Identification of potential serum biomarkers to predict feed efficiency in young pigs


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ABSTRACT: Identification of biomarkers for feed efficiency in livestock will aid in the efficient production of high-quality protein to meet the demands of a growing population. The overall objective of this research was to identify biomarkers in serum for swine feed efficiency and to discover pathways affected by divergent selection for residual feed intake (RFI). Serum was collected from young pigs (between 35 and 42 d of age) from 2 lines of pigs that have been genetically selected to be either more efficient (low-RFI) or less efficient (high-RFI). After blood collection, during finishing, pigs from each line were placed on either a low-energy/high-fiber diet or a traditional high-energy/low-fiber diet to test for any diet effects on RFI. Subsets of 6 pigs per line within each diet were used in 3 independent experiments. Pigs with extreme RFI phenotypes from the low-energy/high-fiber diet were used to confirm the results from the first 2 comparisons. Two-dimensional difference in gel electrophoresis and mass spectrometry were used to identify proteins with different abundances between RFI line or finishing diet. Three proteins had consistent and significant (P < 0.05) RFI line differences for both diets: gelsolin, vitronectin, and serine protease inhibitor A3 (serpinA3). Abundance of gelsolin, a protein with roles in actin filament assembly and immune response, was greater in the more efficient low-RFI pigs (9 to 39%). Vitronectin was also more abundant in the low-RFI pigs (39 to 56%) and has known roles in blood homeostasis and may regulate adiposity. SerpinA3 is a member of a very large family of proteins referred to as serine protease inhibitors. A total of 14 spots that were more abundant in the low-RFI line, some at least twice as abundant, were identified as serpinA3. Multiple isoforms of serpinA3 have been reported (serpinA3-1 to serpinA3-4 in pigs and serpinA3-1 to serpinA3-8 in cattle) with serpinA3 having many different functions dependent on isoform. Gelsolin, vitronectin, and serpinA3 are 3 proteins that may play direct and important biological roles in the pathways that control RFI and, ultimately, feed efficiency through energy utilization and homeostasis. These data demonstrate that serum proteins can be a useful source of potential biomarkers for feed efficiency and provide information on pathways with distinct expression patterns between animals that differ in feed efficiency.

Key words: 2D-DIGE, biomarkers, feed efficiency, pigs, residual feed intake

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A greater understanding of the molecular, metabolic, and physiological components of feed efficiency is needed to develop robust biomarkers. At Iowa State University (Ames, IA), pigs from lines that have been divergently selected for residual feed intake (RFI) have been used as a model to study the biology underlying feed efficiency (Cai et al., 2008; Young et al., 2011). Residual feed intake is the difference between observed feed intake and expected feed intake based on the production measures of ADG and backfat (Cai et al., 2008; Boddicker et al., 2011; Grubbs et al., 2013b; Vincent et al., 2015). Pigs with a low RFI consume less feed than expected and pigs with a high RFI consume more feed than expected. Pigs in the low-RFI line tend to have less backfat and a lower ADG (Young et al., 2011).

To achieve the ultimate goal of more efficient pork production, suitable biomarkers for feed efficiency must first be identified. These biomarkers should be abundant, easy to quantify, and supported by physiological pathways differing between the RFI lines. Therefore, the objectives of this research were to 1) identify protein biomarkers for swine feed efficiency in the serum of young pigs and 2) identify proteins and pathways that are different between pigs that differ in RFI to provide new knowledge and insight in how these pathways regulate feed efficiency.

**MATERIALS AND METHODS**

**Animals and Serum Collection**

All animals were treated in accordance with procedures approved by the Iowa State University Animal Care and Use Committee (number 11-1-4996-S). Pigs from the eighth generation of the Iowa State RFI selection project were used for this study (Cai et al., 2008; Arkfeld et al., 2015; Mpetile et al., 2015). SST Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) were used to collect blood samples from the jugular vein of pigs between 35 and 42 d of age. Samples were allowed to clot and stored in a cooler on ice no more than 4 h after collection before centrifugation. Serum was collected and stored at −80°C until use. At the time of sampling, pigs were fed a standard corn–soybean–dried distillers' grain with solubles diet that met or exceeded nutrient and energy requirements for this size pig (NRC, 1998).

