RESEARCHREPORT

ANIMAL SCIENCE — Nutritional Efficiency Consortium

Title: Evaluating the contribution of ion pumps and protein turnover towards feed efficiency in finisher pigs selected for low and high residual feed intake **– NPB #10-009** **Revised**

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Industry Summary. Feed costs are on the rise and are a major variable cost in swine production. As such, the overall objective of this proposal was to determine the extent to which energy sensing, insulin signaling and markers of muscle protein turnover contributes to feed efficiency (FE) in pigs divergently selected for residual feed intake (RFI). Residual feed intake is the residual portion of feed intake which indicates the extent to which animals deviate from their predicted or expected level of feed intake, with efficient animals having lower RFI index. Thus, RFI is a unique measure of FE because it represents true differences in the ability of animals to use feed energy for the metabolic processes of maintenance and growth. Using Iowa State University's unique RFI pig lines, we hypothesized that pigs selected for low RFI are more efficient at nutrient utilization and lean tissue accretion because they have reduced protein degradation and turnover compared to higher RFI pigs. To test this hypothesis, we examined the extent to which liver and muscle AMP activated protein kinase (AMPK, energy sensor of tissues) contributes to enhanced metabolic efficiency in pigs selected for low RFI**.** Furthermore, we evaluated protein markers of muscle insulin signaling and protein turnover (degradation and synthesis) to understand the biological basis for the differences in RFI and FE in finisher pigs. Twelve high RFI and 12 low RFI finisher gilts were individually housed and used for this study. Muscle and liver samples were collected and AMPK, insulin signaling intermediates, protein degradation markers and markers involved in translation initiation of muscle protein synthesis were assessed by enzyme assays or western blots analysis of protein expression. The results of this project indicate that selection for improved FE and low RFI may select for reduced protein degradation and turnover. This is explained by the evidence that low RFI skeletal muscle had greater calpastatin activity, lower calpain and ubiquitin-proteasome activity compared to that of high RFI, less efficient pigs. No differences in insulin signaling intermediate markers and proteins involved in translation control of protein synthesis (mTOR pathway) were found in between our selection lines. Together, these data indicates that protein degradation and turnover may play a critical role in FE in swine. Nutritional and pharmacological strategies could be developed to attenuate protein turnover and degradation activity in finisher pigs to improve FE gains. Please contact Nicholas Gabler at Iowa State University for further detail (ngabler@iastate.edu).

Keywords: Residual feed intake, feed efficiency, protein degradation, protein synthesis

Scientific Abstract. Residual feed intake (RFI) is defined as the difference in the observed and expected feed intake, while accounting for growth and backfat. However, little is known about why pigs selected for reduced RFI (low RFI) are more efficient at converting feed into body weight gain compared to the high RFI pig. To this end, a line of Yorkshire pigs divergently selected for reduced RFI over seven generation was used. The objective of this proposal was to determine the extent to which energy sensing, insulin signaling and muscle protein turnover (synthesis and degradation) contributes to the improved feed efficiency in finisher pigs. We hypothesized that pigs selected for low RFI have lower feed intake and are more efficient at nutrient utilization and lean tissue accretion because they have reduced protein degradation and turnover compared to high RFI pigs. To test this, 12 low RFI and 12 high RFI line gilts were paired by age and weight (68.4±3.49kg) and randomly assigned to individual pens for six weeks. After this period, pigs were euthanized and muscle and liver samples collected and analyzed for enzyme activities and protein expression of energy metabolism, insulin signaling, and protein synthesis and degradation markers. As expected, ADFI was significantly lower in the low RFI compared to the high RFI pigs (2.0 vs 2.4 kg/d, $P<0.0001$). However, as expected the ADG remained the same between the two lines. Additionally, compared to the high RFI line, G:F was significantly higher in the low RFI pigs (0.29 vs 0.35, $P<0.001$, respectively). Muscles from gilts animals selected for low RFI had less 20S proteasome, μ- and m-calpain activities and greater calpastatin activity compared to that of high RFI, less FE pigs ($P<0.05$). Furthermore, 20S proteasome activity was lower in low RFI muscle compared to the high RFI muscle ($P=0.026$). Significant moderate positive correlations were observed between RFI and muscle 20S proteasome activity, but not with liver activity. No differences in muscle insulin signaling intermediates and translation initiation signaling proteins (mTOR pathway) were found ($P>0.05$). However, tissue specific differences in AMPK activities were observed. In conclusion, selection for reduced RFI decreases feed intake with no significant difference in growth performance. Altogether, these data indicate lower skeletal muscle protein degradation. Therefore, protein turnover (particularly degradation) may be an important aspect to FE in swine.

