Reprod. Nutr. Dev. 38 (1998) 391–400



**Original article**

# Pancreatic secretion of zinc and carboxypeptidase A and B in growing pigs

Mette S. Hedemann[a], Vince M. Gabert[a,c], Torben Larsen[b]

[a] Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, 8830 Tjele, Denmark
[b] Department for Animal Health and Welfare, Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, 8830 Tjele, Denmark
[c] Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

(Received 6 October 1997; accepted 20 June 1998)

**Abstract** – The secretion of zinc and the level of carboxypeptidase A and B activity in pancreatic juice were studied in three pigs fitted with a pancreatic pouch re-entrant cannula (PM pigs) and three different pigs with a catheter surgically implanted in their pancreatic duct (CM pigs). The zinc in the pancreatic juice appeared to be primarily associated with carboxypeptidase A and B. Both the concentration of zinc in pancreatic juice and the daily secretion of zinc in PM pigs were greater than in CM pigs. However, compared to the daily intake of zinc, its secretion in pancreatic juice was low. The specific activity levels of carboxypeptidase A and B in pancreatic juice collected from PM pigs were higher than in CM pigs. The total activity of carboxypeptidase A in pancreatic juice did not differ between collection methods. The total activity of carboxypeptidase B was higher in pancreatic juice collected from PM than from CM pigs. The differences in zinc and carboxypeptidase secretion between PM and CM pigs were probably due to physiological changes induced following the different surgical preparations of the animals. © Inra/Elsevier, Paris

**carboxypeptidase / pancreatic secretion / pigs / zinc**

**Résumé – Sécrétion pancréatique de zinc et carboxypeptidases A et B chez le porc en croissance.** La sécrétion de zinc et le niveau d'activité des carboxypeptidases A et B dans le suc pancréatique ont été étudiés sur trois porcs munis de canules pancréatiques ré-entrantes (porcs PM) et sur trois porcs porteurs d'un cathéter implanté par voie chirurgicale dans le canal pancréatique (porcs CM). Le zinc du suc pancréatique est apparu associé de façon primaire aux carboxypeptidases A et B. La concentration en zinc du suc pancréatique et la sécrétion journalière de zinc chez les porcs PM étaient plus élevées que chez les porcs CM. Cependant, comparée à l'apport journalier de zinc, la sécrétion dans le suc pancréatique est faible. Les activités spécifiques des carboxypeptidases A et B étaient plus fortes

* Correspondence and reprints
E-mail: Mette.Hedemann@agrsci.dk

chez les porcs PM que chez les porcs CM. L'activité totale de la carboxypeptidase A dans le suc pancréatique était la même par les méthodes de collecte, tandis que l'activité de la carboxypeptidase B était supérieure chez les porcs PM par rapport aux porcs CM. Les différences de sécrétion de zinc et de carboxypeptidsase entre les porcs PM et CM étaient probablement dues à des modifications physiologiques induites consécutives aux différentes techniques chirurgicales utilisées. © Inra/Elsevier, Paris

**carboxypeptidase / sécrétion pancréatique / porc / zinc**

## 1. INTRODUCTION

Pancreatic juice is a complex solution that contains various enzymes, electrolytes and metal ions. Many studies have investigated how exocrine pancreatic secretion changes in response to changes in diet composition, age and feeding regimen (e.g. [4, 14]). However, there is little information available on the secretion of electrolytes and metal and the factors which influence their concentration and role in pancreatic juice.

The concentration of zinc and the activity levels of both carboxypeptidase A and B are highly correlated in pancreatic juice collected from rats. In the rat, feeding zinc deficient diets decreases carboxypeptidase activity. The activity is, however, restored by feeding diets that contain adequate levels of zinc [17]. The activity levels of carboxypeptidase A and B have been measured in pancreatic juice collected from pigs fed diets which varied in nutrient composition [28, 30]. The concentration of zinc in pancreatic juice from pigs was determined in normal and zinc-deficient pigs [23, 24]. However, the correlation between the carboxypeptidase A and B activity levels and the concentration of zinc in pancreatic juice has not been determined in the pig and may be of physiological significance. In addition, the diurnal pattern of zinc secretion and carboxypeptidase A and B activity have not been determined.

It is suggested that exocrine pancreatic secretion plays an important role in zinc homeostasis in humans. This is, in part, because zinc is continuously secreted from the exocrine pancreas and after which it can be reabsorbed, thereby creating an enteropancreatic circulation of zinc [15, 16]. It is suggested that zinc in pancreatobiliary secretion plays a minor role in maintaining zinc homeostasis in the rat [5]. The importance of its role remains to be investigated in the pig.

