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The exocrine pancreatic secretion in pigs and its hormonal regulation as influenced by carbohydrates and fats given *per os* or infused intraduodenally

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1. INTRODUCTION

It is necessary for the living organism to digest feed and assimilate the various nutrients in order to fulfil its nutritional requirements. The digestive system of omnivore, monogastric animals as the pig is highly developed and allows the animal to adapt to different nutritional sources. This ability is of great importance for modern agricultural production, as due to economic pressure pig feed has to be designed variably in order to adapt to varying market and animal requirements. The pancreas is a major part of the digestive system since it represents the main source of digestive enzymes and bicarbonate. The understanding of the physiological processes of the pancreas is crucial in order to optimise feeding strategies. Moreover, the pig becomes more and more important as a model in human biomedicine due to the development of surgical techniques suitable for preparation of chronic animal models that allow long-term *in vivo* investigation of different physiological and metabolic processes.

1.1. Pancreatic secretions in pigs

The pancreas produces more protein per gram of tissue than any other organ (Lowe, 1994b) and contains 90 to 95% of exocrine tissue and about 2 to 3% of endocrine tissue (Brannon, 1990). According to Fredirick and Jamieson (1994) the pancreas is mainly composed of acinar cells (> 80%); the major function of the acinar cells is to synthesise and to secrete a variety of digestive enzymes, water and diverse electrolytes into the duodenum.

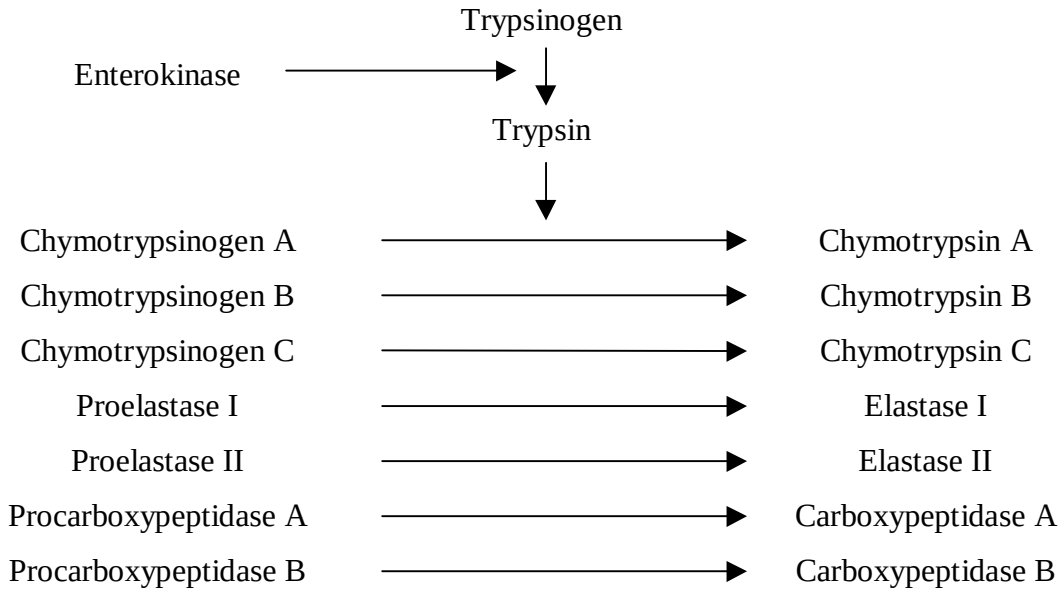
1.1.1. Enzyme secretion of the exocrine pancreas

The exocrine pancreas secrets hydrolytic enzymes into the duodenum which are essential for digestion and absorption of various nutrients to be utilised in the intermediary metabolism. Among these, proteolytic, amylolytic and lipolytic enzymes are considered to be the most important (Ohlsson et al., 1982).

Proteolytic enzymes

It is well known that pancreatic proteases are secreted as inactivated zymogens. Activation of these zymogens is initiated by a cascade mediated by enterokinase, a protein synthesised in the intestinal epithelium. Enterokinase is important for the transformation of trypsinogen to trypsin which activates the zymogens of all proteolytic enzymes (Lowe, 1994b). The activation cascade of proteolytic enzymes is illustrated in Figure 1.

Figure 1: The porcine pancreatic proteolytic enzymes and its activation cascade



after Ohlsson et al., 1982; Lowe, 1994b

Activated proteolytic enzymes act both as endopeptidases or exopetidases as they cleave proteins at specific sites along the protein chain. Trypsin hydrolyses peptide bonds between ARG (arginine) and LYS (lysine), whereas chymotrypsin cleaves the peptide bonds between LEU (leucine) and MET (methionine) and at aromatic amino acids as PHE (phenylalanine), TYR (tyrosine) and TRP (tryptophane). Similar to trypsin and chymotrypsin, elastase hydrolyses peptide bonds within the protein molecule containing ALA (alanine), VAL (valine), GLY (glycine), TYR, PHE and LEU. The carboxypeptidases are exopeptidases and hydrolyse cleavages at the carboxyl-terminal end of the protein molecule at PHE, TYR, ARG and LYS residues (Ohlsson et al., 1982; Lowe, 1994b).

Glycosidase

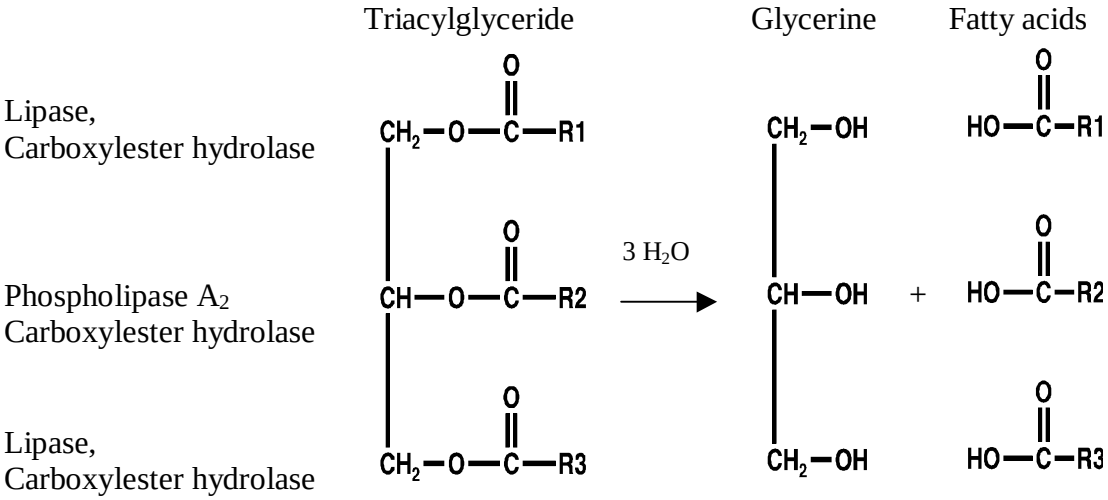
Alpha-amylase represents the only glycosidic enzyme of the exocrine pancreas. It cleaves 1,4-glycoside bonds in dietary starch (Lowe, 1994b) and breaks down complex starch molecules into small maltose complexes, which are hydrolysed to glucose by maltase located in the brush-border membrane of the mucosa (Kirchgessner, 1987).

Lipolytic enzymes

Most of the dietary fat is digested by lipolytic enzymes secreted by the exocrine pancreas, although especially in younger animals a minor part of the lipids is digested in the stomach by gastric lipase (Jensen et al., 1997b). Fats are non-soluble in water which explains why dietary fat has to be emulsified by means of bile salts and phospholipids secreted into the duodenum before being hydrolysed (Rathelot et al., 1975). In total, three lipolytic enzymes are secreted by the exocrine pancreas into the duodenum: lipase, carboxylester hydrolase and phospholipase A_2. In addition, colipase as an essential cofactor in lipid digestion is also secreted into the duodenum (Rinderknecht, 1993). All lipolytic enzymes have in common that they hydrolyse triacylglycerides to fatty acids and to glycerine, mono- or diacylglycerides.

Pancreatic lipase is the main fat cleaving enzyme; it cleaves triacylglycerides in position one and three only. Carboxylester hydrolase is a non-specific lipolytic enzyme which cleaves ester linkages at positions one, two and three of triacylglycerides (Jensen et al., 1997b). Phosholipase A_2 hydrolyses triacylglycerides specifically in position two after activation of its zymogen prophospholipase A_2 by trypsin phospholipids such as phoshatidylcholine (lecithin) and sphingomyelin (Rinderknecht, 1993; Lehninger et al., 1994; Lowe, 1994a; Lowe, 1994b). The mode of action of the different lipolytic enzymes is shown in Figure 2:

Figure 2: Lipolytic enzymes and position of hydrolysis



An important cofactor of lipid digestion is colipase. Colipase is secreted by the exocrine pancreas in an inactivated form as procolipase; it is activated by trypsin. Colipase is essential to accomplish the attachment of lipase to emulsified fats (Rinderknecht, 1993; Lowe, 1994a; Lowe, 1994b). Several studies (Pierzynowski et al., 1995; Jensen et al., 1997a) showed that the secretion of lipase and colipase is highly correlated.

Other enzymes

In addition to the enzymes involved in the hydrolysis of proteins, carbohydrates and fats some other pancreatic enzymes are secreted into the duodenum.

Kallikrein becomes an active enzyme following the activation of Kallikreinogen by trypsin. It is a minor component of exocrine pancreatic secretions since it accounts for 0.4% of the total protein content in pancreatic juice only (Lowe, 1994b). Kallikrein is a very specific enzyme whose main function is the release of kinins from kinogens (Orstavik, 1983; Borges, 1992).

Nucleases represent another source of enzymes secreted by the exocrine pancreas. Both desoxyribonuclease (DNase) and ribonuclease (RNase) are secreted into the duodenum as active enzymes. DNase as well as RNase catalyse the cleavage of nucleotides (Lowe, 1994b).

1.1.2. Non-enzyme secretions of the exocrine pancreas

As the pH-optima for pancreatic enzymes to be active are in the range between pH 7.5 to 10.5 (Makkink, 1993) it is necessary to buffer the digesta passing from the stomach into the duodenum. Pancreatic juice has a relatively high pH of 8.5 due to the secretion of bicarbonate (406 to 679 mmol/d) (Gabert et al., 1996). As a result, it neutralises gastric hydrochloric acid and generates a slightly alkaline environment in the duodenum (Kidder and Manners, 1987). Moreover, the exocrine pancreas secrets water, mucins, urea, sodium, potassium and chloride into the duodenum, which contribute to the supply of the gastrointestinal tract with mucopolysaccharides, nitrogen and essential electrolytes (Rinderknecht, 1993; Gabert, 1997).

1.2. Response of the exocrine pancreas to feeding regimen and to dietary modifications

Feeding regimen

It has been shown that the exocrine pancreas adapts to the frequency of feeding. Pigs fitted with a permanent pancreatic fistula were fed once, twice or three times a day (Hee et al., 1988b). When feeding the animals twice or three times a day, the postprandial values for the volume of secretion as well as for protein-, trypsin, chymotrypsin and α-amylase were elevated compared to pre-prandial values. This increase was less pronounced when pigs were fed once a day only. The daily volume of secretion increased ($P<0.05$) by 500 ml with each additional meal; the α-amylase secretion increased ($P<0.05$) by 100% with each meal. However, no influence of the frequency of feeding was observed on the total secretion of protein, trypsin and chymotrypsin (Hee et al., 1988b).

Type of diet

A considerable effect on exocrine pancreatic secretions is mediated by the type of diet. Semi-synthetic as well as synthetic diets which consist mainly of purified ingredients such as corn starch, saccharose, cellulose or isolated proteins evoked a lower pancreatic secretion compared to diets containing natural feed ingredients (Partridge et al., 1982; Mosenthin and Sauer, 1991).

Dietary protein

The exocrine pancreatic secretion in pigs adapts to the source and level of dietary protein consumed. After supplementing a protein-free diet up to a level of 30 % with protein, the specific activities of trypsin and chymotrypsin increased whereas the volume of secretion and specific protein contents in pancreatic juice were not affected (Corring and Saucier, 1972). According to Hee et al. (1988a) an increase in the protein level in diets for young pigs from 0.3% to 14.5% led to an 100% increase ($P<0.05$) in the total activities of trypsin and chymotrypsin, which confirms previous reports by Corring (1977). Moreover, it can be derived from studies by Valette et al. (1988) that the source of protein may affect the volume and enzyme secretion as well. It has been shown in the rat, that a higher intake of proteins with a more favourable amino acid balance may result in elevated specific chymotrypsin activities (Brannon, 1990). The consumption of rapeseed concentrate as a protein source led to a decrease in the volume of pancreatic juice secreted, but to an increase in the protein concentration of pancreatic juice when compared to casein (Valette et al., 1992).

Dietary carbohydrates

Several studies showed that pancreatic α-amylase secretion reacts very sensitive with respect to the amount of starch in the diet. Corring and Chayvialle (1987) observed in the pig an 2.3-fold increase in total α-amylase activities when the daily intake of dietary starch was increased by 400%. This increase in α-amylase activity was observed 1 to 2 h postprandially (Corring et al., 1989). However, no changes in specific α-amylase activities were observed, when dietary starch was replaced by monomeric carbohydrates such as glucose or dextrose (Corring, 1977).

Studies conducted by Mosenthin and Sauer (1991) and by Mosenthin and Sauer (1993) showed that the replacement of starch by cellulose or straw meal evoked in tendency ($P<0.1$) a decrease in the total activity of α-amylase. A substitution of starch by pectin resulted in a decrease ($P<0.05$)in the total α-amylase activity in pancreatic juice. However, the authors did not report an influence of cellulose, straw meal or pectin on the total secretion of nitrogen or on total trypsin, chymotrypsin and lipase activities in pancreatic juice.

Dietary fats

Most of the studies which have been carried out to investigate the influence of different fats on the exocrine pancreatic secretion have been conducted with rats (Bucko and Kopec, 1968; Deschodt Lanckman et al., 1971; Gidez, 1973; Sabb et al., 1986). It was shown that exocrine pancreatic secretions are correlated with the level of fat in the diet (Bucko and Kopec, 1968). Gidez (1973) suggested that the carbohydrate / fat relation in a diet has a strong impact on the lipase activity, whereas Deschodt Lanckman et al. (1971) pointed out that the biosynthesis of lipase is more efficiently stimulated by unsaturated fatty acids than by saturated fatty acids. This was confirmed in studies by Ballesta et al. (1990) who showed in dogs that a diet containing higher levels of polyunsaturated fatty acids (sunflower oil) evoked in pancreatic juice higher total activities of α-amylase and lipase as well as higher protein concentrations compared to a diet containing a fat source (olive oil) with a relatively high content of monounsaturated fatty acids.

Only a few studies have been carried out with pigs that focus on the influence of level, quality and composition of fat on exocrine pancreatic secretions. Previous studies by Corring (1980) showed that specific lipase activity in pancreatic juice increased 7-fold after increasing the triacylglyceride intake from 30 to 200g. Mourot and Corring (1979) observed similar results when the dietary fat content was increased from 5% to 25%, as the specific lipase activity increased ($P<0.001$) by 83%. The source of dietary fat may also influence lipase activities as was shown by Simoes Nunes (1986). Higher specific lipase activities in pancreatic tissue homogenate were obtained when fats such as lard with higher contents of saturated fatty acids were fed as compared to sunflower oil containing more polyunsaturated fatty acids. However, Gabert et al. (1996) and Jensen et al. (1997a) observed in pigs that fatty acid composition of different oils (coconut, canola and fish oil) had only minor effects on exocrine pancreatic secretion of growing pigs fitted with permanent pancreatic cannulas allowing for chronic sampling of pancreatic juice.

1.3. Endocrine regulation of the exocrine pancreas

Several gastrointestinal hormones, such as cholecystokinin (CCK) and secretin, are involved in the regulation of the exocrine pancreas via endocrine or nervous pathways. Gastrointestinal hormones that may affect exocrine pancreatic secretions due to a variety of different mechanisms are summerised in Table 1.

Table 1: Gastrointestinal hormones involved in the regulation of the exocrine pancreas

Hormone/Peptide	Effect on pancreas	Dietary stimuli for release / inhibtion	Releasing tissue	Reference
Secretin	stimulates secretion of fluid and bicarbonate	unbuffered H^+ in the duodenum	duodenal and jejunal mucosa	(Mössner, 1990a)
Cholecystokinin (CCK)	stimulates secretion of enzymes (main effect on proteolytic enzymes)	protein, carbohydrates, fat	duodenal and jejunal mucosa	(Douglas et al., 1988; Greenberg, 1993; Liddle, 1995)
Pancreatic Polypeptide (PP)	inhibits enzyme, protein and bicarbonate secretion, minor effect on volume of secretion	fat, fatty acids	small intestine	(Lonovics et al., 1981; Owyang et al., 1983; Fried et al., 1984; Langlois et al., 1989)
Peptid YY (PYY)	inhibits enzymatic and volume secretion	fat, fatty acids, protein	distal ileum, colon	(Greeley et al., 1989a; Greeley et al., 1989b; Guan et al., 1991; Lin et al., 1996)
Neurotensin (NT)	stimulates enzyme, protein and bicarbonate secretion	fat, fatty acids	ileum	(Walker et al., 1985; Gomez et al., 1986; Mössner, 1990b)
Bombesin	stimulates protein and volume secretion	?	gastric mucosa	(Holmgren et al., 1982; Lilja et al., 1984; Ami et al., 1993)
Enterostatin	inhibits pancreatic secretion	fat, protein	pancreatic juice	(Holmgren et al., 1982; Lilja et al., 1984; Erlanson-Albertsson et al., 1991)
Enteroglucagon	inhibits pancreatic secretion	nonabsorbed nutrients in the ileum	?	(Dowling et al., 1985; Sagher et al., 1991; Holst, 1997)

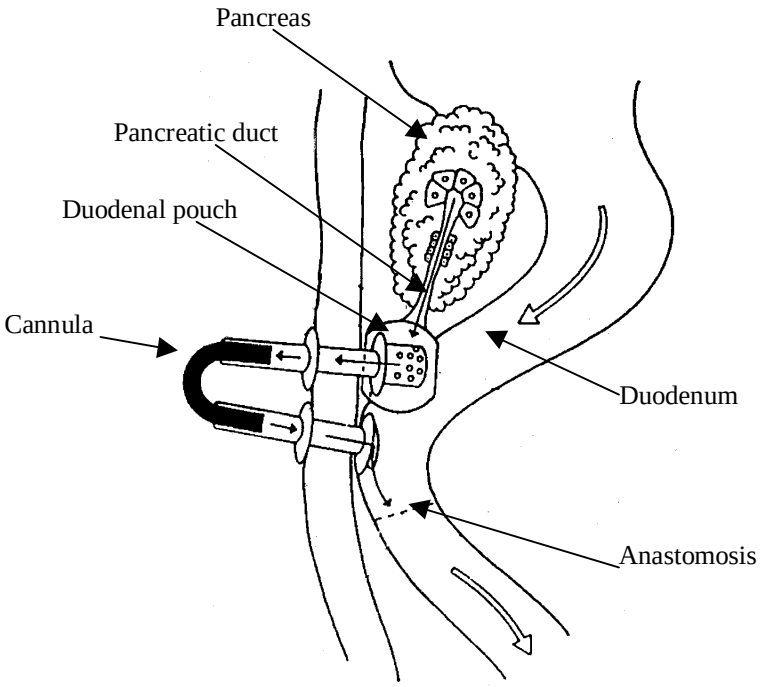
1.4. Surgical preparation of pigs with permanent pancreatic cannulas

The development of surgical methods to prepare animals with permanent cannulas that allow for chronic sampling of pancreatic juice, and therefore long-term studies under *in vivo* conditions, was an essential prerequisite in order to study the complex physiological processes of the exocrine pancreas. Several methods have been developed. The "Pouch Method" and the "Catheter Method" are the most commonly used methods in pigs. As was pointed out by Zabielski et al. (1997) there exists no ideal method that fulfils all requirements, i.e. each method has its specific advantages and disadvantages. Nevertheless, these methods permit a deeper insight in the physiology or pathophysiology of the exocrine pancreas than acute animal models.

The Pouch Method

The Pouch Method for the collection of pancreatic juice in dogs was originally introduced by Dragstedt et al. in 1930. In this method a large pouch from the upper duodenum including the mouth of the pancreatic duct was prepared and the duodenum was connected with the pylorus. This invasive method allowed for the sampling of pancreatic secretions via an intestinal cannula and it was modified several times (Preshaw et al., 1965; Herrera et al., 1968; Hee et al., 1985). The Pouch Method has been used for collection of pancreatic juice in ruminants (Ternouth and Buttle, 1973; St-Jean et al., 1992) and pigs (Zebrowska et al., 1983; Hee et al., 1985; Hee et al., 1988a; Gabert et al., 1996; Jensen et al., 1997a). A schematic illustration of this method is given in Figure 3.

Figure 3: Pancreatic cannula according to the Pouch Method (Hee et al., 1985).



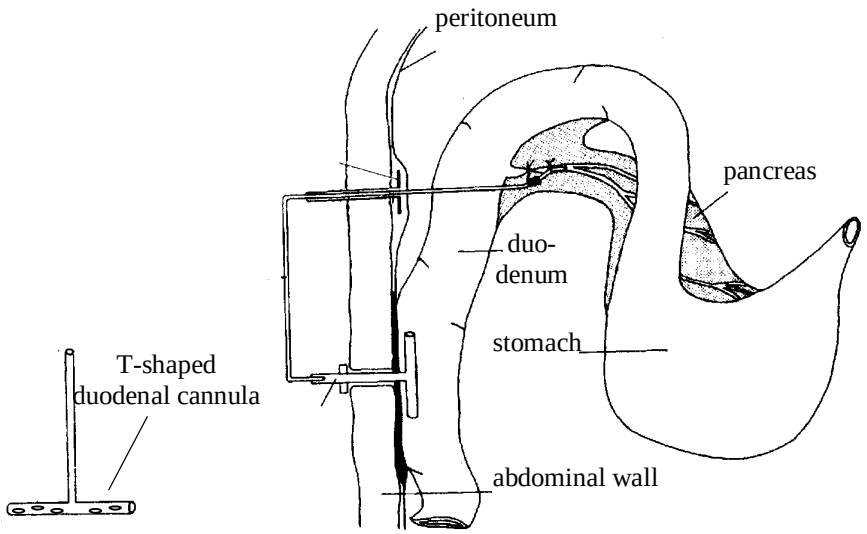
Postoperative problems associated with this method can arise due to the extensive surgical procedure. For example, the postoperative mortality is high (25 to 85% in dogs) and other pathological signs as avascular necrosis, ulceration or leakage of the pouch may occur (Zabielski et al., 1997). Gabert et al. (1997) showed that almost 100% of the enzymes in the pancreatic juice collected by means of the Pouch Method were activated as the duodenal mucosa of the pouch produced enterokinase. Moreover, as for the surgery anastomosis is required duodenal-pancreatic neural reflexes may be disturbed. However, the maintenance of animals fitted with a duodenal pouch is easy as flushing with saline once or twice a week is sufficient to avoid blockages of the cannula. Animals can be kept without major physical restraints during sampling periods; especially for long-term studies this method is recommended (Gabert et al., 1997).

The Catheter Method

The Catheter Method was originally developed by Routley et al. (1952). It is based on the chronic implantation of an elastic catheter into the pancreatic duct combined with a

ligation of the pancreatic duct close to the sphincter of oddi. This method is used for studies on exocrine pancreatic secretions in several animals such as dogs (Routley et al., 1952), calves (Zabielski et al., 1990; Zabielski et al., 1992), cows (Pierzynowski et al., 1988a), sheep (Pierzynowski and Barej, 1984), piglets (Pierzynowski et al., 1988b; Jensen et al., 1997b) and growing pigs (Botermans and Pierzynowski, 1999). The Catheter Method was modified by Pierzynowski et al. (1988a) and Thaela et al. (1995) who used silicon instead of plastic tubings as cannulas; in addition, the T-shaped duodenal cannula was perforated for smooth re-introduction of the pancreatic juice into the duodenum (Figure 4).

Figure 4: Routley's Catheter Method modified according to Pierzynowski et al. (1988b) and Thaela et al. (1995)



Animals fitted with a pancreatic duct catheter require much more maintenance and postoperative care than pigs surgically modified according to the Pouch Method, as due to the thin tubings blockages occur frequently. Moreover, the ligation of the pancreatic duct proximal to the sphincter of oddi disabled the sphincter. However, this method provides several advantages compared to the Pouch Method. The reduction of possible post-surgical traumata due to a minor invasion allows a fast recuperation and early postoperative feeding of the animals. According to Zabielski et al. (1997) the Catheter Method is especially suitable for young animals. Moreover, pancreatic juice collected with a pancreatic duct catheter contains exclusively inactivated, pure zymogens (Gabert et al., 1997).

Other surgical methods

Another common method for collecting pancreatic juice is the so called “Thomas- method” as it was described first by Thomas (1941) and by Thomas and Crider (1946). It is still often used, mostly in studies with dogs. In this method the pancreatic duct (Wirsung’s duct) is ligated and a wide cannula is implanted in the greater curve of the duodenum directly in front of the minor duodenal papilla. Before the start of the collection of pancreatic juice the cannula is opened and a tube (glass or plastic) can be inserted into the duct. Although this method allows sampling of pure, inactivated pancreatic juice, it is not recommended for young, mobile animals since the risk of damaging the cannula is high (Zabielski et al., 1997).