Introduction. Compared with growth and development, our understanding of the molecular biology and physiological characteristics regulating feed efficiency is relatively limited. However, with feed cost on the rise, and feed being a major variable cost in livestock production, the focus of the scientific community needs to shift towards elucidating the underlying physiology that functions to regulate efficiency gains in finisher pig growth. There are many different ways feed efficiency (FE) can be quantified. Traditionally, gain to feed, feed to gain, and feed conversion ratios (FCR) are used in the livestock industry. Alternatively, Koch et al. (1963) suggested in the 1960's the concept of residual feed intake (RFI) or net feed intake to measure feed efficiency in cattle. The residual portion of feed intake indicates the extent to which animals deviate from their predicted or expected level of feed intake, with efficient animals having lower RFI. Thus, RFI is a unique measure of FE because it represents true differences in the ability of animals to use feed energy for the metabolic processes of maintenance and growth. Hence, differences in FE through RFI are not captured by selection on growth and backfat. This is significant, because over 30% of differences in feed intake between and among pigs are not related to growth and composition, but results from differences in energy required for processes such as maintenance, immune system activation, activity, and digestive and metabolic efficiency. Although past selection for lean growth has led to substantial increases in feed efficiency, further increases are limited by differences in feed intake that are not related to growth and backfat, i.e. by differences in RFI. RFI has been shown to be moderately heritable in pigs (ranging from 0.15 to 0.58 heritability) and genetic selection for RFI has been shown to result in a reduction in feed intake in cattle (Koch et al., 1963; Crews, 2005), pigs (Cai et al., 2008) and poultry (van Eerden et al., 2004).

The primary biological factors that contribute to differences in RFI have been quantified in poultry (Luiting et al., 1991; Luiting, 1998) and beef cattle (Richardson and Herd, 2004; Herd and Arthur, 2009), but not in swine. However, similar biological factors as identified in poultry and beef cattle are expected to contribute to RFI in pigs based on our own findings and literature. Many of these key factors include physical activity, feed intake patterns and behavior, stress, digestibility, protein turnover, and metabolism. AMP-

activated protein kinase (AMPK) is a metabolic master switch that regulates cellular energy homeostasis in response to metabolic or non-metabolic stress. This kinase is activated by an increase in the intracellular AMP:ATP ratio, by nutrient sensing, or during times of stress.

Since protein turnover is the sum total of protein degradation and synthesis, it is also import to measure markers of protein synthesis. Such markers include measuring the phosphorylation of proteins in the insulin/mammalian target of rapamycin (mTOR) pathway. This pathway is well characterized and activation is well-known to lead to translation initiation, protein synthesis and muscle growth (Hay and Sonenberg, 2004). Our fundamental understanding of the metabolic and cellular responses that determine efficiency with which pigs are able to utilize feed is largely unknown. Considering that feed is a major cost in pig production, the limited knowledge of the pigs' metabolic and cellular responses is reducing the ability of producers and nutritionists to optimally manage the physiology to maximize pork production and reduce feed costs.

