Effects of crude red kidney bean lectin (phytohemagglutinin) exposure on performance, health, feeding behavior, and gut maturation of pigs at weaning

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ABSTRACT: The aim of this study was to obtain information that could help to ease the weaning transition in commercial pig production. Before weaning, phytohemagglutinin (PHA) in the form of a crude preparation of red kidney bean lectin was fed by gavage to 24 crossbred [(Swedish Landrace × Yorkshire) × Hampshire] piglets, whereas 24 control piglets were fed α-lactalbumin by gavage, to study the effect on growth, occurrence of postweaning diarrhea, feeding behavior, and some anatomical and physiological traits of the gastrointestinal tract. Within the litter, piglets were randomly assigned to PHA treatment or control and remained in the same pen from the beginning (PHA exposure at 7 d before weaning) until the end of the experiment (14 d postweaning). Weaning took place at the age of 31 to 34 d. Pigs treated with PHA grew faster (P = 0.013) during the first week postweaning and tended to have lower total diarrhea scores (P = 0.10) than did control pigs. On d 5 after weaning, piglets treated with PHA spent more time eating (P = 0.028) than control pigs. No immunostimulating effect of PHA, measured by plasma immunoglobulin G, could be detected. An increase in the intestinal barrier properties before weaning, as a response to PHA treatment, was demonstrated in intestinal absorption studies using Na-fluorescein and BSA as gavage-fed markers. Less uptake (measured as plasma concentrations) of the marker molecule Na-fluorescein occurred during a 24-h study period, and numerically lower levels of BSA were observed compared with studies in control pigs of the same age. A total of 12 pigs (6 control, 6 PHA-treated) were euthanized on the day of weaning for analyses of gastrointestinal properties. The PHA-treated pigs tended to have a longer total small intestinal length (P = 0.063) than that of the control pigs. The enzyme profile of the jejunal epithelium responded to PHA exposure with a decrease in lactase activity and an increase in maltase and sucrase activities, which is similar to changes normally observed after weaning. No differences were found in the size of the pancreas or in its contents of trypsin and amylase. In conclusion, exposing piglets to crude, red kidney bean lectin for 3 d during the week before weaning led to changes in performance and small intestinal functional properties that would be expected to contribute to a more successful weaning.

Key words: feeding behavior, lectin, maturity, performance trait, pig, weaning

INTRODUCTION

Pigs reared under commercial conditions in Sweden are normally weaned at 4 to 5 wk of age. The first few weeks after weaning are a sensitive period, partly because of the change of nutritional source from milk to dry feed. A growth check is often seen (Pluske et al., 1997), as well as postweaning diarrhea, which is a common problem in many production herds, with the most serious type being associated with haemolytic Escherichia coli bacteria (Svendsen et al., 1974). Undigested feed accumulated in the gastrointestinal (GI) tract, as a result of inefficient digestion, constitutes the media for the growth of these haemolytic E. coli bacteria (Kenworthy and Crabb, 1963; Palmer and Hulland, 1965).

Phytohemagglutinin (PHA) is a lectin obtained from red kidney beans (Phaseolus vulgaris). A study on suck-
ling pigs showed that exposure to a crude preparation of PHA led to a decrease in the size of the intestinal villi and an increase in the crypt depth in the epithelium of the small intestine, as well as an increase in the activities of intestinal sucrase and maltase (Rådberg et al., 2001). Using pure PHA fed to suckling rats accelerated growth and induced precocious functional maturation of the GI tract (Linderoth et al., 2005a). These responses were similar to the structural and functional changes seen at weaning (Aumaitre and Corring, 1978; Pluske et al., 1997). The effect of PHA on GI maturation is not fully elucidated. Working with suckling rats, Linderoth et al. (2006) found that PHA binding to the GI epithelium resulted in accelerated crypt cell proliferation, gut growth, and emergence of more adult-like enterocytes.

In the current study, the GI tract of suckling piglets was exposed to PHA shortly before weaning. The hypothesis was that stimulating gut maturational processes before weaning would reduce postweaning problems and improve pig performance and health after weaning, expressed as better growth and less occurrence of postweaning diarrhea.