The objective of the present study was to determine the diurnal pattern of exocrine pancreatic secretion of zinc and the zinc-dependent metalloenzymes, carboxypeptidase A and B, in growing pigs. The animals were fed a grower–finisher diet supplemented with zinc at a level that exceeded Danish recommendations for growing pigs. Furthermore, these studies made it possible to estimate the importance of pancreatic zinc secretion to zinc homeostasis by comparing the daily pancreatic secretion of zinc to the daily intake of zinc.

This present study was part of a study, conducted to evaluate the effect of fat source on exocrine pancreatic secretion in growing pigs. The results concerning the effect of fat source have been reported previously [6].

## 2. MATERIALS AND METHODS

### 2.1. Animals and diets

Two experiments were performed with a total of six crossbred barrows (obtained from the Danish Institute of Agricultural Sciences' swine herd). The pigs were kept individually in stainless steel metabolic crates and surgically prepared for the total collection of pancreatic juice. In experiment one, three barrows (initial BW 37 kg) were fitted with a pancreatic pouch re-entrant cannula for collection and subsequent return of pancreatic juice (pouch method, PM) according to previously described procedures [7, 9]. In

experiment two, three barrows (initial BW 32 kg) had a catheter surgically placed into the pancreatic duct as well as a simple T-cannula in the duodenum, for the complete collection and reinfusion of pancreatic juice (catheter method, CM) [19, 26].

In both experiments, the pigs were fed one of three diets that contained 40.3 % wheat starch, 22.3 % fish meal and 15.0 % of fish oil, rapeseed oil or coconut oil. The diets also contained 10.0 % wheat bran, 10.0 % sucrose and 2.4 % minerals and vitamins [6]. The amount of zinc, in the form of zinc oxide, supplied by the premix was 80 mg/kg diet and the final zinc content of the diets was 110 mg/kg diet. The pigs were fed 1.65 kg/d in three meals of equal amounts at 0800, 1600 and 2400 hours; therefore, the daily intake of zinc was 182 mg. The experiment was performed according to a 3 × 3 Latin square design. Each experimental period consisted of 7 d: 5 d adaptation to the experimental diet followed by 2 d collection of pancreatic juice.

Pancreatic juice was collected for a total of 24 h, continuously from 1600 to 2400 hours on day 6, from 0800 to 1600 hours on day 7 and from 2400 hours on day 7 to 0800 hours on day 8 (the end of the experimental period). Pancreatic juice was collected at hourly intervals. After each hour of collection, the volume of pancreatic juice secreted was recorded and 5 % of the total volume sampled was stored at –80 °C until analyses. The volume removed was replaced with 154 mM sodium chloride and the pancreatic juice was continuously reinfused into the duodenal T-cannula with a peristaltic pump (Model 2650, Ole Dich Instrument Makers, Hvidovre, Denmark). The pump was adjusted frequently so that the rate of pancreatic juice return was as close as possible to the rate of secretion. Pancreatic juice from the last hour of collection of the most recent 8-h sampling period was infused during the first hour of the next collection.

The protocol used in both experiments complied with the Danish Ministry of Justice, Law no. 382 (10 June 1987), Acts no. 739 (6 December 1988) and no. 333 (18 May 1990) concerning animal experimentation and care of experimental animals.

## 2.2. Chemical analyses and enzyme activities

Prior to being analysed, the hourly pancreatic juice samples were thawed in warm water (approximately 30 °C) and immediately placed in an ice bath. The samples were proportionally pooled over 2 h to give a sample size of 6 mL. The juice was pipetted into acid washed tubes. From the pooled 2 h sample, two 1 mL samples were pipetted into vials for the determination of protein and carboxypeptidase A and B activities. The remaining 4 mL of pancreatic juice were subsequently wet ashed prior to the determination of zinc.

The procedure for determining the concentration of protein in pancreatic juice [13] was modified and performed in a 96-microwell plate, using bovine serum albumin (code A7638, Sigma Chemical, St. Louis, MO) as a standard.

Zinc concentration was determined by atomic absorption spectrometry (PU 9400 X, Philips Scientific, Cambridge, UK). Samples were wet ashed by adding concentrated nitric acid (14 M, 1:1 by vol) followed by exposure to increasing temperatures. Measurements were calibrated on Tritisol (a prepared standard, Merck, Darmstadt, Germany) and standard curves were verified by independently produced zinc chloride solutions before each analysis.