Objectives. The overall objective of this proposal is to determine the extent to which energy sensing and muscle protein turnover contribute to the increased feed efficiency that has been observed in pigs selected for reduced residual feed intake (RFI). Our central hypothesis was that pigs selected for low RFI have lower feed intake and are more efficient at nutrient utilization and lean tissue accretion because they have reduced protein turnover compared to the higher RFI pigs. We tested this central hypothesis and accomplish the overall objective of this application by addressing the following specific research objectives:

Objective 1: *Identify the extent to which AMP activated protein kinase contributes to altered metabolism, reduced feed intake and enhanced metabolic efficiency in pigs selected for low RFI.* We hypothesized that tissue ATP requirements in muscle, liver and intestines (small and large) are lower in low RFI pigs compared to the higher RFI control pigs. We also expected to see differences in the activity of AMP-activated protein kinase (AMPK; dubbed the cellular fuel gauge, because it is activated by a drop in the energy status of the cell).

Objective 2: *Evaluate the contribution of muscle protein turnover (degradation and synthesis) to the biological basis for genetic differences in RFI and FE in finisher pigs*. We hypothesized that protein turnover will be reduced (less protein degradation) in the low RFI versus control pigs.

Materials & Methods. *Study design and animals.* All animal procedures were approved by the Animal Care and Use Committee of Iowa State University. Twelve low RFI and twelve control line pigs of higher RFI weighing 68.4±3.49 kg BW were selected from the 7th generation of the Iowa State Yorkshire swine residual feed intake selection project. Paired by line, based on age and weight, each pair was randomly assigned to individual pens. Pigs were allowed to acclimate for three days and then were placed onto ad libitum feeding for six weeks. At all times, pigs had free access to water and were fed a standard corn-soybean diet that meet or exceeded NRC (2009) swine requirements. All pigs were weighed weekly to calculate average daily gain and feed intake was recorded based on feed offered minus feed refused for each pig divided by the amount of days. Ultrasonic measurements of 10th-rib BF and loin eye area (LEA) were collected on d 0 and 42 of the test period. Two 10th rib images were collected by a National Swine Improvement Federation certified technician 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). These data was used to then calculate measures of RFI for individual pigs. RFI indexes were obtained as the residuals from analysis of ADFI using a model with BF and ADG included as additional covariates (Young et al., 2009). Residual feed intake was computed over the whole test period only. At the end of the test period, pigs were euthanized by captive bolt and immediately exsanguinated. Thereafter, muscle and liver samples were collected and frozen in liquid nitrogen for later analysis.

Objective 1. ***AMPK activities:*** AMPK activity as expressed by phosphorylation to total ratio's from skeletal muscle (longissimus dorsi) and liver will be determined in the 12 low RFI and 12 high RFI pigs. Frozen tissue samples will be thawed and homogenized. Whole muscle and liver protein lysates were prepared using Buffer A (50 mM Tris–HCl pH 7.5, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride and 10% glycerol) containing 1% Triton X-100 and 5 μM aprotinin, leupeptin and pepstatin. The homogenates were centrifuged at 6000×g for 20 min at 4°C to remove insoluble materials. The protein concentrations were determined using BCA reagents (Pierce, Rockford, IL). Total eluent protein was separated by SDS-PAGE using a 10% resolving gel. Proteins were transferred to a nitrocellulose membrane and probed overnight with primary antibody for total AMPKα or phosphorylated Thr172-AMPKα (Cell Signaling) at a dilution of 1:1000. After the overnight primary antibody treatments, all membranes were incubated with a 1:20,000 dilution of goat–anti-rabbit IgG complexed to horseradish peroxidase (Pierce) for 1 h at room temperature. Blots were developed using the SuperSignal West Pico Chemiluminescent Substrate System (Pierce) and exposed to film. Semi-quantitative band analysis and densitometry was performed using the Quantity One 1-D analysis software (Bio-Rad, Hercules, CA).

