Gilts selected for low residual feed intake have potential for decreased protein degradation

Shannon M. Cruzen, Amanda J. Harris, Katrin Hollinger, Joshua T. Selsby, Nicholas K. Gabler,

Steven M. Lonergan and Elisabeth Huff-Lonergan

Department of Animal Science, Iowa State University, Ames, Iowa, 50011 United States

Abstract – The objective of this study was to evaluate the contribution of muscle protein turnover (degradation and synthesis) to the biological basis for genetic differences in finisher pigs selected for residual feed intake (RFI). RFI is defined as the difference between expected feed intake (for the individual pig's achieved rate of gain and backfat depth) and the individual's observed feed intake. We hypothesized that protein turnover would be reduced in low RFI pigs. Twelve low RFI (7th generation of selection for low RFI) and high RFI (2nd generation of selection for high RFI) gilts were paired by age and weight and fed a standard corn-soybean diet for 6 weeks. Pigs were euthanized, muscle and liver samples collected, and insulin signaling, protein synthesis, and protein degradation proteins were analyzed for expression and activities. Muscle from low RFI pigs had less μ- and m-calpain activities, greater calpastatin activity, and lower 20S proteasome activity compared to their high RFI counterparts (P<0.05). No differences in insulin signaling intermediates and translation initiation signaling proteins (mTOR pathway) were observed (P>0.05). These data indicate less protein degradation occurs in pigs selected for reduced RFI and may account for a significant portion of the increased efficiency observed these animals.

Key Words – residual feed intake, calpain system, proteasome.

I. INTRODUCTION

Feed costs are currently the single major cost in swine production. Selecting for pigs which more efficiently utilize feed is one way to decrease these costs. In a population, it is reasonable to calculate an expected feed intake required for growth performance (average daily gain; ADG) and composition (backfat depth; BF). The difference between this expected value and the individual pig's actual feed intake is termed residual feed intake (RFI). First suggested by Koch et al. [1], RFI is a unique and effective way to measure feed efficiency. Iowa State University is currently in the seventh generation of a selection project for low RFI (more efficient) pigs and in the second generation of divergently selecting for high RFI (less efficient) pigs. These lines provide a useful model to study the genetics and physiology responsible for swine feed efficiency differences.

Therefore, we are using the RFI selection project to elucidate the physiological functions that are responsible for the observed differences in feed efficiency. Many of these factors have been quantified in poultry and beef, including physical activity, feed intake patterns and behavior, stress, digestibility, protein turnover, and metabolism [2-3]. Richardson and Herd [4] estimated that 37% of the variation in cattle for RFI was due to protein turnover, tissue metabolism, and stress, and it is reasonable to suggest that this may be true for swine as well. Therefore, the objective of this study was to evaluate the contribution of muscle protein turnover (degradation and synthesis) to the biological basis for genetic differences in RFI. Compared to the high RFI line, we hypothesized that protein turnover would be reduced in pigs selected for reduced RFI.

II. MATERIALS AND METHODS

A. Study Design and Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University. Twelve 7th generation low RFI and twelve high RFI Yorkshire gilts (2nd generation selection for high RFI) weighing 68.4±3.49 kg BW were selected from the Iowa State University RFI project population. Gilts were paired by line, based on age and weight, and

each pair was assigned to an individual pen. Pigs were fed a standard corn-soybean diet for 6 weeks, and feed intake data (ADFI) were collected. Weights were taken weekly in order to calculate ADG. Ultrasound measurements of BF and loin eye area were performed on d 0 and 42. These data were used to calculate RFI measures for each pig. RFI indices were obtained as the residuals from analysis of ADFI using a model with BF and ADG included as covariates [5]. At the end of the test period, pigs were euthanized by captive bolt and subsequent immediate exsanguination. Muscle and liver samples were collected and either analyzed immediately or frozen in liquid nitrogen for later analysis.

B. Protease Activities

Calpain and calpastatin activity determination were performed on the *longissimus* and *semitendinosus* (n=22). The *semitendinos*us was divided into red (RST) and white (WST) portions. Sarcoplasmic protein was extracted according to the method of Melody et al. [6]. Supernatant protein samples were loaded onto a 20 ml Q-Sepharose Fast Flow anion exchange column equilibrated with TEM. After washing, calpastatin, µ-calpain, and m-calpain were eluted using a linear gradient of 0-400 m*M* KCl in TEM. Calpastatin eluted in two separate peaks (calpastatin I and II, at 50-90 m*M* KCl and 120-190 m*M* KCl, respectively, Figure 1), followed by µ-calpain (180-240 m*M* KCl) and m-calpain (300-400 m*M* KCl).

The activities of μ- or m- calpain or calpastatin-containing fractions were determined using casein as a substrate, using a modified method of Koohmaraie [7]. Protein content of the original sample was determined in order to calculate activity on a total protein basis. Estimation of crude protein (Nitrogen x 6.25) was done using an Automated LECO Nitrogen Analyzer (LECO-TruSpec® N, LECO Corp., St. Joseph, MI, USA).