**MATERIALS AND METHODS**

**Animals**

The study was approved according to rules of the Swedish Central Committee for Laboratory Animals.

The study was carried out at the Department of Agricultural Biosystems and Technology at Odarslöv Research Farm, which has a closed herd of about 50 sows. A total of 48 crossbred (Swedish Landrace × Yorkshire) × Hampshire pigs from 6 litters (2 litters of 8 piglets per group for 3 consecutive groups) were included in the study. Piglets were 24 to 27-d of age at the beginning of the study and were randomly divided within the litter into 4 control pigs (total n = 24) and 4 PHA-treated pigs (total n = 24). Piglets were kept with the sow in conventional farrowing pens for loose-housed sows until weaning at 31 to 34 d of age (7 d after the beginning of the experiment), at which time the sow was removed, and the piglets stayed in the same pen during the entire experimental period (1 litter/pen). Room temperature in the farrowing unit was maintained at about 21°C, and piglets had access to a well-bedded creep area with a heating lamp. Lights were on approximately 0800 to 1630.

Before weaning, piglets were not given creep feed, and they did not have access to the sow’s feed. From weaning, piglets were given ad libitum access to the standard starter diet Pigfor Växett (Lantmännene, Stockholm, Sweden; content per kilogram: 13.0 MJ of ME; 161 g of CP; 63 g of crude fat; 44 g of crude fiber; 12.0 g of lysine; 7.8 g of methionine + cysteine; 7.0 g of threonine) from a feeder with 2 feeding places per pen.

On the day of weaning, 1 pig/treatment and pen (a total of 12 pigs) was euthanized and used for the measurements and analyses on the GI tract.

**Experimental Procedure**

**PHA Exposure.** For 3 d during the week before weaning (−7, −6, and −5 d in relation to weaning), piglets in the PHA group were fed by gavage a crude preparation of PHA (400 mg/kg of BW) dissolved in 0.9% NaCl, using a soft stomach tube made of polyvinyl chloride plastic. Kidney bean albumins were water/acetate acid-extracted from finely grounded, commercially available, red kidney beans (Phaseolus vulgaris) according to Pusztai and Watt (1974). Using a haemagglutination assay, the lectin content was established at approximately 25% (i.e., the dose of PHA that the pigs received, which was estimated to 100 mg/kg of BW). The other 2 main components of the preparation were trypsin inhibitor (of the Bowman-Birk type) and alpha-amylase inhibitor. Control pigs were given the same amount of α-lactalbumin (400 mg/kg of BW) dissolved in 0.9% NaCl. The gavage feeding was carried out in a quick, standardized, and nonpainful manner, to minimize the stress for the animals.

**Blood Sampling.** Blood samples (2 mL) were obtained from the cranial vena cava using disposable syringes containing 1.5 mg of EDTA and 1,000 kIU of Trasylol (Bayer, Leverkusen, Germany; a protease inhibitor, added routinely to minimize peptide and protein degradation), as described by Rantzer et al. (1995). Blood samples for analysis of total protein and plasma immunoglobulin G (IgG) were taken at about 0900 in the morning on d −7, −5, 0, 2, 7, 14, and 28 in relation to weaning. Blood samples for intestinal absorptive capacity were taken as described below. Samples were immediately cooled on ice and then centrifuged at 3,000 × g for 15 min at 4°C. Plasma was harvested and stored at −20°C until further analyses.

**Intestinal Absorptive Capacity In Vivo.** On the morning of the day before weaning (i.e., d −1), pigs were fed by gavage a marker molecule solution containing 2 molecules with different properties: BSA (Sigma, St. Louis, MO) with high molecular mass (approximately 66,000 Da) and requiring energy and specific transport proteins to cross cell membranes; and Na-fluorescein (NaF; Merck, Darmstadt, Germany) with low molecular mass (376 Da) and that diffuses passively through cell membranes. The marker molecules had been dissolved in 0.9% NaCl and were given by stomach tube as a cocktail of 4 mL/kg of BW, containing 500 mg of BSA/kg of BW and 10 mg of NaF/kg of BW. Blood samples for analyses of marker molecules were taken, using the technique described above, at 0 h and at 0.5, 1, 2, 4, 8, and 24 h after administration, respectively.

