Use of Transcriptional Profiling and Assessment of Blood Parameters to Understand Biological Mechanisms Controlling Feed Intake and Efficiency in Pigs

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Summary and Implications

In this study, using transcriptional profiling of key tissues, we aimed to identify genetic mechanisms differing between control pigs and pigs that have been under selection for low residual feed intake (RFI) for three generations. A further aim was to determine the pathways responding to feed restriction within these lines and any line x treatment interactions resulting in gene expression differences. Preliminary results indicate that 2,809 genes in fat ($p<0.04$, $q<0.2$) and 61 genes in liver ($p<0.001$, $q<0.2$) showed differential expression in response to feed restriction. Also, 1,247 genes ($p<0.02$, $q<0.2$) showed differential expression between low RFI and control pigs and 38 genes ($p<0.001$, $q<0.2$) showed a line x feed interaction in liver. In addition, we measured the concentration of some of the important feed intake regulators in the blood such as leptin, triglyceride, and glucose. We found that the average blood leptin level to be significantly higher in the control *ad libitum* (CA) pigs than the control restricted (CR) group. Interestingly, the selected line of pigs on both restricted (SR) and *ad libitum* (SA) feed had similar blood leptin levels as found in the CR group pigs. Serum glucose levels were higher in CR than CA, however, we observed an opposite trend in the selected group. Combined with the transcriptional profiling results, blood hormone parameters may help us understand potential pathways that control FI and FE in pigs.

Introduction

Feed is the major variable cost in pork production, totaling up to 44% of the entire cost. Determination of genetic mechanisms that control feed intake (FI) and feed efficiency (FE) remains a major challenge for improvement of FE and FI. Although feed is associated with production traits such as growth and composition (Luiting 1998), considerable variation in FE and FI exists that is independent of these traits. This variability is called Residual Feed Intake (RFI) (Luiting, 1990), which is measured as the feed consumed above what is on average required for growth and maintenance. Cai et al (2006) reported successful development of lines that differ in RFI at ISU and estimated heritability of RFI to be 0.30.

Pigs from these lines that differ in RFI are likely to display differential genetic mechanisms that underly their effectiveness in depositing and maintaining fat and lean tissue. Investigation of differences in gene expression, feed intake regulators and blood parameters that are indicators of energy store status may provide insight into the mechanisms that underly these differences.

Leptin is a 16kDa protein hormone secreted from white adipocytes and its role is implicated in many areas, including food intake and energy expenditure. The functional, long form of the leptin receptor ObRb is found in the hypothalamic area and is thought to be responsible for a leptin mediated decrease in feed intake and an increase in energy expenditure. The short form of the leptin receptor proteins is expressed in many tissues, yet their physiological roles are unclear. When subjected to a feed restriction, the level of glucose in the blood decreases. This results in activation of gluconeogenesis and glycogenolysis, primarily in the liver, which increases the level of blood glucose. Subsequently, lipids are mobilized from adipocytes and transported to tissues that require energy from fatty acid oxidation. Ninety percent of lipids are stored in the form of triacylglycerol or triglyceride (TG) and under energy constraint, they are broken down to free fatty acids and glycerol.

Against this background, the objectives of this study were to study differences in gene expression and in blood parameters related to energy metabolism in pigs that differ in RFI and that are subjected to restricted versus *ad libitium* feeding.

Materials and Methods

Transcriptional profiling: Low and high RFI gilts from the lines described in Cai et al. (2006) were allowed feed *ad libitum* or were feed restricted to 80% of maintenance for 7 days in a complete 2 x 2 factorial design. Four pigs were evaluated per treatment combination. Total RNA was isolated from liver and fat tissues and analyzed using hybridization to the Affymetrix Porcine Genechip™, which allows assessment of the level of expression of over 24,000 transcripts for genes across the genome. A mixed linear model was fit to each tissue and each gene using SAS Proc Mixed to identify genes that differed in expression between treatments.

Blood parameter assays: Blood samples were collected at sacrifice and put on ice upon collection and kept at 4°C overnight to aid coagulation. Blood was centrifuged at 1000 x *g* for 15 min and the serum was isolated and stored at -20°C. Blood leptin (n=23) and cortisol (n=23) were measured using radioimmunoassays by collaborator R. Barb et al. (USDA-ARS-Russell Agricultural Research Center, Athens, Georgia). Blood glucose (n=44) and TG (n=45) levels were determined using Hexokinase (Roche Diagnostics) and TG (Diagnostic Chemicals Limited) kits.

Results and Discussion

Growth and FI related parameters: Pre-treatment RFI values along with growth and feed intake values during treatment were evaluated (Figs. 1, 2, 3 respectively). The assessment of body fat content via backfat thickness at slaughter was summarized (Fig. 4).

Figure 1.

