OF PIGLETS,
DIETARY PROTEINS,
AND PANCREATIC PROTEASES

Promotor: **Dr Ir M.W.A. Verstegen**
buitengewoon hoogleraar op het vakgebied van de veevoeding,
in het bijzonder de voeding van de eenmagigen

Co-promotor: **Dr Ir L.A. den Hartog**
directeur van het proefstation voor de varkenshouderij te Rosmalen

OF PIGLETS,
DIETARY PROTEINS,
AND PANCREATIC PROTEASES

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Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr H.C. van der Plas,
in het openbaar te verdedigen
op donderdag 3 juni 1993
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

VOORWOORD

Dit proefschrift is het resultaat van 4½ jaar onderzoek bij de vakgroep Veevoeding. In die 4½ jaar vol mee- en tegenvallers is mij duidelijk geworden dat steun en gezelligheid van collega's onontbeerlijk zijn voor het behoud van 'lol in je werk'. Gelukkig heeft het hieraan bij Veevoeding nooit ontbroken.

Enige mensen, die, elk op hun eigen wijze, een bijdrage hebben geleverd aan de totstandkoming van dit proefschrift, wil ik met name noemen:

Allereerst natuurlijk mijn promotor, prof. dr ir M.W.A. Verstegen. Martin, jouw suggesties en ideeën (hoewel vaak onverstaanbaar of onleesbaar) bleken (na ontcijfering) altijd erg waardevol te zijn. Vat het niet persoonlijk op dat ik je tips voor stellingen ("Melk moet mogen") niet heb benut.

Dr ir L.A. den Hartog, mijn co-promotor. Leo, jij bent vanaf het begin betrokken geweest bij het onderzoek en je was van onschatbare waarde bij het na tegenslagen weer 'op de rails zetten' van mij en m'n onderzoek.

Toen Leo vertrok naar Rosmalen nam dr ir B. Kemp veel van zijn klussen over, waaronder mijn begeleiding. Bas, jouw felle inbreng, vooral op het statistische vlak heeft mij veel verder geholpen. Uit resultaten, waar ik vaak kop noch staart aan kon ontdekken, wist jij altijd listige theorieën te destilleren.

Ook Bas verliet de vakgroep na enige tijd (zou het aan mij liggen?) en werd opgevolgd door dr ir M.W. Bosch. Marlou, jij hebt de laatste fases van het onderzoek meegemaakt en daarmee waarschijnlijk ook de leukste discussies. Jouw praktische en concrete instelling heeft me erg aangesproken, bovendien had dit proefschrift zonder jouw inzet waarschijnlijk een hoofdstuk minder geteld...

Bij de proeven met gecannuleerde biggen heeft het ILOB een grote rol gespeeld. Met name Joop Huisman, Piet van Leeuwen, Dick van Kleef en Kasper Deuring wil ik hierbij hartelijk danken voor hun hulp. Martin van Baak en zijn lab-medewerkers hebben veel werk verricht om de enzymanalyses van de grond te krijgen. Ook Martje Fentener van Vlissingen heeft door haar bemoeienissen een belangrijke bijdrage geleverd aan mijn ontwikkeling en aan dit proefschrift.

De medewerkers van proefaccomodatie De Haar waren altijd bereid mee te denken met de praktische invulling van de proeven. De inzet van Jan Hagens, Ries Verkerk, André Jansen en de anderen heb ik erg gewaardeerd.

Mijn paranimfen, Josien van de Kraats en Annemarie Zijlmans waren altijd beschikbaar voor een praatje en een pafke, zodat ik mijn kleine frustraties en irritaties weer snel kwijt raakte.

Ook alle andere medewerkers van de vakgroep hebben hun steentje bijgedragen aan dit proefschrift door hun assistentie bij het slachten van ca 150 biggen en door hun aandacht en steun in voor- en tegenspoed. Bedankt hiervoor!

STELLINGEN

I

Verschillen in schijnbare verteerbaarheid tussen ondermelkpoeder, sojaprodukten en vismeel bij jonge biggen zijn voornamelijk te wijten aan verschillen in endogene stikstof-verliezen.

(dit proefschrift)

II

De exocriene pancreas is niet de belangrijkste bron van endogene stikstof-verliezen bij jonge biggen.

(dit proefschrift)

III

Onvoldoende overeenstemming met betrekking tot monster-bewaaromstandigheden en enzymanalyses bemoeilijkt de interpretatie van literatuur-gegevens.

(dit proefschrift)

IV

Bij jonge biggen is de voeropname na het spenen belangrijker dan de eiwitbron in het voer voor de ontwikkeling van pancreas-protease-activiteiten.

(dit proefschrift)

V

Het door Szabó *et al.* (1976) beschreven effekt van omgevingstemperatuur op (pancreas) enzymactiviteiten bij *ad lib* gevoerde varkens moet waarschijnlijk geinterpreteerd worden als een effekt van voeropname.

(Szabó *et al.* (1976) Magyar Állatorvosok Lapja 31(5):325-328)

(dit proefschrift)

VI

"Het kweken van tomaten en het fokken van varkens gebeurt in Nederland op meer wetenschappelijke basis dan het voeren van overheidsbeleid".

(A. Hoogerwerf, bestuurskundige TU Twente, in 'NOVA', 19 maart 1993)

VII

De theorie, dat bepaalde karaktereigenschappen de kans op het krijgen van (ernstige) ziektes kunnen vergroten, is vooral op humane gronden verwerpelijk.

VIII

Positieve discriminatie van vrouwen bij sollicitaties leidt tot discriminatie van mannen die een baan zoeken en tot discriminatie van vrouwen die een baan hebben.

IX

Het bezit van kennis zonder het vermogen tot kennisoverdracht is nutteloos.

X

Proefdieren schijnen te streven naar maximale variatie binnen proefgroepen, waardoor veel dierfysiologische experimenten, gericht op verschillen tussen proefbehandelingen in de war gestuurd worden.

Stellingen behorende bij het proefschrift van C.A. Makkink, getiteld 'Of piglets, dietary proteins, and pancreatic proteases'.

Wageningen, 3 juni 1993

OP EEN BIG

EEN BRITSE BIG WIENS NAAM WAS PORK
LIEP ALTIJD ROND MET MES EN VORK.
"IK DRAAG DIT BIJ MIJ", SPRAK HET BEEST,
"ZOALS EEN IEDER, DIE DIT LEEST
EN BRITS VERSTAAT, TERSTOND ZAL SNAPPEN,
FOR IF THE *WORST* SHOULD COME TO *HAPPEN*".
                                        (TRIJNTJE FOP)

Makkink, C.A., 1993. Of piglets, dietary proteins, and pancreatic proteases (Van biggen, voereiwitten en pancreas proteases).
Newly weaned piglets often show digestive disorders, frequently resulting in diarrhoea. These disorders may be related to the dietary protein source, since young piglets are less capable of digesting proteins of vegetable origin than older pigs. This study was undertaken to investigate the development of protein digestive capacity in weaned piglets. In the first part of the thesis it was established that the difference in nitrogen digestibility between milk, soya and fish proteins was mainly due to differences in endogenous nitrogen losses and not to differences in true digestibility. In the second part, the pancreatic protease activities of newly weaned piglets fed various dietary protein sources was investigated. It was found that dietary protein source affected trypsin and chymotrypsin activities in pancreatic tissue and jejunal digesta, however, these differences could not explain the differences in endogenous nitrogen losses. It is more likely that other sources of endogenous nitrogen, e.g. the small intestinal wall, are responsible for the differences in protein digestibility observed in young piglets. Furthermore, it was concluded from the experiments described in this thesis, that post-weaning feed intake has a greater effect on the development of pancreatic enzyme activities than dietary protein source *per se*. Young piglets are capable of increasing their pancreatic secretion in response to appropriate stimuli. Stimulating post-weaning feed intake may help to smooth the development of protein digestive capacity in young piglets. In this respect, feed intake patterns may be at least as important as quantitative feed intake.
Ph.D. thesis. Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands.

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GENERAL INTRODUCTION

General

The first food that a newborn piglet ingests is colostrum from the sow. Until weaning milk is the major source of nutrients. Young piglets are very well capable of digesting nutrients from colostrum and milk immediately after birth.

Half a century ago, piglets were normally weaned between 8 and 12 weeks of age (Linton, 1927), gradually increasing their solid feed intake. Nowadays, weaning has moved to an earlier age (3 to 4 weeks) so as to increase sow productivity. Developments in the preparation of weaner diets (milk replacers) have enabled the piglet to be weaned at this age.

However, after weaning at 3 to 4 weeks of age, growth checks and digestive disorders are often experienced in modern piglet rearing. The causes of these problems concerning newly weaned piglet performance are many-fold. Weaning includes various stress factors for the young piglet. The change to a new environment imposes climatic stress factors upon the young piglet. Especially the nutritional challenge is a crucial aspect of weaning. The withdrawal of sows milk is an important factor in the process of weaning, not only from a nutritional point of view (composition of sows milk with respect to easily digestible nutrients) but also in a behavioral and immunological sense (Stanton and Mueller, 1976).