**Performance and Health.** Pigs were individually weighed on d −7, −6, −5, −2, −1, 0, 3, 7, 14, and 28 (weaning = d 0). Diarrhea scores were registered on d −2, 0 to 7, 9, 12, and 14, using the following scale: 0 = no signs of diarrhea; 1 = some loose fecal consistency; 2 = very loose fecal consistency; and 3 = watery, spurtting feces (Rantzer et al., 1996). Pigs with a diarrhea score of 3 were treated by giving antibiotic injections (Borgal
vet., Intervet International, Schwabenheim, Germany) for 3 d. Fecal samples were taken on d −2, 0, 5, 7, and 14, by using a cotton swab inserted into the rectum.

**Feeding Behavior.** Pens with the pigs were continuously videotaped from weaning to 5 d thereafter using a time-lapse video recorder (Panasonic AG-6720A) and a surveillance camera (Panasonic WV-CL300, STV Video Data, Malmö, Sweden). Tapes from d 1 and 5 after weaning were used to determine the time spent eating by each pig. A pig was considered to be eating when its nose was pointed downward at the feeder. Eating was not registered when this lasted less than 5 s, and interruptions shorter than 5 s during eating were not noted. For further analysis of the feed intake pattern, time spent feeding was divided into meals, or bouts. Bouts were separated by between-bout intervals of at least 5 min with no feeding activity. Intervals of less than 5 min between feeding occasions were considered as within-bout intervals, and thus 1 bout was defined as the sum of the feeding time for which not more than 5 min had passed with the pig not eating.

**Analyses from Euthanized Animals**

**Tissue Collection.** On the day of weaning, before the sow was removed, 1 PHA-treated and 1 control pig from each litter (a total of 6 PHA-treated and 6 control pigs) were anesthetized using i.v. injection of a barbiturate solution. The abdomen of the anesthetized animals was opened by an incision along the midline, and the pancreas and the small intestine were dissected free. Immediately after dissection, segments for pancreatic and intestinal protein analysis were obtained and weighed, after which the pigs were euthanized using an overdose of the barbiturate solution. The intestine was placed on a steel table fitted with 2 rows of pegs 1 m apart. The small intestine was placed from peg to peg without stretching and measured (including the previously removed segments of the jejunum).

**Pancreatic and Intestinal Protein and Enzyme Activity.** For enzyme activity analyses, the pancreas and a 20-cm long segment from the cranial (beginning 25 cm caudal to the Treitz ligament), middle (measured at half of the total distance from the Treitz ligament to the ileo-cecal ligament), and caudal (ending 25 cm cranial to the ileo-cecal ligament) portion of the jejunum, respectively, were quickly dissected, frozen in liquid nitrogen, and stored at −80°C. A portion of the pancreas, weighing about 5% of the whole gland, was homogenized on ice in 0.2 M Tris-HCl buffer + 0.05 M CaCl$_2$, pH 7.8, at a ratio of 1:10 (wt/vol) using a glass/glass homogenizer with a motor-driven pestle. The homogenate was then centrifuged at 15,000 × g for 1 h (4°C), and the supernatant was used for analyses of total protein, and of trypsin and amylase activities.

Total protein was determined using the Lowry method (Lowry et al., 1951), modified for 96-well microplates (Pierzynowski et al., 1990), and using BSA (Sigma) as a standard. Pancreatic trypsin activity was measured in a microplate by using a modification (Pierzynowski et al., 1990) of the original method of Fritz et al. (1966), with the substrate N-a-benzoyl-DL-arginine-p-nitroanilide (Sigma). Pancreatic amylase activity was analyzed using blue starch as a substrate (Phadebas Amylase Test, Pharmacia, Uppsala, Sweden), according to the manufacturer’s instructions.

