Pancreatic Exocrine Secretion During the First Days After Weaning in Pigs

D. Rantzer,* P. Kiela,‡ M-J. Thaela,§ J. Svendsen,* B. Ahrén,§
S. Karlsson,§ and S. G. Pierzynowski‡,‡

*Department of Agricultural Biosystems and Technology, Swedish University of Agricultural Sciences, S-230 53 Alnarp, Sweden; ‡Department of Animal Physiology, Warsaw Agricultural University, Nowoursynowska 166, 02-766 Warsaw, Poland; §Department of Animal Physiology, University of Lund, Helgonavägen 38, S-223 62 Lund, Sweden; and Department of Medicine, University Hospital of Malmö, University of Lund, S-205 02 Malmö, Sweden

ABSTRACT: Feed replacement at weaning plays an important role in the induction of pancreatic maturation. To understand the changes in the exocrine pancreas at weaning and the relation to postweaning problems, we studied the function of the exocrine pancreas and changes of intestinal hemolytic Escherichia coli in four pigs. The pigs were chronically fitted with pancreatic duct catheters and T-shaped cannula inserted into the duodenum for reintroduction of pancreatic juice. One day before weaning (at 30 d of age), pancreatic juice was collected for 1 h before and 1 h after a morning and an evening suckling. The pigs were not creep fed, but from weaning the pigs received a standard weaning diet ad libitum. On d 1, 2, 3, and 5 after weaning, pancreatic juice was collected continuously for the 24-h period. The total pancreatic secretion was measured at hourly intervals, 1.5-mL samples were taken for analysis, and the remaining juice was returned to the animal. On these days, samples from the duodenum, ileum, and rectum were also taken for analyses of hemolytic E. coli. From the day before to 5 d after weaning, a gradual increase in pancreatic secretion was observed concerning volume (P < .001) and protein (P < .01) and trypsin (P < .02) levels. An increase (P < .01) in hemolytic E. coli in the duodenal contents was also documented during this period. We assume that the gradual increase in the measured variables of pancreatic secretion is related to the increasing consumption of solid feed. However, the appearance of E. coli and disappearance of milk components from the gastrointestinal tract could be other factors stimulating the exocrine pancreas.

Key Words: Pancreas, Pigs, Weaning, Trypsin, Escherichia coli

Introduction

Most studies on the development of pancreatic enzymes in pigs concern enzyme concentrations in pancreatic tissue (for reviews see Cranwell and Moughan, 1989; Moughan et al., 1992). The relative amount of trypsin in pancreatic tissue remained unchanged before weaning (at 4 wk of age), whereas an increase occurred 2 wk after weaning (Lindeman et al., 1986; Owsley et al., 1986). However, Corring et al. (1987) showed that this increase also occurred at 6 wk of age in creep-fed and unweaned pigs. This does not agree with the data on pancreatic trypsin secretion in chronically catheterized pigs (Pierzynowski et al., 1990, 1993b). Significant increases in basal output and postprandial responses did not occur until after weaning, irrespective of weaning at 4 or 6 wk of age (Pierzynowski et al., 1993b). Following weaning, however, there was a steady increase in pancreatic secretion (Pierzynowski et al., 1995).

In the present study, we monitored the development of pancreatic function (exocrine and endocrine) during the first 5 d after weaning. We also correlated the levels of immunoreactive cationic trypsin (IRCT) in plasma to pancreatic trypsin output. Moreover, considering the presence of antibacterial activity of the pancreatic juice (Pierzynowski et al., 1992; Pierzynowski et al., 1993a), studies of the intestinal bacterial flora relationships and amounts at weaning were performed.
Materials and Methods

Surgical Procedures and Housing

Four purebred Swedish Landrace pigs from one litter were obtained from a production herd (Odarslöv’s Research Farm, Department of Agricultural Biosystems and Technology, Swedish University of Agricultural Sciences, Lund, Sweden), where complete management and health records were maintained (Olsson and Svendsen, 1989).

