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Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs

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Abstract
The objective of this study was to determine the extent to which genetic selection for residual feed intake (RFI) impacts electron leakage and reactive oxygen species (ROS) production in mitochondria from muscle and liver tissue. Understanding how genetic selection for RFI impacts animal physiology and growth efficiency is of the utmost importance as the world population increases. Production efficiency is tied directly to energy use. Mitochondria were used in this study because they produce 90% of the ATP in the body and use a large majority of dietary energy. Mitochondria were isolated from both muscle and liver tissue from pigs genetically selected for RFI (n = 8 per RFI line; 34 ± 4 kg). A 2,7-dichlorofluorscein diacetate assay was used to detect differences in hydrogen peroxide production between the more efficient low RFI line and the less efficient high RFI line. Our hypothesis was that greater efficiency would be linked to less ROS production from the mitochondria. There was less ROS production in mitochondria from the white portion of the semitendinosus in the low RFI line compared with the high RFI line, when both NADH and Flavin Adenine Dinucleotide (FADH2) energy substrates were used (glutamate and succinate, respectively). Additionally, mitochondria from the red portion of the semitendinosus in the low RFI line had less ROS production when succinate was used as an energy substrate ($P < 0.05$). A positive correlation was observed between RFI and ROS in mitochondria from the LM. These data indicate genetic selection for RFI may influence mitochondrial ROS production and efficiency of pork production.

Keywords
electron transport, mitochondria, reactive oxygen species, residual feed intake

Comments
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Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs[1]

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ABSTRACT: The objective of this study was to determine the extent to which genetic selection for residual feed intake (RFI) impacts electron leakage and reactive oxygen species (ROS) production in mitochondria from muscle and liver tissue. Understanding how genetic selection for RFI impacts animal physiology and growth efficiency is of the utmost importance as the world population increases. Production efficiency is tied directly to energy use. Mitochondria were used in this study because they produce 90% of the ATP in the body and use a large majority of dietary energy. Mitochondria were isolated from both muscle and liver tissue from pigs genetically selected for RFI (n = 8 per RFI line; 34 ± 4 kg). A 2,7-dichlorofluorscein diacetate assay was used to detect differences in hydrogen peroxide production between the more efficient low RFI line and the less efficient high RFI line. Our hypothesis was that greater efficiency would be linked to less ROS production from the mitochondria. There was less ROS production in mitochondria from the white portion of the semitendinosus in the low RFI line compared with the high RFI line, when both NADH and Flavin Adenine Dinucleotide (FADH2) energy substrates were used (glutamate and succinate, respectively). Additionally, mitochondria from the red portion of the semitendinosus in the low RFI line had less ROS production when succinate was used as an energy substrate ($P < 0.05$). A positive correlation was observed between RFI and ROS in mitochondria from the LM. These data indicate genetic selection for RFI may influence mitochondrial ROS production and efficiency of pork production.

Key words: electron transport, mitochondria, reactive oxygen species, residual feed intake

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INTRODUCTION

Conservation of natural resources is important as the population of the world nears the expected 9 billion in 2050, as projected by the United Nations (2011). In meat production, the largest use of natural resources is through the production of feed. Therefore, improving feed efficiency of livestock is an important goal for sustainability and profitability. Residual feed intake (**RFI**) is a measure of production efficiency that is calculated by determining the difference between the observed feed intake and expected feed intake of an individual animal based on performance (Koch et al., 1963; Boddicker et al., 2011). Animals with a low RFI are more feed efficient than animals with a high RFI and maintain similar growth performance. The RFI can be considered to be a predictive measure of energy use (Herd and Arthur, 2009; Reynolds et al., 2011).