The small intestinal sections (cranial, middle, and caudal) were thawed, mucosal scrapings were obtained using a microscope slide and then were homogenized in 25 volumes (wt/vol) of 0.9% NaCl at 0°C. Analyses of intestinal disaccharidases; i.e., maltase, sucrase, and lactase activities, were performed according to Dahlqvist (1984). Enzyme activities were expressed as units, with 1 unit defined as the amount of enzyme transforming 1.0 mmol of substrate/min at 25°C.

**Analyses from Live Animals**

**Plasma Levels of Marker Molecules.** The concentration of marker molecules in plasma samples from the absorption experiments was analyzed according to Nejdfors et al. (2000). The amount of Na-fluorescein in plasma, in relation to standard dilutions of Na-fluorescein dissolved in PBS buffer, was measured in a 96-microwell plate (Nunc, Roskilde, Denmark) by spectrofluorometry (CytoFluor 2300, Millipore, Bedford, MA), using a filter set of 485 nm for excitation and 530 nm for emission. Bovine serum albumin was quantified by electroimmunoassay (Laurell, 1966) using purified BSA (Sigma) diluted in swine serum as a standard, and using rabbit antibodies against BSA (Dako, Glostrup, Denmark) for detection.

**Total Protein and IgG in Blood Plasma.** Plasma samples taken on d −7, −5, 0, 2, 7, 14, and 28 were analyzed for total protein (mg/mL) according to the Lowry method (Lowry et al., 1951), and for IgG with radial immunodiffusion (Fahey and McKelvey, 1965) using porcine serum IgG (Sigma) as a standard and rabbit antibodies to porcine IgG (Carlsson et al., 1980).

**Bacteriology.** The cotton swabs with the fecal samples were placed in test tubes containing 10 mL of sterile, 0.85% NaCl solution at 8°C. Test tubes were kept at room temperature for about 2 h and then shaken in a shaker (REAX 2000, KEBO-Lab, Lund, Sweden) for 30 s at approximately 1,500 rpm, vibration orbit 5 mm. Using serial dilutions and inoculation on blood agar plates, the proportion of haemolytic *E. coli* with respect to the total aerobic bacteria was determined by counting. Where *E. coli*-appearing haemolytic colonies dominated (>50%), verification was performed by testing for glucose digestion, lactose digestion, indole production, motion, and gram color (Rantzler et al., 1995).

**Statistics**

All data were tested for normality using the univariate normal procedure (SAS Inst. Inc., Cary, NC). Parameters that were normally distributed (BW gain,
time spent feeding, concentrations of plasma protein and IgG, pancreatic and intestinal weights and enzymes) were then analyzed with the GLM procedure using the model

$$Y_{ijk} = \mu + g_i + f_j + e_{ijk},$$

where $Y$ = the response of a specific trait; $\mu$ = the overall mean; $g_i$ = the effect of litter/sow ($i = 1, 2, 3, 4, 5, 6$); $f_j$ = the effect of treatment ($j = 1, 2$); and $e_{ijk}$ = the residual random term. When analyzing BW gain, the effect of gender was also included in the model. Because the experiment was conducted as repetitions for 3 consecutive groups, an effect of batch was included in the model from the beginning but was removed when it was found that it had no effect on the outcome. Parameters that were not normally distributed (diarrhea score, feeding bouts, uptake of marker molecules, total intestinal length, concentration of pancreatic protein) were tested with the 1-sided, Wilcoxon 2-sample test. Logistic regression was used for analyzing bacteriology data (proportion of pigs with haemolytic E. coli dominating; i.e., >50% of total aerobic bacterial flora). Data in the text are presented as means ± SEM.

**RESULTS**

No signs of aversive effects, such as vomiting, were observed in response to the gavage feeding of PHA or $\alpha$-lactalbumin solutions to the animals. No symptoms of diarrhea or other illnesses were recorded during the daily observations of the pigs from the day the treatments began until weaning.

**Body Weight Gain.** Pigs in the 2 experimental groups (PHA, control) had the same initial mean weight ($7.9 \pm 0.27$ kg; Figure 1). From the beginning of the experiment (7 d before weaning) until the day of weaning, PHA-treated pigs gained less weight ($P = 0.003$) than control pigs (Table 1). During the first 7 d postweaning, however, the PHA-treated pigs had greater BW gain than control pigs ($P = 0.01$); in fact, control pigs lost BW during this period. From d 7 to 28 postweaning, no differences in BW gain were observed between the 2 groups.