1.4.1. Comparison of methods

Gabert et al. (1996) and Jensen et al. (1997a) showed in a comparative study that in pigs fitted with permanent cannulas which allow for chronic sampling of pancreatic juice, results might be affected by the surgical method used to collect pancreatic juice. The authors showed that exocrine pancreatic secretions in pigs fitted with a cannula according to the Pouch Method (Hee et al., 1988a) were not influenced by the source of dietary fat (coconut, canola or fish oil). However, pigs fitted with a pancreatic duct catheter according to Pierzynowski et al. (1988b) showed elevated ($P<0.05$) total chymotrypsin activities after consumption of a diet containing coconut oil and decreased carboxylester hydrolase activities ($P<0.05$) after fish oil was included in the diet. Moreover, pigs fitted with a pancreatic duct catheter secreted more pancreatic juice which had a higher pH, and substantial higher total trypsin, carboxylester hydrolase and colipase activities as compared to corresponding values obtained with the Pouch Method. However, total α-amylase activities were lower in pancreatic juice of pigs surgically modified according to the Catheter Method.

1.5. Hypotheses of this thesis

The hypotheses worked on in this thesis are as follows:

Hypothesis 1:
Both the oral and intraduodenal administration of fibre in the form of isolated potato fibre affect the exocrine pancreas via the gastrointestinal hormones cholecystokinin and secretin by stimulating the volume of secretion as well as the enzyme secretion in pancreatic juice.

Hypothesis 2:
Purified fat sources differing in chain length affect the exocrine pancreas and its hormonal regulation differently when infused intraduodenally under prandial conditions.

Hypothesis 3:
Vegetable oils differing in chain length and degree of saturation affect the exocrine pancreas differently when infused intraduodenally under prandial conditions.

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2. CARBOHYDRATES AND EXOCRINE PANCREATIC SECRETIONS IN PIGS

2.1. Summary

The response of the pancreas on nutritional and dietary factors and the divers mechanisms controlling the exocrine pancreas are of particular interest. In this review, the effect of dietary carbohydrates including different fibre sources on quantitative and qualitative aspects of pancreatic secretion will be addressed. The importance of describing dietary fibre (DF) in as much chemical and physical detail as possible needs to be emphasised since the lack of information makes comparisons of most published studies on the effect of DF on the pancreatic secretion extremely difficult. Starch is hydrolysed in the intestinal lumen by pancreatic α-amylase into maltose, triose and α-dextrins. Pancreatic adaptation of piglets to dietary starch starts immediately after weaning . Studies carried out with growing pigs and rats showed that the production of pancreatic α-amylase is very sensitive to changes in the content of dietary starch as increasing starch contents in a diet evoked increased α-amylase activities. The effect of NSP on the exocrine pancreas remains unclear, as some authors reported stimulatory effects whereas other authors obtained equivocal results. For example, an increase in the volume of secretion of pancreatic juice and total nitrogen is reported when the crude fibre content of the diet originated from native sources (wheat bran) rather than pure cellulose. This is in support of the idea that the type of diet and source of DF, i.e. diets made up of natural rather purified components, stimulate the exocrine pancreas. Differences due to methodological sources of variation must be taken into consideration when comparing results obtained with different surgical techniques to collect pancreatic juice. For example, the pouch technique showed a higher secretion of volume but a lower α-amylase activity when compared with the catheter method.

2.2. Introduction

The understanding of digestive processes and physiological mechanisms is essential for developing optimal feeding strategies for pigs. This may be a key factor in the prevention of nutritional diseases and moreover, it is crucial for the application of feeding strategies to protect the environment. In this context, the role of the pancreas as a major source of enzyme production is of specific interest. The development of pancreatic fistulation techniques (Wass, 1965; Pekas et al., 1966; Aumaitre, 1972; Corring et al., 1972; Partridge et al., 1982; Zebrowska et al., 1983; Hee et al., 1985; Pierzynowski et al., 1988b) allows for long term *in vivo* studies in several species including the pig. The response of the pancreas to nutritional and dietary factors and the different mechanisms controlling the secretions of the exocrine pancreas are of particular interest. In this review, the effect of dietary carbohydrates, including different fibre sources on quantitative and qualitative aspects of pancreatic secretion will be addressed.

2.3. Definition and classification of dietary fibre

It is still a matter of controversy how to define dietary fibre (DF) and several definitions have been suggested. The terms crude fibre (CF), neutral-detergent fibre (NDF), acid-detergent fibre (ADF) or non-starch polysaccharides (NSP) have been used interchangeably. Trowell et al. (1976) defined the term DF ”as the sum of the polysaccharides and lignin which are not digested by the endogenous secretions of the gastrointestinal tract”. This definition covers both chemical and physiological aspects of DF, but from an analytical point of view it is too imprecise to devise routine methods for fibre estimation. A common and widely accepted chemical definition of DF is ”the sum of all non-starch polysaccharides and lignin”. However, this basic and reductionist approach does not take into account many other dietary components including starch resistant to amylase (resistant starch, RS), several non-digestible oligosaccharides (NDO) and some protein and lipid fractions (Englyst et al., 1987) which, in the large intestine, behave similarly to some sources of NSP and might be included within the

definition of those taking an holistic view. An overview over the classification of carbohydrates present in feedstuffs including feed additives is presented in Table 1:

Table 1: Classification of carbohydrates

Category	Monomeric residues	Source
Non-starch polysaccharides (NSP)		
Cell Wall NSP		
Cellulose	Glucose	Most feedstuffs
Mixed linked β-glucans	Glucose	Barley, oats, rye
Arabinoxylans	Xylose, arabinose	Rye, wheat, barley
Arabinogalactans	Galactose, arabinose	Cereal by-products
Xyloglucans	Glucose, xylose	Cereal flour
Rhamnogalacturans	Uronic acid, rhamnose	Hulls of peas
Galactans	Galactose	Soybean meal, sugar-beet pulp
Non-cell wall NSP		
Fructans	Fructose	rye
Mannans	Mannose	Coconut cake, palm cake
Pectins	Uronic acids, rhamnose	Sugar beet pulp
Galactomannans	Galactose, mannose	Guar gum
Non-digestible oligosaccharides (NDO)		
α-Galacto-oligosaccharides	Galactose, glucose, fructose	Soybean meal, peas, rapeseed meal
Fructo-oligosaccharides	Fructose	Cereals, feed additives
Transgalacto-oligosaccharides	Galactose, glucose	Feed additives, whey and other milk products
Resistant starch (RS)		
Physical inaccessible starch	Glucose	Peas, faba beans
Native starch	Glucose	potatoes
Retrograded starch	Glucose	Heat-treated starch products

(Bach Knudsen, 1997)

Finally, another approach to define DF is to divide DF into a soluble and insoluble fraction. This differentiation is made due to its physiochemical properties and its nutritional effects. Soluble fibre may evoke viscous conditions in the stomach and the small intestine where they may affect digestion and absorption whereas the insoluble fibre fractions exert their effects usually in the large intestine (bulking effect). Consequently, many analytical procedures have

been developed to differentiate between soluble and insoluble fibre fractions. However, as was pointed out by Graham and Åman (1991), this distinction is often designed to fit into an analytical procedure rather than to correspond to actual physiological conditions since the fibre complex is continuously modified during gastrointestinal transport.

The importance of describing DF in as much chemical and physical detail as possible needs to be emphasised since the lack of information makes comparisons of most published studies on the effect of DF on the pancreatic secretions extremely difficult.

2.4. The response of the exocrine pancreas to dietary starch

Starch is the principal dietary carbohydrate. Apart from RS, starch is hydrolysed in the intestinal lumen by pancreatic α-amylase into maltose, triose and α-dextrins (Corring, 1980). The remaining dissacharides are hydrolysed into monomeric sugars by intestinal enzymes such as maltase, lactase and saccharase.

Pancreatic adaptation of piglets to dietary starch starts immediately after weaning. Young pigs weaned at 35 d of age showed a sharp increase in α-amylase activity 7 d after the diet was changed from milk to a diet with high starch content (Aumaitre, 1972). These results were confirmed by Flores et al. (1988) who found an increased specific activity of α-amylase after substitution of fat with starch in 7 to 10-wk old piglets. The process of adaptation to changes in the level of dietary starch takes 5 to 7 d in growing pigs (Corring, 1980).

There is convincing evidence that the pancreas adapts to the level of starch in the diet. Studies with growing pigs (Ozimek et al., 1985) and rats (Forman and Schneeman, 1980) showed that the production of pancreatic α-amylase is very sensitive to changes in the content of dietary starch. Corring and Chayvialle (1987) observed a 2.3 fold increase in specific α-amylase activity in growing pigs after a 3 fold increase of starch in the diet. Studies by Ozimek et al. (1985) showed a 50% decrease in total α-amylase production when 15% corn starch was replaced by 15% fat in a 15% crude protein corn starch-based diet. Mosenthin and

Sauer (1993) included 7.5% pectin in a diet for growing pigs at the expense of corn starch. There was a decrease ($P<0.05$) in the total activity of α-amylase. As pointed out by Corring (1980), the total secretion of α-amylase in both treatments exceeded by far the amounts theoretically required for intestinal hydrolysis of starch under optimal conditions. Therefore, the physiological importance of the observed decrease remains unclear. Hansen (1986) pointed out that maldigestion in humans is likely to occur only if the pancreatic enzyme secretion drops below 10% of the normal output. A possible explanation could be a carbohydrate related feedback mechanism which was reported by Jain et al. (1991) in humans. These authors infused carbohydrates (a solution of rice starch and glucose) at different rates (0; 12.5; 25; 50; 100 mg/min) into the ileum of human subjects. As the amount of unabsorbed carbohydrates in the ileum increased, the ratio of α-amylase to trypsin secretion increased ($P<0.005$) as well. It is suggested that the increase in α-amylase secretion following infusion of carbohydrates into the ileum is regulated via a feedback mechanism at the ileal rather than the duodenal level because at the same time there was a decline in the rate of passage of dietary carbohydrate from the stomach to the duodenum.

It may be assumed that these changes in the volume of pancreatic secretion and activity of enzymes are dependent on the level of glucose in the blood. Glucose administered to the jugular vein of pigs evoked a significant decrease in the secretion of pancreatic juice, protein and, in addition, a decrease in the total activities of α-amylase, chymotrypsin and lipase (Simoes Nunes and Corring, 1981). However, Rudick and Janowitz (1970) observed in humans a higher α-amylase output after elevation of the blood glucose level, whereas Karlsson et al. (1995) could not show an effect on the exocrine pancreas of pigs after intravenous 2-deoxy-D-glucose infusion, although the plasma levels of glucagon and insulin were elevated ($P<0.01$). Pierzynowski and Barej (1984) suggested that insulin enhances the stimulatory action of the vagus nerve on the pancreatic secretion of sheep and a very good correlation between changes in the plasma insulin concentration and the secretion of pancreatic enzymes was observed in cows (Pierzynowski et al., 1988a). The picture of the response of the exocrine and endocrine pancreas remains unclear. Moreover, it seems like that the duration of glucose infusions has an influence on the pancreatic response as short term infusions decrease and long term infusion stimulate the enzymatic secretion

(Pierzynowski, 1999). A feedback mechanism is likely controlling the pancreatic secretion and blood glucose level, but further investigation regarding exocrine and endocrine pancreas should be carried out.

2.5. The response of the exocrine pancreas to dietary NSP and dietary fibre

The exocrine pancreas of pigs adapts its secretion not only to the type and level of starch in the diet, but also the type and level of NSP in the diet.

In studies reported by Mosenthin and Sauer (1993) four barrows (initial BW 70 kg), fitted with permanent pancreatic cannulas according to the ”pouch method” (Hee et al., 1985), were fed two corn starch-based diets, containing 16% crude protein from soybean meal, without or with 7.5% pectin included at the expense of corn starch. The pigs were fed twice daily, pancreatic juice was collected continuously at 1 h-intervals for a total of 24 h. The inclusion of pectin at the expense of corn starch had no effect ($P>0.05$) on the rate of secretion of pancreatic juice and specific activities of trypsin, chymotrypsin, α-amylase or lipase. In addition, total activities of trypsin, chymotrypsin and lipase were not affected by the dietary treatments. However, there was a reduction ($P<0.05$) in the total activity of α-amylase when corn starch was replaced by pectin. To our knowledge, no reports have been published yet on the effect of pectin or other gel-forming polysaccharides on pancreatic secretions in pigs. On the other hand, several studies have been conducted with rats. It is difficult to draw conclusions from these studies because the results obtained are equivocal, which may partly be attributed to the different techniques used to measure pancreatic secretions. According to Forman and Schneeman (1980) and Calvert et al. (1985) there is only little evidence that pectin might affect the exocrine pancreas either by affecting the secretion via hormonal pathways (cholecystokinin and secretin) or via a negative feedback mechanism, as described in detail by Owyang (1994) and Miyasaka and Funakoshi (1998). However, it must be considered that these studies were done on slaughtered animals, which may distort the obtained results compared to results of studies done with chronically fistulated animals.

Several studies have been carried out that focus on the effect of different sources of NSP on exocrine pancreatic secretions. As was pointed out by Mosenthin and Sauer (1991), differences in the source of fibre could, in part, explain differences both in ileal amino acid digestibilities between feedstuffs and among different samples of the same feedstuff, as described by Sauer and Ozimek (1986). These differences may be attributed to changes in the rate of secretion of protein and digestive enzymes in the pancreatic juice following consumption of feedstuffs rich in fibre.

Mosenthin and Sauer (1991) determined the effect of source of fibre on the rate of secretion of protein, trypsin, chymotrypsin, α-amylase and lipase. Six barrows (initial BW 50 kg) were fitted with a permanent pancreas re-entrant cannula according to Hee et al. (1985). The animals were fed three different corn starch-based diets: a basal diet containing 49.9% corn starch and two experimental diets in which 10% corn starch were replaced by 10% Alphafloc (cellulose), and 10% straw meal, respectively. The inclusion of Alphafloc had no effect ($P>0.05$) on the secretion of pancreatic juice, nitrogen and the specific as well as total activities of trypsin, chymotrypsin, α-amylase and lipase. In addition, the secretion of pancreatic juice and that of nitrogen was not ($P>0.05$) affected by the inclusion of straw but there was a decrease ($P<0.05$) in the specific activities of chymotrypsin and α-amylase. However, most likely because of the higher volume of secretion of pancreatic juice (although not significant, $P<0.10$) in pigs fed the straw-containing diet, there were no differences ($P<0.05$) between the total enzyme activities.

The results of Mosenthin and Sauer (1991) and Mosenthin et al. (1994) who reported no effect of level and source of fibre on the total activities of enzymes secreted in pancreatic juice, are in agreement with those of Zebrowska et al. (1983) and Fevrier et al. (1992). The results of a study by Zebrowska and Low (1987) revealed that substitution of 50% of the wheat in a wheat-based diet (88.7% wheat) by 50% wheat bran or by 50% wheat flour, respectively, did not affect the volume of secretion. However, a level of 20% NSP in the diet originating from wheat and wheat bran induced a 78% higher secretion ($P<0.01$) of pancreatic juice compared to the diet based on wheat and wheat flour containing 5% NSP. Despite the higher secretion of

volume, total enzyme activities were not significantly affected by the different dietary treatments.

However, these results are in contrast to these obtained by Langlois et al. (1987) in growing pigs fitted with a pancreatic duct cannula. The control group was fed a cereal-based diet without wheat bran whereas the experimental group received a diet containing 40% wheat bran included at the expense of wheat. Wheat bran induced an increase ($P<0.05$) in the volume (+115%) and protein content (+36%) of pancreatic juice during a 24-hour period. Moreover, in contrast to Zebrowska and Low (1987) total enzyme activities were enhanced ($P<0.05$) when wheat bran was included in the diet. This study confirms observations made by Jakob et al. (1999) in piglets. Three 8 wk old piglets with a BW of 12.4 kg at surgery were fitted with a chronic pancreatic duct catheter and a re-entrant duodenal fistula according to Pierzynowski et al. (1988b). After a post-operative recovery period of 7 d the pigs were fed two diets according to the following experimental design: all pigs received for a period of 7 d a commercial weaner diet as a control diet, followed by a period in which the same diet supplemented with 2% potato fibre was fed . Thereafter, the control diet without potato fibre was fed for another 7 d. The chemical composition of potato fibre is presented in Table 2. The volume of pancreatic juice, the protein secretion and both the total and specific trypsin, lipase and α-amylase activities increased ($P<0.05$) after adaptation to the diet supplemented with potato fibre. After re-adaptation to the control diet without potato fibre supplementation no decrease in the parameters measured to the initial levels was observed.

Table 2: Chemical composition of Potato Fibre (%)

crude fibre	cellulose	lignin	pectin + hemicellulose	starch	protein	fat
70%	23%	2%	45%	10%	7%	0.3%

In previous growth trials (Pierzynowski, 1999) positive effects of potato fibre supplementation to a diet for growing pigs on production traits were observed.. This improvement in performance may be related to the increased secretion of pancreatic enzymes due to potato fibre supplementation, which, in turn, may have a positive effect on nutrient digestibility, resulting in a better nutrient supply to the pig. A possible explanation is given by Botermans

and Pierzynowski (1999) who showed that better performance of piglets compared to litter mates is related to higher protein content and trypsin activities in pancreatic juice.

This stimulatory effects of DF on the secretion of pancreatic enzymes observed by Langlois et al. (1987) and Jakob et al. (1999), are in contrast to the results obtained by Zebrowska and Low (1987), Mosenthin and Sauer (1993) and Mosenthin et al. (1994). Langlois et al. (1987) reported a stimulatory effect of DF after replacement of starch by DF, whereas Mosenthin et al. (1994) obtained a decrease in α-amylase activity after the replacement of starch by DF. One explanation for this can be derived from the studies by Zebrowska and Low (1987) who suggested that the volume of secretion of pancreatic juice as well as the protein secretion apparently are more related to the content of NSP in the diet than to the crude fibre content. This emphasises the importance of a precise definition of DF when comparing the results of different studies .

This necessity for a clear definition of DF can be derived from studies by Partridge et al. (1982) and Zebrowska and Low (1987). These authors could show that semi-synthetic diets based on either starch and casein or wheat flour and casein induced a distinctly lower ($P<0.05$) pancreatic juice secretion compared to cereal-based diets. Enzyme activities were not affected by the source of fibre. Especially the results reported by Zebrowska and Low (1987) support the idea that the type of diet and source of DF, i.e. diets made up of natural rather purified components, stimulate secretions of the exocrine pancreas. These authors reported an increase ($P<0.01$) in the volume of secretion of pancreatic juice and total nitrogen when the crude fibre content of the diet originated from natural sources (wheat bran) rather than pure cellulose. Literature results on the volume of secretion and protein secretion in pancreatic juice secretion in growing pigs in response to different dietary treatments are summarised in Table 3.
Part of the variation between studies related to the effect of DF on exocrine pancreatic secretions may be attributed to different techniques used to collect pancreatic juice. Whereas Zebrowska et al. (1983), Zebrowska and Low (1987), Mosenthin and Sauer (1991) and Mosenthin and Sauer (1993) used the ”pouch method” (Hee et al., 1988a) to collect pancreatic juice, the animals in the studies of Langlois et al. (1987) and Jakob et al. (1999) were fitted with a pancreatic duct cannula as described by Corring et al. (1972) or

Pierzynowski et al. (1988b) and Zabielski et al. (1997), respectively. Langlois et al. (1987) reported after replacement of 50% wheat by wheat bran in a cereal- based diet an increase in total protein secretion despite a decrease in protein concentration. Gabert et al. (1996a) reported considerable differences between surgical methods used to collect pancreatic juice for the volume of secretion and enzyme activities. Jensen et al. (1997) found, in a comparative study, far considerable differences in the volume of pancreatic juice secreted and in the chemical and enzyme composition of pancreatic juice when comparing the ”pouch method” and the ”catheter method”. For example, the concentration of protein in pancreatic juice from pigs prepared with the ”pouch method” was higher ($P<0.001$) than in pigs fitted with a pancreatic duct catheter. In addition, specific and total α-amylase activities were increased ($P<0.01$) in pigs fitted with a fistula according to the ”catheter” method. Moreover, the volume of secretion was enhanced ($P<0.05$) in pigs prepared with the pancreatic duct catheter. In conclusion, differences due to the method for collection of pancreatic juice must be taken into consideration when comparing results (Table 3).

Table 3: Influence of type of diet on daily volume of secretion of pancreatic juice and protein-secretion in pigs

Reference	Feed intake (kg/d)	Volume (l)	Protein (g)	Body weight (kg)	Surgical procedure
Zebrowska et al., 1983	1.5 barley / soybean meal	2.2	12.1	40	pouch
	1.5 starch /casein	1.2	10.9	40.	pouch
	1.5 starch / soybean meal	3.8	14.4	35 - 50	pouch
Zebrowska and Low, 1987	1.4 wheat	4.1	17.9	34*	pouch
	1.4.wheat / wheat bran	4.6	19.0	34*	pouch
	1.4 wheat / wheat flour	2.6	15.8	34*	pouch
	1.4 wheat flour / cellulose	1.8	13.0	34*	pouch
Langlois et al., 1987	1.6 no wheat bran	1.7	14.6	38	duct
	1.6 40% wheat bran	3.6	19.7	38	duct
Mosenthin and Sauer, 1991	1.8 starch	3.7	26.9	60	pouch
	1.8 cellulose	3.2	22.8	59	pouch
	1.8 straw meal	4.6	28.5	69	pouch
(Mosenthin and Sauer, 1993	1.8 0% pectin	3.8	25.5	70	pouch
	1.8 7.5% pectin	4.7	27.0	70	pouch
Jakob et al., 1999	0.5 0% potato fibre	1.2	11.7	12.4*	duct
	0.5 2% potato fibre	1.9	20.2	12.4*	duct

* BW at surgery

In addition, when comparing results from different studies, the effect of different breeds on pancreatic secretion must be considered. Fevrier et al. (1992) did not observe an effect of both different breeds (Large white and Mei Shan) and the level of wheat bran (0%, 20%, 51.8%) in the diet on enzyme activities in pancreatic juice. On the other hand, Freire et al. (1996) reported differences when different levels of wheat bran (0% or 15%) were fed to different breeds of pigs. Total activities of pancreatic lipase, trypsin and α-amylase were 2.0, 1.5 and 5.0 fold higher in Alentejano compared to Large White piglets. Both groups were weaned at the age of 21d. It should be mentioned, that in the studies of Freire et al. (1996) and Jakob et al. (1999) piglets (12kg) were used whereas in the other studies animals weighing between 35 and 70kg were used. As reviewed by Makkink and Verstegen (1990a) it is evident that

pancreatic secretion (volume, protein and enzyme activities) increases with age and, moreover, dietary changes may interact with development of the exocrine pancreas. Thus, the effect of age on pancreatic secretion must also be considered when comparing results of different studies.

2.6. Conclusions

The results of studies that relate to the effect of different sources and levels of dietary carbohydrates on exocrine pancreatic secretions in pigs show considerable variations. According to Partridge et al. (1982) this variation is of biological origin rather than an artefact. The nutritional implications of these studies, however, may be minor as long as the quantity of pancreatic enzymes secreted is sufficient for digestion. Corring (1980) states that under physiological conditions the quantity of pancreatic enzymes secreted is sufficient for digestion of approximately ten times the amount of food usually consumed. Moreover, as was pointed out by Imbeah et al. (1988), comparing results from different studies relating to pancreatic secretion in pigs is difficult, because these comparisons are confounded by differences in feed intake, feeding regimen, diet composition, body weight and the method used to collect pancreatic juice.

The effect of dietary fibre and its mode of action in piglets still remains open. Further studies are warranted to clarify possible physiological implications in the nutrition of piglets. However, comparison of results between different research groups require standardisation of methods used to collect pancreatic juice and to determine pancreatic enzyme activities. Furthermore, a clear description and definition of DF is necessary in order to obtain conclusive results.

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3. THE INFLUENCE OF LIPIDS ON EXOCRINE PANCREATIC SECRETIONS IN PIGS

3.1. Summary

The characteristics of the exocrine pancreatic secretion in pigs and its hormonal regulation as influenced by dietary lipids are reviewed. There is clear evidence that the secretion of lipolytic enzymes is positively correlated with the amount of fat consumed by the pig. For example, there was an increase in the specific lipase activity by 83% after the dietary fat content was increased from 5% to 25%. Moreover, it was shown that also the quality of fat has an influence on exocrine pancreatic secretions. Peroxidized canola oil stimulated total lipase secretion much more than non-peroxidized oil. The influence of fatty acid composition on exocrine pancreatic secretions is discussed equivocally. Some authors showed that saturated fats stimulated the exocrine pancreatic secretions more than unsaturated. Others showed that the chain length of fatty acids had a strong influence on pancreatic secretions as well. Due to the different surgical methods used for sampling of pancreatic juice and wide variety of fats and oils used in these studies, direct comparisons between studies are extremely difficult to make.

Plasma levels of hormones such as cholecystokinin (CCK), neurotensin (NT) and peptide YY (PYY) are influenced by the nutrient composition of the diet. With increasing amounts of fat present in the small intestine, the release of these hormones was stimulated. There is evidence that CCK release is dependent on the chain length of the fatty acids. Medium chain triglycerides stimulated the CCK release more than long chain triglycerides. Neurotensin was released more by unsaturated than by saturated fatty acids; similar results were observed for the PYY release. However, results are contradictory and further investigations are warranted that focus on the underlying mechanisms involved in the regulatory response of the exocrine pancreas to lipids of different origin.

3.2. Introduction

The understanding of the complex physiological digestive processes plays a major role in optimizing feeding strategies for farm animals. As the pancreas is an important part of the digestive system and the main source of digestive enzymes, knowledge about its physiological processes is important. The pancreas consists between 90 to 95% of exocrine and between 2 to 3% of endocrine tissue (Brannon, 1990); it secretes enzymes for digestion of lipids, carbohydrates and proteins either in an active or inactive form, as well as bicarbonate for the neutralisation of hydrochloric acid from the stomach and other components (Table 1).