Pigs (n = 78 low-RFI and n = 80 high-RFI) were individually fed to market weight (121 ± 7.2 kg; Arkfeld et al., 2015). During the finishing phase, each line of pigs was divided into 2 groups; one-half was fed a high-energy/low-fiber diet and the other half was fed a low-energy/high-fiber diet (Table 1). Feed intake data were collected during finishing, and RFI was calculated for each animal by adjusting ADFI for the covariates of ADG, backfat, metabolic BW, on-test weight, on-test age, off-test weight, and off-test backfat (Cai et al., 2008). To account for differences in feed requirements between lines and diets, each of these covariates was fitted as an interaction with line and diet.

**Table 1.** Pigs from the eighth generation of the Iowa State University (Ames, IA) residual feed intake (RFI) selection project were used. Pigs from 2 lines that were divergently selected for RFI during finishing were placed on either a high-energy/low-fiber diet or a low-energy/high-fiber diet during finishing. Serum samples were collected between 35 and 42 d of age

<table>
<thead>
<tr>
<th>Diet</th>
<th>High RFI</th>
<th>Low RFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High energy/low fiber</td>
<td>n = 40</td>
<td>n = 41</td>
</tr>
<tr>
<td>(n = 81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low energy/high fiber</td>
<td>n = 40</td>
<td>n = 37</td>
</tr>
<tr>
<td>(n = 77)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Animal Selection**

Proteome profiles of serum from young pigs were compared based on the RFI performance during finishing. A total of 3 comparisons were made between the high- and low-RFI pigs (n = 36; Table 1). Animals selected for these 3 comparisons were from the larger cohort of pigs described above (n = 158). The first 2 comparisons were made within the high-energy/low-fiber diet and within the low-energy/high-fiber diet. To create large contrasts in RFI, for each diet, 6 pigs with lowest RFI within the low-RFI line were compared with 6 pigs with highest RFI within the high-RFI line (Table 2). Subsequently, however, RFI was recalculated for each pig based on a joint analysis with pigs from a second replicate of the line × diet trial to improve adjustments of feed intake for growth and backfat to compute RFI. This resulted in some reranking of animals within line and diet such that the animals used in the first 2 comparisons did not represent the extremes within line and diet anymore. Therefore, based on these recalculated RFI values, a third comparison was made within the low-energy/high-fiber diet, using 6 pigs with lowest new RFI values from the low-RFI line and 6 pigs with highest new RFI values from the high-RFI line. This third experiment was used to confirm observations in the first 2 comparisons after RFI values were recalculated.

**Sample Preparation**

Protein concentration was determined on each serum sample using a Quick-Start Bradford Protein Assay (Bio-Rad, Hercules, CA). Serum samples were...
Table 2. Three comparisons using 2-dimensional difference in gel electrophoresis (2D-DIGE). Average residual feed intake (RFI) of the evaluated animals is shown for the less efficient high-RFI line and the more efficient low-RFI line under the 2 diets (n = 6 pigs per line/diet) in each of the 3 comparisons. Residual feed intake for each pig was based on a joint analysis of 2 replicates of a larger line × diet trial, with covariate adjustment of feed intake for growth and backfat by line and diet to derive RFI.

<table>
<thead>
<tr>
<th>Item</th>
<th>High RFI (SE), kg feed/d</th>
<th>Low RFI (SE), kg feed/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>High energy/low fiber comparison</td>
<td>1.437 (0.136)</td>
<td>–0.596 (0.040)</td>
</tr>
<tr>
<td>Low energy/high fiber comparison</td>
<td>0.255 (0.155)</td>
<td>–1.823 (0.063)</td>
</tr>
<tr>
<td>Low energy/high fiber extreme</td>
<td>0.555 (0.041)</td>
<td>–2.301 (0.084)</td>
</tr>
</tbody>
</table>