**Diarrhea Score and Bacteriology.** For the entire experimental period, PHA-treated pigs tended ($P = 0.10$) to have a lower sum of diarrhea scores ($65 \pm 0.7$) than control pigs ($107 \pm 1.1$). When expressed as the mean diarrhea score per pig per day, PHA-treated pigs had lower scores on d 3 ($P = 0.04$; Figure 2).

The proportion of pigs showing dominant haemolytic E. coli, i.e., >50% of total aerobic bacterial flora in the rectal swabs, is shown in Figure 3. No differences between the 2 treatment groups were observed. During the experimental period, 2 pigs from the control group (same litter) died from postweaning diarrhea on d 8 after weaning.

![Figure 1. Body weight (means ± SEM) of phytohemagglutinin (PHA)-treated pigs (n = 18) and $\alpha$-lactalbumin-fed control pigs (n = 18) from the beginning of the experiment (PHA pigs received 400 mg/kg of BW of a crude red kidney bean preparation, and control pigs were given the same amount of $\alpha$-lactalbumin via stomach tube on d −7, −6, and −5 in relation to weaning) until 14 d after weaning (weaning at 31 to 34 d of age).](image-url)

**Table 1.** Weight gain of phytohemagglutinin (PHA)-treated pigs (n = 18) and $\alpha$-lactalbumin-fed control pigs (n = 18 to d 7 and n = 16 thereafter because of 2 pigs dying from postweaning diarrhea) during the periods of the experiment

<table>
<thead>
<tr>
<th>Weight gain, kg</th>
<th>Treatment$^1$</th>
<th>Control</th>
<th>PHA</th>
<th>SE$^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>d −7 to 0</td>
<td>1.77</td>
<td>1.29</td>
<td>0.10</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>d 0 to 7</td>
<td>−0.12</td>
<td>0.52</td>
<td>0.17</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>d 7 to 14</td>
<td>1.43</td>
<td>1.74</td>
<td>0.17</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>d 14 to 28</td>
<td>5.56</td>
<td>5.59</td>
<td>0.35</td>
<td>&gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Phytohemagglutinin (in the form of a crude red kidney bean preparation) and $\alpha$-lactalbumin (400 mg/kg of BW) were fed by gavage on d −7, −6, and −5 in relation to weaning.

$^2$Pooled SE are shown after d 7 due to unequal numbers of observations in treatment means.
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Figure 2. Diarrhea scores (means ± SEM) of phytohemagglutinin (PHA)-treated (n = 18) and control pigs (n = 18) during the experimental period. The PHA pigs received 400 mg/kg of BW of a crude red kidney bean preparation, and control pigs were given the same amount of α-lactalbumin via stomach tube on d −7, −6, and −5 in relation to weaning. 0 = no signs of diarrhea; 1 = some loose fecal consistency; 2 = very loose fecal consistency; and 3 = watery, spurting feces. *P < 0.05.

Figure 3. Proportion of phytohemagglutinin (PHA)-treated and control pigs with haemolytic *Escherichia coli* dominating; i.e., >50% of total aerobic bacterial flora, in the rectal swabs taken during the experimental period. The PHA pigs received 400 mg/kg of BW of a crude red kidney bean preparation, and control pigs were given the same amount of α-lactalbumin via stomach tube on d −7, −6, and −5 in relation to weaning.

Figure 4. Feed intake pattern expressed as time spent feeding (means ± SEM) during 4-h intervals on (A) d 1 and (B) d 5 after weaning for phytohemagglutinin (PHA)-treated (n = 18) and control pigs (n = 18). The PHA pigs received 400 mg/kg of BW of a crude red kidney bean preparation, and control pigs were given the same amount of α-lactalbumin via stomach tube on d −7, −6, and −5 in relation to weaning. *P < 0.05; **P < 0.01.

0030 to 0430: PHA 5 min 50 s ± 1 min 12 s, control 2 min 29 s ± 0 min 51 s, *P = 0.004*.