Proteasome activity was measured in the LD and liver using a commercial kit (Chemicon International). Immunoblotting was used to measure levels of ubiquitinated proteins, which indicate tagging for proteasome degradation.

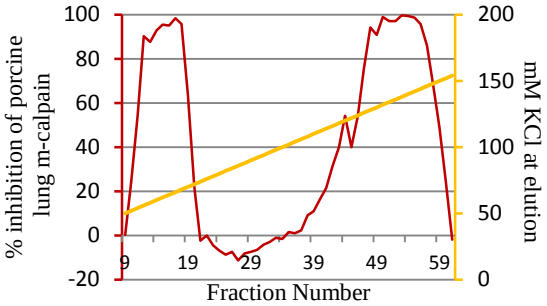

Figure 1 Representative example of calpastatin isoform separation during elution using a Q-Sepharose ion exchange column.

C. Expression of Protein Synthesis Markers

Longissimus dorsi samples were prepared for immunoblotting. Expression of total and phosphorylated Akt, insulin receptor, insulin-like growth factor receptor, total and phosphorylated mammalian target of rapamycin (mTOR, Ser2448), S6K1 (Thr389), 4EBP1 (Thr70) were assessed using immunoblotting [8].

D. Statistical Analysis

Data were analyzed as a complete randomized design using the MIXED procedure in SAS (v. 9.2, SAS Inst., Cary, NC). The model included fixed effects of line and replicate, and the covariate of off-test BW. Phenotypic correlations were computed based on residuals derived using the CORR procedure of SAS.

III. RESULTS AND DISCUSSION

Gilts from the low RFI line had an average RFI index of -0.21±0.07, whereas high RFI gilts had an RFI index of -0.02±0.07 (P=0.06).

Across muscles, µ- and m-calpain activity were greater (P<0.05) in muscle from high RFI pigs compared to low RFI pigs. In contrast, calpastatin activity was greater (P<0.05) in muscle from low RFI pigs (Figure 2), which is in agreement with Smith et al. [9]. This combination of reduced calpain activities and increased calpastatin activity in low RFI pigs indicates decreased capacity for protein degradation. Furthermore, because µ-calpain and calpastatin play a large role in the proteolysis that occurs during meat aging, these results also indicate potentially reduced tenderness in pork from these animals.

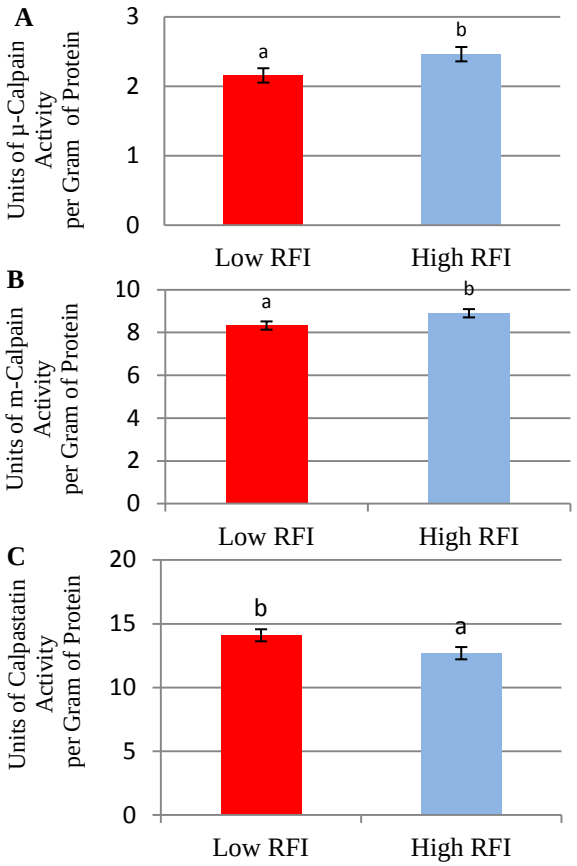

Figure 2 µ-Calpain (A), m-calpain (B) and calpastatin (C) activities in gilts selected for either low or high residual feed intake (RFI). Combined values from *longissimus dorsi* and red and white *semitendinosus*. n=11 pigs per line. P<0.05. Bars represent standard errors of the means.

The ubiquitin-proteasome pathway is another major proteolytic system found in the cytoplasm. Proteins selected for degradation are ubiquitinated and then targeted to the proteasome, where proteolysis occurs. The 20S subunit is the catalytic core of the proteasome. No differences in 20S proteasome activity or protein ubiquitination were observed in liver samples of low versus high RFI pigs (Table 1, P>0.05). However, in the LD, low RFI pigs had less 20S proteasome activity (P<0.05) compared to high RFI pigs. In addition, LD 20S proteasome activity was highly correlated with RFI index values (R^2=0.60, P<0.05). Liver 20S proteasome activity, while not significantly correlated with RFI index (R^2=0.49, P=0.10), was significantly correlated with BF (R^2=0.56, P<0.05) and ADFI (R^2=0.59, P<0.05). No differences were observed

Table 1 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	0.03
Ubiquitin (AU)[2,3]	1.30	1.10	0.090	0.15

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

in LD ubiquitination (Table 1, P>0.05). Decreased 20S proteasome activity is further evidence of reduced protein degradation in pigs selected for low RFI.