The piglet must learn to consume a solid weaning diet of a completely different texture, temperature, smell, taste and composition compared to sows' milk.

Protein is the most expensive nutrient in pig feeds, especially when milk proteins are fed. Because milk products will be less available in the future, attempts are made to decrease the proportion of milk proteins and increase the amount of vegetable protein sources in post-weaning pig diets. The incorporation of large amounts of non-milk proteins, however, often leads to digestive disorders in young piglets (Cline, 1991).

The adaptation of the piglet's digestive system to different protein sources is a very complex process. Adaptation must occur with respect to pH regulation (acid secretion in the stomach and alkaline secretions from the pancreas, gall-bladder and small intestine), enzyme secretions (stomach, pancreas, small intestine), motility (gastric emptying, passage rate of digesta in different parts of the gastrointestinal tract) and absorption (transport mechanisms) (Schnabel, 1983; Sève, 1985; Cline, 1991).

The digestion of the diet depends not only on dietary factors (energy/protein content, antinutritional factors, (non)essential amino acids) but also on factors concerning the animal that consumes the diet (age, breed, weaning circumstances, health status, sex). Environmental factors (temperature, humidity, hygiene) also play a part in digestion and performance.

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In current practice, the piglets are introduced to creep feed from one or two weeks of age onwards. Creep feed is usually based on milk proteins and contains easily digestible carbohydrates and fats.

About one week before, or sometimes just after weaning, other protein sources are incorporated in the piglets' diet. The shift from milk protein to other dietary proteins can put too great a strain on the piglets' immature digestive system. This can lead to growth depression and/or (post weaning) diarrhoea.

Digestion of Different Protein Sources in Piglets of Different Ages

Protein digestion is usually measured as apparent faecal nitrogen digestion. Especially with newly weaned piglets, not many data are available on ileal nitrogen digestibility, let alone on true nitrogen digestibility. Individual housing and intestinal surgery are more difficult for young piglets than for growing swine and this complicates ileal and true digestibility studies in piglets.

In this thesis the term 'digestibility' will apply to apparent faecal digestibility unless stated otherwise.

In young piglets, the difference in digestibility values between vegetable and animal protein is considerable. Soy proteins, e.g., are not digested to the same extent as milk proteins (Wilson and Leibholz, 1981c). Later in life, pigs are more capable of digesting protein sources from plant origin (Combs *et al.*, 1963; Van de Kerk, 1982). The fact that the difference in digestibility between animal protein and vegetable protein sources decreases with advancing age of the piglets (Table 1) leads us to believe that the 'developmental or adaptational state' of the piglet is the bottle neck in the digestion of protein. If we want to look into the causes of depressed digestion more closely, we have to consider the process of protein digestion in more detail.

There are different sites in the gastrointestinal tract of the pig where problems can occur when overall protein digestion is disturbed. Firstly, feed intake can be depressed as often occurs during the early post-weaning period. Feed intake is affected by palatability of the feed, texture of the feed (pellets, meal, slurry), physical properties (pellet size and hardness) and generally by familiarity of the feed to the animal and by well-being of the animal.

Also, the interaction between the solid diet and the piglet's digestive tract is important: the physical and chemical characteristics of the post-weaning diet may affect the integrity of the small intestinal mucosa leading to villus atrophy and malabsorption. This gut wall damage may then lead to depressed feed intake.

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An important role in protein digestion is played by the stomach. In the stomach, acid and proteolytic enzymes are added to the feed and clot formation occurs depending on the type of ingested protein. Gastric emptying patterns determine the release of gastric contents into the small intestine and thus affect the intestinal digestion of protein.

In the duodenum, pancreatic juice and bile are added to the chyme. Pancreatic juice contains bicarbonate, which serves to buffer the acid contained in the digesta leaving the stomach. The intestinal pH needs to be elevated because the optimum pH for activity of the pancreatic proteases lies around 8. The pancreatic proteases continue the breakdown of proteins which was initiated in the stomach. Pancreatic enzyme synthesis, secretion and activation should respond to the substrates (e.g., type of dietary protein) presented in the digesta so that adequate digestion of nutrients is ensured.

The protein digestion by intestinal peptidases in the small bowel coincides largely with absorption since many intestinal proteases are attached to the gut wall so that products of hydrolysis can be absorbed directly. An intact gut wall mucosa is of course a prerequisite for undisturbed absorption of nutrients. Gut wall damage is a common finding during the early post-weaning period in pigs (Nabuurs, 1991). Villus atrophy and crypt hyperplasia hamper digestion and absorption in the small intestine of newly weaned piglets.

A disturbed digestion and absorption leads to an excess of nutrients in the lower small intestine. This may lead to serious alterations in the pig's microflora, resulting in proliferation of pathogens and probably digestive disorders.

Thus, it is clear that the causes of inadequate protein digestion can be many-fold, since the digestion of protein is the result of many processes occurring during the passage of food through the digestive tract. These processes cannot be considered independently since their interrelationships form the basis for proper protein digestion.

In the next paragraph a description is given of the process of protein digestion in the digestive tract of the pig.

Table 1   Apparent faecal digestibility (protein/N) of diets based on different protein sources in young piglets.

ref.	age at weaning	(weeks or days) or weight (kg) of the piglets: during exp.	protein source: skimmed milk powder	casein	soya- bean meal	soya protein conc.	isolated soya protein	fish meal
1	15 d.	3-4 wks	79.1		73.8			70.7
	15 d.	5-6 wks	81.6		75.9			69.6
	15 d.	7-8 wks	85.7		85.0			83.7
2	8 d.	25-29 d.		94.47				
	3 d.	25-29 d.		93.97				
	3 d.	25-29 d.		94.19				
3	21 d.	3-5 wks		86.3	82.3			
4	4-5 d.	28 d.	98.6		82.2		89.1	
	4-5 d.	14 d.	96.8		93.7			
	4-5 d.	35 d.	98.3		95.7			
5		4.7 kg		83		79.7	83.6	
6	21 d.	28 d.	80		72			
	21 d.	41 d.	86		78			
7	5 wks	5-7 wks	91.75					
8	?	20-22 kg				76		
9	21 d.	4 wks		89.89		71.42		
	21 d.	8 wks		95.72		88.12		
	7 d.	2 wks		68.27		65.64		
	7 d.	4 wks		88.16		69.23		
	7 d.	6 wks		89.22		74.58		
10	10 d.	18-23 d.	95		77			
	10 d.	38-43 d.	96		83			
11	21 d.	32-35 d.	96.09				93.20	
		39-42 d.	96.35				93.15	
		46-49 d.	95.91				89.96	
		53-56 d.	93.96				94.24	
		32-56 d.	95.57				93.63	
12	5-11 d.	12-16 d.	93.0					
	5-11 d.	16-20 d.	94.6					
	5-11 d.	20-24 d.	92.2					
	5-11 d.	24-28 d.	92.1					
	5-11 d.	28-32 d.	94.0					
	5-11 d.	32-36 d.	93.1					
	5-11 d.	36-40 d.	95.5					
	5-11 d.	12-40 d.	93.5					
13	2 d.	23-51 d.	93.7		74.0			
14		10-14 d.	95.6					
15	21 d.	25-35 d.	92.61		80.06	90.69	90.95	
16	21 d.	42 d.		83.7	76.2	80.8	80.6	

References: 1=Combs et al. (1963), 2=Watkins & Veum (1986), 3=Etheridge et al. (1984), 4=Wilson & Leibholz (1981c), 5=Li et al. (1989), 6=Christison & Parra de Solano (1982), 7=Moughan et al. (1989), 8=Green & Kiener (1989), 9=Pekas et al. (1964), 10=Hays et al. (1959), 11=Sewell & West (1965), 12=Decuypere et al. (1981), 13=Sherry et al. (1978), 14=Zamora et al. (1979), 15=Sohn & Maxwell (1990), 16=Walker et al. (1986)

The Course of Protein Digestion

A simplified diagram of protein digestion in monogastrics is given in Figure 1.
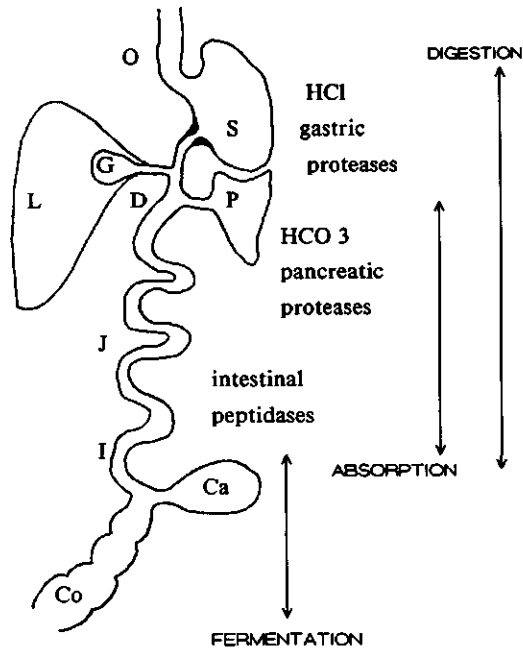

Figure 1.   A schematic overview of the sites of digestion and absorption of protein in the digestive tract of pigs. O=Oesophagus, S=Stomach, P=Pancreas, L=Liver, G=Gallbladder, D=Duodenum, J=Jejunum, I=Ileum, Ca=Caecum, Co=Colon.