Evaluating feeding behavior according to feeding bouts, no differences were observed between treatment groups in the length of the bouts. However, a tendency...
Table 2. Length and number of feeding bouts on d 1 and 5 after weaning (31 to 34 d of age) in phytohemagglutinin (PHA)-treated (n = 18) and control (n = 18) pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Control</th>
<th>PHA</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of bouts, min</td>
<td>d 1</td>
<td>1.92</td>
<td>2.32</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>d 5</td>
<td>3.62</td>
<td>3.36</td>
<td>0.21</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Number of bouts</td>
<td>d 1</td>
<td>17.44</td>
<td>17.78</td>
<td>1.71</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td></td>
<td>d 5</td>
<td>13.39</td>
<td>19.78</td>
<td>1.47</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1Phytohemagglutinin (in the form of a crude red kidney bean preparation) and α-lactalbumin (400 mg/kg of BW) were fed by gavage on d −7, −6, and −5 in relation to weaning.

to longer bouts was noted for PHA-treated pigs on d 1 (P = 0.09), and PHA-treated pigs had a significantly greater number of bouts on d 5 after weaning (P = 0.05; Table 2).

Blood Plasma Concentrations of Total Protein and IgG. No differences in plasma concentrations of total protein between the 2 treatment groups could be observed during the experimental period, although control pigs consistently showed somewhat greater values than did PHA-treated animals (Figure 5). Also in the case of IgG, no differences between treatment groups could be detected, although the control group consistently showed greater levels, except for the last sample taken (d 28).

Intestinal Absorption In Vivo. The PHA-treated pigs generally showed less uptake of marker molecules, administered at 1 d before weaning, compared with that of control pigs. This was most evident in the case of the low molecular weight marker NaF, where the total uptake (sum of concentrations in the 7 blood samples) was less (P = 0.01), and for 3 individual samples (0.5 h, P = 0.02; 1 h, P = 0.03; and 2 h, P = 0.03), respectively (Figure 6). Uptake of BSA was less in the blood sample taken 1 h after administration (P = 0.03; Figure 6) in the PHA-treated pigs compared with control pigs.

Intestinal and Pancreatic Parameters, In Vitro Studies. Total length of the small intestine of PHA-treated pigs tended to be longer (PHA 106.2 ± 5.66 cm/kg of BW; control 93.5 ± 4.06 cm/kg of BW; P = 0.06) than that of the control pigs. In addition, PHA treatment resulted in a change in the concentrations of the intestinal disaccharidases in the jejunum (Table 3) compared with those found for untreated control pigs. Thus the PHA group tended to have less lactase activity (in the middle section, P = 0.08) compared with controls, whereas maltase activity tended to be greater (in the cranial section, P = 0.10), and the sucrase activity was greater in the cranial section (P = 0.006) for PHA-treated pigs. No effect of treatment was found in size or enzymatic contents of the pancreas (Table 4).

DISCUSSION

The aim of this experiment was to provide information that could be used to make weaning a less difficult process in commercial pig production. Treatment of pigs with a crude preparation of PHA for 3 d during the week before weaning was expected to prevent the commonly observed growth check after weaning and to reduce occurrence of postweaning diarrhea. Improved growth
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Figure 6. Blood plasma levels (means ± SEM) of the marker molecules Na-fluorescein (NaF) and BSA during 24 h after marker gavage feeding to phytohemagglutinin (PHA)-treated pigs (n = 24) and control pigs (n = 24). The PHA pigs received 400 mg/kg of BW of a crude red kidney bean preparation, and control pigs were given the same amount of α-lactalbumin via stomach tube on d −7, −6, and −5 in relation to weaning. Marker molecules were administered on d −1. *P < 0.05.

Table 3. Disaccharidase activities in the cranial, middle, and caudal1 regions of the jejunum from phytohemagglutinin (PHA)-treated (n = 6) and control pigs (n = 6) at weaning (31 to 34 d old).