Two-Dimensional In Gel Electrophoresis

Using whole serum (Grubbs et al., 2015), three 2-dimensional difference in gel electrophoresis (2D-DIGE) comparisons were performed using methods previously described (Grubbs et al., 2013b, 2014). Fifty micrograms of protein from each serum sample was labeled with CyDye 3 or 5 dyes for minimal labeling according to the manufacturer’s directions (GE Healthcare, Piscataway, NJ). CyDyes were alternated between samples from the high- and low-RFI group to avoid bias for dye binding. CyDye 2 for minimal labeling was used to label the pooled reference sample (containing an equivalent amount from each sample within a comparison) for each gel. For the first dimension, immobilized pH gradient (IPG) strips (11 cm, pH 4–7; GE Healthcare) were used. Each strip was loaded with a total of 45 μg of labeled protein, 15 μg of protein labeled with CyDye 3 or 5, and 15 μg of the pooled reference (CyDye 2). DeStreak Rehydration Solution (GE Healthcare) with 2.5 mM DTT was added to the protein mixture to the volume specified by the strip manufacturer (200 μL). The labeled protein mixture was added to individual wells in a reswelling tray along with the IPG strips and allowed to passively rehydrate overnight at room temperature in a humidified chamber. Isoelectric focusing was performed on an Etan IPGphor isoelectric focusing system (GE Healthcare) for a total of 14,000 Vh (step: 500 V for 1,500 Vh; gradient: 1,000 V for 800 Vh; gradient: 6,000 V for 8,800 Vh; and step: 6,000 V for 2,900 Vh).

After isoelectric focusing, strips were equilibrated using two 15-min washes, first with equilibration buffer (50 mM Tris-HCl [pH 8.8], 6 M urea, 30% glycerol, 2% SDS, and trace amounts of bromophenol blue) with 65 mM DTT and second with equilibration buffer with 135 mM iodoacetamide. Equilibrated strips were loaded onto 12.5% SDS-PAGE (26 by 20 cm) gels (acylamide: N,N′-bis-methylene acrylamide 100:1, 0.1% SDS, 0.05% tetramethylethylenediamine, 0.05% ammonium persulfate, and 0.5 M Tris-HCL, pH 8.8) using agarose as an overlay. An Etan DALT SIX system (GE Healthcare) was used to resolve isoelectrically focused proteins. Gels were run at 100 V for approximately 2,400 Vh. Each gel was run in duplicate, with separate isoelectric focusing steps and SDS-PAGE steps. Gels were imaged using an Etan DIGE Imager (GE Healthcare). Images were processed and analyzed using DeCyder 2D software version 6.5 (GE Healthcare). For each 2D-DIGE comparison, 2 technical replications were performed. Statistical differences for each spot within a comparison were determined in DeCyder by a Student’s t-test. The abundance of each individual spot was normalized using the pooled CyDye 2 sample present on each gel in the comparison. The logarithm of the standardized protein abundance of each spot was compared in a t-test using the DeCyder 2D Software (version 6.5) Biological Variation Analysis module (GE Healthcare) as described by Grubbs et al. (2013b) and Cruzen et al. (2013).

Protein Identification

Following image analysis, additional 2-dimensional SDS PAGE gels were run in a similar fashion as previously described (pH 4–7, 12.5% SDS-PAGE gels). These gels were loaded with 800 μg of protein and stained with colloidal Coomassie stain (Grubbs et al., 2014). After staining, protein spots that were determined to be significantly (P < 0.05) different in abundance between high- and low-RFI pigs in the DeCyder analysis in at least 1 of the 3 comparisons were excised for protein identification. Protein identification was performed at the Iowa State University Protein Facility (Ames, IA) using liquid chromatography–tandem mass spectrometry (MS/MS) on a Q-Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo-Fisher Scientific, Waltham, MA). Protein in the excised gel plugs was digested via tryptic digest followed by fractionation of peptides.
via liquid chromatography and MS/MS. Data from the MS/MS were analyzed using Proteome Discoverer software (Thermo-Fisher Scientific) and processed using a MASCOT database search (Grubbs et al., 2013b) to determine protein identities.

**Western Blotting and Phosphoprotein Staining**

Two-dimensional western blots for serpinA3 were used to confirm the identity of serpinA3 in the serum, using methods previously described (Grubbs et al., 2013a). Briefly, 80 μg of serum protein was loaded on a 7-cm pH 4 to 7 IPG strip (total 7,250 Vh [step: 300 V for 1.5 h; gradient: 1,000 V for 300 Vh; gradient: 5,000 V for 4,500 Vh; and step: 5,000 V for 2,000 Vh]). The second dimension was performed on 12.5% SDS-PAGE gels (10 by 10.5 cm) run at 120 V for approximately 330 Vh. Traditional western blot protocols were followed (Melody et al., 2004). Blots were probed with a primary anti-serpinA3 polyclonal antibody at a concentration of 1:1,000 (Abnova Corporation, Walnut, CA; catalog number H00000012-D01) followed by a secondary goat anti-rabbit antibody at a concentration of 1:10,000.