Objective 2. Markers of muscle protein turnover (degradation and synthesis): To assess skeletal muscle protein metabolism, longissimus dorsi, triceps brachii, and semimembranosus samples from the high and low RFI pigs were analyzed utilizing several techniques. Calpain proteases (μ- and m-calpain) were hypothesized to contribute to protein turnover in muscle (Smith and Dodd, 2007). Calpastatin is the endogenous inhibitor of the calpains and thus is thought to regulate these activities. Calpain activity was determined with casein zymography (Melody et al., 2004) and calpastatin activity was measured against a purified m-calpain standard (Lonergan et al., 2001). Because these proteins are susceptible to rapid postmortem alterations and because freezing disrupts calpain and calpastatin activity, these assays were done on fresh tissue. Proteasome activity was measured using a commercial kit for detecting 20S Proteasome activity (Chemicon International). Liver and longissimus dorsi protein expression of total and phosphorylated Akt, insulin receptor (IR), insulin-like growth factor receptor (RGFR), total and phosphorylated mTOR (Ser2448), S6K1 (Thr389), 4EBP1 (Thr70) was assessed by western blot as markers of protein synthesis between the low and high RFI pigs as previously described (Kimball et al., 2003). Furthermore, liver and skeletal muscle westerns blots were also used to determine ubiquitinated proteins as protein degradation markers between the two lines pigs. Western blots were ran as previously described above.

Statistical analysis

Data was analyzed using MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). The model included fixed effects of line, rep and the covariate of off-test BW. Additionally, phenotypic correlations between production traits, RFI and molecular markers of degradation were computed based on residuals derived using the CORR procedure of SAS.

Results: *Growth Performance.* There was no difference in start (68.0 vs. 67.1 kg, $P>0.10$) or end (83.4 vs. 82.3 kg, $P=0.26$) body weights over the performance collection period between the high and low RFI pigs, respectively. As expected, ADFI was significantly lower in the low RFI compared to the high RFI pigs (2.0 vs 2.4 kg/d, $P<0.0001$). However, as expected, the ADG remained the same between the two lines. Additionally, compared to the high RFI line, G:F was significantly higher in the low RFI pigs compared to the high RFI pigs (0.29 vs 0.35, $P<0.001$, respectively).

AMP Activated Protein Kinase Activity. AMP-activated protein kinase (AMPK), a major sensor of energy status within the cell was assessed in muscle and liver in the two pig lines. Its activity is correlated to its phosphorylation state and based on semi-quantitative Western blot analyses for the total and phosphorylated (Thr172) AMPK proteins in tissue homogenates (Table 1). The abundance of total AMPK protein was not different between the pig lines in either the muscle or liver homogenates. Interestingly, phospho-AMPK was

approximately 110% higher in the low versus high RFI liver samples (P=0.03, Table 1). However, the ratio of phosphorylated to total AMPK expression was not different in this tissue across the two lines (P=0.17). Compared to the high RFI muscle, the phosphorylated to total AMPK protein ratio (indicator of AMPK activity) was increased by 170% in the low RFI tissue (P=0.030, Table 1).

Table 1. Protein expression of AMP activated protein kinase (AMPK)

	Low RFI	High RFI	*P-value*
Longissimus Dorsi[1]			
Phospho-AMPKα (Thr172)[2]	0.96 ± 0.178	0.66 ± 0.091	0.16
Total AMPKα[3]	1.25 ± 0234	1.27 ± 0.150	0.93
Phospho:Total AMPKα[2]	0.98 ± 0.108	0.57 ± 0.115	0.030
Liver[1]			
Phospho-AMPKα (Thr172)[2]	0.99 ± 0.059	0.80 ± 0.046	0.030
Total AMPKα[3]	0.76 ± 0.067	0.66 ± 0.077	0.23
Phospho:Total AMPKα[2]	1.89 ± 0.263	1.45 ± 0.121	0.17

[1]Data are least square means ± standard errors (protein band density arbitrary units).
[2]n=12 (6 Low RFI, 6 High RFI pigs).
[3]n=24 (12 Low RFI, 12 High RFI pigs).

Protein Degradation. Irrespective of muscle type (Longissimus dorsi, semi tendinosis red or white), the select low RFI line had less µ-calpain activity, which may indicate less protein turnover (Figure 1, P=0.033). In muscle, µ-calpain is considered primarily responsible for proteolysis during aging. Furthermore, m-Calpain shows exactly the same relationship (P=0.023, data not shown). Therefore, lower µ-calpain activity represents a decrease in protein turnover. The select low RFI line also had increased calpastatin activity compared to the high RFI controls (P=0.036, Figure 2).

