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Enteral exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs[1]

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ABSTRACT: The present investigation characterized the effect of red kidney bean lectin exposure on gut maturation and function in young piglets. Eleven suckling pigs were given by stomach tube a crude red kidney bean lectin preparation (containing about 25% lectin, 400 mg/kg BW) (lectin-treated pigs) at 10, 11, and 12 d of life, and an additional 16 pigs (control pigs) were given saline instead. On the next day, the intestinal absorptive capacity was determined in vivo, and on the 14th d of life the piglets were killed and organs and small intestine samples were collected for analyses and in vitro permeability experiments. The lectin-treated pigs showed an increase in stomach weights and mucosa thickness, whereas no weight effect was found for the small intestine, spleen, liver, or adrenals. Morphometric analyses of the small intestine in lectin-treated pigs showed a decrease in villus heights, an increase in crypt depths and crypt cell mitotic indices, and fewer vacuolated enterocytes per villus and reduced vacuole size. Lectin treatment also resulted in a decrease in the absorption of different-sized marker molecules after gavage feeding, a decrease in intestinal marker permeability, and a change in small intestinal disaccharidase activities, with increased maltase and sucrase activities. The size of the pancreatic acini was also greater in the lectin-treated pigs, but no increases in enzyme content or pancreatic weight could be determined. In addition, the blood plasma levels of cholecystokinin were higher in the lectin-treated than in the control pigs. The results indicate that exposure to crude red kidney bean lectin induces structural and functional maturation of the gut and pancreatic growth in young suckling piglets. This possibility of inducing gut maturation may lead to an improvement in the piglets' ability to adapt to weaning and to an increase in the growth and health of these animals.

Key Words: Disaccharidase, Lectins, Mophometrics, Pancreas, Pancreozymin, Permeability

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Introduction

The gastrointestinal (**GI**) tract and its accessory glands undergo major development during the neonatal period in mammals. In piglets, this is illustrated by the shift in expression of the enterocyte brush border disaccharidases with decreased lactase and increased sucrase and maltase activities. Moreover, enterocytes with a high endocytotic activity (fetal-type) are gradually replaced during the first 3 wk of life by new adult-type cells with markedly reduced endocytosis (Klein, 1989). These maturational changes gradually progress with age and become evident at weaning, when the gut has to change its digestive and absorptive abilities to effectively utilize the weaning diet instead of the more easily digestible milk. Extensive structural changes are seen in the intestine at this time, with a decreased villi height and increased crypt depth. In addition, the stomach acid secretion amplifies and the amount of gut enzymes increases, reflecting an elevated pancreatic function (Henning et al., 1994). However, due to the early, abrupt weaning that is generally practiced in today's pig production, a natural development of the gut is not allowed. Thus, weaning is a period of transition hazardous to an animal's health in relation to the gut disturbances that occur due to lack of luminal stimula-

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tion following inadequate feed intake and due to immature gastrointestinal structure and function (Pluske et al., 1997).

Lectins from the red kidney bean (*Phaseolus vulgaris*) have been shown in adult rats to bind to carbohydrate structures on the gut epithelium and thereby potently promote the growth of the GI tract and the pancreas (Pusztai, 1993). The aim of the present study was to explore whether gavage feeding of a crude preparation of kidney bean lectin may have similar growth-promoting effects, accelerating the structural and functional maturation of the gut well before weaning, with the aim of better preparing the pigs to digest and absorb the postweaning feed.

Materials and Methods

Animals

The study was approved by the Lund University Ethical Review Committee for Animal Experiments and conducted according to the European Community regulations concerning the protection of experimental animals. The study included 27 crossbred (Swedish Landrace × Yorkshire) × Hampshire pigs from four different litters, obtained from the Swedish University of Agricultural Sciences (Odarslöv Research Farm, Alnarp, Sweden). The piglets were 10 d old at the start of the study and were randomly divided within litters into control pigs (n = 16) and lectin-treated pigs (n = 11). All piglets were kept with the sow and were not creep-fed at any time during the study.

Experimental Procedure

Lectin Exposure. In the morning, when the pigs were 10 d of age, the control pigs were given vehicle alone (4 mL 0.9% NaCl/kg BW) by stomach tube, whereas the lectin-treated pigs were given a crude preparation of red kidney bean lectin (red kidney bean albumin, 400 mg/kg BW dissolved in 0.9% NaCl). The treatment was repeated at 11 and 12 d of age. The purification of red kidney bean albumin was performed according to Pusztai and Watt (1974), and the preparation contained about 25% lectin. The dose of kidney bean lectin that the pigs received was estimated to be about 100 mg/kg BW. Blood samples were obtained from the left or right subclavian vein just before gavage-feeding lectin and at 4 h and 1 to 4 d after. The samples were centrifuged at 3,000 × *g* for 15 min at 4°C, and the plasma was harvested and stored at –20°C until analyses.

Intestinal Absorptive Capacity In Vivo. When the piglets were 13 d of age, intestinal absorptive capacity was determined in vivo. In the morning, the pigs were fed via stomach tube a marker molecule solution containing bovine serum albumin (BSA, 67 kDa, Sigma Chemical Co., St. Louis, MO) (500 mg/kg), 1-deamino-8-D-arginine vasopressin (**dDAVP**, 1,067 Da; Ferring, Malmö, Sweden) (0.01 mg/kg), and Na-fluorescein (376 Da, Merck, Darmstadt, Germany) (9.4 mg/kg). The marker molecules had been dissolved in 0.9% NaCl and given to the pigs as a cocktail of 4 mL/kg BW. For marker molecule analysis, 4-mL blood samples were taken from the left or right subclavian vein just before (0 h) and at 0.5, 1, 2, 4, 8, and 24 h after administration of the marker cocktail.