Two-dimensional staining for phosphorylated peptides was performed to determine if proteins were post-translationally modified via phosphorylation (Grubbs et al., 2014). Two-dimensional electrophoresis was performed by loading 200 μg of protein on 11-cm pH 4 to 7 IPG strips for the first dimension followed by running 12.5% SDS-PAGE gels. Gels were stained for phosphoproteins using ProQ Diamond Stain (Molecular Probes, Eugene, OR). Gels were then stained for total protein using SyPro Ruby total protein stain (Molecular Probes). Images were collected using an Etta DIGE imager and processed using DeCyder for visual confirmation of phosphorylated and total protein.

**RESULTS**

The pigs used for the 2D-DIGE comparisons in the current experiments were from a larger study, as shown in Table 1. In each of the three 2D-DIGE comparisons, average RFI was different between high- and low-RFI groups (Table 2). In the low-energy/high-fiber diet comparison, 372 protein spots were observed across at least 50% of the gel images. Of these 372 spots, 42 were more abundant in the pigs from the high-RFI group and 52 were greater in the low-RFI group ($P < 0.05$). In the high-energy/low-fiber diet comparison, 427 protein spots were observed in at least 50% of the gel images. A total of 63 spots were greater in the high-RFI group and 49 were greater in the low-RFI group ($P < 0.05$). For the extreme RFI phenotype comparison, in the low-energy/high-fiber diet comparison, a total of 492 proteins spots were observed in at least 50% of the gel images. A total of 89 protein spots were different between the high- and low-RFI groups ($P < 0.05$) with 48 spots increased in the low-RFI group and 41 increased in the high-RFI group.

A total of 29 protein spots were chosen for identification using mass spectrometry. Proteins were selected based on being statistically different between 2 groups in at least 1 of the 3 comparisons and on sufficient abundance as visually assessed in the pick gel. Peptides, generated by tryptic digest, were sequenced to determine the parent protein identity. Identifications were possible on 18 of the 29 spots submitted (Table 3; Fig. 1). Proteins identified included serpinA3 (14 spots), gelsolin (2 spots), and vitronectin (2 spots). Differences in protein abundance between the high- and low-RFI groups across the 3 comparisons are reported in Table 4. Identification of the same protein in multiple spots indicates presence of different isoforms of same protein due to variation in AA sequence or post-translational modification. This provides an explanation for the observation of identical peptides being identified across multiple spots. For example, R.RIDALHLP.R was found in 4 of the 5 spots identified as serpinA3-6 (Table 3).

Two spots identified as gelsolin (192 and 194) were more abundant in the low-RFI line across all 3 comparisons (9 to 39%; $P = 0.01$ to 0.036; Table 4). The abundance of vitronectin varied in response to RFI line across the 3 comparisons. For the 2 spots identified as vitronectin (346 and 354), no difference in abundance was observed for the traditional high-energy/low-fiber diet ($P > 0.05$), whereas for the high-fiber diet, a greater abundance (55–56%; $P < 0.005$) in the low-RFI group was observed. In the extreme RFI comparison, spot 346 tended to be greater in abundance in the low-RFI group ($P = 0.075$) and spot 354 was not different ($P > 0.1$).

Fourteen spots were identified as serpinA3, which was confirmed using a 2-dimensional western blot (Fig. 2). Eight of the 14 spots identified as serpinA3 were at least twice as abundant in the low-RFI group in at least 1 of the 3 comparisons (Table 4). SerpinA3 expression was variable in response in each of the 3 comparisons. For the traditional high-energy/low-fiber diet, 7 of the 14 spots were significantly ($P < 0.1$) or tended to be more abundant in the low-RFI group and 6 were not different. For the high-fiber/low-energy diet, 8 of the 14 spots were significantly greater in the low-RFI group, 1 was less abundant in the low-RFI group, and 4 did not differ between groups. In the extreme RFI comparison, 5 spots were more abundant in the low-RFI group, 2 were less abundant in the low-RFI group, and 7 did not differ between groups.
DISCUSSION

