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Donaldson, Janine

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GROWTH PERFORMANCE AND DIGESTIBILITY IN EXOCRINE PANCREATIC INSUFFICIENT PIGS SUPPLEMENTED WITH A PANCREATIC ENZYME PREPARATION

Janine Donaldson

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science

Johannesburg, 2008

DECLARATION

I declare that the work contained in this dissertation is my own, with all assistance acknowledged. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

…….

(Janine Donaldson)

…… day of …… 2008

ABSTRACT

Exocrine pancreatic insufficiency (EPI) is a major complication of cystic fibrosis. Conventional treatment involves the replacement of pancreatic enzymes and intake of a low fat diet. However, contrary to previous therapeutic strategies, a high fat diet may be beneficial in EPI patients. The present study investigated the effects of dietary supplementation with Creon 10 000 a pancreatic enzyme preparation, in conjunction with a high-fat diet, on growth performance, digestibility and absorption of fat in a pig model of EPI by the surgical ligation of the pancreatic duct in 6 male pigs (Swedish Landrace X Yorkshire X Hampshire). Following surgery, and for the duration of the experimental period, pigs were fed a high fat diet (twice daily). The experimental period lasted for 15 days during which blood, urine and faecal samples were collected. In the last 7 days of the experimental period (days 8-14), Creon 10 000 was included in the high fat meals. Urine and faecal samples were analysed for dry matter, crude protein and fat content. Plasma was used to assess the lipaemic index and the plasma lipid profiles. Treatment with Creon 10 000 significantly increased body mass ($P = 0.016$) and the digestibility of dry matter, crude protein as well as the co-efficient of fat absorption were also significantly improved following treatment ($P<0.05$). Creon 10 000 improved the lipaemic index values and significant changes in plasma free fatty acid and triglyceride concentrations were observed but not in cholesterol or high and low density lipoproteins. This study supports previous reports that the administration of pancreatic enzyme preparations together with a high fat meal is a beneficial strategy for the nutritional management of EPI.

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Table of Contents

DECLARATION ii

ABSTRACT iii

ACKNOWLEDGEMENTS iv

LIST OF FIGURES vii

LIST OF ABBREVIATIONS viii

Chapter 1- Introduction 1

1.1. The exocrine pancreas 2

1.1.1. General structure and function 2

1.1.2. Regulation of exocrine pancreatic secretion 4
i) General Introduction 4
ii) Regulation of pancreatic bicarbonate (HCO_3^-) secretion 6
iii) Regulation of pancreatic digestive enzyme secretion 8
a) Release of pancreatic digestive enzymes 8
c) Feedback regulation of pancreatic enzyme secretion 11
1.1.3. The exocrine pancreatic digestive enzymes 13

1.2. The exocrine pancreas and digestion 16

1.2.1. Factors other than the exocrine pancreas affecting digestion 16
1.2.2. The importance of the exocrine pancreas in the digestion of fat 18
i) Dietary fat processing and the role of pancreatic lipase 18
ii) Effects of reduced pancreatic lipase secretion 22

1.3. Growth and the role of the exocrine pancreas 25

1.4. Exocrine pancreatic insufficiency (EPI) 31

1.4.1. General introduction on EPI 31
1.4.2. Exocrine pancreatic insufficiency in cystic fibrosis 32
1.4.3. Models available to study EPI 34
1.4.4. The pig model of EPI 38

1.5. Aims 40

Chapter 2 - Experiment 41

2.1. Experiment 42

2.1.1. Methods 42
i) Experimental design 42
ii) Animals 42
iii) Surgery 43
a) Pancreatic duct ligation and jugular vein catheterization 43
b) Post-surgical management 45
iv) Feeding 45
v) Experimental procedure 46
a) General experimental procedure 46
b) Dosing with the Creon 10 000 48
c) Feed sample processing 48
d) Collection and processing of faecal and urine samples 49
e) Blood sampling and plasma processing 49
vi) Sample Analysis 50
a) Feed, faecal and urine samples 50
b) Blood samples 51
vii) Data Analysis 53
2.1.2. Results 54
i) Body mass 54
ii) Dry matter and apparent digestibility 54
a) Dry matter (Faecal and Urine) 54
b) Nitrogen balance, crude protein (faecal and urine) 59
c) Fat 63
iii) Plasma lipids 66
a) Turbidimetry 66
b) Plasma lipid profile 69
2.1.3. Discussion 73

Chapter 3 – Conclusion 84

Chapter 4 – References 87

LIST OF FIGURES

Page

Figure 1: Body mass (kg) of pigs measured during the control period and on the first (day 8) and last (day 14) days of treatment with the pancreatic enzyme preparation..... 56

Figure 2: Faecal dry matter content (g) measured during the control and treatment periods 57

Figure 3: Apparent digestibility of dry matter during the control and treatment periods 58

Figure 4: Crude protein content (g) in faeces during the control and treatment periods . 61

Figure 5: Apparent digestibility of crude protein on each of the three collection days during the control and treatment periods 62

Figure 6: Fat content (g) in the faeces measured during control and treatment periods .. 64

Figure 7: CFA values calculated on each of the three collection days during the control and treatment periods..... 65

Figure 8: Calculated lipaemic index values for each blood sample taken across the 24hr blood sampling period, before treatment (day 6), and on the first (day 8) and last (day 14) days of treatment..... 68

Figure 9: Free fatty acid (FFA) concentration ($mmol.l^{-1}$) measured during the control period (day 6) and on the first (day 8) and last days (day 14) of treatment 71

Figure 10: Triglyceride (TG) concentration ($mmol.l^{-1}$) measured during the control period (day 6) and on the first (day 8) and last days (day 14) of treatment 72

LIST OF ABBREVIATIONS

Arachidonic acid	AA
Bicarbonate	HCO_3^-
Central nervous system	CNS
Cholecystokinin	CCK
Co-efficient of fat absorption	CFA
Cyclic adenosine monophosphate	cAMP
Cystic fibrosis	CF
Cystic fibrosis transmembrane regulator	CFTR
Docosahexaenoic acid	DHA
Enteric nervous system	ENS
Essential fatty acid deficiency	EFAD
Exocrine pancreatic insufficiency	EPI
Free fatty acids	FFA
Gastrointestinal tract	GIT
High density lipoproteins	HDL
Low density lipoproteins	LDL
Long-chain polyunsaturated fatty acids	LCPUFA
Pancreatic acinar atrophy	PAA
Pancreatic enzyme replacement therapy	PERT
Pancreatic polypeptide	PP
Peptide YY	PYY

Triglycerides	TG
Vasoactive intestinal peptide	VIP

Chapter 1- Introduction

1.1. The exocrine pancreas

1.1.1. General structure and function

The pancreas is one of the largest glands associated with the gastrointestinal tract and is situated retroperitoneally in the abdomen, close to the second and third lumbar vertebrae. The head of the human pancreas lies in the concavity of the duodenum, with its body extending towards the posterior wall of the abdomen and its tail towards the hilus of the spleen (Egerbacher & Bock, 1997; Motta *et al*., 1997). The pig pancreas consists of a right and left lobe. The right lobe or duodenal lobe which is located in the first duodenal loop corresponds to the head of the human pancreas. The left lobe or splenic lobe, which extends towards the spleen, corresponds to the tail of the human pancreas. The bile duct of the pig enters into the duodenum and the accessory pancreatic duct, if found, leads from the duodenal lobe of the pig pancreas to the duodenum, about 10-13cm behind the opening of the bile duct (Xu *et al*., 1999).

The pancreas is composed of two distinct sections which differ from one another in both morphology and function. They are: the endocrine pancreas which produces hormones mainly involved in the regulation of carbohydrate metabolism and thus, blood sugar levels and the exocrine pancreas which produces pancreatic juice

containing bicarbonate (HCO_3^-) and digestive enzymes necessary for digestion (Motta *et al*., 1997).

The endocrine cells of the pancreas comprise about 2-3% of the total pancreatic cell mass and are located within the Islets of Langerhans. The Islets of Langerhans contain four main types of endocrine cells, all secreting different hormones. The cell types include: the α-cells which produce glucagon, the β-cells which produce insulin, the δ-cells which produce somatostatin and the PP-cells which produce pancreatic polypeptide (Kim & Hebrok, 2001; Konturek *et al.*, 2003). These endocrine hormones first perfuse the surrounding acinar cells before reaching the general circulation, thus also playing a role in the regulation of digestive enzyme synthesis, transport and secretion (Konturek *et al*., 2003). The exocrine component of the pancreas makes up about 95% of the total pancreatic cell mass and consists of two major functional parts namely, the ductal cells (5%) and the acinar cells (90%). Together these exocrine cells are responsible for the secretion of the pancreatic juice which is released in response to the presence of acidic chyme and digestive products within the duodenum as well as a variety of cephalic and gastric signals. The ductal cells are largely responsible for the secretion of both HCO_3^- and water which form part of the aqueous component of the pancreatic juice. The aqueous component, the release of which is stimulated primarily by the hormone secretin, functions in the neutralization of duodenal contents as well as providing an optimal pH for the activity of both the pancreatic and intestinal brush border digestive enzymes (Konturek *et al*., 2003). The acinar cells function in the synthesis and storage of

digestive enzymes which are released into the duodenum in response to various secretagogues such as cholecystokinin (CCK), neuropeptides and neurotransmitters. These secretagogues act via the activation of specific receptors on the membrane of the acinar cells to cause the fusion of the zymogen granules (in which the digestive enzymes are stored) with the acinar cell membrane resulting in the release of the digestive enzymes by exocytosis (Motta *et al*., 1997).

1.1.2. Regulation of exocrine pancreatic secretion

i) General Introduction

The regulation of exocrine pancreatic secretion is a complex process involving both neural and hormonal controls, with various mechanisms contributing to pancreatic regulation still to be elucidated.

The pancreas is innervated by both sympathetic and parasympathetic nerve fibres which together form a significant intrinsic nerve plexus which enables the pancreas to act independently from both the central nervous system (CNS) and the gut (Niebergall-Roth & Singer, 2001). These unmyelinated nerve fibres are distributed throughout the interlobular connective tissues making up the pancreas, innervating both the glandular cells and the pancreatic vessels (Konturek *et al.*, 2003). The majority of the preganglionic parasympathetic nerve fibres terminate on the pancreatic ganglia, whereas the postganglionic sympathetic fibres are distributed to

pancreatic ganglia as well as the pancreatic islets, blood vessels and ducts. The innervation of the exocrine pancreas by the sympathetic nervous system is modest compared to that of the islets and pancreatic blood vessels, thus the parasympathetic nervous system plays the major role in directly regulating exocrine pancreatic secretion (Niebergall-Roth & Singer, 2001). The sympathetic regulation of the exocrine pancreas is indirect, inhibiting pancreatic secretion by decreasing blood flow and inhibiting transmission in the pancreatic ganglia (Love *et al.*, 2007).

The pancreatic neurons are targets for all classes of extrinsic nerves which innervate the pancreas, including the vagal efferent nerves. The entero-pancreatic neurons of the stomach and duodenum also terminate in the pancreatic ganglia, thus allowing local control by the gut. The pancreatic neurons are then responsible for the transmission of signals via afferent fibres of external autonomic nerves to the enteric (ENS) or central (CNS) nervous systems, and then from the ENS or CNS via efferent autonomic nerves to the secretory cells of the pancreas (Konturek *et al.*, 2003; Love *et al.*, 2007). Thus, it is clear that both entero-pancreatic reflexes and vagovagal reflexes function in the regulation of the exocrine pancreas.

In addition to the neural components affecting exocrine pancreatic secretion, various other peptide hormones are also involved in the control of pancreatic exocrine secretion. The pancreatic endocrine hormones first supply the exocrine portion of the pancreas before reaching the circulation thus contributing to the regulation of pancreatic enzyme synthesis, transport and secretion (Konturek *et al.*, 2003).

When examining the regulation of the exocrine pancreas it is easier to assess the regulatory control in terms of the two main functional parts of the exocrine pancreas, namely the ductal and acinar cells and their respective secretions.

ii) Regulation of pancreatic bicarbonate (HCO_3^-) secretion

The ductal cells are responsible for the secretion of the aqueous component of the pancreatic juice, containing both HCO_3^- and water. The aqueous component is responsible for the neutralization of duodenal contents as well as providing an optimal pH for the activity of both the pancreatic and intestinal brush border digestive enzymes. The regulation of both HCO_3^- and water secretion vary slightly with different animal species. Humans, pigs, dogs and cats display relatively low levels of HCO_3^- and water secretion under basal conditions with increased secretion in response to secretin stimulation (Konturek *et al*., 2003). Secretin is a 27 amino acid peptide hormone secreted from the duodenal S endocrine cells in response to the presence of acidic chyme in the duodenum (Davis *et al*., 2004). It acts on receptors on the basolateral membrane of the pancreatic ductal cells to bring about its effects. The secretion of HCO_3^- by the pancreatic ductal cells in response to secretin stimulation is mediated by a number of Cl^-/ HCO_3^- anion exchangers which operate in conjunction with the cystic fibrosis transmembrane regulators (CFTR's). The HCO_3^- produced within the ductal cells is transported into the intestinal lumen in exchange for Cl^- ions, which are supplied to the lumen by the CFTR's (Ishiguro *et al*., 2002; Konturek

et al., 2003). This mechanism can only account for the secretion of HCO_3^- in species such as rats, it can not account for the much higher levels of HCO_3^- secreted in species such as humans. Previous studies have shown that secretin is able to stimulate a HCO_3^- rich secretion from the pancreas after the intestinal lumen was injected with a solution containing high HCO_3^- concentrations and low Cl^- concentrations, a situation in which the active Cl^-/ HCO_3^- anion exchangers would then facilitate the absorption of HCO_3^- rather than the secretion of HCO_3^- (Ishiguro *et al*., 2002). Furthermore, it has been demonstrated that the Cl^-/ HCO_3^- anion exchangers are inhibited under conditions of high luminal HCO_3^- concentrations, thus in order for HCO_3^- to be transported into the lumen it must do so against a very steep concentration gradient (Ishiguro *et al*., 2002). Ishiguro *et al*. (2001) demonstrated the importance of a large electrochemical gradient which in fact drives the HCO_3^- across the membrane into the lumen despite the high HCO_3^- concentrations inside the intestinal lumen. The electrochemical gradient is largely attributed to Na^+/ HCO_3^- and Na^+/ H^+ pumps located within the pancreatic ducts (Ishiguro *et al*., 2001).