Poor nutrient and energy use by livestock may be accounted for by changes in mechanisms responsible for regulation of mitochondria function. In the mitochondria, an increase in electron leakage from electron transport chain can occur with the loss of mitochondrial regulatory mechanisms, leading to a potential reduction in ATP synthesis capacity. Furthermore, excessive reactive oxygen species (**ROS**) are generally produced with an increase in electron leakage, diverting dietary energy from growth toward cellular repair and/or autophagy

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mechanisms. The ROS production from the mitochondria was first reported by Boveris and Chance (1973). Reports in chickens (Bottje et al., 2002) and cattle (Kolath et al., 2006) support the hypothesis that ROS production, measured by the production of hydrogen peroxide, is influenced by feed efficiency (chickens) and RFI (cattle). Therefore, our objective was to establish the extent to which genetic selection for RFI in pigs influences ROS production in muscle and liver mitochondria and oxidative stress. The rationale for this objective is that muscle and liver ROS production are linked to variations in energy use through mechanisms designed to protect tissues and the animal from mitophagy and protein turnover. Differences in ROS production in tissues from the high and low RFI pig lines may partially explain the energetic and feed efficiency differences associated with selection.

MATERIALS AND METHODS

All animal procedures were approved by the Animal Care and Use Committee (1–11–7058-S) at Iowa State University (**ISU**).

Animals

Eight high RFI and 8 low RFI gilts from the eighth generation of the ISU RFI selection project (Cai et al., 2008), matched by age and BW (34 ± 4 kg BW), were selected and placed into randomly assigned individual metabolism pens for 12 wk. Pigs had free access to a standard corn and soybean meal diet (Table 1) that was formulated to meet or exceed the nutrient requirements for this size of pig (NRC, 1998). Body weight and feed intake (**FI**) were recorded weekly throughout the experiment. The 10th rib backfat and loin eye area were measured on all pigs at the beginning and end of the study by ultrasound, using an Aloka 500V SSD ultrasound machine fitted with a 3.5-MHz, 12.5-cm, linear array transducer (Corometrics Medical Systems Inc., Wallingford, CT). For each individual pig, ADG, ADFI, and G:F were calculated, using BW, FI, and backfat data collected throughout the entire experiment. Residual feed intake indices for individual pigs were analyzed, according to statistical methods, previously described by Cai et al. (2008). The RFI indices were calculated by this equation (Cai et al., 2008):

$$\text{RFI} = \text{ADFI} - \beta1\ (\text{on-test BW deviation}) + \beta2\ (\text{off-test BW deviation}) + \beta3\ (\text{metabolic mid-BW}) + \beta4\ (\text{ADG}) + \beta5\ (\text{off-test backfat}).$$

Tissue Collection

At the end of the experimental period (wk 12), gilts were euthanized in pairs (n = 1 per line) via captive bolt stunning, followed by exsanguination, over 8 d. A section of the LM from the lumbar region, a portion of the liver, and both entire semitendinosus muscles were collected and placed on ice for transport back to the laboratory (1 h postmortem). Liver was first washed in PBS to minimize blood contamination in the sample. The semitendinosus muscle was divided into red and white portions (**STR** and **STW**, respectively).

Mitochondria Isolation

Mitochondria were isolated via differential centrifugation (Iqbal et al., 2004; Smith, 1967). Briefly, ~100 g of each muscle or 80 g of liver tissue were homogenized in at least 5 volumes of Buffer A (220 mM Mannitol, 70 mM Sucrose, 2 mM HEPES, 1 mM EGTA, and 0.5 mg/mL fatty acid free BSA, pH 7.4). Slurry was centrifuged and pellet containing cellular debris was discarded (twice at 600 × g for 10 min at 4°C). The supernatant was strained through cheesecloth after each centrifugation. Mitochondrial pellets were formed by centrifuging at 7,750 × g for 20 min at 4°C. The supernatant was decanted and pellets were washed 3 times with Buffer B (220 mM Mannitol, 70 mM Sucrose, 2 mM HEPES, and 0.5 mg/mL fatty acid free BSA, pH 7.0).

Washed mitochondria were resuspended in 2 to 3 mL of Buffer B. A modified Lowry protein assay (Lowry et al., 1951) was performed to determine protein concentration (BioRad Laboratories, Hercules, CA). Mitochondrial protein yield was determined and there were no differences between lines in yield (Fig. 1). Mitochondria were then diluted to a protein concentration of 2 mg/ml with Buffer B and stored at 4°C until use.

