

Three-Day Enteral Exposure to a Red Kidney Bean Lectin Preparation Enhances the Pancreatic Response to CCK Stimulation in Suckling Pigs

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Key Words

Weaning · Development · Trypsin · Amylase · Lipase · Insulin · CCK receptors

Abstract

Background: A reason for the digestive problems that often occur around early weaning in piglets could be that the pancreas is not yet fully developed and the enzymes required for degradation of the solid food are not secreted in enough amounts. **Objectives:** The aim of the study was to investigate the possibility of inducing pancreas maturation with enhanced enzyme secretion. **Methods:** 10-day-old suckling pigs were gavage fed with a red kidney bean lectin preparation for 3 days, and the pancreatic response to intravenous infusion of CCK-33 was measured in the anaesthetized animals fitted with pancreatic duct catheters. **Results:** The pancreatic fluid secretion, protein output, and the trypsin and amylase outputs were significantly increased in response to CCK stimulation after the lectin treatment, as compared to those of the control littermates ($p \leq 0.05$). In addition, the plasma insulin basal levels and those observed during CCK-33 stimulation were lower in the lectin-treated piglets. **Conclusion:** The results suggested that the lectin treatment led to an increase in the capacity for pancre-

atic enzyme secretion in the suckling piglets. An enhanced pancreatic function might help to ameliorate the problems that may appear in modern pig production which are associated with weaning.

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Introduction

The digestive tract and its accessory glands undergo major developmental changes during the neonatal period in mammals. These maturational changes gradually progress with age, and in nature, with gradual weaning, permit the digestive tract to keep pace with the demands placed on it due to the gradual change in diet. Extensive structural changes are seen in the digestive tract at this time, and the amount of enzymes present in the intestinal tract increase, reflecting an elevated pancreatic function [1].

In young pigs, pancreatic weight (relative to body weight) and content of digestive enzymes has been shown to increase with age [2–4]. In studies with chronically catheterized piglets, it has been demonstrated that pancreatic secretion – both the basal secretion, the secretory response to milk consumed, and the secretagogue stimulation by CCK and secretin – was low throughout the suckling period [5]. However, after weaning, a marked

increase in the pancreatic fluid secretion and the output of protein and digestive enzymes was observed, both basally and after stimulation [5, 6].

Lectins are carbohydrate-binding proteins found in high amounts, especially in leguminous plants, that will bind to and aggregate cells in the body [7]. In adult rats, dietary kidney bean (*Phaseolus vulgaris*) lectin has been shown to bind to the gut epithelium and thereby potently promote the growth of the gastrointestinal tract and the pancreas [7–9]. In addition, pancreatic secretion and intestinal luminal enzyme (trypsin, chymotrypsin and amylase) levels were reported to increase after lectin exposure [10, 11]. Apart from these effects in the adult rat, we have shown that enteral exposure to red kidney bean lectin stimulates the growth and maturation of the gut and pancreas in young suckling rats [12]. In addition, although no increase was observed in pancreas weight, an increase in the area of the pancreatic acini has been reported after exposing suckling pigs to a preparation of red kidney bean lectin [13].

The aim of the present study was to determine if enteral exposure to a kidney bean lectin preparation might accelerate the functional maturation of the pancreas in suckling animals. To do this, the same suckling pig animal model was used as in previous studies; animals 10 days of age were gavage fed with a preparation of red kidney bean lectin for 3 days [13], whereafter the secretory response before and during secretagogue stimulation was studied in the animals anaesthetized and fitted with pancreatic duct catheters.

Materials and Methods

Animals

In the study, six cross-bred ((Swedish Landrace × Yorkshire) × Hampshire) piglets from 3 different litters were obtained from the research herd of the Swedish University of Agricultural Sciences in Alnarp (Odarslöv Research Farm). This herd is closed and batch farrowing is practised. The animals in this herd are well defined since all animals are followed from birth to slaughter with an individual journal including data of production, rearing system, eventual disease treatments and production data. The piglets were divided into lectin-treated pigs ($n = 3$) and control littermates ($n = 3$), but kept together with the sow and allowed to suckle with no restrictions. Animal treatments and experiments were conducted according to the European Community regulations concerning the protection of experimental animals, and the Lund University Ethical Review Committee on Animal Experiments approved the study.