The digestion of protein is accomplished by the action of different enzymes along the gastrointestinal tract. An overview is given in Table 2.

Table 2   Proteolytic enzymes secreted from different sites along the gastrointestinal tract.

site	enzyme	pH optimum	specificity
mouth/saliva[1]	-	-	-
stomach[2]	chymosin	6.3	milk clotting
		3.5	proteolysis
	pepsin	6.3	milk clotting
		2.0	proteolysis at N-side of LEU, ASP, GLU, PHE, TYR and TRP, not at C-side of PRO
	gastricsin	6.3	milk clotting
		3.0	proteolysis
duodenum[3,4,5] (pancreas)	trypsin	7.5-8.5	proteolysis at C-side of LYS, ARG (and AECYS), not at N-side of PRO
	chymotrypsin	8	proteolysis at C-side of PHE, TRP, TYR, MET, GLU, ASP and LEU, not at N-side of PRO
	carboxy-peptidases	7.5-8	proteolysis at N-side of C-terminus (PHE, TYR, TRP, ARG and LYS)
	elastase	7.5-10.5	proteolysis at C-side of ALA and GLY (aliphatic amino acids)
small intestine[6] (gut wall)	endo-peptidases	7.0-7.5	proteolysis, near hydrophobic aminoacids
	amino-peptidase	7.0-7.5	proteolysis, neutral amino acids at N-terminus
	carboxy-peptidases	7-9	proteolysis, mainly PRO, at C-terminus

[1] Kidder and Manners, 1972; Low and Zebrowska, 1989
[2] Sangild, 1990
[3] Rinderknecht, 1986; Kidder and Manners, 1972; Wood et al., 1974
[4] Walsh, 1970
[5] Wilcox, 1970
[6] Rérat and Corring, 1991

According to Kidder and Manners (1972) and Low and Zebrowska (1989) pig saliva does not contain any proteolytic enzymes. Protein digestion in pigs starts in the stomach through the combined action of acid and proteolytic enzymes. Young (suckling) piglets secrete only small amounts of pepsinogen and hydrochloric acid (Sangild, 1990). Initially, the only protein source for the suckling piglet is sow's milk. Gastric digestion of milk proteins is a specific process, characterized by the clotting of the milk (casein) by chymosin (rennin) action. Non-milk proteins will not coagulate in the stomach although some acid-induced precipitation may occur. Milk (lactose) is also fermented by

lactobacilli in the young piglet's stomach, resulting in lactic acid accumulation (Cranwell, 1990).

An undisturbed gastric function depends on animal factors (acid and enzyme secretion, gastric emptying) and on aspects of the feed (buffering capacity, bulkiness, solubility, clot formation). These factors cannot be considered separately because they interact, e.g., a high dietary buffering capacity increases the requirements for acid secretion.

The digestion of protein is continued by the proteolytic action of enzymes secreted by the pancreas. The pH of intestinal contents is raised through the secretion of HCO_3^- by the pancreas. The secretion of pancreatic juice is regulated by nervous and endocrine mechanisms (Solomon, 1987).

Some hormones and peptides affecting pancreatic secretion are listed in Table 3.

Table 3   Some hormones and peptides affecting pancreatic secretion (Solomon, 1987; Langlois *et al.*, 1989; Brannon, 1990; Croom *et al.*, 1992)

hormone/peptide	effect	stimulus
secretin	stimulates secretion of fluid and bicarbonate	unbuffered H^+ in duodenum
cholecystokinin-PZ	stimulates secretion of proteases and growth of pancreas	intraluminal peptides, H^+, fatty acids, cations, trypsin inhibitors
pancreatic polypeptide	decreases pancreatic enzyme and bicarbonate output, no effect on juice flow	?
gastrin	stimulates secretion of enzymes	stomach distention, intragastric peptides or amino acids (?)
chymodenin	stimulates chymotrypsinogen secretion	?
(entero)glucagon	inhibits pancreatic secretion	?
vasoactive intestinal peptide	stimulates secretion of bicarbonate	vagus nerves (+)
somatostatin	decreases gastrointestinal tract motility, blocks HCl and bile secretion, inhibits absorption	vagus nerves (-) splanchnic activity (+)

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Secretin and CCK-PZ (cholecystokinin-pancreozymin) are the most important hormones in the regulation of pancreatic secretion (Solomon, 1987; Croom *et al.*, 1992). The end products of pancreatic protein digestion are mainly peptides.

It is generally accepted that activation of pancreatic zymogens occurs through the action of enterokinase secreted by the duodenal wall and of trypsin from the pancreas itself. The presence of end products of pancreatic protein digestion in the intestinal lumen is recognized and communicated to the exocrine pancreas through various hormones and peptides. Feedback mechanisms are very important in pancreatic regulation but the precise (inter)actions of various hormones and peptides remains to be unravelled.

Small intestinal digestion of peptides is performed by the action of brush-border peptidase enzymes (Low and Zebrowska, 1989). Absorption occurs simultaneously because digestion is mainly established through peptidases attached to the intestinal epithelium. Peptides can be hydrolysed at the luminal site and transported as amino acids or absorbed as peptides and hydrolysed in the cytoplasm of the enterocyte or absorbed as peptides and transported into the circulation as such (Friedrich, 1989).

Nutrients that remain undigested and/or unabsorbed until the terminal terminal ileum are subjected to fermentation by intestinal microbes. Excessive bacterial activity may lead to formation of toxins and to proliferation of pathogenic bacteria.

Animal Factors Affecting Protein Digestion

1. pH

The pH in the different parts of the digestive tract influences the course of digestion of nutrients in pigs and other animals.

Precursors of digestive enzymes are activated at specific pH-values. Digestive enzymes have specific pH-optima at which their activity is highest (Table 2). Gastric proteolytic enzymes have two pH-optima: 6.3 for milk-clotting and 2.0-3.5 for proteolysis (Table 2). The pH in the digestive tract also affects microflora proliferation and thereby fermentation of feed components (Banwart, 1981; Cranwell, 1990).

Appropriate regulation of pH in the gastrointestinal tract is a prerequisite to ensure optimal digestion of dietary protein and other nutrients.

The maintenance of an optimal pH in a certain part of the tract depends on the buffering capacity of the feed or chyme entering that part of the tract and on the secretory capacity of the pH regulating organs (stomach, pancreas, liver, small intestine).

2. Enzyme secretion

The synthesis and secretion of proteolytic enzymes needs to be sufficient to digest the amount and type of dietary protein offered to the animal. In the stomach of suckling piglets, the clotting of milk-casein is an important aspect of digestion. Milk clotting influences gastric emptying and thus ensures a more regular release of nutrients into the gut (Sangild, 1990). Other protein sources (soya, fish) are hydrolysed differently: they do not coagulate in the stomach and therefore their digestion probably depends more on pancreatic enzymes (Pekas et al., 1964). Gastric proteolysis is low in young piglets and increases with advancing age (Leibholz, 1981, 1986).

During suckling, exocrine pancreatic secretion remains low and is not stimulated by milk ingestion (Pierzynowski, 1991). After weaning, the development of pancreatic enzyme secretion is less clear: Some authors (Lindemann et al., 1986; Owsley et al., 1986) report an initial decline in post-weaning trypsin and chymotrypsin activities in pancreatic tissue and intestinal contents, presumably caused by 'post-weaning stress' or by low post-weaning solid feed intake. Others (Pierzynowski, 1991; Efird et al., 1982) find a substantially higher pancreatic enzyme secretion or activity in the small intestine from weaning onwards. Pierzynowski (1991) also found that the ingestion of solid feed after weaning increases pancreatic secretion while milk ingestion during the suckling period does not affect basal secretion. The effect of post-weaning feed intake (level and pattern) on pancreatic enzyme synthesis and secretion is insufficiently clear yet.

3. Motility

The motility of the digestive tract is an important factor in digestion. Stomach emptying and intestinal passage rate are affected by frequency of feeding (Braude et al., 1970) and by dietary protein source (probably through clotting properties) (Maner et al., 1962; Newport and Keal, 1983).