Pigs from the RFI selection project at Iowa State University have been extensively studied (Boddicker et al., 2011; Young et al., 2011; Cruzen et al., 2013; Arkfeld et al., 2015). Genetic control of differences in feed efficiency has been demonstrated through this RFI selection program. Data provided from these projects have yielded a large knowledge base to suggest potential biological pathways that are impacted by selection for RFI. Pigs from the more efficient low-RFI line have been shown to produce less mitochondrial reactive oxygen species, leading to less cellular oxidative damage and a shift in metabolism from repair and replacement to protein accretion (Grubbs et al., 2013a). The protein profile of the mitochondria of low-RFI pigs corroborates these data; mitochondria from low-RFI pigs had a greater ability to handle cellular stress through a greater abundance of heat shock protein 60 and 70 and of many metabolic enzymes related to the tricarboxylic acid cycle and ATP synthesis (Grubbs et al., 2013b, 2014). Our current working hypothesis is that the greater protein accretion of pigs from the low-RFI line is due to the molecular changes and metabolic response to selection for improved RFI (Harris et al., 2012; Cruzen et al., 2013; Grubbs et al., 2013a). These data demonstrate that pigs from the Iowa State University RFI selection lines differ not only in feed efficiency but also in the biology underlying feed efficiency. This model provides documented

Table 3. Protein identification and peptides of identified spots submitted for mass spectrometry

<table>
<thead>
<tr>
<th>Spot ID</th>
<th>Protein ID</th>
<th>Species</th>
<th>Accession</th>
<th>pI</th>
<th>Coverage, %</th>
<th>Mass, kDa</th>
<th>Peptides</th>
<th>Mouse score</th>
</tr>
</thead>
<tbody>
<tr>
<td>192</td>
<td>Gelsolin</td>
<td>Sus scrofa</td>
<td>gi</td>
<td>350579587</td>
<td>9.17</td>
<td>29.6</td>
<td>8</td>
<td>K.HVVPNVEAQV_R.R.AEVPWSNFRG</td>
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<tr>
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<td>Gelsolin</td>
<td>Sus scrofa</td>
<td>gi</td>
<td>350579587</td>
<td>9.17</td>
<td>29.6</td>
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<tr>
<td>312</td>
<td>SerpinA3-4</td>
<td>Bos taurus</td>
<td>gi</td>
<td>313471467</td>
<td>6.32</td>
<td>46.0</td>
<td>5</td>
<td>R.GPTLEIEGLK_F.K.DTQSIIFLGK_V</td>
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<tr>
<td>316</td>
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<td>Sus scrofa</td>
<td>gi</td>
<td>194038353</td>
<td>5.75</td>
<td>47.4</td>
<td>7</td>
<td>R.RIDALHLPR_F.R.KSFENFIV_R.FDPSPLFATVLR_D</td>
</tr>
<tr>
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<td>gi</td>
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<td>5.75</td>
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<td>R.RIDALHLPR_F.R.FDPSPLFATVLR_D</td>
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<td>gi</td>
<td>1754491</td>
<td>5.48</td>
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<td>Vitronecin</td>
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</tr>
<tr>
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<td>Sus scrofa</td>
<td>gi</td>
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<td>49.0</td>
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<td>5.75</td>
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<td>K.LLDKFIQODAR.E.R.KSFENFIV_R.R.RIDALHLPR_F</td>
</tr>
</tbody>
</table>

1ID = identity.
2pI = isoelectric point.
molecular, metabolic, physiological, and performance response to selection for RFI, which provides opportunities to use this model to discover and define robust protein biomarkers for efficiency. Using this model, biomarkers can be identified that are abundant and easy to quantify. Moreover, biomarkers that prove to be consistent with differences in protein accretion, growth performance, and RFI are likely to be robust markers for swine performance outside of our model.

Accurate prediction of feed efficiency of pigs at an early age will accelerate the speed of genetic improvement and increase production efficiency. Serum from blood is well documented as a source of biomarkers due to the dynamic nature of proteins in the serum and their relationship to biological status (Ahn and Simpson, 2007). Ultimately, serum could be collected from young pigs and analyzed for a biomarker or biomarkers to classify the pigs as either low or high efficiency more quickly without the costs of traditional methods to measure feed efficiency.

