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Relationship of Mitochondrial Biogenesis and Phenotypic Expression of Residual Feed Intake in Fat-Tailed Lambs

Research Article

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ABSTRACT

Residual feed intake (RFI) is a popular measure of feed efficiency in livestock animals. The functionality of mitochondrial metabolism has been shown to be associated with variation in feed efficiency. In this study, mitochondrial biogenesis was determined by measuring the mitochondrial DNA (mtDNA) copy number in skeletal muscle of two groups of fat-tailed lambs divergently ranked on phenotypic expression of RFI (high- and low-RFI). A quantitative real-time PCR was performed to determine the relative mRNA levels of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α) and NAD$^+$-depended deacetylase sirtuin1 (SIRT1), as two major genes involved in the regulation of mitochondrial biogenesis. The level of mtDNA in skeletal muscle of low-RFI lambs was higher than those ranked as high-RFI. Higher mRNA level of PGC-1α was also detected in low-RFI lambs in compared with the high-RFI group. However, no significant difference was observed between the RFI groups for mRNA expression of SIRT1. The results showed a negative relationship between phenotypic expression of RFI with mitochondrial biogenesis and PGC-1α mRNA level in skeletal muscle of fat-tailed lambs. Overally, our results suggest that the increased mitochondrial dynamics has a potential to regulates bioenergetic efficiency and energy expenditure in low-RFI lambs.

KEY WORDS feed efficiency, mitochondrial DNA, PGC-1α, SIRT1, skeletal muscle.

INTRODUCTION

In areas of the world where religion or ethnicities preclude the consumption of beef or pork, sheep meat provide a major source of high-quality animal protein. Iran ranks fifth in the world sheep population, with the majority of sheep being fat-tailed and belonging to non-genetically improved native breeds. With the increasing production costs and continuing degradation of natural pastures, along with the preservation of germplasm of native domestic animals, it is also important to improve feed efficiency.

Residual feed intake (RFI) is a popular measure of feed efficiency, which can be defined as the difference between actual feed consumed and expected feed intake required for the rate of gain and body weight (Arthur *et al.* 2001). Variation in RFI is known to exist in a range of animal species including beef cattle (Archer *et al.* 1999), pigs (De Haer *et al.* 1993) and sheep (Rajaei Sharifabadi *et al.* 2012; Rajaei Sharifabadi *et al.* 2016). Over the past decade, researchers have paid considerable efforts to characterize the physiological mechanisms responsible for the variation in RFI. Many different physiological processes such as metabolism,

feed intake, digestibility, activity and thermoregulation have been suggested to be associated with the phenotypic expression of RFI (Herd and Arthur, 2009). Recently, several genome-wide association studies and transcriptome profiling have also been conducted to explore the molecular basis of RFI (Tizioto *et al.* 2015; Kong *et al.* 2016b). These genomic studies highlight multiple genes from various metabolic pathways that may explain the variation in RFI. There is a need to increase understanding of the molecular mechanisms underlying RFI that will aid in the identification of biomarkers for use in the breeding programs as well as optimization of nutritional strategies.

Mitochondria play central roles in the cellular energy provision as well as the generation of reactive oxygen species Tizioto *et al.* (2015) in eukaryotic cells. At a cellular level, mitochondrial dysfunction leads to decreased efficiency of energy production from the substrate (e.g. NADH) and is associated with increased oxidative damage to cellular macromolecules mediated by reactive oxygen species (ROS) (Bottje and Carstens, 2009). Therefore, mitochondrial functionality may influence the capacity of an animal to efficiently convert feed to body mass. The relationship between mitochondrial function and feed efficiency was firstly reported in broiler chickens by Bottje and colleagues (Bottje *et al.* 2002). Further studies in the ruminant animals also showed a negative correlation between phenotypic expression of RFI with the mitochondrial function (Kolath *et al.* 2006) and activity of mitochondrial respiratory chain complexes (Rajaei Sharifabadi *et al.* 2012; Zamiri *et al.* 2016). There is evidence that the mitochondrial metabolism-related genes were differentially expressed in breast muscle between high and low feed efficiency pedigree broiler males (Bottje and Kong, 2013) and that the mitoproteome was elevated in the high feed efficiency phenotype (Kong *et al.* 2016b). However, the molecular regulation of mitochondrial function and biochemistry in low- and high-RFI animals has additional features that are not completely defined, particularly in sheep.