Hydrogen Peroxide Production Assay

Hydrogen peroxide (**H_2O_2**) production from the mitochondria was used to determine ROS production via 2,7 dichlorofluorescin diacetate (**DCFH**), as previously describe by Iqbal et al. (2001), with modifications. All assay reagents were made fresh each day from either frozen stock solutions or raw chemical. A H_2O_2 standard curve was used to calculate production values from the mitochondria. A BioTek Synergy H4 microplate reader (BioTek U.S., Winooski, VT) was used to detect fluorescence of DCFH at an excitation/emission wavelength of 480/530 nm.

Each sample and standard were run in triplicate on a black 96 well plate. Superoxide dismutase (Sigma-Aldrich, St. Louis, MO) was added (20 units) to each well, along with 45 μL of assay buffer containing 51 μM

Table 1. IIngredients and chemical composition of the diet (as-fed basis)

Item	Content, %
Ingredients	
Corn	80.18
Soybean meal, 46.5%	16.72
L-lysine	0.25
DL-methionine	0.01
L-tryptophan	0.05
Vitamin-mineral premix[1]	0.30
Monocalcium phosphate	1.06
Limestone	0.93
Salt	0.50
Calculated composition	
ME, Mcal/kg	3.32
NE, Mcal/kg	2.46
DM, %	89.4
Crude protein, %	14.7
Crude fat, %	3.6
Crude fiber, %	2.6
SID[2] lysine, %	0.80
Available phosphorus, %	0.28

[1]Supplied per kilogram of diet: vitamin A, 8364 IU; vitamin D3, 1533 IU; vitamin E, 45 IU; vitamin K, 2.2 IU; choline, 6.5 mg; riboflavin, 4.2 mg; niacin, 21 mg; pantothenic acid, 17 mg; vitamin B-12, 28 mcg; biotin, 1.6 mcg; folic acid, 0.0005 mg; Zn, 112 mg as zinc sulfate and zinc oxide; Mn, 54 mg as manganous oxide; Fe, 145 mg as ferrous carbonate and ferrous sulfate; Cu, 20 mg as copper chloride; I, 0.76 mg as ethylenediamine dihydriodide; Se, 0.25 mg as sodium selenite.

[2]Standard ileal digestablility.

DCFH. In addition to DCFH, either 8 μM glutamate or succinate were added to provide an energy substrate for electron transport complexes (I and II, respectively). Individual electron transport complexes were inhibited by 10 μM rotenone, 8 μM 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione (**TTFA**), 13 μM antimycin A, or a combination of the 3. To each well, either the H_2O_2 standards or 90 μg of mitochondrial protein were added. The plate was incubated at 38°C and readings taken at 0, 5, 10, 15, and 20 min. Blank wells containing no mitochondria were used to calculate background fluorescence.

Statistical Analysis

All data were analyzed using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC). Growth and performance statistical analysis used line as a fixed effect, with a covariate of harvest date. Hydrogen peroxide production was analyzed with the fixed effect of line, a random effect of harvest date, and covariate of BW was used, blocking by muscle or tissue. Simple Pearson correlations were calculated using the PROC CORR procedure of SAS. Differences were considered significant at $P < 0.05$ and a tendency at $P < 0.10$.

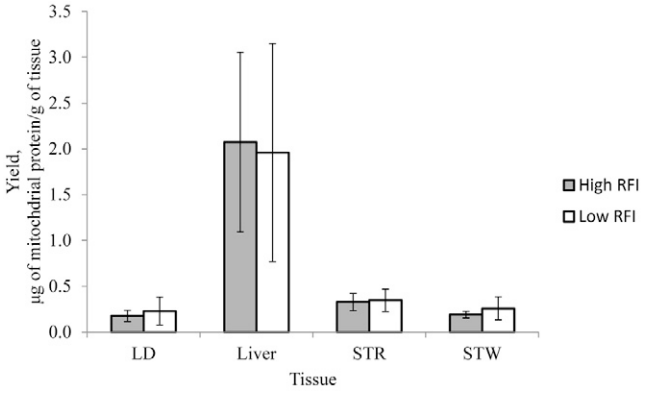

Figure 1. Mitochondrial yield from individual tissues between residual feed intake lines (RFI), measured in mg of mitochondria per g of tissue used in the extraction. Longissimus dorsi (LD), liver, red portion of the semitendinosus (STR), and the white portion of the semitendinosus (STW). No differences between lines in extractable mitochondrial protein ($P > 0.15$).