Based on the 2D-DIGE data and the 18 protein spots identified in this study, 3 proteins have been identified
that could serve as potential serum biomarkers for RFI and thereby feed efficiency in pigs: gelsolin, vitronectin, and serpinA3. These proteins are abundant in sera, allowing ease of quantification, and most importantly, they are involved in known biological pathways that contribute to variation in feed efficiency through energy utilization and homeostasis. Gelsolin is a protein commonly found in the blood and associated with actin filament assembly and immune response (Cohen et al., 2011). Vitronectin, also commonly found in the blood, stabilizes plasminogen activator inhibitor-1 (PAI-1) to inhibit plasminogen-initiated proteolysis (Czekay et al., 2003), indicating a role in blood hemostasis and, potentially, nutrient management. Serine protease inhibitor A3, also known as α-1-antichymotrypsin or serpinA3, is part of a larger family of proteins that contains in excess of 700 members. Physiological functions of serpinA3 have been linked to many disease states such as Alzheimer’s disease and cancers and have been shown to have roles in immune system function and inflammatory response (Baker et al., 2007).

**Gelsolin**

Gelsolin, also known as brevin, actin-depolymerizing factor, and AGEL, is known as a regulator of actin filament assembly and as an immune system protein (Cohen et al., 2011) and is primarily produced in the liver and muscle tissue. There are 2 isoforms of gelsolin, cytoplasmic and plasma, which have molecular weights of 90 and 93 kDa, respectively (Yin et al., 1984; Kothakota et al., 1997). These 2 isoforms are from a

![Figure 2. Confirmatory 2-dimensional western blot for serine protease inhibitor A3 (serpinA3). The first dimension was run on a 7-cm pH 4 to 7 immobilized pH gradient strip. The second dimension was run on a 12.5% SDS-PAGE gel. Primary antibody was an anti-serpinA3 (Abnova, Walnut, CA; 1:1,000) with a goat anti-rabbit conjugated to horseradish peroxidase (1:10,000) secondary antibody. MW = Molecular Weight.](image-url)
Biomarkers for feed efficiency in pigs

Vitronectin

Vitronectin is known to interact with serpins in the blood and play a role in blood homeostasis (Ma et al., 2004). Vitronectin associates with the B, C, and E classes of serpins, namely serpin B2 (Ehrlich et al., 1990) and C1 and E1 (Czekay et al., 2003). Vitronectin has a high affinity for PAI-1 (Czekay et al., 2003), and such binding extends the active life of PAI-1 (Zhou et al., 2003). Plasminogen activator inhibitor-1 is encoded by the serpin E1 gene in mammals (Czekay et al., 2003). Plasminogen activator inhibitor-1 has been linked with thrombosis and fibrosis (Landin et al., 1990) as well as with insulin resistance and obesity (Lijnen et al., 2003).

The ability to inhibit thrombin, a known inflammatory mediator, is increased 200-fold when PAI-1 is bound to vitronectin (Lijnen, 2005). Inhibition of thrombin would allow less disruption of interendothelial junctions in blood vessels (Mehta and Malik, 2006). The greater abundance of vitronectin in the low-RFI pigs and vitronectin’s control of thrombin through PAI-1 are in agreement with the lower production of reactive oxygen species by the mitochondria of pigs from this line (Grubbs et al., 2013a), indicating that the low-RFI line may be under less overall oxidative stress, at both inter- and extracellular sites. Lower inflammatory stress on endothelial cells could indicate an improved nutrient movement into the cell, providing for more efficient growth. The lower fat composition of the low-RFI line (Harris, 2012), coupled with the greater vitronectin abundance and lower oxidative stress, may indicate a role of vitronectin in the biological pathways associated with feed efficiency.