Enhanced stimulation of the ductal cells by secretin results in a raised HCO_3^- concentration within the pancreatic juice and in turn a higher pH and decreased Cl^- concentration. Besides secretin, there is another peptide which also acts on the receptors present on the basolateral membrane of the pancreatic ductal cells to bring about similar effects, namely vasoactive intestinal peptide (VIP). In both pigs and guinea pigs, a large number of VIP neurons are present within the pancreas which

release large amounts of VIP during vagal stimulation thus evoking HCO_3^- secretion in a manner similar to that of secretin (Konturek *et al*., 2003).

Since the HCO_3^- and water secretion by the pancreatic ductal cells plays an important role in the maintenance of an optimal duodenal pH, it is clear that duodenal pH is in fact the major regulator of secretin release. However, there is a threshold pH value for secretin release of 4.5, below which the HCO_3^- secreted is related to the increase in plasma secretin concentration which depends upon the amount of acid chyme that reaches the duodenum (Konturek *et al*., 2003).

iii) Regulation of pancreatic digestive enzyme secretion

a) Release of pancreatic digestive enzymes

The pancreatic digestive enzymes are synthesised and stored within the pancreatic acinar cells and are released by a process of exocytosis in response to the binding of various secretagogues to receptors on the acinar cell surface. The receptors involved produce their effects through the interaction with a G-protein complex resulting in the stimulation of phospholipase C activity which in turn causes the formation of inositol-1,4,5-triphosphate. Inositol-1,4,5-triphosphate then binds to various intracellular receptors resulting in the release of Ca^{2+} from intracellular stores. The increased Ca^{2+} concentration within the acinar cells causes the fusion of the zymogen granules (in which the enzymes are stored) with the plasma membrane, releasing the

digestive enzymes into the lumen by exocytosis. Some of the secretagogues involved in the release of the pancreatic digestive enzymes from the acinar cells include CCK, neuropeptides and neurotransmitters (Konturek *et al*., 2003; Niebergall-Roth & Singer, 2001).

CCK is considered to be the most important mediator of exocrine pancreatic secretion (Niebergall-Roth & Singer, 2001). CCK is produced and released by the intestinal I-cells and neurones within the duodenum and jejunum in response to the presence of the digestive products of both proteins and lipids from ingested food (Pierzynowski *et al*., 2005). The most potent stimulator of CCK release with respect to lipid hydrolysis products are fatty acids with longer acyl chains, whereas tryptophan and alanine are the most potent stimulants of the protein breakdown products (Wang & Cui, 2007). CCK exerts its effects via two known receptors, namely CCK1R and CCK2R which are members of the G-protein-coupled receptor family. CCK1R binds only CCK, whereas the CCK2R receptor is able to bind both CCK and gastrin (Rengman *et al*., 2007). CCK1R receptors are found in the gall bladder, the exocrine pancreas and in some areas of the CNS and are selective for the sulphated CCK molecules, whereas CCK2R receptors are selective for both sulphated and non-sulphated CCK molecules and are widely dispersed throughout the stomach and the brain (Wang & Cui, 2007). CCK is able to exert its effects on exocrine pancreatic secretion either by the direct stimulation of the CCK receptors on the surface of pancreatic acinar cells or via various neural pathways. The mechanism by which CCK brings about the exocrine pancreatic secretion differs with different animal species. In the rat, CCK stimulation

of both the neural pathways as well as the direct stimulation of the pancreatic acinar cells results in the exocrine pancreatic secretion. Whereas in humans, CCK acts mainly via the neural pathways in order to bring about exocrine pancreatic secretion as there are no significant CCK binding sites present on the human pancreatic acinar cells (Wang & Cui, 2007; Yamamoto *et al*., 2005).

b) Pre- and postprandial pancreatic digestive enzyme secretion

Secretion of the digestive enzymes from the pancreas occurs continuously throughout both the fasting state as well as in the digestive state. During the fasting state, when the upper GIT does not contain any food, in most animal species pancreatic enzyme secretion is somewhat cyclic and is thought to be a result of cholinergic activation, resulting in raised plasma motilin levels and the activation of the duodenal migrating myoelectric complex cycle, which in turn produces a cyclic basal pancreatic secretion. Basal pancreatic enzyme secretion is relatively low compared to the response elicited by the exocrine pancreas after food is ingested (Konturek *et al*., 2003). Upon the ingestion of food, the digestive state of exocrine pancreatic secretion is initiated and can be divided into three different phases, coinciding with the phases of digestion. They are: the cephalic phase, the gastric phase and the intestinal phase and contribute to about 20%, 10% and 70% of the pancreatic postprandial response to a meal, respectively (Ishiguro *et al*., 2001; Konturek *et al*., 2003). The cephalic phase of pancreatic secretion is stimulated primarily by the sight, smell and taste of food as well as the chewing and swallowing actions upon ingestion of food. The cephalic

response is said to be mediated by the vagus nerve, as previous studies have shown that the effect could be abolished with the cooling of the vagi (Zabielski & Naruse, 1999). The next phase is referred to as the gastric phase and is brought about by a vagally-mediated reflex which occurs as a result of the distension of the stomach with ingested food. The last phase of the pancreatic postprandial response is known as the intestinal phase, which accounts for the majority of the pancreatic secretion. The intestinal phase is mediated primarily by the hormones CCK and secretin, the release of which is stimulated by the presence of acidic chyme and digestive products in the duodenum (Zabielski & Naruse, 1999).

c) Feedback regulation of pancreatic enzyme secretion

Negative feedback systems in the control of pancreatic enzyme secretion have been observed in a number of different animal species including chickens, rats and pigs (Owang *et al.*, 1986). By preventing the pancreatico-biliary juice from entering the proximal intestine a significant increase in secretion of the pancreatic enzymes was observed and then when the pancreatico-biliary juice was infused into the duodenum, the pancreatic enzyme secretion was suppressed (Owang *et al.*, 1986). Previous studies also observed that dietary trypsin inhibitors were able to provoke excessive pancreatic enzyme secretion in rats (Fushiki & Iwai, 1989). The feedback regulation of pancreatic enzyme secretion which exists in man is different to that in rats and pigs. The basal amounts of trypsin in the duodenum of man, are too low to be able to exert any inhibitory effects, however the intraduodenal perfusion of trypsin inhibits

pancreatic enzyme secretion (Owang *et al.*, 1986). The exact mechanism by which feedback regulation of pancreatic enzyme secretion occurs is still unknown. Previous studies had suggested that the process is mediated by a hormone secreted by the proximal small intestine, which was later established to be CCK. Reduced levels of trypsin and chymotrypsin result in increased levels of circulating CCK which in turn increases pancreatic enzyme secretion (Fushiki & Iwai, 1989; Owang *et al.*, 1986). Pierzynowski *et al.* (2007), observed that pancreatic juice, bile and bile salts when infused into the ileum, inhibited prandial pancreatic secretion. Thus, confirming the existence of inhibitory mechanisms located in the ileum, which regulate exocrine pancreatic secretions, these inhibitory mechanisms are referred to as the "ileal brake". A variety of substances present in the ileal lumen are able to induce a reduction in pancreatic secretion, thus the "ileal brake" refers to a common non-specific mechanism by which pancreatic enzyme secretion is regulated (Pierzynowski *et al.*, 2007).

The presence of a feedback regulation control system of pancreatic enzyme secretion in humans has important clinical implications specifically in the treatment of pain experienced by patients with chronic pancreatitis (Fushiki & Iwai, 1989). Patients with pancreatitis or exocrine pancreatic insufficiency have elevated plasma CCK concentrations due to failure of the feedback system to regulate CCK release in the absence of pancreatic enzyme secretion. The elevated CCK levels in turn cause the pancreas to be over stimulated and the patient experiences pain (Owang *et al.*, 1986). Thus in order to alleviate the pain experienced by patients, the stimulation of the

pancreas by CCK should be reduced which would then reduce pancreatic secretions and thus decrease ductal pressure and pain. Previous studies have shown that large doses of pancreatic extracts rich in proteases have relieved pain in patients with chronic pancreatitis (Fushiki & Iwai, 1989).

1.1.3. The exocrine pancreatic digestive enzymes

The pancreatic digestive enzymes released from the acinar cells play an essential role in the digestion of all major food classes (Beck, 1973; Konturek *et al.*, 2003). Digestion by pancreatic enzymes accounts for approximately half of the overall digestion which occurs within the digestive tract (Brannon, 1990).

The three major classes of exocrine pancreatic enzymes are: the proteases, lipases and amylase (Lavau *et al*., 1974).

The pancreatic proteases all have similar three dimensional structures, containing a reactive serine residue in the active site and form part of the family of endopeptidases. Trypsin and chymotrypsin are the main pancreatic proteases. Trypsin is responsible for the cleavage of peptide bonds between basic amino acids such as lysine and arginine and the next amino acid. Trypsin is secreted in its inactive form as trypsinogen and consists of 223 amino acid residues; it is then activated by active trypsin through the cleavage of the N-terminal octapeptide. Chymotrypsin is responsible for the cleavage of the peptide bonds between aromatic residues such as

phenylalanine, tryptophan and tyrosine and the next residue. Both trypsin and chymotrypsin have several isozymes depending on different species (Beck, 1973; Brannon, 1990; Konturek *et al.*, 2003). Another enzyme which also plays a role in the breakdown of proteins is carboxypeptidase. Carboxypeptidase is secreted by the exocrine pancreas and is responsible for the hydrolysis of alimentary proteins and peptides from their COOH-terminal residues. There are two different types of carboxypeptidases which are classified according to the type of terminal residues they hydrolyse. The A-type has a preference for apolar COOH-terminal residues and the B-type has a preference for basic COOH-terminal residues (Joshi & St. Leger, 1999).

Pancreatic lipase is produced by the exocrine pancreas and is secreted as an active enzyme consisting of 449 amino acid residues including a complex glycan chain (Miled *et al.*, 2000). Pancreatic lipase has a histidine residue in its catalytic site and a serine residue in its active binding site and acts at the lipid/water interface to hydrolyse and convert triglycerides to diglycerides and then to monoglycerides and fatty-acids, through the cleavage of ester bonds. The monoglycerides and free fatty-acids which are partitioned into micelles then cross the unstirred water layer at the lipid/water interface and are absorbed by the enterocyte (Brannon, 1990; Konturek *et al*., 2003). Colipase also plays a very important role in the digestion and absorption of dietary fats. Colipase is secreted in its inactive form as procolipase and then tryptic activity results in the cleavage of the peptide from procolipase to become colipase. Colipase is essential in the functioning of pancreatic lipase as it stabilises the lipase in the presence of inhibitors such as bile salts, phospholipids and dietary proteins (D'

Agostino *et al*., 2002; Lowe, 1997). Procolipase was also demonstrated to play an important role in the regulation of body mass. Studies involving the peptide which is cleaved from procolipase have demonstrated that when the peptide is injected into rabbits the rabbits experienced weight loss. The peptide is now known as enterostatin and has been proven to decrease the voluntary intake of dietary fats and thus has been shown to play a role in long term weight loss (D' Agostino *et al*., 2002; Lowe, 1997).

Amylase produced by the exocrine pancreas is secreted in its active form. It is responsible for hydrolysing α-1,4-glucosidic bonds in oligosaccharides of four units or more, forming maltose and smaller oligosaccharides in the process (Brannon, 1990).

1.2. The exocrine pancreas and digestion

1.2.1. Factors other than the exocrine pancreas affecting digestion

In addition to the role the pancreatic enzymes play in the digestion of ingested food, a host of other factors affect the process of digestion, including specific dietary properties of ingested food.

Previous studies focussing on the effects of dietary carbohydrates on postprandial lipid metabolism have found that several steps within the complex processing of dietary lipids can be altered depending upon whether the diet is rich in digestible or indigestible carbohydrates (Lairon *et al*., 2007). Diets rich in digestible carbohydrates result in lower levels of lipases secreted by both the gastric mucosa and the pancreas, whereas diets rich in indigestible carbohydrates or dietary fibre have been shown to cause increased lipase levels and output into the duodenum (Lairon *et al*., 2007). Thus, if one consumes a diet rich in digestible carbohydrates, lower levels of lipases are available to take part in the hydrolysis of dietary fats and therefore less fat is digested and absorbed. Whereas if one consumes a diet rich in indigestible carbohydrates, more lipases are available to take part in the hydrolysis of dietary lipids, thus more lipids are digested and ultimately more of the ingested dietary lipids are absorbed.

Besides the components ingested together with the dietary lipids, the form in which the lipids themselves are ingested can also affect the digestive process. In weanling pigs, it has been shown that by increasing the level of fat in the diet, an improved overall daily weight gain and feed efficiency results. However, this phenomenon primarily occurs during the later stages of the nursery period and is believed to be due to the fact that piglets have a particularly low fat digestibility in the early nursery phase (Cera *et al*., 1988; Cera *et al*., 1989). Studies focusing on the effect of emulsification and fat encapsulation on weanling performance and nutrient digestibility have found that by supplementing emulsifiers into the high fat diet of weanlings, fat digestibility is improved and through the encapsulation of the fat in the diet, the structure of the fats are altered thus improving their utilization during the early nursery period (Xing *et al*., 2004).