In mammals, the mitochondrial genome encodes only 13 proteins that are all subunits of respiratory chain enzyme complexes (Anderson *et al.* 1981), whereas the regulation of mitochondrial metabolism and dynamics is governed by several nuclear transcription networks (Ventura-Clapier *et al.* 2008). As the typical mitochondrial dynamics, mitochondrial biogenesis is the process by which cells increase their individual mitochondrial mass and copy number (Ventura-Clapier *et al.* 2008). This process is accompanied by dramatic changes in the function and oxidative capacity of the mitochondria (Austin and St-Pierre, 2012). Peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α), a nuclear-encoded protein, is the master regulator of mitochondrial biogenesis that potentially initiates a transcriptional cascade and eventually leads to the mitochondrial biogenesis (Fernandez-Marcos and Auwerx, 2011; Nisoli *et al.* 2003). Elevated expression of PGC-1α has also been shown to positively affect the cellular redox status by up-regulation of the ROS-detoxifying enzymes (Austin and St-Pierre, 2012). However, the activity of PGC-1α is strongly controlled by several post-translational modifications such as methylation, phosphorylation and deacetylation. The latter is mediated by NAD^+- dependent deacetylase Sirtuin 1 (SIRT1) and positively regulates the activity of PGC-1α (Fernandez-Marcos and Auwerx, 2011). Therefore, mitochondrial biogenesis and the expression of its regulators may serve as the foundation for further in-depth studies of the molecular basis of variation in RFI.

In this study, we hypothesized that the variation in RFI is partly due to differences in the regulation of mitochondrial dynamics. Therefore, the mRNA expression of the master regulator of mitochondrial biogenesis (PGC-1α) was determined in skeletal muscle of low- and high-RFI lambs. To explore the activity of PGC-1α, we assessed the copy number of mitochondrial DNA (mtDNA) in each cell as an index of the mitochondrial biogenesis and analyzed the deacetylation status of PGC-1α by measure the mRNA level of SIRT1.

MATERIALS AND METHODS

Animal management and tissue sampling

Animals in this study (58 Kurdi ram lambs, 6 months old, 32.1±4.2 kg body weight) were randomly selected from spring-born flock of the Jovain Industrial and Agricultural company (Khorasan-e-Razavi, Iran) and reared at the Animal Production Site of the company. The lambs were individually housed in 1 × 1.2 m cages and had free access to water and a pelleted diet through the experiment. After a 10 d acclimation period, an *ad libitum* feeding strategy was performed for 42 d and average daily dry matter intake (DMI) was calculated using the total dry matter intake for 42 d. The lambs were weighed weekly and two consecutive day body weight (BW) records were measured at the beginning and end of the experimental period. Average daily gain (ADG) and mid-test metabolic BW (MMBW) of individual lambs were estimated by linear regression, as described by (Ceacero *et al.* 2016). Residual feed intake was also calculated as the residual of the linear regression equation of DMI on ADG and MMBW. Following equation was fitted to calculate RFI:

$$DMI = \alpha + \beta 1\ (ADG) + \beta 2\ (MMBW) + \varepsilon$$

Where:
α: intercept.

$\beta1$ and $\beta2$: partial regression coefficients of DMI on ADG and MMBW, respectively.
ε: RFI.

At the end of the experimental period, animals with RFI value less or higher than the mean minus or plus 0.5 standard deviation were grouped as low- or high-RFI phenotypes, respectively. Surgical biopsy sampling was performed from *Longissimus dorsi* muscle for six lambs per phenotypic group as described by Zamiri *et al.* (2016). The biopsy samples were flash-frozen in liquid nitrogen and kept at -80 ˚C for subsequent analyses.