RESULTS

As expected, gilts selected for low RFI had a 10% lower ADFI ($P = 0.018$) for the same rate of growth ($P = 0.67$) compared with their high RFI counterparts. This translated into a significant ($P = 0.017$) improvement in G:F (Table 2). These performance phenotypes (Table 2) were consistent with previously published data from generation 5 of this experiment (Smith et al., 2011). On-test and off-test BW were not different between the lines ($P \geq 0.10$). Ultrasound back fat was greater in the less efficient high RFI line when compared with the more efficient low RFI line ($P \leq 0.05$). However, loin eye area was not different across lines (27.2 vs. 24.9 cm^2, respectively, $P = 0.11$, Table 2). As expected, calculated RFI index between the low and high lines were different (–0.14 vs. 0.03 kg feed/day, respectively, $P < 0.001$, Table 2).

To assess the effect of RFI selection on mitochondria ROS production in various tissues, ex vivo hydrogen peroxide production rate was measured. Although not statistically different, there was a consistent trend for mitochondria from the LM in the low RFI line to have 30 to 34% less hydrogen peroxide production, when compared with the high RFI line, when using glutamate as an energy substrate with no inhibitors or complex I and II inhibitors ($P = 0.14$ and 0.18, respectively, Fig. 2). Moreover, RFI and H_2O_2 production were positively correlated with both uninhibited electron transport and electron transport blocked at complex I ($r = 0.493$ and 0.485, respectively, $P = 0.05$, Table 3). When blocking electron transport at complex III, using antimycin A, no significant correlation existed ($P = 0.17$). When succinate was used as a FADH2-linked energy substrate for complex II, coupled with the addition of rotenone to prevent electron back flow to complex I, high RFI animals had a numerical in-

Table 2. Growth and performance data on pigs (n = 8 per line) genetically selected for low and high residual feed intake (RFI) during the testing period

Item	Low RFI	High RFI	SEM	*P*-value
	n = 8	n = 8		
Performance data				
On test age, d	148	146	3.2	0.67
Off-test BW, kg	94.2	98.3	3.33	0.238
Ultrasound backfat, mm	13.0	16.7	1.68	0.043
Loin eye area, cm^2	27.2	24.9	1.39	0.11
ADFI, kg/d	1.60	1.86	0.101	0.0175
ADG, kg/d	0.739	0.754	0.034	0.67
G:F	0.47	0.41	0.024	0.0167
Calculated RFI, kg/d[1]				
Average index within line	–0.138	0.032		<0.001
Greatest index within line	0.152	0.244		
Lowest index within line	–0.326	–0.136		

[1]Residual feed intake (RFI) index = ADFI-β_1 (on-test BW deviation) + **β**2 (off-test BW deviation) + **β**3 (metabolic mid-BW) + **β**4 (ADG) + **β**5 (off-test backfat)

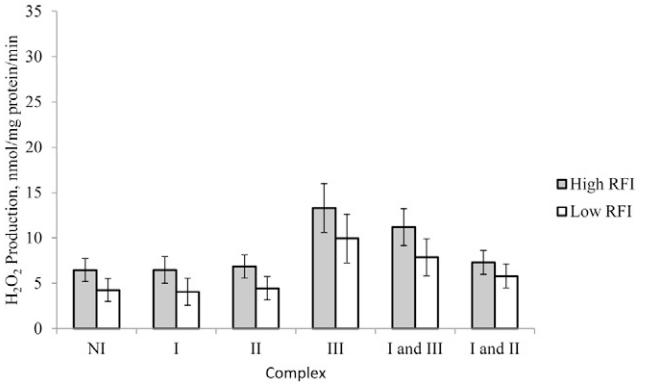

Figure 2. Comparison of hydrogen peroxide (H2O2) production rate in mitochondria from longissimus dorsi of pigs (n = 8 per line) divergently selected for residual feed intake (RFI). Glutamate provided as an energy source. No inhibition (NI) along with rotenone to block Complex I (I), 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione (TTFA) to block complex II (II), antimycin A to block complex III (III), and rotenone and antimycin A (I and III), rotenone and TTFA (I and II).