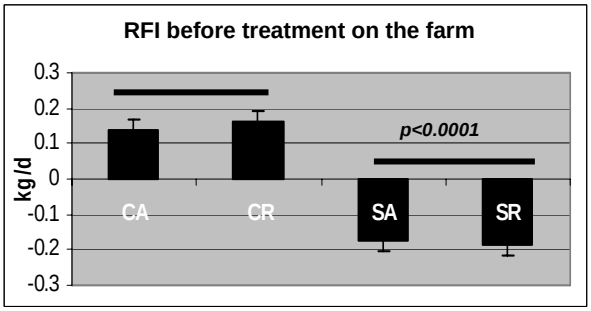


Figure 2.

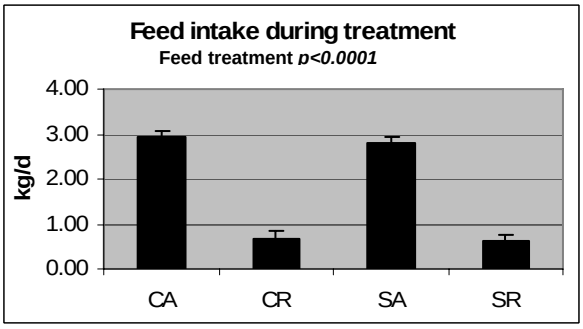


Figure 3.

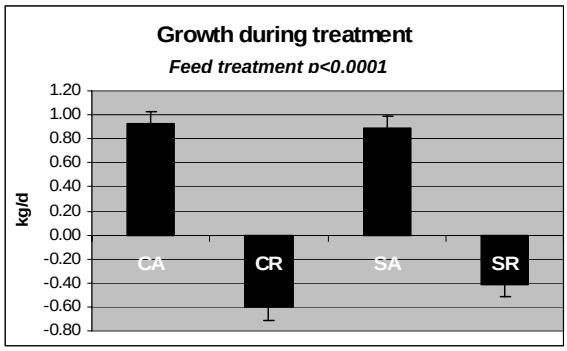
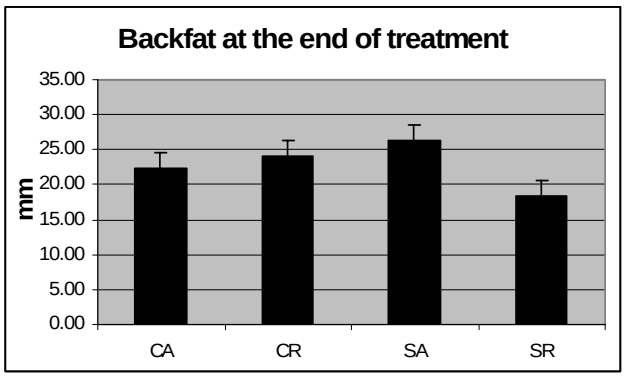


Figure 4.



Microarray results: Preliminary results for transcriptional profiling of liver and fat tissues are summarized in **Table 1.** Pigs that differed in RFI showed differences in gene expression levels in the liver. Also in the liver, 38 genes showed a significant Line x Feed interaction. Results suggest that selection for low RFI has resulted in alterations in the expression of some genes. We are currently adding more animals and tissues for transcriptional profiling to further investigate these differences.

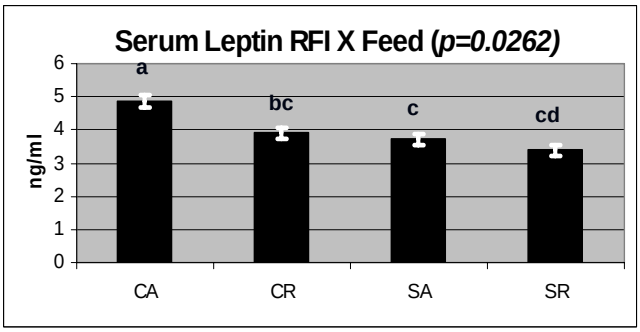
Table 1. Summary of genes that were differentially expressed between feeding treatments (Feed), high versus low RFI pigs, or their interaction, based on a false discovery rate of 20%.

Tissue	Factor	Significance level (p)	Number of Genes
Fat	Feed	0.04	2809
Liver	Feed	0.001	61
Liver	RFI	0.02	1247
Liver	Feed * RFI	0.001	38

Blood parameters: Leptin levels in the blood indicate the level of body fat stores and strength of signal to decrease feed intake. Results in Figure 5 show that, while the CA group had higher serum leptin than the CR group, low RFI pigs did not show a difference in leptin levels in response to feed restriction. The level of serum leptin under restricted or *ad libitum* feeding in low RFI pigs was not significantly different from that in high RFI animals that were under restriction. Of most interest is the finding that the low RFI pigs had lower circulating leptin levels ($p<0.0001$) than the high RFI animals when fed similar amount of *ad libitum* feed (Fig. 2). Leptin levels do not correspond with backfat thickness for SA and CA groups (Fig. 4), which implies that the difference seen in leptin level is not due to increased fat stores. Higher leptin level in CA did not decrease feed intake in CA (Fig 2). Leptin also acts to inhibit lipogenesis in pigs (Ramsay, 2003) which may imply that lower leptin

level in SA result in more lipogenesis than in CA and this seem to be supported, in this case, by backfat thickness being higher in SA than CA (Fig 4).