Procarboxypeptidase A was activated to carboxypeptidase A with trypsin. Pancreatic juice was diluted ten times in a buffer (5 mM tris (hydroxymethyl) aminomethane (code 8382, Merck, Darmstadt, Germany), 45 mM sodium chloride and pH 7.5). Subsequently, 50 µL of a trypsin solution (50 mg bovine trypsin (code T8253, Sigma Chemical) dissolved in 50 mL buffer) were added to 1 mL of diluted pancreatic juice and the solution was incubated at 37 °C for 15 min. A titremetric method was used to determine carboxypeptidase A activity [29]. Activated pancreatic juice (100 µL) was added to 5 mL of a substrate solution containing 5 mM hippuryl-DL-phenyllactic acid (code H9755, Sigma Chemical). The hydrolysis was followed by continuous titration with 0.1 M NaOH at 25 °C using a Titralab pH stat (Radiometer, Copenhagen, Denmark).

The inactive procarboxypeptidase B in pancreatic juice was activated to carboxypeptidase B with trypsin. The pancreatic juice was diluted 5 to 20 times in a buffer (27.5 mM tris (hydroxymethyl) aminomethane (code 8382, Merck, Darmstadt, Germany), 110 mM sodium chloride and pH 7.65). Following this, 50 µL of a trypsin solution (50 mg trypsin (code T8253, Sigma Chemical) dissolved in 50 mL buffer) was added to 1 mL of diluted pancreatic juice and the solution was incubated for 20 min at 25 °C. Carboxypeptidase B activity was measured using hippuryl-arginine as a substrate [1].

Enzyme activity in the pancreatic juice was expressed as units (U) per liter (specific activity) and per 2 or 24 h (total activity). One U of enzyme activity was defined as the hydrolysis of 1 μmol of substrate in 1 min. Total enzyme activity levels were calculated as specific activity × volume of pancreatic juice secreted per 2 or 24 h.

### 2.3. Statistical analyses

Analysis of variance (ANOVA) was carried out to examine the effect of period, pig and diet on the daily volume of pancreatic juice secreted, concentration and daily secretion of protein and zinc and the specific and total 24 h activity levels of carboxypeptidase A and B in the pancreatic juice collected from the PM and CM pigs. To compare the pouch and the catheter methods, the data were pooled within method and a one-way ANOVA was carried out with collection method as the source of variation. The 2 volume of pancreatic juice, concentration and secretion of protein and zinc and specific and total activity levels of carboxypeptidase A and B observed in the PM and CM pigs were subjected to ANOVA at each 2 h collection interval of the 24 h collection period. The General Linear Model (GLM) procedure [21] was used and the sources of variation were period, pig and diet. In order to compare the 2 h volume of secretion and the 2 h secretion and concentrations of protein and zinc and specific and total 2 h activity levels of carboxypeptidase A and B in pancreatic juice collected from the PM and CM pigs, the data were pooled within collection method and a one-way ANOVA was conducted with collection method as the source of variation.

Pearson correlation [21] was used to evaluate the relationships between the concentrations of protein, zinc and the activity levels of carboxypeptidase A and B and between the 2 h secretion of protein and zinc and total 2 h activity levels of carboxypeptidase A and B in pancreatic juice collected from the PM and CM pigs.

## 3. RESULTS

The pigs remained healthy and consumed their meal allowances throughout each experimental period. Post-mortem examination, carried out at the conclusion of each experiment, revealed no intestinal adhesions or other abnormalities. Within each surgical group there was no effect ($P > 0.05$) of diet (fat source) or experimental period on either the volume of pancreatic juice secreted, the protein and zinc concentrations or on the specific or total activity levels of carboxypeptidase A and B; therefore the pigs were pooled within surgical method. The diurnal patterns of the 24 h secretion of pancreatic juice, protein and zinc are shown in *figure 1*. The volume of pancreatic juice secreted by the CM pigs was sometimes higher ($P < 0.05$) and was more variable than the volume of pancreatic juice secreted by the PM pigs. The diurnal patterns of the secretion of proteins and zinc in pancreatic juice collected from both groups of pigs peaked within 2 h of feeding. Protein and zinc secretions (mg/2 h) were usually higher ($P < 0.05$) in the pancreatic juice from the PM than from the CM pigs.

The diurnal pattern of the secretion of carboxypeptidase A and B is shown in *figure 2*. Carboxypeptidase A activity was highest within 2 h of feeding and was numerically higher in pancreatic juice from PM pigs than from CM pigs.