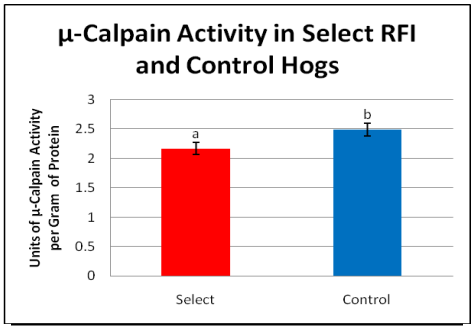

Figure 1. Muscle calpain activity in pigs selected for low or high residual feed intake (RFI).

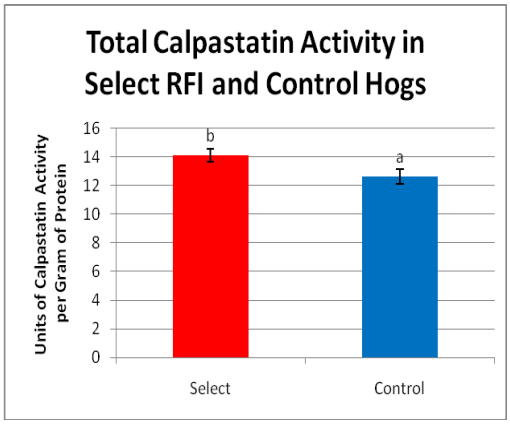

Figure 2. Total calpastatin activity in muscle from pigs selected for high (control) or low (select) residual feed intake (RFI).

The ubiquitin-proteasome is the major proteolytic system in the cytoplasm of cells that catalyze the selective degradation of proteins. The 20S proteasome is the catalytic core of the proteasome complex responsible for the breakdown of these proteins. We observed no differences in 20S proteasome activity in the liver samples between our high and low RFI pigs (Table 2, P<0.12). However, there was a significant (P=0.024), 24% reduction in 20S proteasome activity in the muscle of low RFI pigs, compared to their high RFI counterparts (Table 2).

Table 2. Least squares means for 20S proteasome activity and ubiquitin protein expression in liver and *Longissimus dorsi* (LD) samples from pigs selected for low or high residual feed intake (RFI).

	High RFI	**Low RFI**	**SEM**[4]	***P-value***
Liver				
20S Proteasome Activity (RFU)[1,3]	151.5	128.2	13.84	0.12
Ubiquitin (AU)[2,3]	1.30	1.26	0.280	0.86
LD				
20S Proteasome Activity (RFU)[1,3]	63.8	48.3	5.90	0.026
Ubiquitin (AU)[2,3]	1.30	1.10	0.090	0.15

[1]Relative fluorescence units.
[2]Arbituary units based on densitometry intensity of lanes expressing ubiquitin tagged proteins.
[3]n=7 pigs per line.
[4]Standard error of means.

Insulin Signaling Cascade and Protein Synthesis Markers. To determine the effects of selection for residual feed intake on insulin signaling intermediates and those involved in translation initiation of muscle protein synthesis, western blots analysis of *Longissimus dorsi* were performed (Table 3). Akt phosphorylation on Ser473 and Thr308 and their ratio to total Akt protein expression tended to decrease by approximately 30-60% in pigs selected for low RFI compared to their high RFI counterparts (P<0.10). However, the insulin receptor (IR), insulin receptor substrate and the insulin-like growth factor receptor expression in muscle was not affected by genetic selection for RFI in these finisher pigs (Table 3). Similarly, mTOR, SK61 and 4EBP1 total and phosphorylation expression was not different between muscles isolated from both low and high RFI gilts (Table 3).

Table 3. *Longissimus dorsi* (LD) muscle expression of key insulin signaling and protein synthesis cascade proteins in pigs selected for low and high residual feed intake (RFI).