RNA extraction and reverse transcription

Total RNA was extracted from the biopsy samples (100 mg) by Trizol reagent (Thermo Scientific, Rockford, IL) according to the manufacturer's recommendations. The integrity of RNA was assessed using 1% agarose gel electrophoresis and RNA concentration and purity were determined for each sample by Take 3 Micro-Volume plate using Epoch Microplate reader (BioTek, Winooski, VT). The RNA samples were diluted to an equal concentration of 500 ng/µL in DEPC-water and 1 µg of RNA (2 µL of RNA suspension) was reverse-transcribed to complementary DNA (cDNA) in a final volume of 20 µL by using AccuPower® CycleScript RT PreMix (dN6) kit (Bioneer, South Korea) according to manufacturer's protocol. Five microliters of cDNA suspension from each sample were pooled together (pooled cDNA) to make a serial dilution and remained cDNA samples (15 µL) were diluted 10 times by adding 135 Ml nuclease-free water.

Nuclear and mitochondrial DNA extraction

Nuclear and mitochondrial DNAs were simultaneously extracted using the method described by Sambrook *et al.* (1987) with some modification. The concentration of DNA was determined using Epoch Microplate reader (BioTek,Winooski, VT) and adjusted to an equal concentration of 1000 ng/µL. Five microliters of DNA suspension from each sample were pooled together (pooled cDNA) to make a serial dilution and remained DNA samples were diluted 10 times by adding nuclease-free water (diluted DNA).

Quantitative real time polymerase chain reaction (qRT-PCR)

The quantities of PGC-1α and SIRT1 mRNA were determined by qRT-PCR. Briefly, 5 µL of diluted cDNA (5 ng/µL cDNA) was amplified in a 20 µL PCR reaction medium containing 10 µL SYBR Green (Maxima SYBR Green/ROX qPCR Master Mix 2X, Thermo Scientific, USA), 1 µL of both forward and reverse primers (100 nM) and 8 µL nuclease-free water. The reaction was initiated at 95 ˚C for 5 min, followed by 40 cycles of 95 ˚C for 30 s, 60 ˚C for 30 s and 72 ˚C for 30 s. The amplification was monitored using the 7300 Real-Time PCR machine (Applied Biosystems, USA). The mRNA quantities of PGC-1α and SIRT1 were normalized to beta-actin (ACTB) mRNA as a housekeeping gene (Dervishi *et al.* 2010). Summary details for the oligonucleotides used in this study are presented in Table 1. To determine the amplification efficiency, a 4-fold serial dilution (10^1, 10^0, 10^{-1} and 10^{-2}) provided from the pooled cDNA was also subjected to real-time quantitative-polymerase chain reaction (qRT-PCR). Relative mRNA levels of the target genes were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Mitochondrial DNA copy number

A real-time PCR analysis was conducted to measure the relative copy number of mtDNA per diploid nuclear genome (nDNA). Five microliters of the diluted DNA (5 ng/µL) were amplified in 20 µL PCR reaction medium, following the same thermal condition as above. Oligonucleotides primers were designed to amplify genomic DNA region for beta-actin (G-ACTB) and mitochondrial DNA region for cytochrome *b* (Cyt-b) as reliable markers of nDNA (Davinelli *et al.* 2013) and mtDNA (Addabbo *et al.* 2009) copy number, respectively. The amplification efficiency was determined by 4-fold serial dilution (10^1, 10^0, 10^{-1} and 10^{-2}) of pooled DNA. The copy number of mtDNA per nDNA was calculated as $2^{\Delta Ct}$, where ΔCt is the difference between Ct values of Cyt-b and G-ACTB.

Statistical analysis

Data were analyzed as a completely randomized design with individual lambs as the experimental unit and phenotypic groups (low- and high-RFI) as the fixed effect using PROC GLM of SAS (2004) and the following model:

$$Y_{ij}= \mu + P_i + \varepsilon$$

Where:
μ: overall mean.
P_i: fixed effect of phenotypic groups.
ε: residual error.