Table 1. Composition of pancreatic juice

Enzymes		
	Proteases	Trypsinogen 1, 2, 3
		Chymotrypsinogen A, B, C
		Proelastase 1, 2
		Kallikreinogen
		Procarboxypeptidase A1, A2, B1, B2
	Glycosidase	α-Amylase
	Lipases	Triglyceride lipase
		Colipase
		Carboxylester hydrolase
		Phospholipase A2
	Nucleases	DNase I
		RNase
Electrolytes		
		Chloride
		Sodium
		Potassium
Bicarbonate		
Mucins		
Urea		

after: Kidder and Manners (1987); Schulz, (1987); Lowe, (1994b)

The development of pancreatic fistulation techniques for several animal species including the pig (Wass, 1965; Pekas et al., 1966; Aumaitre, 1972; Corring et al., 1972; Partridge et al., 1982; Zebrowska et al., 1983; Hee et al., 1985; Pierzynowski et al., 1988) gave researchers the opportunity to study *in vivo* the various physiological mechanisms which regulate pancreatic secretions. The response of the pancreas to dietary factors and different mechanisms

controlling the secretions of the exocrine pancreas is of particular interest. This review will focus mainly on the effects of lipids of different origin on quantitative and qualitative aspects of pancreatic secretions in pigs and on the regulation of the exocrine pancreas mediated by gastrointestinal hormones.

3.3. Chemical composition of lipids and lipolytic

Lipids have a very high energy density. Consequently, they are a valuable component of pig diets. In addition, because of the high energy content of lipids, there is a margin for inclusion of other components. This is of special interest from an economical point of view as other dietary components can be chosen to lower feed costs and/or to increase the nutritive value of the diet. Lipids contain three essential fatty acids, namely linoleic, γ-linolenic and arachidonic acid which are important for the biosynthesis of phospholipids. These lipids are important components of cell membranes and essential for the formation of prostaglandins, which are involved in the regulation of various metabolic processes (Kirchgessner, 1987).

Lipids used in animal nutrition are triacylglycerols with fatty acids in positions one, two and three of glycerol. Lipids used in animal nutrition differ widely in chemical structure with respect to fatty acid composition. For example, vegetable oils, such as olive, soybean, canola or sunflower, consist mainly of unsaturated fatty acids with a chain length of C18 , whereas tropical plant oils, such as palm and coconut oil, contain saturated fatty acids with a chain length of C12 to C14. Fats derived from marine animals, such as fish oil, contain polyunsaturated fatty acids with a chain length longer than C20, whereas lard or tallow contain saturated fatty acids with a chain length of C16 and C18 (Table 2). These differences in fatty acid profiles of fats and oils may influence the physiology of the pancreas in different ways.

Jensen et al. (1997) showed that most of the lipids in diets for piglets are digested by enzymes secreted by the exocrine pancreas; in younger animals gastric lipase is capable to hydrolyze lipids. Fats are non-soluble in water and therefore fat must be emulsified before being cleaved. Digestion is carried out by means of bile salts and phospholipids which are secreted with bile

into the duodenum. The exocrine pancreas secrets three different lipolytic enzymes into the duodenum: lipase, carboxylester hydrolase and phospholipase A_2. Lipase is the most important fat-cleaving enzyme. This enzyme is capable to cleave linkages at positions one and three whereas carboxylester hydrolase cleaves all linkages. Prophospholipase A_2 is activated by trypsin to phospholipase A_2 which cleaves phospholipids such as phosphatidylcholine (lecithin) and sphingomyelin specifically at position two (Rinderknecht, 1993; Lehninger et al., 1994; Lowe, 1994a; Lowe, 1994b).

Colipase, secreted by the pancreas, is an essential cofactor involved in the digestion of lipids as it catalyses the attachment of lipase to emulsified lipids (Rinderknecht, 1993).

Table 2. Fatty acid composition (% of total fatty acid content) of lipids present in feedstuffs

Fatty acid	Corn oil	Sunflower oil	Rapeseed oil	Olive oil	Lard	Fish oil	Coconut oil
C 8:0							5-10
C 10:0							5-10
C 12:0							44-51
C 14:0	0.5-3	0-1	0-1.5		1-4	1-8	13-19
C 16:0	8-15	4-8	1-6	12	21-31	10-28	7-12
C 16:1	0.2-0.5	0.1-1	0-2		1-5	7-13	
C 18:0	1-4	2-5	1-4	2	11-21	0-3	1-4
C 18:1	27-43	14-50	11-39	61	40-52	6-24	5-8
C 18:2	35-62	33-77	10-22	15	2-8	1-12	1-2
≥ C 20 unsaturated			32-57			22-58	

after: Kirchgessner, (1987); Yago et al. (1997c).

3.4. Effect of level of fat in the diet on the secretions of the exocrine pancreas

Several authors have demonstrated that an increase in dietary fat level is closely correlated with a higher secretion of lipase (Corring et al., 1989). According to Sabb et al. (1986) the specific pancreatic lipase activity in the young rat adapts primarily to the amount of dietary fat. A diet with a high fat content (> 57% energy from fat) increased specific lipase activity by 200% compared to diets in which less than 47% of the energy was derived from fat. Mourot and Corring (1979) showed similar results in pancreatic tissue of pigs. The animals were fed a

diet containing either 5 or 25% peanut oil. The specific lipase activity was 83% higher when the diet containing 25% fat was fed. An increase in the specific lipase activity by 700% was observed by Corring (1980) in pigs after increasing the triacylglyceride intake from 30 to 220 g. Hee et al. (1988) also showed in pigs that the total lipase activity increased 6-fold when the level of dietary fat (tallow) was increased from 2 to 10%. Ozimek et al. (1995) reported in studies with pigs an increase in the total lipase activity by 340% after 15% starch in the diet was replaced by 15% canola oil. However, this adaptation of the exocrine pancreas to the amount of dietary fat was not observed in dogs fed either a high fat or a high starch-containing diet (Manas et al., 1996). In the rabbit it was shown that pancreatic lipase activity increased 2-fold when the amount of dietary fat was increased from 2.7% to 12% (Borel et al., 1991).

3.5. Effect of quality of fat on the exocrine pancreas

There is a scarcity of information on the effect of quality of fat on the secretory activity of the pancreas in pigs. It is known that hydroperoxides, which are the primary products resulting from oxidative processes of unsaturated fat during storage and processing, are involved in the production of rancidity, odours, bad flavours and even toxic compounds. Ozimek et al. (1995) compared the effect of peroxidized versus non-peroxidized canola oil in the diet of growing pigs. After replacement of 15% canola oil by 15% peroxidized canola oil (heated at 180°C for 12h) the total lipase activity increased 2.5-fold.

3.6. Effect of fatty acid composition on the exocrine pancreas

Several studies have been conducted to investigate the response of the exocrine pancreas to changes in the fatty acid profile of dietary or intraduodenally infused lipids. However, most of these studies were carried out with slaughter investigations in particular with rats. These studies do not allow for the measurement of total enzyme activities since long-term collections of pancreatic juice are not possible. According to Sauer and Mosenthin (1999) only results expressed in total rather than specific activities are a true reflection of the effect of dietary

treatments on the exocrine pancreas since differences in specific activities may simply reflect dilution by pancreatic juice.

The results obtained in studies with rats are contradictory with respect to the influence of the degree of saturation and/or chain length of fatty acids on lipase activities. Deschodt Lanckman et al. (1971) showed that the lower the degree of saturation, the higher the specific lipase activity. Corn oil with a high content of saturated fatty acids (polyunsaturated/saturated (p/s) ratio was 0.1) had a less pronounced effect on the specific lipase secretion in the rat than the same amount of sunflower oil in the diet (p/s ratio is 6.5). These observations were confirmed by Sabb et al. (1986) and Ricketts and Brannon (1994). According to these authors the inclusion of polyunsaturated fatty acids increased specific lipase activities more than saturated fatty acids. However, as was pointed out by Saraux et al. (1982), specific lipase and colipase activity was not affected by the degree of saturation or the chain length of fatty acids when rats were fed a diet containing 40% fat. It should be emphasized, however, that estimates of specific enzyme activities in pancreatic homogenates do not provide information on the diurnal variation. From experiments with fistulated calves (Zabielski et al., 1993; Zabielski et al., 1997a) it was shown that the secretory response of the exocrine pancreas can change within minutes. This has to be taken into consideration when interpreting results obtained by means of slaughter investigations.

Only a few studies have been conducted with pigs in which the effect of fatty acid composition on pancreatic secretion of lipolytic enzymes was determined. Simoes Nunes (1986) investigated the influence of sunflower oil and lard on exocrine pancreatic secretions in the growing pig. A control group received a starch-based diet whereas two experimental groups were fed diets in which 21% lard or 21% sunflower oil were included at the expense of starch. The pigs were slaughtered on d 12 after the start of the experiment and pancreatic tissue homogenates were obtained. Although the pancreatic protein content was similar in all groups, the specific lipase activity was 60% higher in the pigs fed lard and about 300% higher in the pigs fed sunflower oil compared to the control treatment. These significant differences between the treatments indicate that the degree of saturation or the chain length of the fatty acids may influence specific lipase activity.

Only one study in which the influence of fats differing in chain length and degree of saturation on exocrine pancreatic secretions was conducted with pigs fitted with permanent pancreatic cannulas allowing the determination of both specific and total enzyme activities. Gabert et al. (1996) conducted two experiments using two different surgical procedures to collect pancreatic juice. Three barrows were fitted with a pancreatic duct catheter according to Pierzynowski et al. (1988) and three barrows according to the pouch method as described by Hee et al. (1988). The animals of each group received three different wheat-based diets containing 15% fish oil, rapeseed oil and coconut oil, respectively. In pigs fitted with the pouch no differences between the parameters measured were observed. The coconut and fish oil treatment evoked an increase in total activity of chymotrypsin and carboxylester hydrolase, however, this was only observed in pigs fitted with a pancreatic duct catheter. As considerable differences between both surgical methods exist, Gabert et al. (1996) claimed that these differences may be explained by different physiological changes induced by the two methods. The implantation of a catheter into the pancreatic duct bypasses the *sphincter oddi* and the formation of a duodenal pouch involves anastomosis of the duodenum and duodenal-pancreatic neural reflexes may be distorted (Zabielski et al., 1997b). However, it should be mentioned that the number of observations was relatively small in this study which, in turn, limits the interpretation of these results. Studies on the influence of different lipids on exocrine pancreatic secretions in different species, including humans, are summarized in Table 3.

Table 3. Effect of different lipids on exocrine pancreatic secretions in different species.

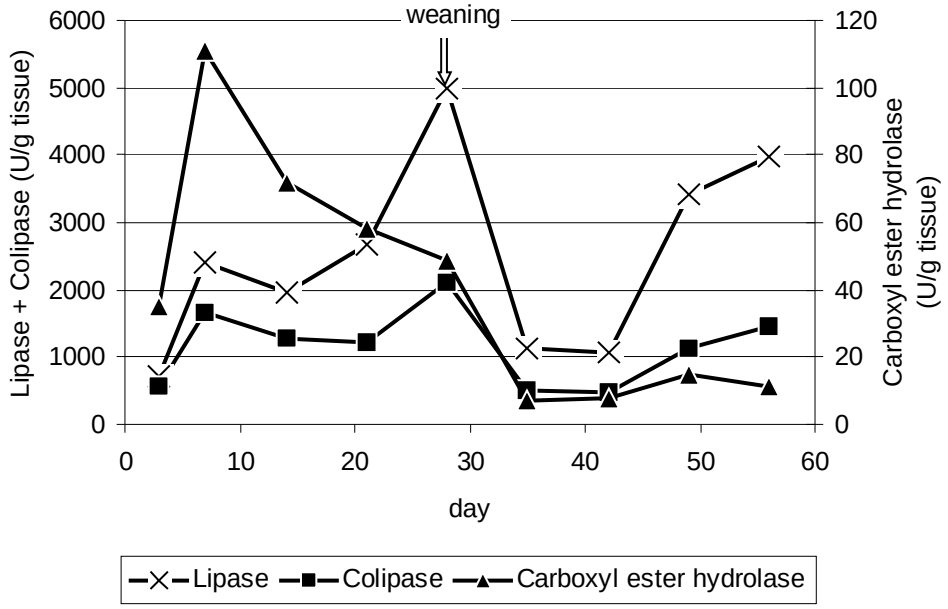
Species	Lipids	Pancreatic secretion	Reference
Pig	sunflower oil, lard	unsaturated long chain fatty acids (sunflower oil) increased specific lipase activities more than lard	Simoes Nunes, 1986
	fish-, rapeseed-, coconut oil	unsaturated, long chain fatty acids (fish oil) increased total carboxylester hydrolase activities more than rapeseed and coconut oil	Gabert et al., 1996
Dog	sunflower, olive oil	polyunsaturated fatty acids (sunflower oil) increased total lipase activities more than saturated fatty acids (corn oil)	Ballesta et al., 1990
Rat	sunflower oil, corn oil	polyunsaturated fatty acids (sunflower oil) increased specific lipase activities more than saturated fats (corn oil)	Deschodt Lanckman et al., 1971
	safflower-, corn,- olive-, coconut oil, butter, lard	polyunsaturated fatty acids (safflower oil) increased specific lipase activities more than corn,- olive-, coconut oil, butter and lard	Sabb et al., 1986
	medium chain triglycerides (C8-C10), coconut oil	no influence of degree of saturation or chain length	Saraux et al., 1982
	safflower oil, lard	polyunsaturated fatty acids (safflower oil) increased specific lipase activities	Ricketts and Brannon, 1994
Human	sunflower-, olive oil	monounsaturated fatty acids (olive oil) increased total lipase activities more than saturated fatty acids (sunflower oil)	Yago et al., 1997a

3.7. Dietary fat and stage of development

Jensen et al. (1996) showed in the suckling piglet that the level of pancreatic lipase is relatively low and increases with age of the piglet until weaning (Figure 1). However, Cranwell and Moughan (1989) reported that suckling piglets are able to digest sows milk very efficiently; they reported an apparent fat digestibility of 96%. This supports the idea by Jensen

et al. (1996) that during the suckling period gastric lipase may play a major role in the hydrolysis of fat. Moreover, Jensen et al. (1996) observed an increase in lipolytic enzyme activity until weaning. They concluded that the low pancreatic lipase activities in suckling piglets are compensated by high carboxylester hydrolase activities (Figure 1). Interestingly, carboxylester hydrolase is similar in structure to the bile-salt stimulated lipase, which is found in human milk and plays an important role in the nutrition of premature born infants (Hernell and Blackberg, 1994a; Hernell and Blackberg, 1994b).

Figure 1. Development of lipolytic enzyme activities in pancreatic tissue of piglets (after: Jensen et al. (1997)).



3.8. Hormonal regulation of pancreatic secretions mediated by different lipids

It has been shown that intestinal perfusions with fatty acids stimulate pancreatic secretions (Solomon, 1987). In addition, there is evidence that they mediate the release of hormones and regulatory peptides (Olsen et al., 1989). The gastrointestinal hormones secretin and cholecystokinin (CCK) are considered to be the most potent stimulators of the secretions of the exocrine pancreas. Whereas secretin mediates mainly the secretion of bicarbonate, water and electrolytes, CCK stimulates the acini of the pancreas, which release pancreatic enzymes (Brannon, 1990).

CCK is released after contact with digesta in the duodenum. Several authors showed in studies with different species (rats, dogs and pigs) that CCK release is stimulated after contact of the duodenal mucosa with either protein, carbohydrate or fat (Corring et al., 1986; Rhodes et al., 1988; Corring et al., 1989; Lluis et al., 1989; Greenberg, 1993; Jakob et al., 1999). Comparative studies with rats showed that fat and carbohydrate stimulate the exocrine pancreatic secretion less than protein (Douglas et al., 1988). In contrast, Hopman et al. (1985) showed in studies with humans that the consumption of equal amounts of fat and protein increased plasma CKK concentrations to the same extent, whereas starch consumption stimulated the release of CCK to a lesser extent than other nutrients. Corring and Chayvialle (1987) demonstrated, in pigs fitted with permanent pancreatic cannula, adaptation of the specific lipase activity to the amount of dietary fat, but there was no effect on plasma CCK levels.

It can be concluded from results of Douglas et al. (1990) that medium-chain triglycerides with a chain length smaller than C12 stimulated CCK release more than long chain triglycerides. The consumption of medium-chain triglycerides (caprylic acid) evoked a 2.8-fold higher CCK release than the consumption of long-chain triglycerides (corn oil). In dogs with pancreatic cannulas no differences in plasma CCK levels were observed after consumption of diets containing either olive or sunflower oil (Yago et al., 1997b).

Other gastrointestinal hormones are considered to be influenced by fat in the diet as well. Lluis et al. (1989), in studies with dogs, reported an increase in the level of neurotensin (NT) after intraduodenal application of corn oil. Sagher et al. (1991) demonstrated that the composition of fat may influence the level of NT in the distal part of the small intestine to a different extent. Rats were fed for 8 wk three different experimental diets in which 40% of the energy content was derived from butter (mainly saturated fatty acids), olive oil (mainly unsaturated fatty acids) and corn oil (polyunsaturated fatty acids), respectively. The control diet was low in fat (10% of the energy from fat). The consumption of olive and corn oil resulted in an increase in the concentrations of NT compared to the control treatment. However, this increase in NT plasma levels was not observed after consumption of butter. The authors postulate that the increased levels of NT after consumption of olive and sunflower oil could be explained with a better absorption of unsaturated fatty acids. In contrast to results obtained in studies with dogs and pigs, Wood et al. (1988) showed in studies with rats, that NT, injected at three different levels subcutaneously, increased the fresh weight of the pancreas by 16%. However, an increase in the specific lipase activity was not observed. This was confirmed by Beck et al. (1992) in studies with rats who also did not show an effect of a diet high in fat on plasma levels of NT.

The hormone peptide YY (PYY) is considered to inhibit pancreatic secretions via a feedback mechanism. It is released from the ileum to the portal circulation approx. 30 min postprandially (Greeley et al., 1989b); it inhibits pancreatic secretions via a negative feedback mechanism (Mössner, 1990; Guan et al., 1991; Lin et al., 1996). Studies with dogs fitted with pancreatic duct catheters showed that a diet containing sunflower oil increased total activities of α-amylase and lipase compared to dogs fed a diet containing olive oil (Ballesta et al., 1990). It was demonstrated in humans that the consumption of a diet containing olive oil resulted in elevated PYY levels compared to the consumption of a diet containing sunflower oil (Yago et al., 1997b). These results were confirmed by Serrano et al. (1997). In addition various other hormones interact with the exocrine secretions of the pancreas which emphasizes the complexity of the regulatory mechanisms involved in the secretory response of the pancreas to dietary fat (Table 4).

Table 4. Gastrointestinal hormones regulating the exocrine pancreatic secretion

Hormone/peptide	Effect on pancreas	Dietary stimuli for release	Releasing tissue	Reference
Cholecystokinin (CCK)	stimulates secretion of enzymes (mainly proteolytic enzyme activity)	protein, carbohydrates, fat	duodenal and jejunal mucosa	Douglas et al., 1988; Greenberg, 1993; Liddle, 1995
Pancreatic Polypeptide (PP)	inhibits enzyme, protein and bicarbonate secretion, minor effect on volume of secretion	fat, fatty acids	small intestine	Lonovics et al., 1981; Owyang et al., 1983; Fried et al., 1984; Langlois et al., 1989
Peptid YY (PYY)	inhibits enzyme activity and volume secretion	fat, fatty acids, protein	distal ileum, colon	Greeley et al., 1989a; Greeley et al., 1989b; Guan et al., 1991; Lin et al., 1996
Neurotensin (NT)	stimulates enzyme, protein and bicarbonate secretion	fat, fatty acids	ileum	Walker et al., 1985; Gomez et al., 1986; Mössner, 1990
Bombesin	stimulates protein and volume secretion	not known	gastric mucosa	Holmgren et al., 1982; Lilja et al., 1984; Ami et al., 1993
Enterostatin	inhibits pancreatic secretion	fat, protein	pancreas, fragment of pro-colipase after its activation by trypsin	Holmgren et al., 1982; Lilja et al., 1984; Ami et al., 1993
Enteroglucagon	inhibits pancreatic secretion	unabsorbed nutrients in the ileum	not known	Dowling et al., 1985; Sagher et al., 1991; Holst, 1997

3.9. Conclusions

It can be concluded that level as well as type and origin of dietary fat is probably the most important factor affecting the secretion of lipolytic enzymes. A close positive correlation between the amount of dietary fat and lipase activity could be shown. Moreover, there is evidence that not only the level of fat in the diet influences the exocrine pancreatic secretions, but also the type and origin of fat has to be considered as an important factor. However, results are contradictory so far and no final conclusion can be drawn if the degree of saturation and/or the chain length of fatty acids is the most effective factor involved in the regulation of the exocrine pancreas.

Furthermore, gastrointestinal hormones such as CCK, NT, PYY and secretin mediate the secretory response of the exocrine pancreas to fat supplementation, however, most of the underlying pathways are not known yet.

Thus, further investigations are warranted to elucidate the effects of fats of different composition both on the secretions of the exocrine pancreas and on the underlying hormonal regulatory processes. It has to be emphasised that most of the studies do not allow for the determination of total volume secretion total protein output and total enzyme activities as well due to methodological constraints (slaughter investigations). The application of surgical techniques that permit permanent collection of pancreatic juice will provide more detailed information on the effect of dietary lipids on the function of the exocrine pancreas. Pigs are appropriate animal models not only with respect to animal nutrition, since they may also be used in human biomedicine.

3.10. References

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4. THE INFLUENCE OF POTATO FIBRE ON EXOCRINE PANCREATIC SECRETIONS AND ON PLASMA LEVELS OF INSULIN; SECRETIN AND CHOLECYSTOKININ IN GROWING PIGS

4.1. Summary

The effect of a potato fibre preparation on exocrine pancreatic secretions and on gastrointestinal hormone levels in plasma was studied in three 8 wk old piglets that were surgically fitted with a jugular vein catheter for blood sampling, a pancreatic duct catheter and a T-shaped duodenal cannula for collection of pancreatic juice. The animals were fed for 2 wk a control diet (experimental period 1), thereafter for 2 wk the control diet supplemented with 2% potato fibre (experimental period 2) and for another 2 wk the control diet again (experimental period 3). Additionally, intraduodenal (i.d.) infusions of the experimental diet, the control diet and potato fibre as well as intraveneous (i.v.) infusions of a solution containing Cholecystokinin (CCK) and secretin were administered.

Potato fibre in the diet evoked in tendency ($P<0.1$) an increase in the volume of secretion of pancreatic juice and a significant ($P<0.05$) increase both in the mean values of the total protein content and total activities of lipase, trypsin and α-amylase when compared to the control diet. The i.d. infusion of the control diet , experimental diet and fibre infusate as well as the i.v. administration of the hormone infusate led to a spontaneous secretory response of the exocrine pancreas. Besides gastrointestinal hormones, such as CCK, other factors such as short chain fatty acids may be involved in the regulation of the exocrine pancreas.

4.2. Introduction

It can be derived from various studies related both to animal and human nutrition that the dietary inclusion of plant fibre will affect the function of the gastrointestinal tract and the health status of the whole organism as well. Since the exocrine pancreas represents a major source of endogeneous secretions into the gastrointestinal tract, several studies have been

performed that focus on the effect of dietary fibre (DF) on the secretions of the exocrine pancreas in different species including humans.

In studies with growing pigs, Mosenthin and Sauer (1991) and Mosenthin et al. (1994) reported no effect of level and source of dietary fibre (DF) on the total activities of enzymes secreted in pancreatic juice when 10% cellulose, 10% strawmeal or 7.5% pectin were included in the diets. These results are in agreement with those of Zebrowska and Low (1987). The authors found that the replacement of 50% of wheat in a wheat-based diet (88.7% wheat) by 50% wheat bran did not affect the volume of secretion and the total activities of trypsin, chymotrypsin, carboxypeptidase A and B and of α-amylase in pancreatic juice.

However, these results are in contrast to those obtained by Langlois et al. (1987) in growing pigs who found an increase ($P<0.05$) in the volume (+115%) and protein content (+36%) of pancreatic juice when 40% wheat bran was included in a cereal-based diet at the expense of wheat. In contrast to the results reported by Zebrowska and Low (1987) total enzyme activities in pancreatic juice were increased ($P<0.05$) when wheat bran was included in the diet.

The effect of DF on exocrine pancreatic secretions in humans is contradictory. Dukehart et al. (1989) observed no influence of DF on exocrine pancreatic secretions, whereas Sommer and Kasper (1980) reported a decrease in the volume of pancreatic secretion ($P<0.025$) when carrageenan and guar meal were included in the diets; there was a trend ($P<0.1$) towards a lower secretion of protein and the specific α-amylase activity. A possible explanation of these results was provided by Dunaif and Schneeman (1981) in *in vitro* experiments. The incubation of human pancreatic juice with cellulose or xylan resulted in a substantial loss in the activities of all enzymes that were estimated. Similarly, incubation with wheat bran as well as with oat bran caused a decrease in the specific activities of α-amylase and chymotrypsin whereas the incubation with pectin increased ($P<0.05$) the specific activities of these enzymes. However, possible mechanisms underlying the regulatory effect of DF on enzyme activities under *in vitro* conditions are still unknown.

Potato fibre as a source of DF in diets for growing pigs was used in recent studies by Siljander-Rasi et al. (1998).The supplementation of a basal diet with 2% potato fibre reduced daily body weight gain (Siljander-Rasi et al., 1998). However, the influence of potato fibre on exocrine pancreatic secretions remains unclear and it can be speculated that potato fibre may stimulate the production of pancreatic enzymes thus facilitating digestion and subsequent absorption of nutrients; Botermans and Pierzynowski (1999) reported that an increase in body weight gain in growing pigs was positively correlated with an increase in exocrine pancreatic secretions.

The first objective of this study was to obtain further information on the effect of potato fibre in the diet of growing pigs on the exocrine pancreatic secretions. The second objective was to study the spontaneous response of the exocrine pancreas as influenced either by i.d. infusion of different dietary substrates including potato fibre or by i.v. infusion of gastrointestinal hormones, such as cholecystokinin (CCK) and secretin, that are known to stimulate exocrine pancreatic secretions .

