal muscle mitochondria proteomes are altered in pigs divergently selected for residual feed intake. **Journal of Animal Science,** v. 92, p. 1995–2007, 2014.

GRUBBS, J. K. et al. Selection for residual feed intake alters the mitochondria protein profile in pigs. **Journal of Proteomics**, v. 8, p. 334-345, 2013.

HERD, R. M.; ARCHER, J. A.; ARTHUR, P. F. Reducing the cost of beef production through genetic improvement of feed intake: Opportunity and challenges to application. **Journal of Animal Science**, v. 81, p. 9–17, 2003.

HERD, R. M.; ARTHUR, P. F. Physiological basis for residual feed intake. **Journal of Animal Science**, v. 87, p. 64–71, 2009.

HOLLUNG, K. et al. Application of proteomics to understand the molecular mechanisms behind meat quality. **Meat Science**, v. 77, p. 97–104, 2007.

HOQUE, A. A. et al. Genetic parameters for carcass traits of field progeny and their relationships with feed efficiency traits of their sire population for Japanese Black (Wagyu) cattle. **Livestock Production Science**, v. 99 p. 111–118, 2006a.

HOQUE, A. A. et al. Genetic relationship between different measures of feed efficiency and its component traits in Japanese Black (Wagyu) bulls. **Livestock Science**, v. 100, p. 251–260, 2006b.

JING, L. et al. Transcriptome analysis of mRNA and miRNA in skeletal muscle indicates an important network for differential Residual Feed Intake in pigs. **Scientific Reports,** v. 5, p. 1-14, 2015.

KARISA, B. K. et al. Candidate genes and single nucleotide polymorphisms associated with variation in residual feed intake in beef cattle. **Journal of Animal Science,** v. 9, p. 3502–3513, 2013.

KERLEY, S. Nutrition and Feed Efficiency of Beef Cattle. In: HILL, R. A. **Feed efficiency in the beef industry**. New York: John Wiley and Sons Inc., 2012. p. 75-122.

KLEIBER, M. Efficiency of Energy Utilization in Farm Animals. **American Journal of Clinical Nutrition**, v. 8, p. 614-620, 1960.

KOCH, R. M. et al. Efficiency of feed use in beef cattle. **Journal of Animal Science**, v. 22, p. 486–494, 1963.

KOOHMARAIE, M. et al. Meat tenderness and muscle growth: is there any relationship. **Meat Science**, v. 62, p. 345–352, 2002.

KRAFT, G. et al. Portal glucose delivery stimulates muscle but not liver protein metabolism. **Animal Journal of Physiology and Endocrinology Metabolism**, v. 303, p. 1202–1211, 2012.

LANCASTER, P.A. et al. Phenotypic and genetic relationships of residual feed intake with performance and ultrasound carcass traits in Brangus heifers. **Journal of Animal Science**, v.87, p. 3887–3896, 2009.

LAWRENCE, T.L.J.; FOWLER, V.R. **Growth of Farm Animals**. Wallingford: CAB International, 2002.

MALTIN, C.; PLASTOW, G. Functional Genomics and Proteomics in Relation to Muscle Tissue. In: TE PAS, M.F.W.; EVERTS, M.E.; HAAGSMAN, H.P. **Muscle development of livestock animals:** physiology, genetics, and meat quality. Wallingford: CAB International, 2004, p. 267-296.

MARCONDES, M, I. et al. Predicting efficiency of use of metabolizable energy to net energy for gain and maintenance of Nellore cattle. **Journal of Animal Science**, v. 91, p. 4887–4898, 2013.

MOORE, S.; MUJIBI, F.D.; SHERMAN, E.L. Molecular basis for Residual Feed Intake in beef cattle. **Journal of Animal Science**, v.87, p.41-47, 2009.

MULLEN, A. M. et al. Understanding meat quality through the application of genomic and proteomic approaches. **Meat Science**, v. 74, p. 3–16, 2006.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrients requirements of beef cattle**. Washington: National Academy Press, 1996, 242p.

OKANISHI, T. et al. Genetic analysis of residual feed intakes and other performance test traits of Japanese Black cattle from revised protocols. **Animal Science Journal**, v. 79, p. 291–296, 2008.

OLIVEIRA, P. S. N. et al. Identification of genomic regions associated with feed efficiency in Nelore cattle. **BMC Genetics**, v. 15, p. 1-10, 2014.

ROBINSON, D. L; ODDY, V. H. Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. **Livestock Production Science**, v.90, p. 255–270, 2004.

ROLFE, K.M. et al. Genetic and phenotypic parameter estimates for feed intake and other traits in growing beef cattle and opportunities for selection. **Journal of Animal Science**, v.89, p. 3452–3459, 2011.

SCHENKEL, F.S.; MILLER, S.P; WILTON, J.W. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. **Canadian Journal of Animal Science**, v. 84, p. 177–185, 2004.