In pre-ruminant calves the composition of the diet is associated with small intestinal motility patterns and diarrhoea (Duvaux, 1985). Feeding of cows milk resulted in a normal motility pattern and no diarrhoea. Soy protein feeding resulted in diarrhoea and abnormal motility patterns (increased number of regular spiking activity (RSA) episodes). When milk was fed in combination with sucrose, calves developed diarrhoea and the number of RSA's decreased while long periods of irregular spiking activity (ISA) were found (Duvaux, 1985).

A relation between feed intake patterns and intestinal motility in newly weaned piglets has been proposed by Sissons (1989) but experimental proof is still lacking.

4. Absorption

The intestinal absorption of protein hydrolysis products has recently been reviewed by Webb (1990). He concludes that small peptides are absorbed from the small intestine more rapidly than free amino acids.

Temporary changes in intestinal wall structure (decrease in villus length and increase in crypt depth) and malabsorption are common findings in newly weaned pigs (Hampson and Kidder, 1986; Löfstedt, 1986; Nabuurs, 1991). The villus atrophy leads to an increase in endogenous nitrogen losses and to malabsorption. The causes of villus atrophy and crypt elongation are not sufficiently clear yet. Pre- and post-weaning feeding practices may be associated with changes in gut wall morphology. Creep feeding resulted in deeper crypts and partly prevented the shortening of villus and the decreased absorption after weaning (Nabuurs, 1991). Deprez *et al.* (1987) managed to reduce post-weaning villus atrophy in newly weaned piglets by providing liquid (slurry) in stead of dry feed. Relations between gut wall morphology and feed intake of newly weaned piglets are not established conclusively yet.

It is clear that animal factors are of major influence on protein digestion. The different factors mentioned in the foregoing paragraph are affected by the physiological status of the pig (age, stress, health and disease).

Dietary Factors Affecting Protein Digestion

1. Buffering Capacity of Feedstuffs

The buffering capacity of feedstuffs is important for the pH regulation in the stomach. The pH in the stomach should reach low values (2 to 4) to promote pepsinogen activation and to inhibit bacterial growth. The stomach should excrete enough hydrochloric acid to provoke this decrease of pH. Certain feedstuffs, especially protein sources and mineral mixtures, require large amounts of acid to reach low pH values (see Table 4).

If the stomach is deficient with respect to acid secretion, highly buffering agents in the feed should be avoided.

In young animals, the lowering of pH in the stomach is supported by lactic acid production by bacteria present in the stomach. Lactic acid bacteria grow on lactose, which is an important ingredient of (sows) milk. This is one of the reasons why milk products are well digested by young piglets, despite of their high protein content (and thus high buffering capacity).

Table 4   Buffering capacity of different feedstuffs (mmol HCl consumption required to
               reach pH 4 per 100 g original matter). (Bolduan *et al.*, 1988)

feedstuff	buffering capacity
skim milk (acid)	3.07
skim milk (fresh)	7.12
skim milk (dried)	66.37
wheat	8.99
barley	9.97
yeast	30.10
soyabean meal (extracted and toasted)	50.68
fish meal	60.38
mineral mixture	1260.50
starter for weaner piglets	30.00

In the stomach of unweaned piglets, enzymes are secreted which induce the clotting of
milk-casein (Sangild, 1990).

When the buffering capacity of the feed is too high, or when the acid production in the
stomach is too low, pH in the stomach will remain high, causing proliferation of bacteria
and insufficient activation of pepsinogen. Thus, the digestion of non-milk protein will be
depressed and bacteria can travel through the digestive tract exerting deleterious effects
further down the gut.

To prevent overburdening of the stomach during difficult periods (e.g. just after weaning)
diets should be formulated with relatively low buffering capacities or with organic acids
added (Bolduan *et al.*, 1988). Increasing the frequency of feeding can also prevent
digestive problems in the stomach.

2. Protein structure

The structure of a protein can be described in terms of primary, secondary, tertiary and
quaternary structure (Pomeranz, 1991).

- primary structure: amino acids composition and sequence.
- secondary structure: spatial configuration by hydrogen
        bonds.
- tertiary structure: three-dimensional organization by
        disulphide bonds, hydrophobic bonds, hydrogen bonds between side chains,
        electrostatic interactions, dipole-dipole interactions, and charge-dipole interactions.
- quaternary structure: aggregation of protein subunits.

The amino acid composition of the protein determines its susceptibility to enzymatic
degradation, as illustrated in Table 2.

The structure and spatial organization of a protein determines its functional properties (solubility, stability over pH and temperature ranges) and therefore affects its digestibility (solubility, accessability towards enzymes).

3. Antinutritional factors

Antinutritional factors (ANF's) can affect the digestion of nutrients through various mechanisms. Trypsin inhibitors bind to trypsin and thus inhibit the digestion of protein by removing active trypsin from the gut, by suppressing the activation of other zymogens and by increasing the amount of endogenous nitrogen losses. Tannins bind to protein molecules thus preventing the digestion of these proteins. Lectins bind to the gut wall and hamper absorption of digestive end products. The effects of ANF's on dietary protein utilization have recently been summarized by Nitsan (1991).

4. Interactions between nutrients

A nutrient may interfere with the digestion of other nutrients in the diet. Carbohydrates are known to interact with protein digestion (Sewell and West, 1965). Eggum (1973) showed that lactose may improve protein utilization as well as intestinal pH, microflora and intestinal vitamin synthesis. Thus, lactose influences protein digestion both directly and indirectly.

From the foregoing paragraphs it can be concluded that protein digestion is affected by dietary factors, animal factors and interactions between animal and dietary factors.
In this chapter different aspects of protein digestion have been outlined with special reference to newly weaned piglets. The digestion of dietary protein is a multi-factorial process affected by various animal and dietary factors. Digestive adaptation to the change in dietary composition constitutes a major event during the early post-weaning period in young pigs.
This thesis describes investigations performed with young piglets to elucidate the physiological adaptations occurring at weaning with respect to the digestion of different dietary protein sources.

Scope of the Thesis

The performance of young piglets (growth, feed intake, feed/gain ratio) depends on several factors, e.g., age of piglets, environmental conditions (e.g. temperature), social conditions (social order), feeding conditions (amount of feed, composition of feed) and

stress factors.

An important aspect of the feed is the protein source: especially young piglets respond differently to different protein sources. Proteins of animal origin are dealt with more easily than vegetable proteins (Wilson and Leibholz, 1981a,b,c). This difference in protein digestion diminishes with advancing age of the animals (Combs *et al.*, 1963; Van de Kerk, 1982).

> The research described in this thesis was undertaken to study the development of the protein digestive capacity of young piglets during the post-weaning period.

From the foregoing paragraphs it can be derived that the proximal digestive tract (stomach and duodenum + pancreas) is of major importance for the digestion of dietary protein. Digestive disorders resulting from poor protein digestion may be exhibited further down the gut, e.g., bacterial proliferation due to excessive amounts of substrate at the terminal ileum or diarrhoea at the faecal level. However, these (sub)clinical signs will be preceded by digestive disturbances occurring at the upper digestive tract. Therefore, it is postulated that the proximal digestive tract organs (stomach and pancreas) play a key role during the post-weaning adaptation period to a new dietary (protein) regime. The process of adaptation of the stomach and pancreas with respect to enzyme secretion and pH regulation will determine the development of (dietary protein-related) digestive disorders often observed during the early post-weaning period. Extending our knowledge on the digestive adaptation of newly weaned piglets may provide nutritional tools to improve post-weaning pig performance.

To investigate the role of the stomach and the pancreas in the development of post-weaning protein digestive capacity of young piglets, the following aspects have been taken into account:

- Endogenous nitrogen losses at the faecal and ileal level have been determined in young piglets fed different protein sources to assess the causes of differences in apparent nitrogen digestibility between protein sources (Chapter II).
- Literature data on the role of the pancreas and its adaptation to various animal and feed characteristics have been collected (Chapter III).
- Some analytical aspects of the determination of pancreatic trypsin and chymotrypsin activity have been investigated (Chapter III and IV).
- The development of pancreatic trypsin and chymotrypsin activity in pancreatic tissue and small intestinal digesta of newly weaned piglets fed different dietary protein sources has been studied (Chapter V and VI).

- Gastric protein breakdown and digestive tract pH related to the development of protein digestive capacity have been examined (Chapter V and VI).
- Jejunal wall morphology has been studied with respect to villus length and crypt depth (Chapter VI).
- Pancreatic secretion has been measured in response to intravenous and intraduodenal stimuli (Chapter VII).
- Interrelationships between gastric protein breakdown, gastrointestinal pH, pancreatic enzyme activities and nitrogen digestibility have been described (Chapter VIII).
- The role of gastric function, pH, pancreatic enzymes and feed characteristics in post-weaning piglet performance has been discussed (Chapter VIII).