crease ROS production (Table 4). As with glutamate, there was a tendency for a positive correlation between RFI and ROS production (P = 0.10) with succinate.

No differences in H_2O_2 production were observed for STR when electron transport occurred through complex I with the use of glutamate as an energy substrate (Fig. 3). Conversely, there was a significant decrease in hydrogen peroxide production when succinate was used as an energy source for electron transport for complex II. The electron transport chain from complex II to IV, in low RFI pigs, had a tendency to have less ROS production than the high RFI line (P = 0.085, Table 4). This was confirmed when ROS production from complex II, through the use of the inhibitor TTFA, was increased in high RFI gilts (P = 0.035, Table 4).

In the STW mitochondria of high RFI pigs, H2O2 production was increased or tended to be increased, depending on inhibitor treatment (Fig. 4 and Table 4). With the use of succinate for complex II associated electron transport, there was a positive correlation between RFI and ROS production, with the inhibition of complexes I, II, and III (r^2 = 0.53, P = 0.032, Table 3). No differences in ROS production were observed between high and low RFI mitochondria isolated from the liver (Fig. 5 and Table 4).

DISCUSSION

The role of mitochondria in energy biogenesis and ROS production in swine has been poorly characterized in the context of feed efficiency. Therefore, gilts from divergently selected lines in RFI and feed efficiency (**FE**) were used as a model to study mitochondria ROS production in muscle and liver tissues. The feed conversion ratio of low RFI pigs from the Iowa State selection project has been reduced by 0.22 g/d over 8 generations when

Table 3. Selected Pearson Correlation Coefficients for hydrogen peroxide production from mitochondria isolated from LM and semitendinosus, white portion[1]

Item	Whole	I	II	III	I and III	I and II
			LM, glutamate			
Residual feed intake index	0.493	0.495	0.385	0.351	0.437	0.357
P-value	0.052	0.052	0.14	0.18	0.091	0.18
			LM, succinate			
	I	I and II	I and III	I, II, and III		
Residual feed intake index	0.423	0.451	0.403	0.418		
P-value	0.10	0.079	0.12	0.11		
			Semitendinosus, white portion, succinate			
Residual feed intake index	0.350	0.351	0.460	0.537		
P-value	0.17	0.19	0.073	0.32		

[1]Treatments were whole electron transport (Whole), Complex I inhibition (I) with rotenone, Complex II inhibition (II) with 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione, Complex III inhibition (III) with antimycin A, and combinations (n = 8 per line).

compared with the high RFI line. In the more efficient low RFI line used in this study, there was a decrease in ADFI and ultrasound backfat, when compared with the less efficient high RFI gilts. Similar results were seen in the fifth generation of this same selection project (Smith et al., 2011) and reports from another selection experiment (Barea et al., 2010; Lefaucheur et al., 2011). These phenotypes, differences in ADFI, in particular, may be partially explained by changes in mitochondrial function.

