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EXPRESSION OF MITOCHONDRIAL RESPIRATORY COMPLEX GENES IN LIVER TISSUE OF CATTLE WITH DIFFERENT FEED EFFICIENCY PHENOTYPES

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SUMMARY

Feed efficiency is an economically important trait that is likely influenced by complex molecular mechanisms. We utilized the NRC Beef Cattle Model to predict feed intake based on observed gain (and gain based on observed intake), where the NRC model also accounts for breed type, sex and season. The difference between this NRC-predicted feed intake and observed intake is termed model predicted residual consumption (MPRC). Associations between feed efficiency and mitochondrial respiration have been previously reported in the literature. From a study of 177 animals, RNA was extracted from liver samples from 18 animals at each extreme of the MPRC tails (36 samples). Following microarray analysis, quantitative realtime RT-PCR (qPCR) was used to examine expression of several respiratory complex genes including mitochondrial genes *COX1*, *COX2*, *COX3* (Complex IV) and *CYTB* (Complex III), and nuclear genes *COX4*, *COX6A1*, *COX7A2*, *COX7B*, *COX7C* (Complex IV), *SHDB* (Complex II) and *NQO2* (Complex I). Although expression for some genes was influenced by sire and family, no relationship between expression of any of these genes was found to be associated with feed efficiency phenotype.

INTRODUCTION

Cattle producers may derive economic benefit from selection of feed efficient animals, but molecular mechanisms that affect feed efficiency phenotype have not been clearly elucidated. Previously, Mukherjee *et al*. (1970) reported an association between feed efficiency and mitochondrial respiration in chickens. A number of studies confirmed the link (reviewed by Bottje *et al*. 2004), but the results of those experiments were influenced by differences in breed and/or diet until Bottje *et al*. (2002) observed the same association in male chickens of a single broiler strain fed the same diet. Several parameters of respiration were measured in mitochondria from leg and breast muscle of high and low feed efficiency (FE; the ratio of gain-to-feed) birds, and a correlation between FE and efficient coupling of electron transport was hypothesized. Subsequent studies (Bottje *et al*. 2004; Iqbal *et al*. 2004, 2005; Ojano-Dirain *et al*. 2004, 2005) supported that hypothesis, suggesting that activity of all 5 respiratory complexes was decreased in low FE chickens.

More recently, Kolath *et al*. (2006) isolated mitochondria from the longissimus muscle of Angus steers with low or high residual feed intake (RFI). Mitochondria from steers with low RFI (that is, the more feed-efficient animals) exhibited higher respiration rates than those from steers with high RFI, but mitochondrial function was not different between the two groups.

In the current study, we compared expression of respiratory complex genes by measuring mRNA quantity in liver from steers with feed efficient or inefficient phenotypes. Liver tissue was chosen for this study because of the physiological role of the liver in metabolic processes.

MATERIALS AND METHODS

Animals. Data were collected on 177 Nellore-Angus F_2 steers produced by embryo transfer from 10 Nellore-Angus F_1 donor females and 4 Nellore-Angus F_1 sires (Amen 2007). Individual feed intake was evaluated as previously described by Amen (2007). Briefly, steers were fed and intake

was measured by use of a Calan gate system, beginning at an average age of 11 to 13 months until slaughter at age 17-18 months. Liver samples were collected at time of harvest and snap-frozen in liquid nitrogen, then stored at -70°C until processed. As described by Amen (2007) and based on the NRC (2000) model, daily feed intake was predicted based on observed weight gain for each animal and standardized input for animal type, age, sex, condition, and breed. The model predicted dry matter intake (MDMI) was subtracted from observed DMI and the difference defined as MPRC, such that those animals that consumed less than predicted (and thus, were more efficient) had negative MPRC. This method was used instead of traditional RFI so that data from multiple contemporary groups could be used simultaneously. Liver RNA samples from the 18 steers at each extreme of MPRC were used in the microarray analysis (n=36). MPRC values for these steers ranged from 1.2 to 2.8 standard deviations from the mean of the group as a whole.