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ENDOGENOUS N LOSSES AT THE TERMINAL ILEUM OF YOUNG PIGLETS FED DIETS BASED ON FOUR DIFFERENT PROTEIN SOURCES

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Summary

Fourteen piglets, initially weighing 8 kg, were each fitted with a PVTC (Post Valvular T Caecum) cannula and with two catheters, one in a jugular vein and one in a carotid artery. Each piglet received one of four experimental diets containing either skimmilk powder (SMP), soybean meal (SBM), soy protein isolate (SI) or fish meal (FM) as the sole protein source. The diets were given in equal amounts at 12 h intervals. ^{15}N-L-Leucine was intravenously infused continuously at a rate of $\pm$ 40 mg ^{15}N-L-Leucine per kg body weight per day. Apparent faecal nitrogen (N) digestibilities were 94.0, 84.0, 90.8 and 89.1 % for the SMP, the SBM, the SI and the FM diet, respectively. Daily endogenous faecal N losses were 315, 963, 715 and 697 mg for diets SMP, SBM, SI and FM, respectively. True faecal N digestibilities were 97.3, 93.5, 98.1 and 96.4 % for diets SMP, SBM, SI and FM, respectively. Apparent ileal nitrogen (N) digestibilities were 84.4, 76.5, 78.4 and 73.0 % for the SMP, the SBM, the SI and the FM diet, respectively. Daily endogenous ileal N losses were 786, 1422, 1970 and 1558 mg for diets SMP, SBM, SI and FM, respectively. True ileal N digestibilities were 92.7, 90.6, 98.4 and 89.3 % for diets SMP, SBM, SI and FM, respectively. It was concluded that true N digestibilities were high for all four protein sources, both at the ileal and at the faecal level. Differences in apparent N digestibility were mainly caused by differences in endogenous N secretion.

Introduction

Apparently digested nitrogen at the terminal ileum is considered to be a good measure for nutritional value of protein in the diet. The apparently undigested part, however, can arise from either the feed protein or from endogenous protein (secreted enzymes, mucus, sloughed off mucosal cells). To distinguish between endogenous and exogenous nitrogen, different methods have been proposed (Souffrant, 1991). Until recently, protein free diets and regression techniques have been used to measure endogenous nitrogen losses and thus calculate 'true' compared to apparent digestibilities (Souffrant, 1991). However, protein free diets and different protein sources may itself lead to different levels of endogenous secretion (Huisman, 1990, De Lange *et al.*, 1989). To obtain results on endogenous secretion in normally fed pigs, the feed nitrogen or nitrogen in the animal body can be labelled with a stable isotope in order to distinguish between endogenous and exogenous nitrogen. From that true nitrogen digestibility (also referred to as 'real' nitrogen digestibility) can be calculated (Souffrant, 1991).

Recently, the ^{15}N infusion method for labelling the animal body nitrogen has been introduced and evaluated (Souffrant *et al.*, 1981, De Lange *et al.*, 1990). With this technique a distinction

can be made between endogenous and exogenous nitrogen at the faecal or ileal level. Endogenous nitrogen originates from different sources: saliva, gastric and pancreatic juice, bile, gut wall secretions, sloughed off gut wall cells and bacteria. The precursor pools for all these endogenous nitrogen sources cannot be analyzed separately. Therefore, it is important to have an indicator which reflects the endogenous precursor pool. Urine is not suitable as such, because the amount and composition of nitrogen containing substances in urine depends on the utilization of orally ingested nitrogen by the animal. Whole blood and plasma contain also larger molecules (proteins) which will not be used for the production of compounds to be secreted into the lumen. The TCA-soluble fraction of the blood plasma is considered to be the precursor (amino acids, small peptides, urea) pool for synthesis of endogenous nitrogen. The secretion of nitrogenous compounds originates thus from both intermediary protein conversion and from absorbed nutrients (Herrmann et al., 1986, Souffrant et al., 1981, 1986, 1991). The endogenous nitrogen in digesta or faeces can be calculated through isotope dilution and hence true nitrogen digestibilities can be calculated. Since secreted nitrogenous compounds can also be formed in the enterocytes directly from absorbed resources, the endogenous nitrogen losses as measured using the TCA-soluble plasma as the precursor pool will generally be somewhat underestimated. The technique assumes comparable absorption of labelled and unlabelled amino acids. Also, the extent of labelling of the endogenous nitrogen secretion should not change significantly during the course of the experiment. However, because of the continuous (albeit slow) rise in body protein labelling due to recycling of labelled amino acids, calculated endogenous N values tend to be underestimated.

The measurement of endogenous N losses can give information on the causes of low apparent digestibilities (either low digestibility of the feedstuff or high endogenous secretion by the animal).

Especially with young animals, low apparent (nitrogen) digestibilities are sometimes found at the ileal and the faecal level, especially when vegetable protein (e.g., soy protein) is fed in stead of protein of animal origin (e.g., milk proteins).

Not much is known about the causes of low (apparent) digestibilities of different dietary protein sources in young piglets. Digestibility of soy protein is lower for piglets than for older pigs, digestibility of milk protein is high for pigs of all ages (Wilson & Leibholz, 1981a,b,c).

For the development of feeds for newly weaned piglets it is important to know endogenous nitrogen losses and apparent nitrogen digestibility after weaning. An experiment was undertaken to compare endogenous nitrogen losses in young piglets fed diets based on different protein sources.

The method of infusion of ^{15}N-L-leucine was used to assess the endogenous N losses in the faeces and at the distal ileum in young piglets. The ^{15}N label will also appear in other (though

not all) amino acids due to transamination.

Due to different levels of transamination, it is not feasible to measure the separate endogenous amino acid losses.

Materials and Methods

Animals, diets and experimental scheme

Fourteen castrated male piglets (Great York, initial live weight 7.5 - 8.5 kg) were used to measure apparent and true ileal nitrogen digestibilities of four different diets. Piglets did not receive any creep feed during the suckling period. Piglets were weaned between 3 and 4 weeks of age and housed individually on mesh floors, tethered to prevent cannula and catheter damage.

Piglets were fed twice daily at 8.00 h and 20.00 h. Two piglets were fed the diet based on skimmilk powder (SMP), three piglets were fed the diet based on soybean meal (SBM), six piglets (four experimental and two spare piglets) were fed the diet based on soy isolate (SI) and three piglets were fed the diet based on fish meal (FM). The diets were fed at a level of 380 g per day throughout the experiment (approx. 4% of live weight per day).

Composition of the protein sources and diets is given in Table 1 and 2. The diets were balanced with regard to net energy, crude protein, crude fat, crude fibre and essential amino acids. Chromium oxide at a level of 0.1 % was included as a marker for digestibility calculation.

After a 6 day 'cage adaptation' period and an overnight fasting period, the piglets were anaesthetized through inhalation anaesthesia (N_2O, O_2, halothane) and fitted with a 'Post Valvular T Caecum' cannula (Van Leeuwen et al., 1991) at an age of 4 to 5 weeks (approximately 8 kg live weight). Immediately before surgery, lidocaine with epinephrine was administered, subcutaneously and intramuscularly in the area of the incision. The flank area was shaved and disinfected with a general disinfectant. An incision was made in the right abdominal wall in the entera-paralumbar area and some distance above the mammary tissue. The caecal apex and the terminal portion of the ileum were located, exteriorized and the caecum was removed. The ileum and the large intestine at the site of the removed caecum was exteriorized. The cannula barrel (internal diameter 19 mm, external diameter 24 mm) was placed through the opening which was made not larger than necessary to aid in securing and positioning the cannula barrel. Thus, surgery consisted of a caecectomy in which an opening for the cannula appeared directly opposite the ileocaecal valve (Van Leeuwen et al., 1991).

