Differential composition of proteomes in sow colostrum and milk from anterior and posterior mammary glands

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ABSTRACT: Piglets obtaining milk from anterior and middle mammary glands (MG) grow faster than those suckling posterior MG, but the underlying mechanisms are not clear. The purpose of this study was to investigate the differential proteomes of colostrum and milk secreted by anterior and posterior MG. Six healthy primiparous sows with 7 pairs of MG were used; the first and the second pairs were defined as anterior MG and the sixth and seventh pairs as posterior MG. Colostrum and milk were collected at d 1 and 14 after parturition, respectively. Comparative proteomics analysis was performed to identify the differentially expressed proteins in colostrum and milk secreted by anterior and posterior MG. Results show that protein composition in colostrum and milk varied markedly with the anatomical location of MG. Immunoglobulins, lactadherin, and haptoglobin were upregulated (P < 0.05) in colostrum from anterior MG compared with posterior MG. Concentrations of immunoglobulins and lactoferrin in milk from anterior MG were greater (P < 0.05) than milk from posterior MG. Moreover, concentration of proteins from somatic cells was greater (P < 0.05) in milk from posterior MG compared with anterior MG. Most proteins, in which abundance was upregulated in colostrum and milk from anterior MG, contribute to passive immunity, intestinal development of suckling piglets and epithelial integrity, and the health of MG. Collectively, these results indicate that in comparison with posterior MG, anterior MG are more active in protein synthesis and produce more immunoglobulins and lactoferrin in colostrum and milk.

Key words: anterior mammary gland, colostrum, milk, posterior mammary gland, proteome, sow

INTRODUCTION

For suckling piglets, maternal colostrum and milk are the sources of very digestible nutrients (Lin et al., 2009) and various forms of bioactive compounds, including immunological defense factors, hydrolytic enzymes, hormones, and growth factors (Michaeliclou and Steijns, 2006). The notion that bioactive compounds in milk can influence physiological development of the suckling young is widely accepted (Walzem et al., 2002; Politis and Chronopoulou, 2008). Therefore, the sufficient provision of milk is crucial for the immature newborns.

In pig production, it is important to produce healthy, heavy, and uniform piglets at weaning. Within the first few hours after birth, competition for mammary glands (MG) occurs among newborn piglets, the anterior and middle MG are preferred, and the offspring that acquire milk from the anterior and middle MG grow faster than piglets suckling posterior MG (Puppe and Tuchscherer, 1999; Kim et al., 2000). The underlying mechanisms are not clear although several explanations have been proposed, which include differences in anatomical structures (Algers, 1993; Nielsen et al., 2001) and amounts of milk production (Skok et al., 2007) between anterior and posterior MG. However, other workers have reported no difference in milk production between anterior and posterior MG (Hartman et al., 1962).

We hypothesized that proteomes in colostrum or milk from porcine anterior and posterior MG are differentially expressed, which may explain, in part, why piglets suckling anterior MG grow faster than their littermates suckling posterior MG. This hypothesis was tested using 2-dimensional gel electrophoresis (2-DE) coupled with matrix-assisted laser desorption/ioniza-
tion-time of flight (MALDI-TOF) mass spectrometry (MS); Wang et al., 2006a, 2009a,b).

**MATERIALS AND METHODS**

All animal procedures were performed according to protocols approved by the China Agricultural University Animal Care and Use Committee.

**Animals and Experiment Design**

Six primiparous sows (Dalland, Sino-Dutch Animal Husbandry Training and Demonstration Center, Beijing, China) were used in the present study. Mean litter size was 8.7 piglets per sow with a range from 7 to 11 piglets. Sows with 14 MG were selected for this study. Mammary glands were categorized as described by Kim et al. (2000). The first and second pairs of MG were defined as anterior MG, the third, fourth, and fifth pairs of MG were defined as middle MG, and the sixth and seventh pairs of MG were defined as posterior MG. Fresh colostrum or milk samples from anterior and posterior MG were collected at 0800 h on d 1 and 14 after parturition. Colostrum and milk samples were immediately frozen at −70°C until analyzed. Milk samples from middle MG were not obtained because middle glands behaved similarly to anterior glands according to our previous study (Kim et al., 2000).