Lectin Exposure

The treatment started when the piglets were 10 days old and was repeated for 3 days. In the morning, the lectin-treated pigs were gavage fed with a crude preparation of lectin obtained from red

kidney beans (*P. vulgaris*; red kidney bean albumin, 400 mg/kg dissolved in 0.9% NaCl) by a stomach tube. The purification of red kidney bean albumin was performed according to Pusztai and Watt [14]. The preparation contained approximately 25% lectins, and the dose of kidney bean lectin that the pigs received per day was thus estimated to be 100 mg/kg. The control pigs were gavage fed with the vehicle (5 ml/kg 0.9% NaCl).

Experimental Procedure

After the treatment period when the pigs were 13 days of age, they were sedated with azaperone (Stresnil; Janssen Pharmaceutica, Beerse, Belgium), weighed and transported to the surgical facilities within 30 min. The piglets were then anaesthetized, intubated and anaesthesia was kept during surgery using halothane (2-bromo-2-(1,1,1-trifluoroethane), Halothane, SIC Chemicals Ltd., Avonmound, England). The right and left external jugular veins were catheterized with silicon tubing (Silastic, Dow Corning Corp. Midland, Mich., USA) for the intravenous infusion of anaesthetic and hormones, and for blood sampling. A linea alba laparotomy was then performed, and the pancreatic duct was exposed and catheterized with a silicon tubing for collection of pancreatic juice. Since halothane completely inhibits pancreatic juice secretion, the anaesthesia was changed to metomidate (Hypnodil, Janssen Pharmaceutica, Sweden, 10–20 mg kg⁻¹ h⁻¹) after surgery, and the piglets were allowed to stabilize for a 30-min period [15].

During the entire experiment, secretin (Ferring AB, Malmö, Sweden, 20 pmol kg⁻¹ h⁻¹, in saline with 0.5% bovine serum albumin (BSA), Sigma Chemical Co., St. Louis, Mo., USA) was continuously infused intravenously to maintain a background pancreatic fluid secretion. To evaluate any differences between the control and lectin-treated pigs, pancreatic juice was collected both before (basal), during and after stimulation with CCK. Thus, the pancreatic juice was first collected for 3 × 10 min; then CCK-33 (Ferring AB, Malmö, Sweden) dissolved in saline with 0.5% BSA was infused at a dose of 700 pmol kg⁻¹ h⁻¹ for 60 min, and the pancreatic juice was collected in 6 × 10 min periods. An additional 3 × 10 min collection of the pancreatic juice was performed after the end of the CCK infusion. The pancreatic juice was sampled in plastic tubes on ice, measured for volume and then frozen at –20°C until the analyses were performed.

Analyses

In the pancreatic juice, the total protein concentration was analysed using the method of Lowry et al. [16], with purified BSA (A-7638; Sigma Chemical Co., St. Louis, Mo., USA) as a standard. The trypsin activity was measured after activation by the addition of enterokinase (Sigma) to the pancreatic juice, using Na-benzoyl-DL-arginine-*p*-nitroanilide (Sigma) as a substrate. Amylase activity was analysed using blue starch as a substrate (Phadebas Amylase Test, Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions. Lipase activity was determined by pH stat titration (Mettler components DK10, DK11 and V11) using tributyrin as substrate [17]. Enzyme activities were expressed as units (U), with 1 U defined as the amount of enzyme transforming 1.0 mmol of substrate per minute at 25°C.

Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ¹²⁵I-labelled human insulin as tracer and rat insulin as standard (Linco Research, St. Charles, Mo., USA). Plasma glucose levels were determined with the glucose oxidase method.