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Table 1.   Composition of the protein sources, nutrients, amino acids and trypsin inhibitor activity

protein source:	SMP skimmilk powder	SBM soybean meal	SI soy protein isolate	FM fish meal
dry matter (DM), %	96.2	87.5	95.2	94.4
ash (% of DM)	8.3	7.1	4.1	13.8
crude protein (CP, % of DM)	35.4	52.6	89.5	75.8
ether extract (% of DM)	0.5	1.7	3.6	10.3
crude fibre (% of DM)	0.0	7.1	1.3	0.1
N-free extracts (% of DM)	55.8	31.5	1.5	0.0
Lysine (% of CP)	8.24	5.84	6.01	7.52
Threonine (% of CP)	4.23	3.72	3.66	4.04
Methionine (% of CP)	2.70	1.39	1.21	3.01
Cystine (% of CP)	1.00	1.61	1.12	1.02
Tryptophan (% of CP)	1.30	1.22	1.04	0.96
Trypsin inhibitor (mg inhibited trypsin per g product)	–	3.64	6.27	–
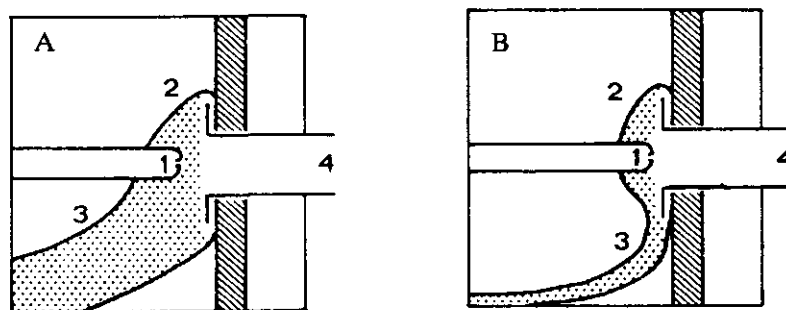
Figure 1.   Post Valvular T Caecum cannula in place (Van Leeuwen et al., 1991).
A. directly after opening the cannula,
B. about 15 minutes after opening of the cannula
     (ileocaecal valve protrudes into the cannula)
1. ileocaecal valve   2. caecum   3. colon   4. PVTC cannula

Table 2.   Diet composition

diet:	SMP skimmilk powder	SBM soybean meal	SI soy protein isolate	FM fish meal
ingredient (% of the diet)				
skimmilk powder (35.3 % CP)	45.50	-	-	-
soybean meal (48 % CP)	-	34.40	-	-
soy isolate	-	-	18.20	-
fish meal	-	-	-	22.15
maize starch	29.60	39.84	52.79	52.91
dextrose	15.00	15.00	15.00	15.00
sunflower/soy oil	2.00	2.00	2.00	0.50
cellulose	5.00	2.85	5.00	5.00
vit/min premix	1.00	1.00	1.00	1.00
ground limestone	0.80	1.35	1.40	0.75
mono Ca phosphate	0.50	2.10	2.20	0.60
salt	-	0.50	0.50	0.10
$KHCO_3$	0.10	-	1.50	1.00
$NaHCO_3$	0.30	0.40	-	0.80
L-Lysin-Hcl	-	0.16	0.10	-
DL-Methionine	0.10	0.20	0.17	0.03
L-Threonine	-	0.10	0.04	0.05
L-Tryptophan	-	-	-	0.01
Cr_2O_3	0.1	0.1	0.1	0.1
analyzed composition (%)				
dry matter (DM)	91.0	89.8	90.7	89.6
nutrients (% in dry matter)				
crude protein (CP)	16.9	18.4	17.6	18.4
crude fibre	4.1	5.7	4.2	4.4
LYS	1.39	1.21	1.14	1.39
MET	0.56	0.48	0.40	0.59
CYS	0.17	0.30	0.20	0.19
THR	0.71	0.79	0.69	0.81
TRP	0.22	0.22	0.18	0.19
metabolizable energy (MJ/kg DM)	16.63	16.51	17.00	17.30
net energy (MJ/kg DM)	11.64	11.56	11.90	12.11

This cannula allows nearly quantitative collection of ileal chyme (Köhler *et al.*, 1992). Figure 1 shows the location of the cannula. After surgery, a 7 days recovery period was allowed before blood vessel catheterization. Catheters were placed in the external jugular vein (for continuous ^{15}N infusion) and in the carotid artery (for blood sample collection). After catheterization, another 4 days recovery period followed before ^{15}N infusion started.

The continuous intravenous ^{15}N-L-Leucine infusion was performed at a rate of approximately 40 mg ^{15}N-L-Leucine (95 % [15]N enrichment) per kg body weight per day (Perfusor[R]-Secura-Dauerinfusionsgerät, Fa. B. Braun, D-3508 Melsungen). Two additional piglets on the SI diet were excluded from ^{15}N infusion.

Sample collection and analysis
From the start of ^{15}N infusion quantitative faeces and urine collections were made daily. During the first 6 days of infusion the PVTC cannula was closed. Apparent and true faecal N digestibilities were determined from the ingested feed and the excreted faeces during these 6 days. Faeces were collected in plastic bags attached to the animals by means of a colostomy system as used in humane medicine (Combihesive[R]-system, Squibb BV, NL 2285 VL Rijswijk). Quantitative urine collections were made and twice daily aliquots were taken and frozen for nitrogen determination. At day 7, 9 and 11 after the start of ^{15}N infusion, ileal chyme was collected for 24 hours per day. Digesta were collected, weighed and frozen hourly, pooled per animal per day. Digesta and faeces were freezedried and ground (1 mm) before analysis. Feed samples were also ground before analysis. Apparent ileal and faecal N digestibilities were determined from the concentration of marker (Cr_2O_3) in feed and in digesta or faeces. Cr_2O_3 was analyzed in feed, digesta and faeces through flame atom absorption spectrophotometry (Perkin-Elmer 300).
Trypsin inhibitor contents were analyzed according to Van Oort et al. (1989).
Blood samples were taken twice daily from the carotid catheter during feeding at 8.00 and 20.00 hours. After centrifugation (2500 rpm, 10 minutes), the supernatant was collected and blood plasma protein was precipitated using 20% trichloroacetic acid (TCA). Total N was analyzed in supernatant, precipitate, digesta, urine and faeces according to the Kjeldahl method. After Kjeldahl-N-analysis, the amount of ^{15}N was measured in the NH_4Cl-solutions using an emission spectrometer (Isonitromat 5201 or NOI-6, Fa. Statron, Fürstenwalde, Germany).
The corrections for ileal and faecal endogenous nitrogen were made from the ^{15}N enrichment excess in TCA soluble fraction of blood plasma and in digesta or faeces during the days of ^{15}N infusion.The contribution of endogenous to total N in ileal chyme or faeces can be calculated from the ratio of ^{15}N enrichment excess in ileal chyme or faeces and in the blood TCA soluble fraction, assuming that the ^{15}N excess in the endogenous N and in the blood TCA soluble fraction is similar. The calculations are carried out according to Souffrant et al. (1986) using the following formula:

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$$N_{en} = N_{tot} \times \frac{N_{ex\ c/f}}{N_{ex\ bl}}$$

N_{tot}	= total N in chyme or faeces (g/day)
N_{en}	= endogenous N in chyme or faeces (g/day)
$N_{ex\ c/f}$	= ^{15}N excess in chyme or faeces
$N_{ex\ bl}$	= ^{15}N excess in the TCA soluble fraction of the blood

True nitrogen digestibilities were calculated from the apparent digestibilities by subtracting the endogenous nitrogen from the total nitrogen recovered from faeces or ileal chyme.

Statistical analysis was done by one-way analysis of variance and Tukey multiple comparisons ($\alpha=0.05$) were used to compare means of experimental groups (Snedecor, 1956; Rasch et al., 1978, SAS, 1985).

Results

In Figure 2, ^{15}N enrichment excess in digesta, faeces, urine and TCA-soluble blood plasma during ^{15}N-L-leucine infusion is shown. After 6 days (=144 hours) of infusion, the percentage enrichment levels off indicating sufficient labelling of animal body protein.

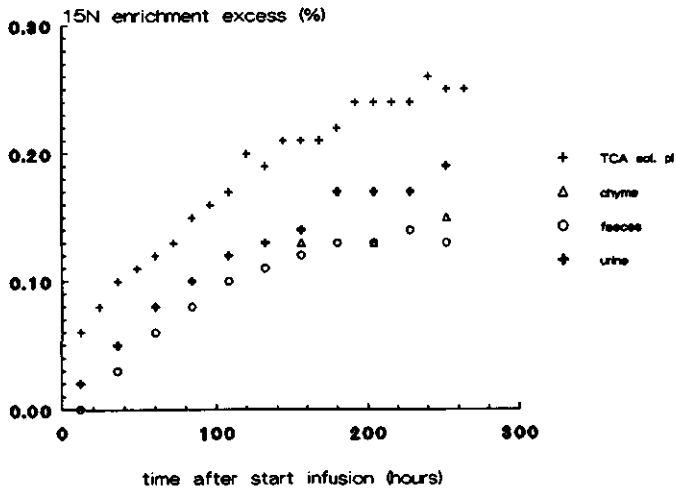Results on faecal digestibilities and losses are presented in Table 3.

Figure 2. ^{15}N enrichment excess (%) in urine, faeces, ileal chyme and TCA-soluble plasma, soybean meal diet (means of three animals).