At the age of 23 d, the pigs (5.3 ± 0.3 kg BW) were surgically modified. Neuroleptic analgesia was induced with azaperone (2 mg/kg BW i.m.; Stresnil, Janssen & Pharmaceutica, Beerse, Belgium). Pigs were then anesthetized with halothane (ISC Chemicals Ltd., Avonmouth, England) in air (1.5 to 2%, vol/vol) and laparotomized.

A Silastic® (Dow Corning, Midland, MI) catheter, .76 mm i.d. and 1.65 mm o.d., with a Silastic ring (placed 5 to 7 cm from the catheter tip and movable for 2 to 3 cm distance) was inserted into the pancreatic duct and exteriorized percutaneously in the last right intercostal space according to a modified (Thaela et al., 1995) method of Pierzynowski et al. (1988). When the incision was closed, the ring was placed between abdominal muscle layers. The pancreatic catheter was interconnected externally with a nonperforated Silastic T-shaped cannula inserted into the duodenum, for the reintroduction of pancreatic juice during resting conditions. Another Silastic catheter was implanted in the jugular vein and exteriorized percutaneously in the neck. Between blood samplings, the catheter was filled with heparinized saline (50 IU/mL) (Heparin, Pharmacia, Stockholm, Sweden). Two of the four pigs were fitted with an additional cannula in the ileum for sampling the contents. After recovery from anesthesia (approximately 12 h), the pigs were returned to their litters. To prevent the sow and litters from interfering with the catheters and to avoid bending, the catheters were secured using adhesive medical tape (Micropore, 3M Medical-Surgical Division, St. Paul, MN).

The pigs were nourished only by suckling until weaning at 30 d of age, and no solid supplemental (creep) feed was given. The sows and their litters were housed in standard farrowing pens with solid flooring, chopped straw bedding, and heating lamps to ensure a good environment for the pigs. To prevent the pigs from ingesting any solid feed before weaning, the sow was fed in a separate “restaurant” pen once a day between 0800 and 2000. The cages were also provided with water nipples and heating lamps.

Experimental Procedures

Effect of Morning and Evening Milk (Suckling) Before Weaning. One day before weaning (29 d of life) at 0800 and 2000, all pigs were taken from the sow, and the experimental pigs were slightly restrained in collection slings. Pancreatic juice was collected in four 15-min periods; then, all pigs were returned for 30 min to the sow and allowed to suckle. The experimental animals were transferred back to the slings, and four additional 15-min collections of pancreatic juice were performed.

All the pancreatic juice was collected in plastic tubes placed on ice. The volume of the pancreatic juice collected was measured, and the collected juice was reintroduced into the duodenum with a syringe, except for 1.5-mL samples that were stored at −20°C until analysis.

Blood (2 mL) was collected promptly before suckling and after the pigs returned to the slings (after suckling). The blood was transferred into plastic tubes containing 1.5 mg of EDTA and 1,000 KIU of aprotinin (TrasyloL; Bayer, Leverkusen, Germany). After centrifugation, the plasma was separated and stored at −20°C until analyses.

Weaning Experiment. On the day of weaning, pancreatic juice sampling started at 0800 and continued continuously for 3 d, with 1-h collection periods. Another 24-h experiment was performed on d 5 after weaning. The collected pancreatic juice was treated as before weaning.

During the experimental days, blood (2 mL) was sampled every 6 h and treated as before weaning.

Beginning on d 1 after weaning, samples of duodenal (four pigs) and ileal (two pigs) contents were collected once daily at 0900 on the experimental days via implanted cannulae into the test tubes. Additionally, fecal samples were obtained using a cotton swab inserted in the rectum.

Analysis

Pancreatic Juice. Total protein was estimated using the Lowry method (1951) modified to be performed on 96-well microplates, with bovine serum albumin (Sigma Chemical, St. Louis, MO) as a standard.