SZABO, Z et al. Mass spectrometry-based label free quantification of gel separated proteins. **Journal of Proteomics,** v. 75, p. 5544-5553, 2012.

TIZIOTO, P. C. et al. Global liver gene expression differences in Nellore steers with divergent residual feed intake phenotypes. **BMC Genomics**, v. 16, p. 1-14, 2015.

VINCENT, A. et al. Divergent selection for residual feed intake affects the transcriptomic and proteomic profiles of pig skeletal muscle. **Journal of Animal Science**, v. 93, p. 2745–2758, 2015.

WOLCOTT, M.L. et al. Genetics of meat quality and carcass traits and the impact of tenderstretching in two tropical beef genotypes. **Animal Production Science, v.** 49, p.383–398, 2009.

WULFHORST, J.D. et al. Producer Awareness and Perceptions about Feed Efficiency in Beef Cattle. In: HILL, R. A. **Feed efficiency in the beef industry**. New York: John Wiley and Sons Inc., 2012. p. 29-48.

SECOND CHAPTER – ARTICLE

Proteomic profile of skeletal muscle and liver tissue of high and low residual feed intake identified Nellore cattle

Article formatted according to Journal of Animal Science

ABSTRACT: Residual feed intake (RFI) has been extensively used to evaluate feed efficiency, however the molecular underlying differences in this trait remains unclear. In order to characterize mechanisms driving differences on energy metabolism of beef cattle skeletal muscle and liver tissue proteome was analyzed. From a group of 120 Nellore young bulls identified for RFI, cattle with the highest (n=9) and lowest (n=9) values were selected for protein abundance studies. After slaughter, samples were collected immediately after slaughter and subjected to two-dimensional gel electrophoresis (2-DE) followed by mass spectrometry to identify differentially abundant proteins associated with RFI values. Two differentially abundant spots were identified in skeletal muscle of high RFI group (Alpha actin 1 e 14-3-3 protein epsilon), one was identified in muscle of low RFI group (Heat shock protein beta-1) and one was identified in liver tissue of low RFI group (10-formyltetrahydrofolate dehydrogenase) ($P<0.05$). These data indicates that genetic selection for RFI tends to cause changes in skeletal muscle and liver protein profile, suggesting that differences in low and high RFI cattle may be due skeletal muscle protein turnover and liver energy metabolism.

Key words: Feed efficiency, 2D electrophoresis, Heat shock protein beta-1, Alpha actin 1, 10-formyltetrahydrofolate dehydrogenase.

1. Introduction

Feed efficiency is one of the most important parameters within a beef production system affecting the production costs and environmental damage. Among several ways to estimate feed efficiency, the residual feed intake (RFI) has been reported as a better trait for selection than feed conversion ratio (Herd et al., 2003) since it is phenotipically independent of growth rate and body weight in growing (Arthur et al., 2001; Archer et al., 2002; Baker et al., 2006). Although RFI has been extensively used to evaluate feed efficiency, the physiological basis of the RFI remains unclear. According to Herd and Arthur, (2009) at least five major processes are involved in variation of efficiency: feed intake, feed digestion, activity, thermoregulation, and energy metabolism.

Considering that the turnover of body proteins accounts for 30% of the energy spent for cattle maintenance, the metabolism of skeletal muscle and liver is an important factor affecting feed efficiency, since these tissues accounts for about two thirds of whole body protein turnover in mammals (Frayn, 2009). However, most of the studies regarding molecular mechanisms underlying differences in feed efficiency in Nellore cattle have focused their efforts at changes in the transcriptome level (Oliveira et al.,

2014; Fonseca et al., 2015; Tizioto et al., 2015) while information regarding differences at proteomic level is scarce. Therefore, the objective of this study was to evaluate the consequences of divergent feed efficiency Nellore cattle identified by RFI on protein profile of skeletal muscle and liver tissues.

2. Material and Methods

The animal procedures were carried out at *Centro APTA Bovinos de Corte, Instituto de Zootecnia (IZ), Sertãozinho*, São Paulo, Brazil. Procedures were approved by the Ethics Committee on Animal Use - CEUA/IZ, (approval number: 213-15).

Identification of High and Low Residual Feed Intake Animals

A contemporary group of one hundred and twenty Nellore young bulls were subjected to a growth period of 98 days receiving the same diet formulated to meet the requirements of 1 kg/day gain according to CNCPS (2012). Cattle were fed using a GrowSafe automated feeding system (GrowSafe Systems Ltd, Airdrie, Canada).

Residual feed intake (RFI) was calculated during the growth period using the following model:

$$SFI = \beta_0 + \beta_P * BW^{0,75} + \beta_G * ADG + \varepsilon\ (RFI)$$

where SFI = standardized daily feed intake, β_0= regression intercept, β_P= regression coefficient of SFI on metabolic mid-weight, β_G = regression coefficient of SFI on average daily gain and ε = residual error in SFI.