The ROS production from the mitochondria was first reported by Boveris and Chance (Boveris and Chance, 1973; Chance et al., 1979). Electron leakage, a source of ROS, from the electron transport chain mainly occurs through complex I and III. If leakage is excessive, oxidative damage of DNA, lipids (Yu, 1994), and proteins may occur (Bottje et al., 2002; Droge, 2002). Identifying site-specific complex differences for deviations in ROS production would aid in the development of strategies to ameliorate electron leakage. Chickens undergoing metabolic stress, pulmonary hypertension syndrome (**PHS**), have increased ROS production from the mitochondria isolated from the lungs (Iqbal et al., 2001). An increase in mitochondrial ROS from metabolic stresses prevalent in disease states (examples include cancer and diabetes) and aging (Droge, 2002; Valko et al., 2007) may be similar to selection for high RFI in pigs. In the PHS study (Iqbal et al., 2001), electron leakage and ROS production were found to occur primarily at complexes I and III. Electron transport in feed efficiency studies from mitochondria from breast muscle of chickens also indicates site-specific defects at complex I and III (Bottje et al., 2002). Mitochondria from STW in the present study exhibited similar patterns of ROS production, with differences between RFI lines being the greatest when inhibiting complexes I and III with rotenone and antimycin A when using both glutamate and succinate as an energy source. These patterns suggest complexes I and III may be responsible for a majority of electron leakage under different types of stress, both metabolic and biological.

The effect of FADH2- or NADH-linked energy substrates on mitochondrial ROS formation also aids in identifying defects in electron transport. Glutamate is a complex I NADH-linked energy substrate, whereas suc-

Table 4. Hydrogen peroxide production in selected tissues using succinate as an energy source for complex II of electron transport[1]

	H_2O_2 production (nmol/mg protein/min)			
Complex	High residual feed intake	Low residual feed intake	SEM	*P*-value
LM				
I	7.68	6.10	2.41	0.53
I and II	7.87	5.70	2.10	0.41
I and III	17.27	14.41	5.31	0.61
I, II, and III	18.07	15.14	5.50	0.61
Semitendinosus, red portion				
I	20.07	13.41	3.24	0.085
I and II	21.39	13.16	3.05	0.035
I and III	53.68	43.53	8.82	0.29
I, II, and III	56.78	47.04	9.76	0.36
Semitendinosus, white portion				
I	11.11	7.58	1.79	0.096
I and II	11.34	7.59	1.84	0.087
I and III	21.44	14.01	2.12	0.013
I, II, and III	22.57	14.91	2.46	0.021
Liver				
I	60.37	55.78	8.19	0.60
I and II	33.44	34.56	9.35	0.91
I and III	35.85	38.16	5.67	0.70
I, II, and III	24.50	31.03	6.13	0.33

[1]Rotenone was added to prevent blackflow of electrons into complex I. Additional inhibitors used included 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione for complex II and antimycin A for complex III, or a combination of the 3 (n = 8 per line).

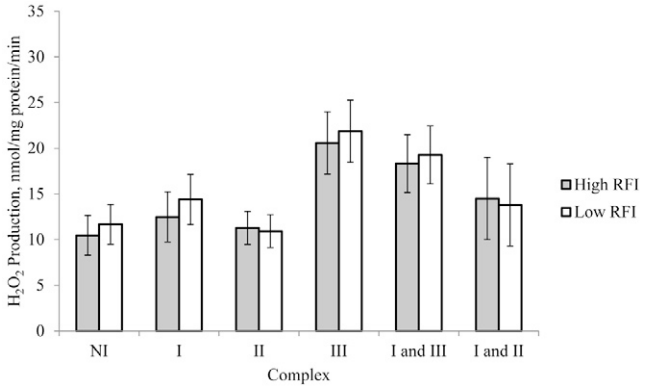

Figure 3. Comparison of hydrogen peroxide (H_2O_2) production rate in mitochondria from semitendinosus red portion of pigs (n = 8 per line) divergently selected for residual feed intake (RFI). Glutamate provided as an energy source. No inhibition (NI) along with rotenone to block Complex I (I), 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione (TTFA) to block complex II (II), antimycin A to block complex III (III), and rotenone and antimycin A (I and III), rotenone and TTFA (I and II).