Carboxypeptidase B secretion also reached its highest levels within 2 h of feeding and was often substantially higher ($P < 0.05$) in pancreatic juice collected from the PM pigs than from CM pigs. The diurnal patterns of total carboxypeptidase A and B activities were similar to those of protein and zinc (*figures 1* and *2*).

The volume of pancreatic juice and concentrations and flows of zinc and protein and specific and total carboxypeptidase A and B activities in pancreatic juice from pigs prepared with the pouch or catheter method are shown in *table I*. The daily volume of pancreatic juice from CM pigs was higher ($P < 0.05$), 4.1 versus 2.6 L/24 h, respectively, than from PM pigs. The average concentration of protein and the daily secretion of protein were greater ($P < 0.01$) in the pancreatic juice collected from PM pigs. The average zinc concentration, 1.00 and 0.41 mg/L, respectively, and daily secretion of zinc, 2.54 and 1.50 mg/24 h, respec-

tively, in the pancreatic juice were also larger ($P < 0.0001$) in PM pigs than in CM pigs. In the present experiment, the percentage of daily zinc intake that was secreted in pancreatic juice was calculated to be 1.40 and 0.82 % for PM and CM pigs, respectively.

The specific activity levels of carboxypeptidase A were greater ($P < 0.0001$) in pancreatic juice collected from PM pigs than

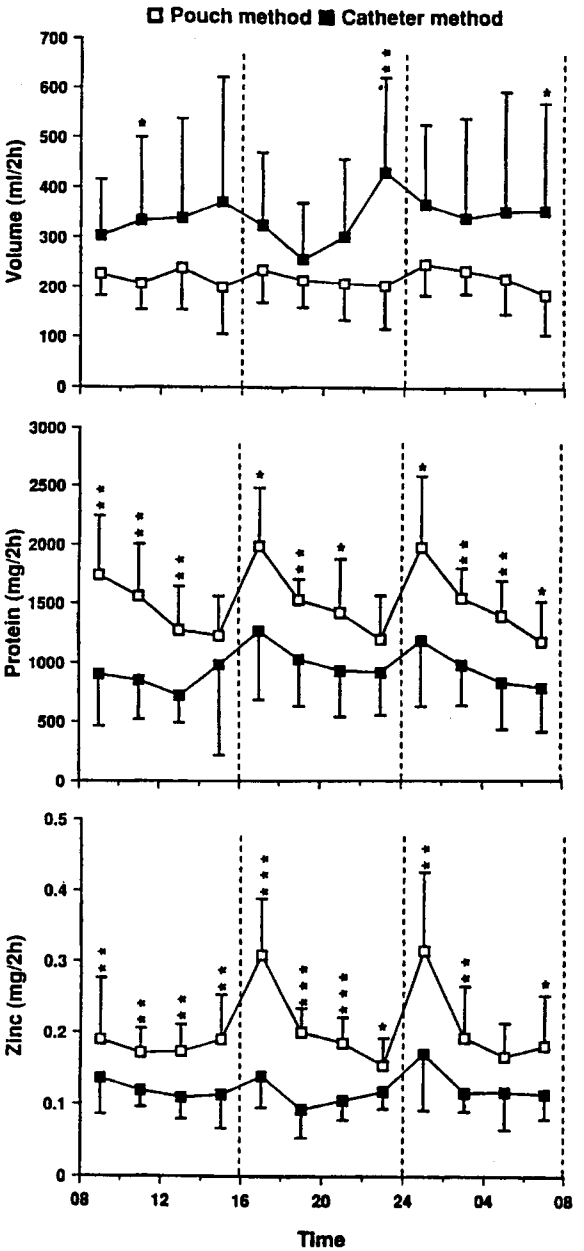


**Figure 1.** Diurnal pattern of the volume of pancreatic juice secreted and the secretion of protein and zinc in pancreatic juice collected from pigs prepared with the pouch or catheter method. Secretion rates differ between methods: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Each point is the mean of nine observations and the standard deviation. The dashed vertical lines indicate feeding times.