In addition to the structure of ingested fats being able to alter the digestive process, the amount of fat ingested also affects the digestive process. In fact, previous studies have shown that the distribution of all the pancreatic enzymes changes in response to the varying amounts of components ingested within the diet, thus the ingested amount of proteins etc. also play a role in determining the digestive processes and pancreatic enzyme levels which follow (Behrman & Kare, 1969). In a study involving the changes in distribution of canine pancreatic enzymes in response to varying diet composition, it was found that by increasing the level of dietary fat ingested a significantly increased lipase activity within the pancreatic juice was observed. In the same study it was also observed that the increase in dietary fat level had no effect on

the protease activity, however by increasing the dietary protein intake, increased levels of protease activity were also observed (Behrman & Kare, 1969). Another study involving the levels of pancreatic lipase in the rat also demonstrated an increased level of pancreatic lipase in response to an increased dietary fat content. The mechanism by which the increase in lipase levels arises is still unclear but it has been thought to involve an increase in the rate of biosynthesis of pancreatic lipase, however how the increased dietary fat levels affect the enzyme synthesizing systems to bring about the increase is unknown (Gidez, 1973; Lavau *et al*., 1974).

Thus, it is clear that in addition to the pancreatic digestive enzymes themselves, a number of other factors also affect the digestive process specifically the dietary components. In particular the amount of nutrients ingested within the diet (i.e. the amount of carbohydrates, lipids, proteins etc.) as well as the form in which they are ingested plays an important role in the digestive process.

1.2.2. The importance of the exocrine pancreas in the digestion of fat

i) Dietary fat processing and the role of pancreatic lipase

Digestion as a result of the pancreatic digestive enzymes accounts for approximately half of the overall digestion which takes place within the digestive tract (Beck, 1973; Brannon 1990).

In the present study, I will be focussing on the digestion of fat, as previous studies involving the relationship between pancreatic lipase output and malabsorption in patients with severe pancreatic insufficiency have demonstrated that steatorrhea (the presence of fat in the faeces) only occurs when the lipase output is 10% of the normal level (DiMagno *et al.,* 1973). These observations in turn led DiMagno *et al.* (1973) to conclude that the exocrine pancreas has a large reserve capacity for enzyme secretion, thus secreting more digestive enzymes than that required for normal digestion. Additionally, it has been noted that the decrease in lipase activity is one of the most critical events in the course of chronic pancreatic disorders and even with standard pancreatic enzyme replacement therapy, the normalisation of fat digestion does not occur (Carriere *et al.*, 2005).

Digestion and absorption of fat are highly efficient processes which enable as much as 95% of the dietary lipids ingested by humans to be absorbed. Numerous organs are involved in the processing of dietary lipids including the stomach, liver, small intestine and pancreas. The process of the digestion of fat can be divided into a number of different stages, including hydrolysis, emulsification, micellization and the uptake of lipids by the enterocytes (Miled *et al*., 2000). Fat digestion is initiated in the stomach by the preduodenal lipases, which include lingual lipase which is secreted by the serous glands of the tongue and gastric lipase which is secreted by the chief cells of the stomach (Aoubala *et al*., 1995; Lairon *et al*., 2007). These preduodenal lipases, together with the peristaltic action of the stomach hydrolyse a small amount of the dietary triglycerides (Huggins *et al*., 2003; Hui & Howles, 2002). The remaining

undigested lipids are then delivered to the small intestine where the lipids are emulsified. Emulsification of the dietary lipids involves the conversion of large lipid droplets into smaller lipid droplets in order to increase the surface area available to the water-soluble lipolytic enzymes. In order to prevent the smaller lipid droplets from coalescing, stabilizers in the form of bile salts and lecithin are required (Borgstrom, 1975; Miled *et al*., 2000). Once in the small intestine, it is the pancreatic lipase present in the pancreatic juice which is responsible for the hydrolysis of the bulk of the ingested dietary lipid. Colipase, which is secreted by the pancreas in its inactive form as procolipase, is activated by trypsin in the intestinal lumen. It then binds to the pancreatic lipase as well as a bile acid and in doing so allows the lipase to act at the oil-water interface to hydrolyse the lipids within the emulsion droplet (Borgstrom, 1975; Huggins *et al*., 2003). Pancreatic lipase is in fact inhibited by physiological concentrations of bile salts in the duodenum and thus is dependent on colipase for its activity in the presence of bile salts (Lowe, 1997). Lipolysis can be divided into three distinct steps. Firstly, the triglyceride molecule is converted to a diglyceride and a single fatty acid. Secondly, the diglyceride molecule is converted to a monoglyceride by splitting off another fatty acid. After two of the fatty acids have been removed, the third fatty acid remains attached to the glycerol. Lastly the remaining fatty acid is removed from the monoglyceride, yielding a single free fatty acid and glycerol. Monoglycerides and free fatty acids are the main products of triglyceride lipolysis and they, together with bile salts, lecithin, fat-soluble vitamins and cholesterol form micelles (Beck, 1973).

The formation of micelles is a vital step to ensure the absorption of lipid digestion products as it enables the fat-soluble molecules to diffuse across the brush border membrane. Previous studies have demonstrated that the uptake of fatty acids by the enterocyte is dependent on the micellar concentration of the fatty acids in the aqueous phase of the intestinal lumen (Hofmann & Borgstrom, 1964). However, although the micelles greatly facilitate the uptake of fatty acids, the entire micelle does not diffuse across the brush border membrane, the micelle merely diffuses to the surface of the brush border membrane and the lipid soluble contents leave the micelle and diffuse passively or are actively transported across the membrane (Hofman & Simmonds, 1971). Micellar solubilization is essential in the uptake of fatty acids as the brush border membrane of enterocytes is actually separated from the bulk of the luminal contents by an unstirred fluid layer. Thus, the molecules in the bulk phase of the intestinal lumen can only come into contact with the brush border membrane once they have diffused across the unstirred fluid layer. The formation of micelles greatly enhances the concentration of fatty acids available for uptake by the enterocyte as the micelles are able to diffuse across the unstirred fluid layer, whereas the solubility of individual fatty acids in the bulk aqueous phase is low and thus few fatty acid molecules would be able to gain access to the brush border membrane (Tso *et al*., 2004). Most of the absorption of the lipid digestion products takes place in the duodenum and jejunum, by passive diffusion or by active protein-facilitated processes; with most of the ingested fat being absorbed by the time the chyme reaches the middle section of the jejunum (Lairon *et al*., 2007; Staarup & Hoy, 2000).

Once the products of fat digestion have been absorbed, they are transported to the smooth endoplasmic reticulum of the intestinal epithelial cells, where the free fatty acids and monoglycerides are resynthesized into triglycerides (Hui & Howles, 2002). Together with the other products of lipid digestion, the triglycerides are packaged into lipid droplets known as chylomicrons, with phospholipids covering the external surface of the lipid droplet. The chylomicrons are then released from the epithelial cell via exocytosis and are transported to the systemic circulation from the lymphatic system via the thoracic duct. The chylomicrons are then transported by the blood to both adipose and muscle tissue, where free fatty acids and monoglycerides are released and enter the adipose and muscle tissue. The remnants of the chylomicrons are then transported to the liver for degradation (Huggins *et al*., 2003; Hui & Howles, 2002).

ii) Effects of reduced pancreatic lipase secretion

Now that I have focussed on the processing of dietary lipids and the involvement of pancreatic lipase in that process, it is clear that any disturbance in the function of pancreatic lipase would cause a disruption in the entire process of dietary lipid processing and have numerous consequences in terms of the lipids made available to the body.

Lipids have many beneficial effects within the body. They form integral components of cell membranes, act as carriers for fat-soluble vitamins and act as thermal

insulators and shock absorbers. Lipids also serve as an energy substrate and are precursors for eicosanoids which aid in the regulation of blood pressure, blood clotting and immune functions (D'Agostino & Lowe, 2004; Mattes, 2005). Thus, in the case of a condition such as exocrine pancreatic insufficiency (EPI), the reduced or absent pancreatic lipase secretion would result in reduced dietary fat digestion and thus reduced fat absorption, resulting in less fat made available to the body to carry out its vital functions and more dietary fat excreted in the faeces (steatorrhea).

In addition to the ingested fat playing an important role within the body, the actual process of digesting fat is also important, resulting in a number of different responses within the body. Many previous studies have shown that the infusion of fat into the duodenum results in the slowing of gastric emptying, reduced hunger and thus reduced food intake (Heddle *et al*., 1989). However, recent studies involving both humans and animals have suggested that the effects brought about by the intraduodenal infusion of fat are in fact dependent on the lipolysis of dietary triglycerides to fatty acids. Thus, conditions such as EPI, with a form of lipase inhibition, are associated with rapid gastric emptying of fat resulting in reduced interaction of the ingested fat molecules with the intestinal receptors involved in triggering the normal responses to fat. This in turn leads to various gastrointestinal side effects and reduced feed back signals to slow gastric emptying and decrease appetite (Raybould *et al*., 1998).

Other studies involving the effects of intraduodenal lipid infusion on ghrelin, pancreatic polypeptide (PP) and peptide YY (PYY) release have shown that the fat-induced stimulation of PYY and PP and the suppression of ghrelin is in fact dependent on the lipolysis of ingested dietary lipids (Feinle-Bisset *et al*., 2005). Ghrelin has been shown to play a role in meal initiation, thus stimulating appetite and subsequently increasing food intake. Orally ingested fat suppresses ghrelin secretion thus suppressing appetite and food intake. This suppressive effect of ingested fat on ghrelin secretion is found to be modulated by the interaction of fat digestion products with the gut (Broglio *et al*., 2004; Feinle-Bisset *et al*., 2005; Feinle *et al*., 2003). Thus, EPI patients with reduced pancreatic lipase levels and in turn, reduced fat digestion and absorption should experience an increased appetite and increased food intake.

1.3. Growth and the role of the exocrine pancreas

The term ‘growth’ can be described as a variety of processes by which an individual increases in weight, height, organ size as well as in their ability to function and adapt to various environmental stresses (Washburn, 1950). Human growth, viewed as a long term process, is reasonably regular and is characterised by a pattern of changing height velocity from infancy to adulthood. There are four distinctive human growth phases namely, the foetal growth phase, infancy, childhood and puberty, each with altering height velocities. Following birth a large increase in height velocity is observed with a rapid deceleration in velocity when the child reaches about 3 years old. After 3 years of age, the child experiences a period of slow and lowered height velocity until reaching puberty. Puberty then begins with an increasing height velocity which continues until the individual reaches the age of peak height velocity, after which a deceleration is observed until growth actually ceases (Boersma & Wit, 1997). Previous studies involving the various growth phases have shown that the four human growth phases are not necessarily independent of one another and that it is possible that the growth experienced during a single growth phase may affect the growth experienced in subsequent growth phases. For example if a child does not gain a lot of height during the childhood phase, this might result in an increased gain in height during the puberty phase (Luo & Karlberg, 2000).

In order for growth to take place within the lifespan of the individual, the millions of cells making up the individual's body must be supplied with sufficient nutrient material to enable them to multiply and reproduce new cells. The chemicals required by the cells to accomplish their various activities are referred to as "essential foods" and must be ingested in the diet of the individual to ensure that the growth process does not fail or become distorted. If however, for some reason the individual experiences a period of malnutrition, the growth of that specific individual will be transiently inhibited (Washburn, 1950). Following periods of growth inhibition, a phenomenon referred to as "catch-up" growth is usually observed. Catch-up growth refers to an increased height velocity to above those normal values for individuals at a specific age or maturity level. The purpose of this 'catch-up' growth period is an attempt to return the child to its pre-retardation growth curve (Boersma & Wit, 1997). Three different types of catch-up growth phases have been identified. The first type involves a significant increase in height velocity to such an extent that the deficit in growth is quickly eliminated. This type of catch-up growth occurs mainly during infancy and childhood growth phases. The second type of catch-up growth is somewhat delayed following the growth retardation, however the growth continues for a longer period of time thus ultimately eliminating the growth deficit. This specific type of catch-up growth is seen mainly during adolescence. The last type of catch-up growth is a mixture of both previous types of catch-up growth, where there is a rapid increase in height velocity following the growth deficit as well as a delay in the growth process thus causing it to be prolonged (Boersma & Wit, 1997). With respect to the "essential foods" required in order for growth to take place, some

studies have demonstrated the importance of specific nutrients for infant growth. Previous studies on infant growth have demonstrated a relationship between perinatal fatty acid metabolism and early human growth. These studies observed an inverse relationship between the birth weight of infants and the plasma lipid content of long-chain polyunsaturated fatty acids (LCPUFA) such as arachidonic acid (AA) and docosahexaenoic acid (DHA) (Koletzko, 2001). At first these results were considered not to support the hypothesis that these fatty acids played a role in early human growth, however once the results were revisited it became clear that the low plasma levels of AA and DHA could reflect the greater disappearance of LCPUFA's from the plasma to be incorporated into the growing tissues, thus resulting in lower plasma concentrations (Koletzko, 2001).

Thus one can see that a number of different factors affect the growth and developmental processes during one's lifetime and if any of these contributing factors are disturbed in any way, the growth process could be affected and even transiently inhibited.

As with the human growth phases, the pig also experiences different growth phases in which the role of the exocrine pancreas in terms of growth performance varies within the various production phases of the pig, adjusting to the altering biological needs of the pig as it matures. During suckling, exocrine pancreatic secretion is relatively low; with the most likely reason being due to the piglet consuming only milk. The milk does not require advanced digestion and is thus perfectly suited to the immature gut

and low level of pancreatic enzymes released during this phase of pig development. Also, the flow of digesta into the intestine is relatively constant as a result of the frequent feedings that take place during suckling, and the exocrine pancreas is constantly stimulated thus resulting in reduced sensitivity and low pancreatic secretions. Despite the low pancreatic secretion during suckling, the pigs have a large capacity to grow but only utilise about 40% of this growth capacity due to the sow regulating their feeding patterns and therefore the piglets can not consume the milk *ad libitum* (Van den Borne *et al*., 2007). After weaning, there is a gradual increase in exocrine pancreatic secretion, with a significant increase 5 days post weaning, when the sow's milk is absent from the GIT and the pig pancreas is adapted to a solid feed diet. Despite the increased level of exocrine pancreatic secretions during this post-weaning period, it seems that the exocrine secretions are still insufficient for the required digestion and absorption of nutrients and the piglet usually experiences postweaning diarrhea. The development of postweaning diarrhea is associated with the overgrowth of the bacteria *Escherichia coli* within the digestive tract, as the GIT is bombarded with undigested food. Thus it is possible that the exocrine pancreas could limit pig growth during this period (Rantzer *et al*., 1997).