	High RFI[1,3]	**Low RFI**[1,3]	**SEM**[2]	***P-value***
Insulin Signaling Cascade				
Phospho-Ser Akt (AU)[2]	1.00	0.76	0.125	0.20
Phospho-Thr Akt (AU)[2]	1.00	0.32	0.256	0.09
Total Akt (AU)[2]	1.00	1.03	0.147	0.90
Ser:total Akt (AU)[2]	1.10	0.73	0.134	0.07
Thr:total Akt (AU)[2]	1.16	0.41	0.293	0.10
Insulin Receptor (AU)[2]	1.00	0.82	0.172	0.46
Insulin Receptor Substrate (AU)[2]	1.00	0.88	0.178	0.64
Insulin-like Growth Factor Receptor (AU)[2]	1.00	0.89	0.120	0.54
Protein Synthesis Pathway				
mTOR (AU)[2]	0.95	0.95	0.027	0.99
Phospho-Ser2448 mTOR (AU)[2]	0.97	0.97	0.012	0.99
S6K1 (AU)[2]	0.95	0.95	0.027	0.99
Phospho-Thr389 S6K1 (AU)[2]	0.97	1.04	0.356	0.09
4EBP1 (AU)[2]	0.97	0.97	0.013	0.99
Phospho-Thr70 4EBP1 (AU)[2]	0.98	1.02	0.025	0.14
Ser:total mTOR (AU)[2]	1.01	1.01	0.027	0.99
Thr:total S6K1 (AU)[2]	1.03	1.09	0.059	0.28
Thr:total 4EBP1 (AU)[2]	1.01	1.05	0.034	0.29

[1]Least qsquared means of arbituary units based on band densitometry intensity of expressed proteins.
[2]Standard error of means.
[3]n=7 pigs per line.

Residual correlations. Residual correlations of performance traits with RFI and protein degradation markers were generally low (Table 4). Correlations of ADFI and BF with RFI were high, positive, and significant ($P < 0.001$). However, as expected, RFI was significantly negatively correlated with FE (-0.90, $P<0.001$). Correlations of calpains and calpastatin with performance traits and RFI indices were weak and not significant. Interestingly, total muscle calpastatin was negatively correlated with RFI and backfat (Table 4). Finally, muscle 20S proteasome activity was moderately positively correlated with RFI ($P=0.038$) and negatively with FE ($P=0.10$) and ADG ($P=0.11$).

Table 4. Residual correlations between protein degradation markers, residual feed intake index and performance traits in finisher pigs[1]

	RFI index	Backfat	ADG	ADFI	FE
RFI index	1.00				
Backfat	0.66	1.00			
	0.021				
ADG	-0.20	-0.25	1.00		
	0.53	0.42			
ADFI	0.93	0.82	-0.02	1.00	
	<0.0001	0.001	0.96		
FE	-0.90	-0.80	0.53	-0.84	1.00
	<0.001	0.002	0.07	<0.001	
Liver 20S proteasome	0.49	0.56	-0.01	0.59	-0.45
	0.10	0.049	0.98	0.042	0.14
Muscle 20S proteasome	0.60	0.34	-0.21	0.48	-0.50
	0.038	0.28	0.52	0.11	0.10
Muscle m-Calpain	-0.07	-0.03	0.40	0.04	0.15
	0.82	0.94	0.22	0.90	0.67
Muscle µ-Calpain	0.07	0.17	0.15	0.14	-0.09
	0.86	0.64	0.67	0.70	0.80
Muscle total Calpastatin	-0.38	-0.25	0.10	-0.31	0.40
	0.25	0.45	0.77	0.35	0.23
Muscle Ubiquitin	0.13	0.43	0.31	0.48	-0.22
	0.66	0.12	0.66	0.07	0.45

[1]Upper row = residual correlations. Bottom row = *P*-values.