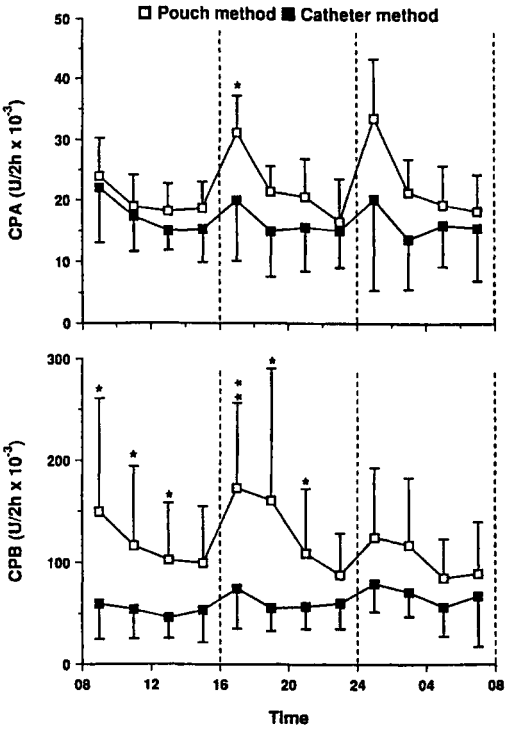


**Figure 2.** Diurnal pattern of the secretion of carboxypeptidase A (CPA) and carboxypeptidase B (CPB) in pancreatic juice collected from pigs prepared with the pouch or catheter method. Secretion rates differ between methods: $*P < 0.05$, $** P < 0.01$. Each point is the mean of nine observations and the standard deviation. The dashed vertical lines indicate feeding times.

**Table I.** The volume of pancreatic juice secreted and concentrations and daily flows of protein, zinc and carboxypeptidase A and B activities in pancreatic juice from pigs fitted with a pancreatic pouch re-entrant cannula (pouch method) or a catheter in the pancreatic duct (catheter method)*.

| Item | | Pouch method | Catheter method | SE | $P <$ |
|---|---|---|---|---|---|
| Volume | L/24 h | 2.63 | 4.09 | 0.47 | 0.05 |
| Protein | g/L | 7.15 | 3.25 | 0.20 | 0.0001 |
| | g/24 h | 18.4 | 11.9 | 1.24 | 0.01 |
| Zinc | mg/L | 1.00 | 0.41 | 0.03 | 0.0001 |
| | g/24 h | 2.54 | 1.50 | 0.12 | 0.0001 |
| Carboxypeptidase A | U/L x $10^{-3}$ | 107.0 | 69.5 | 4.1 | 0.0001 |
| | U/24 h x $10^{-3}$ | 270.5 | 237.5 | 13.5 | 0.11 |
| Carboxypeptidase B | U/L x $10^{-3}$ | 571.8 | 208.2 | 30.2 | 0.0001 |
| | U/24 h x $10^{-3}$ | 1 450.8 | 757.4 | 160.3 | 0.01 |

* Standard error of the mean; $n = 108$ except for 24 h means where $n = 9$.

from CM pigs. However, the total activity level of carboxypeptidase A did not differ between both groups of pigs. The total activity of carboxypeptidase B in pancreatic juice collected from PM pigs was higher ($P < 0.001$) than in the pancreatic juice from CM pigs.

The correlation coefficents between zinc, specific and total protein and carboxypeptidase A and B activities are presented in *table II*. For PM pigs, zinc and protein, both the concentrations and secretion, were highly correlated. Specific activity of carboxypeptidase A was highly correlated to both protein and zinc concentrations. Likewise, the total activity level of carboxypeptidase A was highly correlated to total secretion of zinc and protein. The highest correlation coefficients for CM pigs were observed between zinc concentration and specific carboxypeptidase A activity, protein and zinc concentration and protein concentration and specific carboxypeptidase B activity.

## 4. DISCUSSION

The differences observed between the two collection methods in terms of the volume of pancreatic juice secreted (*figure 1* and *table I*) may relate to an effect of the surgical method on the regulation of pancreatic secretion. When a catheter is placed in the pancreatic duct, the duct is ligated just before entry into the duodenum and the duodenal papilla is bypassed. Removal of the function of the duodenal papilla may increase secretion rate; however, this aspect remains to be investigated. In the construction of an isolated duodenal pouch, most of the extrinsic nerves leading from the stomach to the pancreas via the duodenum will be cut; regulatory mechanisms may be altered and pancreatic secretion may be reduced [19, 27].