Trypsin activity was measured using a modification of the original method of Erlanger et al. (1961). Fifty microliters of juice and 150 µL of .2 M Tris-HCl buffer,
The effect of morning and evening suckling on pancreatic exocrine secretion in pigs (29 d of age) a

<table>
<thead>
<tr>
<th></th>
<th>Before suckling</th>
<th>After suckling</th>
<th>Paired t-test</th>
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<tr>
<td>Volume, mL/(h·kg)</td>
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<tr>
<td>Morning</td>
<td>.38 ± .30</td>
<td>.73 ± .54</td>
<td>P &lt; .13</td>
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<tr>
<td>Evening</td>
<td>.97 ± .57</td>
<td>.68 ± .46</td>
<td>P &lt; .14</td>
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<tr>
<td>Paired t-test</td>
<td>P &lt; .14</td>
<td>P &lt; .49</td>
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<td>Protein output, mg/(h·kg)</td>
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<tr>
<td>Morning</td>
<td>1.24 ± 1.28</td>
<td>3.09 ± 3.28</td>
<td>P &lt; .25</td>
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<tr>
<td>Evening</td>
<td>1.14 ± .81</td>
<td>2.11 ± 1.54</td>
<td>P &lt; .02</td>
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<tr>
<td>Paired t-test</td>
<td>P &lt; .87</td>
<td>P &lt; .53</td>
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<td>Trypsin output, U/(h·kg)</td>
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<tr>
<td>Morning</td>
<td>.29 ± .34</td>
<td>.78 ± .84</td>
<td>P &lt; .24</td>
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<tr>
<td>Evening</td>
<td>.39 ± .31</td>
<td>.82 ± .67</td>
<td>P &lt; .007</td>
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<tr>
<td>Paired t-test</td>
<td>P &lt; .44</td>
<td>P &lt; .88</td>
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aValues are means ± SD. P-values indicate the result of comparison with paired Student’s t-test within rows or columns.

pH 7.8, containing .05 M CaCl₂ were placed in microplate wells. The juice was activated by preincubation of the plate for 20 min at 37°C and 15 min at ambient temperature with .12 mg/mL enterokinase (Sigma) added to the Tris buffer. The reaction was then started by adding 100 µL of substrate solution containing 1 mg/mL Nα-benzoyl-DL-arginine-p-nitroanilide (Sigma). The change in absorbency at 405 nm was followed for 45 s at ambient temperature on a spectrophotometer with a built-in kinetic program (Uniscan II; Lab-systems OY, Helsinki, Finland). The trypsin activity was expressed as units (U) and defined as the amount of enzyme that hydrolyzed 1 µmol of substrate per minute.

Plasma Insulin, Glucagon, Glucose, and Immunoreactive Trypsin Levels. Plasma levels of insulin were determined with RIA using a guinea pig anti-insulin antibody (Linco Research, St. Louis, MO), 125I-labeled porcine insulin (Novo Research, Bagsvaerd, Denmark) as a tracer, and porcine insulin (Novo) as a standard.

Plasma glucagon concentrations were determined radioimmunologically using a guinea pig antiguclagon antibody (Linco), 125I-labeled glucagon as a tracer and porcine glucagon as a standard.

Plasma glucose concentrations were determined with the glucose oxidase method (Bruss and Black, 1978).

Plasma IRCT concentrations were determined with an ELISA method (Sandström et al., 1986). The method is based on competition between immobilized porcine trypsin (Novo) and trypsin in a standard series or in the unknown plasma samples for specific rabbit antibodies to cationic porcine trypsin produced in our laboratory (Ohlsson et al., 1982).

Hemolytic E. coli Intestinal Contents. One gram of the intestinal digesta was mixed with 9 mL of sterile .9% NaCl solution, and 10-fold dilutions were made. Samples of .1 mL were spread on blood agar plates that were incubated aerobically at 37°C for 18 h.

The swab with the fecal sample was placed in a test tube containing 10 mL of sterile .9% NaCl solution at 8°C. The test tube was held at ambient temperature for approximately 1 h and then shaken in a shaking machine for 30 s. Ten-fold dilutions were made, and samples (.1 mL) were spread on blood agar and incubated as previously described (Rantzer et al., 1995). The E. coli colonies were routinely tested for glucose and lactose digestion, indole production, motion, and Gram staining.

In all samples, the proportion of hemolytic E. coli bacteria was evaluated with respect to the total aerobic flora. The total number of hemolytic E. coli colonies grown from the duodenal samples was calculated and expressed per gram of digesta.