Intestinal Tissue Permeability In Vitro. On the next day (i.e., when the piglets were 14 d of age), intestinal tissue permeability in vitro was evaluated. The pigs were anesthetized with pentobarbiturate (Pentobarbitalnatrium, Apoteksbolaget, Umeå, Sweden; 40 mg/kg, i.v.) and the abdomen was opened by an incision along the midline. Two 20-cm-long segments of the small intestine (jejunum), one proximal taken 5 cm posterior to the Treitz ligament, and one distal taken 5 cm anterior to the ileo-cecal ligament, were excised and stripped from the serosa-muscle layer. These segments were immediately immersed in room-tempered, oxygenated modified Krebs-Ringer buffer, pH 7.4 (NaCl, 110.0 mmol/L; $CaCl_2$, 3.0 mmol/L; KCl, 5.5 mmol/L; KH_2PO_4, 1.4 mmol/L; $NaHCO_3$, 29.0 mmol/L; Na-pyruvate, 5.7 mmol/L; Na-fumarate, 7.0 mmol/L; Na-glutamate, 5.7 mmol/L; and glucose, 13.4 mmol/L), and further divided to provide three proximal and three distal segments from each animal. The segments were cut along the mesenteric border and mounted as tissue sheets in Ussing diffusion chambers (Precision Instrument Design, Los Altos, CA) with an exposed area of 1.78 cm^2, in accordance with Grass and Sweetana (1988) and Nejdfors et al. (2000). The chambers on the mucosal and serosal sides were each filled with 5 mL of buffer, which was continuously oxygenated with carbogen (95% O_2 and 5% CO_2), circulated by gas lift, and kept at 37°C throughout the experiments. At the start of the experiments (t = 0) (i.e., within 30 min of the induction of anesthesia), the buffer on the mucosal side was replaced with 5 mL of buffer containing the marker molecules ovalbumin (**OVA**, 45 kDa, Sigma) (25 mg/mL), FITC-dextran 4400 (**FD4**, 4,400 Da, Sigma) (1 mg/mL), and ^{14}C-mannitol (182 Da, NEN Life Sci. Products, Brussels, Belgium) (1.38 μ*M*). Buffer samples of 1 mL for marker analyses were taken from the serosal receiver side and replaced with an equal volume of buffer after 20, 40, 60, 80, 100, or 120 min of incubation. Under these conditions, the intestinal samples were considered to be viable for 2 h (Pantzar et al., 1993; Nejdfors et al., 2000).

Tissue Collection and Histology. Directly after the removal of the intestinal segments for the permeability experiments, the remaining small intestine, the pancreas, spleen, stomach, liver, and adrenals were removed and weighed/measured, after which the pigs were killed. For enzyme activity analyses, the pancreas, stomach, and 20-cm-long segments from the proximal (beginning 25 cm posterior to the Treitz ligament), middle, and distal (ending 25 cm anterior to the ileo-cecal ligament) portions of the small intestine were quickly collected, frozen in liquid nitrogen, and stored at –80°C.

For histological analyses, 2-cm-long, whole-thickness segments were taken from the small intestine at the following locations: duodenum, 2 cm proximal to the pancreatic duct orifice; proximal jejunum, 65 to 70 cm distal to the ligament of Treitz; middle jejunum; distal jejunum, 40 cm proximal to the ileo-cecal ligament; and ileum, 10 cm proximal to the ileo-cecal junction. Samples of the right lobe of the pancreas (1-cm^3 blocks) and stomach tissue (1 cm^2 whole wall from the antrum, close to the greater curvature) were also collected. The tissue samples were fixed in Bouin's solution for 5 to 7 d and then stored in 70% ethanol before further preparation. The samples were then dehydrated and embedded in paraffin and serial sections 5 μm in thickness were stained with hematoxylin and eosin for morphometric analysis under a light microscope. In addition, periodic acid shiff and alcian blue staining were performed for mucopolysaccharide analysis. In the small intestinal preparations, the villus height and width, the crypt depth, and the tunica mucosa thickness were measured. In each slide, 30 well-oriented villi and crypts lying outside the area with Peyer's patches were measured at low magnification with a Nikon optical binocular microscope attached to a Nikon Camera and a PC computer with MultiScan v6.08 software (MultiScan, Warsaw, Poland) (Biernat et al, 1999). Villus height was measured as the distance from the crypt opening to the tip of the villus, whereas crypt depth was measured from the base of the crypt to the level of the crypt opening. The enterocytes of 30 well-oriented villi were analyzed for the presence of supranuclear vacuoles (Baintner, 1986) and the number was calculated as a percentage of all villi enterocytes. In addition, the area of the vacuoles (μm^2) was measured using a planimetric feature of the MultiScan v6.08 software. The mitotic index was estimated in the jejunal crypts by calculating the percentage of mitotic cells in 1,000 to 1,200 counted cells. For the detection of apoptotic cells in the jejunal epithelium, an improved TUNEL method (Labat-Moleur et al., 1998) and staining with 7-aminoactinomycin D was used. The rate of apoptosis was estimated on the basis of chromatin condensation under a fluorescence microscope. In the stomach sections, the thickness of the tunica mucosa was measured. In the pancreatic specimens, the size of the pancreatic acini (cross-sectional area) and the number of acinar cells per acinus were measured.