4.3. Materials and Methods

4.3.1. Animals

A total of three 8 wk old piglets were obtained 4 wk after weaning from a Swedish Landrace herd (Odarslov's Research Farm, Swedish University of Agricultural Sciences, Lund). The average body weight (BW) was 12.4 kg at the time of surgery. The pigs were housed individually in pens under 12 h light : 12 h dark cycles (lights were on from 08.00 h to 20.00 h).

4.3.2. Surgical procedures

The pigs were surgically fitted with a chronic pancreatic duct catheter and a T-shaped duodenal cannula for collection and subsequent return of pancreatic juice into the duodenum according to Pierzynowski et al. (1988) and modified as described by Thaela et al. (1995). Additionally, a permanent jugular vein catheter for blood sampling was implanted according to procedures adapted from Pierzynowski et al. (1988).

4.3.3. Experimental procedures

The pigs were fed semi-ad libitum twice daily at 10.00h and 16.00h. Two different diets, a barley-based control diet (Växfor, Lantmännen, Stockholm, Sweden) and an experimental diet based on the control diet and supplemented with 2% potato fibre preparation (Povex^{TM}, Lyckeby Stärkelsen, Lyckeby, Sweden) were fed. The animals had free access to water. The chemical composition of the control diet and the potato fibre preparation is shown in Table 1 and 2, respectively. The chemical composition of the control diet was determined according to Naumann et al. (1976).

Table 1 Chemical composition of the control diet:

	Nutrients (g/kg DM)
Organic matter	937.0
Crude protein	177.8
Crude fat	52.1
Crude fibre	45.8
N- free extract	661.3
Starch	415.0
NDF	243.9
ADF	63.0
ADL	13.7

Table 2 Chemical composition of potato fibre (g/kg DM)[1]

Crude fibre	Cellulose	Pectin + hemicellulose	Lignin	Starch	Protein	Fat
700	**230**	**450**	**20**	**100**	**70**	**3**

[1]Data from Lyckeby Stärkelsen, Lyckeby, Sweden

After surgery, the pigs were allowed an 8-d recuperation period followed by three experimental periods, each lasting 14d. The experimental design is illustrated in Figure 1. The control diet was fed to all 3 pigs during the first and third experimental period whereas the experimental diet (control diet supplemented with potato fibre) was provided exclusively during the second period. Each of the experimental periods consisted of an 8-d adaptation period to the diet. Thereafter, within a period of 6 d, the secretory response of the exocrine pancreas to the i.d.. infusion of different dietary substrates and the i.v. infusion of a solution of two gastrointestinal hormones was studied. The infusates that were infused i.d. consisted of (1) 2% potato fibre and 98% saline (w/v), (2) 20% of the control diet and 80% saline (w/v) and (3) 20% of the experimental diet and 80% saline (w/v), which are referred to as the fibre, control diet and experimental diet infusates, respectively. In addition, a solution containing of 1 IDU (Ivy Dog Unit) CCK-33 (corresponding to 254 pmol CCK-33) and 1 CU (Clinical Unit) secretin (corresponding to 110 pmol secretin) dissolved in saline with 0.5% BSA (bovine serum albumin, Sigma, St. Louis, MO, US) was prepared which in the following is referred to as hormone infusate. According to Pierzynowski et al. (1999) the level of hormones in the infusate corresponds to physiological concentrations with the potential to stimulate the exocrine pancreas up to 50% of its capacity.

During the first and third experimental period the control diet, fibre and the hormone infusates were infused. However, during the second experimental period in which the experimental diet (with potato fibre) was fed, the control diet infusate was replaced by the experimental diet infusate. Within each experimental period the infusion treatments followed a randomised order with two repetitions.

The pigs received the last meal 17 h before the infusions started at 09.00h on d 9 to d 14 of each experimental period. The dietary infusates were infused i.d. over a period of 30 min at a rate of 5 ml/kg BW/h. Before the start and after the completion of these infusions pure saline was infused as control infusion at the same rate over a period of 60 min each (Figure 2). The hormonal infusate was infused i.v. over a period of 30 min and at a rate of 2 ml/kg BW/h. A control infusion containing saline with 0.5% BSA (Sigma, St. Louis, MO, US) was infused at the same rate over a period of 60 min before and 60 min after the hormonal infusion was received. The infusions were carried out by means of a syringe pump (Pompa Infuzyina Typ 340B, Unipan, Warsaw, Poland).

Pancreatic juice was collected quantitatively during both control infusion periods (2 x 60 min) and over a period of 30 min when the dietary and hormonal infusates were administered. It was collected by free drainage into a glass bottle at the right side of the animals attached to a belt allowing the animal to move freely during collections. The volume of secretion was recorded and the whole samples were stored at –20°C until analyses. Blood samples of 2 ml were taken 45, 90 and 150 min after the start of the control infusion. After the addition of 4 mmol EDTA and 1000 KIU (Kallikrein Inhibitor Units) Trasylol (Bayer, Leverkusen, Germany) as a proteinase-inhibitor, the blood samples were ice-chilled immediately and centrifuged at 4000 rpm. The plasma samples were stored at –20° C until analyses.

Figure 1 Experimental design (Experimental periods)

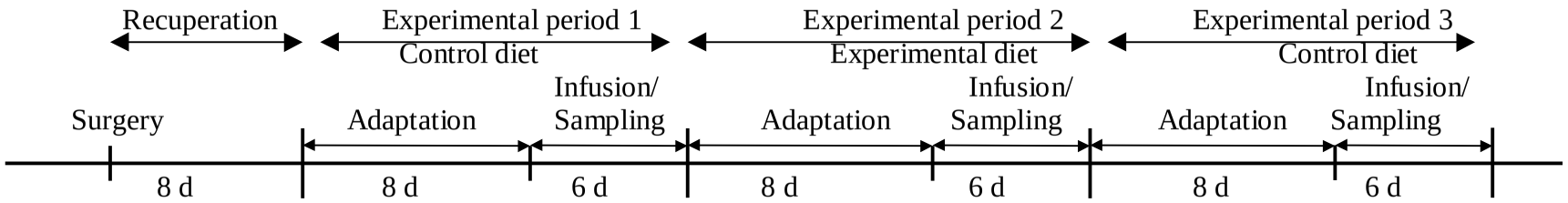
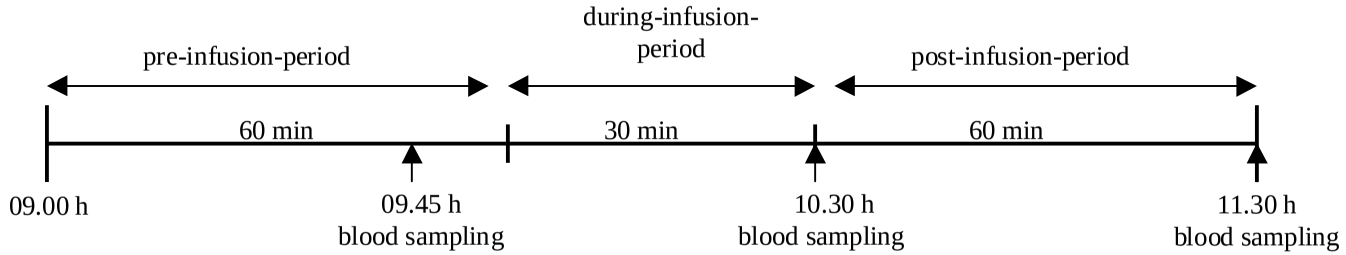


Figure 2 Experimental design (Infusions)



4.3.4. Chemical Analyses

Pancreatic juice samples were analysed for total protein content using the Lowry method (Lowry et al., 1951), performed on 96-well microwell plates, and BSA (Sigma, St. Louis, MO, USA) as a standard. Trypsin activities were estimated after enterokinase (Sigma, St Louis, MO, US) activation using N-α-benzoyl-DL-arginine-p-nitroanilide (Sigma, St Louis, MO, US) as a substrate (Pierzynowski et al, 1990). Lipase activities were determined by a pH-stat titration method using tributyrin as a substrate, as described by Borgström and Hildebrand (1975). Activities of α-amylase were determined by the method of Ceska et al. (1969) using the Phadebas amylase reagent as a substrate (Pharmacia Diagnostics, Uppsala, Sweden). One unit (U) of enzyme activity is defined as the amount of enzyme hydrolysing 1 µmol substrate per min. Total enzyme activities in pancreatic juice were expressed as U per 1 h of secretion per kg metabolic BW (U/h/$kg^{0.75}$). Blood samples were analysed for the plasma insulin levels using a radio-immuno-assay (RIA) with guinea pig antiporcine insulin (Milab, Malmö, Sweden), ^{125}I-labelled insulin and porcine insulin as a standard (Novo Novo Nordisk A/S, Bagsvaerd, Denmark) according to a method by Thaela et al. (1995). Plasma secretin concentrations were measured with a RIA according to Schaffalitzky de Muckadell and Fahrenkrug (1977). CCK levels in plasma were determined with a RIA according to Cantor and Rehfeld (1985). Plasma glucose levels were analysed by the glucose oxidase method described by Bruss and Black (1978).

4.3.5. Statistical analyses

Data were analysed using Statview software (1992, Abacus Concepts, Berkeley, CA, US). with 2-factorial ANOVA, Tukey range test (with experimental period in the model) and Student's t-test (with infusates in the model). The results were expressed as mean values + SEM.

4.4. Results

Pigs fed the diet containing potato fibre (experimental period 2) showed in tendency (P>0.1) an increase in the volume of secretion of pancreatic juice and a significant (P<0.05) increase both in the mean values of the total protein content and total activities of lipase, trypsin and α-amylase when compared to corresponding values in period 1 (Table 3). These values remained at that level after feeding the pigs the control diet in period 3 resulting in an 1.5-fold increase in the volume of secretion, although not significant, and in a 2.2-fold increase (P<0.05) in the total protein content compared to those values obtained in period 1. Similar differences were obtained for total trypsin and lipase activities which increased (P<0.05) 2.2-fold and 2.4-fold, respectively. No significant differences between period 2 and 3 were obtained for the volume of secretion, total protein content and total activities of trypsin and lipase. The total α-amylase activity increased (P<0.05) 2.8-fold from period 1 to period 2 and decreased (P<0.05) 1.9-fold in period 3 as compared to period 2. However, the total α-amylase activity in period 3 is still 1.5-fold higher (P<0.05) than in period 1.

Table 3 The influence of diet on the volume of secretion, protein secretion and total enzyme activities in pancreatic juice in experimental periods 1, 2 and 3

Experimental Period	1		2		3	
Diet	Control		Experimental		Control	
	Mean	SEM[1]	Mean	SEM	Mean	SEM
Volume, (ml/h/kg$^{0.75}$)	3.9	0.8	6.3	0.8	5.7	0.8
Protein, (mg/h/kg$^{0.75}$)	6.3^{a}	0.4	10.9^{b}	0.6	13.9^{b}	2.0
Trypsin, (U/h/kg$^{0.75}$)	4.6^{a}	0.3	8.6^{b}	0.8	10.0^{b}	1.4
Lipase, (U/h/kg$^{0.75}$)	1.2^{a}	0.3	2.6^{b}	0.4	2.9^{b}	0.3
α-amylase, (U/h/kg$^{0.75}$)	320^{a}	20	890^{b}	30	480^{c}	180

[1] Standard error of the mean within a experimental period

[a,b,c] Means in the same row not followed by the same superscript are significantly different (P<0.05)

As is shown in Table 4, the mean values for plasma insulin tended to be higher (P<0.1) in pigs adapted to the diet supplemented with potato fibre; there was a 2.1-fold increase compared to period 1 and 1.3-fold increase in comparison to period 3. The plasma glucose and secretin levels were not affected (P>0.1) by the different dietary treatments whereas the CCK levels decreased (P<0.05) following feeding of pigs with the control diet in period 3.

Table 4 The influence of diet on the plasma levels of insulin, glucose, secretin and cholecystokinin (CCK)

Experimental Period	1		2		3	
Diet	control		experimental		control	
	Mean	SEM[1]	Mean	SEM	Mean	SEM
Glucose, mmol, L	3.82	0.2	4.00	0.2	3.78	0.1
Insulin, pmol/l	8.3^{A}	2.0	17.3^{B}	1.0	13.6^{A}	2.0
Secretin, pmol/L	8.0	3.1	9.4	3.5	9.9	4.3
CCK, pmol/L	4.3^{a}	0.6	3.8^{ab}	0.5	2.9^{b}	2.9

[1] Standard error of the mean within a experimental period

^{A,B} means in the same row not followed by the same superscript are (P<0.1)

^{a,b} means in the same row not followed by the same superscript are different (P<0.05)

The time of infusion had a major effect on the volume of secretion of pancreatic juice in all three periods; the mean values of the infusates were higher (P<0.05) when measured during the period (30 min.) of infusion of the different infusates than during the pre- and post-infusion periods when the control infusions with saline were administered (Table 5). Moreover, the hormone infusate induced in period 1 during all three infusion periods a higher (P<0.05) volume of secretion of pancreatic juice as compared to the control diet infusate and also in comparison to the fibre infusate except for the pre-infusion period. However, during periods 2 and 3 this stimulatory effect of the hormone infusate was less pronounced and in most cases not significant (P>0.05).

In period 2 the mean values for total protein content in pancreatic juice were higher (P<0.05) during the period (30 min.) in which the different infusates were administered compared to the

pre- and post-infusion periods with saline as control; they were also higher ($P<0.05$) compared to the pre-infusion period in period 3 (Table 6).

In periods 1-3 total trypsin activities were numerically higher for all infusates compared to the control infusions in the pre- and post-infusion periods (Table 7). These differences were significant ($P<0.05$) for period 2. In addition, the control diet infusate induced in period 1 higher ($P<0.05$) total trypsin activities when determined during the period of infusion as compared to the pre- and post-infusion periods.

Total lipase activities were higher ($P<0.05$) in periods 2 and 3 for all infusates compared to the control infusions in the pre- and post-infusion periods (Table 8). Furthermore, the fibre infusate caused higher ($P<0.05$) total lipase activities in pancreatic juice as compared to the control diet in period 1.

As shown in Table 9, the mean values for total α-amylase activities in pancreatic juice were equal or higher during the period of infusion of the different infusates than during the pre- and post-infusion periods, the difference being significant ($P<0.05$) for period 2. Extremely low total α-amylase activities for the post- and pre-infusion period of the control diet infusate were obtained in periods 1 and 3, respectively. These differences were significant ($p<0.05$) compared to corresponding values for the fibre and hormone infusate obtained in the post-infusion period of period 1 and the pre-infusion period of period 3.

Table 5 The effect of time period of infusion of control diet, experimental diet, fibre or hormone infusate on the volume of secretion of pancreatic juice in pigs.

Experimental Period	1			2			3		
Infusion Period	Pre-	During-	Post-	Pre-	During-	Post-	Pre-	During-	Post-
Infusate									
Control diet[1]	2.1^{ab}	2.8^{b}	1.3^{a}				6.3^{cd}	5.0^{c}	6.3^{cd}
Experimental diet[1]				5.2^{bcd}	5.6^{cd}	4.1^{c}			
Fibre[1]	3.9^{bc}	3.8^{bc}	5.2^{c}	6.5^{cd}	7.9^{cd}	6.1^{d}	4.3^{ab}	6.2^{cd}	3.1^{bc}
Hormone[1]	4.5^{c}	8.5^{d}	3.6^{b}	5.6^{bcd}	10.8^{de}	4.6^{abc}	5.2^{c}	10.3^{e}	4.6^{c}
Mean[2]	3.5^{a}	5.0^{bc}	3.4^{a}	5.8^{bc}	8.1^{e}	4.9^{bc}	5.3^{bc}	7.1^{de}	4.7^{bc}
SEM[3]	0.7	1.0	0.6	0.4	0.9	0.3	0.6	0.9	0.6

[1] Mean values for pre-infusion, during-infusion and post-infusion periods (ml/h/kg$^{0.75}$) within an infusion period
[2] Mean values of control diet, experimental diet, fibre and hormone infusates (ml/h/kg$^{0.75}$) within an infusion period
[3] Standard error of the mean within an infusion period
[a,b,c,d,e] Means in the same row or in the same column not followed by the same superscript are different ($P<0.05$)

Table 6 The effect of time period of infusion of control diet, experimental diet, fibre or hormone infusate on total protein content of pancreatic juice in pigs.

Experimental Period	1			2			3		
Infusion Period	Pre-	During-	Post-	Pre-	During-	Post-	Pre-	During-	Post-
Infusate									
Control diet[1]	5.1 ^{ab}	8.7 ^{bc}	3.5 ^{a}				14.9 ^{cd}	15.0 ^{cd}	21.6 ^{e}
Experimental diet[1]				7.9 ^{bc}	14.3 ^{cd}	10.1 ^{c}			
Fibre[1]	8.3 ^{bc}	5.6 ^{ab}	6.7 ^{b}	10.0 ^{c}	14.8 ^{cd}	10.5 ^{c}	9.8 ^{bc}	17.2 ^{de}	9.0 ^{bc}
Hormone[1]	6.3 ^{b}	7.8 ^{b}	4.6 ^{a}	8.8 ^{bc}	14.9 ^{cd}	6.6 ^{bc}	11.5 ^{cd}	17.2 ^{de}	9.1 ^{bc}
Mean[2]	6.6 ^{a}	7.4 ^{a}	4.9 ^{a}	8.9 ^{b}	14.7 ^{c}	9.1 ^{b}	12.1 ^{c}	16.4 ^{d}	13.3 ^{cd}
SEM[3]	0.9	0.6	0.6	0.6	0.1	0.7	1.5	0.4	2.4

[1] Mean values for pre-infusion, during-infusion and post-infusion periods (mg/h/kg$^{0.75}$) within an infusion period
[2] Mean values of control diet, experimental diet, fibre and hormone infusates (mg/h/kg$^{0.75}$) within an infusion period
[3] Standard error of the mean within an infusion period
[a,b,c,d,e] Means in the same row or in the same column not followed by the same superscript are different ($P<0.05$)

Table 7 The effect of time period of infusion of control diet, experimental diet, fibre or hormone infusate on total trypsin activity of pancreatic juice in pigs.

Experimental Period	1			2			3		
Infusion Period	Pre-	During-	Post-	Pre-	During-	Post-	Pre-	During-	Post-
Infusate									
Control diet[1]	4.1^{ab}	7.5^{c}	2.9^{a}				10.5^{cd}	10.3^{cd}	15.6^{e}
Experimental diet				5.9^{abc}	10.8^{cd}	7.3^{bcd}			
Fibre[1]	5.9^{bc}	4.5^{ab}	5.0^{b}	8.9^{bcd}	12.5^{cd}	8.7^{cd}	7.0^{cd}	14.7^{de}	6.8^{b}
Hormone[1]	4.2^{ab}	5.7^{bc}	2.6^{a}	7.7^{bcd}	11.0^{cd}	5.2^{abc}	7.3^{bcd}	11.7^{cd}	5.8^{bc}
Mean[2]	4.7^{b}	5.9^{b}	3.5^{a}	7.5^{c}	11.4^{d}	7.1^{c}	8.3^{cd}	12.2^{d}	9.4^{cd}
SEM[3]	0.6	0.5	0.5	0.9	0.3	0.7	1.1	0.8	2.0

[1] Mean values for pre-infusion, during-infusion and post-infusion periods (U/h/kg$^{0.75}$) within an infusion period
[2] Mean values of control diet, experimental diet, fibre and hormone infusates (U/h/kg$^{0.75}$) within an infusion period
[3] Standard error of the mean within an infusion period
[a,b,c,d,e] Means in the same row or in the same column not followed by the same superscript are different ($P<0.05$)

Table 8 The effect of time period of infusion of control diet, experimental diet, fibre or hormone infusate on total lipase activity of pancreatic juice in pigs.

Experimental Period	1			2			3		
Infusion Period	Pre-	During-	Post-	Pre-	During-	Post-	Pre-	During-	Post-
Infusate									
Control diet[1]	0.7^{a}	0.4^{a}	0.2^{a}				3.2^{bd}	2.8^{bd}	3.4^{bd}
Experimental diet[1]				2.5^{b}	2.2^{b}	1.2^{ab}			
Fibre[1]	2.1^{b}	1.7^{b}	1.4^{b}	3.1^{bc}	3.8^{bc}	3.1^{bc}	1.5^{b}	3.7^{bc}	1.8^{b}
Hormone[1]	1.1^{ab}	1.5^{ab}	1.1^{ab}	2.2^{b}	3.2^{bc}	1.7^{ab}	3.0^{bd}	4.7^{c}	1.9^{b}
Mean[2]	1.3^{a}	1.3^{a}	0.9^{a}	2.6^{b}	3.1^{c}	2.0^{b}	2.6^{b}	3.7^{c}	2.4^{b}
SEM[3]	0.4	0.2	0.2	0.3	0.3	0.3	0.5	0.3	0.3

[1] Mean values for pre-infusion, during-infusion and post-infusion periods ($U/h/kg^{0.75}$) within an infusion period
[2] Mean values of control diet, experimental diet, fibre and hormone infusates ($U/h/kg^{0.75}$) within an infusion period
[3] Standard error of the mean within an infusion period
[a,b,c,d] Means in the same row or in the same column not followed by the same superscript are different ($P<0.05$)

Table 9 The effect of time period of infusion of control diet, experimental diet, fibre or hormone infusate on the total α-amylase activity of pancreatic juice in pigs.

Experimental Period	1			2			3		
Infusion Period	Pre-	During-	Post-	Pre-	During-	Post-	Pre-	During-	Post-
Infusate									
Control diet[1]	450^{bc}	460^{bc}	80^{a}				70^{a}	170^{ab}	150^{b}
Experimental diet[1]				670^{bcd}	1470^{cd}	560^{bc}			
Fibre[1]	590^{bc}	290^{b}	340^{bc}	920^{cd}	1080^{cd}	810^{cd}	450^{bc}	950 ^{bc}	440^{bc}
Hormone[1]	280^{b}	560^{bc}	240^{b}	820^{cd}	1170^{cd}	490^{bc}	420^{bc}	1260^{cd}	450^{bc}
Mean[2]	440^{b}	440^{b}	220^{a}	800^{c}	1240^{d}	620^{b}	310^{ab}	800^{bc}	350^{ab}
SEM[3]	90	50	50	70	100	50	120	200	70

[1] Mean values for pre-infusion, during-infusion and post-infusion periods (U/h/kg$^{0.75}$) within an infusion period
[2] Mean values of control diet, experimental diet, fibre and hormone infusates (U/h/kg$^{0.75}$) within an infusion period
[3] Standard error of the mean within an infusion period
[a,b,c,d] Means in the same row or in the same column not followed by the same superscript are different ($P<0.05$)

4.5. Discussion

It has been shown that the principal effect of dietary fibre on the exocrine pancreas of pigs is an increase in the volume of secretion (Zebrowska and Low, 1987, Mosenthin and Sauer, 1991). The results of the present study confirm that native potato fibre stimulates the secretion of pancreatic juice when the pigs were changed from the control diet in period 1 to the experimental diet in period 2. These results support findings by Mosenthin et al. (1994) who also reported a higher secretion of pancreatic juice when pectin as highly viscous fibre source was fed to growing pigs. Moreover, the feeding of the control diet without potato fibre resulted in a significant decrease in α-amylase activity in period 3 compared to period 2. However, the volume of secretion, the total protein content as well as the total activities of trypsin and lipase remained at the same level when the pigs were switched back from the experimental diet in period 2 to the control diet in period 3. As the volume of secretion in growing pigs increases with age (Makkink, 1993) it can be speculated if the higher volume of secretion in period 3 compared to period 1 is related to an increase in BW and/or age of the pigs.

In general, an uniform pattern both in the secretion of pancreatic juice, and the total output of protein and enzyme activities (trypsin, lipase, α-amylase) was obtained when either different substrates (control diet infusate, experimental diet infusate, fibre infusate) were administered into the duodenum or when gastrointestinal hormones such as CCK and secretin were infused i.v. These infusates stimulated the exocrine pancreas by inducing a spontaneous secretory response of the pancreas during the time period of infusion. Consequently, the volume of secretion, the total output of protein, trypsin, lipase and α-amylase were consistently and in most cases lower ($P<0.05$) in the pre- and post-infusion periods than the corresponding values determined during the infusion of the different infusates. This spontaneous response to the infusion treatments corresponds to the immediate postprandial response after feeding as reported by Thaela et al. (1995).

It can be further derived from the results of this study that the presence of substrates in the duodenum *per se* has a much more pronounced effect on the pattern of secretion of the exocrine pancreas than the source of substrates itself. The time period when the different infusates were infused was uniformingly characterised by an increased secretion of pancreatic

juice, protein and enzymes, irrespective of the source of substrate (control diet infusate, experimental diet infusate, fibre infusate) administered. These results could indicate that during this phase the acinar (producing enzymes) and ductal (producing fluid) cells of the pancreas were stimulated, probably hormonally via CCK and secretin (Pierzynowski et al., 1999) and neurally via the vagus nerve (Solomon, 1987). Considering the increased secretion of pancreatic juice, protein and enzymes during the i.v. infusion of CCK and secretin, it can be concluded that the secretory response of the pancreas to the infusion of different substrates is controlled via feed back mechanisms mediated by the plasma levels of those gastrointestinal hormones (Owyang, 1994) that are involved in the stimulation of the acinar and ductal cells of the pancreas.

According to Botermans and Pierzynowski (1999) higher exocrine pancreatic secretions are positively correlated to daily weight gain. If under the experimental conditions described herein the positive response of the exocrine pancreas to potato fibre supplementation will have a similar effect needs to be verified.