Discussion. A significant part of the variation associated with RFI is thought to be related to the level of protein turnover, i.e. synthesis and degradation. AMP-activated protein kinase (AMPK) is a metabolic master switch that regulates cellular energy homeostasis in response to metabolic or non-metabolic stress. This kinase is activated by phosphorylation as a result of an increase in the intracellular AMP:ATP ratio. Thus, phosphorylation is positively correlated to its enzyme activity. More recently, AMPK activation has also been shown to inhibit Na^+, K^+-ATPase activity and decrease the sodium pump cell surface abundance in L6 skeletal muscle cells (Benziane et al., 2009). Furthermore, activation of AMPK causes an inhibition of protein synthesis via the mammalian target of rampamycin (mTOR) pathway (Bolster et al., 2002; Horman et al., 2002). These authors indicate that AMPK activation of eukaryotic translation elongation factor 2 (eEF2) kinase, results in reduced phosphorylation of Akt/protein
kinase B on Ser(473), mTOR on Ser(2448), ribosomal protein S6 kinase at Thr(389), and eukaryotic initiation factor 4E-binding protein on Thr(37) and the phosphorylation and inactivation of eEF2. Ultimately, this causes reduced signaling through the mTOR pathway, culminating in the inhibition of translation initiation of protein synthesis. Insulin signaling and the mTOR pathway play a pivotal role in skeletal muscle protein synthesis via regulating translation initiation (Hay and Sonenberg, 2004; Orellana et al., 2007). Intermediates such as Akt (or protein kinase B) promote activation of the mTOR cascade leading to the downstream initiation of protein synthesis. In our current study, the low RFI muscle had increased AMPK activity. These was accompanied with no change in mTOR pathway signaling between the lines and reduce Akt insulin signaling protein expression in the low RFI pigs. These data are in agreement with Bolster et al., (2002) and Horman et al., (2002).
Protein turnover is energetically expensive and differences in protein metabolism have been reported between cattle with high and low RFI (Richardson and Herd, 2004). In sheep, protein turnover may account for 19% of the increment in ATP expenditure (Gill et al., 1989). Further, higher RFI is attributed to higher turnover and

increased rates of degradation, thereby giving rise to decreased efficiency of protein gain. This is in agreement with the data presented in this paper in which RFI was positively correlated with ubiquitin-proteasome activity. We have also previously found several indicators in our RFI lines, that selection for low RFI may have altered protein degradation, but not synthesis, and thus protein turnover (unpublished data). Additionally, we have shown that the amount of intact desmin is correlated with RFI (r= -0.18, P=0.02) in our selection lines (Smith et al., 2011). Desmin is an intermediate filament that links adjacent myofibrils and it can undergo rapid proteolysis. Therefore, the higher amount of intact desmin in low RFI pigs indicates less protein degradation.

Furthermore, calpastatin, the endogenous inhibitor of the ubiquitous calpain proteases (μ- and m-calpain) has been shown to be up regulated in our low RFI pig compared to the high RFI muscles (Smith et al., 2011). This is also in agreement with the current study in which heavier pigs were used. In feedlot steers that were divergently selected for one generation based on RFI, it was also reported that the level of skeletal muscle calpastatin higher in high efficient (low RFI) compared to the less efficient steers (McDonagh et al., 2001).

Muscles in animals selected for low residual feed intake had less μ- and m-calpain activity compared to that of high RFI animals, which may also indicate less protein turnover. Calpains are proteolytic enzymes which are inhibited by calpastatin and this is another indication of lower protein turnover. Therefore, decreased μ-calpain and increased calpastatin activity could result in decreased tenderness. Altogether, pigs selected for low RFI and improved feed efficiency have less μ- and m-calpain activity and greater calpastatin activity compared to that of control high RFI pigs. These data indicate that selection for feed efficiency also may select for reduced protein turnover. No differences in insulin signaling protein intermediates and proteins involved in translation control of protein synthesis (mTOR pathway) were found in between our selection lines. Together, these results warrant further examination of protein turnover as it relates to FE in swine.