RESULTS AND DISCUSSION

Residual feed intake is a measure of feed efficiency that refers to variation in feed intake between animals at the same level of production and maintenance requirement (Herd and Arthur, 2009). As we observed in this study, the lambs in high- and low-RFI groups were similar in MMBW and ADG, but consumed different amounts of feed (Table 2).

Table 1 Primer sets used for analysis of *PGC-1α* and *SIRT1* genes expression as well as mitochondrial biogenesis

Primer names	Sequence of primer forward (F) and reverse (R)	Acc. No. GenBank NCBI	Amplicon (bp)	AT (°C)
Gene expression				
PGC-1α	F:5' ATTGCCCTCATTTGATGCGC 3' R:5' TAGCTGAGTGTTGGCTGGTG 3'	AY957611	154	59
SIRT1	F:5' GTGCGAAAGTGACGAAGATG 3' R:5' GGATCTGTGCCAATCATGAG 3'	XM015104377	117	59
ACTB	F:5' GGACTTCGAGCAGGAGATGG 3' R:5' GGTAGTTTCGTGAATGCCGC 3'	U39357	172	60
Mitochondrial biogenesis				
Cyt-b	F:5' TAACACTCCCCCTCACATCG 3' R:5' AGAGGATTAGGGCGAGGACG 3'	NC_001941	153	60
G-ACTB	F:5' TACTAACCCTGGCTCGTGTG 3' R:5' CGTCATACTTGGGACACCAC 3'	NC_019481	199	60

AT: Annealing temperature; PGC-1α: peroxisome proliferator-activated receptor γ coactivator 1 α; SIRT1: sirtuin1; ACTB: beta-actin; Cyt-b: mitochondrial DNA region for Cytochrome b and G-ACTB: genomic DNA region for beta-actin.

Table 2 Growth performance of lambs with the divergent phenotypic expression of residual feed intake (RFI)

Traits (kg)	Phenotype groups		SEM	P-value
	High-RFI	Low-RFI		
No.	6	6		
Initial BW	34.98	33.77	1.21	0.49
Final BW	46.42	43.89	1.50	0.26
MMBW	14.96	14.45	0.35	0.32
ADG	0.27	0.23	0.02	0.22
DMI	2.35	1.80	0.09	< 0.01
RFI	0.19	-0.21	0.04	< 0.01

High-RFI: RFI ≥ mean + 0.5 standard deviation and Low-RFI: RFI ≤ mean – 0.5 standard deviation.
BW: body weight; MMBW: med-test $BW^{0.73}$; ADG: average daily gain and DMI: dry matter intake.
SEM: standard error of the means.

The variation in feed intake has been previously reported in beef cattle (Arthur *et al.* 2001; Durunna *et al.* 2011) and sheep (Rajaei Sharifabadi *et al.* 2012; Rajaei Sharifabadi *et al.* 2016), where the animals were divergently ranked on the basis of phenotypic expression of RFI. This variation may be attributed to metabolic efficiency and energy expenditure (Herd and Arthur, 2009).

Here, we firstly evaluated the copy number of mtDNA as an index of mitochondrial biogenesis in skeletal muscle of high- and low-RFI lambs. The results showed a negative relationship between mitochondrial biogenesis and phenotypic expression of RFI (Figure 1A).

When compared with high-RFI lambs, the greater copy number of mtDNA in low-RFI lambs indicates an increased level of mitochondrial biogenesis that may enhance the capacity of cells for generation of ATP (Austin and St-Pierre, 2012).