It is likely possible that potato fibre affects the microbial activity of the large intestine and, in consequence, the production of short chain fatty acids (SCFA). Kato et al. (1989) and Mineo et al. (1990) could show that the i.v. infusion of SCFA stimulated both the exocrine and endocrine pancreas in ruminants. In pigs, SCFA are involved in the regulation of stomach emptying (Malbert et al., 1994). It can be speculated if the stimulating effect of potato fibre on the pancreas could also be attributed, at least in part, to the production of SCFA in the large intestine. Moreover, SCFA are potent stimulators of insulin release in ruminants (Manns and Boda, 1967). Therefore, a stimulation of the exocrine pancreas via a well described insulin-pancreatic acinar axis is possible (Williams and Goldfine, 1985, Pierzynowski, 1990). Moreover, the plasma levels of CCK were lower ($p<0.05$) in pigs adapted to the experimental diet in period 2 and also in pigs fed the control diet again in period 3. It can be derived from these result that an increase in enzyme secretion as observed in these periods is not necessarily associated with a higher CCK level in plasma. A possible stimulating effect of SCFA on the interdigestive, postprandial and gut hormone stimulated pancreatic secretion in pigs warrants further investigations.

4.6. Conclusions

The secretory response of the exocrine pancreas can be stimulated by the presence of potato fibre in the diet. Moreover, a spontaneous secretory response of the pancreas following the i.d. infusion of different dietary substrates and the i.v. infusion of CCK and secretin resulted in higher levels of volume of secretion, protein and enzymes in pancreatic juice. Obviously, higher enzyme activities are not necessarily associated with higher CCK levels in plasma; a possible stimulating effect of SCFA on the exocrine pancreas warrants further investigation.

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5. FATS INFUSED INTRADUODENALLY AFFECT THE POSTPRANDIAL SECRETION OF THE EXOCRINE PANCREAS AND THE PLASMA LEVELS OF GASTROINTESTINAL HORMONES IN GROWING PIGS

5.1. Summary

In pigs, the spontaneous secretion of the exocrine pancreas and the release of cholecystokinin (CCK) and peptide YY (PYY) in response to the intraduodenal infusion of fully saturated synthetic fats differing in chain has not been studied yet under prandial conditions. Six growing pigs (BW 13.6 kg) were surgically prepared with pancreatic duct catheters and duodenal re-entrant T-cannulas. Blood samples were obtained by means of a catheter placed in the external jugular vein. The animals were fed twice daily at a rate of 2% of BW. Beginning with the morning feeding, a medium chain triglyceride (mct: glyceroltricaprylat), a long chain triglyceride (lct: glyceroltristearat) or saline as a control was infused intraduodenally at a rate of 0.1% of BW over a period of 1 h according to a 3 x 2 latin square design. Pancreatic juice was collected halfhourly over a period of 4 h, beginning 1 h preprandially (0900 h) till 3 h postprandially (1300 h); blood samples were obtained 15 min preprandially and 15, 45, 90 and 150 min postprandially. The infusion of mct evoked a change in the trend of the curve for the volume of secretion of pancreatic juice. Moreover, both lct and mct infusions induced a change in the trends of the curves for protein and trypsin output. Lipase and colipase contents and outputs were influenced by mct infusions. There were no changes in the trends of the curves for CCK and PYY levels. A difference between the trends of the curves for the saline and mct treatment was observed for the volume of secretion, protein output, lipase content and output, trypsin and colipase output in pancreatic juice. Moreover, a difference in the trends of the curves between mct and lct was obtained for the outputs of protein, lipase and colipase. Plasma CCK levels were decreased in the mct treatment as compared to the saline and lct treatment. The results implicate an immediate, distinguished response of the exocrine pancreas towards fats different in chain length.

5.2. Introduction

The secretions of the exocrine pancreas are required for hydrolysis of nutrients present in food and feed (Rinderknecht, 1993). Enzyme secretion is highly dependent on diet composition, age and feeding regimen (Corring et al., 1989. This is well described for different species including the rat (Bucko and Kopec, 1968; Gidez, 1973), dog (Behrmann and Kare, 1969) and the pig (Corring, 1980; Makkink and Verstegen, 1990; Ozimek et al., 1995). For example, Mourot and Corring (1979) observed with increasing levels of fat in the diet higher lipase contents in porcine pancreatic tissue. Deschodt Lanckman et al. (1971) and Ricketts and Brannon (1994), in rats, showed an increased lipase contents when polyunsaturated fatty acids were included in the diet. Simoes Nunes (1986) fed diets to pigs containing either 21% sunflower oil or lard. The author showed that sunflower oil evoked a higher ($P < 0.05$) lipase content than lard. In studies by Gabert et al. (1996) pigs were prepared with a pancreatic duct catheter and adapted to diets containing either coconut oil, rapeseed oil or fish oil. The authors found a higher ($P < 0.05$) chymotrypsin secretion in pancreatic juice of pigs fed a diet supplemented with coconut oil, and higher ($P < 0.05$) secretion of carboxylester hydrolase in pigs fed a diet containing fish oil.

The gastrointestinal hormones peptide YY (PYY) and cholecystokin (CCK) are considered to be major regulative hormones of the exocrine pancreas. Several authors could show in dogs that fat is stimulating the release of PYY (Aponte et al., 1985; Pappas et al., 1985; Lluis et al., 1989) and the release of CCK in dogs (Shiratori et al., 1989) and cats (Backus et al., 1995) as well. The effect of dietary fat on plasma CCK levels in pigs is discussed equivocally as Corring and Chayvialle (1987) could not observe any effect whereas Cuber et al. (1990) reported a stimulatory effect of fats. Moreover, Yago et al. (1997a) demonstrated in humans that not only the quantity of fat consumed but also the composition of dietary fat influenced plasma CCK and PYY levels. A diet based on olive oil with a higher degree of saturation than sunflower oil evoked higher hormone levels compared to the diet supplemented with sunflower oil.

In most studies dietary changes exhibited a fast response in enzyme adaptation and the secretion of gastrointestinal hormones which is completed within one week (Bucko and Kopec, 1968; Deschodt Lanckman et al., 1971; Corring, 1980). There is evidence for the

existence of such a mechanism in rats (Bucko and Kopec, 1968; Deschodt Lanckman et al., 1971), pigs (Corring and Chayvialle, 1987; Hee et al., 1988) and dogs (Yago et al., 1997b).

However, there is still a scarcity of information on the existence of a spontaneous adaptation of the exocrine pancreas when fully saturated fats different in chain length are fed to pigs. The objectives of the present study were to examine the effect of purified fat sources, namely glyceroltricaprylate (C 8:0) and glyceroltristearate (C 18:0), (1) on the spontaneous exocrine pancreatic secretion in pigs and (2) on plasma levels of the gastrointestinal hormones CCK and PYY.

5.3. Materials and Methods

5.3.1. Animals

The studies were carried out with six piglets (Swedish Landrace x (Yorkshire x Hampshire)) obtained from a production herd (Odarslöv's Research Farm, Swedish University of Agricultural Sciences, Lund) with an average BW of 13.6 kg at the beginning of the experiment. The pigs were housed individually and freely moving in pens (1 x 2 m), had free access to water and were kept under 12 h light / 12 h dark cycles (light on from 08.00 h to 20.00 h). Treatments and experiments were conducted according to the European Community regulations concerning the protection of experimental animals and Lunds University Ethical Committee Allowance.

5.3.2. Surgical procedures

The pigs were sedated with azaperone (Stresnil, Janssen Pharmaceutica, Beerse, Belgium; 2 mg/kg BW) and anesthetised with Halothane (ISC Chemicals Ltd., UK; 3% air). Surgery was performed under aseptic conditions. The pigs were surgically fitted with a chronic pancreatic duct catheter and a T-shaped duodenal cannula for collection and subsequent return of pancreatic juice into the duodenum according to Pierzynowski et al. (1988) and modified as described by Thaela et al. (1995). Additionally, a catheter for blood sampling was implanted into the *vena jugularis* according to Pierzynowski et al. (1988).

5.3.3. Experimental procedures

The piglets were fed twice daily (1000h and 1600h) a barley-based starter diet with 17.7% crude protein and 5.2% crude fat (Växfor, Lantmännen, Stockholm, Sweden) at a rate of 2% of BW. After a post-surgical recuperation period of 7 d, beginning with the morning feeding (10.00h), a medium chain triglyceride (mct: glyceroltricaprylat, Fluka, Deisenhofen, Germany) or a long chain triglyceride (lct: glyceroltristearat, Fluka, Deisenhofen, Germany) or saline as a control was infused directly into the duodenum of the piglets via the duodenal T-cannula. The daily amount of fat infused into the duodenum amounted for 0.1% of BW which corresponds to 5% fat supplementation to the diet. The fats were filled into syringes and saline was added to a final volume of 36 mL. They were kept under heating lamps at body temperature and were emulsified by means of vigorous shaking just before the infusions started. The fat treatments and the control infusion with saline (36 mL) were administered in small boluses of 3 mL per bolus every 5 min over a period of 1 h (1000 h to 1100 h). The fats were provided according to a 3 x 2 Latin square design; the fatty acid composition of the fats infused is shown in Table 1:

TABLE 1 Fatty acid composition of mct[1] and lct[2]

Fatty acid	Carbon	% Fatty acid	
		mct	lct
Caprylic	**C 8:0**	**100**	**0.15**
Capric	C 10:0		0.17
Lauric	C 12:0		1.32
Myristic	C 14:0		3.61
Palmitic	**C 16:0**		**26.29**
Stearic	**C 18:0**		**61.61**
Oleic	C 18:1		0.44
Arachidic	C 20:0		1.84

Pancreatic juice was collected over a period of 4 h, beginning 1 h preprandially (0900 h) and lasting for 3 h postprandially (1300 h). The volume of secretion was recorded in 30 min intervals, an aliquot (1 mL) was obtained for analyses and immediately stored at –20°C. The

[1] mct = medium chain triglyceride (glyceroltricaprylate)
[2] lct = long chain triglyceride (glyceroltristearat)

remainder was re-infused into the duodenum via the duodenal cannula in small doses every 5 min over a period of 30 min at a level that corresponded to the rate of secretion of pancreatic juice.

Additionally, blood samples of 5 mL were obtained 15 min pre- and 15, 45, 90 and 150 min postprandially. The samples were taken by means of syringes containing 4 mmol EDTA and 1000 KIU (Kallikrein Inhibitor Unit) Trasylol (Bayer, Leverkusen, Germany) as a proteinase-inhibitor. The blood samples were immediately ice-chilled and centrifuged at 4000 rpm. The plasma obtained was stored at –20°C until analyses.

5.3.4. Analytical procedures

The fats infused were analysed for their fatty acid composition by means of a GLC-procedure according to Naumann et al. (1976). Pancreatic juice samples were analysed for protein using the Lowry method (Lowry et al., 1951), performed on 96-well microwell plates, and using bovine serum albumine (BSA, Sigma, St. Louis, MO, US) as a standard. Intra- and interassay CV for the protein determination were 3.1 and 3.6%, respectively. Trypsin (EC 3.4.21.4) activities were estimated after enterokinase (Sigma, St Louis, MO, US) activation using N-α-benzoyl-DL-arginine-p-nitroanilide (Sigma, St Louis, MO, US) as a substrate (Pierzynowski et al., 1990). Intra- and interassay CV for the trypsin determination were 2.8 and 3.2%, respectively. Lipase (EC 3.1.1.3) activities were determined by a pH-stat titration method using tributyrin as a substrate, as described by Borgström and Hildebrand (1975). Interassay CV for the lipase activity was 4.2%. One unit (U) of enzyme activity is defined as the amount of enzyme hydrolysing 1 µmol substrate per min. A competitive ELISA was used for measuring pancreatic colipase. The estimation was adapted to a procedure described for measuring enterostatin (Mei et al., 1993). Antiserum was obtained by immunising a rabbit (3BI-16) with porcine procolipase (purified from porcine pancreas according to the method of Erlanson et al. (1973)). Ninety-six-well microtiter plates were coated over night with 0,2µg/mL procolipase (purified, (Erlanson et al., 1973)). The antibody against procolipase was diluted 1:5000, the secondary biotin conjugated antibody (Sigma, St Louis, MO, US) was diluted 1:6000 and the streptavidin-alkaline phosphatase (Sigma, St Louis, MO, US) was diluted 1:6000. The plate was developed by the addition of p-nitrophenyl phosphate (Sigma, St Louis, MO, US) and a standard curve ranging from 500 µg/mL to 0.7 µg/mL was used in

this assay. A RIA kit was used for the determination of plasma CCK levels (Eurodiagnostica, Malmö, Sweden) and for plasma PYY levels (Peninsula Lab., St. Helens, UK). The methods recommended by the manufacturers were used except for minor modifications for the PYY estimation: Before extracting the peptides out of the sample solutions, the recommended Sep-Pak C_{18} (Sep-Pak Vac 3cc, Waters, Milford, MA, US) cartridges were pretreated with 100% acetonitrile (Merck, Darmstadt, Germany). After conditioning the columns, they were loaded with 2 mL sample solution. Intraassay CV and recovery were 16% and 80% for CCK and 14% and 67% for PYY, respectively.

Protein contents in pancreatic juice were expressed as mg per mL (mg/mL), protein outputs were expressed as mg per 1 h of secretion per kg BW (mg/(h•kg)). Enzyme contents in pancreatic juice were expressed as U per mL (U/mL), enzyme outputs were expressed as U per 1 h of secretion per kg BW (U/(h•kg)). Colipase contents in pancreatic juice were expressed as µg per mL (µg/mL), colipase outputs were expressed as µg per 1 h of secretion per kg BW (µg/(h•kg)). Plasma CCK levels were expressed as pmol per L (pmol/L), plasma PYY levels were expressed as pg per mL (pg/mL).

5.3.5. Statistical analyses

Data were analysed with Statview software (vers. 4.57, Abacus Concepts, Ca, USA) using repeated measures ANOVA with time, treatment and time x treatment interaction in the model. Post Scheffe's test was performed to compare treatment means of the pooled data. The results were expressed as mean ± SEM (standard error of mean). The level of significance was set at 5% ($P < 0.05$).

5.4. Results

The pigs recovered well from surgery and started to gain BW (300 to 450 g/d) 3 to 5 d postsurgically. The pigs remained clinically healthy and consumed their meal allowances of the diet within 10 to 15 min. Postmortem examinations after the experiment revealed no intestinal adhesions or other abnormalities.

As illustrated in Figure 1, the infusion of mct evoked a postprandial decrease in the volume of secretion from 2.6 mL/(h•kg) (30min postprandially) to a value of 0.25 mL/(h•kg) (2 h postprandially). There was a minor increase 4 h postprandially which amounted to 1.3 mL/(h•kg). As a result, for the volume of secretion, the trend of the curve for the mct treatment changed ($P < 0.01$) whereas no change ($P > 0.2$) for the lct and saline infusion was obtained. A difference ($P < 0.02$) between the diurnal patterns of the curves for the mct and saline treatment was observed, whereas the curves for mct and lct treatment showed in tendency ($P < 0.10$) different trends of the curves. No difference ($P > 0.3$) between the trends of the curves for saline and lct infusions was obtained.

The protein content in pancreatic juice showed non-directional trends of the curves for all three treatments. The diurnal patterns did not change ($P > 0.2$) for these treatments and no differences ($P > 0.4$) between the trends of the curves were observed (Figure 2a). However, based on protein output in pancreatic juice, mct infusions induced a change ($P < 0.01$) in the trend of the curves whereas the control infusion with saline and the lct treatment did not evoke ($P > 0.2$) a change in the diurnal patterns (Figure 2b). The trend of the curve for the mct infusions differed ($P < 0.01$) from the trend of the curve for the lct infusion, as both curves showed 30 min after feed consumption a prandial increase in the protein output from 6.2 to a value of 15.9 mg/(h•kg) for the mct treatment and from 4.8 to 8.2 mg/(h•kg) for the lct treatment. Values for the saline and the lct treatment remained at levels of 8 to 10 mg/(h•kg) up to 4h postprandially, whereas protein outputs for the mct treatment decreased 2 to 3h postprandially under preprandial levels resulting in 1.35 mg/(h•kg). The protein output of the mct treatment recovered to preprandial values 4 h postprandially.

As illustrated in Figure 3a, the diurnal patterns of trypsin contents did not change ($P > 0.1$) for the saline and mct infusions, whereas the lct infusions evoked a change in the trend of the curve ($P < 0.01$). However, no differences ($P > 0.05$) between the trends of the curves were observed. A prandial increase in the outputs of trypsin was obtained for all three treatments. However, the trend of the curve for the mct treatment decreased under preprandial values (from 3.78 to 0.99 U/(h•kg)) 90 min postrandially and recovered to preprandial values 4 h postprandially (Figure 3b). The diurnal patterns of the mct ($P < 0.01$) and lct ($P < 0.03$) infusions changed whereas the control infusion with saline did not have any effect ($P > 0.4$) on trypsin output. Moreover, the trends of the curves for the trypsin outputs differed between

lct and mct treatments as well as between the saline and the mct treatments ($P < 0.01$). No difference ($P > 0.2$) between the diurnal patterns of the saline and lct infusion was obtained.

Lipase contents showed a non-directional trend of the curves for saline and mct infusions ($P > 0.2$), whereas the lct infusions evoked a change ($P < 0.05$) of the trend of the curve; a prandial peak (2.5-fold increase compared to preprandial value) 30 min postprandially was observed (Figure 4a). The diurnal patterns of lct and mct infusions differed ($P < 0.02$) from each other, whereas no difference ($P > 0.1$) was found between the saline and lct as well as between saline and mct treatment. The trend of the curve for lipase outputs did not change ($P > 0.4$) for the saline treatment, whereas there was a tendency towards a change ($P < 0.06$) in the trend of the curve for the mct treatment and a highly significant ($P < 0.01$) change in the diurnal pattern of the lct infusion (Figure 4b). Lipase outputs increased threefold and 2.6-fold for the lct and for the mct treatment, respectively, 30 min postprandially compared to preprandial values. Whilst lipase outputs decreased under preprandial values for the mct infusion, lipase outputs for the lct treatment remained on elevated levels ranging from 2.5- to threefold over preprandial values. The trends of the curves between mct and lct treatments were different ($P < 0.03$) whereas no difference between saline and mct ($P > 0.2$) as well as between saline and lct treatments ($P > 0.3$) were observed.

The infusion of mct evoked a change ($P < 0.05$) in the trend of the curve for the colipase content in pancreatic juice and in tendency ($P < 0.09$) there was also a change in the trend of the curve for the lct treatment. No change ($P > 0.5$) was obtained for the saline treatment.(Figure 5a). A postprandial increase of the colipase content was observed for both the mct and lct treatment, peaking 1 h postprandially at 56.3 U/µl and 58.7 U/µl, respectively. No differences ($P < 0.2$) between the trends of the curves were obtained. The diurnal patterns of the colipase output changed ($P < 0.01$) for the mct infusion whereas the infusion of saline and lct did not have any effect ($P > 0.1$). Colipase output increased after infusion of lct fourfold compared to preprandial values and remained for 2.5 h on this elevated level. The trends of the curves differed ($P < 0.01$) between the saline and mct treatment as well as between the mct and lct treatment, whereas no difference ($P > 0.7$) between saline and lct infusions was found (Figure 5b).

As illustrated in Figure 6a, plasma CCK levels for saline and lct treatment did not show a change ($P > 0.2$) in their diurnal patterns during the experiment, however, there was a trend (P

< 0.08) for a change in the mct treatment. Although the curves do not differ (P > 0.2) from each other, the comparison of treatment means showed that the mct treatment resulted in 35% and 40% lower (P < 0.01) plasma CCK levels compared to the saline and lct treatment, respectively.

The trends of the curves for the plasma PYY levels did not change (P > 0.5) for all three treatments during the experimental period. Moreover, no differences (P > 0.6) were observed between the diurnal patterns of all three treatments; PYY levels remained at constant values of approx. 25 pg/mL (Figure 6b).

FIGURE 1 The diurnal pattern of volume of secretion of pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM

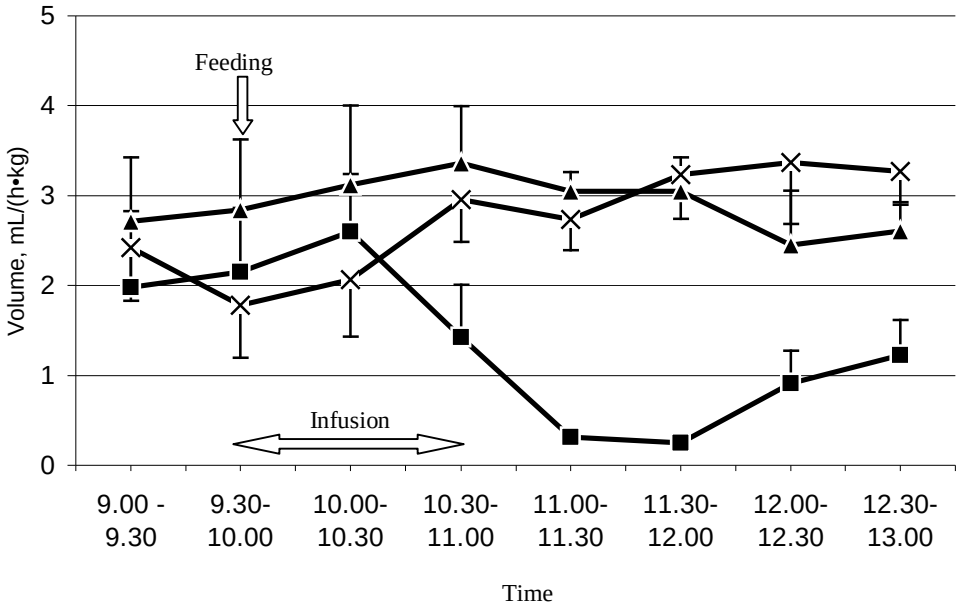


FIGURE 2a The diurnal pattern of protein content in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM

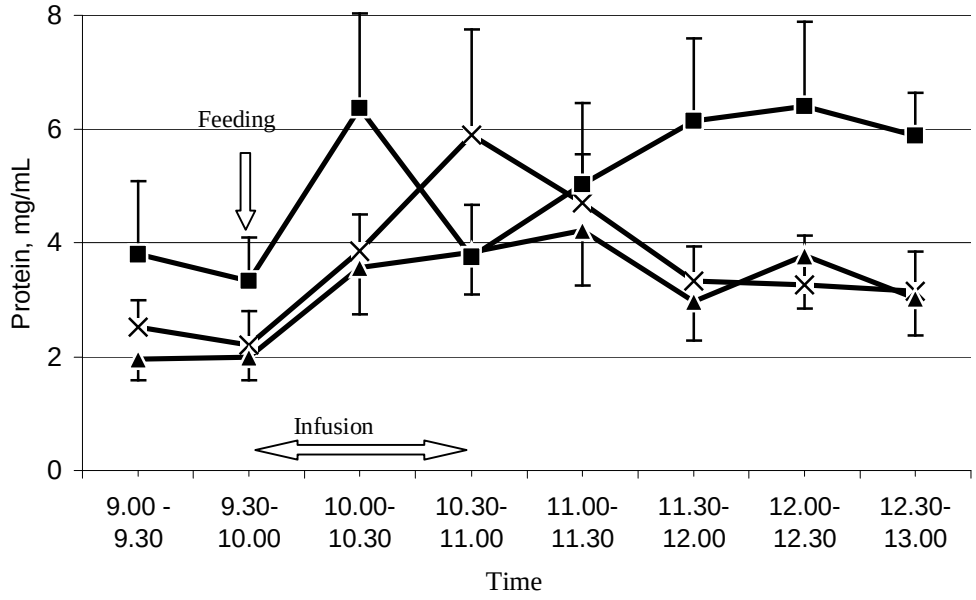


FIGURE 2b The diurnal pattern of protein output in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM

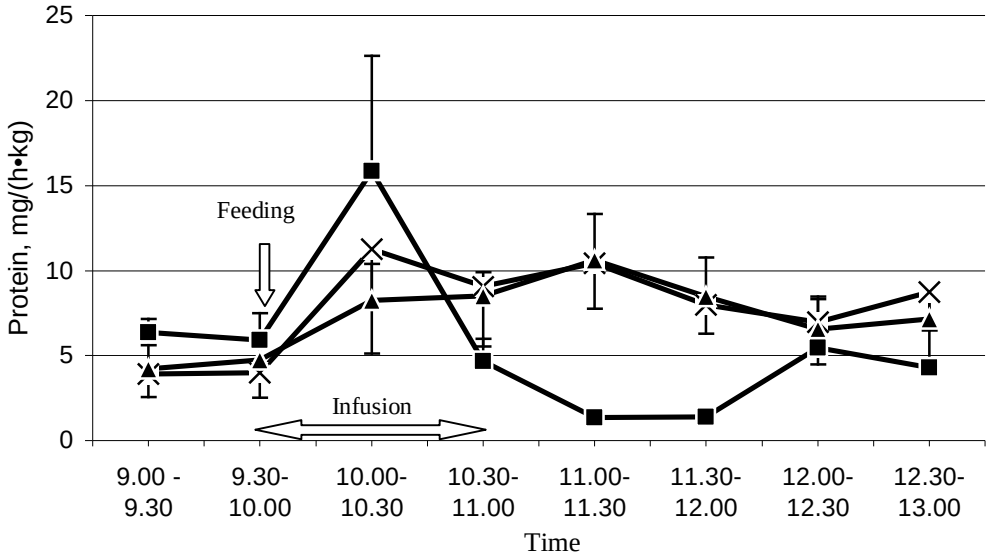


FIGURE 3a The diurnal pattern of trypsin content in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM.