Table 1. List of real-time PCR assays

Gene	Complex	Genome	Accession #	Primers
COX1	IV	mitochondrial	DQ124400	F: 5'-gggaatagtttgggctataatgtc
				R: 5'-gatgtgaagtaggctcgtgtgt
COX2	IV	mitochondrial	DQ124400	F: 5'-tcgtcccgtccaggctta
				R: 5'-aactgtggtttgacccgca
COX3	IV	mitochondrial	DQ124400	F: 5'-ccaccacttcggctttgaag
				R: 5'-ggaaaagtcagactacgtctacgaaa
COX4	IV	nuclear	NM_001001439	F: 5'-atcccgcacacctttga
				R: 5'-ttccactcgttcttgtcgtag
COX6a1	IV	nuclear	NM_001077831	F: 5'-ccctattccataaccctcatgtg
				R: 5'-tccaggttctctttattcgtcttca
COX7a2	IV	nuclear	NM_175807	F: 5'-cggttggtgggtagtaactg
				R: 5'-atggtcctcttagcaatctgac
COX7b	IV	nuclear	NM_175795	F: 5'-tttatgttcaacctcaggatgtttc
				R: 5'-atctgccttgccactgctt
COX7c	IV	nuclear	NM_175831	F: 5'-tgcagccgccatttcttc
				R: 5'-tagcgctgttggacgctcta
CYTB	III	mitochondrial	EF693798	F: 5'-catccgacacaacaacagcatt
				R: 5'-gctccgtttgcgtgtatgtatc
SDHB	II	nuclear	NM_001040483	F: 5'-tactggtggaacggagacaag
				R: 5'-gtgtggcagcggtagaga
NQO2	I	nuclear	NM_001034323	F: 5'-gtgacatcattgaggagcagaaga
				R: 5'-cgggcacgctgaacca
RPS20	control	nuclear	BC103289	F: 5'-accagccgcaacgtgaa
				R: 5'-ccttcgcgcctctgatca

RNA. Liver tissue (about 200 mg/sample) was pulverized under liquid nitrogen, transferred to 2 ml TRI Reagent® (Molecular Resource Center, Cincinnati, OH, USA) and homogenized through an 18 ga needle. RNA was extracted from homogenized tissue with TRI Reagent® and 1-bromo-3-chloropropane (BCP, Molecular Resource Center). The manufacturer's recommended protocol was modified to include additional extractions with 2:1 TRI:BCP and BCP alone. RNA was precipitated in 1 ml isopropanol, washed consecutively with 70%, 95% and 100% ethanol, and resuspended in 100 ul nuclease-free water (Invitrogen, Carlsbad, CA, USA). RNA quality was assessed by capillary electrophoresis through RNA 6000 NanoChips on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) according to the manufacturer's instructions. RNA was purified through RNeasy mini columns (Qiagen, Valencia, CA) using the manufacturer's RNA cleanup protocol, DNase-treated with the DNA-*free*™ kit (Ambion, Austin, TX, USA)

according to the kit instructions, and quantified on a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Real-time PCR. Total RNA (800 ng) was reverse transcribed (RT) in a 40 µl reaction with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). One reaction containing template, but no enzyme, was included as a control. The cDNA was diluted 1:4 in 25 ng/ul yeast tRNA (Sigma-Aldrich, St. Louis, MO, USA). Real-time PCR was performed in a 20 µl reaction containing 2 µl cDNA, 1X SYBR® GreenER™ PCR master mix (Invitrogen) and 300 nM primers. Primer pairs (Table 1) were designed with Oligo 6 software (Molecular Biology Insights, Inc., Cascade, CO). Amplification was carried out in 96-well plates in a 7900 HT real-time thermal cycler (Applied Biosystems, Foster City, CA, USA) with the thermal profile: 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Amplification data were analyzed with SDS software v.2.2.2 (Applied Biosystems). Amplification efficiency was validated for all primer pairs.