From the 120 animals, a total of 18 animals (9 Low and 9 High RFI cattle) were selected and submitted to a finishing period of 125 days receiving the same diet formulated meet the requirements of 1.3 kg/day gain requirements until reach 550 kg of body weight.

Animal slaughter and Samples collection

At the end of the finishing period, cattle were slaughtered in an experimental slaughter house after fasting from solids for 16 h. Samples of longissimus muscle was collected immediately after exsanguination at the 12^{th} rib of the right side of the carcass. Liver tissue samples were collected immediately after the evisceration. Once collected, both skeletal muscle

and liver tissue samples were snap-frozen and powdered in liquid nitrogen, and kept at -80°C for further analyses.

Protein extraction

Laboratory procedures were carried out at *Laboratório de Biotecnologia Animal* and *Núcleo de Análises de Biomoléculas (Nubiomol) of Universidade Federal de Viçosa*, Minas Gerais, Brazil. Samples of 0.05g of liver and skeletal muscle tissue were homogenized using a polytron PT 3100 (Leucerne, Switzerland) on ice for 10s in 1 ml of lysis buffer containing: 7M urea, 2M thiourea, 4% 3-3 [(cholamidopropyl) dimethilammonio] -1- propanesulfonate (CHAPS), 1% dithiothreitol (DTT), 2% immobilized pH gradient (IPG) buffer (pH 3 to 10), 1M phenylmethanesulfonyl fluoride (PMSF) and protease inhibitors. The supernatant obtained after centrifugation for 30 min at 4 °C was used as the protein extract for later electrophoresis analysis. The total amount of proteins was quantified by Quick Start *Bradford* Protein *Assay (Bio-Rad*, Hercules, CA) using *Bovine serum albumin* (BSA) as a standard. One-dimensional electrophoresis was performed to test the integrity of the protein extracted.

Two-dimensional electrophoresis

In the first dimension of electrophoresis, the Immobilized pH Gradient (IPG) strips (GE Healthcare Lifesciences, Uppsala, Sweden) of 24 cm, pH 3-10, were rehydrate overnight at room temperature in 450 µl of "DeStreak" rehydration buffer (GE Healthcare Lifesciences, Uppsala, Sweden), 2% IPG buffer pH 3–10, containing 1200µg of protein.

Then, samples were subjected to isoelectric focusing on an Ettan IPGphor3 system (GE Healthcare Lifesciences, Uppsala, Sweden) at 20°C with the protocol recommended for the specific type of IPG strip (step and hold at 500V for 1h; gradient to 1000V for 0.8kVh; gradient to 10000 V for 16.5 KVh and step at 10000 V for 17.2 KVh) with a current limit of 50 mA/strip.

For the second dimension, strips were equilibrated in 1.5 M Tris–HCl (pH 8.8), 6M urea, 2% sodium dodecyl sulfate (SDS), 30% glycerol, and 0.002% bromophenol blue buffer for 20 min with 1% dithiothreitol (DTT) and 20 min with 2.5% iodoacetamide. The Strips were transferred to a 12.5% acrylamide gel and fixed with an agarose sealing solution. The SDS- polyacrylamide gel electrophoresis (SDS-PAGE) was performed in

a vertical Ettan DALTsix system (GE Healthcare Lifesciences, Uppsala, Sweden) using Laemmli running buffer at a 1× concentration for the anode and a 2× concentration for the cathode. The electrical current for electrophoresis was kept at 20 mA/gel with an initial voltage of 80 V for 45 min to allow proteins to migrate in the gel strip. After this period, the voltage was increased to 500 V, using 40 mA/gel until the sample run over the gel. At the end of the run, gels were stained using a colloidal Coomassie Blue G-250 procedure involving fixation in 10% acetic acid/40% ethanol overnight followed by addition of a solution containing 8% ammonium sulfate, 0.8% phosphoric acid, 0.08% Coomassie Blue G-250, 20% methanol for 72 h and destained by solution of acetic acid 1%. Finally, gels were kept in a solution of acid acetic 2% until subsequent image analysis.

Image analysis

The Two-dimensional electrophoresis (2-DE) gels were scanned with ImageScanner using Lab Scan program (GE Healthcare Lifesciences, Uppsala, Sweden) and analyzed with ImageMaster Platinum software (GE Healthcare Lifesciences, Uppsala, Sweden). Spots with P value ≤ 0.05 obtained by ANOVA were considered differentially abundant.