Analyses

Marker Molecules. The concentrations of marker molecules in the plasma samples from the in vivo absorption experiments and in the samples from the Ussing chamber experiments (in vitro) were analyzed as follows (Nejdfors et al., 2000). The ^{14}C-mannitol levels were determined by liquid scintillation after the addition of scintillation fluid (Ready Safe, Beckman, Fullerton, CA) in a beta scintillation counter. The amount of fluorescence due to the presence of FD4 and Na-fluorescein, respectively, was measured in a 96-microwell plate (Nunc, Roskilde, Denmark) by spectrophotofluorometry (CytoFluor 2300, Millipore, Bedford, MA), using a filter set-up of 485 nm for excitation and 530 nm for emission. The standards for FD4 and Na-fluorescein were dissolved in Krebs and PBS buffers, respectively. The presence of dDAVP was determined using a radioimmunoassay (Lundin et al., 1985); the lowest detectable amount was 2 pmol/L. Ovalbumin and BSA (Sigma) were quantified by electroimmunoassay (Laurell, 1966), using purified OVA or BSA diluted in buffer or swine serum, respectively, as standards, and using rabbit antibodies against OVA or BSA (Dako, Glostrup, Denmark).

Intestinal Disaccharidase Activity. The jejunal sections (proximal, middle, and distal) were thawed and mucosal scrapings were made using a microscope slide and homogenized in 25 volumes (wt/vol) of 0.9% NaCl at 0°C. Analyses of disaccharidases (i.e., maltase, sucrase and lactase activities) were performed according to Dahlqvist (1984).

Pancreatic Protein and Enzyme Activity. A portion of the pancreas weighing about 5% of the whole gland was homogenized in 0.2 *M* Tris-HCl buffer + 0.05 *M* $CaCl_2$, pH 7.8, in the ratio of 1:10 (wt/vol) using a glass/glass homogenizer with a motor-driven pestle at 0°C. The homogenate was then centrifuged at 15,000 × *g* for 1 h at 4°C and the supernate 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 as a standard. Trypsin activity was measured with a microplate modification (Pierzynowski et al., 1990) of the original method of Fritz et al. (1966), using the substrate N-a-benzoyl-DL-arginine-p-nitroanilide (Sigma). Amylase activity was analyzed using blue starch as a substrate (Phadebas Amylase Test; Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions. Enzyme activities were expressed as units (U), with one unit defined as the amount of enzyme transforming 1.0 μmol of substrate per minute at 25°C. The protein and enzyme activities were calculated for the whole organ or for 20-cm intestinal samples and reported per kilogram of BW.

Cholecystokinin in Blood Plasma. Plasma samples were ethanol-extracted (recovery 80%) and assayed by radioimmunoassay using a cholecystokinin (**CCK**) kit, according to the manufacturer's instructions (Eurodiagnostica AB, Malmö, Sweden). All the samples were analyzed in one assay; the lowest detectable concentration was 0.3 pmol/L and the intraassay variation was 5.5% for CCK-8s at 4.4 pmol/L. The specificity (cross-reactivity) was 100% for CCK 26-33s, <0.01% for CCK 26-33n-s, <0.5% for gastrin-17s, and <0.01% for gastrin-17n-s. The CCK 26-33s was used as the standard.

Statistics

Body weights, organ weights, biochemical analyses (marker molecules, enzyme activities, and CCK levels),

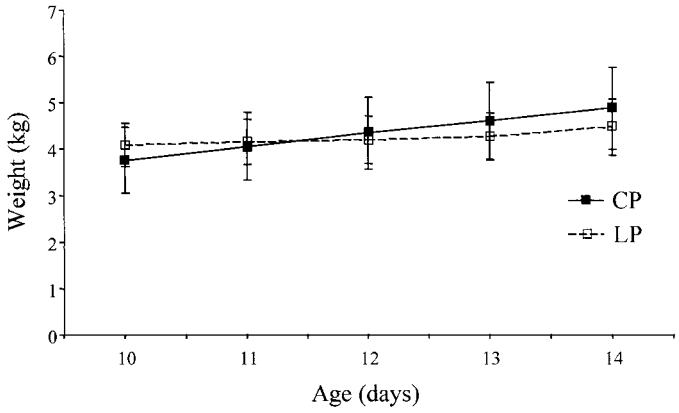

Figure 1. Weight gain of control pigs (CP, n = 16) and lectin-treated pigs (LP, n = 11) at the start of (10 d of age) and during the 3 d of treatment (10 to 12 d of age), until pigs were killed at 14 d of age.

and mitotic index between control and lectin-treated pigs were compared statistically using Student's *t*-test. Data collected over time for body weight, blood plasma levels of marker molecules and CCK, and tissue passage in vitro of marker molecules were also compared and tested with a one-way repeated measures analysis of variance (ANOVA) and Tukey's post hoc test (SigmaStat for Windows v2.0, SPSS Science, Chicago, IL). The results of the morphometric analyses were compared using a nonparametric Mann-Whitney test (InStat for Macintosh v2.03, Graph Pad Software, San Diego, CA). In all statistical analyses, $P < 0.05$ was taken as the level of significance, and all analyses were performed in accordance to Sokal and Rohlf (1995).

Results

Body Weight Gain and Organ Weights

As shown in Figure 1, the control pigs demonstrated a linear BW gain, whereas the lectin-treated pigs gained less weight during the 3 d the kidney bean albumin preparation was administered. From the 1st d of treatment until the day pigs were killed, the weight gain was 9.3% for lectin-treated ($P < 0.05$) and 29.5% for control pigs ($P < 0.05$). However, there were no significant differences in either the initial or final weights between the two groups. Six of the 11 lectin-treated and none of the 16 control pigs showed diarrhea symptoms during the treatment; for lectin-treated pigs the diarrhea stopped within 24 h in every case. At postmortem examination, no obvious differences in the total length and weight of the different small intestinal regions were found between the two groups, except that the weight of the distal jejunum ($P = 0.015$), was lower in lectin-treated pigs (Table 1). In addition, no differences in pancreas, liver, spleen, or adrenal weights were found between the two groups.