Following weaning, during the growing period of the pig production phase, the importance of the exocrine pancreas has been demonstrated using pigs with induced EPI, usually by means of pancreatic duct ligation (Kammlott *et al*., 2005; Gregory *et al*., 1999). However, even though the exocrine pancreas is clearly a limiting factor with respect to pig growth during this period, feed intake also greatly influences

growth performance. Previous studies have shown that a high feed intake during the growing period results in an increased daily weight gain as well as a more efficient feed conversion, thus positively affecting growth performance. During the finishing period of the pig production phase (where the pigs reach between 60-110kg) however, feed intake may limit performance as it could result in an increased fat deposition and a poor feed conversion efficiency (Botermans *et al*., 1999).

When examining the various growth phases of the pig it is clear that the exocrine pancreatic secretions definitely play a role in determining pig growth performance, however the importance of the secretions with respect to overall growth do differ depending on the stage of pig development. Corring & Bourdon (1977) demonstrated that older pigs with weights of approximately 50kg were not significantly affected by the ligation of the pancreatic duct and perform quite well without the exocrine pancreatic secretions in terms of growth performance (Corring & Bourdon, 1977). Thus it is clear that the exocrine pancreatic secretions are more vital in terms of growth performance following weaning and during the growing period, having less of an effect in suckling and finishing pigs (Botermans *et al*., 1999; Corring & Bourdon, 1977).

Parallel to the varying levels of exocrine pancreatic secretion during the different growth phases of the pig, the enzyme content of the exocrine pancreatic juice also changes. After weaning both the levels and activities of trypsin and amylase are increased compared to those present in the pancreatic juice during suckling. These

changes coincide with the adaptation of the pig pancreas to the digestion of solid feed which usually contains up to three times more protein and carbohydrates than fat- compared to that of sow's milk (Pierzynowski *et al.*, 1990). After weaning, the exocrine pancreatic secretion of amylase, lipase, colipase and carboxylester lipase follows a general pattern associated with development - one of increased secretion with increasing age (Pierzynowski *et al*., 1993).

1.4. Exocrine Pancreatic insufficiency (EPI)

1.4.1. General introduction on EPI

Exocrine pancreatic insufficiency (EPI) refers to a state of impaired pancreatic secretion, usually resulting from the destruction of the pancreatic acinar tissue, either through an inflammatory process or by the progressive atrophy of the tissue. The destruction of the pancreatic acinar tissue leads to the inadequate production of pancreatic digestive enzymes resulting in a state of maldigestion and thus, ultimately the malabsorption of essential nutrients. The diagnosis of exocrine pancreatic insufficiency is based on the presence of typical clinical signs including, weight loss, diarrhoea, steatorrhea, voluminous faeces and polyphagia, together with various pancreatic function tests which are carried out when EPI is suspected (Adamama-Moraitou *et al*., 2004; Biourge & Fontaine, 2004; Kim *et al*., 2005).

Conventional treatment of exocrine pancreatic insufficiency involves replacement of the pancreatic enzymes with the goal of relieving the clinical symptoms of EPI (weight loss, diarrhoea, steatorrhea, voluminous faeces and polyphagia) and correcting the various nutritional deficiencies which result. However, previous studies involving enzyme replacement therapy in the treatment of EPI have shown that despite the administration of high doses of pancreatic enzyme extracts, normalisation

of digestion does not always occur and only partial corrections of the malnutrition have been reported (Bernabdeslam *et al.*, 1998; Tabeling *et al.*, 1999).

1.4.2. Exocrine pancreatic insufficiency in Cystic fibrosis

Cystic fibrosis is one of the main causes of EPI with a prevalence of over 90% in all cystic fibrosis sufferers (Littlewood *et al*., 2006). Cystic fibrosis (CF) is one of the most common hereditary disorders among the Caucasian population. It is an autosomal recessive disease involving all exocrine secreting organs such as the exocrine pancreas, the bronchi, liver, testes and gut. It is characterised by abnormally high sweat chloride concentrations as well as increased viscosity of all exocrine secretions. This increased viscosity results in the blockage of most ducts within the exocrine glands, causing reduced secretions of Cl^-, HCO_3^- and water (Couper *et al*., 2002; Lindley, 2006; Naruse *et al*., 2002).

The secretory defects associated with CF result from a mutation in the ABCC7 gene (cystic fibrosis gene) which codes for the cystic fibrosis transmembrane regulator (CFTR): a cAMP-dependent Cl^- channel situated on the apical membrane in epithelial cells of exocrine glands. The most common mutation of the CF gene corresponds to a specific deletion of three base pairs resulting in the loss of a phenylalanine at position 508 in the CFTR protein (Naruse *et al*., 2002). Various mutations of the CF gene exist, causing different abnormalities of the CFTR, leading to the abnormal transport

of both fluid and electrolytes across the epithelial cell membranes of ducts within exocrine glands (Littlewood *et al*., 2006). Since CF involves a mutation of the CF gene, causing abnormalities in the gene product (CFTR) which is abundant in the pancreatic duct epithelia, it makes sense that the majority of CF patients display impaired pancreatic ductular function, resulting in the development of EPI (Bernabdeslam *et al*., 1998; Lindley, 2006). The presence of these CFTR's in the apical membrane of the pancreatic duct epithelial cells is essential for normal pancreatic secretory function and thus, the dysfunction of these Cl^- channels as a result of the CF mutation plays a key role in the development of pancreatic insufficiency during the course of the disease (Littlewood *et al*., 2006; Naruse *et al*., 2002).

In most CF patients the pancreas is primarily affected due to the blockage of the pancreatic ducts as a result of the impaired transmembrane fluid and electrolyte transport. The impaired secretion of anions, water and pancreatic enzymes results in a limited flow of protein-rich pancreatic juice, causing protein precipitation within the duct which eventually results in a blockage. As the pancreatic ducts become increasingly obstructed by both cellular debris and the viscous secretions of the pancreas itself, the lumen begins to dilate with the formation of both intra- and interlobular fibrosis. Eventually the pancreatic acinar cells are replaced with fibrous tissue, fat and small cysts (Abello *et al.*, 1989; Lindley, 2006; Naruse *et al*., 2002). Destruction of the pancreatic acinar cells results in reduced secretion of the pancreatic digestive enzymes resulting in the maldigestion and ultimately the malabsorption of

essential nutrients. The maldigestion resulting from exocrine pancreatic insufficiency is the main gastrointestinal problem in approximately 80-90% of all cystic fibrosis sufferers, resulting in a poor nutritional status due to the loss of energy and nutrients necessary for growth. This in turn contributes to an increased level of morbidity and mortality within cystic fibrosis sufferers. (Benabdeslam *et al.*, 1998; Gan *et al.*, 1994; Gregory *et al*., 1999). Thus, it is important to find good strategies for the nutritional management of EPI in CF patients in order to improve the poor nutritional status of patients.

Even though great advances have been made in the treatment of EPI in both Cystic Fibrosis patients as well as other EPI sufferers, additional studies are necessary in order to further improve therapy in general (DiMagno, 1993).

1.4.3. Models available to study EPI

Various animal models are available for the study of exocrine pancreatic insufficiency, including rat models (Setser *et al.*, 1979), canine models (Kim *et al*., 2005) and pig models (Pierzynowski *et al*., 1988). Each of the animal models of EPI has its own advantages and disadvantages, and achieves a state of EPI via different methods; the models include chronic injections of reserpine, Zein or oleic acid into the common bile duct, as well as the surgical obstruction or ligation of the pancreatic duct.

The chronically reserpinized rat has been used as a method to induce a state similar to that of EPI in CF patients. Reserpinization of adult rats has been shown to produce biochemical, histological and physiological changes in the pancreas, similar to those seen in the pancreas of CF patients (Morton *et al.*, 1980; Setser *et al.*, 1979). Since the human infant with CF may display a somewhat different degree of pancreatic insufficiency to the adult CF patient, a model for the development of EPI similar to that seen in young CF patients was also developed. The reserpinization of the rats was carried out either through once-daily injections of reserpine into pregnant dams or into the newborn pups themselves. Doses of $125\mu g.kg^{-1}$ and $50\mu g.kg^{-1}$ of reserpine were injected into the pregnant dams and newborn rats, respectively (Werlin *et al.*, 1983). Upon studying the effects of chronic reserpine injections on the structure and thus the secretory ability of the immature rat pancreas, results obtained indicated that the reserpinization of the developing rat causes changes not only in the development of the immature pancreas but also in the structure of the pancreas thus resulting in alterations in the overall development of pancreatic function. Thus, the prenatal and neonatal reserpinization of the rats results in pancreatic changes similar to those seen in CF (Werlin *et al.*, 1983).

EPI is commonly induced by the obstruction of the pancreatic duct (Corring & Bourdon, 1977), which can be carried out via a number of different procedures for example, the injection of a Zein solution into the common bile duct of rats. Zein is an alcohol soluble corn protein which is often used as coatings for food as well as in adhesives (Tomita *et al.*, 1988). Injection of the Zein solution into the common bile

duct of the rats resulted in a reduction in total pancreatic protein to about 20% of that of control rats. The pancreas of the Zein-injected rats was also devoid of acinar tissue and was replaced with fibrous and lymphocytic infiltrates (Tomita *et al.*, 1988). Therefore the injection of a Zein solution into the common bile duct of the rats proved to be a safe and effective method to suppress exocrine pancreatic secretion, resulting in the development of EPI. This specific method of duct obstruction is particularly useful in rats compared to ligation of the pancreatic ducts, which is usually quite difficult to achieve due to the major ventral and dorsal ducts which converge at the ventral lobe of the pancreas and the numerous minor ducts which open directly into the common bile duct (Baetens *et al.*, 1979; Takahashi *et al.*, 1977). By using the Zein injection method of duct obstruction, it is possible to almost completely occlude each of the pancreatic lobe ducts of the rat pancreas without obstructing the common bile duct, thus avoiding the development of obstructive jaundice and ultimately the death of the rat (Tomita *et al.*, 1988).

Another example of a non-surgical model of induced duct obstruction involves the injection of oleic acid into the common bile duct of the rat. As with the chronically reserpinized rat model of EPI as well as the Zein injection EPI model, the injection of oleic acid into the common bile duct of rats leads to the development of long-lasting atrophy of the pancreatic acinar cells, thus resulting in reduced exocrine pancreatic function and ultimately EPI (Henry & Steinberg, 1993; Mundlos *et al*., 1986).

In addition to the non-surgical methods of duct obstruction resulting in the development of EPI, different surgical methods are also available. Exocrine pancreatic insufficiency can be created through the ligation of the pancreatic duct or by the complete removal of the pancreas, thus preventing the pancreatic secretions from reaching the contents of the duodenum. The latter however, is not very popular as besides the exocrine pancreatic secretions being absent, the influence of the endocrine pancreas is also removed, which only usually occurs in the very late stages of pancreatic insufficiency (Naruse *et al*., 2002).

Ligation of the pancreatic duct results in the development of EPI in pigs (Abello *et al*., 1989; Pierzynowski *et al*., 1988). Abello *et al*. (1989) showed that by ligating the pancreatic duct in pigs, a reproducible experimental model of total pancreatic insufficiency was created. Basically, the method involved anaesthetising the pigs with halothane and then transecting the pancreatic duct between two ligatures. The accessory pancreatic duct, which is present in about 10% of animals, was also located and ligated. Following the pancreatic duct ligation, upon macroscopic examination of the pancreas, a fibrous and atrophic gland was observed (Abello *et al*., 1989) confirming that ligation of the pig pancreatic duct results in the development of EPI and therefore would provide a useful model in the study of EPI. The minipig is also commonly use as a model of EPI, where the EPI is induced by the ligation of the pancreatic duct. Pancreatic duct ligation resulted in reduced digestibilities of both protein and fat, confirming the presence of EPI with the absence of necessary pancreatic enzymes including amylase and lipase (Mobeler *et al*., 2007).

1.4.4. The pig model of EPI

For the purpose of this study, I made use of the pig model of EPI. In order to study various aspects of pancreatic secretion and the role these secretions play in general, many studies make use of the pig. The pig is widely accepted as a model for humans in both nutrition and related studies as numerous anatomical and physiological similarities exist between pigs and humans. Some of the similarities which exist include: feeding patterns, digestive physiology and dietary habits (omnivore) (Moughan *et al*., 1992). I also made use of young, growing pigs as previous studies have demonstrated that older, heavier pigs are not as sensitive to the lack of exocrine pancreatic secretions in terms of growth performance (Corring & Bourdon, 1977).

In addition to the pig being a widely accepted model for humans, it also offers a number of other technical advantages. Such as the possibility for repeated simultaneous sampling at various levels of the intestine, as well the possibility for repeated venous sampling for hormone determination; advantages which are not presented in most of the current rat models of EPI (Abello *et al*., 1989).

Since this study is particularly focussed on the digestion and absorption of fat, the pig also posed as the ideal model to use as previous studies have shown that the rat has an extraordinary ability to digest fat even in the absence of pancreatic lipase (Henry & Steinberg, 1993). Thus, if we used the rat model it would not have clearly

reflected the relationship between pancreatic lipase output and fat digestion and absorption.