**Whey Preparation**

Whey was prepared from each sample as follows. Colostrum and milk samples were defatted by centrifugation at 7,500 × g and 4°C for 10 min (Wu and Knabe, 1994). The precipitated casein was removed by centrifugation (100,000 × g for 1 h at 4°C), and the resulting whey was stored at −70°C until analyzed. Milk samples from middle MG were not obtained because middle glands behaved similarly to anterior glands according to our previous study (Kim et al., 2000).

**2-Dimensional Gel Electrophoresis**

Protein content in whey was determined by the Bradford Coomassie Blue dye-binding method (Bio-Rad, Hercules, CA) using BSA (Sigma, St. Louis, MO) as a standard (Bradford, 1976). One milligram of protein was used for each analytical gel after suspension in 450-µL solution containing 7 M urea, 2 M thiourea, 4% 3-[3-[(cholamidopropyl)-dimethylammonio]-1-propanesulfonate, 50 mM dithiothreitol, and 0.5% immobilized pH gradient (IPG) buffer of pH 3 to 10 (Wang et al., 2009b)]. After a brief vortex, samples were subjected to gel-rehydration loading for 12 h at 20°C using pH 3–10NL 24-cm-long IPG strips (GE Healthcare, Piscataway, NJ). After full incorporation of the samples, IPG strips were transferred to a IPGphor system (GE Healthcare). Isoelectric focusing was then performed at 20°C by a series of increasing voltage steps as follows: 30 V for 1 h, 300 V for 2 h, 500 V for 2 h, 1,000 V for 3 h, linear gradient to 10,000 V over 3 h, and 10,000 V, which was held constant until a total of 100,000 voltage hours (Görg et al., 2000).

After the first dimension, IPG strips were equilibrated twice for 15 min under gentle stirring with a solution containing 6 M urea, 50 mM Tris-HCl pH 8.8, 30% (vol/vol) glycerol, and a trace of bromophenol blue. To the first equilibration solution, 1% dithiothreitol was added. To the second equilibration solution, 2.5% iodoacetamide was added. The second dimension was performed by the use of 12.5% SDS-PAGE gels. The IPG strips were put on top of the SDS gels, which were run at 30 mA/gel for 30 min and then at 50 mA/gel until the bromophenol blue came out of the gels. Analytical gels were stained with Coomassie Brilliant Blue G-250 (Wang et al., 2005).

High-resolution gel images (400 dpi) were obtained using an image scanner (model PowerLook 2100XL ImageScanner, UMAX Technologies, Atlanta, GA), and image analysis was performed (Image-Master 2D Platinum Version 6.01, GE Healthcare; Wang et al., 2009b). After normalizing the quantity of each spot by total valid spot intensity, differentially expressed protein spots with a variation over 1.5-fold in the relative percent volume were selected for further identification, as recommended (GE Healthcare).

**In-Gel Digestion of Protein and Mass Spectrometric Analysis**

Differentially expressed protein spots were cut from the gel and in-gel protein digestion was performed (Wang et al., 2005). Briefly, the destained gel pieces were completely dried by vacuum centrifugation (200 × g, 30 min, room temperature; Eppendorf Concentrator 5301, Hamburg, Germany). Then, 2 µL of trypsin (10 µg/mL) in 25 mM ammonium bicarbonate solution was added to each gel piece. The mixture was incubated at 4°C for 1 h to rehydrate in the trypsin solution and then incubated at 37°C for 12 h. The resulting peptides were extracted 3 times at 37°C by 8-µL aliquots of 5% (vol/vol) trifluoroacetic acid (TFA) for 1 h, 2.5% (vol/vol) TFA in 50% (vol/vol) acetonitrile (ACN) for 1 h, and 100% (vol/vol) ACN for 1 h. The peptide solution was then dried in a vacuum centrifugation (200 × g, 3 h, room temperature) and resolubilized in 2 µL of 0.5% (vol/vol) TFA for MS analysis.

The peptide fragments produced from each protein spot were used to generate peptide mass fingerprinting (PMF) data (Bruker Reflex MALDI-TOF MS analysis, Bruker Daltonik, Bremen, Germany) at the reflection mode setting (Wang et al., 2005). Briefly, a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% (vol/vol) ACN and 0.1% (vol/vol) TFA was used as matrix. One microliter of the matrix solution was added to each gel piece. After air-drying, the gel pieces were then analyzed in a Bruker Reflex MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) at the reflection mode setting (Wang et al., 2005). Briefly, a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% (vol/vol) ACN and 0.1% (vol/vol) TFA was used as matrix.
and 1 µL of sample solution were mixed and loaded onto the Score384 target well. The variables used for the PMF analysis were 20 kV accelerating voltage and 23 kV reflecting voltage.