Pancreatic juice from PM pigs contained more protein than pancreatic juice from CM pigs (*figure 1* and *table I*). This was likely due to the presence of sloughed epithelial

**Table II.** Correlation coefficients between protein concentration and flows of protein, zinc and carboxypeptidase A and B activities in pancreatic juice from pigs fitted with a pancreatic pouch reentrant cannula (pouch method) of a catheter in the pancreatic duct (catheter method).

| | Pouch method | Catheter method |
|---|---|---|
| | Protein (g/L) | |
| Zinc (mg/L) | 0.68 | 0.62 |
| Carboxypeptidase A (U/L) | 0.64 | 0.53 |
| Carboxypeptidase B (U/L) | 0.43 | 0.62 |
| | Zinc (mg/L) | |
| Carboxypeptidase A (U/L) | 0.79 | 0.83 |
| Carboxypeptidase B (U/L) | 0.45 | 0.60 |
| | Protein (g/2 h) | |
| Zinc (mg/2 h) | 0.68 | 0.46 |
| Carboxypeptidase A (U/2 h) | 0.64 | 0.42 |
| Carboxypeptidase B (U/2 h) | 0.38 | 0.54 |
| | Zinc (mg/2 h) | |
| Carboxypeptidase A (U/2 h) | 0.73 | 0.54 |
| Carboxypeptidase B (U/2 h) | 0.36 | 0.53 |

* $n = 108$ for the pouch method and the catheter method, respectively, all correlation coefficients are significant ($P < 0.001$).

cells and mucin secreted into the pouch [8, 25, 31]. Pancreatic juice collected from PM pigs was more viscous and contained more cell debris than pancreatic juice from CM pigs. Similar results have been reported previously [8].

The concentrations of zinc determined in the pancreatic juice collected from CM pigs in this study (*table I*) were within the range of those determined in an experiment with 5-kg pigs, where the pigs were anaesthetized and had pancreatic secretion stimulated with an injection of secretin. The concentrations of zinc in the pancreatic juice and bile were 0.52 and 0.35 mg/L, respectively [24]. However, these studies are difficult to compare given the different body weights of the pigs and methodology used. In a previous study in which growing pigs were prepared for total collection of pancreatic juice with the pouch method, the total daily secretion of zinc in pancreatic juice was 4.0 mg and the total dietary intake of zinc was 60 mg [3]. A similar daily rate of excretion was observed in this experiment with pigs prepared with the pouch method (*table I*) even though the zinc intake was three times as high (182 mg/d).

In the present study, the pattern of zinc secretion in pancreatic juice from growing pigs was parallel to the secretion of protein and the zinc-dependent enzymes carboxypeptidase A and B (*figures 1* and *2*). In short-term studies, a close relationship between protein, zinc and carboxypeptidase A and B in the pancreatobiliary fluid from rats was observed after duodenal infusion of a protein source [23]. Following the intravenous injection of $^{65}Zn$ into growing pigs with a catheter in the pancreatic duct, a very close relationship between hourly concentration of protein, up to 7 h following the injection, and $^{65}Zn$ counts was observed suggesting that the zinc was bound to proteins in pancreatic juice [18].

High correlation coefficients between zinc concentration, protein concentration and carboxypeptidase A and B activities have been observed in previous experiments with rats [2, 11]. It was concluded that zinc is associated primarily with carboxypeptidase A and B in pancreatobiliary fluid [2, 11].

However, the aforementioned correlation coefficients were lower in the present study (*table II*). The cited experiments carried out with rats were of short duration, 2 h of collection, and the experimental conditions were standardized. In the present experiment, the periods of collection were longer and the volume of secretion as well as the concentration of protein and zinc and carboxypeptidase A and B activities were quite variable (*figure 1* and *2*).

Increased pancreatic secretion of digestive enzymes following feeding in pigs has been observed in several studies [10, 26] and was also observed in this study (*figure 2*). This is the first study in which the diurnal patterns of zinc and carboxypeptidase A and B secretion have been investigated for a total of 24 h. It is important to consider both the basal and stimulated secretion levels of zinc and carboxypeptidase A and B and not to use only short-term observations to predict the daily secretion. This statement is supported by the results of this study which demonstrated that the secretion rates change throughout the day and are quite variable in pigs.

The difference in zinc secretion in pancreatic juice between PM and CM pigs (*figure 1* and *table I*) was likely related to physiological changes induced by the two surgical methods used to prepare pigs for the in vivo collection of pancreatic juice. The higher concentration of zinc in the pancreatic juice collected from PM pigs was probably due to the presence of sloughed epithelial cells. No attempt was made to filter or purify the samples of pancreatic juice from PM pigs, but it would be useful to quantify the amount of zinc associated with sloughed epithelil cells originating from the duodenal pouch.