<table>
<thead>
<tr>
<th>Enzyme concentration and region</th>
<th>Treatment2</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactase, units/mg of protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranial</td>
<td>0.045</td>
<td>0.010</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Middle</td>
<td>0.055</td>
<td>0.009</td>
<td>0.09</td>
</tr>
<tr>
<td>Caudal</td>
<td>0.008</td>
<td>0.004</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Maltase, units/mg of protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranial</td>
<td>0.111</td>
<td>0.023</td>
<td>0.10</td>
</tr>
<tr>
<td>Middle</td>
<td>0.154</td>
<td>0.027</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Caudal</td>
<td>0.160</td>
<td>0.028</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Sucrase, units/mg of protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranial</td>
<td>0.015</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>Middle</td>
<td>0.032</td>
<td>0.015</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Caudal</td>
<td>0.041</td>
<td>0.011</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

1Cranial region beginning 25 cm caudal to the Treitz ligament, middle region measured half of the total distance from the Treitz ligament to the ileo-cecal ligament, and caudal region ending 25 cm cranial to the ileo-cecal ligament.

2Phytohemagglutinin (in the form of a crude red kidney bean preparation) and α-lactalbumin (400 mg/kg of BW) were fed by gavage on d −7, −6, and −5 in relation to weaning.

was observed for PHA-treated pigs compared with control pigs during the week immediately after weaning. The PHA-treated pigs were, to some extent, affected by treatment because they grew slower than control pigs during the week before weaning (the week of treatment). This effect has previously been noted in pigs (Rådberg et al., 2001) and rats (Linderoth et al., 2005a). However, in the current study, 1 possibility was that control pigs in fact had improved growth during this period due to the nutrient surplus of the α-lactalbumin, given as a control substance. Alpha-lactalbumin was used as a control substance instead of saline because it was considered to be more relevant to use a protein in comparison to the tested lectin PHA. The PHA-treatment did not lead to long-lasting effects on pig weights because differences between the 2 experimental groups had disappeared after d 7 postweaning.

Both treatment groups were kept together, with 4 PHA-treated and 4 control pigs/litter. Therefore individual feed consumption or feed consumption per treatment could not be determined. Instead, pigs were videotaped, and the measure of time spent standing with the nose pointing downwards in the feeding trough was used to compare treatment groups. Feeding behavior of the individual pig may have been affected by the other pigs in the group, because feed intake is strongly influenced by social facilitation (Hsia and Wood-Gush, 1983). The advantages of being able to compare treated (PHA pigs) and controls within the same litter were

Table 4. Weight of pancreas and pancreatic content of total protein and enzymes (trypsin and amylase activities) in relation to BW from phytohemagglutinin (PHA)-treated pigs (n = 6) and control pigs (n = 6) at weaning (31 to 34 d old).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas weight, g/kg</td>
<td>Control</td>
</tr>
<tr>
<td>Protein, mg/kg</td>
<td>1.41</td>
</tr>
<tr>
<td>Trypsin, units/kg</td>
<td>103.4</td>
</tr>
<tr>
<td>Amylase, units/kg</td>
<td>14.3</td>
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1Phytohemagglutinin (in the form of a crude red kidney bean preparation) and α-lactalbumin (400 mg/kg of BW) were fed by gavage on d −7, −6, and −5 in relation to weaning. Values within a row do not differ (P > 0.10).
important because pigs with the same genetic background could be studied under exactly the same conditions. The disadvantage of dividing and moving the litter could be avoided when pigs were allowed to stay in the same litter and pen where they were born. Because feeding behavior immediately after weaning was the subject for observation, we believe that moving pigs at weaning would have affected their behavior for a few days when adapting to their new environment. Videotaping also made it possible to study how the pigs distributed the feeding time during the 24 h of the day and night. This showed that PHA-treated pigs spent more time at the feeder and divided their total feeding time into more bouts, which should enable them to better utilize their feed-degrading capacity. The underlying mechanisms for this behavior will, however, require more research to be explained.

Previous studies have shown that PHA-treatment of nursing rats (Linderoth et al., 2005a,b) resulted in growth of the gut and pancreas. Except for a tendency to a longer total intestinal length in PHA-treated pigs, no growth effects were observed in the current study.