**Protein Identification**

The PMF analysis was performed [MASCOT search engine (http://www.matrixscience.com), Matrix Science, London, UK]. Monoisotopic peptide masses were used to search the databases. A peptide mass accuracy was 0.2 Da. One missed cleavage was allowed. The carbamidomethylation of cysteine and oxidation of methionine were set as variable modifications. For protein identification by PMF, peptide masses were searched against the database for other mammalian species (MSDB 20060831, Imperial College London, London, UK).

**Western Blotting**

Extracted proteins (30 µg/sample) were separated by electrophoresis (Bio-Rad, Richmond, CA) on 12.5% SDS-PAGE before transferred electrophoretically to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA). After blocking with Tris-buffered saline-Tween-20 (TBST; 0.05% Tween 20, 100 mM Tris-HCl, and 150 mM NaCl, pH 7.5) containing 5% fat-free dry milk at 4°C overnight, the membranes were incubated with primary antibodies (i.e., anti-lactoferrin, anti-IgA, and anti-serum albumin in dilution of 1:500, 1:300, and 1:300, respectively), for 2 h. The membranes were rinsed in TBST and incubated with a secondary antibody (horseradish peroxidase-labeled antirabbit IgG diluted in 1:5,000) for 2 h. The protein bands were visualized with a chemiluminescence substrate using a gel-imaging system (Tanon Science and Technology, Shanghai, China), and then the films were scanned. The optical densities of proteins were analyzed with software (Quantity One 1-D Analysis Software, Bio-Rad).

**Statistical Analyses**

Optical densities of proteins are expressed as means ± SEM. The relations of particular protein expression between anterior MG and posterior MG were analyzed using Student’s t-test. The P-value of <0.05 was used to indicate statistical significance.

**RESULTS**

**Expression of Proteins in Sow Colostrum from Anterior and Posterior MG**

The 2-DE run for 6 independent biological replicates of colostrum and milk from anterior and posterior MG detected differentially expressed protein spots (P < 0.05 and ratio >1.5 or −1.5). Seventeen protein spots were identified through the MALDI-TOF-MS analysis. There are 8 differentially expressed protein spots between colostrum from anterior and posterior MG (Figures 1 and 2). Proteins such as serum albumin, Ig µ-chain, Ig γ-chain precursor, lactadherin, haptoglobin, Ig γ-chain, Ig α-chain C region, C8: Ig α-chain C region; M1: lactoferrin; M2: Ig α-chain C region; M3: Ig κ-chain VJ region; M4: myosin-4; M5: lymphocyte cytosolic protein 1; M6: creatine kinase B-type; M7: pregnane X receptor; M8: phosphoglycerate kinase; M9: 14-3-3 protein zeta chain.

**Expression of Proteins in Sow Milk from Anterior and Posterior MG**

Nine proteins are differently expressed between milk from anterior and posterior MG (Figures 1 and 2). Proteins, such as lactoferrin, Ig α-chain C region, and Ig κ-chain VJ region, had increased expression in mature milk from anterior MG compared with that from posterior MG. Concentrations of proteins such as myosin-4, lymphocyte cytosolic protein 1, creatine kinase B-type, pregnane X receptor, phosphoglycerate kinase, and 14-3-3 protein zeta chain were decreased in mature milk from anterior MG compared with posterior MG. The biochemical information about these proteins is summarized in Table 1.
Validation of Proteomics Data by Western Blotting

Western blot analysis was performed for 3 proteins (lactoferrin, IgA, and serum albumin) randomly selected from Tables 1 and 2 for validation of proteomics data. Serum albumin was obtained from colostrum, whereas lactoferrin and IgA were obtained from mature milk (14 d after parturition). The expression of these proteins from anterior MG was greater than that from posterior MG ($P < 0.05$; Figure 3). This result is consistent with the finding from the proteomics analysis (Tables 1 and 2).