In-gel digestion of proteins

The differentially abundant spots between the two extremes for RFI of both tissues were cut out from gels using pipet tips of 1,000µL and placed in 1.5 mL tubes. The trypsinization was performed using a modified method based on Shevchenko et al (2006). The gels pieces were destained through washes of solution containing 50% acetonitrile and 25 mM ammonium bicarbonate (Ambic), pH 8.0, and dried at room temperature. Subsequently, the solution was removed and the samples were dehydrated in 200 µl of 100% ACN. The reduction reaction was made with 100 µl of 65 mM DTT and 100 mM Ambic and the alkylation was made with 100 µl of 200 mM iodoacetamide and 100 mM Ambic. For sample cleavage, 20µl of Trypsin Porcine - Mass Spectrometry Grade (Promega, Madison, USA) was added to the fragments and stored at ice for 45 min, to allow trypsin to penetrate the gel fragments. The recovery of tryptic peptides was made with the addition of a solution containing 5% formic acid and 50% ACN and removal of the supernatant. The samples were desalting using Zip Tip C18 micro columns (Millipore, Billerica, MA) to decrease interference in

crystallization of the samples with the matrix used in mass spectrometer analysis, and were then stored at -20 °C until subsequent analysis.

Protein identification

Peptide masses of the samples were obtained using an Ultraflex III MALDI TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) and then were searched against the Bovidae database deposited in UniProt using the MASCOT program (Matrix Science, London, UK) and MASCOT Peptide Mass Fingerprinting database search. An accuracy of 0.5 Da was used in the search criteria. Trypsin was set as enzyme with one allowed miscleavage. Fixed modification and variable modification used were carbamidomethyl and oxidation, respectively. The numbers of peptide matches, sequence coverage, molecular weight (MW), and *isoelectric point (*pI) were used to evaluate the database search results. The Scaffold program (Proteome Software, Portland, OR) was used to validate the proteins identified by Mascot program, with the identity for both proteins and peptides equal to or above 90%.

3. Results

The 2-D gels include proteins in the molecular mass region of 14.4 to 97 kDa and the pH range between 3 and 10. Significance testing by ANOVA showed that 13 spots were different in abundance ($P \leq 0.05$) in skeletal muscle and 8 spots were different in abundance in liver tissue between the RFI+ (less efficient) and RFI- (more efficient) groups. Representative gels with the position of the spots are shown in Figures 1 and 2.

From the 13 spots that had different abundance in skeletal muscle tissue of RFI+ and RFI- groups, three were able to be identified via mass spectrometry. In the liver tissue, from the 8 spots differentially abundant between RFI+ and RFI- groups, only one was able to be identified via mass spectrometry. The failure to identify all the spots may be due to decreased protein concentration or the lack of Bovidae protein databases.

From the 3 spots identified in skeletal muscle tissue, two had higher abundance in RFI+ (spots 1 and 2), which were identified as Actin Alpha 1 and 14-3-3 Protein Epsilon. One spot had higher abundance in RFI– (spot 3), and was identified as Heat Shock Protein Beta 1.

In the liver tissue, we have observed a higher abundance of one spot (spot 4) The spot differentially abundant in the liver tissue was identified as 10-formyltetrahydrofolate dehydrogenase, being more abundant in RFI-animals. The identification and information related to the validity of search results are shown in Tables 1 and 2.

The protein name, accession number, Mascot score, matched peptides, % sequence coverage, source and theoretical pI and MW were derived from database. All four spots were identified by matching peptide data to *Bos taurus* protein sequences in the Uniprot database. Similarity between the experimental molecular weights and the theoretical molecular weights indicated that all of the identified proteins were full-length proteins.

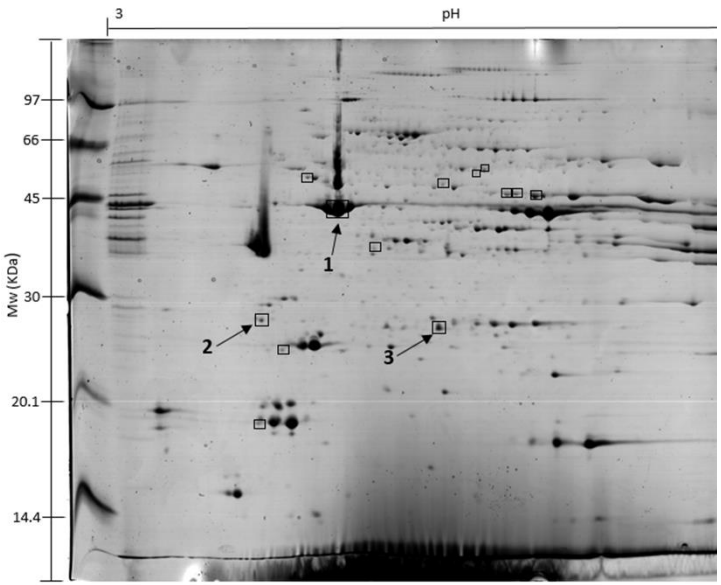

Figure 1. Representative 2-dimensional gel image of skeletal muscle of one Nellore bull. The proteins are indicated by spot number, which corresponds to those identified as differentially abundant between the different RFI groups.