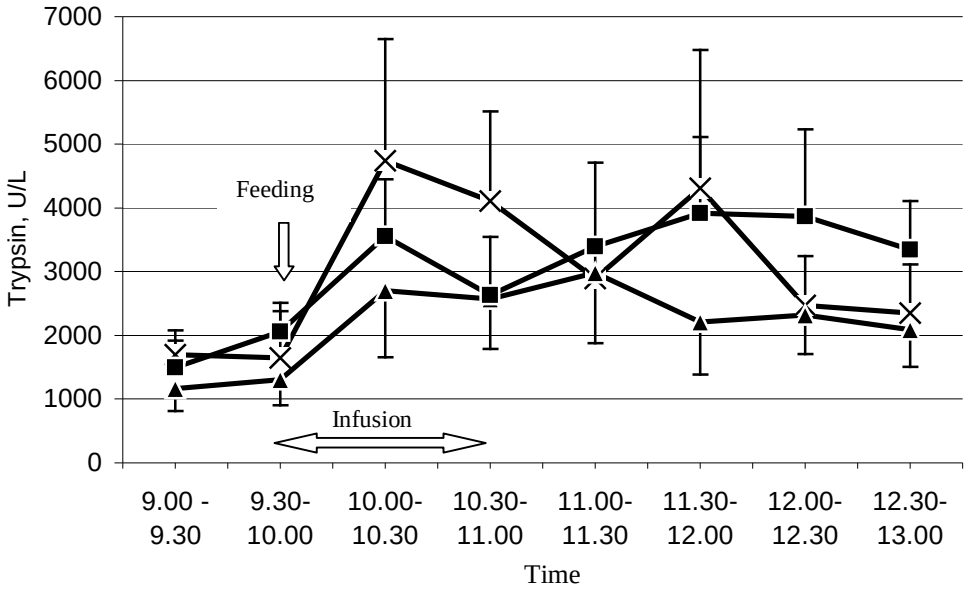


FIGURE 3b The diurnal pattern of trypsin output in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM

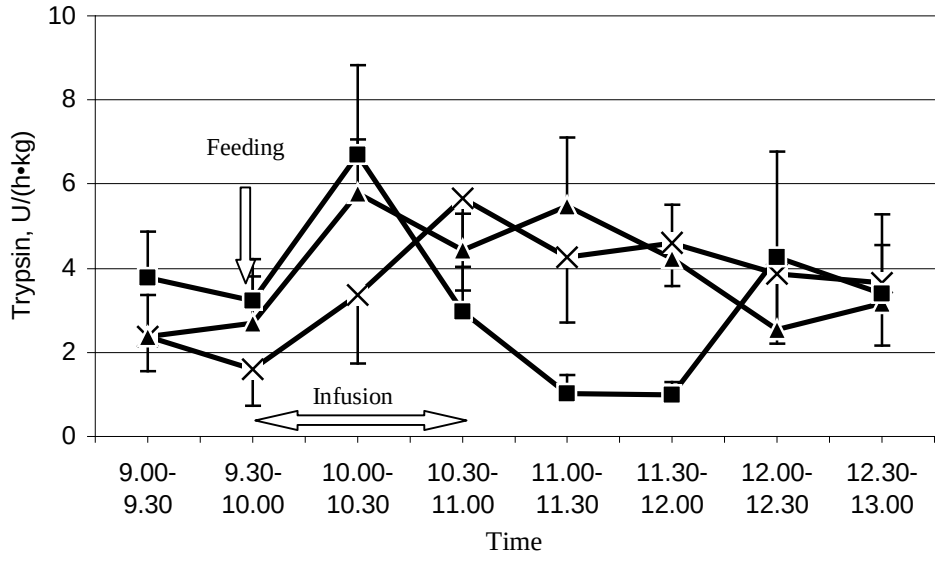


FIGURE 4a The diurnal pattern of lipase content in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM.

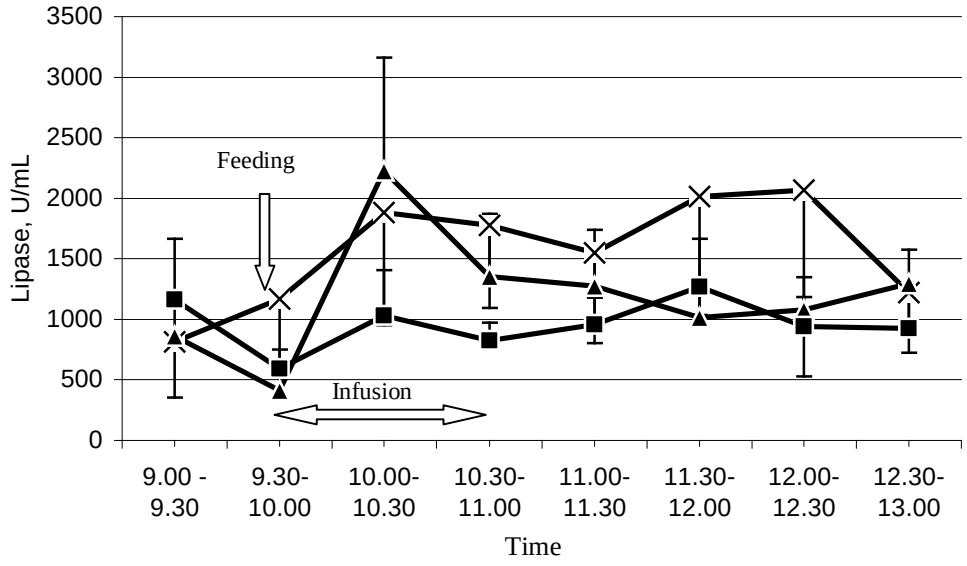


FIGURE 4b The diurnal pattern of lipase output in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM.

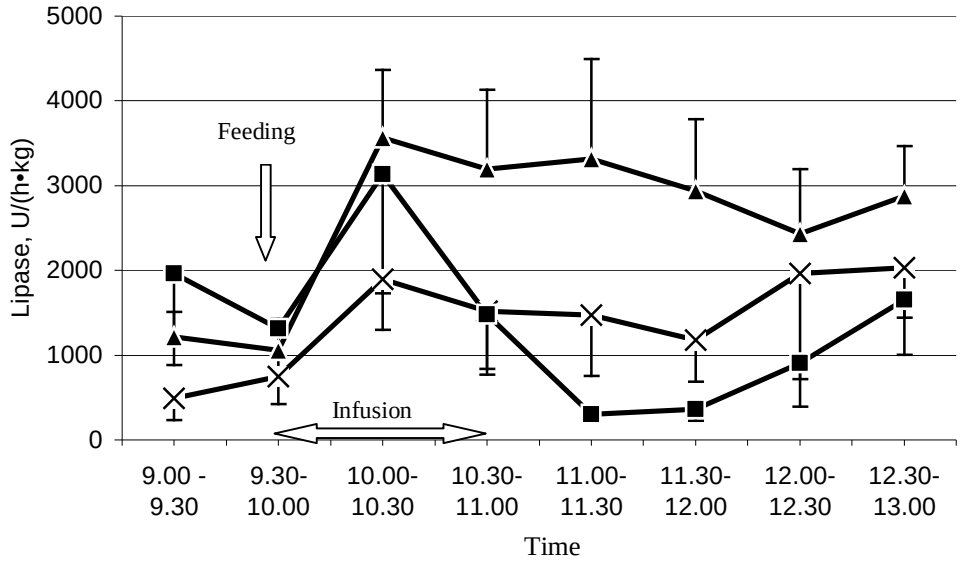


FIGURE 5a The diurnal pattern of colipase content in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM

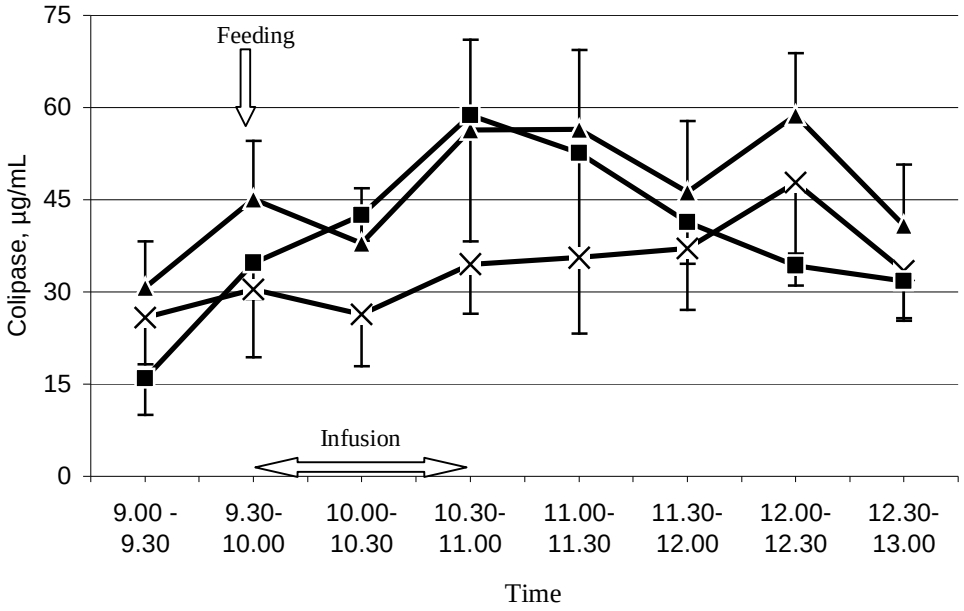


FIGURE 5b The diurnal pattern of colipase output in pancreatic juice after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM

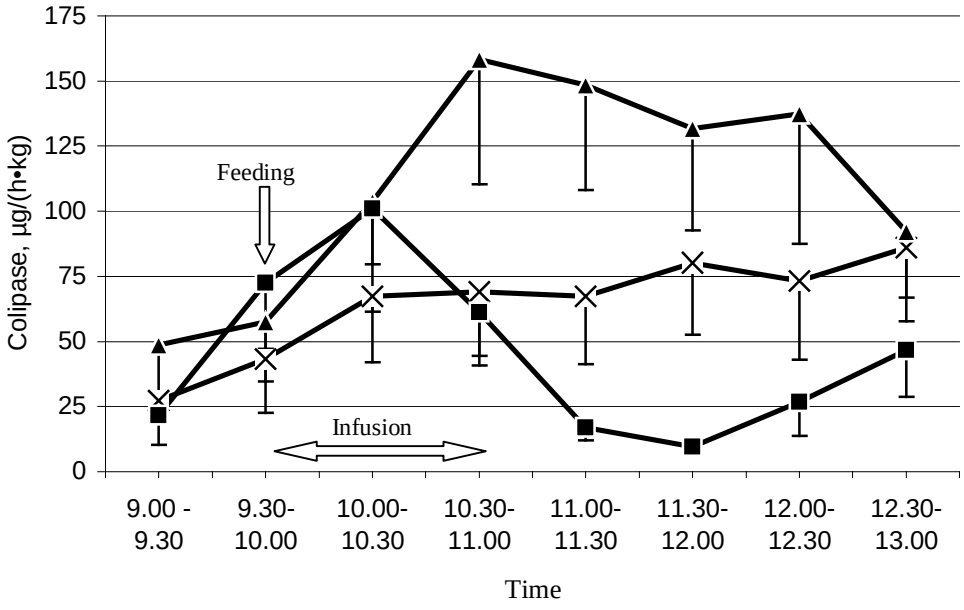


FIGURE 6a The diurnal pattern of plasma CCK (cholecystokinin) levels after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM

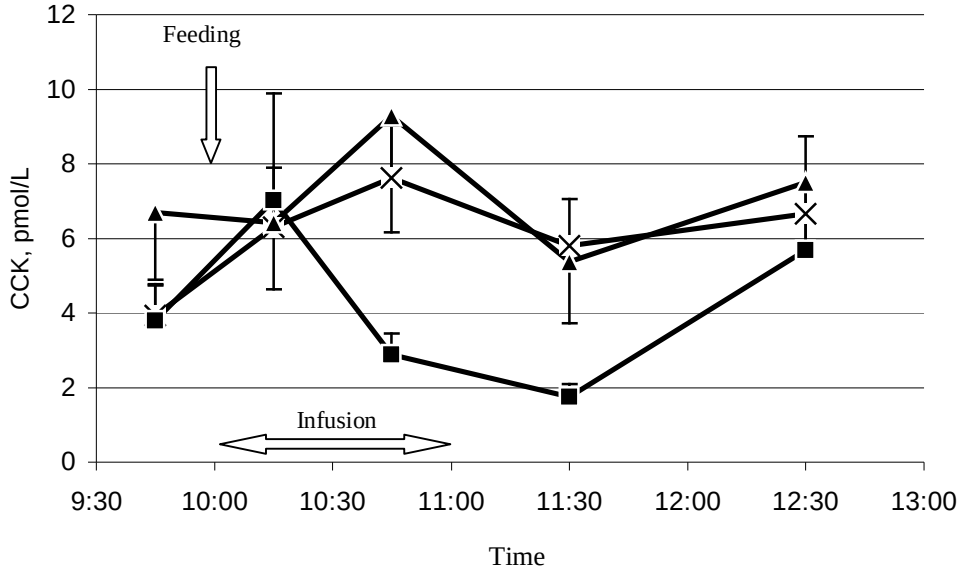
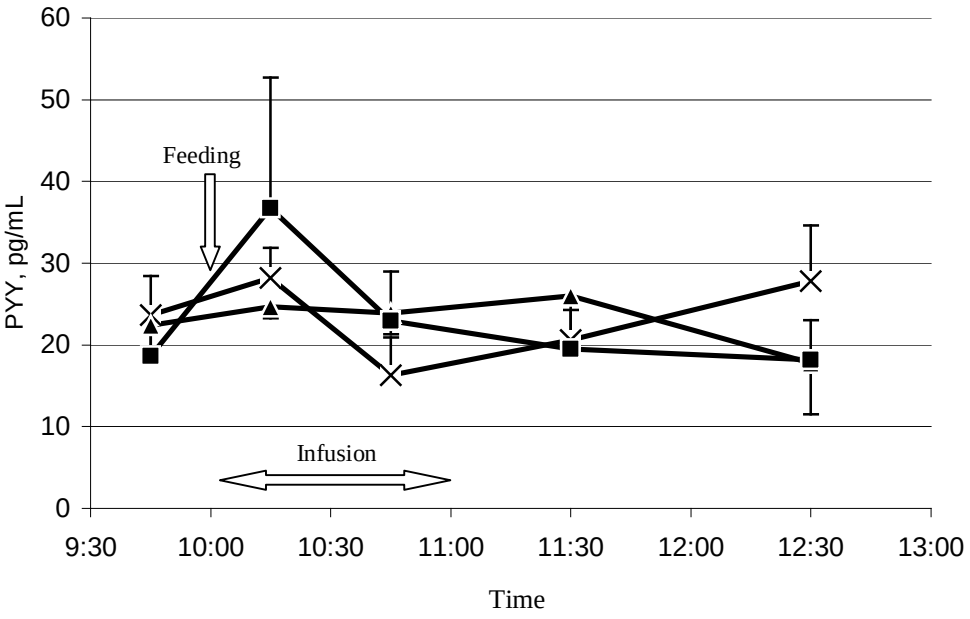


FIGURE 6b The diurnal pattern of plasma PYY (peptid YY) levels after intraduodenal infusion of saline (x), mct (■) and lct (▲), mean + SEM



5.5. Discussion

All variables estimated except for colipase contents showed an immediate response of the exocrine pancreas to feed intake and infusions of fat. This prandial response to feed intake is reflected by an immediate increase in the volume of secretion, in enzyme activities and in plasma CCK levels. Similar responses to feed intake have been described earlier in studies with pigs by Hee et al. (1988) and Thaela et al. (1995). Protein and trypsin outputs showed very similar diurnal patterns, which might be related to the fact that in pig pancreatic juice the ratio between trypsin and protein is fourfold higher compared to values obtained in pancreatic juice of rats. This implicates that trypsin is a major component of the protein fraction in pancreatic juice of pigs (Harada et al., 1982). Pancreatic lipase is most important for fat digestion in the small intestine. However, based on lipase contents in pancreatic juice, the results for the different infusion treatments are equivocal and no clear evidence for the influence of fats differing in chain length on pancreatic lipase secretion exists. Based on lipase outputs, however, three different trends of the curves for the three different infusion treatments were obtained. Lipase outputs did not change in the diurnal pattern after saline infusion whereas the mct treatment evoked a prandial peak and a postprandial decrease under preprandial values. On the other hand, after lct infusions the lipase outputs remained at the prandial elevated level. Moreover, this pattern for each of the infusion treatments is also similar for colipase contents and outputs.

The different infusion treatments had only minor effects on plasma PYY levels, which confirms observations by Aponte et al. (1985) who showed that infusion of either lauric (C 12:0) or oleic (C 18:1) acid into the proximal duodenum did not evoke a PYY release. In contrast, ileal and combined duodenal and ileal infusions of lauric or oleic acid produced similar significant increases in plasma PYY levels. The authors concluded that plasma PYY levels are not influenced by the chain length of fatty acids. It cannot be excluded that in the present study the absolute amounts of triglycerides infused intraduodenally were not sufficient to stimulate the PYY release at the ileal level. Moreover, Serrano et al. (1997) pointed out that the degree of saturation of fatty acid must be considered as an important stimuli for PYY release. In humans, the consumption of a diet containing olive oil with high levels of monounsaturated fatty acids evoked higher plasma PYY levels than the consumption of a diet containing sunflower oil with high levels of polyunsaturated fatty acids. This may

explain that no differences in plasma PYY levels were observed in the present study since the fats infused were both fully saturated.

The plasma CCK levels for the saline and lct treatments did not differ from each other which confirms observations in pigs (Corring and Chayvialle, 1987). These authors did not find a difference in plasma CCK levels after consumption of either high-fat or high-starch diets compared to a balanced control diet. However, in the present study, the plasma CCK concentrations decreased after the start of the mct infusions. This decrease follows the same diurnal pattern as was obtained for enzyme outputs during the experimental periods. CCK is known to be a potent stimulus for the pancreatic secretion in the pig (Pierzynowski et al., 1995; Houe et al., 1997), and different CCK mediated feedback mechanisms, as described recently by Pierzynowski et al. (1999), are responsible for the close relationship between enzyme secretion and plasma CCK levels.

In addition, there are indications that the CCK release might be influenced by plasma PYY levels. Fifteen min after the infusion of mct the plasma PYY showed a great variation (mean 36.7 pg/ml, SEM 16.0) which implicates that at least in some animals the PYY release was highly stimulated. Coincidentally, the plasma CCK level started to decrease 90 min after the beginning of the mct infusions. A possible explanation for this interaction is provided by Lluis et al. (1988). These authors showed in adult dogs that a suppression of CCK release was linked to an increase in plasma PYY levels. The authors concluded that the CCK release was inhibited by an increased PYY release.

The reason for the decrease in volume of secretion after mct infusions, and as a result, similar decreases in enzyme outputs, remains unclear. One possible explanation was provided by Layer et al. (1990) who could show in humans that small quantities of nutrients (e.g. fat) that were perfused into the ileum decreased pancreatic enzyme secretion by more than 80% ($P < 0.001$) in comparison to perfusions with saline. Moreover, Furuse et al. (1992) demonstrated that mct are absorbed via the blood and the lymphatic system whereas lct are absorbed exclusively via the lymphatic system. This difference could mediate different hormonal feedback mechanisms. Furthermore, mct might be absorbed at a higher rate than lct resulting in lower quantities reaching the ileum of pigs.

In conclusion, the infusions of different fats into the duodenum under prandial conditions evoked different responses. It can be assumed that the chain length of the fats infused will have an influence on the release of CCK and therefore on exocrine pancreatic secretions. There is no clear evidence that PYY is mediating the regulation of exocrine pancreatic secretions with respect to fat digestion. Furthermore, the results of the present study clearly show that enzyme and protein contents do not reflect physiological conditions; therefore studies based on the slaughter method must be reviewed critically. This method does not allow for the measurement of enzyme outputs since long-term collections of pancreatic juice are not possible. According to Sauer and Mosenthin (1999) only results expressed in outputs rather than contents are a true reflections of the effect of dietary treatments on the exocrine pancreas since differences in contents may simply reflect dilution by pancreatic juice.

Further investigations are warranted to identify the factors that may be responsible for the changes in the volume of secretion, enzyme secretion and the release of gastrointestinal hormones after mct infusions. Further studies should focus on gastrointestinal hormones involved in the regulation of the exocrine pancreas, such as CCK, PYY but also neurotensin and secretin. Especially the determination of the diurnal pattern of secretin in plasma is of interest, as secretin is considered to be the major regulative hormone of the volume of pancreatic secretion.

5.6. References

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6. INFLUENCE OF INTRADUODENALLY INFUSED OLIVE AND COCONUT OIL ON POSTPRANDIAL EXOCRINE PANCREATIC SECRETIONS OF GROWING PIGS

6.1. Summary

The effect of dietary vegetable oils that were infused directly into the duodenum on exocrine pancreatic secretions in pigs has not been studied yet. The objective of the present study was to determine the spontaneous response of the exocrine pancreas to different vegetable oils under prandial conditions. Six growing pigs (BW 13.2 kg) were surgically prepared with pancreatic duct catheters and duodenal re-entrant T-cannulas. The animals were fed twice a day (1000 and 1600) a commercial weaner diet at a rate of 2% of BW. Beginning with the morning feeding, olive oil, coconut oil or saline as a control were infused in boluses every 5 min in total 0.1% of BW over a period of 1 h directly into the duodenum according to a 3 x 2 Latin Square Design. Pancreatic juice was collected over a period of 4 h, beginning 1 h preprandially (0900) till 3 h postprandially (1300). A time effect was observed after the infusion of olive oil on the volume of secretion, on protein contents and outputs as well as on lipase contents and outputs and on colipase contents. The infusion of saline and coconut oil changed the trends of the curves for lipase and colipase outputs. No time x treatment interactions were observed regarding volume of secretion, protein contents and outputs, trypsin contents and outputs and lipase outputs. The trends of the curves for lipase contents were different between the olive oil and saline treatment and between the olive oil and the coconut oil treatment. The trends of the curves for the olive oil and saline treatment differed from each other regarding colipase contents. Pooled values of colipase outputs were elevated after coconut oil treatment and a positive correlation between trypsin and colipase contents was found. It is suggested that under prandial conditions the exocrine pancreas responds differently in its spontaneous secretion to different vegetable oils.

6.2. Introduction

The secretions of the exocrine pancreas are required for hydrolysis of nutrients present in food and feeds (Rinderknecht, 1993). Enzyme secretion is highly dependent on diet composition, age and feeding regimen (Corring et al., 1989; Makkink and Verstegen, 1990). This is well described for different species including the rat (Bucko and Kopec, 1968; Gidez, 1973), dog (Behrmann and Kare, 1969) and the pig (Corring, 1980; Makkink and Verstegen, 1990; Ozimek et al., 1995). For example, Mourot and Corring (1979) observed with increasing levels of fat in the diet greater lipase contents in porcine pancreatic tissue. Deschodt Lanckman et al. (1971) and Ricketts and Brannon (1994), in rats, showed an increased lipase content when polyunsaturated fatty acids were included in the diet. Simoes Nunes (1986) fed diets to pigs containing either 21% sunflower oil or lard. The author showed that sunflower oil evoked a greater ($P < .05$) lipase content than lard. In studies by Gabert et al. (1996) pigs were prepared with a pancreatic duct catheter and adapted to diets containing either coconut oil, rapeseed oil or fish oil. The authors found a greater ($P < .05$) chymotrypsin secretion in pancreatic juice of pigs fed a diet supplemented with coconut oil, and greater ($P < .05$) secretion of carboxylester hydrolase in pigs fed a diet containing fish oil.

It has been reported that dietary changes exhibit a fast response in enzyme adaptation and the secretion of gastrointestinal hormones which is completed within one week (Bucko and Kopec, 1968; Deschodt Lanckman et al., 1971; Corring, 1980).

Moreover, there is evidence for the existence of a spontaneous adaptation of the exocrine pancreas, observed in species such as dogs (Yago et al., 1997), rats (Bucko and Kopec, 1968; Deschodt Lanckman et al., 1971) and pigs (Hee et al., 1988b; Corring and Chayvialle, 1987).

There is still a scarcity of information on the existence of a spontaneous adaptation of the exocrine pancreas when fats different in chain length and degree of saturation are given to pigs. The objective of the present study was to examine the effect of vegetable oils different in fatty acid composition, namely olive oil (containing mainly unsaturated long-chain fatty acids) and coconut oil (containing mainly saturated medium-chain fatty acids), on exocrine pancreatic secretions in pigs.

6.3. Materials and Methods

6.3.1. Animals

The studies were carried out with six piglets (Swedish Landrace x (Yorkshire x Hampshire)) obtained from a production herd (Odarslöv's Research Farm, Swedish University of Agricultural Sciences, Lund) with an average BW of 13.2 kg at the beginning of the experiment. The pigs were housed individually and freely moving in pens (1 x 2 m), had free access to water and were kept under 12 h light / 12 h dark cycles (light on from 08.00 h to 20.00 h). Treatments and experiments were conducted according to the European Community regulations concerning the protection of experimental animals and Lunds University ethical committee allowance.

6.3.2. Surgical procedures

The pigs were sedated with azaperone (Stresnil, Janssen Pharmaceutica, Beerse, Belgium; 2 mg/kg BW) and anaesthetised with Halothane (ISC Chemicals Ltd., UK; 3% air). Surgery was performed under aseptic conditions. The pigs were surgically fitted with a chronic pancreatic duct catheter and a T-shaped duodenal cannula for collection and subsequent return of pancreatic juice into the duodenum according to Pierzynowski et al. (1988) and modified as described by Thaela et al. (1995).

6.3.3. Experimental procedures

The piglets were fed twice daily (1000 and 1600) a barley-based weaner diet containing 17.7% crude protein and 5.2% crude fat (Växfor, Lantmännen, Stockholm, Sweden) at a rate of 2% of BW. After a post-surgical recuperation period of 7 d, beginning with the morning feeding (1000), olive oil or coconut oil or saline as a control was infused directly into the duodenum of the piglets via the duodenal T-cannula. The amount of fat infused into the duodenum amounted to 0.1% of BW which corresponds to 5% fat supplementation to the diet and can be considered as a physiologically adequate dose. The fats were filled into syringes and saline was added to a final volume of 36 mL. The syringes were kept under heating lamps

at body temperature and were emulsified by means of vigorous shaking just before the infusions started. The fats and the control infusion with saline (36 mL) were administered in small boluses of 3 mL per bolus every 5min over a period of 1 h (1000 to 1100). The fat treatments were arranged according to a 3 x 2 Latin Square Design; the fatty acid composition of the fats infused is shown in Table 1.