Overall, the insulin signaling and protein synthesis markers measured were not affected by RFI line (Table 2). However, Akt phosphorylation on Thr308 and the ratio of Akt phosphorylation on either Thr308 or Ser473 to total Akt protein expression tended to decrease by approximately 30-60% in pigs selected for low RFI compared high RFI pigs (P<0.10). Phosphorylation, and especially the ratio of phosphorylated to unphosphorylated Akt, is an indication of activation. Akt is upstream of several pathways involving protein synthesis and prevention of protein degradation, including activation of mTOR [10-11]. Reduced Akt phosphorylation should therefore result in decreased protein synthesis; however, no protein markers in the mTOR pathway were significantly different between the two lines.

IV. CONCLUSION

There is substantial evidence that reduced protein turnover due to decreased activity of protein degradation systems within muscle may contribute to the increased efficiency associated with pigs selected for reduced RFI. However, there are concerns that, due to the reduced levels of µ- calpain and increased calpastatin activity,

Table 2. *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)	1.00	0.76	0.125	0.20
Phospho-Thr Akt (AU)	1.00	0.32	0.256	0.09
Total Akt (AU)	1.00	1.03	0.147	0.90
Ser:total Akt (AU)	1.10	0.73	0.134	0.07
Thr:total Akt (AU)	1.16	0.41	0.293	0.10
Insulin Receptor (AU)	1.00	0.82	0.172	0.46
Insulin Receptor Substrate (AU)	1.00	0.88	0.178	0.64
Protein Synthesis Pathway				
mTOR (AU)	0.95	0.95	0.027	0.99
Phospho-Ser2448 mTOR (AU)	0.97	0.97	0.012	0.99
S6K1 (AU)	0.95	0.95	0.027	0.99
Phospho-Thr389 S6K1 (AU)	0.97	1.04	0.036	0.09
4EBP1 (AU)	0.97	0.97	0.013	0.99
Phospho-Thr70 4EBP1 (AU)	0.98	1.02	0.025	0.14
Ser:total mTOR (AU)	1.01	1.01	0.027	0.99
Thr:total S6K1 (AU)	1.03	1.09	0.059	0.28
Thr:total 4EBP1 (AU)	1.01	1.05	0.034	0.29

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

decreased tenderness and therefore reduced quality may result from these changes. Future research will investigate tenderness and sensory attributes in generation eight of the Iowa State RFI line.

ACKNOWLEDGEMENTS

The authors would like to thank Jack Dekkers, Martha Jeffrey, and staff from the ISU swine farms for their help with this project. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2010-65206-20670 from the USDA National Institute of Food and Agriculture and the Iowa Pork Producers Association Grant no. 10-009.

REFERENCES

1. Koch R. M., Swiger L. A., Chambers D., et al. (1963) Efficiency of Feed Use in Beef Cattle. J Anim Sci 22(2):486-494.
2. Herd R. M., Arthur P. F. (2009) Physiological basis for residual feed intake. J Anim Sci 87(14):E64-E71.
3. Luiting P. (1990) Genetic variation of energy partitioning in laying hens: causes of variation in residual feed consumption. World Poultry Sci J 46(02):133-152.
4. Richardson E. C., Herd R. M. (2004) Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust J Exp Agr 44(5):431-440.
5. Young J. M., Cai W., Dekkers J. C. M. (2011) Effect of selection for residual feed intake on feeding behavior and daily feed intake patterns in Yorkshire swine. J Anim Sci 89(3):639-647.
6. Melody J. L., Lonergan S. M., Rowe L. J., et al. (2004) Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. J Anim Sci 82(4):1195-1205.
7. Koohmaraie M. (1990) Quantification of Ca2(+)-dependent protease activities by hydrophobic and ion-exchange chromatography. J Anim Sci 68(3):659-665.
8. Kimball S. R., Orellana R. A., O'Connor P. M. J., et al. (2003) Endotoxin induces differential regulation of mTOR-dependent signaling in skeletal muscle and liver of neonatal pigs. Am J of Physiol-Endoc M 285(3):E637-E644.
9. Smith R. M., Gabler N. K., Young J. M., et al. (2011) Effects of selection for decreased residual feed intake on composition and quality of fresh pork. J Anim Sci 89(1):192-200.
10. Otto A., Patel K. (2010) Signalling and the control of skeletal muscle size. Exp Cell Res 316(18):3059-3066.
11. Braun T., Gautel M. (2011) Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nat Rev Mol Cell Bio 12(6):349-361.12(6):349-361.