The contribution of pancreatic zinc secretion to the maintenance of zinc homeostasis

may be relatively low. Following studies with calves fed a diet containing 34 mg zinc/kg diet and fitted with a catheter in the pancreatic duct, it was concluded that zinc excretion by the pancreas was not the major source of endogenous zinc loss as it only contributed one-fourth or less of the total endogenous loss of zinc [22]. In an experiment in which pigs were given a single intravenous injection of $^{65}Zn$, 75 to 90 % of the zinc injected was excreted in the feces. Fecal excretion of zinc was, however, not greatly affected when the pancreatic duct was ligated thereby preventing pancreatic secretion into the intestine [18]. The concentration of zinc in pancreatobiliary fluid from rats was slightly elevated when the dietary level of zinc was increased by 100 times [20].

These studies suggest that pancreatobiliary secretion of zinc is not likely to be an important contributor to zinc homeostasis in the rat.

However, the role of pancreatic secretion in zinc homeostasis may depend on the dietary intake of zinc, especially for extremely low zinc intakes, and the importance of this may be different in different species. In humans it has been determined that pancreatobiliary secretion of zinc is a major contributor to endogenous zinc and is important in maintaining zinc status [12, 16]. Pancreatic secretion of zinc in pigs likely has a minor role in maintaining zinc homeostasis based on the relatively low level of daily zinc secretion in pancreatic juice compared to daily zinc intake.

In conclusion, the surgical method used to prepare the pigs for the total collection of pancreatic juice had a large effect on the concentration of zinc in the pancreatic juice and also on the activity levels of carboxypeptidase A and B. In addition, daily zinc secretion in pancreatic juice was relatively low compared to daily zinc intake in pigs fed diets which met the zinc requirement and may therefore have a minor role in maintaining zinc homeostasis. The zinc secretion in the pancreatic juice paralleled the secretion of protein and carboxypeptidase A and B. Zinc in the pancreatic juice appeared to be primarily associated with carboxypeptidase A and B.

## ACKNOWLEDGEMENT

The authors wish to thank Karl H. Jakobsen, Peter Sørensen, Morten Smed, Marie L. Nielsen and Dorte Agnholt for their technical assistance. Financial support provided by the Danish Veterinary and Agricultural Research Council is gratefully acknowledged.

## REFERENCES

[1] Appel W. Carboxypeptidases, in: Bergmeyer U. (Ed.), Methods of Enzymatic Analysis, Vol. 2, Acadenic Press, New York, 1974, pp. 986–999.

[2] Berger J., Schneennan B.O., Stimulation of bilereatic zinc, protein and carboxypeptidase secretion in response to various proteins in the rat, J. Nutr. 116 (1986) 265–272.

[3] Chavez E., Li S., Sauer W.C., Mineral contribution of pancreatic secretion in growing pigs fed two dietary protein levels, in: Fischer P.W.F., L'Abbé M.R., Cockell K.A., Gibson R.S. (Eds.), Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals, NRC Research Press, Ottawa, Canada, 1997, pp. 319–820.

[4] Corring T., Juste C., Lhoste E., Nutritional regulation of pancreatic and biliary secretions, Nutr. Res. Rev. 2 (1989) 161–180.

[5] Finley J., Johnson P.E., Relative influence of amount of dietary zinc and infusion of protein into the duodenum on the amount of zinc in biliary/pancreatic secretions, Nutr. Res. 12 (1992) 1217–1228.

[6] Gabert V.M., Jensen M.S., Jørgensen H., Engberg R.M., Jensen S.K., Exocrine pancreatic secretions in growing pigs fed diets containing fish oil, rapeseed oil or coconut oil, J. Nutr. 126 (1996) 2076–2082.

[7] Gabert V.M., Sauer W.C., Li S., Fan M.Z., Rademacher M., Exocrine pancreatic secretions in young pigs fed diets containing faba beans (*Vicia faba*) and peas (*Pisum sativum*): Nitrogen, protein and enzyme secretion, J. Sci Food Agric. 70 (1996) 247–255.

[8] Hee J.H., Pancreatic secretions in the growing pig, M.Sc. thesis, University of Alberta, Edmonton, AB, 1984.

[9] Hee J.H., Sauer W.C., Berzins R., Ozimek L., Permanent re-entrant diversion of porcine pancreatic secretions, Can. J. Anim. Sci. 65 (1985) 451–457.

[10] Hee J., Sauer W.C., Mosenthin R., The effect of frequency of feeding on the pancreatic secretions in the pig, J. Anim. Physiol. Anim. Nutr. 60 (1988) 249–256.