Calculations and Statistics

In the experiments before weaning, with natural suckling, the results from the 15-min pancreatic juice collections were calculated for a 1-h basal (before suckling) and 1-h postprandial (after suckling) period. To compare these results with the mean daily pancreatic secretion after weaning, the data obtained before and after the suckling in the morning and the evening were averaged.

Results were statistically evaluated with repeated measures ANOVA, with pig and day as factors, and average of pig per day as experimental unit. If differences were significant, the Student-Newman-Keuls multicomparison test was used to compare changes between different days.

Results

Pancreatic Juice Secretion Before Weaning. Before weaning, the basal juice outflow and the protein and trypsin outputs did not differ significantly between the evening and morning observations (Table 1). After 30
min of suckling, the volume of pancreatic juice was not significantly changed, whereas protein \((P < .02)\) and trypsin \((P < .007)\) outputs increased after the evening suckling but not after the morning.

**Body Weight and Feed Intake During the First Five Days After Weaning.** No body weight gain was documented in the experimental pigs throughout the study \((\text{means} \pm \text{SD} \text{ were } 5.5 \pm .4 \text{ kg at weaning}, 5.2 \pm .4 \text{ kg on d 3}, \text{ and } 5.3 \pm .4 \text{ kg on d 5 after weaning})\), but in nonoperated littermates a small body weight gain was observed. Solid feed consumption increased gradually after weaning from \(42 \pm 26 \text{ g/pig on d 1 to } 110 \pm 85 \text{ g/pig on d 2, } 133 \pm 51 \text{ g/pig on d 3, and } 223 \pm 104 \text{ g/pig on d 5. However, these data may not reflect the true feed intake, because the pigs tended to spill the concentrate. Water was allocated ad libitum, the pigs were accustomed to the nipple drinkers from the day of birth, and all were observed to drink.**

**Pancreatic Juice Secretion After Weaning.** After weaning \((30 \text{ d of life; 0800})\), the pancreatic secretion gradually increased during the study period \((5 \text{ d after weaning})\). This rise was linear and concerned volume \((\text{Figure 1a, } P < .001)\), protein output \((\text{Figure 1b, } P < .01)\), and trypsin output \((\text{Figure 1c, } P < .02)\). The mean protein and trypsin concentrations of pancreatic juice did not change significantly throughout the study \((\text{Figures 2a and 2b})\), although the trypsin concentration tended to rise \((P < .06)\).

**Glucose, Insulin, and Glucagon Levels.** Mean plasma glucose concentrations were similar \((P < .52)\) throughout the experiment, and weaning did not significantly affect the plasma glucose concentration. Even though the feed consumption on d 1 was negligible, there was no significant drop in glucose concentration on that day \((\text{Figure 3a})\). This could probably be attributed to the low plasma insulin concentration on the first day after weaning \((\text{Figure 3b})\). However, plasma glucagon and insulin concentrations did not change significantly throughout the study period \((\text{Figures 3b and 3c})\).

**Plasma Immunoreactive Cationic Trypsin.** The mean daily concentrations of plasma IRCT varied among the animals but remained similar \((P < .70)\) throughout the study \((\text{Figure 4})\). There was no positive correlation between the trypsin concentration or the trypsin activity output in pancreatic juice and plasma IRCT \((\text{correlation coefficients .09 and .04, respectively; } P > .05 \text{ and } P > .05, \text{ respectively})\).

**Hemolytic E. coli Intestinal Contents.** The total number of hemolytic E. coli in the duodenum showed a significant increase by d 5 after weaning \((\text{Figure 5})\). The domination of hemolytic E. coli in the total aerobic bacterial flora seemed to begin in the ileum and then spread to the descending duodenum. On d 5 after weaning, E. coli was dominant in the duodenum and ileum of all the pigs, but in rectum this was only observed for one of the four pigs \((\text{Figure 5})\).
Discussion

Immature function of the exocrine pancreas in postnatal mammals has been reported by several authors (Lee and Lebenthal, 1983; Henning, 1987; Githens, 1990). The quantitative and qualitative changes in exocrine pancreatic secretion around weaning in pigs are dependent on age (Corring et al., 1978; Weström et al., 1987), but other factors (stress, feed changes) may also be important. At weaning in pigs, when the entire diet profile changes abruptly from milk to solid feed, the exocrine pancreas increases its function (Pierzynowski, 1991; Pierzynowski et al., 1993b, 1995). It could be speculated that the increase in pancreatic secretion is correlated with an age-dependent maturation of the neurohormonal regulatory reflexes. However, the effect of dietary changes

Figure 2. Total protein (a) and trypsin (b) concentrations in pancreatic juice before and during the first 5 d after weaning. The SD and the results of repeated measures ANOVA are indicated.