Stomach

Treatment with the crude kidney bean lectin significantly increased the stomach weight ($P < 0.001$) and the thickness of the mucosa ($P = 0.03$) in the suckling animals. The H^+ ion concentration of the stomach contents tended to be higher ($P = 0.058$) for lectin-treated than for control pigs (Table 2).

Intestinal Morphology

Morphometric analysis of the small intestine showed differences between the two groups with regard to crypt and villus sizes (Table 3) as well as to the degree of enterocyte vacuolization (Table 4), although no differences in intestinal weight and total length could be detected (Table 1). In the lectin-treated pigs, the villus height had decreased significantly in all parts ($P = 0.001$) of the small intestine except the duodenum ($P = 0.28$), whereas the villus width had not changed significantly. Lectin treatment significantly increased the crypt depth in the proximal jejunum ($P = 0.02$), and the crypt cell mitotic index was significantly increased over the entire jejunum ($P < 0.001$) (Table 3). The apoptosis rate was slightly higher in the epithelium of the proximal jejunum (10.8% in control vs 16.5% in treated pigs), whereas no difference was found in the middle jejunum (15.6% in controls vs 16.2% in trated pigs). In the lectin-treated pigs the mucosal thickness was reduced in the entire small intestine, and the reduction was significant for most of the intestine (Table 3). In the control group, numerous enterocytes containing large vacuoles were observed in the distal jejunum and ileum (Figure 2). These vacuolated enterocytes were observed all along the villi, whereas no vacuolated cells were observed in

Table 1. Weight and length of organs (mean ± SEM) from control pigs (CP, n = 8) and lectin-treated pigs (LP, n = 8)[a]

	Small intestine							
Group	Length, cm/kg	Proximal jejunum, g/20 cm	Middle jejunum g/20 cm	Distal jejunum, g/20 cm	Pancreas, g/kg	Liver, g/kg	Spleen, g/kg	Adrenal, g/kg
CP	144 ± 9	3.4 ± 0.1	4.0 ± 0.3	6.0 ± 0.3	1.1 ± 0.09	30.5 ± 1.3	3.0 ± 0.2	0.14 ± 0.01
LP	136 ± 6	3.3 ± 0.2	4.1 ± 0.2	4.9 ± 0.3*	1.2 ± 0.09	28.2 ± 1.3	3.1 ± 0.2	0.15 ± 0.01

*$P < 0.05$.

Table 2. Empty stomach weight, thickness of the gastric tunica mucosa, pH and [H^+] of gastric content from control pigs (CP, n = 8) and lectin-treated pigs (LP, n = 8)[a]

Group	Stomach weight, g/kg	Mucosa thickness μm	pH	[H^+] mmol/mL
CP	4.6 ± 0.2	423 ± 34	4.1 ± 0.1	0.06 ± 0.009
LP	5.7 ± 0.1***	503 ± 12**	3.9 ± 0.07	0.08 ± 0.006

[a]Presented as means ± SEM.
**$P < 0.01$.
***$P < 0.001$.

the intestinal crypts. The vacuoles were located supranuclearly, in the upper and middle part of the enterocyte, and did not contain mucus, as evidenced by the negative results of staining with PAS and alcian blue. In the lectin-treated pigs, the number of vacuolated enterocytes and the size of the vacuoles were reduced in comparison to those of the control pigs, and the vacuolated enterocytes were restricted to the upper half of the villi.

Intestinal Disaccharidase Activities

In the middle and distal jejunum, lectin treatment led to an increase in the specific activities (U/g protein) of sucrase ($P = 0.020$ vs 0.000) and maltase ($P = 0.027$ vs 0.000), whereas the activity of lactase was not significantly changed. In the proximal jejunum, lectin treatment resulted in a tendency for all the enzyme activities to decrease (Figure 3). The same pattern was seen when the total digestive enzyme capacity (U/kg BW) was determined.

Intestinal Tissue Permeability

In general, the mucosal to serosal passage of the markers in the Ussing chambers increased with time ($P < 0.05$), with the exception of OVA in the distal jejunum. The passage of the relatively small marker molecule ^{14}C-mannitol was approximately 10 and 100 times greater than for the larger FD4 and OVA, respectively. In all piglets, a regional difference could be found, and the passage of the three markers was higher in the proximal than in the distal part of the jejunum. The intestines of the lectin-treated pigs showed reduced marker passage compared to that of the control pigs for all markers ($P < 0.05$ to $P < 0.01$) (Figure 4).

Pancreas

Although no change in pancreatic weight due to treatment with the crude lectin preparation was found, histological examination revealed a marked increase in the length ($P = 0.032$), width ($P = 0.004$), and area (P

Table 3. Morphometric measurements of the small intestinal mucosa and crypt cell mitotic index of control pigs (CP, n = 8) and lectin-treated pigs (LP, n = 8)[a]

Intestinal region and group	Crypt depth, μm	Mitotic index, %	Villus length, μm	Villus width, μ	Mucosa thickness, μm
Duodenum					
CP	186 ± 8		585 ± 55	121 ± 5	817 ± 58
LP	189 ± 8		494 ± 28	123 ± 4	711 ± 27*
Proximal jejunum					
CP	149 ± 5	3.1 ± 0.1	579 ± 13	103 ± 1	755 ± 17
LP	177 ± 8*	4.5 ± 0.1***	476 ± 38**	105 ± 4	679 ± 48***
Middle jejunum					
CP	155 ± 7	3.8 ± 0.1	773 ± 69	107 ± 5	986 ± 74
LP	157 ± 8	5.0 ± 0.1***	627 ± 31**	103 ± 3	815 ± 45
Distal jejunum					
CP	137 ± 3	3.5 ± 0.1	688 ± 122	113 ± 4	849 ± 116
LP	136 ± 6	4.3 ± 0.1***	424 ± 45**	112 ± 4	585 ± 52*
Ileum					
CP	130 ± 5		474 ± 51	108 ± 3	646 ± 50
LP	137 ± 5		375 ± 26**	109 ± 3	508 ± 29