This finding, however, confirms previous evidence that finds a relationship between feed efficiency and expression of genes involved in the mitochondrial biogenesis (Kelly *et al.* 2011). To further understanding of the molecular mechanism that differentially regulates the mitochondrial biogenesis in high- and low-RFI lambs, we analyzed the expression of PGC-1α as the master regulator of mitochondrial biogenesis at mRNA level. In skeletal muscle, upregulation of PGC-1α stimulates mitochondrial biogenesis through initiate a sequence of events leading to fission and fusion of mitochondria (Ventura-Clapier *et al.* 2008). Thus, over-expression of PGC-1α is likely be associated with increased mitochondrial biogenesis. In agreement with the result for mtDNA copy number, we also observed a higher level of PGC-1α mRNA in low-RFI lambs than those ranked as high-RFI group (Figure 1B). Similar to our finding, Kelly *et al.* (2011) reported a negative correlation between RFI and mRNA expression of PGC-1α in muscle of Limousin × Friesian beef heifers. However, no significant difference was reported in another study between low- and high-RFI Nellore cattle for mRNA expression of PGC-1α in both skeletal muscle and liver tissues (Fonseca *et al.* 2015).

These contradictory results may be due to differences in the tissues type and method of tissue sampling. In both Kelly *et al.* (2011) and the present study, the expression of PGC-1α was measured in biopsies of the *Longissimus dorsi* muscle, whereas the muscle samples used in the study of Fonseca *et al.* (2015) harvested from masseter muscle after slaughtering.

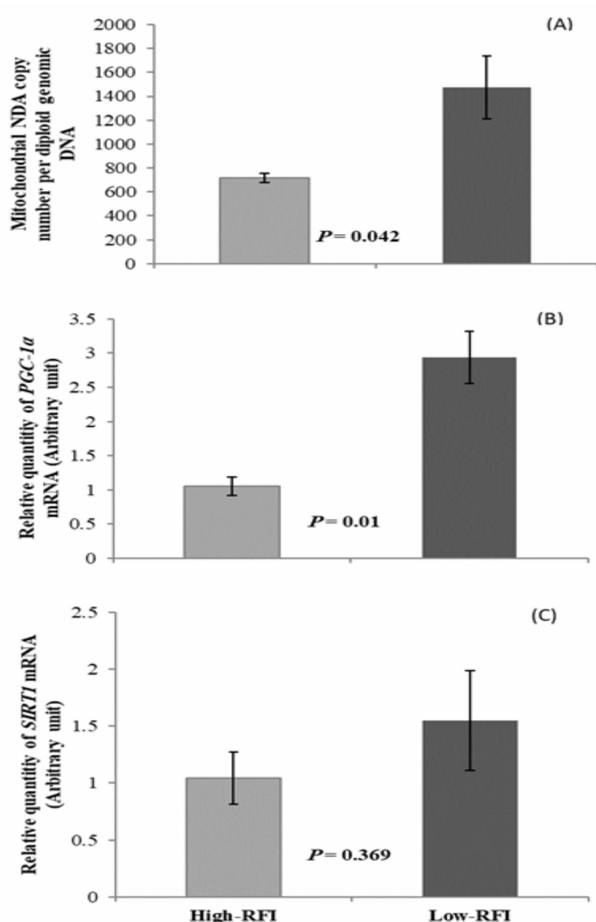

Figure 1 Copy number of mitochondrial DNA per diploid genomic DNA (A) and relative expression of *PGC-1α* (B) and *SIRT1* (C) genes in the muscle of lambs with the divergent phenotypic expression of residual feed intake (RFI)

However, the increased expression of PGC-1α in low-RFI animals observed in this study may be associated with higher mitochondrial functionality that was previously reported in lambs with negative RFI (Rajaei Sharifabadi *et al.* 2012; Zamiri *et al.* 2016). In a proteomics study, PGC-1α was predicted to be activated in pedigree male broilers exhibiting high compared to low feed efficiency (Kong *et al.* 2016a).

There is strong evidence that the cascade mediated by PGC-1α improves the mitochondrial oxidative phosphorylation (Austin and St-Pierre, 2012). Therefore, it seems that the increased mitochondrial biogenesis and function in efficient lambs is partially attributed to the over-expression of PGC-1α.

The role of SIRT1 in expression and activity of PGC-1α was also analyzed in the biopsies samples. Although SITR1 mRNA expression in the muscle of low-RFI lambs was numberically higher, there was no significant difference (P=0.369) between the phenotypic groups for mRNA expression of SIRT1 (Figure 1C).