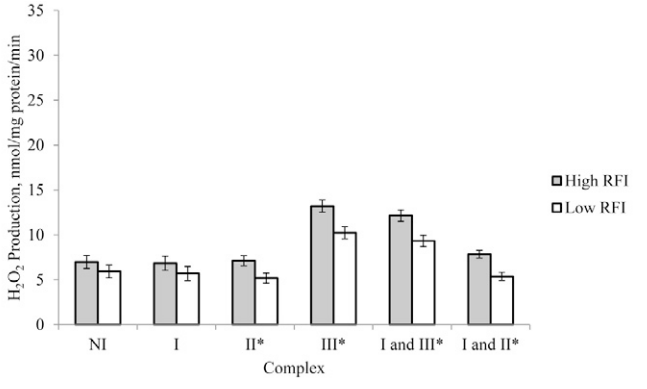

Figure 4. Comparison of hydrogen peroxide (H2O2) production rate in mitochondria from semitendinosus white portion of pigs (n = 8 per line) divergently selected for residual feed intake (RFI). Glutamate provided as an energy source. No inhibition (NI) along with rotenone to block Complex I (I), 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione (TTFA) to block complex II (II), antimycin A to block complex III (III), and rotenone and antimycin A (I and III), rotenone and TTFA (I and II).* $P \leq 0.05$

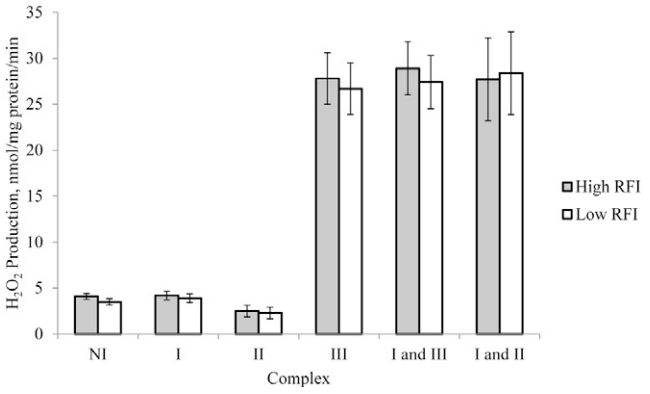

Figure 5. Comparison of hydrogen peroxide (H2O2) production rate in mitochondria from liver of pigs (n = 8 per line) divergently selected for residual feed intake (RFI). Glutamate provided as an energy source. No inhibition (NI) along with rotenone to block Complex I (I), 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione (TTFA) to block complex II (II), antimycin A to block complex III (III), and rotenone and antimycin A (I and III), rotenone and TTFA (I and II).

cinate is an energy source for complex II. When succinate was used as a complex II FADH2-linked energy substrate, rotenone, an inhibitor for complex I, was used to block electron backflow into complex I. Differences between energy substrates (glutamate and succinate) were observed within STR. This result reveals information regarding the location of electron leakage. In STR, no differences were observed with the use of glutamate as an energy source. However, a tendency for increased electron leakage was observed when using succinate and blocking electron back flow into complex I. These differences indicate that electron leakage could be occurring primarily between complex II and complex III in pig tissues. Determining specific locations of electron leakage in the electron transport chain may lead to development of potential amelioration strategies.

Selection line differences in ROS production in mitochondria from STW and STR support the hypothesis that selection for low RFI results in reduced electron leakage from muscle mitochondria. These data show that genetic selection for low RFI attenuates H_2O_2 production in the muscle mitochondria, limiting free radical formation. One outcome of decreasing ROS production could be a decrease in oxidative protein damage, leading to a decrease of mitophagy and protein turnover (Cruzen et al., 2012). Muscle from broilers with (comparable to high RFI project) low FE have been shown to have an 81% increase in protein carbonyl content (Iqbal et al., 2004). This ultimately may contribute to a shift in energy use from growth to cellular repair, thus decreasing FE in growing pigs.

Feed efficiency of avian and livestock has been linked to electron leakage and H_2O_{2v} production. Bottje et al. (2002) demonstrated a positive association between feed efficiency and mitochondrial function from isolated mitochondria, from breast and leg muscles of broilers. A positive correlation with RFI and ROS production, in the form of H_2O_2, in the LM muscle of our gilts in the mitochondria isolated from the LM was observed. This positive correlation indicates genetic selection for RFI has the potential to influence ROS production from the mitochondria.