Table 3.   Results of ^{15}N infusion experiment, digestibility and endogenous losses, faecal data.

diet	SMP	SBM	SI	FM	rootMSE
number of piglets	2	3	5*	3	
apparent faecal N digestibility (%)	94.0^a	84.0^b	90.7^a	89.1^a	1.75
number of piglets	2	3	4	3	
endogenous faecal N mg/day	315^b	963^a	715^{ab}	697^{ab}	204.7
endogenous faecal CP g/100 g DM intake	0.56^b	1.77^a	1.30^{ab}	1.28^{ab}	0.372
endogenous faecal N g/100 g N intake	3.4^b	9.6^a	7.4^{ab}	7.2^{ab}	2.06
endogenous faecal CP mg/kg LW	174^b	561^a	425^{ab}	404^{ab}	120.8
true faecal N digestibility (%)	97.3^a	93.5^b	98.1^a	96.4^a	0.78

Means within a row bearing the same superscript do not differ (Tukey, p>0.05)
* different number of piglets for apparent digestibility is caused by inclusion of one of the two spare piglets without ^{15}N-infusion on the SI-diet.

Apparent faecal N digestibility was highest in piglets fed the SMP diet (94.0%) and lowest for the SBM diet (84.0%). Apparent faecal N digestibilities for the SI and FM diets were intermediate (90.8 and 89.1%, respectively). Endogenous N losses at the faecal level were lowest for the SMP diet (315 mg/d) and highest for the SBM diet (963 mg/d). Endogenous N losses on the SI and FM diets were intermediate (715 and 697 mg/d, respectively). True faecal N digestibility was highest for the SI and SMP diets (98.0 and 97.3%, respectively) and lowest for the SBM diet (93.5%). True faecal N digestibility was lowest on the SBM diet (93.5%). True faecal N digestibility on the FM diet was intermediate (96.4%). True faecal N digestibilities were high (> 90%) on all diets.

Data on ileal digestibilities and N losses are given in Table 4.

Apparent ileal N digestibility was highest with the SMP diet (84.4 compared to 78.4, 76.5 and 73.0 % for the SI, the SBM and the FM diet respectively). Endogenous N losses at the terminal ileum were about twice as high with the SBM diet and the FM diet compared to the SMP diet, resulting in almost similar true ileal N digestibilities for the three diets (92.7, 90.6 and 89.3 % with the SMP, the SBM and the FM diet, respectively).

Table 4. Results of ^{15}N infusion experiment, digestibility and endogenous losses, ileal data.

diet	SMP	SBM	SI	FM	rootMSE
number of piglets	2	3	6[*]	2[**]	
apparent ileal N digestibility (%)	84.4[a]	76.5[b]	78.4[ab]	73.0[b]	2.67
number of piglets	2	3	4	2[**]	
endogenous ileal N mg/day	786[b]	1422[ab]	1970[a]	1558[ab]	359.3
endogenous ileal CP g/100 g DM intake	1.41[b]	2.61[ab]	3.56[a]	2.86[ab]	0.641
endogenous ileal N g/100 g N intake	8.3[b]	14.1[ab]	20.4[a]	16.2[ab]	3.69
true ileal N digestibility (%)	92.7[b]	90.6[b]	98.4[a]	89.3[b]	1.29

Means within a row bearing the same superscript do not differ (Tukey, $p>0.05$)
[*] different number of piglets for apparent digestibility is caused by inclusion of two spare piglets without ^{15}N-infusion.
[**] one piglet on FM diet was excluded due to aberrant values on nitrogen content in ileal digesta.

With the SI diet endogenous losses were about 2.5 times higher compared to the SMP diet. The true ileal N digestibility for the SI diet was 98.4 %.
Apparent N digestibilities (faecal and ileal) did not differ between SBM and SI diet ($p>0.05$, Table 3 and 4), however, true digestibilities (faecal and ileal) did differ ($p<0.05$, Tables 3 and 4). The SMP caused higher apparent N digestibilities (faecal and ileal) than the other protein sources.
In general, differences in true N digestibilities between protein sources were smaller than differences in apparent N digestibilities.
With the soy-based diets (SBM and SI) standard deviations of parameters were generally larger than with the animal protein diets (FM and SMP). This indicates that large differences between individual piglets were noticed when soy-based diets were fed.
The soy-based diets caused the highest endogenous nitrogen losses (SI at the terminal ileal level and SBM at the faecal level). The true ileal digestibility of the SI protein was higher than of the other protein sources.
Nitrogen balance data and organ weights are given in Table 5.

Table 5.   Organ weights and N balance data, from day 1 till day 6.

	SMP	SBM	SI	FM	rootMSE
number of animals (n)	2	3	5[*]	3	
live weight (kg)	11.3[a]	10.7[a]	10.4[a]	10.8[a]	0.52
pancreatic weight (g)	19.3[ab]	22.1[ab]	23.0[a]	15.9[b]	2.53
small intestinal weight (g)	317[a]	375[a]	401[a]	355[a]	75.4
N intake (mg/day)	9438[c]	10048[a]	9646[b]	9626[b]	27.0
N urine (mg/day)	1935[ab]	2014[b]	3409[a]	2171[ab]	576.7
N urine as % of N intake	20.5[ab]	20.0[b]	35.4[a]	22.6[ab]	6.07
N faeces (mg/day)	568[b]	1611[a]	1040[ab]	1048[ab]	304.6
N faeces as % of N intake	6.0[b]	16.0[a]	10.8[ab]	10.9[ab]	3.12
N balance (mg/day)	6936[a]	6423[ab]	5198[b]	6407[ab]	582.4
N balance as % of N intake	73.5[a]	63.9[ab]	53.9[b]	66.6[ab]	5.91

Means within a row bearing the same superscript do not differ (Tukey, p>0.05)
[*] including one spare piglet without ^{15}N-infusion.

Table 6.   Results of comparisons of means (T-tests) for ileal vs faecal and true vs apparent data for each protein source. diff. = difference between faecal and ileal or between apparent and true. When p<0.05 'diff.' is significantly different from 0.

FAECAL-ILEAL	diet: SMP diff. (p)	SBM diff. (p)	SI diff. (p)	FM diff. (p)
App. N dig.:	9.65 (0.0099)	7.50 (0.0053)	12.75 (0.0060)	15.90 (-)
End. N loss (mg/day):	-471.(0.0041)	-459 (0.0158)	-1256 (0.0297)	-880 (0.1065)
Endogenous CP (g/100g DM int.):	-0.85 (0.0038)	-0.84 (0.0156)	-2.27 (0.0284)	-1.62 (0.1035)
Endogenous N (g/100g N int.):	-4.95 (0.0064)	-4.57 (0.0147)	-13.00 (0.0168)	-9.15 (0.1001)
End. CP (mg/kg LW):	-260.5 (0.0041)	-268.0 (0.0184)	-733.6 (0.0338)	-503.7 (0.1246)
True N dig.:	4.60 (0.0277)	2.90 (0.0151)	-0.33 (0.7471)	6.75 (0.1437)

APPARENT-TRUE	SMP diff. (p)	SBM diff. (p)	SI diff. (p)	FM diff. (p)
ileal N dig.:	-8.35 (0.0267)	-14.17 (0.0221)	-20.48 (0.0031)	-14.17 (0.0221)
faecal N dig.:	-3.30 (0.0768)	-9.57 (0.0356)	-7.40 (0.0053)	-7.10 (0.0448)

No differences in live weights between diets were noticed (p>0.05). Pancreatic weights did differ between diet SI and diet FM (p<0.05). Small intestinal weights did not differ between diets (p>0.05).

Nitrogen intake differed between diets, due to different crude protein contents in the diets. Urinary N excretion was highest with the SI diet and lowest with the SMP diet. Faecal N excretion was highest with the SBM diet and lowest with the SMP diet. The difference in N balance between SMP and SI was significant (p<0.05).

In Table 6 comparisons are given between faecal and ileal data and between apparent and true digestibilities.

At the ileal level and at the faecal level, true N digestibility was higher than apparent N digestibility with all protein sources (Table 7). The ranking for apparent ileal N digestibility (SMP-SI-SBM-FM) was not similar to the ranking for true ileal N digestibility (SI-SMP-SBM-FM).

Discussion

Endogenous nitrogen in faeces and ileal chyme as measured using the continuous ^{15}N-L-leucine infusion technique can be underestimated for two reasons: Firstly, endogenous protein is synthetized not only from free amino acids in the TCA-soluble fraction of the plasma, but also directly from absorbed amino acids in the enterocytes of the intestinal wall. Secondly, the labelling of body protein will continue to rise (slowly) during continuous infusion of ^{15}N-leucine, due to recycling of labelled amino acids. This will also result in an underestimation of the amount of endogenous nitrogen (De Lange *et al.*, 1990).

Wilson & Leibholz (1981c) studied apparent and true N digestibilities of diets containing milk or soybean protein with piglets (28 days of age) using a nitrogen free diet. For the milk protein diet they found apparent and true ileal N digestibilities of 86 and 92 % respectively, which closely resembles our values of 84 and 93 % respectively. Apparent nitrogen digestibility was expected to be higher for the milk protein diet because the newly weaned pig is supposed to be adapted to this protein source. The relatively low digestibility could be due to differences between cow's milk protein and sow's milk protein.