1.5. Aims

Restriction of dietary fat intake in the treatment of EPI is commonly suggested, however recent studies have demonstrated that the consumption of a high-fat diet may be well tolerated by EPI patients and can restore optimal body mass and nutritional status of the patients (Biourge & Fontaine, 2004). Thus, the main objective of this study was to corroborate previous findings on the inclusion of fat in the diet of EPI patients by investigating the effects of dietary supplementation with Creon 10 000, together with a high-fat diet, on short term growth performance, apparent digestibility and fat digestion and absorption, using the pig as a model of EPI.

An additional objective of the study was to make use of turbidimetry and the lipaemic index as an approach to measuring and analysing the plasma lipid content before and after treatment.

Chapter 2 - Experiment

2.1. Experiment

2.1.1. Methods

i) Experimental design

The study was performed with 6 pigs in total, which were divided into two groups of three pigs each for ease of handling and animal welfare monitoring. Both groups were subjected to the same experimental procedures.

ii) Animals

6 castrated, new born, male pigs (Swedish Landrace X Yorkshire X Hampshire) weighing approximately 1.5 kg each, were randomly selected from the University herd at Odarslöv, Swedish Agricultural University and used in the study. Prior to the experimental period, pigs were weaned at four weeks of age and then housed at Odarslöv in individual pens (1.0 x 1.5m) with perforated plastic flooring and wood chips as bedding. All pens were equipped with a dry feeding trough, a drinking nipple and a constant heating lamp (150 W). Pigs were allowed to move freely within their pens and had visible contact with each other. During the experimental period pigs were housed in modified metabolic cages at the animal unit of the Department of Cell and Organism Biology, Lund University, Sweden. Metabolic cages were also equipped with a drinking nipple and a constant heating lamp (150 W). The study was

approved by the Lund University Ethics Review Committee on Animal Experiments, Sweden (Ethics clearance no: M 142-06) and the Animal Ethics Screening Committee of the University of the Witwatersrand, South Africa (Ethics clearance no: 2006/70/05).

iii) Surgery

a) Pancreatic duct ligation and Jugular vein catheterization

First Surgery: Pancreatic Duct Ligation

To artificially induce a state of pancreatic insufficiency, when the pigs were 8 weeks old, pancreatic duct ligation was performed as follows: All pigs were fasted for 12hrs prior to surgery. Azaperone (Stresnil, LEO, Helsingborg, Sweden) at $4mg.kg^{-1}$ I.V was administered as a premedication; pigs were then given a full body bath using surgical soap. Following the body bath, pigs were intubated endotracheally and placed on a surgical table, which was disinfected with an iodine solution (Jodopax; Ferrosan, Malmö, Sweden) and 70% ethanol. The pigs were then anaesthetised using a 0.5-1.5% air mixture of Fluothane (Zeneca, Gothenburg, Sweden) and carrier O_2 at approximately $0.5l.min^{-1}$. All surgery was performed under aseptic conditions.

The abdominal region was shaved clean and a 10cm incision was made posterior to the sternum, along the *linea alba.* Once the pancreatic duct was located, it was isolated and ligated using two silk sutures (Silk 0-3 Ethicon, Johnson and Johnson)

and then cut between the sutures. During surgery, all pigs were thoroughly examined to ensure that there were no accessory pancreatic ducts present. Ampicillin (Doktacillin; Astra Läkemedel, Södertalje, Sweden) was administered as a post-surgical antibiotic at 15mg.kg^{-1} I.V and 50mg into the wound. The wound was then sutured closed; using absorbable sutures for the muscle layers and non-absorbable sutures for the suturing of the skin.

Second Surgery: Jugular vein catheter implantation

8 weeks after pancreatic duct ligation surgery (pigs: 16 weeks old), external jugular vein catheters were implanted. All surgical conditions were the same as for the pancreatic duct ligation surgery. In brief: following anaesthesia, the region of the right external jugular vein was shaven clean and a 5cm incision was made between the brachial joint and the mandibular angle. Once the right external jugular vein was located, a small incision was made within the vessel and a Silastic catheter (1.02mm internal diameter and 2.16mm outer diameter) was placed inside. The catheter was secured to the vessel using two silk sutures (Silk 0-3 Ethicon, Johnson and Johnson) and then brought to the exterior under the skin of the dorsal part of the neck. Ampicillin (Doktacillin; Astra Läkemedel, Södertalje, Sweden) was administered as a post-surgical antibiotic at 15mg.kg^{-1} I.V and 50mg into the wound. The wound was then sutured closed. The catheter was then rinsed with saline and blocked with a plastic stopper.

b) Post-surgical management

Following both surgeries, the pigs were closely monitored (feed intake and wound healing) and treated with Ampicillin (Doktacillin; AstraZeneca, Södertalje, Sweden), prophylactically at 15mg.kg^{-1} I.V for 3 days.

iv) Feeding

Prior to the pancreatic duct ligation surgery the pigs were fed a standard pig diet ("Lantmännens enhetsfoder för smågrisar" 53910 SOLO 330) twice a day (5% body mass per meal) at 08:00-09:00 hrs and 16:00-17:00 hrs.

Following the first and second surgeries, as well as for the entire experimental period, the pigs were fed the standard pig diet ("Lantmännens enhetsfoder för smågrisar" 53910 SOLO 330) enriched with +/- 15% extra fat in the form of a mixture composed of 30% rape oil ("Rapsolja", Karlshamn) and 60% cream from cow's milk ("Vispgrädde", 40 % fat content), twice a day (5% body mass per meal) at 08:00-09:00 hrs and 16:00-17:00 hrs.

Table 1 shows the constituents of the standard pig feed, before the addition of the extra fat. After addition of the extra fat to the standard pig feed, the fat content of the diet was increased to +/- 18%.

Standard pig feed constituents (as supplied by manufacturer) listed in table below:

Constituents	**%**
Fat	3.5
Protein	17.6
Ash	5.12
Water	12.4
Carbohydrates	52.0
Total Energy	**12.6MJ.kg^{-1}**

Table 1: Constituents (%) and the total energy content (MJ.kg^{-1}) of standard pig feed.

v) Experimental procedure

All the monitoring and feeding of the pigs, the preparation and administration of the pancreatic enzyme preparation as well as the collection of all faecal, urine and blood samples were performed by me.

a) General experimental procedure

Following the pancreatic duct ligation surgery, pigs were allowed 8 weeks recovery period during which they were closely monitored. After the recovery period, the jugular vein catheters were implanted and the pigs were placed in metabolic cages and allowed an adaptation period of 7 days prior to starting the feeding trial experimental procedure, whilst continuing to receive the high fat diet.

The feeding trial experimental period comprised of 15 days in total:

- Day 1: pigs were weighed to obtain a ‘before treatment’ body mass, all weighing took place on an animal facility balance and was performed before administration of the morning meal.
- Days 2-4: pigs remained in the metabolic cages, whilst continuing to receive the high fat diet.
- Day 5: faecal and urine samples were collected for 24hrs.
- Day 6: faecal and urine samples were collected for 24hrs. Blood samples for ‘base-line’ readings were also collected for 24hrs, the collection of which will be further explained below.
- Day 7: faecal and urine samples collected for 24hrs.
- Day 8: treatment with Creon 10 000 (Solvay Pharmaceuticals GmbH, Hannover, Germany) was commenced (dosing of which will be further explained), the pigs were weighed to obtain a ‘start of treatment’ body mass and blood samples for ‘start of treatment’ readings (24hrs) were collected.
- Day 9-11: treatment with Creon 10 000 was continued together with the high fat diet.
- Day 12: faecal and urine samples collected for 24hrs.
- Day 13: faecal and urine samples collected for 24hrs.
- Day 14: faecal and urine samples collected for 24hrs and blood samples for ‘end of treatment’ readings were obtained (24hrs).
- Day 15: pigs were weighed to obtain an ‘end of treatment’ body mass.

Following completion of the feeding trial, all pigs were killed using an intravenous anaesthetic overdose of Pentobarbital (Mebumal, Nordvacc, Stockholm, Sweden).

b) Dosing with the Creon 10 000

12 Creon 10 000 capsules were administered together with the morning meal and then another 12 capsules with the evening meal. The dose of Creon 10 000 used in this study has previously been shown to improve digestibility in pancreatic-duct ligated pigs (Tabeling *et al*. 1999). Each Creon 10 000 capsule contains 150mg pancreatin which in turn contains 10 000 active lipase units, 8000 active amylase units and 600 active protease units. Dosing with the active compound was performed together with 20g of Vanilla yoghurt (3% fat, 4% protein, 12% sugar, the remainder of the yoghurt's composition consists of water and other organic and inorganic constituents, however a full proximate analysis was not performed; Skane Mejerijet AB, Lund, Sweden) and 20g of the pig's meal. Once the Creon 10 000 preparation mixture was consumed, the pigs were given the remaining portion of the meal.

c) Feed sample processing

Samples (100g) of the feed mixture administered to the pigs were taken daily during the experimental procedure in order to determine selected nutrient intake.

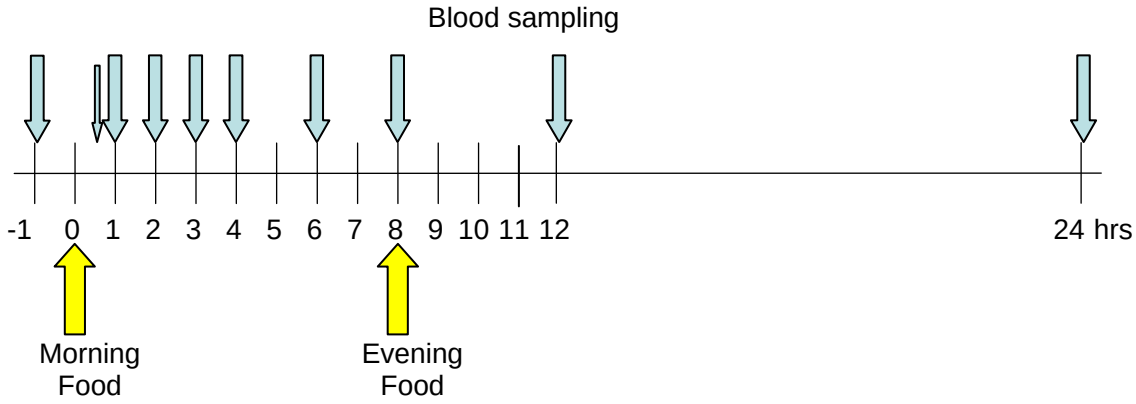
d) Collection and processing of Faecal and Urine samples

Faecal samples collected on each of the six collection days were stored in separate containers for each pig, on each specific day. Upon completion of the collection on each day, total sample weight was measured and recorded and then faecal samples were homogenised using a standard kitchen blender and stored at -20ºC until further analysis.

Urine samples collected on each of the six collection days were also stored in separate containers for each pig, on each collection day. Sulphuric acid was added to samples during collection to keep pH below 3. Upon completion of the collection on each day, total sample weight was measured and recorded and the urine samples were stored at -20 ºC until further analysis.

e) Blood sampling and plasma processing

The blood samples were obtained as follows: 5ml blood samples were taken via the jugular vein catheters approximately one hr prior to administration of the morning meal. Subsequently, samples were then obtained 30mins after administration of food, and then at 1, 2, 3, 4, 6, 8, 12, and 24hrs after morning food administration (see timeline below).



Blood samples were collected into 10ml glass vials containing 0.5ml of Trasylol (aprotinin 10000 KIE.ml^{-1}; Bayer and EDTA 0.04g.ml^{-1} of Trasylol; Merck) and placed on ice until they were centrifuged at 3000G, at 4 ºC for 15mins, approximately 30-60mins after withdrawal. 1ml aliquots of all plasma samples were then prepared and the samples were then stored at -20 ºC until further analysis.

vi) Sample Analysis

a) Feed, faecal and urine samples

All feed, faecal and urine samples were analysed by a certified specialist laboratory, Lantmännen Analycen AB, Lidköping, Sweden, for dry matter, nitrogen and fat content, using standard AOAC procedures:

Dry matter content: Both feed and faecal samples were analysed for dry matter content using desiccation (drying out) at 103 ºC for 5hrs

Nitrogen content: Feed, faecal and urine samples were analysed for raw nitrogen content using the Kjeldahl method (Bradstreet, 1954) with an N factor of 6.25.

Fat content: Both feed (with pre-extraction) and faecal (without pre-extraction) samples were analysed for fat content using the standard gravimetric method for fat analysis, using a Tecator manual Kjeltec Auto Sampler, 1035 Analyzer (Tecator AB, Sweden).

b) Blood samples

The blood samples obtained were used to analyse both the lipaemic index of the plasma samples as well as the plasma lipid profile, using standard turbidimetry and clinical chemistry methods, respectively. Turbidimetry procedures were carried out by me in the Department of Cell and Organism Biology, Lund University, Sweden. I used the lipaemic index as a measure to indicate the changes observed in the lipid composition after treatment with the pancreatic enzyme preparation. The lipaemic index which is calculated using the absorbencies of the lipid samples at both 660nm and 700nm, serves as an indication of the amount of lipid in the samples being measured. The wavelengths that are used to calculate the lipaemic index (660nm and

700nm) are used as it has been shown that the colour of the lipids in solution correspond well to those wavelengths- i.e they are the wavelengths of the light spectrum at which the lipid solutions absorb maximally at. The more lipid that is present in the sample, the lower the transmittance of light through the sample and thus, the greater the absorbance of the light by the lipids in the sample. Therefore, the greater the absorbance of the sample, the greater the lipid content of the sample and thus, the higher the lipaemic index (De Haene et al., 2006).

The clinical chemistry methods used to determine plasma lipid profiles were carried out in a specialist laboratory, Medilab, Tarnaby, Sweden.