**DISCUSSION**

Proteomics plays an important role in discovery research to identify unexpected proteins in complex biological samples (e.g., colostrum and milk; Wang et al., 2009a). Proteins in human and bovine whey preparations have been investigated using the proteomics approach (Galvani et al., 2001; Yamada et al., 2002; Palmer et al., 2006; Fong et al., 2008). There were also studies of whey proteins in human milk at different phases of lactation (Murakami et al., 1998). However, a comparison of whey proteins from MG of different anatomical locations has not previously been reported for any species.

The lactating mammary gland secretes a wide array of proteins and has an important role in piglet growth (Kim and Wu, 2009). Piglets suckling anterior MG grow faster than those nursed by the remaining MG (Puppe and Tuchscherer, 1999; Kim et al., 2000). To help understand the underlying mechanisms, the present study investigated differential proteomes in colostrum and milk secreted by anterior and posterior MG. The novel and important finding from this work is that the proteomes of colostrum and milk were expressed differentially between porcine anterior and posterior MG. Eight proteins were upregulated in colostrum from anterior MG; 5 of them were immunoglobulin fractions (Ig µ-chain, poly-Ig receptor precursor, Ig γ-chain precursor, Ig α-chain C region, and Ig λ-chain C region). Another 2 proteins that were abundant in colostrum from anterior MG with antimicrobial and immunomodulatory activities were lactadherin and haptoglobin. However, only 3 of these 9 proteins were upregulated in mature milk (d 14 after parturition) from anterior MG compared with posterior MG: IgA (Ig κ-chain VJ region and Ig α-chain C region) and lactoferrin. The remaining proteins, which were downregulated in anterior MG compared with posterior MG, were all derived from milk-borne cells. The fact that only a few differentially expressed proteins were identified between anterior and posterior MG does not necessarily negate the significance of this study. Rather, the finding helps narrow our focus on potentially important proteins that may have crucial roles in the growth of sow-reared piglets.

Components of the immune system are present in fetal pigs immediately before farrowing and the fetus can synthesize IgG in response to an antigen challenge in utero (Sinkora et al., 2002). However, piglets are born with virtually no IgG in plasma mainly because the epitheliocorial nature of the porcine placenta prevents the transfer of maternal immunoglobulins to the fetus and fetuses are generally not exposed to antigens. After birth, proteins in colostrum can be absorbed directly by suckling piglets before gut closure to provide neonates with passive immune protection (Sangild et al., 1997; Danielsen et al., 2006). Sow colostrum is characterized by increased concentrations of IgG and reduced con-

**Figure 2.** Abundance of differentially expressed protein spots in 2-dimensional gels of colostrum and mature milk from anterior mammary glands (A) and posterior mammary glands (B) of lactating sows. C1: serum albumin; C2: Ig µ-chain; C3: poly-Ig receptor precursor; C4: lactadherin; C5: haptoglobin; C6: Ig γ-chain; C7: Ig α-chain C region; C8: Ig λ-chain C region; M1: lactoferrin; M2: Ig α-chain C region; M3: Ig κ-chain VJ region; M4: myosin-4; M5: lymphocyte cytosolic protein 1; M6: creatine kinase B-type; M7: pregnane X receptor; M8: phosphoglycerate kinase; M9: 14-3-3 protein zeta chain.
Table 1. Differentially expressed protein spots between sow colostrum from anterior and posterior mammary glands (MG)