[a]The results are presented as means ± SEM.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

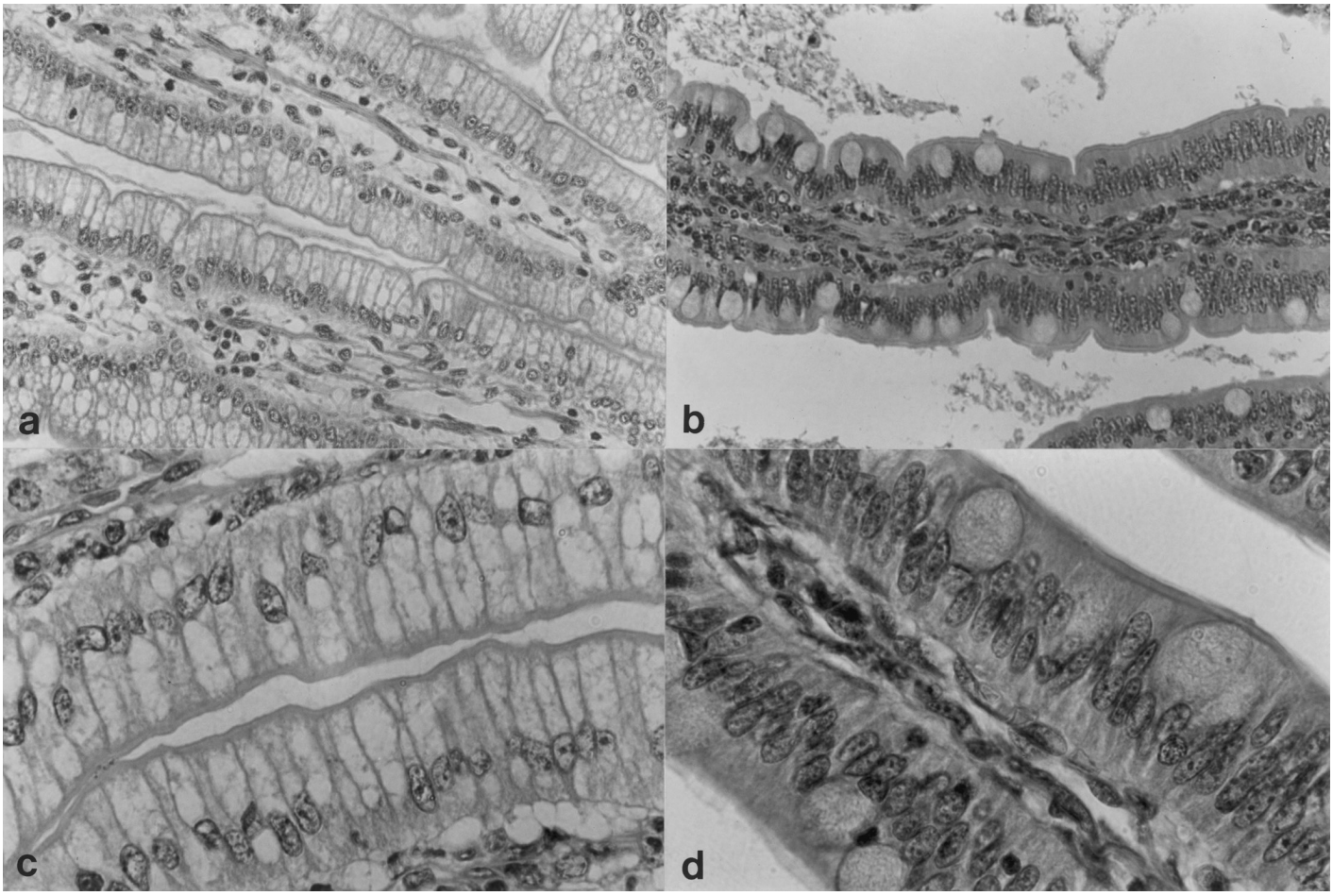

Figure 2. Photomicrograph of intestinal villi at the level of the distal jejunum in control (left) and lectin-treated (right) piglets. Note the abundance of large vacuoles in the enterocytes of the control piglets and the lack of large vacuoles in enterocytes of the lectin-treated piglets (magnification: top × 58, bottom, × 145).

= 0.031) of the pancreatic acini after the lectin administration. Analysis of pancreatic homogenates showed no differences between groups with respect to protein content or the content of the individual enzymes trypsin and amylase (Table 5).

Intestinal Absorption In Vivo

After gavage feeding, the levels of the markers increased in the blood in both groups ($P < 0.05$). The

Table 4. The proportion of vacuolated enterocytes in the intestinal villi and the area of the large vacuoles (LV) in the distal jejunum of control pigs (CP, n = 8) and lectin-treated pigs (LP, n = 8)[a]

Group	Enterocytes with LV, %	Area of the LV, μm^2
CP	97	103 ± 2
LP	25	49 ± 2***

[a]The results are presented as percentages and means ± SEM.
***$P < 0.001$.