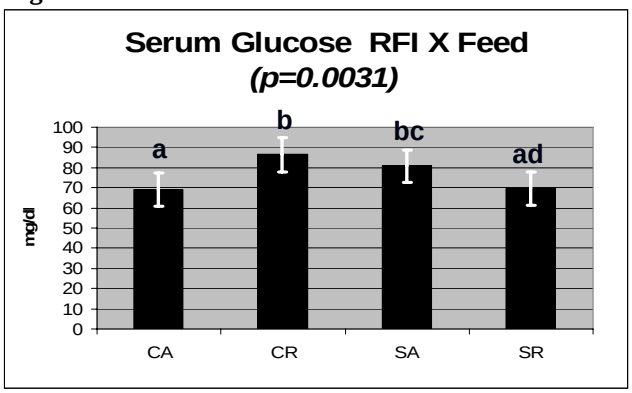
Figure 5.



Differing letters show statistical difference. ab: p=0.0004; ac: p<0.0001; ad: p<0.0001; bd: p=0.0270. (n=23 CA=6, CR=5, SA=6, SR=6)

Blood glucose levels increase shortly after consumption of a meal. During feed restriction, glucose in the blood is first depleted due to the lowered amount of feed, and then brought back to normal levels via hormonal signals such as glucagon that activate glycogenolysis and gluconeogenesis. Our results show that, whilst feed restriction increased the glucose level in the high RFI pigs (CR>CA), the opposite was observed in the low RFI pigs (SA>SR) (Fig. 6). The level of blood glucose in SA pigs was similar to those in the CR group, which paralleled the leptin pattern seen previously. CR group may have been more responsive to energy restraint than SR group, thus, turning on gluconeogenesis or glycogenolysis to recover the blood glucose level to normal. Moreover, *ad libitum* fed low RFI animals have higher serum glucose than low RFI counterparts, suggesting that low RFI group may deplete blood glucose slower than the high RFI group.

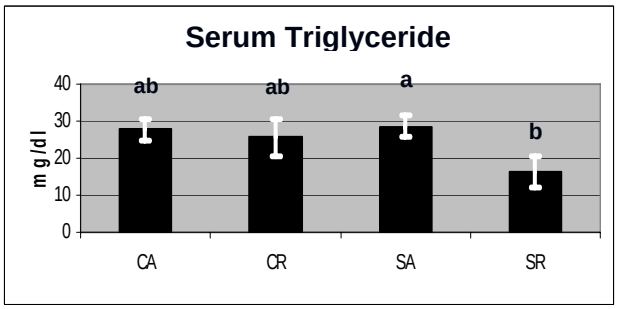
Figure 6.



Differing letters show statistical difference ab: p=0.0098; ac: p=0.0122; bd: p=0.0023; cd: p=0.0288. (n=45 CA=14, CR=8, SA=15, SR=8)

During lipid assimilation, TGs are enveloped in chylomicrons in the intestinal epithelial cells and put into blood and lymph. Subsequently, chylomicrons are recognized by adipocyte membrane receptors and stored for energy. Under feed restriction, free fatty acids and glycerol are mobilized (lipid mobilization) from adipocytes to be sent to tissues that are in need of energy for oxidation. Our results indicate that the level of serum TG did not change due to feed restriction in the control animals (Fig. 7). On the other hand, the level of TG was lower in the SR than SA, which seemed to indicate that under feed restriction, the low RFI pigs may store lipids as fat stores more efficiently than the *ad libitum* selected group. Based on backfat thickness, the selected group under feed restriction had the lowest level of fat store at the end of the treatment whereas the high RFI animals under feed restriction did not decrease body fat stores compared to the *ad libitum* group (Fig. 4). Selected group animals may efficiently store lipids and utilize lipids as energy source in the face of feed restraint. Further studies of direct lipid mobilization assays are necessary to elucidate this question.

Figure 7.



Differing letters show statistical difference bc: p=0.0414 (n=44 CA=14. CR=7. SA=15. SR=8)

We see evidence of differences in the pattern of genetic expression and the levels blood parameters that are indicative of energy store status between pigs that are selected for low RFI and those that are high RFI animals. Our results may allow a better understanding of the underlying biological and genetic mechanisms for feed intake and feed efficiency.