Table 1 Fatty acid composition of olive and coconut oil

Fatty acid	Carbon	% Fatty acid	
		Coconut oil	Olive oil
Caprylic	C 8:0	6.72	
Capric	C 10:0	8.85	
Lauric	**C 12:0**	**45.95**	
Myristic	**C 14:0**	**18.12**	
Palmitic	C 16:0	9.92	11.59
Palmitoleic	C 16:1		1.01
Stearic	C 18:0	3.78	2.83
Oleic	**C 18:1**	**7.31**	**71.64**
Linoleic	C 18:2	1.83	9.67
Linolenic	C 18:3		0.77
Arachidic	C 20:0		0.48

Pancreatic juice was collected over a period of 4 h, beginning 1 h preprandially (0900) lasting for 3 h postprandially (1300). The volume of secretion was recorded in 30 min intervals, an aliquot (1 mL) was obtained for analyses and immediately stored at -20°C. The remainder was re-infused into the duodenum via the duodenal cannula in small doses every 5 min over a period of 30 min.

6.3.4. Analytical procedures

The fatty acid composition of the olive and coconut oil was determined by a GLC method according to Naumann et al. (1976). Pancreatic juice samples were analysed for protein using the Lowry method (Lowry et al., 1951), performed on 96-well microwell plates and using bovine serum albumin (BSA, Sigma, St. Louis, MO, US) as a standard. Intra- and interassay

CV for the protein determination were 3.1 and 3.6%, respectively. Trypsin (EC 3.4.21.4) activities were estimated after enterokinase (Sigma, St Louis, MO, US) activation using N-α-benzoyl-DL-arginine-p-nitroanilide (Sigma, St Louis, MO, US) as a substrate (Pierzynowski et al., 1990). Intra- and interassay CV for the trypsin determination were 2.8 and 3.2%, respectively. Lipase (EC 3.1.1.3) activities were determined by a pH-stat titration method using tributyrin as a substrate, as described by Borgström and Hildebrand (1975). Interassay CV for the lipase activity was 4.2%. One unit (U) of enzyme activity is defined as the amount of enzyme hydrolysing 1 µmol substrate per min. A competitive ELISA was used for measuring pancreatic colipase. The determination was adapted to a procedure described earlier for measuring enterostatin (Mei et al., 1993). Antiserum was obtained by immunizing a rabbit (3BI-16) with porcine procolipase (purified from porcine pancreas according to the method of Erlanson et al. (1973)). Ninety-six-well microtiter plates were coated over night with 0.2 µg/mL procolipase (purified, (Erlanson et al., 1973)). The antibody against procolipase was diluted 1:5000, the secondary biotin conjugated antibody (Sigma, St Louis, MO, US) was diluted 1:6000 and the streptavidin-alkaline phosphatase (Sigma, St Louis, MO, US) was diluted 1:6000. The plate was developed by the addition of p-nitrophenyl phosphate (Sigma, St Louis, MO, US) and a standard curve ranging from 500 µg/mL to 0.7 µg/mL was used in this assay.

Protein contents in pancreatic juice were expressed as mg per mL (mg/mL), protein outputs were expressed as mg per 1 h of secretion per kg BW (mg/(h•kg)). Enzyme contents in pancreatic juice were expressed as U per mL (U/mL), enzyme outputs were expressed as U per 1 h of secretion per kg BW (U/(h•kg)). Colipase contents in pancreatic juice were expressed as µg per mL (µg/mL), colipase outputs were expressed as µg per 1 h of secretion per kg BW (µg/(h•kg)).

6.3.5. Statistical analyses

Data were analysed with Statview software (vers. 4.57, Abacus Concepts, Ca, USA) using repeated measures ANOVA with time, treatment and time x treatment interaction in the model. Post Scheffe's-test was performed to compare treatment means of the pooled data. The relationship between trypsin content (U/L) and colipase content (µg/mL) was tested with correlation analyses. Furthermore, to determine whether the correlation coefficient was

statistically different from 0, Fisher's r to z transformation was performed on the correlation. The results were expressed as mean ± SEM. The level of significance was set at 5% ($P < .05$), a level of 1% ($P < .01$) was defined as highly significant.

6.4. Results

The pigs recovered well from surgery and started to gain BW (300 to 450 g/d) 3 to 5 d postsurgically. The pigs remained clinically healthy and consumed their meal allowances of the diet within 10 to 15 min. Postmortem examinations after the experiment revealed no intestinal adhesions or other abnormalities.

The volume of secretion of pancreatic juice peaked 30 min postprandially at 5.3 mL/(h•kg) for the saline and olive oil infusions and at 4.9 mL/(h•kg) for the coconut infusion treatment and then returned back to the corresponding preprandial values for the saline- and coconut infusions (Figure 1). The trends of the curves for both treatments did not show a change over time ($P > .2$), whereas the volume of secretion following olive oil infusion showed a time effect ($P < .01$) resulting in a decrease to 2.2 mL/(h•kg) 1 h postprandially and a return to preprandial values 2 h postprandially. No differences were observed between the curves of pancreatic juice outflow for the saline and coconut oil treatment. A tendency ($P < .07$) to a higher volume of secretion for the saline compared to the olive oil treatment and a difference ($P < .04$) between the coconut and olive oil treatment was obtained.

The trend of the curve for the protein content and output changed for all three treatments (saline, $P < .01$; coconut, $P < .01$; olive, $P < .04$) over time. However, there were no differences ($P > .4$) between the trends of the curves (Figure 2a,b).

The trends of the curves for the trypsin contents and outputs are similar to those observed for the protein content and output. A time effect was observed ($P < .05$) for all treatments, however no differences ($P > .2$) between the curves were obtained (Figure 3a,b).

All treatments including saline infusion evoked a time effect ($P < .01$) effect in lipase outputs (U/mL). As is shown in Figure 4a, following infusion of olive oil the lipase activity peaked at 1417 U/mL, whereas for the saline and the coconut oil treatment only a moderate increase

was recorded . A time x treatment interaction was observed between the trends of the curve for the olive oil and saline ($P < .01$) and between the olive oil and coconut treatments ($P < .04$). A time effect for lipase outputs was observed for the saline ($P < .04$) and coconut oil ($P < .03$) treatments, whereas no time effect ($P > .3$) was found for the olive oil treatment (Figure 4b). No differences ($P > .9$) between the curves were obtained for lipase outputs.

The colipase content (µg/mL) in pancreatic juice of pigs receiving an intraduodenal infusion of coconut oil increased within 30 min after the start of the infusion to a value of 51 µg/mL and remained for 1 h on this level before it decreased to 34 µg/mL, whereas the colipase content for the olive oil treatment peaked at 55 µg/mL 1.5 h postprandially. All treatments evoked a time effect ($P < .01$) and a tendency ($P < .1$) for a time x treatment interaction was observed between the trends of the curves for the olive oil and the coconut oil treatment (Figure 5a). A time effect for the colipase output (µg/(h•kg)) was obtained for the saline ($P < .01$) and coconut oil ($P < .01$) treatment, whereas no effect ($P > .3$) was found for the olive oil treatment. No differences ($P > .3$) existed between the trends of the curves. The pooled colipase output after the coconut oil infusion was greater ($P < .02$) compared to corresponding values obtained for the saline and olive oil treatment (Figure 5b).

A positive linear correlation between the trypsin (U/L) and colipase content (µg/mL) was found for each infusion treatment. The correlation for the saline treatment was small ($r = 0.53$, $P < .001$), whereas closer correlations were found for the coconut oil ($r = 0.76$, $P < .001$) and olive oil infusion treatments ($r = 0.85$, $P < .001$).

Figure 1. The diurnal pattern of volume of secretion of pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

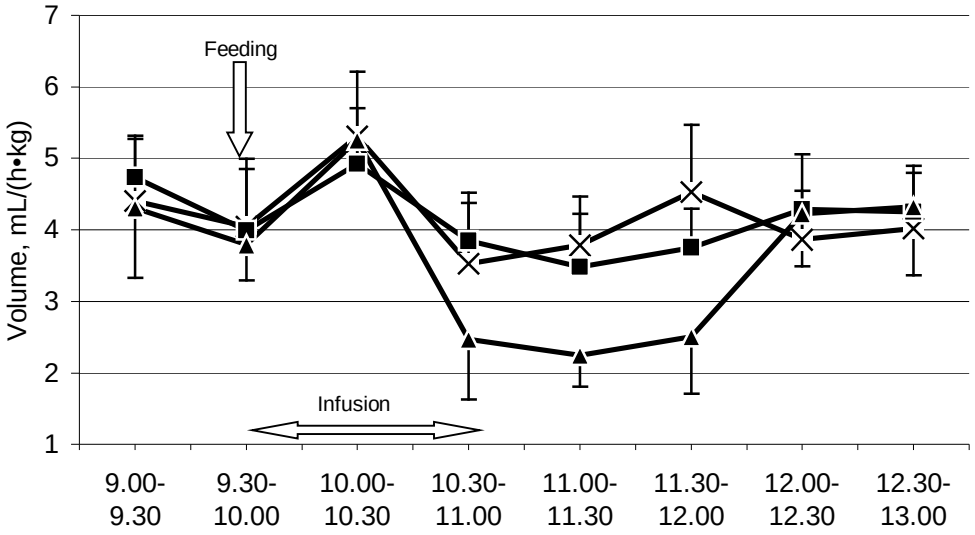


Figure 2a. The diurnal pattern of protein content in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

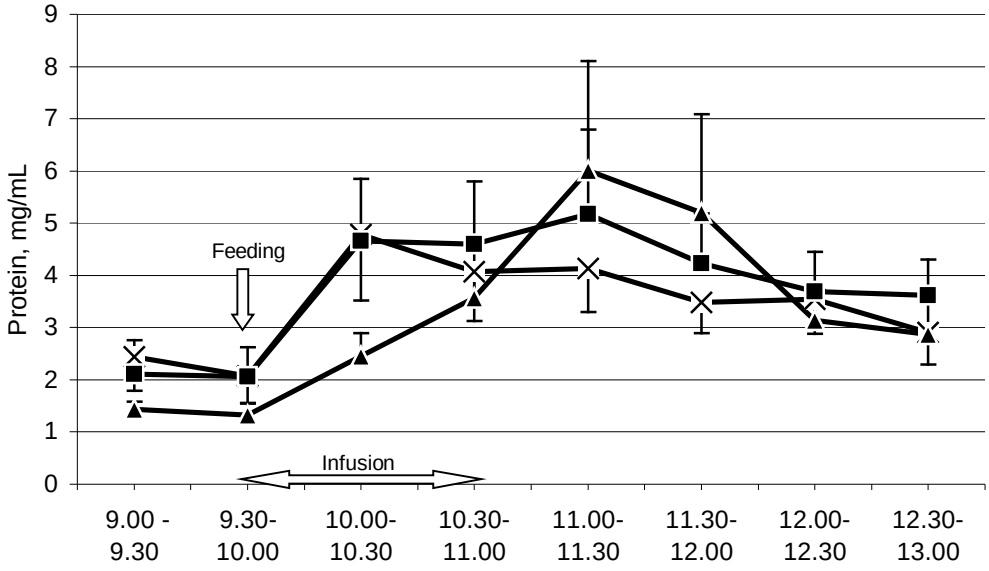


Figure 2b. The diurnal pattern of protein output in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

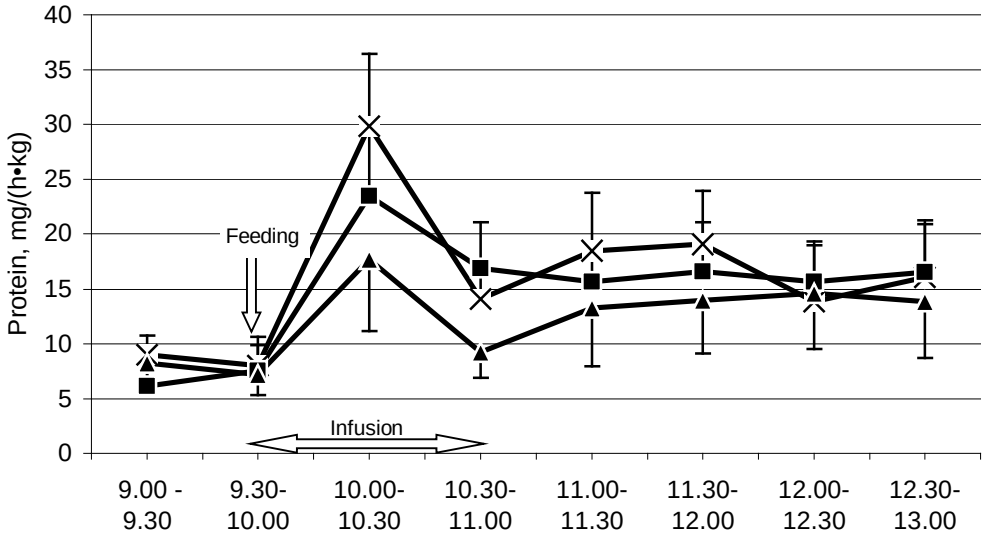


Figure 3a. The diurnal pattern of trypsin content in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

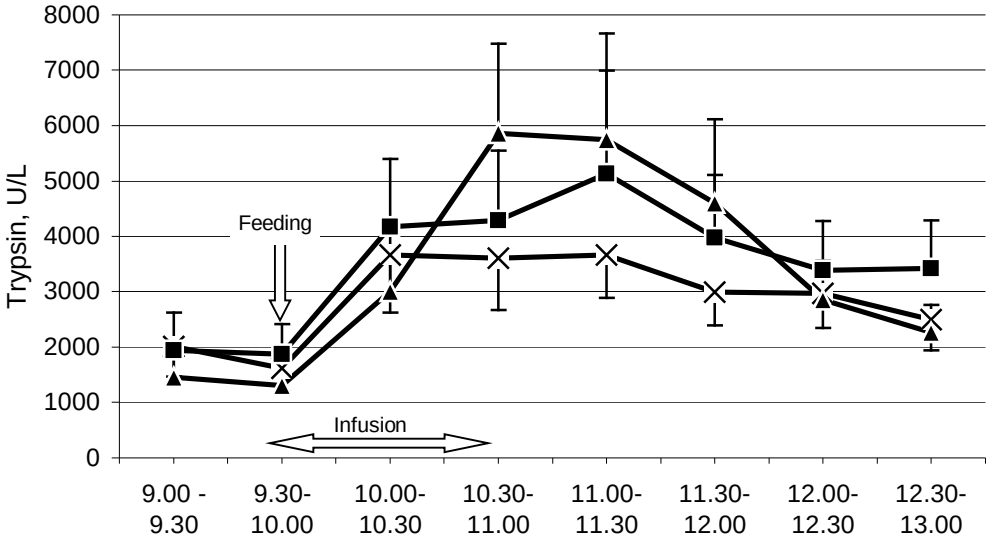


Figure 3b. The diurnal pattern of trypsin output in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

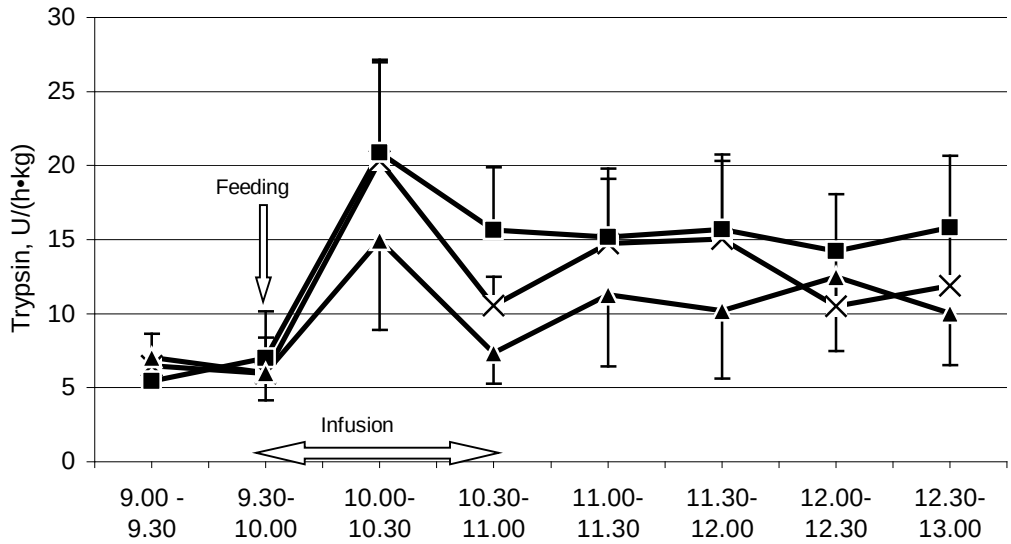


Figure 4a. The diurnal pattern of lipase content in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

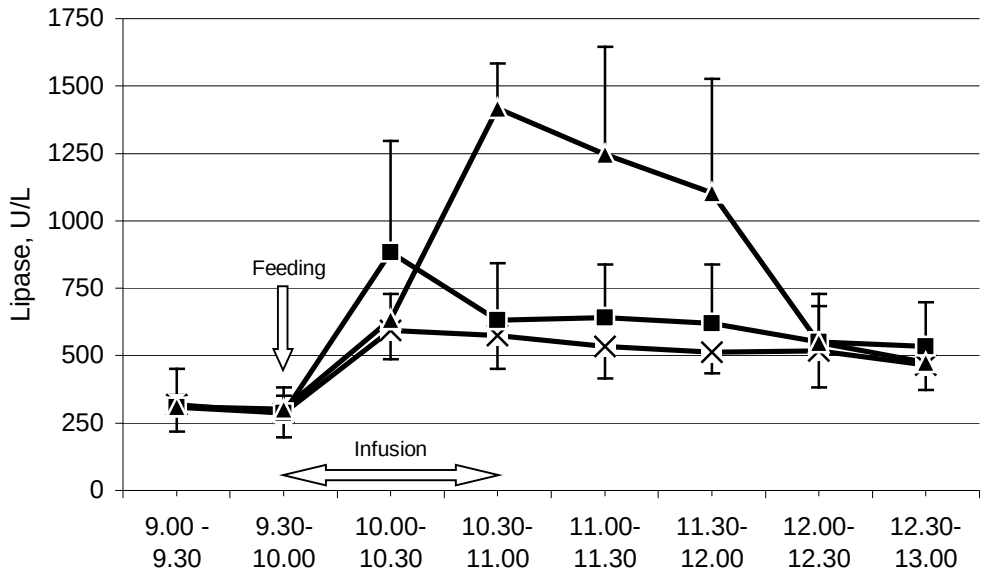


Figure 4b. The diurnal pattern of lipase output in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

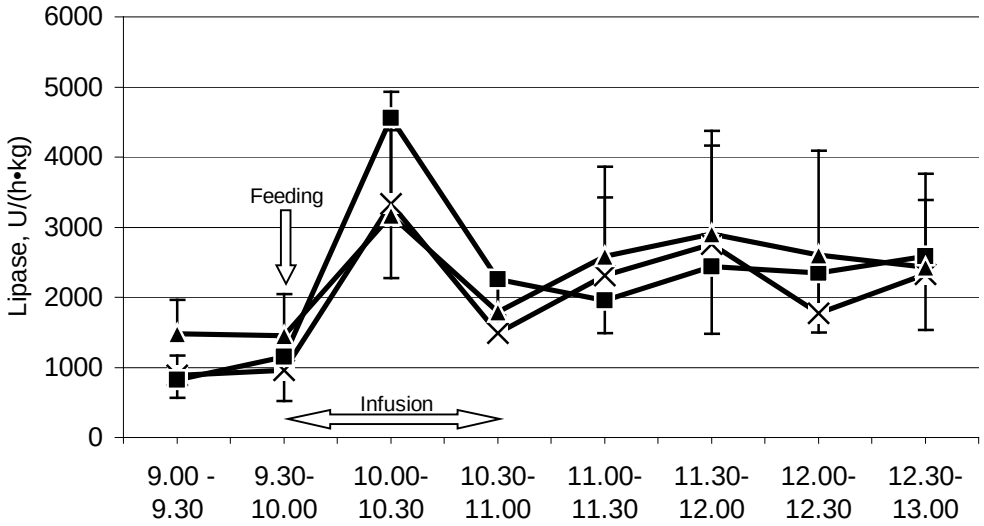


Figure 5a. The diurnal pattern of colipase content in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM

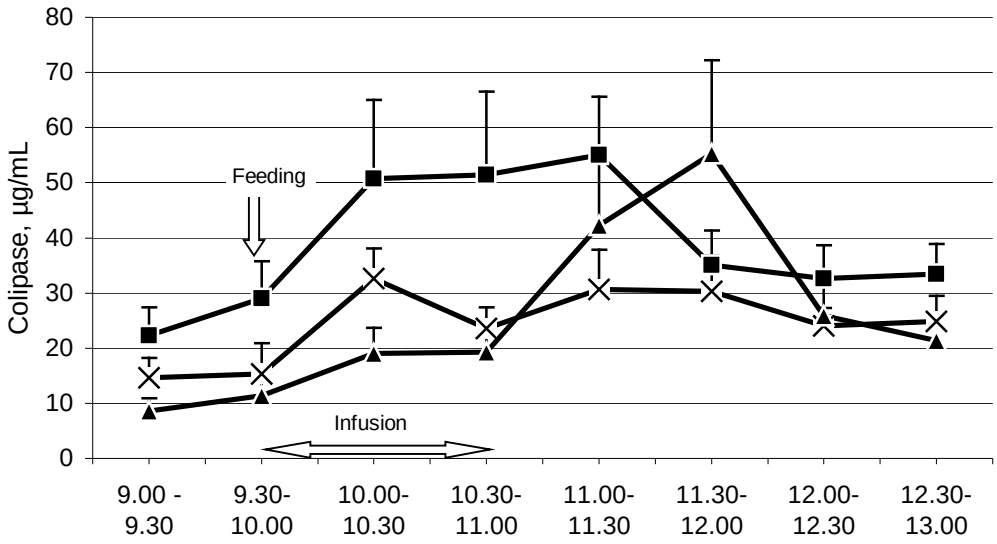
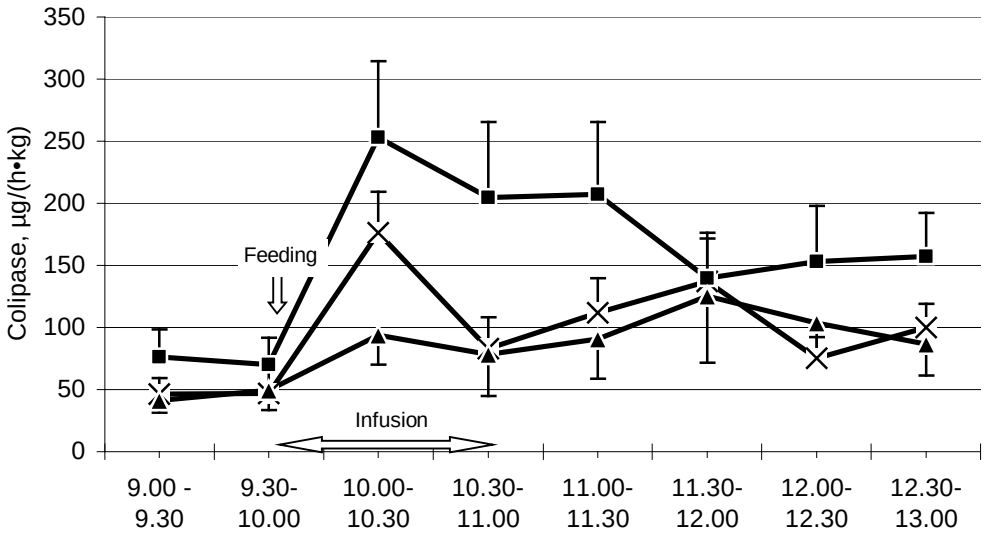


Figure 5a. The diurnal pattern of colipase output in pancreatic juice after intraduodenal infusion of saline (x), coconut oil (■) and olive oil (▲), mean + SEM



6.5. Discussion

It can be derived from the results of this study that there exists an immediate response of the exocrine pancreatic secretion during and after feed consumption and the simultaneous intraduodenal infusion of coconut oil, olive oil and of saline. The immediate prandial increase of protein and trypsin outputs is most likely a response to feed intake *per sé*, which confirm reports by Hee et al. (1988b) and Thaela et al. (1995) who determined an immediate prandial increase in the contents and outputs of protein and trypsin. Protein and trypsin outputs in pancreatic juice showed very similar diurnal patterns, which might be related to the fact that the ratio between trypsin and protein in pancreatic juice of pig is fourfold greater compared to that in pancreatic juice of rat. This implicates that trypsin is a major component of the protein fraction secreted by the exocrine pancreas as was reported by Harada et al. (1982). No differences between the infusion treatments were obtained for the protein and trypsin outputs when comparing the trends of the curves over time (Figure 2b and 3b). It can be concluded that the infusion treatments did not stimulate the exocrine pancreas differently with respect to protein and trypsin outputs. Moreover, the pooled treatment means for the protein content and output as well as the content and output of trypsin did not differ from each other, which confirms observations by Gabert et al. (1996) in growing pigs who also did not obtain a difference when diets containing either coconut, rapeseed or fish oil were fed.