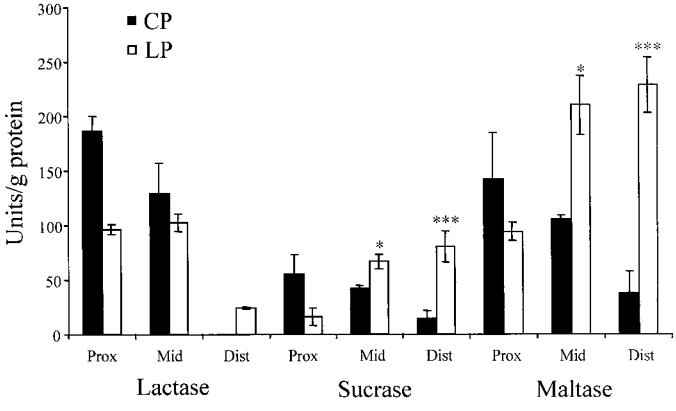

Figure 3. Mucosal disaccharidase activities (mean ± SEM) for lactase, sucrase, and maltase in the proximal (prox), middle (mid), and distal (dist) jejunum from control pigs (CP, n = 8) and lectin-treated pigs (LP, n = 8). *$P < 0.05$ and ***$P < 0.001$.

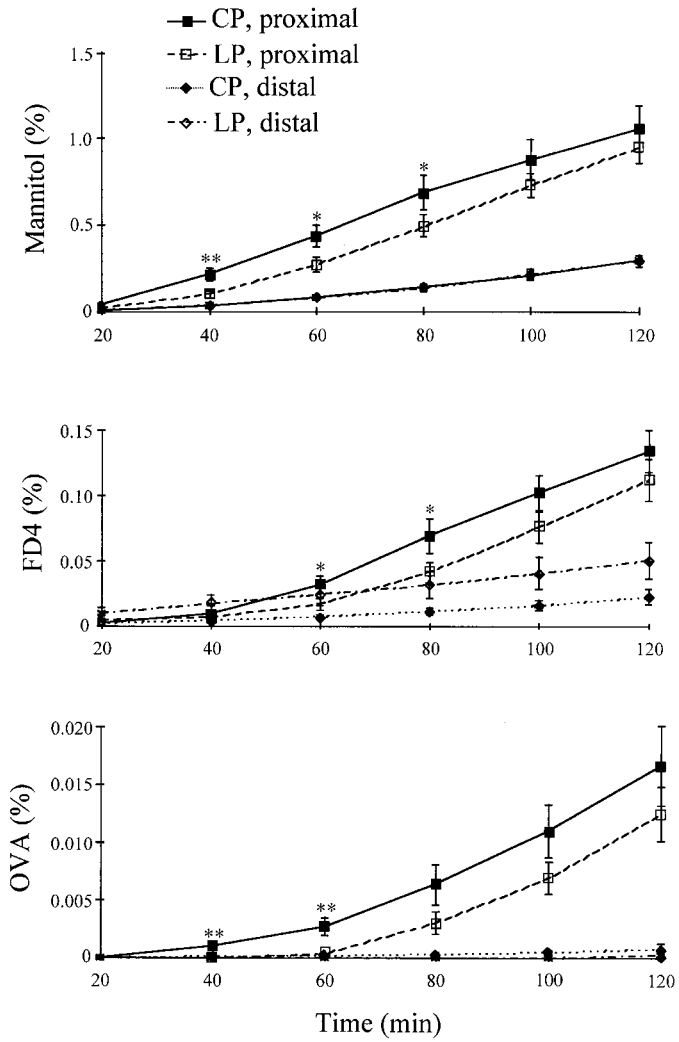

Figure 4. Tissue passage *in vitro* of the different-sized marker molecules, ^{14}C-mannitol, FITC-dextran 4400 (FD4), and ovalbumin (OVA), of control pigs (CP, n = 8) and lectin-treated pigs (LP, n = 8) in proximal and distal jejunum. The permeation of the markers from the mucosal to the serosal side in Ussing diffusion chambers is expressed as a percentage of the serosal concentration (mean ± SEM). *$P < 0.05$ and **$P < 0.01$ indicate significant differences in marker passage between the proximal regions of CP and LP.

peak blood plasma concentrations differed between the marker molecules in a size-dependent manner, and the peaks were seen after 0.5 h for Na-fluorescein, 2 h for dDAVP, and 4 h for BSA. For all these markers, the plasma levels were lower in the treated than in the control group ($P < 0.05$ to $P < 0.001$) (Figure 5), indicating that there had been less intestinal absorption in the treated group.

Blood Plasma Levels of Cholecystokinin

Following lectin treatment, the CCK plasma levels differed between groups after 3 d ($P = 0.009$) and 4 d ($P = 0.051$). However, the CCK levels within the lectin-treated pigs did not increase significantly due to great individual variation ($P > 0.05$). In the control group, the CCK levels remained at the same level from the 1st d of the experiment until pigs were killed, 5 d later (Table 6).

Discussion

The ingestion of a diet containing red kidney beans in rats leads to a reduction in body weight, and the administration of high doses of purified red kidney bean lectin damages the gut mucosa. However, lectin in lower doses (0.01 to 0.2 g/kg BW) does not manifest antinutritional effects in these animals and leads to a reversible dose-dependent hyperplastic growth of the gut wall (Bardocz et al., 1996). Here, the lectin binds to complex carbohydrate structures present on the mucosal cell surface and induces crypt cell proliferation (Pusztai, 1993). In addition to growth-promoting effects in the gut of adult rats (Pusztai et al., 1999), purified kidney bean lectin can stimulate the growth and development of the gut in young suckling rats (Biernat et al., 2000). Thus, it is to be expected that the administration of kidney bean lectin would also stimulate maturation of the GI tract in suckling piglets, and because it is difficult and expensive to obtain pure lectin in adequate amounts for feeding, the use of a crude preparation seems to be appropriate in practice. Preliminary experiments in rats comparing the effect of this crude preparation with that of purified lectin have not shown any difference in effect between them (unpublished data).