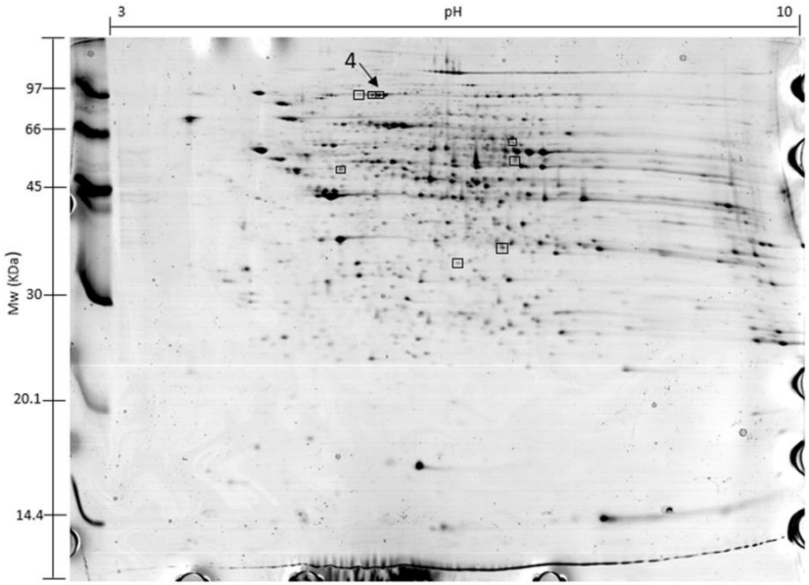

Figure 2. Representative 2-dimensional gel image of liver tissue of one Nellore bull. The proteins are indicated by spot number, which corresponds to those identified as differentially abundant between the different RFI groups.

Table 1. Proteins identified in gels of Muscle Tissue

Spot[a]	Protein	UniProt accession number	Group of greater abundance	Mascot Score	% Protein identification probability[b]	% Protein Coverage[c]	Theoretical		Experimental		Matched peptides[d]
							MW	PI	MW	PI	
1	**Actin, alpha 1, skeletal muscle** OS=Bos taurus GN=ACTA1 PE=2 SV=1	A4IFM8_BOVIN	High RFI	586	100%	18	42051	5.23	42943	5.27	5
2	**14-3-3 protein epsilon** OS=Bos taurus GN=YWHAE PE=2 SV=1	W5PRN8	High RFI	174	100%	16	29174	4.63	26444	3.98	3
3	**Heat shock protein beta-1** OS=Bos taurus GN=HSPB1 PE=3 SV=2	E1BEL7	Low RFI	649	100%	44	22393	5.98	24205	5.58	7

[a] Numbers shown in Figure 1.
[b] Probability for validation by Scaffold of proteins identified by Mascot.
[c] Protein coverage calculated by Scaffold (identified amino acids/total amino acids).
[d] Number of peptides identified in Mascot and validated by Scaffold.

Table 2. Proteins identified in gels of Liver Tissue

Spot[a]	Protein	UniProt accession number	Group of greater abundance	Mascot Score	% Protein identification probability[b]	% Protein Coverage[c]	Theoretical		Experimental		Matched peptides[d]
							MW	PI	MW	PI	
4	**10-formyltetrahydrofolate dehydrogenase** OS=Bos taurus GN=ALDH1L1 PE=2 SV=1	E1BMG9	Low RFI	130	98%	2	98738	5.63	10218	5.78	1

[a] Numbers shown in Figure 2.
[b] Probability for validation by Scaffold of proteins identified by Mascot.
[c] Protein coverage calculated by Scaffold (identified amino acids/total amino acids).
[d] Number of peptides identified in Mascot and validated by Scaffold.

4. Discussion

Proteins differentially abundant in skeletal muscle tissue

The balance between the amount of muscle protein synthesized and the amount of muscle protein degraded determines muscle size. There are three scenarios that can lead skeletal muscle mass accretion: Increased protein synthesis and decreased protein degradation; decreased protein synthesis and decreased protein degradation (most efficient method to increase muscle growth rate); and increased protein synthesis and increased protein degradation (least efficient method to increase muscle growth rate) (Koohmaraie et al., 2010). The poor efficiency attributed to this third scenario is due to the energy expended in the processes of protein turnover. Protein turnover is a very energetically expensive cellular process, either by the amount of ATP required to operate the proteasome system and the loss of energy expended in synthesizing the proteins, which may represent a major contributing process in the phenotypic expression of poor feed efficiency in animals (Cruzen et al., 2013).

In the present study, we have found a greater abundance of the protein alpha actin in skeletal muscle of RFI+ cattle. This protein belongs to the group of myofibrillar proteins, which constitute 55 to 60% of total

protein in muscle tissue. Together actin and myosin are the two main proteins that constitute the myofibril or contractile structure in skeletal muscle, and both are susceptible to degradation by the proteasome (Goll et al., 2008). In addition, levels of alpha-actin are positively correlated with synthesis of muscle fiber proteins and muscle growth (Murgiano et al., 2010). The higher abundance of alpha actin found in skeletal muscle tissue of RFI+ cattle may indicate a increase on skeletal muscle turnover which would lead to a lower efficiency of these animals due to a greater energy expenditure.