Turbidimetry Methods:

200µl of each plasma sample was added to a 96-well plate (96F MicroWell^{TM} Plates, Product no: 269620,Nunc, Denmark) using a pipette (Biohit Proline, Finland), the plate was then loaded into a Spectra Max M_2 plate reader (Molecular Devices, USA) and the absorbance of the plasma samples was measured at wave lengths of 660 and 700 nm. Absorbance results were processed using a SoftMax Pro 4.6 processor (Molecular Devices, USA).

Clinical chemistry methods:

Plasma lipid profiles, including total cholesterol, LDL, HDL, TG and FFA content before and after treatment with the pancreatic enzyme preparation were determined from the frozen plasma samples sent to Medilab, Tarnaby, Sweden; using standard

calorimetric kits (RocheDiagnostic, Switzerland and Wako Chemicals, Neuss, Germany) on a Hitachi 912 Multianalyzer (RocheDiagnostic, Switzerland).

vii) Data Analysis

All data are expressed as mean (SD). Faecal and urine profiles, nitrogen digestibility, coefficient of fat absorption (CFA), blood profiles as well as body mass data were analysed using a repeated measures ANOVA. A Tukey post hoc test was used when significant differences or effects were detected by the repeated measures ANOVA. All statistics were performed using GraphPad Instat version 3.00 for Windows 95 (GraphPad Software, San Diego California USA). $P<0.05$ was considered significant.

2.1.2. Results

i) Body mass

Figure 1 shows the body mass of pigs measured before treatment with the Creon 10 000 preparation, on the first day of treatment (day 8) and on the last day of treatment (day 14). The base-line body mass measurement and those made on day 1 of treatment with the Creon 10 000 preparation were not significantly different. However, administration of the Creon 10 000 supplement for 7 days significantly increased body mass. ($P = 0.016$)

ii) Dry matter and Apparent Digestibility

a) Dry matter (Faecal and Urine)

Figure 2 shows the total daily faecal dry matter content (g) during the control and treatment periods. The faecal dry matter during the treatment period (day 12, day 13 and day 14) was significantly lower than the faecal dry matter during the control period (day 5, day 6 and day 7). ($P < 0.001$)

Figure 3 shows the apparent dry matter digestibility values from all three of the collection days during the control period and all three of the collection days during the treatment period. The dry matter digestibility (%) was calculated using the following formula:

$$\text{digestibility (\%)} = \frac{(\text{DM intake}) - (\text{DM faeces})}{(\text{DM intake})} \times 100$$

The dry matter digestibility values calculated from the last two collection days during the treatment period (day 13 and day 14) were significantly higher than the digestibility value calculated from the first day of control collections (day 5). ($P = 0.0166$).

Urine dry matter content (g) during the control and treatment periods were not significantly different from one another. ($P = 0.1183$).

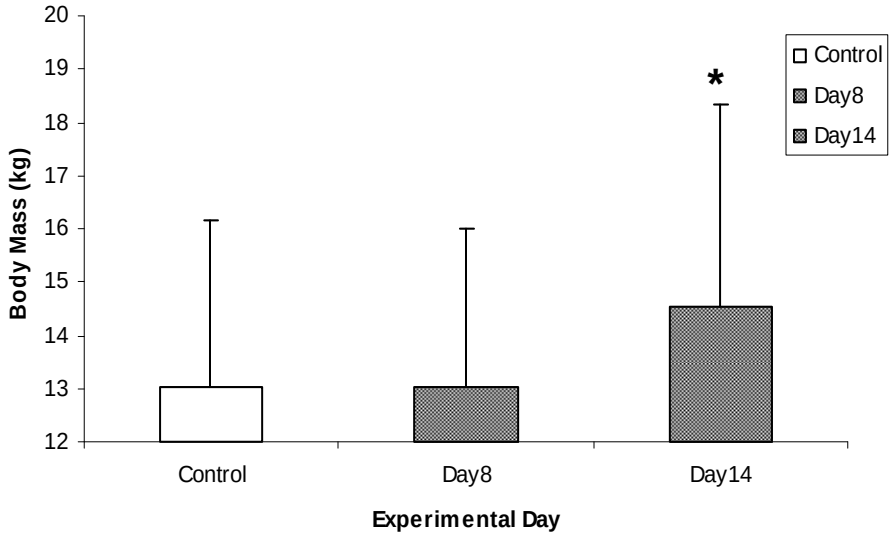


Figure 1: Body mass (kg) of pigs measured during the control period and on the first (day 8) and last (day 14) days of treatment with the pancreatic enzyme preparation

* $P < 0.05$

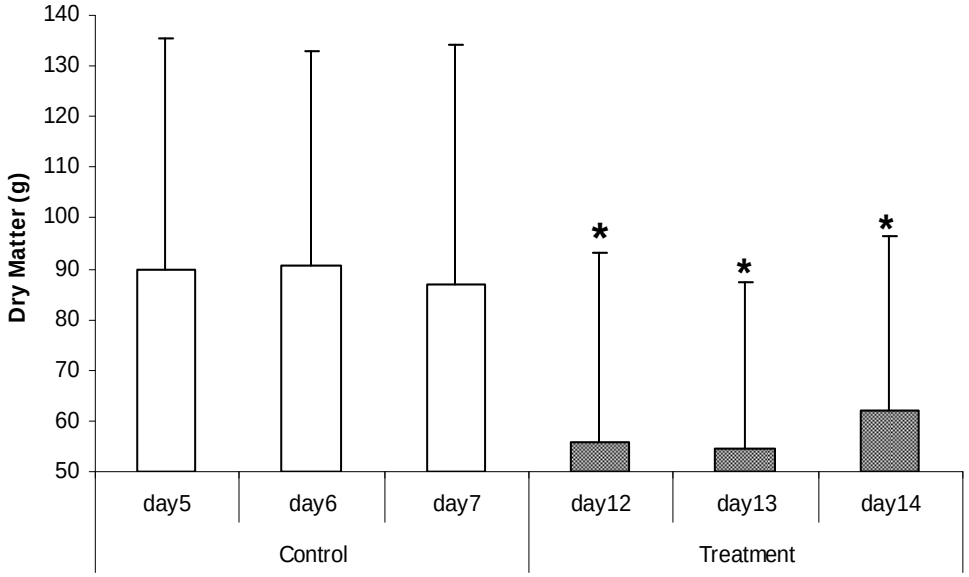


Figure 2: Faecal dry matter content (g) measured during the control and treatment periods

* $P < 0.05$

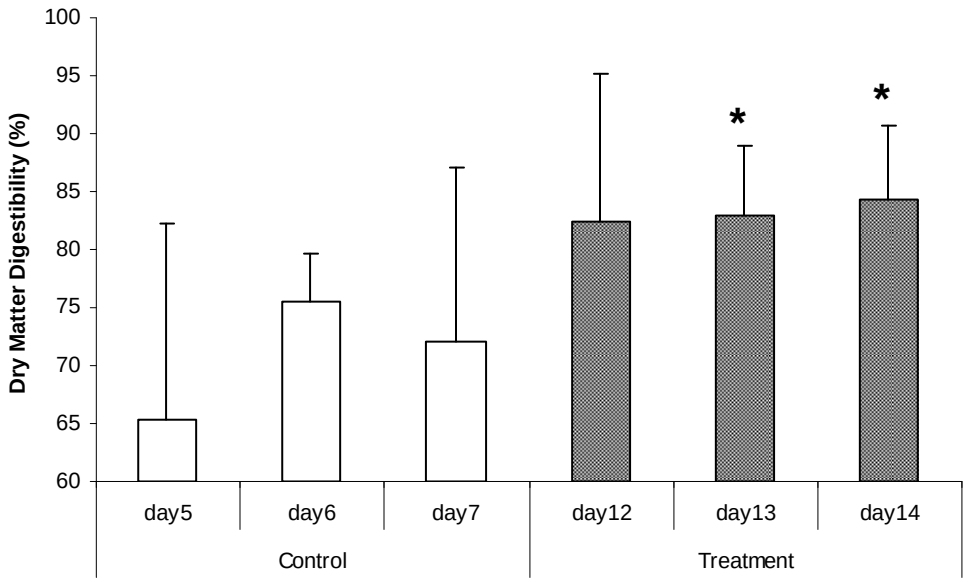


Figure 3: Apparent digestibility of dry matter during the control and treatment periods

* $P < 0.05$ day 5 vs. days 13 and 14.

b) Nitrogen balance, Crude protein (Faecal and Urine)

The nitrogen balance values from the faecal samples were used to calculate the overall crude protein content (g) of the faeces. The nitrogen balance values from the urine samples were referred to as 'urinary nitrogen'.

$$\text{Crude Protein} = \text{Nitrogen} \times 6.25$$

Figure 4 shows the faecal crude protein content (g) during control and treatment periods. The faecal crude protein content on the first two collection days during the treatment period (day 12 and day 13) was significantly lower than the faecal crude protein content during the control period (day 5, day 6 and day 7). ($P = 0.005$).

The crude protein digestibility was calculated using the following formula:

$$\text{digestibility (\%)} = \frac{\text{(CP intake)-(CP faeces)}}{\text{(CP intake)}} \times 100$$

For overall crude protein digestibility during the control and treatment periods significant differences were observed. Figure 5 shows the calculated crude protein digestibility values from all three of the collection days during the control period and all three of the collection days during the treatment period. The crude protein digestibility values calculated from all three of the collections during the treatment

period (day 12, day 13 and day 14) were significantly higher than the digestibility value calculated from the first day of control collections (day 5). ($P = 0.013$).

Urinary nitrogen content (g) during the control and treatment periods were not significantly different from one another. ($P = 0.326$).

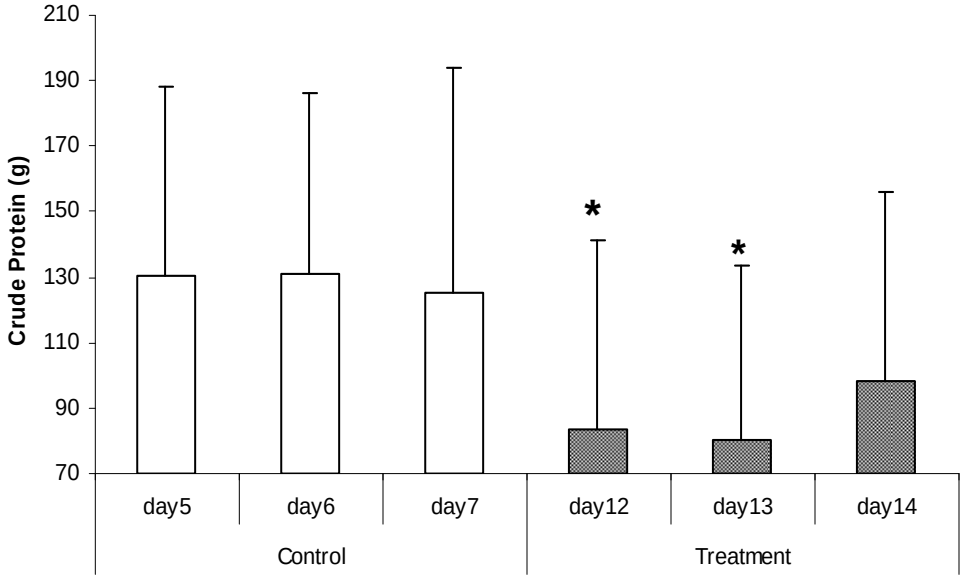


Figure 4: Crude protein content (g) in faeces during the control and treatment periods

* P < 0.05 days 5, 6 and 7 vs. days 12 and 13.

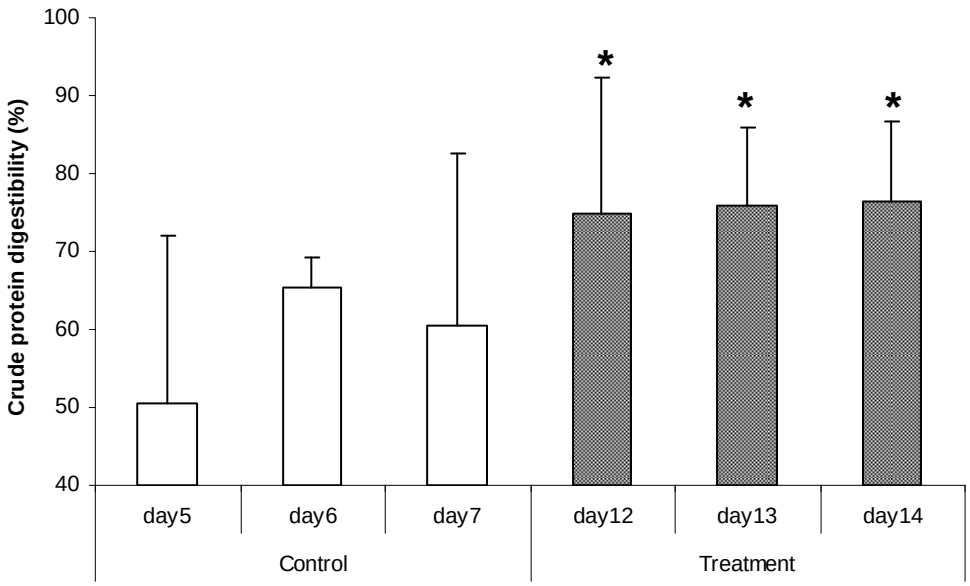


Figure 5: Apparent digestibility of crude protein on each of the three collection days during the control and treatment periods

* $P < 0.05$ day 5 vs. days 12, 13 and 14.

c) Fat

Figure 6 shows the fat content of the faeces (g) measured during the control and treatment periods. The faecal fat content measured on each of the treatment collection days (day 12, day 13 and day 14) was significantly lower than those measured on each of the control collection days (day 5, day 6 and day 7). ($P < 0.0001$)

The co-efficient of fat absorption (CFA) was calculated using the following formula:

$$CFA = \frac{(\text{fat intake}) - (\text{fat in faeces})}{(\text{fat intake})} \times 100$$