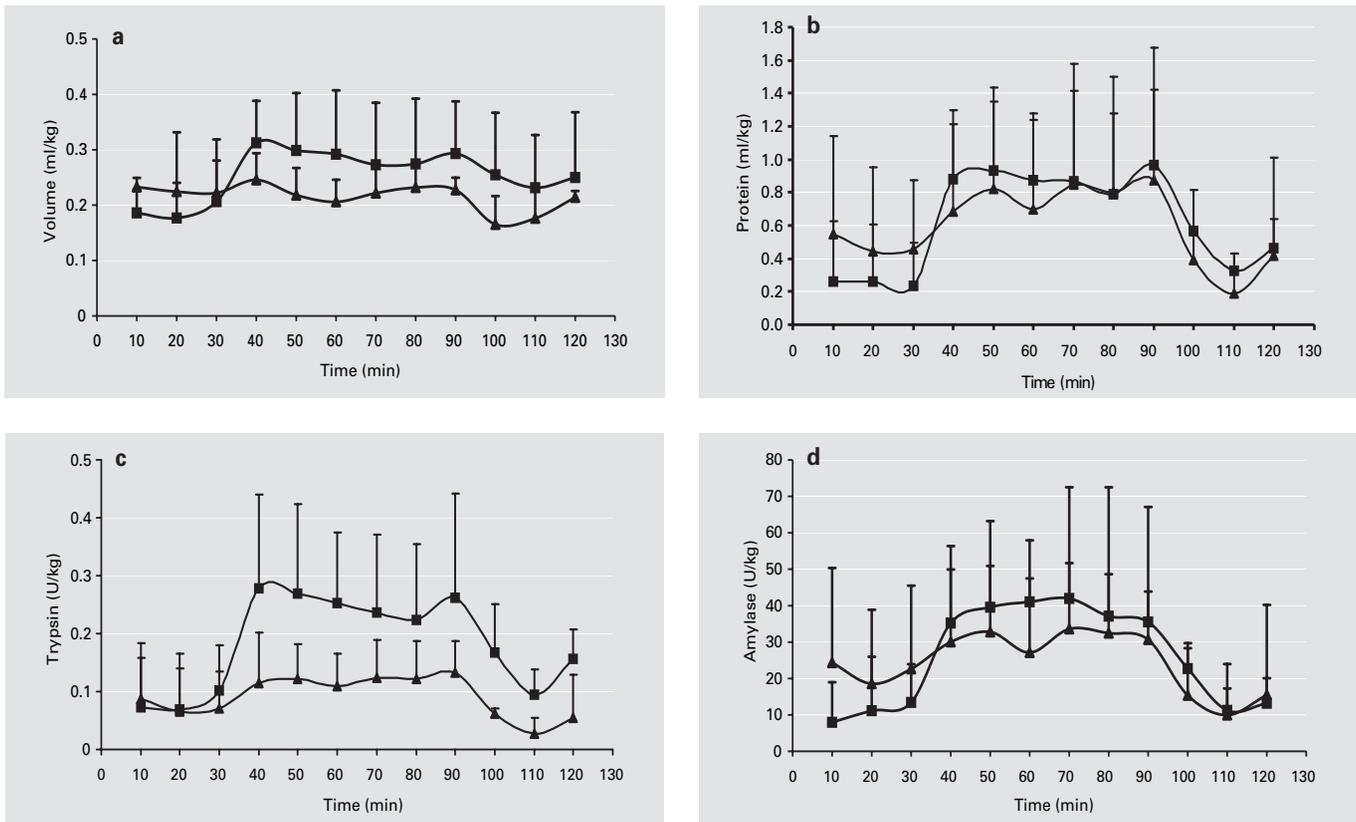


Fig. 1. Pancreatic fluid secretion (a) and output of protein (b), trypsin (c) and amylase (d) measured before (10–30 min), during (30–90 min) and after ended stimulation with an intravenous infusion of CCK-33 (90–120 min) in piglets, either after lectin treatment for 3 days (squares) or control littermates (triangles). During

the period of CCK stimulation (30–90 min), the fluid secretion ($p < 0.001$) and output of protein ($p < 0.01$), trypsin ($p < 0.01$) and amylase ($p < 0.015$) were significantly higher in the lectin-treated pigs than in the control pigs.