Heat shock protein beta 1 (HSPB1), also known as HSP27, a member of the heat-shock family of proteins, is a relatively small molecular chaperone protein associated with cellular development, differentiation and signal transduction (Zhang et al., 2014). Heat shock proteins are essential for normal cellular stress responses (Keady et al., 2013) and may serve to protect cells from otherwise agents (Creagh et al., 2000). Its known role is to bind and stabilize unstable proteins and facilitates their correct assembly, in addition to enhance cell survival by interfering with cellular signal transduction pathways regulating apoptotic cell death (Lomiwes et al., 2014). Small heat shock proteins (sHSPs) bind unfolded polypeptides,

acting as an important defense mechanism against the formation of protein aggregates. (Fischer et al., 2002). This can reduce the binding of proteases responsible for degradation of muscle fibers. According to Kim et al. (2011) down-regulation of HSP27 may increase the degradation of muscle proteins such as actin and myosin.

In the current study we found greater abundance of HSPB1 in RFI– cattle. This may indicate that the defense action of these proteins in skeletal muscle contribute to greater efficiency of the animals due to a lower rate of protein turnover. Moreover, sHSPs have been implicated to play a central role in the structural and functional organization of the three-dimensional intermediate filament and the actin microfilament system (Fischer et al., 2002), thus causing a higher stability and less turnover of actin, which is the opposit mechanism to the observed in the less efficient animals (RFI+).

The 14-3-3 proteins are small (~30 kDa) acidic proteins found in all eukaryotic organisms, with 7 isoforms in mammals. All 14-3-3s are highly conserved both within and across species (Bridges and Moorhead, 2004) and have the ability to bind a large number of proteins causing multiple effects specific proteins (Porter et al., 2006), including kinases,

phosphatases, and transmembrane receptors. Thus, this protein plays an important role in a wide range of vital regulatory processes, such as mitogenic signal transduction, apoptotic cell death, and cell cycle control (Fu et al., 2000). The 14-3-3 epsilon (14-3-3 ε) protein directly prevent apoptosis by binding to mitochondrial apoptotic proteins and apoptotic signal transducing proteins, and is associated with protein kinase C and glycogen synthase kinase 3 and apoptosis-related proteins such as bad and bax, decreasing apoptosis (Jeon et al., 2016). This mechanism may be associated with less energy expenditure with protein turnover in skeletal muscle and higher feed efficiency.

Another function of 14-3-3 ε is its interaction with the insulin-like growth factor I receptor (IGF-I R) and with insulin receptor substrate I (IRS-1) (Craparo et al., 1997). A study performed by Oriente et al. (2005) have shown that insulin action was enhanced following 14-3-3 ε overexpression and was reduced upon antisense depletion of 14-3-3 ε, and overexpression of 14-3-3 ε also induced a reduction in the insulin degradation. This decrease in degradation and increase in insulin action could lead to a higher uptake of insulin by skeletal muscle cells and increased glucose uptake.

Additionally, it has been shown by several studies that high RFI animals have lower efficiency on ATP synthesis which might be due to several factors such as greater mitochondrially derived reactive oxygen species (ROS) production (Bottje and Carstens, 2009), due to greter uncoupling protein 3 (UCP3; triggers mitochondrial proton leak in muscle) mRNA (Ojano-Dirain et al., 2007), compared to low RFI animals. Therefore, the greater abundance of 14-3-3 epsilon protein in RFI+ cattle observed in the current study which may be explained by the need of glucose uptake in the skeletal muscle of these animals due to their lower efficiency to produce ATP. It is noteworthy that the presence of a protein in certain tissue sometimes is not evidence that it is functional. Some important functions of 14-3-3 protein are mediated and regulated by post-translational modification like phosphorylation and it is important to understand its role in the precise regulation of signal transduction (Zi-Jian et al., 2013). Thus, future studies evaluating the phosphorylation of this protein are needed to clarify its activity in relation to factors that determine the feed efficiency.

Proteins differentially abundant in liver tissue

The 10-Formyltetrahydrofolate dehydrogenase (FDH, ALDH1L1) is a cytosolic enzyme that shares significant sequence similarity with enzymes of ALDH family and is expected to possess the same biological function (Strickland et al., 2011). Proteins from ALDH family act as through a antioxidant defense of negative effects of reactive oxygen species (ROS) (Laville et al., 2009). The electron transport chain is a site of ROS production and it is occurs when the protomotive force used to drive ATP synthesis is uncoupled and protons leak back into the mitochondrial matrix generating heat rather than ATP, which will reduce the efficiency of oxidative phosphorylation (Bayir and Kagan, 2008; Bottje and Carstens, 2009). Consequently, ROS production diverts dietary energy from growth towards cellular repair and autophagy mechanisms and may play a role in the phenotypic expression of feed efficiency (Grubbs et al., 2013).