This result is not surprising, because SIRT1 is involved in PGC-1α-mediated regulation of the mitochondrial biogenesis via an energy-sensing pathway and its expression is depended to environment-induced changes in cellular energy status (Rodgers *et al.* 2005; Fernandez-Marcos and Auwerx, 2011). Therefore, increased mitochondrial biogenesis and mRNA expression of PGC-1α in low-RFI lambs observed in this study are independent of SIRT1 gene expression.

CONCLUSION

Our results suggest that the increased mitochondrial dynamics in low-RFI lambs has a potential to regulates bioenergetic efficiency and energy expenditure.

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REFERENCES

Addabbo F., Ratliff B., Park H.C., Kuo M.C., Ungvari Z., Ciszar A., Krasnikof B., Sodhi K., Zhang F., Nasjletti A. and Goligorsky M.S. (2009). The krebs cycle and mitochondrial mass are early victims of endothelial dysfunction: Proteomic approach. *Am. J. Pathol.* **174(1),** 34-43.

Anderson S., Bankier A.T., Barrell B.G., de Bruijn M.H.L., Coulson A.R., Drouin J., Eperon I.C., Nierlich D.P., Roe B.A., Sanger F., Schreier P.H., Smith A.J.H., Staden R. and Young I.G. (1981). Sequence and organization of the human mitochondrial genome. *Nature*. **290,** 457-465.

Archer J.A., Richardson E.C., Herd R.M. and Arthur P.F. (1999). Potential for selection to improve efficiency of feed use in beef cattle: A review. *Australian J. Agric. Res.* **50(2),** 147-162.

Arthur P.F., Archer J.A., Johnston D.J., Herd R.M., Richardson E.C. and Parnell P.F. (2001). Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in angus cattle. *J. Anim. Sci.* **79(11),** 2805-2811.

Austin S. and St-Pierre J. (2012). Pgc1α and mitochondrial metabolism – emerging concepts and relevance in ageing and neurodegenerative disorders. *J. Cell Sci.* **125(21),** 4963-4971.

Bottje W. and Carstens G.E. (2009). Association of mitochondrial function and feed efficiency in poultry and livestock species. *J. Anim. Sci.* **87(14),** 48-63.

Bottje W. and Kong B.W. (2013). Feed efficiency: Mitochondrial function to global gene expression. *J. Anim. Sci.* **91(4),** 1582-1593.

Bottje W., Iqbal M., Tang Z.X., Cawthon D., Okimoto R., Wing T. and Cooper M. (2002). Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. *Poult. Sci.* **81(4),** 546-555.

Ceacero T.M., Mercadante M.E., Cyrillo J.N., Canesin R.C., Bonilha S.F. and de Albuquerque L.G. (2016). Phenotypic and genetic correlations of feed efficiency traits with growth and carcass traits in nellore cattle selected for postweaning weight. *PLoS One*. **11,** e0161366.

Davinelli S., Sapere N., Visentin M., Zella D. and Scapagnini G. (2013). Enhancement of mitochondrial biogenesis with polyphenols: Combined effects of resveratrol and equol in human endothelial cells. *Immun. Ageing.* **10(1),** 28-33.

De Haer L.C.M., Luiting P. and Aarts H.L.M. (1993). Relations among individual (residual) feed intake, growth performance and feed intake pattern of growing pigs in group housing. *Livest. Prod. Sci.* **36(3),** 233-253.

Dervishi E., Serrano C., Joy M., Serrano M., Rodellar C. and Calvo J.H. (2010). Effect of the feeding system on the fatty acid composition, expression of the δ9-desaturase, peroxisome proliferator-activated receptor alpha, gamma, and sterol regulatory element binding protein 1 genes in the semitendinous muscle of light lambs of the rasa aragonesa breed. *BMC Vet. Res.* **6(1),** 40-49.

Durunna O.N., Plastow G., Mujibi F.D., Grant J., Mah J., Basarab J.A., Okine E.K., Moore S.S. and Wang Z. (2011). Genetic parameters and genotype × environment interaction for feed efficiency traits in steers fed grower and finisher diets. *J. Anim. Sci.* **89(11),** 3394-3400.