Table 1. Weight gain (kg) of lectin-gavaged piglets and littermate control piglets from 3 different litters (1, 2, 3) during a 3-day treatment period

	Weight gain, kg			
	day 10	day 11	day 12	day 13
Lectin 1	3.3	3.5	3.5	3.5
Lectin 2	4.7	4.8	4.8	4.5
Lectin 3	2.7	2.7	2.8	3.0
Control 1	3.4	3.7	3.9	4.2
Control 2	5.0	5.3	5.6	6.0
Control 3	3.1	3.4	3.5	3.8

Statistics

The results are expressed as mean values and SD. For statistical analysis, the pancreatic juice data were transferred to relative values (% of basal values) and were analysed using multi-factorial ANOVA and a multiple range test (with the experimental periods, before, during and after CCK stimulation, in the model). Student's

t test for unpaired data was used for the statistical evaluation of differences between plasma insulin levels.

Results

During the treatment period of 3 days, the lectin-gavaged piglets gained little weight (–0.2 to 0.3 kg), in comparison to their littermate controls that had gained between 0.7 and 1.0 kg (table 1). After this treatment period and during anaesthesia, the pancreatic juice secretion was measured before, during and after stimulation by the intravenous infusion of CCK-33 in the lectin-treated piglets and compared to that in their control littermates.

In comparison to the secretion before (basal), an intravenous infusion of CCK-33 significantly increased the volume of juice secreted ($p < 0.05$) in the lectin-treated pigs, while the fluid secretion in the control pigs remained unaffected (fig. 1a). In addition, the CCK-33 infusion led

Fig. 2. Pancreatic lipase output measured before, during and after stimulation with an intravenous infusion of CCK-33 in piglets, either lectin treated for 3 days (unfilled columns) or control littermates (filled columns).

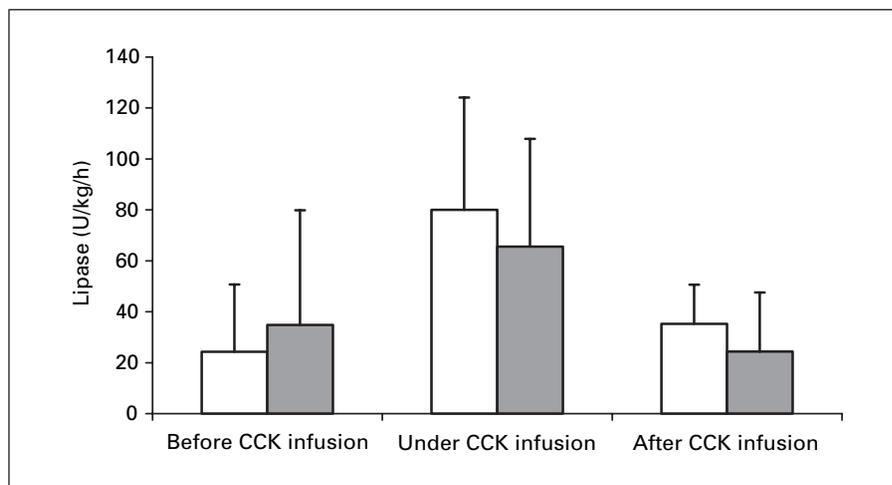
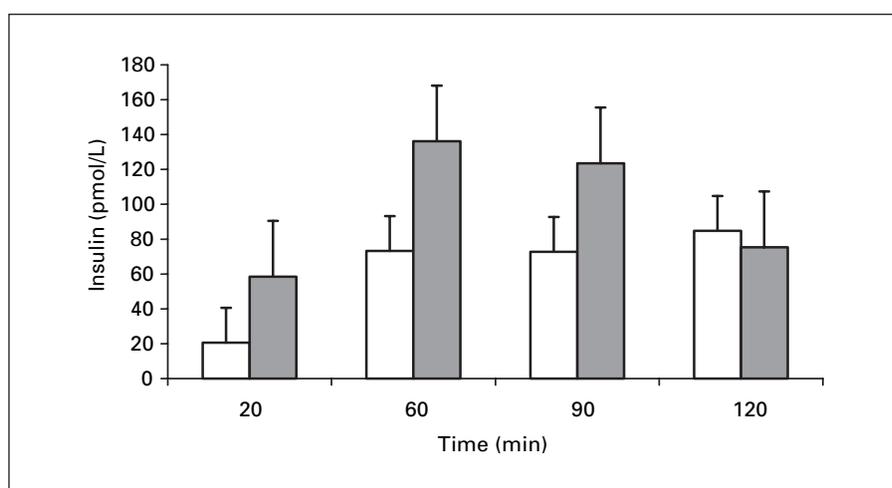