In this context, an unexpected result was reported in previous study where RFI- Nellore cattle (more efficient) presented a greater mRNA abundance of UCP2 (uncoupling protein 2 - one of the proteins that causes the leakage of protons from the mitochondria leading to a production of ROS) in the liver tissue compared to RFI+ cattle (less efficient) (Fonseca

et al., 2015). Thus, the observations of the current study may explain the fact that although a greater production of ROS may happen in the liver tissue of RFI- cattle (Fonseca et al., 2015), it seems that a greater abundance of FDH in liver tissue of RFI- cattle may indicate a mechanism that these animals possess to ameliorate the negative effects of ROS. Consequently, RFI- cattle may have lower energy expenditure with cellular repair which would lower their maintenance requirements leading to a better feed efficiency.

In summary, our data suggest that discrepancy in feed efficiency of cattle identified by RFI may be due to differences in energy expenditure with skeletal muscle protein turnover, showed by the greater abundance of Heat Shock Protein Beta 1 in skeletal muscle of RFI- cattle. Moreover, the greater abundance of Alpha Actin – 1 in less efficient cattle (RFI+) may be associated with higher degradation and subsequent replacement of this protein, which increases their maintenance requirements. In addition to observations in the skeletal muscle, a lower energy expenditure in the liver tissue of higher feed efficiency may be associated with a decrease of negative effects of ROS due to a greater abundance of 10-

Formyltetrahydrofolate dehydrogenase, reducing energy loss with cellular proliferation, also contributing for decrease of maintenance requirements.

LITERATURE CITED

Archer, J., A. Reverter, R. Herd, D. Johnston, and P. Arthur. 2002. Genetic variation in feed intake and efficiency of mature beef cows and relationships with postweaning measurements. In: From the 7th World Congress on Genetics Applied to Livestock Production

Arthur, P. F., G. Renand, and D. Krauss. 2001. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livest. Prod. Sci. 68: 131-139.

Baker, S. D., J. I. Szasz, T. A. Klein, P. S. Kuber, C. W. Hunt, J. B. Glaze, D. Falk, R. Richard, J. C. Miller, R. A. Battaglia and R. A. Hill. 2006. Residual feed intake of purebred Angus steers: Effects on meat quality and palatability. J Anim Sci. 84: 938-945.

Bayir, H., and V. E. Kagan. 2008. Bench-to-bedside review: Mitochondrial injury, oxidative stress and apoptosis – there is nothing more practical than a good theory. Crit. Care. 12: 206-206.

Bottje, W. G., and G. E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. J. Anim. Sci. 87: E48-63.

Bridges, D., and G. B. Moorhead. 2004. 14-3-3 proteins: a number of functions for a numbered protein. Sci. STKE 2004: re10.

Craparo, A., R. Freund, and T. A. Gustafson. 1997. 14-3-3 (ϵ) interacts with the insulin-like growth factor I receptor and insulin receptor substrate I in a phosphoserine-dependent manner. J. Biol. Chem. 272: 11663-11669.

Creagh, E., D. Sheehan, and T. Cotter. 2000. Heat shock proteins-modulators of apoptosis in tumour cells. Leukemia 14: 1161-1173.

Cruzen, S. M., A. J. Harris, K. Hollinger, R. M. Punt, J. K. Grubbs, J. T. Selsby, J. C. M. Dekkers, N. K. Gabler, S. M. Lonergan, and E. Huff-Lonergan. 2013. Evidence of decreased muscle protein turnover in gilts selected for low residual feed intake. J. Anim. Sci. 91: 4007-4016.

Fischer, D., J. Matten, J. Reimann, C. Bönnemann, and R. Schröder. 2002. Expression, localization and functional divergence of αB-

crystallin and heat shock protein 27 in core myopathies and neurogenic atrophy. Acta. Neuropatho. 104: 297-304.

Fonseca, L. F., D. F. Gimenez, M. E. Mercadante, S. F. Bonilha, J. A. Ferro, F. Baldi, F. R. Souza, and L. G. Albuquerque. 2015a. Expression of genes related to mitochondrial function in Nellore cattle divergently ranked on residual feed intake. Mol. Biol. Rep. 42. 42: 559-65

Frayn, K. N. 2009. Metabolic regulation: a human perspective. John Wiley & Sons.

Fu, H., R. R. Subramanian, and S. C. Masters. 2000. 14-3-3 proteins: structure, function, and regulation. Annual review of pharmacology and toxicology 40: 617-647.

Goll, D., G. Neti, S. Mares, and V. Thompson. 2008. Myofibrillar protein turnover: the proteasome and the calpains. J. Anim. Sci. 86: E19-E35.

Grubbs, J. K., A. N. Fritchen, E. Huff-Lonergan, N. K. Gabler, and S. M. Lonergan. 2013. Selection for residual feed intake alters the mitochondria protein profile in pigs. J. Proteomics 80: 334-345.