Fernandez-Marcos P.J. and Auwerx J. (2011). Regulation of pgc-1alpha, a nodal regulator of mitochondrial biogenesis. *Am. J. Clin. Nutr.* **93(4),** 884-890.

Fonseca L.F., Gimenez D.F., Mercadante M.E., Bonilha S.F., Ferro J.A., Baldi F., de Souza F.R. and de Albuquerque L.G. (2015). Expression of genes related to mitochondrial function in nellore cattle divergently ranked on residual feed intake. *Mol. Biol. Rep.* **42(2),** 559-565.

Herd R.M. and Arthur P.F. (2009). Physiological basis for residual feed intake. *J. Anim. Sci.* **87(14),** 64-71.

Kelly A.K., Waters S.M., McGee M., Fonseca R.G., Carberry C. and Kenny D.A. (2011). Mrna expression of genes regulating oxidative phosphorylation in the muscle of beef cattle divergently ranked on residual feed intake. *Physiol. Genom.* **43(1),** 12-23.

Kolath W.H., Kerley M.S., Golden J.W. and Keisler D.H. (2006). The relationship between mitochondrial function and residual feed intake in angus steers. *J. Anim. Sci.* **84(4),** 861-865.

Kong B.W., Lassiter K., Piekarski-Welsher A., Dridi S., Reverter-Gomez A., Hudson N.J. and Bottje W.G. (2016a). Proteomics of breast muscle tissue associated with the phenotypic expression of feed efficiency within a pedigree male broiler line: I. Highlight on mitochondria. *PLOS ONE* **11**(5), e0155679.

Kong R.S.G., Liang G., Chen Y., Stothard P. and Guan L.L. (2016b). Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. *BMC Genom.* **17(1),** 592-601.

Livak K.J. and Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. *Methods* **25(4),** 402-408.

Nisoli E., Clementi E., Paolucci C., Cozzi V., Tonello C., Sciorati C., Bracale R., Valerio A., Francolini M., Moncada S. and Carruba M.O. (2003). Mitochondrial biogenesis in mammals: The role of endogenous nitric oxide. *Science*. **299,** 896-899.

Rajaei Sharifabadi H., Naserian A.A., Valizadeh R., Nassiry M.R., Bottje W.G. and Redden R.R. (2016). Growth performance, feed digestibility, body composition, and feeding behavior of high- and low-residual feed intake fat-tailed lambs under moderate feed restriction. *J. Anim. Sci.* **94(8),** 3382-3388.

Rajaei Sharifabadi H., Zamiri M.J., Rowghani E. and Bottje W.G. (2012). Relationship between the activity of mitochondrial respiratory chain complexes and feed efficiency in fat-tailed ghezel lambs. *J. Anim. Sci.* **90(6),** 1807-1815.

Rodgers J.T., Lerin C., Haas W., Gygi S.P., Spiegelman B.M. and Puigserver P. (2005). Nutrient control of glucose homeostasis through a complex of pgc-1alpha and sirt1. *Nature* **434,** 113-118.

Sambrook J., Maniatis T. and Fritsch E.F. (1987). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SAS Institute. (2004). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R., Chaves A.S., Lanna D.P., Zerlotini-Neto A., Mudadu M.A., Taylor J.F. and Regitano L.C. (2015). Global liver gene expression differences in nelore steers with divergent residual feed intake phenotypes. *BMC Genom.* **16(1),** 242-250.

Ventura-Clapier R., Garnier A. and Veksler V. (2008). Transcriptional control of mitochondrial biogenesis: The central role of pgc-1alpha. *Cardiovasc. Res.* **79(2),** 208-217.

Zamiri M.J., Mehrabi R., Kavoosi G.R. and Sharifabadi H.R. (2016). Relationships between the activity of respiratory-chain complexes in pre- (biopsy) or post-slaughter muscle samples and feed efficiency in random-bred ghezel lambs. *Anim. Prod. Sci.* **57(8),** 1674-1681.