[11] Hempe J.M., Cousins R.J., Cysteine-rich intestinal polypeptide binds zinc during transmucosal zinc transport, Proc. Natl. Acad. Sci. 88 (1991) 9671–9674.

[12] Lee H.H., Hill G.M., Sikha VKNM, Brewer G.J., Prasad A.D., Owyang G., Pancreaticobiliary secretion of zinc and copper in normal persons and patients with Wilson's disease, J. Lab. Clin. Med. 16 (1990) 283–288.

[13] Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J., Protein measurement with folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[14] Makkink C.A., Verstegen M.W.A., Pancreatic secretion in pigs, J. Anim. Physiol. Anim. Nutr. 64 (1990) 190–208.

[15] McClain C.J., The pancreas and zinc homeostasis, J. Lab. Clin. Med. 116 (1990) 275–276.

[16] McClain C.J., Adams L., Shedlofsky S., Zinc and the gastrointestinal system, in: Prasad A.S. (Ed.), Essential and Toxic Trace Elements in Health and Disease (the First International Meeting of the International Society for Trace Element Research in Human), Alan R. Liss, New York, 1988, pp. 55–73.

[17] Mills C.F., Quarterman J., Williams R.B., Dalgarno A.C., The effect of zinc deficiency on pancreatic carboxypeptidase activity and protein digestion and absorption in the rat, Biochem, J. 102 (1967) 712–718.

[18] Pekas J.C., Zinc 65 metabolism: gastrointestinal secretion by the pig, Am. J. Physiol. 211 (1966) 407–413.

[19] Pierzynowski S.G., Westrom B.R., Karlsson B.W., Svendsen J., Nilsson B., Pancreatic cannulation of young pigs for long-term study of exocrine pancreatic function, Can . J. Anim. Sci. 68 (1988) 953–959.

[20] Reinstein N.H., Lonnerdal B., Keen C.L., Schneeman B.O., Hurley L.S., The effect of varying dietary zinc levels on the concentration and localization of zinc in rat bile-pancreatic fluid, J. Nutr. 117 (1987) 1060–1066.

[21] SAS Institute, Inc., SAS/STAT user's guide (release 6.03), SAS Institute Inc., Cary, NC, 1988.

[22] Stake P.E., Miller W.J., Blackmon D.M., Gentry R.P., Neathery M.W., Role of pancreas in endogenous zinc excretion in the bovine, J. Nutr. 104 (1974) 1279–1284

[23] Sullivan J.F., Burch R.E., Quigley H.J., Magee D.F., Zinc deficiency and decreased pancreatic secretoryresponse, Am. J. Physiol. 1227 (1974) 105–108.

[24] Sullivan J.F., Williams R.V., Wisecarver J., Etzel K., Jetton M.M., Magee D.F., The zinc content of bile and pancreatic juice in zinc-deficient swine, Proc. Soc. Exp. Biol. Med. 166 (1981) 39–43.

[25] Ternouth J.H., Buttle H.L., Concurrent studies on the flow of digesta in the duodenum and of exocrine pancreatic secretion in calves, Br. J. Nutr. 129 (1973) 387.

[26] Thaela M.-J., Pierzynowski S.G., Jensen M.S., Jakobsen K., Westrom B.R., Karlsson B.W., The pattern of the circadian rhythm of pancreatic secretion in fed pigs, J. Anim. Sci. 73 (1995) 3402–3408.

[27] Thomas J.E., Methods for collecting pancreatic juice, Gastroenterol. 36 (1959) 362.

[28] Valette P., Malouin H., Corring T., Savoie L., Gueugneau A.M., Berot S., Effects of diets containing casein and rapeseed on enzyme secretion from the exocrine pancreas in the pig, Br. J. Nutr. 67 (1992) 215–222.

[29] Yamasaki M., Brown J.R., Cox D.J., Greenshields R.N., Wade R.D., Neurath H., Procarboxypeptidase A-S6, Further studies of its isolation and properties, Biochemistry 2 (1963) 859–866.

[30] Zebrowska T., Low A.G., The influence of diets based on whole wheat, wheat flour and wheat bran on exocrine pancreatic secretion in pigs, J. Nutr. 117 (1987) 1212–1216.

[31] Zebrowska T., Low A.G., Zebrowska H., Studies on gastric digestion of protein and carbohydrate, gastric secretion and exocrine pancreatic secretion in the growing pig, Br. J. Nutr. 49 (1983) 401–410.