The B-cells interact with T-cells to produce antibodies during antigen exposure (Porter, 1986). Because PHA is an effective T-cell stimulator (Roitt, 1988) but also a powerful oral immunogen that induces a high titer of antiPHA antibody of the IgG isotype (Pusztaí, 1993), it was assumed that PHA-treatment would also influence IgG concentrations in treated pigs. No differences in total plasma IgG level between the 2 treatment groups could be detected; however, it would have been useful to also analyze for specific antiPHA IgG antibodies. The increase in the IgG levels observed after weaning in both treatment groups must be considered an effect of weaning and a response to the newly introduced feed antigens.

In the small intestine, morphological and functional changes also take place at weaning. Villi height decreases, crypt depth increases (see review by Pluske et al., 1997), and enzyme profile changes with a decrease in the activity of the brush-border enzyme lactase and increase in activities of maltase and sucrase (Aumaitre and Corring, 1978). Weaning stimulates pancreatic development and its enzymatic output, but there is a delay until the different enzymes reach efficient levels (Cera et al., 1990; Makkink et al., 1994; Rantzer et al., 1997). Thus, when nutritional source is abruptly changed, the pig’s GI tract is not able to either fully digest the macronutrients from the feed or absorb all of what has been digested. This leads to accumulation of undigested and unabsorbed feed, which constitutes media for growth of haemolytic _E. coli_ bacteria in the GI tract (Kenworthy and Crabb, 1963; Palmer and Hulland, 1965), causing postweaning diarrhea.

In the present experiment, exposing piglet preweaning small intestine to red kidney bean lectin induced a similar functional development with respect to the small intestinal brush-border enzymes, measurable even on the day of weaning. This could imply that the feed can be more efficiently degraded by the digestive enzyme system and that less amounts of undigested feed will be accumulated in the GI-tract. If so, this could be the reason why fewer of PHA-treated pigs developed postweaning diarrhea and none died, whereas 2 pigs from the control group died of postweaning diarrhea.

In a previous study on suckling 14-d-old piglets, we found a decreased marker molecule absorption into blood after gavage and thus increased intestinal barrier properties due to exposure to the crude kidney bean lectin (Rådberg et al., 2001). In the current study, the reduced absorption of the inert and passively transported small marker, Na-fluorescein, could be explained by a decreased surface area. A decreased endocytosis capacity of the enterocytes might explain the reduction in BSA absorption after lectin treatment. Taken together, these results indicated a more efficient intestinal barrier function on the day before weaning after lectin treatment. A study of the histological structure of the small intestinal epithelium was also carried out (not shown), but due to large variations between animals, no significant differences between treatment groups were found. However, in a previous study (Rådberg et al., 2001) morphometric analyses of the small intestine in lectin-treated pigs showed a decrease in villi heights, an increase in crypt depths and crypt cell mitotic indices, and decreased vacuolation of the enterocytes in the caudal small intestine, all indicating maturation changes.

In contrast to other studies (Grant et al., 1999; Linderoth et al., 2005a,b), which showed a PHA-induced effect on pancreatic output of digestive enzymes, no effect was observed on pancreatic activities of amylase or trypsin, or on size of the pancreas itself. This may partly be due to large variations between pigs of this age kept under conventional conditions.

When considering all observed changes mentioned above, we concluded that lectin treatment was a successful method of addressing problems around weaning. The digestive and metabolic changes that normally occur after weaning were moved and induced before weaning. A GI tract totally adapted to milk digestion was able to change its function as a result of exogenous treatment. Keeping PHA-treated and control pigs separately could have resulted in more pronounced differences between treatment groups, especially concerning diarrhea occurrence, haemolytic _E. coli_ concentration, and feeding behavior.

In conclusion, 3 d of enteral exposure of PHA to pigs during the week before weaning resulted in more mature properties of the small intestine in the form of more efficient intestinal barrier functions and a change toward a more adult disaccharidase pattern. This change affected performance and behavior after weaning in a positive direction, where PHA-treated pigs had improved BW gain during the week after weaning, less severe postweaning diarrhea, and a feed intake pattern that better utilizes the pigs’ digestive capacity.
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