Table 5. Pancreatic morphometric parameters (means ± SEM), showing acinus area, length, and width and the number of acinar cells per acinus and the pancreatic content of total protein and enzymes (trypsin and amylase activities) from control pigs (CP, n = 8) and lectin-treated pigs (LP, n = 8)

	Acinus						
Group	Length, μm	Width, μm	Area μm^2	Cells/ acinus	Protein, mg/kg	Trypsin, U/kg	Amylase, kU/kg
CP	26 ± 2	16 ± 2	378 ± 43	9.1 ± 0.1	99.3 ± 7.0	5.82 ± 0.53	4.23 ± 0.66
LP	31 ± 2*	21 ± 0.2**	516 ± 46*	9.2 ± 0.1	86.1 ± 7.2	5.46 ± 0.68	3.57 ± 0.36

*$P < 0.05$.
**$P < 0.01$.

Our observations indicated that exposure to a crude preparation of red kidney bean lectin containing about 25% lectin (red kidney bean albumin) for 3 d affected the piglets. These animals showed a depressed growth rate and some developed diarrhea after the first gavage feeding of lectin but recovered rapidly within 24 h. In all other aspects the treated piglets could not be distinguished from the controls and showed normal feeding behavior throughout the experiment.

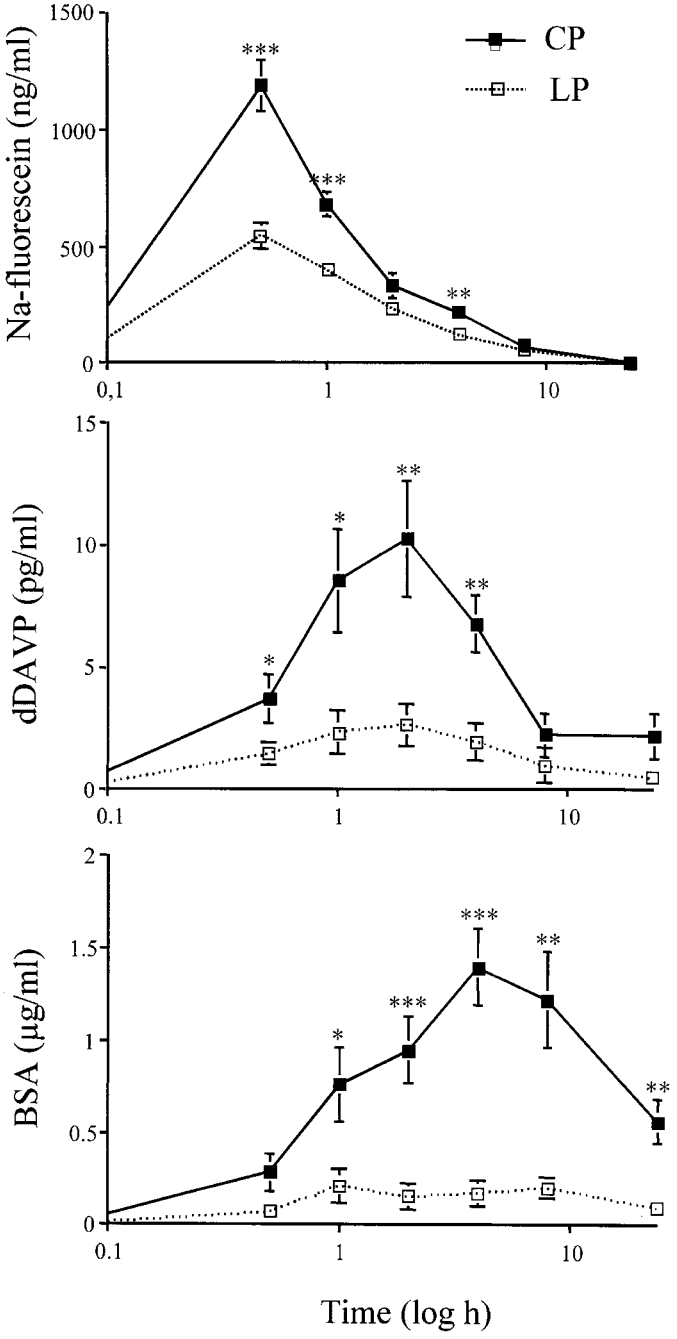

Figure 5. Blood plasma levels (mean ± SEM) of the marker molecules, Na-fluorescein, dDAVP and bovine serum albumin (BSA) during 24 h after marker gavage feeding to control pigs (CP, n = 16) and lectin-treated pigs (LP, n = 11). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table 6. Blood plasma levels of cholecystokinin (CCK; means ± SEM) at 4 h and at 3 and 4 d after the first treatment with crude red kidney bean lectin, as a percentage of the plasma level before the treatment, for control pigs (CP, n = 16) and lectin-treated pigs (LP, n = 11)

	Time after gavage feeding		
Group	4 h, %	3 d, %	4 d, %
CP	103 ± 9	99 ± 12	110 ± 37
LP	132 ± 33	255 ± 73**	511 ± 277

**$P < 0.001$.

The results obtained from many of the variables studied indicated that treatment of young suckling pigs with the crude lectin preparation induced precocious gut maturation. Initially, the treatment might have caused a primary insult to the gut, but no signs of mucosal damage could be detected by histological analysis at 2 d after treatment in either the crypt or the villi regions. Instead, intestinal and organ growth, amplified crypt cell proliferation, altered phenotype of the enterocytes with a more mature disaccharidase pattern, less enterocyte vacuolization, and increased intestinal barrier properties were observed in the lectin-treated animals.

A clear effect on the stomach with growth increment and increased mucosal thickness was seen after lectin treatment. There was also a tendency for an increase in stomach function with a greater HCl secretion. However, the mucosal-specific content of pepsinogen A was unaffected by the lectin treatment (P. T. Sangild [R. Vet. Agric. Univ., Denmark], K. Rådberg, and B. R. Weström, unpublished data).