<table>
<thead>
<tr>
<th>No.</th>
<th>Protein name</th>
<th>Accession No.</th>
<th>Score³</th>
<th>Sequence coverage,⁴ %</th>
<th>No. of matched peptides⁵</th>
<th>Expression level⁶</th>
<th>P-value⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Serum albumin (Sus scrofa)</td>
<td>ABPGS</td>
<td>91</td>
<td>28</td>
<td>12/31</td>
<td>22,500 ± 4,220</td>
<td>ND⁸</td>
</tr>
<tr>
<td>C2</td>
<td>Ig µ-chain (Sus scrofa)</td>
<td>AAC48775</td>
<td>150</td>
<td>58</td>
<td>15/44</td>
<td>88,030 ± 2,480</td>
<td>ND⁸</td>
</tr>
<tr>
<td>C3</td>
<td>Poly-Ig receptor precursor (Sus scrofa)</td>
<td>Q9N2H7</td>
<td>119</td>
<td>30</td>
<td>14/30</td>
<td>71,050 ± 2,280</td>
<td>43,640 ± 430</td>
</tr>
<tr>
<td>C4</td>
<td>Lactadherin (Sus scrofa)</td>
<td>T11743</td>
<td>200</td>
<td>53</td>
<td>18/38</td>
<td>10,610 ± 270</td>
<td>4,940 ± 50</td>
</tr>
<tr>
<td>C5</td>
<td>Haptoglobin (Sus scrofa)</td>
<td>AAM12554</td>
<td>166</td>
<td>55</td>
<td>22/46</td>
<td>119,890 ± 4,930</td>
<td>53,130 ± 1,930</td>
</tr>
<tr>
<td>C6</td>
<td>Ig γ-chain (Sus scrofa)</td>
<td>AAA51295</td>
<td>70</td>
<td>29</td>
<td>9/58</td>
<td>259,920 ± 4,530</td>
<td>168,470 ± 2,530</td>
</tr>
<tr>
<td>C7</td>
<td>Ig α-chain C region (Sus scrofa domestica)</td>
<td>I47175</td>
<td>148</td>
<td>54</td>
<td>14/38</td>
<td>567,630 ± 12,680</td>
<td>217,040 ± 5,580</td>
</tr>
<tr>
<td>C8</td>
<td>Ig λ-chain C region (Sus scrofa)</td>
<td>L1PG</td>
<td>67</td>
<td>68</td>
<td>4/23</td>
<td>975,440 ± 18,480</td>
<td>379,430 ± 7,920</td>
</tr>
</tbody>
</table>

¹Spot numbers refer to sample spot protein numbers that correspond to the labels in Figures 1 and 2.
²Accession No. is the MASCOT result of matrix-assisted laser desorption/ionization-time of flight searched from the MSDB 20060831 database.
³Score is $-10 \times \log (P)$, where $P$ is the probability that the observed match is a random event. A score of ≥59 indicates a significant match ($P < 0.05$) with the named protein.
⁴The minimum coverage of the matched peptides in relation to the full-length sequence.
⁵The number of matched peptides in the database search relative to total peptides from the protein.
⁶Protein expression quantity, expressed as mean ± SEM, n = 3 gels for anterior MG and posterior MG groups. All the identified spots were differentially expressed ($P < 0.05$).
⁷P-value from the Student’s t-test.
⁸ND = not detected.

Table 2. Differentially expressed protein spots between sow mature milk from anterior and posterior mammary glands (MG)

<table>
<thead>
<tr>
<th>No.</th>
<th>Protein name</th>
<th>Accession No.</th>
<th>Score³</th>
<th>Sequence coverage,⁴ %</th>
<th>No. of matched peptides⁵</th>
<th>Expression level⁶</th>
<th>P-value⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Lactoferrin (Sus scrofa)</td>
<td>P14632</td>
<td>278</td>
<td>60</td>
<td>44/97</td>
<td>510,230 ± 14,240</td>
<td>312,210 ± 6,790</td>
</tr>
<tr>
<td>M2</td>
<td>Ig α-chain C region (Sus scrofa)</td>
<td>I47175</td>
<td>90</td>
<td>38</td>
<td>6/21</td>
<td>49,360 ± 4,980</td>
<td>ND⁸</td>
</tr>
<tr>
<td>M3</td>
<td>Ig κ-chain VJ region (Sus scrofa)</td>
<td>AAM76076</td>
<td>63</td>
<td>64</td>
<td>7/68</td>
<td>16,230 ± 1,350</td>
<td>ND⁸</td>
</tr>
<tr>
<td>M4</td>
<td>Myosin-4 (Sus scrofa)</td>
<td>Q9TV62</td>
<td>232</td>
<td>35</td>
<td>53/102</td>
<td>ND⁸</td>
<td>770 ± 70</td>
</tr>
<tr>
<td>M5</td>
<td>Lymphocyte cytosolic protein 1 (Bos taurus)</td>
<td>Q3ZC00</td>
<td>120</td>
<td>31</td>
<td>16/33</td>
<td>350 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>M6</td>
<td>Creatine kinase B-type (Bos taurus)</td>
<td>AAI02334</td>
<td>154</td>
<td>36</td>
<td>16/31</td>
<td>ND⁸</td>
<td>8,180 ± 540</td>
</tr>
<tr>
<td>M7</td>
<td>Pregnan X receptor (Oryctolagus cuniculus)</td>
<td>Q9TU02</td>
<td>79</td>
<td>26</td>
<td>9/65</td>
<td>ND⁸</td>
<td>7,270 ± 290</td>
</tr>
<tr>
<td>M8</td>
<td>Phosphoglycerate kinase (Macropus eugenii)</td>
<td>PC1118</td>
<td>66</td>
<td>31</td>
<td>8/31</td>
<td>15,140 ± 480</td>
<td>26,830 ± 920</td>
</tr>
<tr>
<td>M9</td>
<td>14-3-3 protein zeta chain (Bos taurus)</td>
<td>S65013</td>
<td>138</td>
<td>44</td>
<td>13/29</td>
<td>2,630 ± 140</td>
<td>4,690 ± 320</td>
</tr>
</tbody>
</table>