Herd, R., J. Archer, and P. Arthur. 2003. Reducing the cost of beef production through genetic improvement in residual feed intake: Opportunity and challenges to application. J. Anim. Sci. 81: E9-E17.

Herd, R. M., and P. F. Arthur. 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87: E64-71.

Jeon, S.-J., J.-H. Sung, and P.-O. Koh. 2016. Hyperglycemia decreases expression of 14-3-3 proteins in an animal model of stroke. Neurosci. Lett. 626: 13-18.

Keady, S. M., D. A. Kenny, K. Ohlendieck, S. Doyle, M. G. Keane, and S. M. Waters. 2013. Proteomic profiling of bovine M. longissimus lumborum from Crossbred Aberdeen Angus and Belgian Blue sired steers varying in genetic merit for carcass weight. J. Anim. Sci. 91: 654-665.

Kim, N.-K., D. Lim, S. H. Lee, Y. M. Cho, E. W. Park, C. S. Lee, B. S. Shin, T. H. Kim, and D. Yoon. 2011. Heat shock protein B1 and its regulator genes are negatively correlated with intramuscular fat content in the Longissimus Thoracis muscle of Hanwoo (Korean cattle) steers. J. Agric. Food Chem. 59: 5657-5664.

Koohmaraie, M., M. P. Kent, S. D. Shackelford, E. Veiseth, and T. L. Wheeler. 2002. Meat tenderness and muscle growth: is there any relationship. Meat. Sci. 62: 345–352.

Laville, E., T. Sayd, C. Terlouw, S. Blinet, J. Pinguet, M. Fillaut, J. Glénisson, and P. Chérel. 2009. Differences in pig muscle proteome according to HAL genotype: implications for meat quality defects. J. Agric. Food Chem. 57: 4913-4923.

Lomiwes, D., M. Farouk, E. Wiklund, and O. Young. 2014. Small heat shock proteins and their role in meat tenderness: A review. Meat. Sci. 96: 26-40.

Murgiano, L., A. D'Alessandro, M. G. Egidi, A. Crisa, G. Prosperini, A. M. Timperio, A. Valentini, and L. Zolla. 2010. Proteomics and transcriptomics investigation on longissimus muscles in Large White and Casertana pig breeds. J. Proteome. Res. 9: 6450-6466.

Ojano-Dirain, C., M. Toyomizu, T. Wing, M. Cooper, and W. G. Bottje. 2007. Gene Expression in Breast Muscle and Duodenum from Low and High Feed Efficient Broilers. Poult. Sci. 86: 372-381.

Oliveira, P. S. N., A. S. M. Cesar, M. L. Nascimento, A. S. Chaves, P. C. Tizioto, R. R. Tullio, D. P. D. Lanna, A. N. Rosa, T. S. Sonstegard,

G. B. Mourao, J. M. Reecy, D. J. Garrick, M. A. Mudadu, L. L. Coutinho, and L. C. A. Regitano. 2014. Identification of genomic regions associated with feed efficiency in Nelore cattle. BMC Genet 15.

Oriente, F., F. Andreozzi, C. Romano, G. Perruolo, A. Perfetti, F. Fiory, C. Miele, F. Beguinot, and P. Formisano. 2005. Protein Kinase C-_ Regulates Insulin Action and Degradation by Interacting with Insulin Receptor Substrate-1 and 14-3-3ε. J. Biol. Chem. 280: 40642–40649.

Porter, G. W., F. R. Khuri, and H. Fu. 2006. Dynamic 14-3-3/client protein interactions integrate survival and apoptotic pathways. In: Seminars in cancer biology. p 193-202.

Shevchenko, A., H. Tomas, J. Havlis, J.V. Olsen, and M. Mann. 2006. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat. Protoc. 1: 2856-2860.

Strickland, K. C., N. I. Krupenko, M. E. Dubard, C. J. Hu, Y. Tsybovsky, and S. A. Krupenko. 2011. Enzymatic properties of ALDH1L2, a mitochondrial 10-formyltetrahydrofolate dehydrogenase. Chem. Biol. Interact. 191: 129-136.

Tizioto, P. C., L. L. Coutinho, J. E. Decker, R. D. Schnabel, K. O. Rosa, P. S. N. Oliveira, M. M. Souza, G. B. Mourão, R. R. Tullio, A. S. Chaves, D. P. D. Lanna, A. Zerlotini-Neto, M. A. Mudadu, J. F. Taylor, and L. C. A. Regitano. 2015. Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC Genomics 16: 1-14.

Zhang, Q., H.-G. Lee, S. K. Kang, M. Baik, and Y.-J. Choi. 2014. Heat-shock protein beta 1 regulates androgen-mediated bovine myogenesis. Biotechnol. Lett. 36: 1225-1231.

Zi-Jian, L., H. Xin, P. Chen-Yu, and L. Ning. 2013. Mass Spectrometric Analysis of Phosphorylation Modification in 14-3-3ε Protein. Chin. J. Anal. Chem. 41: 1653-1658.