Fig. 3. Plasma insulin levels measured before (at 20 min), during (at 60 and 90 min) and after (120 min) stimulation with an intravenous infusion of CCK-33 in piglets, either lectin treated for 3 days (unfilled staples) or control littermates (filled staples). The infusion of CCK-33 gave tendency to a lower insulin response in the lectin-treated ($p = 0.065$) as compared to the control pigs.



to significant increases in the total pancreatic protein output ($p < 0.05$) and in the output of the individual enzymes, trypsin, amylase and lipase ($p < 0.05$), respectively, both in the lectin-treated and the control pigs (fig. 1a–d, fig. 2). After stimulation with CCK-33, the volume and enzyme secretion returned towards the basal levels seen before stimulation.

During the period of CCK stimulation, the fluid secretion ($p < 0.001$), protein output ($p < 0.01$) and output of the individual enzymes, trypsin ($p < 0.01$) and amylase ($p < 0.015$), were significantly higher in the lectin-treated pigs than in the control pigs (fig. 1a–d).

Before CCK infusion, the basal plasma levels of insulin were significantly lower ($p < 0.05$) in the lectin-treated piglets than in the controls (fig. 3). The infusion of CCK-33 gave significantly ($p < 0.05$) increased plasma insulin levels in both the control and the lectin-treated animals,

although there was a tendency to a lower response in the lectin-treated group ($p = 0.065$). The plasma glucose levels (6.6 ± 0.3 , control; 8.7 ± 2.6 , lectin treated) were not altered in any of the piglets during CCK stimulation (6.2 ± 1.2 , control; 8.1 ± 1.3 , lectin treated), and no significant difference was observed between the groups.

Discussion

Dietary or experimentally fed lectins have been reported to induce the hyperplastic and hypertrophic growth of the pancreas in both adult [18] and suckling rats [12]. A marked increase in pancreatic acinus size was also reported after experimentally feeding young suckling pigs with a preparation of red kidney bean lectin [13]. The effect of a short-term enteral exposure period of 3 days in

suckling pigs to a kidney bean lectin preparation on the functional capacity of the exocrine pancreas and to some extent on the endocrine pancreas is reported in the present study. Although the number of animals treated and operated on in the study was small, the results were evident when the lectin-treated animals were compared to their control littermates.

Our observations indicated that exposure to a crude preparation of red kidney bean lectin containing about 25% lectin for 3 days affected the piglets. These animals showed a depressed growth rate, even though not significant, and some developed diarrhoea 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 behaviour throughout the experiment.

At 13 days of age, the control pigs showed little response to CCK-33 stimulation (during a background of secretin infusion), whereas the lectin-treated animals showed a significantly enhanced pancreatic fluid secretion. Secretin is classically regarded as the main regulating factor for the electrolyte-rich fluid secreted from the pancreatic ductal cells, with CCK acting synergistically [19]. A similar increase in volume secretion due to CCK-33 infusion during continuous secretin administration has also been seen in older, weaned pigs [20], and in relation to weaning itself [6, 21]. Therefore, the results implied that the effect of CCK on stimulating exocrine fluid secretion might have matured due to the lectin treatment, as seen naturally during the weaning process. This effect, however, is not likely to be mediated directly by the pancreatic duct cells, since CCK receptors have not been found on either the acinar or the ductal cells in pigs [22–26]. Another explanation might be that exposure to lectins stimulates the development of a regulatory pathway between the acinar and ductal cells. Hypothetically, an increase in protein secretion by the acinar cells might stimulate the electrolyte secretion from the ductal cells by an intrapancreatic feedback mechanism [27].