Genetic selection for RFI influences cellular processes, such as electron transport, that affect mitochondrial function and ROS production (Bottje and Carstens, 2009). Phenotypic variations, such as BW, account for 60 to 80% of interanimal variation in FE (Bottje and Carstens, 2009). Therefore, it is concluded that RFI can only account for 20 to 40% of the phenotypic variation by itself in poultry (Bottje and Carstens, 2009). The difference in genetic selection becomes apparent when considering the mitochondrial leakage or ROS production from 3 major meat species. Research in broilers has consistently shown that breast muscle mitochondria from high efficiency birds produce less H_2O_2 than those from less efficient birds (Bottje et al., 2002; Droge, 2002; Bottje and Carstens, 2009).

Our data support the hypothesis that links mitochondria function to ROS production and feed efficiency. However, a comparative analysis suggests that there could be species differences in the linkage between mitochondria function and FE. Kolath et al. (2006) reported that mitochondria from the LM muscle in more efficient cattle produced more total hydrogen peroxide than those from less efficient cattle, though individual complex leakage was not measured. In that study, a single sire was used, so genetic variation was not large. In contrast, a poultry study (Bottje et al., 2002) used the top and bottom 16.6% of birds with measured FE to compare mitochondria function of the 2 groups (0.83 to 0.64 g gain/g feed, high to low FE) and determined more efficient birds had less electron leakage from the mitochondria than less efficient birds. This is supported by Pearson correlations between our RFI and H_2O_2 production data in the current study, in which mitochondria from less efficient gilt tissue generate more H_2O_2.

Electron transport chain assembly should be considered as a source of variation in the results. Both nuclear and mitochondrial DNA control the subunits used to assemble the electron transport chain (Anderson et al., 1981; Bottje et al., 2006). What is not known is how mitochondrial and nuclear DNA work together to form the electron transport chain. Grubbs et al. (2012) used pigs from the seventh generation of this same selection experiment and demonstrated that there may be a shift in mitochondrial protein profile related to oxidative stress in the LM. Additionally, further evidence of this shift in oxidative stress and metabolism was observed in the STR and liver, with increases in

aldehyde dehydrogenase and other metabolically important proteins (Grubbs et al., 2013). These data show electron leakage and ROS formation can be prevalent in mitochondria in muscle and liver tissue from the less efficient high RFI line. Additionally, genetic selection, species differences, and metabolic capacity of the tissue under consideration may influence mitochondria function and ROS production. This model can serve as a platform on which future research can be done on the investigation on the impact of selection for RFI on biological efficiency and energy use in pigs.

In addition to the differences observed between high and low RFI lines, it is important to note the difference of ROS within STN. The STN was separated into its respective red and white portions, providing a within-tissue comparison of differing fiber types. In STR only, succinate was linked with differences between the high and low RFI lines. In STW, both glutamate and succinate were linked with ROS production differences between the high and low RFI lines. These data in pigs are in contrast to observations made by Bottje et al. (2002) in the comparison of leg (primarily red/glycolytic fiber type) and breast (primarily white/oxidative fiber type) muscle.

The data presented provide a comparison of mitochondrial function between pigs genetically selected for high and low RFI. These data show selection for low RFI in pigs reduces the amount of mitochondrial electron leakage and ROS production in muscle. The reason for the difference between lines has not been fully elucidated. However, mitochondrial protein profile may provide insight into the changes in mitochondrial pathways that influence ROS production (Grubbs et al., 2013).

Implications

These data indicate there is a reduction in the amount of electron leakage from mitochondria isolated from muscle of pigs selected for low RFI or improved FE. However, no differences were observed in electron leakage from liver mitochondria between pigs divergently selected from RFI. These results imply electron leakage and ROS production from mitochondria is tissue dependent. Further, genetic selection to decrease RFI improves mitochondrial coupling and function in select tissues and is linked to separate pathways related to tissue physiology. Decreasing ROS production could lead to a decrease in oxidative damage to DNA, lipids, and proteins, leading to a decreased mitophagy and protein turnover. This would then contribute to a shift in energy utilization from cellular repair to improved lean growth of pigs, the ultimate goal in improving production efficiency.

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