In conclusion, to maximize production efficiencies through improved feed conversion and reduced/optimized feed costs, the industry must first develop a greater understanding of the genomic and cellular processes that determine efficient nutrient utilization in the pig. Compared to other species such as cattle and poultry, less is understood about the physiology and cellular processes behind FE and RFI in swine. The research presented here evaluated markers of energy metabolism, insulin signaling and protein synthesis and degradation that may contribute to FE and RFI in pigs. We have identified protein degradation and a key play in muscle metabolism and FE in finisher swine. Optimize nutritional or pharmacological management strategies to mitigate protein degradation systems in muscle we help maximize the potential for lean growth and overall efficiency in finisher pigs. These outcomes will give the US pork industry a competitive advantage by substantially reducing one of the large variable costs associated with pork production, i.e. feed, and help to improve profitability.

References.

Benziane, B. et al. 2009. AMP-activated protein kinase activator A-769662 is an inhibitor of the Na+,K+-ATPase. American journal of physiology. Cell physiology.

Bolster, D. R., S. J. Crozier, S. R. Kimball, and L. S. Jefferson. 2002. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. J. Biol. Chem. 277: 23977-23980.

Cai, W., D. S. Casey, and J. C. Dekkers. 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine. J Anim Sci 86: 287-298.

Crews, D. H., Jr. 2005. Genetics of efficient feed utilization and national cattle evaluation: a review. Genet Mol Res 4: 152-165.

Gill, M., J. France, M. Summers, B. W. McBride, and L. P. Milligan. 1989. Simulation of the energy costs associated with protein turnover and Na+,K+-transport in growing lambs. J Nutr 119: 1287-1299.

Hay, N., and N. Sonenberg. 2004. Upstream and downstream of mTOR. Genes Dev 18: 1926-1945.

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

Horman, S. et al. 2002. Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. Curr. Biol. 12: 1419-1423.

Kimball, S. R. et al. 2003. Endotoxin induces differential regulation of mTOR-dependent signaling in skeletal muscle and liver of neonatal pigs. Am J Physiol Endocrinol Metab 285: E637-644.

Koch, R. M., L. A. Swinger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J Anim Sci 22: 486-494.

Lonergan, S. M., E. Huff-Lonergan, L. J. Rowe, D. L. Kuhlers, and S. B. Jungst. 2001. Selection for lean growth efficiency in Duroc pigs influences pork quality. J Anim Sci 79: 2075-2085.

Luiting, P. 1998. In Regulation of Feed Intake In: D. van der Heide (ed.). p 75-87. CABI Publishing, UK.

Luiting, P., J. W. Schrama, W. van der Hel, and E. M. Urff. 1991. Metabolic differences between White Leghorns selected for high and low residual food consumption. British poultry science 32: 763-782.

McDonagh, M. B. et al. 2001. Meat quality and the calpain system of feedlot steers following a single generation of divergent selection for residual feed intake. Aust J Expt Agric 41: 1013-1021.

Melody, J. L. et al. 2004. Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. J Anim Sci 82: 1195-1205.

Orellana, R. A. et al. 2007. Insulin stimulates muscle protein synthesis in neonates during endotoxemia despite repression of translation initiation. Am J Physiol Endocrinol Metab 292: E629-636.

Richardson, E. C., and R. M. Herd. 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust J Exp Agric 44: 431-440.

Smith, I. J., and S. L. Dodd. 2007. Calpain activation causes a proteasome-dependent increase in protein degradation and inhibits the Akt signalling pathway in rat diaphragm muscle. Experimental physiology 92: 561-573.

Smith, R. M. et al. 2011. Effects of selection for decreased residual feed intake on composition and quality of fresh pork. J Anim Sci 89: 192-200.

van Eerden, E. et al. 2004. Residual feed intake and its effect on Salmonella enteritidis infection in growing layer hens. Poultry science 83: 1904-1910.

Young, J. M., W. Cai, and J. C. Dekkers. 2009. Effect of selection for residual feed intake on feeding behavior and daily feeding patterns in pigs. In: ADSA/ASAS annual meeting, Montreal, Canada