When calculating the co-efficient of fat absorption during the control and treatment periods significant differences were observed. Figure 7 shows the calculated co-efficient of fat absorption (CFA) values from all three of the collection days during the control period and all three of the collection days during the treatment period. The CFA values calculated from all three of the collections during the treatment period (day 12, day 13 and day 14) were significantly higher than the CFA value calculated from the first day of control collections (day 5). ($P = 0.003$)

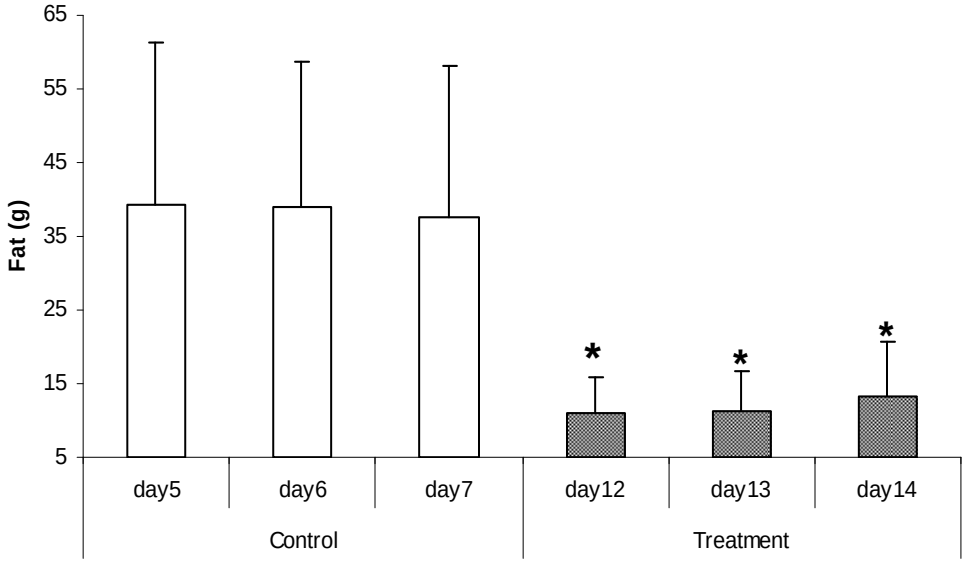


Figure 6: Fat content (g) in the faeces measured during control and treatment periods

* $P < 0.05$

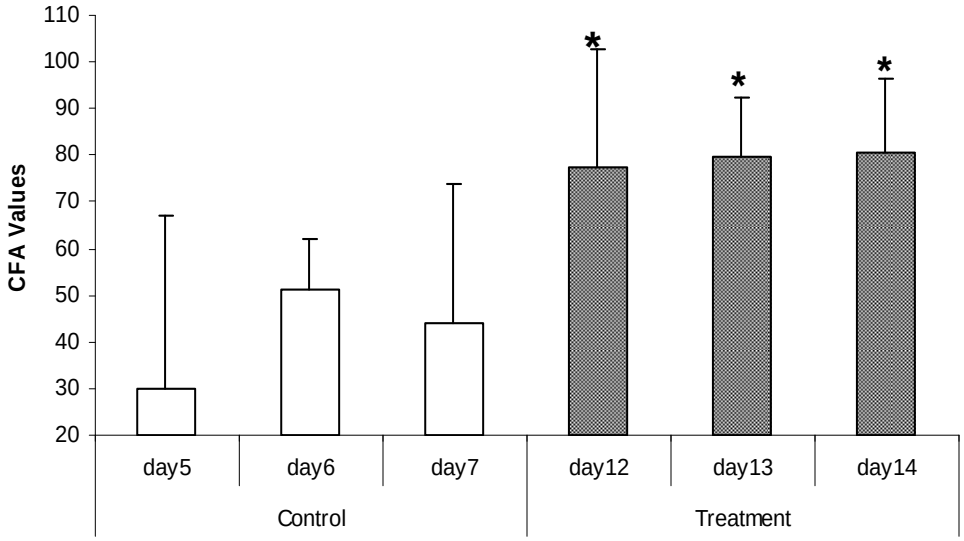


Figure 7: CFA values calculated for each of the three collection days during the control and treatment periods

* $P < 0.05$ day 5 vs. days 12, 13 and 14.

iii) Plasma lipids

a) Turbidimetry

With respect to the analysis of data produced from the turbidimetry process, I decided to calculate the lipaemic index in order to have some sort of measure to focus on, and compare between the control and treatment periods.

Lipaemic index = [(plasma absorbance at 660nm)-(plasma absorbance at 700nm)] x100

(De Haene *et al*., 2006)

Figure 8 shows the calculated lipaemic index values for each blood sample taken across the 24hr blood sampling period, before treatment (day 6) , and on the first (day 8) and last (day 14) days of treatment with the Creon 10 000 preparation.

The calculated lipaemic index values during the control period (day 6) did not differ significantly between the various sampling points and the lipaemic index remained quite constant. On the first day of treatment with the Creon 10 000 preparation (day 8) significant differences between the various sampling points were observed, as well as in the trends observed across the 24hr sampling period compared to that of day 6. Following the first day of treatment (day 8) the lipaemic index increased slowly with time reaching its peak on the 12^{th} hr after morning food administration. On the last day of treatment with the Creon 10 000 preparation (day 14), the lipaemic index also

increased with time and started to increase sooner than that which was observed on day 8, reaching its peak on the 6^{th} hr after morning food administration.

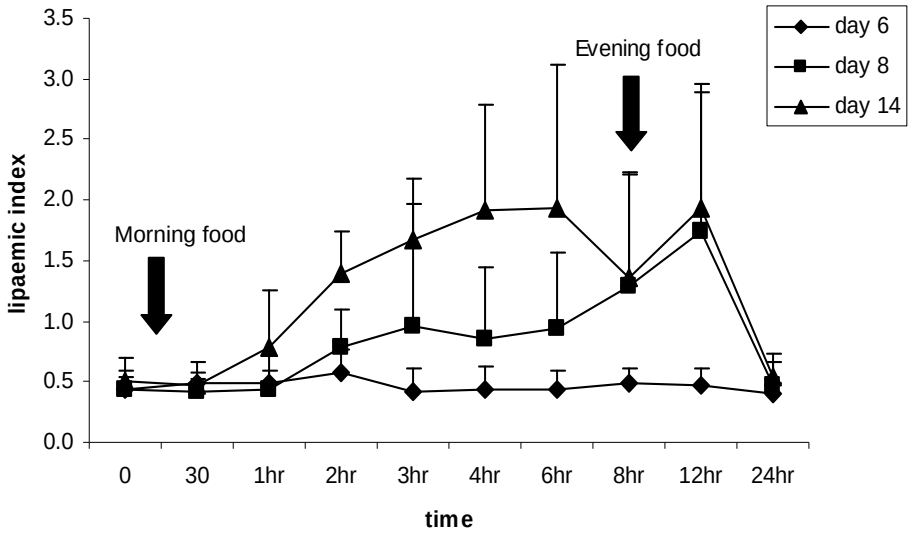


Figure 8: Calculated lipaemic index values for each blood sample taken across the 24hr blood sampling period, before treatment (day 6), and on the first (day 8) and last (day 14) days of treatment

b) Plasma lipid profile

The cholesterol concentration (mmol.l^{-1}) as well as the concentration of both high and low density lipoproteins (mmol.l^{-1}) measured from blood samples collected on day 6 (control), day 8 (first day of treatment) and day 14 (last day of treatment) did not differ significantly between the various blood sampling time points as well as between the different collection days. Significant changes were observed in both the free fatty acid and triglyceride concentration (mmol.l^{-1}) between the various blood sampling time points as well as between the different collection days.

Figure 9 shows the free fatty acid concentration (mmol.l^{-1}) measured during the control period (day 6) and on the first (day 8) and last days (day 14) of treatment with the Creon 10 000 preparation. A similar trend was observed in free fatty acid concentration (mmol.l^{-1}) during both the control period (day 6) and on the first (day 8) and last days (day 14) of treatment with the Creon 10 000 preparation; where the free fatty acid concentration decreased slightly after about 1-2hrs after morning food administration and then slowly began to increase again. However, the time at which the peak free fatty acid concentration was reached, after morning food administration varied significantly between the various sampling days. The peak free fatty acid concentration was reached at the 24th hr after morning food administration during the control period (day 6) and at the 6th hr after morning food administration on the first (day 8) and last days (day 14) of treatment with the Creon 10 000 preparation.

Figure 10 shows the changes observed in the triglyceride concentration ($mmol.l^{-1}$) measured during the control period (day 6) and on the first (day 8) and last days (day 14) of treatment with the Creon 10 000 preparation. The changes observed in triglyceride concentration ($mmol.l^{-1}$) were not as defined as those observed in the free fatty acid concentration; however definite trends were demonstrated on the first and last days of treatment with the pancreatic enzyme preparation (day 8 and day 14).

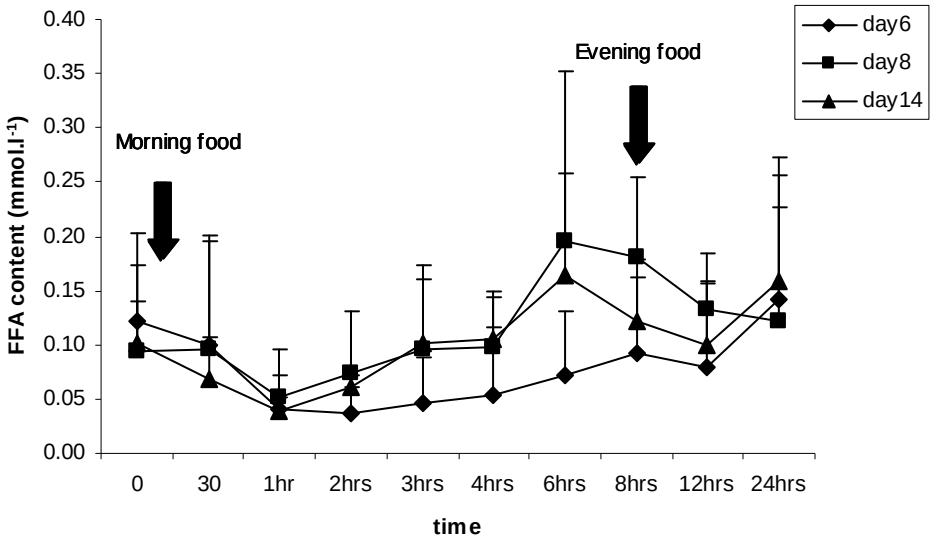


Figure 9: Free fatty acid (FFA) concentration (mmol.l^{-1}) measured during the control period (day 6) and on the first (day 8) and last days (day 14) of treatment

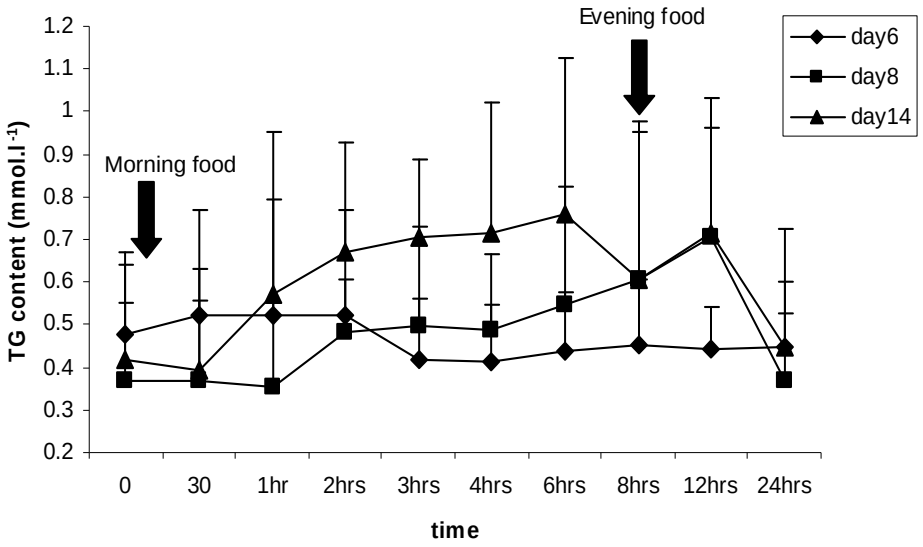


Figure 10: Triglyceride (TG) concentration (mmol.l^{-1}) measured during the control period (day 6) and on the first (day 8) and last days (day 14) of treatment

2.1.3. Discussion

I set out to corroborate the findings of previous studies in which the inclusion of a high fat diet together with the PERT in EPI patients is recommended. I hypothesized that the dietary supplementation of the Creon 10 000 preparation, together with the high fat diet would indeed have beneficial effects with respect to short term growth performance as well as the digestion and absorption of fat in a pig model of exocrine pancreatic insufficiency.

The present study could be improved by including at least a further two control groups, in which the one control group would not be exocrine pancreatic insufficient and would therefore only receive standard pig feed, without any Creon 10 000 supplementation; and the other control group would be exocrine pancreatic insufficient and receive the Creon 10 000 supplementation together with the standard pig feed (no high fat diet). This would allow us to compare the effects of the Creon 10 000 preparation, together with the high fat diet on the various parameters examined to normal values as well as to those values obtained with the supplementation of the Creon 10 000 preparation with standard pig feed. By doing so, we would be able to assess the efficacy of the Creon 10 000 preparation used in the study, in not only improving the parameters measured but also in returning the parameters measured to normal values; as well as the benefit of the inclusion of a high fat diet in the treatment of EPI. The administration of a proton pump inhibitor together with the pancreatic enzyme preparation could also have improved the results

obtained. Due to the absence of pancreatic bicarbonate secretion, the duodenal pH is low and thus sometimes results in the failure of enteric coated enzymes to be released at pH levels < 6 (Proesmans & De Boeck, 2003). Another factor which would have been useful in the present study, would be the measurement of serum amylase, which serves as an assay for pancreatitis and thus would have been informative in assessing the effects of the pancreatic duct ligation surgery on the pancreas itself.