Data were normalized to *RPS20* expression (a gene whose expression was consistent across samples in this study), and relative expression was calculated (Livak and Schmittgen, 2001). The mean of all 36 samples was used as the calibrator value. Expression data were compared by analysis of covariance (SAS® 9.2, PROC GLM; SAS Institute, Inc., Cary, NC, USA). Sire, family, contemporary group, and efficiency phenotype (efficient or inefficient) were modeled as fixed effects with MPRC as a covariate.

RESULTS AND DISCUSSION

This study was conducted to identify whether expression of mitochondrial respiratory complex genes in liver was correlated with efficiency phenotype. Previously published results indicated some association of mitochondrial function with feed efficiency (reviewed in Bottje *et al.* 2006; Bottje and Carstens, 2009). Preliminary microarray experiments (Bovine Oligo Consortium arrays; http://bovineoligo.org) also indicated a possible association between *COX3* and *CYTB*

Table 2. Respiratory complex gene expression, in liver from steers with high and low MPRC values. Data are presented as arbitrary units (AU), relative to mean expression of all samples. Expression is not different between groups.

Gene	Relative gene expression	
	Low MPRC	High MPRC
COX1	0.985 ± 0.225	1.052 ± 0.379
COX2	1.122 ± 0.249	0.919 ± 0.315
COX3	1.137 ± 0.359	0.959 ± 0.381
COX4	0.996 ± 0.220	1.038 ± 0.220
COX6A1	1.008 ± 0.190	1.000 ± 0.155
COX7A2	1.019 ± 0.185	1.008 ± 0.235
COX7B	1.026 ± 0.285	1.001 ± 0.186
COX7C	1.027 ± 0.233	0.983 ± 0.141
CYTB	1.097 ± 0.302	0.969 ± 0.387
SHDB	0.985 ± 0.225	0.985 ± 0.225
NQO2	0.985 ± 0.225	0.985 ± 0.225

(Riggs, 2008). We conducted additional experiments with commercial bovine microarrays and used qPCR to analyze genes representing subunits from all four complexes of the mitochondrial electron transport chain (ETC). We could not validate *COX3* and *CYTB* as differentially expressed

between feed efficiency phenotypes. The initial arrays resulted in weak hybridization signals and may have been affected by the presence of mitochondrial DNA (not shown). Additional DNase treatment was performed prior to qPCR analysis to eliminate potential mitochondrial DNA contamination. Statistical analysis also indicated that expression of *COX2* and *NQO2* appeared to be influenced by sire, and expression of *COX7B* and *NQO2* was affected by family ($p < 0.05$).

CONCLUSIONS

While variation in feed efficiency has been attributed to differences in mitochondrial respiratory function or electron leak due to electron transport defects, differential expression of ETC genes in liver tissue was not associated with differences in feed efficiency in this study. Genetic variation in expression of some of the subunit genes (*COX2*, *COX7B*, and *NQO2*) does appear to exist in the study population, but is not related to feed efficiency. The relationship between mitochondrial functional activity and the quantity of mRNA or protein for individual ETC complex subunits is not clear (e.g. Bottje *et al*. 2004; Ojano-Dirain *et al*. 2005; Garrabou *et al*. 2007). If mitochondrial function is critical for feed efficiency phenotype, other genes that regulate activity may be involved, since differences in expression of mitochondrial genes *COX1*, *COX2*, *COX3* (Complex IV) and *CYTB* (Complex III), and nuclear genes *COX4*, *COX6A1*, *COX7A2*, *COX7B*, *COX7C* (Complex IV), *SHDB* (Complex II) and *NQO2* (Complex I) were not observed between steers with high (inefficient) or low (efficient) MPRC. Expression of these genes was examined only in liver tissue from animals at the tails of the MPRC distribution and not the population as a whole.

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