Figure 3. Concentrations of blood glucose (a) and immunoreactive insulin (b) immunoreactive glucagon (c) in peripheral blood plasma before and during the first 5 d after weaning. The SD and the results of repeated measures ANOVA are indicated.
Figure 4. Concentrations of immunoreactive cationic trypsin (IRCT) in peripheral blood plasma before and during the first 5 d after weaning. The SD and the results of repeated measures ANOVA are indicated.

Figure 5. Proportion of pigs with dominance of hemolytic E. coli in relation to the total aerobic bacterial flora in the duodenum, ileum, and rectum and the log_10 mean counts of hemolytic E. coli per gram of duodenal ingesta during the first 5 d after weaning. The SD and the results of repeated measures ANOVA and multicomparison Student-Newman-Keuls test are indicated.

around weaning on enteropancreatic reflexes is the more probable mechanism, because the above-mentioned increase in pancreatic secretion was not dependent on age at weaning (4 vs 6 wk; Pierzynowski et al., 1993b). It should be noted that the data describing the developmental pattern of the exocrine pancreas around weaning are incomplete because the dynamics of changes during the first hours and days after weaning in comparison with the secretion before weaning have not been described. The earliest observations were performed on pigs during their fourth postweaning day of life (Pierzynowski et al., 1993b).

The results of the present study showed that in suckling pigs, the morning sucking and the evening sucking affected the exocrine pancreas differently; evening feeding stimulated the protein and trypsin outputs significantly, but the morning feeding did not. These findings could explain the failure in previous studies by Pierzynowski (1991) and Pierzynowski et al. (1993b, 1995) to demonstrate a postprandial increase in pancreatic secretion in suckling pigs, because the studies were performed only in the morning.

The mean daily outflow of pancreatic fluid, protein, and trypsin consistently increased during the first 5 d after weaning in comparison to the daily secretion (as approximated by averaging all data obtained from basal plus postprandial periods in the morning plus evening) in the suckling period. In fact, on d 5 after weaning, the secretion of fluid, protein, and trypsin reached the basal secretion levels noted previously in pigs 5 to 13 wk after weaning (Pierzynowski et al., 1995). Thus, the presented observations show that the major developmental quantitative changes in pancreatic exocrine secretion in pigs take place within the first 5 d after weaning. Qualitative changes in pancreatic juice were also indicated, because the tendency for the pancreatic trypsin concentration to increase and the stable total protein concentration could imply that the specific activity of this enzyme increased linearly during the period studied.

It has been proposed that age, frequency of feeding, and nature of the diet are important factors for inducing pancreatic maturation (Cranwell, 1995). However, we consider that age, which generally plays a crucial role in developmental processes, is not the main factor here, because the age differences are negligible and the observed increase in pancreatic secretion was more rapid than could be predicted from the incremental tendency previously observed during the preweaning and late postweaning periods (Pierzynowski, 1991).

Just before weaning (wk 4 of life), the amount of milk consumed daily is approximately 900 g/d (2.5 to 3.0 MJ as ME; Algers, 1989; Williams, 1995). In terms of energy intake, it is comparable with the amount consumed on d 5 after weaning in this study (223 g/pig daily; 2.7 MJ as ME). This suggests that the energy consumed does not determine the level of pancreatic secretion. Thus, the feed as such (composition, protein source, dry matter content, viscosity) seems to be the most important factor responsible for the observed changes.

However, it remains unclear whether it is the withdrawal of milk or the introduction of solid feed that underlies the increase in pancreatic exocrine secretion. One can speculate that milk suppresses pancreatic secretion before weaning, as recently was
documented for gastric secretion in neonatal rats by Rao et al. (1995). This hypothesis is indirectly supported in this study by the fact that during the first postweaning day, when the pigs consumed very little feed, pancreatic secretion did not decline, as has been observed in other experiments on unfed animals (Pierzynowski et al., 1990).