¹Spot numbers refer to sample spot protein numbers that correspond to the labels in Figures 1 and 2.
²Accession No. is the MASCOT (http://www.matrixscience.com, Matrix Science, London, UK) result of matrix-assisted laser desorption/ionization-time of flight searched from the MSDB 20060831 database.
³Score is $-10 \times \log (P)$, where $P$ is the probability that the observed match is a random event. A score of ≥59 indicates a significant match ($P < 0.05$) with the named protein.
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⁵The number of matched peptides in the database search relative to total peptides from the protein.
⁶Protein expression quantity, expressed as mean ± SEM, n = 3 gels for anterior MG and posterior MG groups. All the identified spots were differentially expressed ($P < 0.05$).
⁷P-value from the Student’s t-test.
⁸ND = not detected.
centrations of IgA and IgM. Lin et al. (2009) demonstrated that neonatal piglets can absorb 25% of IgG from colostrum. In contrast, IgA is the predominant protein in mature milk of the sow (Sangild et al., 1997). Interestingly, we found that the concentration of IgG (Ig γ-chain) was greater in colostrum from anterior MG compared with posterior MG. Pigs on anterior MG may obtain adequate IgG before gut closure. Inadequate transfer of maternal IgG to newborns may increase susceptibility to infection during lactation and after weaning (Rooke et al., 2003). Indeed, increased amounts of IgG in plasma at weaning can enhance postweaning performance of piglets (Pierce et al., 2005).

The normal intestinal mucosa prevents the entry of microorganisms and exogenous materials into the blood circulation. The extensive surface barrier is protected by numerous innate mechanisms in cooperation with adaptive mucosal immunity (Li et al., 2007). The main humoral mediators of this local first-line immune system are secretory IgA (sIgA; Ig α-chain C region and poly-Ig receptor precursor) and secretory IgM (sIgM; Ig μ-chain and poly-Ig receptor precursor; Brandtzaeg, 2003; Corthésy, 2007). The concentration of IgA in milk did not decrease as sharply as IgG (Klobasa et al., 1987). The passive mucosal protection of neonatal piglets is dependent on the continuous supply of sIgA and sIgM during the entire lactation period. We suggest that compared with posterior MG, piglets suckling anterior MG are able to acquire more sIgA and sIgM for the passive mucosal immunity during the entire period of lactation to enhance gut immunity.

Another important finding from this study is that the concentrations of some antimicrobial and immunomodulatory proteins were increased in colostrum and milk from anterior MG compared with posterior MG. These proteins include lactadherin and haptoglobin in colostrum and lactoferrin in mature milk (d 14 after parturition). Lactadherin (PAS6/7, MFG-E8) is a glycoprotein originally found in milk and mammary epithelial cells and can bind several cell surface molecules, including phosphatidylserine integrins (Andersen et al., 1997; Silvestre et al., 2005; Dasgupta et al., 2008). Previous investigations demonstrated that endogenous lactadherin promotes Arg-Gly-Asp-dependent cell adhesion via integrins (Taylor et al., 1997) and guards mammary epithelium integrity. Lactadherin can also mediate the removal of apoptotic cells through binding to phosphatidylserine (Dasgupta et al., 2008). Thus, a lack of lactadherin leads to apoptotic cell accumulation and impairs the involution of MG (Hanayama and Nagata, 2005). Notably, lactadherin can facilitate vascular endothelial growth factor-dependent neovascularization via induction of integrin-dependent Akt phosphorylation in endothelial cells (Silvestre et al., 2005). Additionally, the development of MG is enhanced by lactadherin through the intercellular signaling between luminal and myoepithelial cells (Enslin and Shur, 2007). All of these findings may explain, in part, why anterior MG have greater protein and DNA content compared with the remaining MG (Kim et al., 2000). Herein, greater concentrations of lactadherin in milk from anterior MG may indicate that they can produce a greater quality of milk than posterior MG. Furthermore, rotavirus has been recognized as the most common etiological agent of diarrheal disease in neonatal pigs. Lactadherin in milk can prevent rotaviral infection by binding to the host-cell receptors (Kvistgaard et al., 2004) to modulate intestinal epithelial homeostasis and mucosal healing (Bu et al., 2007). Because the intestinal mucosa of neonates is the first-line barrier against infectious agents and the most important site for the absorption of AA (Wu, 2009), epithelial integrity is essential for the well-being of neonates.