SerpinA3

Originally identified in the late 1970s and early 1980s (Chandra et al., 1983), α-1-antichymotrypsin, now known as serine protease inhibitor A3 (serpinA3), was first identified as an acute phase plasma protease inhibitor. Since this initial discovery, over 13 different but closely related gene products have been identified across various tissues and species (Gettins, 2000; Wågsäter et al., 2012). Other common names for serpinA3 include endopin1 and endopin2, depending on the specific gene cluster (Pelissier et al., 2008). Originally identified in mice, serpinA3 has been identified in 2 major animal agriculture species to date, beef cattle and pigs (Baker et al., 2007). Reported concentrations of α-1-antichymotrypsin or serpinA3 in blood vary from 1.0 mg/mL in cattle (Gagaoua et al., 2012) to 2.5 mg/mL in human plasma (Travis and Salvesen, 1983).

SerpinA3 is a member of a 700-plus member family of serine protease inhibitors that are responsible for a wide range of biological processes in many tissue types. SerpinA3 has many proposed biological roles and has several protein isoforms, each coded by a different gene in the same region of a single chromosome (Pelissier et al., 2008; Herrera-Mendez et al., 2009; Gagaoua et al., 2012). Gene expression for serpinA3 has been iden-
Spots identified as serpinA3-8 and serpinA3-6 differed in abundance between the low- and high-RFI groups. The full AA sequences for serpinA3-8 and serpinA3-6 are known for cattle, but in the pig, AA sequence data is limited and the multiple isoforms of serpinA3 are only predicted at this point. The 3 isoforms of serpinA3 we identified were separated by approximately 10 kDa in molecular weight on the 2-dimensional SDS-PAGE gel. Isoforms of serpinA3 are known to vary in overall molecular weight (Pelissier et al., 2008).

Proteins with multiple spots on a gel often indicate some type of post-translational modification (Rogowska-Wrzesinska et al., 2013), mostly through phosphorylation. However, to date, no phosphorylations of serpinA3 have been reported. This was confirmed in the current study through the use of a phosphoprotein stain on a 2-dimensional SDS-PAGE gel, which did not identify presence of phosphoproteins in the known locations of serpinA3 (Fig. 3). To date, there is a limited amount of data on the role of these post-translational modifications (Hwang et al., 1999; Péré-Brissaud et al., 2015).

Spots identified as serpinA3 were consistently greater in abundance in the more efficient low-RFI group of pigs; in many spots, they were more than twice as abundant. This suggests that serpinA3 is a candidate biomarker for feed efficiency. Importantly, both serpinA3-6 and serpinA3-8 were identified. Across the three 2D-DIGE comparisons, serpinA3-8 was consistently greater in abundance (4 spots over 100%) in the low-RFI group. SerpinA3-6 was also consistently more abundant in the low-RFI group, but its difference in abundance was not as large as that of serpinA3-8. These data indicate that some isoforms of serpinA3, specifically serpinA3-8, may serve as more robust biomarker than others.
Bovine serpinA3-1 through serpinA3-6 are potent inhibitors of trypsin. Bovine serpinA3-7 and serpinA3-8 inhibit elastase rather than trypsin (Tassy et al., 2005; Gagaoua et al., 2012). SerpinA3-1 and serpinA3-3 have been shown to play a role in inflammatory response in cattle (Herrera-Mendez et al., 2006). Our data, coupled with previously published data, show that serpinA3 has a role in feed efficiency and is a potential biomarker.

Analysis of gene expression profiles in blood from these same pigs by RNA sequencing did not identify RNA expression of serpinA3 in whole blood (H. Liu and C. K. Tuggle, Iowa State University, personal communication). This highlights the importance of protein versus RNA biomarkers. It is well known that serpinA3 is produced in many tissues, including the liver and muscle (Baker et al., 2007). This tissue-based production of serpinA3 may indicate a direct tissue-specific biological role of serpinA3 in feed efficiency through metabolic energy utilization within tissues such as muscle.

**Implications**

The biological roles of gelsolin, vitronectin, and serpinA3 indicate their potential as biomarkers for feed efficiency and lean growth in pigs. The use of serum from young pigs for this study followed by calculating RFI during finishing demonstrates that biological differences between animals with differing feed efficiencies can be observed early in production. Integration of these data, as well as more targeted approaches that validate the contribution of the 3 proteins identified as well as other proteins not identified in this study to predict feed efficiency, has potential to make pork production more efficient.

**LITERATURE CITED**


Harris, A. J. 2012. Defining physiological differences between gilts divergently selected for residual feed intake. MS Thesis, Iowa State University, Ames, IA.