A larger sample size would also be beneficial in assessing statistical significance of the results obtained; however, many previous studies involving EPI pigs have made use of similar sample sizes (Corring & Bourdon, 1977; Omogbenigun *et al*., 2004; Tabeling *et al*., 1999).

Exocrine pancreatic insufficiency was successfully produced in all six pigs by pancreatic duct ligation. A thorough examination for accessory pancreatic ducts was performed during the pancreatic duct ligation surgery, however over a long period of time accessory pancreatic ducts can develop again, but considering the short duration of my study it is unlikely. However, I did not examine the pancreas at the end of the study to be certain which could have proved useful.

The existence of EPI was confirmed by the presence of steatorrhea and reduced growth performance in all pigs during the 8 week post surgical recovery period following pancreatic duct ligation surgery. Dietary supplementation with the Creon

10 000 preparation, together with the high fat diet had varying results on the parameters examined.

Results obtained with respect to the body mass (kg) of the pigs before and after treatment with the Creon 10 000 preparation indicated a positive relationship between dietary pancreatic enzyme supplementation and growth performance in the pigs with EPI. Body mass (kg) of the pigs on the last day of treatment was significantly higher than that during the control period. My results are in agreement with most other studies involving pancreatic enzyme supplementation in pigs with EPI. Saloniemi *et al.* (1989) observed that pigs with EPI that were not receiving any form of enzyme supplementation displayed a significantly lower weight gain than those pigs with EPI receiving enzyme supplements (Saloniemi *et al*., 1989). Botermans & Pierzynowski (1999) also demonstrated a positive relationship between daily weight gain in pigs and exocrine pancreatic secretion. They observed that an increased exocrine pancreatic enzyme output resulted in an increased daily weight gain in pigs receiving the same daily feed intake (Botermans & Pierzynowski, 1999). Thus demonstrating that sufficient exocrine pancreatic secretion is essential in the digestion of nutrients and good feed utilization and therefore in the growth and development of the animal.

The faecal dry matter content (g) on all three collection days during the treatment period was significantly lower than that on all three collection days during the control period, indicating an increased assimilation in response to the supplementation with Creon 10 000 this was also reflected in the significant improvement in dry matter

digestibility. My results are in agreement with previous studies as Tabeling *et al*. (1999) also observed a significant reduction in the dry matter content of chyme, measured in EPI pigs following treatment with a pancreatic enzyme supplement (Tabeling *et al*., 1999). Following pancreatic duct ligation surgery in pigs, the dry matter content of the chyme was shown to increase dramatically. This increase in dry matter is due to the increased content of undigested nutrients and also due to the lack of dilution of the chyme by the pancreatic juice. Treatment with the pancreatic enzyme supplement improves nutrient digestibility and thus the dry matter content of the chyme (Tabeling *et al*., 1999) and in our case the dry matter content of the faeces is significantly improved.

Similar changes to those observed in the faecal dry matter content and % dry matter digestibility were also observed in the faecal crude protein content and % crude protein digestibility before and after treatment with the Creon 10 000 preparation. Tabeling *et al*. (1999) as well as Kammlott *et al*. (2005) observed that the administration of pancreatic enzymes to EPI pigs dose-dependently increased the total digestibility of nutrients such as crude protein and fat, the digestibilities of which are significantly reduced following pancreatic duct ligation (Kammlott *et al*., 2005; Tabeling *et al*., 1999). My results are in agreement with these findings as I too demonstrated a positive relationship between the percentage crude protein digestibility and the Creon 10 000 preparation supplementation.

Urinary nitrogen content from urine collections during the control period and those during the treatment period were not significantly different from one another. One could speculate that the lack of significant changes in the urinary nitrogen content was probably due to the pigs not being in an extreme state of protein catabolism, therefore they were not excreting any more or less nitrogen than normal. During the control period, the EPI pigs were likely to be conserving nitrogen; however the amount which they are able to conserve was not adequate for optimal growth. This is reflected in the reduced body mass observed in the pigs following the pancreatic duct ligation surgery before commencing treatment with the Creon 10 000 preparation. Following treatment with the Creon 10 000 preparation, the nitrogen digestibility was improved, thus the pigs were able to assimilate more nitrogen which was used to build protein, thus resulting in improved growth performance. The improved growth performance is reflected in the increase in body mass of the pigs following treatment with the Creon 10 000 preparation.

With respect to the faecal fat content, significant differences were observed between control and treatment measurements. There was a significant improvement in fat digestion and absorption in response to the Creon 10 000 preparation supplementation. Kim *et al*. (2005) observed a positive response to treatment with a pancreatic enzyme in a case of canine exocrine pancreatic insufficiency. Following treatment, the steatorrhea was nearly diminished and the faeces became solid and were decreased in volume (Kim *et al*., 2005). Carroccio *et al*. (1992) also observed an

improvement in the steatorrhea status of CF patients receiving a pancreatic enzyme supplement, as well as a reduction in the faecal wet weight (Carroccio *et al*., 1992).

In the present study, the steatorrhea improved following Creon 10 000 preparation supplementation, as reflected by the co-efficient of fat absorption (CFA). The CFA values calculated from all three faecal collections during the treatment period were significantly higher than the CFA value calculated from the first day of control faecal collections, thus indicating improved fat absorption in response to the Creon 10 000 preparation supplementation. Similar results have been obtained in previous studies focusing on the effects of pancreatic enzyme therapy on fat digestion and absorption. Stern *et al.* (2000) also observed increased CFA values in CF patients receiving pancreatic enzyme therapy compared to those receiving a placebo (Stern *et al*., 2000).

Despite the significant improvements in fat digestion and absorption in EPI patients, pancreatic enzyme replacement therapy (PERT) often fails to completely normalize fat absorption (Carroccio *et al*., 1992). PERT is the therapy of choice for most EPI sufferers, with the goal of relieving the clinical symptoms associated with EPI and improving the overall nutritional status of the patient (Dominguez-Munoz *et al*., 2005; Kim *et al*., 2005; Stern *et al*., 2000). Many previous studies involving PERT have displayed improved fat digestion, with reduced loss of fat within the faeces and thus, an improved co-efficient of fat absorption (CFA). However, despite the significant improvements in overall fat digestion, high doses of lipase therapy often fail to normalize fat absorption, posing a therapeutic problem (Carroccio *et al*., 1992).

Failure of PERT can be due to a number of different factors affecting the efficacy of oral pancreatic enzyme supplements, one of which is the fact that the majority of ingested pancreatic enzymes in the original uncoated, conventional preparations were inactivated by gastric acid before reaching the distal duodenum (Carroccio *et al*., 1992). In order to overcome this problem, pH-sensitive preparations containing enteric-coated minimicrospheres were developed and specifically designed to remain intact until they reached the duodenum (target site for drug delivery), where the pH-sensitive coating dissolves to release the digestive enzymes in their active form. In general, patients treated with the enteric-coated minimicrospheres displayed increased CFA, improved stool consistency, decreased stool frequency and an overall greater improvement in disease symptoms (Carroccio *et al*., 1992; Stern *et al*., 2000). In the present study the Creon 10 000 preparation was administered together with 20g of Vanilla yoghurt (slightly acidic) and 20g of the pig's meal, this was done in order to ensure that the enteric-coated minimicrospheres remained stable until reaching the alkaline pH of the duodenum.

Another factor which has been demonstrated to play a role in the efficacy of pancreatic enzyme supplements is the administration schedule of oral pancreatic enzyme supplements. In order to ensure the adequate gastric mixing of the active enzymes with the ingested meal, as well as the simultaneous gastric emptying of the active enzymes with the chyme it is imperative that the pancreatic supplements be administered properly to ensure maximal lipolytic activity (Dominguez-Munoz *et al*., 2005). The oral pancreatic enzyme supplements used by Dominguez-Munoz *et al*.

(2005) in their study were all effective in improving fat digestion irrespective of the administration schedule, however they were more efficient in doing so when administered together with or just after the meal, compared to when administered before the meal (Dominguez-Munoz *et al*., 2005). Thus, when the enzymes are administered together with the meal, the mixing of the exogenous enzymes with the ingested nutrients is optimised. When the enzymes are administered after the meal, even though a portion of the ingested nutrients have been emptied from the stomach, there is still a significant portion of the meal that is properly digested (Dominguez-Munoz *et al*., 2005). In the present study, the Creon 10 000 preparation was administered together with a portion of the pigs' meal, before the majority of the meal was consumed. The method of administration was chosen to ensure consumption of all the Creon 10 000 preparation, before administration of the remaining pig feed. Significant improvements in dietary fat absorption were observed using this particular method of administration, however, the efficacy of the Creon 10 000 preparation needs to be investigated under different schedules and modes of administration.

In addition to optimising the administration schedule of oral pancreatic enzyme preparations, some studies also suggest that altering the diet of the EPI patient could change their response to PERT. These studies focusing on therapeutic strategies for EPI patients have found that the composition of the patients' meals in terms of nutrients may play an important role in alleviating the persistent steatorrhea experienced by the majority of EPI patients. As various nutrients, specifically protein and fat increase the survival of pancreatic enzyme lipolytic activity within the

duodenum and a direct correlation has been found between the CFA and the amount of fat present in the diet (Biourge & Fontaine, 2004; Suzuki *et al*., 1997; Suzuki *et al*., 1999). In fact as the amount of fat in the meals was increased, a greater proportion of fat was absorbed resulting in a higher CFA and reduced steatorrhea (Suzuki *et al*., 1997). These results were somewhat contradictory to previous strategies put forward in the management of EPI, where moderate to low-fat diets were recommended. The rationale behind EPI patients consuming a low-fat diet was due to bacteria being able to metabolise unabsorbed fat to hydroxy-fatty acids which stimulate the secretion of excess fluids in the distal section of the small intestines, thus, possibly aggravating the steatorrhea. However, as previously mentioned high-fat diets are not contra-indicated and are in fact more efficient in the treatment of EPI (Biourge & Fontaine, 2004). The mechanism by which the fat absorption varies depending on the nutrient content of the diet is still under investigation. It is thought to involve the survival of the lipolytic activity of the pancreatic enzymes in response to various nutrients, as well as the interactions of the fat with other undigested nutrients in the lumen (Suzuki *et al*., 1997). In the present study, we administered the Creon 10 000 preparation together with a high fat diet. The high fat diet was well tolerated by the pigs and significant improvements in fat absorption were observed.

In the present study, by making use of turbidimetry, we were able to calculate the lipaemic index for each plasma sample taken at each time point during the control period (day 6), and on the first (day 8) and last days (day 14) of treatment with the Creon 10 000 preparation. The lipaemic index remained relatively constant on the day of control collections (day 6) and significant changes in the lipaemic index were

observed following treatment with the Creon 10 000 preparation (day 8 and day 14). The peak lipaemic index value increased following treatment with the Creon 10 000 preparation and the peak value was reached at a much faster rate following treatment compared to the rate at which it peaked during the control collection period (day 6). No previous studies could be found which evaluated the plasma lipid profile of EPI pigs using turbidimetry and then calculating the lipaemic indices. This approach could thus be employed as a quick screening tool for the indirect assessment of dietary fat assimilation.

The trend in the lipaemic index values as well as in the rate at which the peak values were reached was mirrored in both the plasma fatty acid concentration ($mmol.l^{-1}$) as well as in the plasma triglyceride concentration ($mmol.l^{-1}$) before and after treatment with the Creon 10 000 preparation. This observation led to the conclusion that the changes observed in the calculated lipaemic index values following treatment with the Creon 10 000 preparation were mainly due to the improved absorption of fatty acids and triglycerides, as none of the other blood parameters measured (cholesterol, low density lipoproteins, high density lipoproteins) displayed any significant changes following treatment with the Creon 10 000 preparation. These observations also lead me to conclude that the turbidimetry methods that were used as an approach to analyzing the overall plasma lipid profile of EPI pigs was an effective method and was capable of detecting changes in plasma lipid content. However since the lipid profile of the diet was not determined, I am unable to draw conclusions as to whether the specific changes seen in lipid profile were a normal limitation of the GIT in EPI

pigs to absorb the cholesterol or whether they were due to the diet given not containing significant amounts of the cholesterol.

Chapter 3 – Conclusion

Conclusion:

The present study corroborates the findings of previous studies in confirming that the inclusion of a high fat diet in the treatment of EPI is beneficial at least in the short term. The administration of the Creon 10 000 preparation together with the high-fat diet was well tolerated by the pigs and proved to bring about the desired effects of alleviating some of the detrimental effects of EPI and in doing so, improving the overall nutritional status of the pigs. The dietary supplementation with the Creon 10 000 preparation, in conjunction with a high-fat diet did result in an improved growth performance as well as fat digestion and absorption in our pig model of EPI. Thus, confirming that a high fat diet when supplemented with pancreatic digestive enzymes in EPI patients can play an important role in improving growth performance and fat digestion contrary to some popular beliefs advocating a low fat diet for patients with EPI. The use of turbidimetry as an approach to measuring and analysing the plasma lipid content in EPI pigs, before and after treatment also proved to be effective and is recommended as a quick tool for assessing digestion and absorption of fat in similar future studies.

Recommendations for future studies:

Future studies could be done for a longer time period in order to assess the effects of long-term pancreatic enzyme supplementation, together with a high fat diet in the treatment of EPI and to see whether the enzyme supplementation and the effects thereof can be maintained. Long-term pancreatic enzyme supplementation trials

would be somewhat difficult to do using the pig model of EPI as the pigs can not be kept in the metabolic cages for long periods of time. Perhaps the rat model of EPI would be better to use in long-term studies as they would be easier to handle and maintain within a metabolic cage. Future studies should also assess the importance and effects of various hormones in the control of exocrine pancreatic secretion and how they are affected by the PERT.

Chapter 4 – References

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