The low secretion before weaning could also be related to changes in the endocrine pancreatic function. Glucagon inhibits the synthesis and release of pancreatic enzymes (for review, see Henderson et al., 1981), whereas insulin is a potent stimulator of pancreatic enzyme synthesis (Lee et al., 1990) and is a permissive factor for pancreatic enzyme secretion. However, the plasma concentrations of glucagon and insulin did not change during the 5-d study period, but an increase in plasma insulin has previously been shown 2 to 3 wk after weaning (Pierzynowski et al., 1995). Therefore, insulin or glucagon did not seem to be of great importance for the alteration in exocrine pancreatic secretion during the first few days after weaning. The severe drop of insulin on d 1, which was not associated with glucose changes, is difficult to explain. One possible explanation could be that the metabolism is in a catabolic state during the first day after weaning, when the pigs consumed very little feed.

Hee et al. (1988) and Thaela et al. (1995) postulated that pancreatic secretion displayed a circadian rhythm that was clearly related to the frequency of feeding. In the present study, when weaned pigs were offered feed ad libitum in a strictly controlled 12:12 daylight period, no specificity of secretion during the day and night could be demonstrated, except for a drop in pancreatic secretion for all variables studied (data not shown) that was correlated with turning off the lights at 2000.

The plasma concentration of IRCT has been suggested to be a simple measurable indicator of pancreatic function and development (Sandström et al., 1986). A continual increase in the plasma IRCT in pigs from 3 to 9 wk of age has been demonstrated (Rantzer et al., 1995). However, for the 5 d after weaning, no increase in plasma IRCT was observed. The possibility of plasma IRCT being an indicator of pancreatic function and development was not confirmed in this study, because no positive correlations between trypsin concentration or activity output and plasma IRCT concentration were found during the 5-d postweaning period studied.

The concomitant sampling of ingesta from the duodenum, ileum, and rectum of the same pigs on sequential days after weaning for the purpose of E. coli studies does not seem to have been reported previously. The total number of hemolytic E. coli in the duodenum increased significantly by d 5 after weaning. Other studies, using rectal samples, have shown that the peak in hemolytic E. coli is from d 7 to 10 after weaning (Lysgaard Hale, 1993; Rantzer et al., 1995), so probably the number of hemolytic E. coli continued to increase after the study period. The reason for not presenting the total number of hemolytic E. coli in the ileum was that the ileal catheters had to be flushed to remove the contents, and the samples may therefore have been diluted.

The dominance of the aerobic bacterial flora by hemolytic E. coli seemed to start in the ileum, spreading to the duodenum and probably later to the rectum. On d 5 after weaning, E. coli was dominant in duodenum and ileum in all the pigs, but in rectum it was dominant in only one of the four pigs. These findings agree with earlier studies (Svendsen, 1979; Hampson et al., 1985) and underline the susceptibility of pigs to gastrointestinal infections during this period.

It could be speculated that the great increase in hemolytic E. coli in the upper part of the small intestine in the 5 d studied is an effect of disappearance of milk lysozyme from the intestine. In addition, it is known that the concentration of antibacterial factors in pancreatic juice begins to increase after weaning (Pierzynowski et al., 1992). Thus, the increase in the amount of bacteria in the upper bowels can be an effect of the temporary disruption of the mechanism regulating bacterial homeostasis. It cannot be excluded that a large amount of bacteria in the upper small intestine could eventually provoke an increase in pancreatic juice outflow.

Implications

The present study suggests that the main changes in pancreatic exocrine secretion in pigs take place during the first days after weaning. This more likely corresponds to the disappearance of milk from the gastrointestinal tract than to solid feed consumption. Possibly milk components tonically suppress pancreatic exocrine function during the preweaning period. However, high glucagon levels, which inhibit exocrine pancreas function, may contribute to the low pancreatic secretion in suckling pigs. The great increase in hemolytic E. coli in the small intestine indicates the possibility for the development of E. coli-associated postweaning diarrhea during this period.

Literature Cited