The histological examinations showed structural changes in the intestine of the lectin-treated pigs, including decreased villus height in most of the small intestine and increased crypt depth in the proximal jejunum, but not in the rest of the small intestine. Similar structural changes are associated with weaning (Klein, 1989; Kelly et al., 1992) and lead to decreased gut digestive and absorptive functions (Hampson, 1986). These observed changes in morphology might indicate a higher enterocyte turnover and an increased proliferation of crypt cells due to the lectin treatment; it previously has been shown that the crypt size, the number of cells in the crypts, and the crypt cell proliferation rate are increased after lectin administration in adult rats (Pusztai et al., 1990). In fact, the mitotic index measurements showed markedly elevated crypt cell proliferation in the jejunum. However, the percentage of apoptotic cells in the jejunum was not notably changed. This phenomenon might be explained by the fact that tissue sampling was performed relatively late (48 h) after the lectin treatment.

In the control pigs, numerous vacuolated enterocytes were still present in the distal jejunum and ileum at 2

wk of age, whereas in lectin-treated pigs they were much less frequent and located mainly on the top of the villi. Furthermore, the size of the remaining vacuoles was reduced in the treated pigs. The enterocytes of newborn piglets are characterized by a prominent apical vesicular-tubular system with large cytoplasmic vacuoles. These fetal-type enterocytes are gradually replaced in a proximal-to-distal direction by more mature cells that lack this system and have fewer vacuoles (adult-type) during the first 3 wk of life (Smith and Peacock, 1980; Ekström et al., 1988). Thus, the increased crypt cell proliferation rate and decreased enterocyte vacuolization observed in the treated pigs indicated that there had been an increase in the replacement of fetal-type with adult-type enterocytes (Baintner, 1986) due to the treatment, resulting in a mucosal epithelium that seemed to be more similar to that of older pigs.

A tendency for a decrease in lactase activity and significant increases in sucrase and maltase activities were found following lectin administration. This change was similar to the pattern normally seen during postnatal intestinal development (Henning, 1987). In fact, the proximal-to-distal pattern of sucrase and maltase activity in the lectin-treated pigs was the exact opposite of that in the controls, and this strengthened the probability that lectin altered the phenotype of the enterocytes to a more advanced stage of development.

A spectrum of different-sized marker molecules was used to test the tissue permeability and absorption properties of the gut both in vitro and in vivo. The lectin treatment resulted in decreased intestinal tissue permeability and in lower in vivo absorption, similar to that previously shown postnatally for the absorption of macromolecules in young rats, pigs, and humans (Henning, 1987). The relatively small, hydrophilic marker molecule ^{14}C-mannitol has been shown to pass the gut epithelium by passive diffusion influenced by solvent drag, thus following the net flow of water (Menzies, 1984). The reduced permeability of ^{14}C-mannitol observed in the proximal jejunum after lectin treatment may be explained by a reduction in the absorptive surface area caused by the villi shortening. The observed decrease in the passage of FD4 might have been caused by a combination of decreased surface area, a decreased rate of endocytosis, and a tighter epithelium after the exposure to lectin. The lectin-induced reduction in the passage of the protein marker OVA, which mainly passes the gut epithelium by endocytosis (Sanderson and Walker, 1993), might be explained by the replacement of the fetal-type enterocytes by adult-type enterocytes that lack large vacuoles and have less endocytotic activity (Smith and Peacock, 1980; Baintner, 1986).

The gut maturation induced by lectin administration to suckling pigs was confirmed functionally in absorption studies in vivo after marker molecule gavage feeding. Reduced absorption of the inert and passively transported molecule Na-fluorescein could be explained by decreased surface area (reduced villus size). In addition, the lower absorption of the peptide dDAVP might have been due to a combination of decreased surface area and endocytosis rate, and presumably (though not investigated in the present study) a tighter epithelium following the treatment. The decrease in endocytosis rate due to the presence of more adult-type enterocytes might explain the sharp reduction in BSA (which is absorbed via the endocytotic route) absorption after lectin treatment. In addition, increased proteolysis due to elevated pancreatic enzyme secretion in lectin-treated pigs might also have influenced the amount of intact proteins available in the lumen, and therefore also endocytosis. Taken together, these results indicated that the efficiency of the intestinal barrier function in young pigs had increased after lectin treatment.

Although an increase in pancreatic acini size was observed in the pigs after lectin treatment, no increase in pancreatic growth per se was found, despite an elevation of the plasma levels of CCK, which is a hormone that promotes pancreatic growth in several mammalian species (Nylander et al., 1992; Lee and Lebenthal, 1993). However, the piglet is characterized by relatively high plasma CCK levels during the suckling period, which decrease after weaning (Pierzynowski et al., 1999). Any further increase in the already high CCK levels due to the lectin treatment might have a negligible growth effect on the pancreas because of, for example, receptor saturation. An increase in pancreatic enzyme levels during the first weeks of postnatal life in young pigs has previously been reported (Ohlsson et al., 1987), and an increase in pancreatic secretion has also been observed (Pierzynowski et al., 1990, 1995). However, the lectin treatment did not induce any elevation in pancreatic enzyme levels. Considering pancreatic levels alone, as in the present study, without estimating the real secretion, however, might not give a proper and complete picture of pancreatic function.

Implications

Postweaning diarrhea in pigs is a serious and complex problem from the standpoint of the health of the pigs and of economics. The present study shows the possibility of inducing gut maturation in young piglets by exposure to a crude kidney bean lectin preparation by gavage feeding before weaning. The obvious question is whether these effects of lectin treatment improve the piglets' ability to adapt to weaning and thus improve the growth and health of the animal.

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