Haptoglobin is an acute-phase protein, and its major biological function is to protect the host against risk of an acute phase reaction and the invasion of mammary tissues by pathogenic bacteria (Eckersall et al., 2006). By quenching the respiratory burst (the production of superoxide and hydrogen peroxide; Quaye, 2008) and increasing expression of the antioxidant cytokine IL-10 (Blum et al., 2007), haptoglobin alleviates potential damage to cells and tissues. Therefore, haptoglobin in colostrum and milk may be an important component of the adaptive immune response for suckling pigs to enhance the repair of the MG epithelium and the neonatal intestine in response to infection.

Lactoferrin is an iron-binding glycoprotein of the transferrin family and has a direct antimicrobial role limiting the proliferation and adhesion of microbes or killing them. Compelling evidence shows that oral administration of lactoferrin reduces bacterial infections in the gastrointestinal tract (Di Mario et al., 2003; Artym et al., 2004; Lee et al., 2005; Wang et al., 2006b; Yen et al., 2009), while beneficially promoting the growth of bacteria with lesser iron requirements, such as Lactobacillus and bifidobacteria (Kim et al., 2004; Sherman et al., 2004; Tang et al., 2009). Finally, lactoferrin can protect the host against rotavirus-induced

Figure 3. Western blot analysis of whey proteins, lactoferrin (A), IgA (B), and serum albumin (C) in sow milk. Data are means ± SEM, n = 4. *Differences (P < 0.05) between anterior mammary glands (AMG) and posterior mammary glands (PMG).
Differential proteomes in milk from mammary glands

diarrhea (Pérez-Cano et al., 2008) because of the ability of lactoferrin to sequester iron in biological fluids or to destabilize the membranes of pathogens (Valenti and Antonini, 2005). In support of this view, oral administration of lactoferrin enhances the size of Peyer’s patches in the ileum and concentrations of immunoglobulin and IL-2 in serum, while decreasing concentrations of IL-1 and interferon gamma in blood (Prigomet et al., 2006, 2007; Shan et al., 2007). Thus, we suggest that lactoferrin in milk can help neonatal pigs inhibit the invasion of pathogens such as *Escherichia coli* and rotavirus, thereby maintaining the homeostasis of intestinal microbial population and beneficially modulating immune responses.

Finally, we found that some proteins secreted by MG epithelial cells were upregulated in mature milk from posterior MG compared with anterior MG, such as myosin-4, creatine kinase B-type, pregnane X receptor, phosphoglycerate kinase, and 14-3-3 protein zeta chain. This result may indicate that posterior MG was more abundant in the number of epithelial cells on d 14 of lactation than anterior MG. It is possible that epithelial integrity, anti-infection, and the ability to heal after infection may be weaker in posterior MG than in anterior MG, rendering mature milk from posterior MG to have some somatic cells than milk from anterior MG.

In conclusion, results of this study demonstrate for the first time that proteomes of colostrum and milk are differentially expressed between anterior and posterior MG. Proteins whose expression is upregulated in colostrum and milk from anterior MG contribute to the passive immunity and intestinal development of sucking piglets, as well as epithelium integrity and the health of MG in lactating sows. Additionally, compared with posterior MG, anterior MG can produce more beneficial proteins in colostrum and milk for sucking piglets. Our findings not only explain the beneficial effect of colostrum and milk from anterior MG, but also have important implications for enhancing growth performance and survival of neonatal pigs.

**LITERATURE CITED**