However, the trends of the curves for the lipase content differed from each other, which suggest that vegetable oils containing fatty acids different in chain length and degree of saturation, affect the exocrine pancreas differently. Moreover, compared to saline and coconut oil, the olive oil infusion resulted in a 2.1- ($P < .01$) and 1.5-fold ($P < .04$) increase in the lipase contents, respectively, which is likely related to the fact, that olive oil is dominated by long-chain and unsaturated fatty acids whereas coconut oil contains mainly saturated fatty acids shorter than C 14:0. These findings are in agreement with observations by Simoes Nunes (1986) who reported an increased lipase content when oils containing high levels of unsaturated fatty acids were fed compared to oils containing mostly saturated fatty acids. It was demonstrated in studies with rats that increasing levels of polyunsaturated fatty acids in the diet evoked an increased lipase content in pancreatic tissues (Deschodt Lanckman et al. 1971; Ricketts and Brannon, 1994). It has to be emphasised, however, that the existing differences in treatment means or trends of the curves for lipase contents were nearly compensated when these comparisons were based on lipase outputs, because the volume of

secretion of pancreatic juice showed for all infusion treatments a postprandial decrease, in particular for olive oil. This decrease in volume of secretion observed was also described in dogs after intraduodenal infusion of fats (Stubbs and Stabile, 1989). The increased lipase contents and the decreased volume of secretion remains physiologically unclear. A possible explanation could be, that olive oil contains 72% oleic acid (see Table 1) which, in turn, is known to be a very potent stimulus for the release of the gastrointestinal hormones cholecystokinin (CCK) and Peptide YY (PPY) from the small intestine to the blood circulation (Gabert and Hedemann, 1999). Whereas CCK stimulates the enzyme secretion , PYY is known to inhibit the volume of pancreatic secretion (Onaga et al., 1994). Thus, the results obtained in the present study may be, in turn, a result of the different stimulation of the CCK and PYY release mediated by the different fatty acid composition. Another explanation is provided by Pierzynowski et al. (1999) who pointed out the existence of an intrapancreatic feedback. According to these authors an interaction between the volume of secretion and the enzyme contents may regulate the enzyme outputs. This mechanism explains the effects on the volume of secretion and lipase contents in the present study. This intrapancreatic feedback Pierzynowski et al. (1999) can be considered as a regulative mechanism in order to prevent the pancreas from secreting surplus amounts of enzymes or fluids, thus minimising endogenous losses from exocrine pancreatic secretions. Moreover, it is likely a regulative mechanism to prevent acute pancreatitis deriving from excessive secretion.

The colipase contents and outputs showed a prandial increase; the infusion of coconut oil evoked an elevated secretion which resulted in a different trend of the curve for the colipase output compared to the saline and olive oil treatment. In addition, pooled treatment means were greater ($P < .01$) after coconut infusions compared to saline and olive oil infusions. As the coconut oil treatment resulted in the greatest increase in colipase output, it can be concluded, that fats containing mainly saturated, medium-chain fatty acids, such as coconut oil, stimulate colipase output more than unsaturated, long-chain fatty acids, such as olive oil. It is likely possible that the regulation of the colipase secretion is not mediated by CCK, as oleic acid is known to be the most potent releasing factor of CCK (Schaffalitzky de Muckadell et al., 1986), which stimulates the enzyme secretion of the exocrine pancreas; the olive oil was dominated by oleic acid whereas only small quantities were found in the coconut oil (see Table 1).

Moreover, it is known that pancreatic procolipase is activated by trypsin to give colipase, with a simultaneous formation of enterostatin (Erlanson-Albertsson et al., 1991). In the present study, a positive correlation ($r \geq 53$; $P < .001$) between trypsin and colipase contents was obtained for each individual treatment. This suggests that the secretion of procolipase and trypsinogen may be regulated by a common neuro-endocrine pathway. However, further studies are warranted to discriminate the mechanisms involved.

In the present study, the infusion of olive oil decreased the volume of secretion, combined with a greater colipase output. This increase in colipase output in pancreatic juice suggests a parallel increase of enterostatin in the lumen of the small intestine. Luminal enterostatin has been found previously to decrease exocrine pancreatic secretions by inhibiting the release of CCK (Erlanson-Albertsson et al., 1991). Thus, in the present study it is likely possible that enterostatin is involved in the regulation of the volume of secretion of pancreatic juice. Moreover, the decrease in the volume of secretion mediated by the release of leptin in the stomach, as suggested by Bado et al. (1998), is unlikely due to the intraduodenal infusion of fats.

Further investigations including chemically well defined fats, such as synthetic fats, are warranted to validate the influence of fats containing fatty acids different in chain length and degree of saturation on exocrine pancreatic secretions. Gastrointestinal hormones, such as CCK and PYY are involved in the regulation of enzyme formation and outflow of pancreatic juice (Solomon, 1987), respectively. Further studies should also include measurements of plasma levels of gastrointestinal hormones involved in the regulation of the exocrine pancreas.

6.6. Conclusions

The infusions of different fats into the duodenum under prandial conditions evoked different responses of the exocrine pancreas. It can be assumed that the chain length of fats will affect the spontaneous secretion of the pancreas. The data obtained support the idea of the existence of an intrapancreatic feedback regulating the ratio between volume of secretion and enzyme contents in pancreatic juice.

The results of the present study clearly show that values for enzyme and protein contents do not reflect physiological conditions. According to (Sauer and Mosenthin, 1999) only results expressed in outputs rather than contents are a true reflection of the effect of dietary treatments on the exocrine pancreas because differences in contents may simply reflect dilution by pancreatic juice.

6.7. References

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7. GENERAL DISCUSSION

In the present thesis the state of the art in science with respect to the influence of carbohydrates on exocrine pancreatic secretions in pigs has been reviewed. Moreover, the literature has been reviewed with respect to the influence of lipids on exocrine pancreatic secretions in pigs and its regulation by gastrointestinal hormones.

The effect of a commercial preparation of potato fibre (Povex^{TM}) on the exocrine pancreatic secretion of pigs and its hormonal regulation was studied in order to explain the equivocal picture of the influence of dietary fibre on exocrine pancreatic secretions. Three pigs were fitted with pancreatic duct catheters and fed with diets containing either 0% or 2% potato fibre. Moreover, the effect of potato fibre and of hormone infusates on the spontaneous secretion of the exocrine pancreas was investigated.

Studies were carried out in order to investigate the influence of different synthetic fats infused intraduodenally on the volume of pancreatic juice secreted and the specific and total activities of enzymes secreted with special respect to lipase and colipase. The influence of different synthetic fats on CCK and PPY, hormones regulating the exocrine pancreas, were studied. Another objective was to investigate a possible correlation between trypsin and colipase. Fats infused were glyceroltricaprylat and glyceroltristearat, both synthetic fats. For this study 6 pigs were fitted with pancreatic duct cannulas.

Moreover, the influence of vegetable oils infused intraduodenally on specific and total enzyme activities (with special respect to lipase and colipase) and on the volume of pancreatic juice secreted were studied. Six pigs were fitted with pancreatic duct cannulas. Two different vegetable oils (coconut and olive oil) were infused directly into the duodenum.

7.1. Influence of potato fibre

Several authors showed that the inclusion of dietary fibre into a diet for pigs increases the volume of secretion of pancreatic juice (Zebrowska and Low, 1987; Mosenthin and Sauer, 1991). The study presented in this thesis confirmed this observation as the inclusion of potato fibre into the diet increased the volume of secretion as well.

Moreover, it was demonstrated that the intraduodenal infusion of different substrates as well as the intravenous infusion of gastrointestinal hormones such as CCK and secretin evoked a spontaneous stimulation of the exocrine pancreas. Consequently, the volume of secretion, the total output of protein, trypsin, lipase and α-amylase were consistently and in most cases lower ($P<0.05$) in the pre- and post-infusion periods than the corresponding values determined during the infusion of the different infusates. This spontaneous response to the infusion treatments corresponds to the immediate postprandial response after feeding as reported by Hee et al. (1988) and Thaela et al. (1995).

Moreover, these results indicate that the presence of substrates in the duodenum per se has a much more pronounced effect on the pattern of secretion of the exocrine pancreas than the source of substrates itself. The time period when the different infusates were infused was uniformingly characterised by an increased secretion of pancreatic juice, protein and enzymes, irrespective of the source of substrate administered.

It is likely possible that potato fibre affects the microfloral colonisation of the large intestine and, in consequence, the production of short chain fatty acids (SCFA). Several authors showed that SCFA are involved in the regulation of the exocrine and endocrine pancreas as well as in the regulation of gastric emptying (Kato et al., 1989; Mineo et al., 1990;Malbert et al., 1994). Moreover, SCFA are potent stimulators of insulin release in ruminants (Manns et al., 1967; Manns and Boda, 1967) and insulin is known to stimulate the exocrine pancreas (Williams and Goldfine, 1985). As the insulin level in the present study was elevated, an influence of SCFA deriving from potato fibre on the exocrine pancreas is possible.

7.2. Influence of synthetic fats

A prandial response to feed as described by Hee et al. (1988) and Thaela et al. (1995) was observed for all parameters estimated except for the specific colipase content and the plasma PYY concentrations.

Total lipase activity showed three different slopes of the curves for the three different treatments. Whereas the saline infusion did not change the diurnal pattern, the mct treatment evoked after a prandial increase in total lipase activities a postprandial decrease, whereas the total lipase activities remained at the prandial elevated level. Moreover, this picture is also reflected by the specific and total colipase activities.

No differences were observed in plasma PYY levels, which is in agreement with observations made by Aponte et al. (1985) who did not find differences in the PYY level after infusion of medium or long-chain fatty acids. Another possible explanation for the lack of an effect on the plasma PYY level is given by Yago et al. (1997) who pointed out that the PYY level is closely connected to the degree of unsaturation and the fats applicated in the study presented in this thesis were both fully saturated.

The plasma CCK levels did not differ from each other regarding the saline and lct treatment. However, the plasma CCK level decreased after the start of the mct and the slope of this curves reflects the curve observed for the volume of secretion. As this decrease shows the same diurnal pattern as the decreases in total enzyme activities, studies who showed the close relationship between total enzyme activities and plasma CCK levels (Pierzynowski et al., 1995; Houe et al., 1997; Pierzynowski et al., 1999) were confirmed.

The reason for the decrease observed in the volume of secretion after infusion of mct remains unclear. A possible explanation is given by Layer et al. (1990) who demonstrated in humans that small quantities of nutrients as fats perfused to the ileum decreased pancreatic secretion by greater than 80% compared to saline infusions.

7.3. Influence of vegetable oils

The results of this study show that an immediate response to feed was observed by a prandial increase in total protein contents and total trypsin activities, as formerly observed by Hee et al. (1988) and Thaela et al. (1995). No differences between the slopes of the curves were observed for the protein and trypsin secretion, which confirms observations made by Gabert et al. (1996).

However, the slopes of the curves for the specific lipase activities differed from each other, which suggest that vegetable oils containing fatty acids different in chain length and degree of saturation, affect the exocrine pancreas differently. Moreover, compared to saline and coconut oil, the olive oil infusion resulted in a 2.1- ($P < .01$) and 1.5-fold ($P < .04$) increase in the specific lipase activities, respectively, which is likely related to the fact, that olive oil is dominated by long-chain and unsaturated fatty acids whereas coconut oil contains mainly saturated fatty acids shorter than C 14:0. These findings are in agreement with observations by Simoes Nunes (1986) who reported an increased specific lipase activity when oils containing high levels of unsaturated fatty acids were fed compared to oils containing mostly saturated fatty acids. It was demonstrated in studies with rats that increasing levels of polyunsaturated fatty acids in the diet evoked an increased specific lipase activity in pancreatic tissues (Deschodt Lanckman et al., 1971; Ricketts and Brannon, 1994).

It has to be emphasized, however, that the existing differences in treatment means or slopes of the curves for specific lipase activities were nearly compensated when these comparisons were based on total lipase activities, because the volume of secretion of pancreatic juice showed for all infusion treatments a postprandial decrease, in particular for olive oil. This decrease observed, previously also described in the dog after intraduodenal infusion of fats (Stubbs and Stabile, 1989), and thus compensating for the increased specific lipase activities remains physiologically unclear. A possible explanation is provided by Pierzynowski et al. (1999) who suggests the existence of a feedback compensating for differences in the volume of secretion and specific protein content or specific enzyme activities.

The specific and total colipase content showed a prandial increase; the infusion of coconut oil evoked an elevated secretion which resulted in a different slope of the curve for the total colipase content compared to the saline and olive oil treatment. As the coconut oil treatment

resulted in the greatest increase in total colipase content greatest, it can be concluded, that fats containing mainly saturated, medium chain fatty acids, such as coconut oil, stimulate total colipase secretion more than unsaturated, long chain fatty acids, such as olive oil.

Moreover, it is known that pancreatic procolipase is activated by trypsin to colipase, with a simultaneous formation of colipase and enterostatin (Erlanson-Albertsson et al., 1991). In the present study, a positive correlation between specific trypsin activities and specific colipase contents was obtained for each individual treatment.

7.4. Conclusion and implication

The results of these studies confirm previous conclusions that the response of the exocrine pancreas is affected by diet composition. Moreover, there is clear evidence that in addition to long-term adaptive mechanisms the exocrine pancreas responds spontaneously to the intraduodenal infusion of various nutrients such as potato fibre and lipids of different origin and composition.

It can be speculated if the stimulating effect of dietary fibre on exocrine pancreatic secretions could be attributed to the production of short chain fatty acids (SCFA) in the large intestine. It can be derived from studies in other species that SCFA may stimulate the exocrine pancreas via a well described insulin-pancreatic acinar axis. In addition, the present results provide evidence that higher enzyme activities in pancreatic juice are not necessarily associated with higher CCK levels in plasma. Further studies in pigs are warranted to elucidate possible stimulating effects of SCFA on the interdigestive, postprandial and gut hormone stimulated pancreatic secretion.

Lipids of different composition and origin evoked different responses of the exocrine pancreas when infused intraduodenally under prandial conditions. It can be assumed that differences in chain length and degree of saturation of fats will affects the spontaneous response of the exocrine pancreas differently, mediated by CCK and likely by PYY. There is evidence that the secretion of procolipase and trypsinogen is regulated by a common neuro-endocrine pathway. Further studies are warranted to discriminate the mechanisms involved.

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8. SUMMARY

The exocrine pancreas of pigs secrets with the pancreatic juice digestive enzymes, as α-amylase, lipase and trypsin, bicarbonate and electrolytes to the duodenum. It is known that the exocrine pancreas adapts its secretion to dietary changes. The aim of the present study was to investigate the influence of carbohydrates in the form of potato fibre and of fats differing in their fatty acid composition on the exocrine pancreatic secretion and on the regulative hormonal mechanisms.

Fifteen growing pigs obtained from a Swedish Landrace herd weighing approx. 12kg were surgically fitted with a permanent pancreatic cannula. Therefore the pancreatic duct was catheterised with a silicon tubing, which was excorporised at the right side of the animals. This cannula was connected to a re-entrant duodenal T-shaped silicon cannula to allow a permanent flow of the pancreatic juice. Additionally, in 9 pigs were fitted with a permanent jugular vein catheter to allow chronical blood sampling.

Three animals were fed for 2 weeks a standard weaner diet, then 2 weeks the same diet supplemented with 2% potato fibre. After this dietary treatment, the pigs were fed another two weeks the diet without potato fibre. All measured parameters, e.g. volume of pancreatic secretion, protein output and total trypsin, lipase and amylase activities increased after adaptation to the diet with potato fibre and remained at that level after re-adaptation to the diet without potato fibre supplementation.

Twelve animals were fed twice a day a commercial weaner diet. Two experiments were conducted beginning with the morning feeding: In experiment 1 medium chain triglycerol (mct: glyceroltricaprylat), a long chain triglycerol (lct: glyceroltristearat) or saline was infused, in experiment 2 vegetable oils (olive oil and coconut oil) or saline was infused directly to the duodenum. Pancreatic juice was collected over 4h, beginning 1 h preprandially. Additionally, blood for the estimation of the plasma levels of CCK and PYY was obtained in experiment 1. In 1 experiment a time effect could be shown for the volume of secretion after mct infusion. Moreover, a time effect could be found for the total protein secretion after mct and lct infusion, for the specific trypsin activity after lct treatment and for the total trypsin activities after mct and lct infusions. Specific and total lipase activities as well as specific and total colipase contents were influenced over the time by mct infusions. No time effects on hormonal levels could be shown. Time x treatment interactions are found for the volume of secretion after comparison of the slopes of the curves for saline and mct, for the total protein secretion after comparison of mct with lct and saline. The courses of the curves differed between lct and mct for specific lipase activities and after comparison of mct with saline or lct for total lipase activities and colipase contents. Overall means of CCK were decreased in the mct group as compared to the saline and lct group. In experiment 2 a time effect was observed for the volume of secretion after olive oil treatment and all treatment influenced the specific and total protein secretion. Whereas the specific lipase activity and the specific colipase content was influenced by all treatment, olive oil had no influence on the total lipase activity and total colipase content. No time x treatment interactions were observed regarding volume secretion, specific and total protein secretion, trypsin activities and total lipase activities,

whereas an influence was observed between the slopes of the curves regarding specific lipase activities and speific colipase secretion An influence was obeserved regarding the total colipase secretion between coconut and saline treatment. Overall means were elevated regarding the total colipase secretion after coconut treatment and a positive correlation between specific trypsin activities and colipase secretion was found.
The results show an influence of carbohydrates in the form of potato fibre and of various fats on the exocrine pancreatic secretion. Most likely the high non-starch-polysaccharide (NSP) content of the potato fibre induced the observed effects, as other studies have shown that NSP could stimulate the exocrine pancreas. The intraduodenal infusion of fats differing in chain length and degree of saturation evoked different spontaneous reactions of the exocrine pancreas. This implies that the pancreas is capable to adapt immediately its secretion of pancreatic juice to the composition of fats. This spontaneous adaptation is most likely regulated by gastrointestinal hormones as e.g. CCK.

9. ZUSAMMENFASSUNG

Im Gastrointestinaltrakt des Schweines findet die Verdauung und Assimilation verschiedener Nährrstoffe aus dem Futter statt. Das exokrine Pankreas ist hierbei das wichtigste Verdauungsorgan, da es die Verdauungsenzyme α-Amylase, Lipase, Trypsin und Chymotrypsin sowie Bicarbonat und verschiedene Elektrolyte in das Duodenum sekretiert. Dabei reagiert die Sekretion auf quantitative sowie Veränderungen der Nährstoffzufuhr im Futter. Der Einfluß von isolierten Nicht-Stärke-Polysacchariden (NSP) sowie die Wirkung verschiedener Fette mit unterschiedlicher Fettsäurezusammensetzung auf die exokrine Pankreassekretion ist bisher nicht systematisch untersucht worden. Weiterhin wurden in diesem Zusammenhang keine grundlegenden Untersuchungen durchgeführt, die sich mit möglichen Feedback-Mechanismen beschäftigen, die mittels gastrointestinaler Hormone wie z.B. Cholecystokinin (CCK) oder Peptid YY (PYY) die exokrine Pankreassekretion steuern. Ziel dieser Arbeit war es (1) den Einfluß isolierter NSP aus Kartoffeln sowie (2) den Einfluß von synthetischen und pflanzlichen Fetten unterschiedlicher Herkunft und Zusammensetzung auf die exokrine Pankreassekretion des Schweines und deren hormonelle Steuerung zu untersuchen.
Wachsende Schweine im Gewichtsbereich von ca. 12 kg wurden operativ mit permanenten Pankreasumleitungskanülen versehen. Hierbei wurde der *ductus pancreaticus* mit einem Silikontubus katheterisiert, anschließend wurde der Katheter über eine Silikonkanüle an der rechten Flanke des Tieres ausgeführt. Diese Kanüle wurde mit einer weiteren einfachen T-Kanüle aus Silikon, die im proximalen Duodenum implantiert wurde, verbunden, um einen kontinuierlichen extrakorporalen Fluß des Pankreassaftes von der proximalen zur distalen Kanüle zu gewährleisten. Mit Hilfe eines permanten Katheters in der *v. jugularis* konnten Blutproben während der Versuchsphasen kontinuierlich gezogen werden.

Versuch 1: Ein handelsübliches Alleinfutter für wachsende Schweine wurde 3 Schweinen als Kontrolldiät über einen Zeitraum von 2 Wochen verabreicht. Danach wurde für weitere 2 Wochen eine Versuchsdiät auf der Basis der Kontrolldiät, die mit 2% einer NSP-Matrix aus Kartoffeln ergänzt wurde, gefüttert. Anschließend erhielten die Tiere nochmals über einen Zeitraum von 2 Wochen die Kontrolldiät. Die Aufnahme der Versuchsdiät induzierte eine erhöhte ($P<0.05$) Sekretion an Pankreassaft und steigerte die Menge an Gesamtprotein sowie die Gesamtaktivitäten Trypsin, Lipase und α-Amylase im Pankreassekret. Dieser Anstieg in den Parametern der exokrinen Pankreassekretion konnte auch nach Re-adaptation an die Kontrolldiät beobachtet werden. Gleichzeitig fand tendenziell ($P<0.1$) bei Verabreichung der Versuchsdiät eine Abnahme der CCK-Konzentration im Plasma statt. Nach Re-adaptation an die Kontrolldiät war die CCK-Konzentration sogar signifikant ($P<0.05$) niedriger im Vergleich zur ersten Versuchsperiode bei Verzehr der Kontrolldiät. Die Ergebnisse lassen den Schluß zu, daß NSP aus Kartoffeln einen stimulierende Einfluß auf die exokrine Pankreassekretion ausüben. Diese Stimulation wird jedoch nicht durch CCK induziert, was durch die vergleichsweise niedrigen CCK-Konzentrationen nach Adaptation an die Versuchsdiät und Re-adaptation an die Kontrolldiät dokumentiert wird.
Versuch 2: Für 2 verschiedene Experimente standen insgesamt 12 Tiere mit Pankreasumleitungskanülen zur Verfügung. In beiden Experimenten wurden die Tiere mit einem handelsüblichen Alleinfutter für wachsende Schweine gefüttert. In Experiment 1 wurde alternativ Glyceroltricaprylat (medium chain triglycerol (mct); C 8:0), Glyceroltristearat (long chain triglycerol (lct); C 18:0) oder physiologische Kochsalzlösung als Kontrollinfusion intraduodenal appliziert. In Experiment 2 wurde Olivenöl mit einem hohen Anteil an langkettigen, einfach ungesättigten Fettsäuren (C 18:1), Kokosnußöl mit einem hohen Anteil an mittelkettigen, gesättigten Fettsäuren (C 14:0) sowie physiologische Kochsalzlösung als Kontrollinfusion intraduodenal infundiert.
In beiden Experimenten konnte unabhängig von der Infusionsquelle ein prandialer Peak als direkte Reaktion auf die Futteraufnahme für die Volumensekretion, den Gesamtprotein- und Colipasegehalt sowie für die Gesamtaktivitäten von Trypsin und Lipase beobachtet werden.
Experiment 1: Nach Infusion von mct ließ sich der Verlauf der exokrinen Pankreassekretion in zwei Phasen unterteilen: Während in der ersten Phase ein gleichzeitiger prandialer Anstieg der Volumensekretion, des Gesamtprotein- und Colipasegehaltes sowie der Gesamtaktivitäten an Trypsin und Lipase festgestellt werden konnte, ergaben sich für diese Parameter 60 min postprandial in der zweiten Phase deutlich niedrigere Werte gegenüber den praeprandialen Ausgangswerten. Daraus resultiert ein veränderter Verlauf der einzelnen Kurven über die Zeit ($P<0.05$). Die Verläufe der Kurven für die Gesamtaktivitäten an Lipase unterschieden sich voneinander ($P<0.05$). Der diurnale Verlauf der Plasmakonzentrationen an CCK und PYY war nicht beeinflußt, jedoch war die über 4 h gepoolte CCK-Konzentration nach Infusion von mct im Vergleich zur Infusion von lct und physiol. Kochsalzlösung erniedrigt ($P<0.05$).
Experiment 2: Die Volumensekretion des Pankreassaftes zeigte nach Infusion von Kokosnußöl einen zweiphasigen Verlauf. Während in der ersten Phase ein prandialer Peak beobachtet werden konnte, fiel die Volumensekretion in der zweiten Phase um 100% unter die praeprandialen Ausgangswerte, wodurch eine Veränderung ($P<0.05$) im Verlauf der Kurve induziert wurde. Nach Infusion von Olivenöl stieg die spezifische Lipaseaktiviät um das 5-fache. Diese erhöhte Aktivität blieb über einen Zeitraum von 2.5 h nach der Infusion

bestehen. Bei Infusion von Kokosnußöl sowie von Kochsalzlösung konnte nur ein 2.5-facher prandialer Anstieg beobachtet werden, was die Unterschiede der Kurvenverläufe zwischen Olivenöl und Kokosnußöl ($P<0.04$) sowie zwischen Olivenöl und Kochsalzlösung ($P<0.01$) erklärt. Die spezifische Trypsinaktivität und der spezifische Gehalt an Colipase waren für die jeweiligen Infusionsbehandlungen positiv linear miteinander korreliert ($r>0.6$).
Aus den Ergebnissen dieser Untersuchungen läßt sich ableiten, daß Fette unterschiedlicher Kettenlänge und mit unterschiedlichem Sättigungsgrad einen unterschiedlichen Einfluß auf das exokrine Pankreas ausüben. Da unterschiedliche Fettarten die Lipasesekretion verschiedenartig beeinflussen, ist ein regulativer Mechanismus wahrscheinlich. Die spontane Adaptation des exokrinen Pankreas an die unterschiedlichen Fette wird über einen Feedback-Mechanismus gesteuert wobei diese Steuerung nur untergeordnet durch die Hormone CCK und PYY beeinflußt wird . Daher ist anzunehmen, daß andere Hormone wie z.B. Sekretin regulierend wirken. Erstmalig konnte experimentell gezeigt werden, daß die unterschiedliche, spontane Reaktion des exokrinen Pankreas von der Fettzusammensetzung beeinflußt wird. In vergleichbaren Arbeiten konnte ein derartiger Einfluß nicht nachgewiesen werden, da lediglich gepooltes Probenmaterial ausgewertet wurde. Mögliche Defizite in der Enzymproduktion unter prandialen Bedingungen bleiben bei dieser Methodik unberücksichtigt.

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