In addition, the enzyme secretion was significantly enhanced during CCK-33 stimulation in the lectin-treated piglets in comparison to that of the control pigs. This showed that the trophic effect of lectin treatment on the pancreatic acini, as reported in a previous study [13], is coupled to an increased functional capacity of the exocrine pancreas. Taken together, these results indicated that it was possible to induce the precocious maturation of the exocrine pancreas by the treatment of sucking pigs with a short-term gut exposure to kidney bean lectin. In addition to the increase in zymogen content due to the

increased acinus size, the enhanced reactivity of pancreas to exogenous CCK stimulation in the lectin-treated piglets can be explained by the maturation of the CCK receptors (number and/or affinity) due to the treatment.

A trophic relation between gut exposure to lectins, CCK release and CCK-A receptors in the pancreas has been shown in adult rats, since the stimulated growth of the pancreas after exposure to red kidney bean lectin could be significantly reduced by using a specific CCK-A inhibitor [28], as could the increase in enzyme secretion [29]. However, CCK receptors are not found on the acinar cell of pigs, and thus the explanation for the augmented response to CCK after lectin treatment observed in this study must be different. Our earlier studies rather indicated that CCK-A receptors had played a role – not those on the acinar cells, but those CCK-A receptors shown to be present peripherally to the pancreatic/duodenal region [20]. Exposure to lectins might increase the number of these receptors and thus be responsible for the elevated reactivity of the pancreas after CCK-33 stimulation in piglets. Furthermore, in previous studies of young pigs, an increase in the plasma levels of CCK after short-term exposure to a red kidney bean lectin had been observed [13].

The decrease in the plasma levels of insulin seen in this study has also been reported in earlier studies on adult rats treated with lectins [9, 14, 18]. One possible mechanism for this effect could be that lectins may affect the release of neurotransmitters or hormones, such as the calcitonin gene-related peptide, opioids, substance P or pancreastatin, that can inhibit insulin secretion [30, 31]. Another possibility could be that since lectins can be absorbed into the circulation after oral exposure [18], they might also act as an agonist by binding to the insulin receptor [14]. They would thus exhibit an insulin-like effect and thereby increase insulin sensitivity, which would reduce the secretion of endogenous insulin [32]. This possibility is supported by the presence of unaffected glucose levels in spite of reduced insulin levels.

In conclusion, the results showed that it is possible to induce the precocious maturation of exocrine pancreas function in suckling piglets by short-term gut exposure to kidney bean lectin. In modern pig production, the piglets are weaned at a much earlier state than in nature. The abrupt change from milk to solid food often leads to problems like stress, diarrhoea and the overgrowth of bacteria in the intestinal tract, which in turn leads to malnutrition and early death of the piglets, primarily due to bacterial infection. One reason for the digestive problems that occur around weaning could be the fact that the pancreas of

the piglets is not yet fully developed, and that the enzymes required for degradation of the solid food are either not produced or released to the gastrointestinal tract in high enough amounts.

Lectin exposure might be utilized as a possible pre-treatment in suckling pigs, to overcome some of the unwanted effects due to the inadequate function of the pancreas, as reflected by the low performance of pigs at weaning.

Acknowledgements

The laboratory help of Mrs. Inger Mattsson and Lilian Bengtsson is gratefully acknowledged. This work was supported by grants from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, and the Swedish Agency for Innovation Systems (Vinnova, Eureka projects, No. 2675: Healthy-weaning) and Swedish Medical Research Council.

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