Wilson & Leibholz (1981c) reported apparent and true ileal N digestibilities for the soybean meal diet of 51 and 62 % respectively. In our study we found apparent and true ileal N digestibilities for the SBM diet to be 77 and 91 % respectively. This difference could be due to age of piglets (in our study 35 - 50 days), to nitrogen content of the diet (Wilson & Leibholz

(1981c) used diets containing ± 27 % of crude protein), to quality of soybean meal or to an interaction between age and diet composition. This latter option has been supported by Wilson & Leibholz (1981a) who studied performance of piglets given milk and soybean proteins at different ages. They found an interaction between protein source in the feed and age of the piglets: the performance of piglets fed milk protein did not change between 7 and 35 days of age. Performance of piglets fed soybean protein, however, did increase with increasing age.

Wilson & Leibholz (1981c) studied endogenous N flow at different sites of the gastrointestinal tract of piglets (35 days of age) fed a protein free diet. They reported an endogenous N flow at the terminal ileum of 0.82 g N per day. In our study we found N flows of 0.79 and 1.42 g N per day for piglets fed the SMP diet and the SBM diet respectively. These data indicate that endogenous losses are minimal when milk proteins are fed to young piglets.

Endogenous N losses measured with animals fed protein free diets possibly do not reflect the normal, physiological situation when protein containing diets are fed. The fact that different protein sources in the feed induce different levels of endogenous N secretion stress this aspect. Since the amount of dietary nitrogen could also affect the endogenous nitrogen losses, the regression method also is not very accurate in determining endogenous nitrogen losses in animals fed nitrogen containing diets.

Leibholz (1982) studied the endogenous nitrogen secretion in young pigs (4 weeks of age). She used the regression method to assess endogenous N losses with a feed based on milk protein. By means of regression analysis she calculated endogenous N flow at the terminal ileum to be 3.44 g N per kg dry matter intake, which amounts to 2.2 g endogenous ileal crude protein per 100 g of dry matter intake. In our study we calculated 1.41 g endogenous ileal crude protein per 100 g of dry matter intake for the SMP diet.

The weight of the pancreas is in agreement with data from Le Guen *et al.* (1991) obtained from piglets of 10 to 15 kg live weight fed diets based on casein and fishmeal or pea proteins. The difference in pancreatic weight between SI and FM fed piglets is difficult to explain: it is assumed (Huisman, 1990), that piglets do not show pancreas hypertrophy when fed trypsin inhibitor containing feedstuffs. The reason for the low pancreatic weight of FM fed piglets is unclear.

The low N balance of SI fed piglets is caused by both urinary and faecal N excretion. The high faecal N excretion is caused by both endogenous and exogenous nitrogen. With young piglets (age 35 to 56 days), Newport and Keal (1983) found no effect of dietary protein source (soya bean meal versus combinations of fish meal, skimmilk powder and soybean meal) on nitrogen retention with comparable nitrogen intakes. With diets containing fish meal, soybean meal and skimmilk powder, Zhang *et al.* (1986) found a N retention of 7.1 g/day when 10.9 gram of N was

ingested daily by piglets aged 31 days on average.

From our data we concluded that the differences in apparent ileal N digestibility between the SMP, SBM and FM diet are not caused by differences in the (true) digestibility of the protein sources. From our experiment we also concluded, that true ileal N digestibility of soybean meal is not much different from true ileal N digestibility of skimmilk powder. Therefore, differences between these protein sources with regard to apparent ileal N digestibility and performance of piglets (growth, feed conversion ratio) must be caused by an increase in endogenous N secretion with piglets fed soybean meal. The soy protein itself is highly digestible (90.6 % at the ileal level and 93.5% at the faecal level), but it either stimulates nitrogen secretion by the exocrine glands of the digestive tract or it causes excessive loss of gut wall cells by sloughing off, resulting in endogenous N losses. These endogenous N losses may lead to decreased performance (feed intake, growth) of piglets fed non-milk protein sources after weaning.

In terms of endogenous losses, the expenses for the digestion of vegetable protein are higher than for the digestion of milk protein.

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Chapter III

.

PANCREATIC SECRETION IN PIGS

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Summary

The pancreas plays a major role in digestive processes. Pancreatic secretion controls duodenal pH (buffering capacity) and contains digestive enzymes which adapt to diet composition (substrate content). The development of pancreatic enzymes with age is important when designing feeding strategies for early weaned piglets.

This paper reviews some aspects of the measurement of enzyme activities in pancreatic juice and summarizes the effects of age and diet composition on pancreatic (enzyme) secretion.

The physiological background of adaptation mechanisms is discussed. Some recommendations for further research in this field are also included.

Introduction

Designing optimal feeding strategies for different categories of farm animals requires the understanding of digestive processes.

The development of fistulation and cannulation techniques has facilitated studies on secretion and digestion in vivo.

In this respect the study of pancreatic secretion is important.

The buffering capacity, protein content and enzyme content of pancreatic juice play an important role in the digestive process. Digestive disorders, e.g., post weaning diarrhoea, could be related to pancreas function.

Secretion of pancreatic juice probably adjusts to the pH of digesta leaving the stomach.

Digesta leaves the stomach at a low pH (2 to 4), at which pancreatic enzymes are not active. Therefore, pancreatic juice must be capable of rising digesta pH to 7 à 8 to ensure optimal enzyme activity.

Buffering capacity (acid binding capacity) of feedstuffs is usually defined as the amount of acid (HCl) needed to lower pH of a feed-in-water suspension by one pH-unit.

Buffering capacity of pancreatic juice should be defined as the amount of juice needed to raise pH of digesta leaving the stomach to pH 7 or 8.

Enzymes should be secreted in amounts appropriate for the digestion of the ingested feed components. Therefore, enzyme secretion must adapt to dietary composition.

Furthermore, enzymes are proteins which are secreted into the gut lumen. These enzyme-

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proteins will be partly digested and absorbed. Another part will disappear into the large intestine without being digested or absorbed. This latter part of enzyme-proteins is lost for utilization by the pig.

The loss of these endogenous proteins should be minimized to ensure optimal feed utilization by the animal.

Since the development of pancreatic fistulation techniques (Wass, 1965; Pekas, 1965; Aumaître, 1972; Corring *et al.*, 1972; Corring and Jung, 1972; Corring and Saucier, 1972; Corring, 1974; Corring, 1980; Partridge *et al.*, 1982; Schumann *et al.*, 1983; Zebrowska *et al.*, 1983; Hee *et al.*, 1985; Zebrowska, 1985; Van Leeuwen, 1986; Ozimek *et al.*, 1986; Pierzynowski *et al.*, 1988) pancreatic secretion has become an important research field over the past decades.

Pancreatic juice production (flow rates) has been measured in relation to time of day (Kvastnitskii, 1951), feeding and frequency of meals (Corring *et al.*, 1972; Hee *et al.*, 1988b) and diet composition (Zebrowska *et al.*, 1981; Langlois *et al.*, 1987; Hee *et al.*, 1988a; Imbeah *et al.*, 1988).

Abello *et al.* (1987) showed that in fed pigs, pancreatic and biliary secretions hardly affected intraduodenal pH. In fasted pigs, however, bile and especially pancreatic juice did contribute highly to the neutralization of gastric acid chyme entering the duodenum.

Direct measurements of buffering capacity of bile or pancreatic juice (titration studies) are not known from literature.

Pancreatic juice contains the following digestive enzymes and zymogens: amylase, lipase, colipase, trypsinogen, chymotrypsinogen, pro-carboxypeptidase A and B, pro-elastase, ribonucleases and desoxy-ribonucleases (Bell Davidson Emslie-Smith, 1972b).

The measurement of enzyme activities in pancreatic juice encounters several problems, e.g., standardization of methods of analysis, enzyme activity changes in stored pancreatic juice and interference by microbial contamination of the juice.

Methods for Collecting Pancreatic Juice

The collection of pancreatic juice from fistulated animals has several advantages: secretion can be measured directly in response to several factors, e.g., diet composition. Repeated collections can be made on the same animal, development of secretion with age can be measured and the juice can be analyzed for protein content, buffering capacity, enzyme

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activities etc.

The use of fistulation techniques should be subject to critical evaluation: surgery should be relatively simple to perform and measurements should be representative for normal, healthy animals.
Basically, two different techniques are being used for fistulation of the pancreas in pigs. The first method, developed by Corring *et al.* (1972) involves fistulation of the pancreatic duct (see Figure 1).
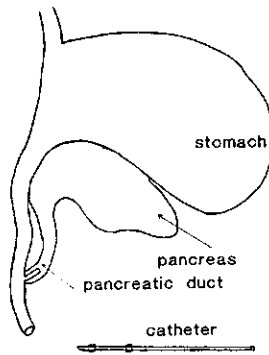
Figure 1.   Pancreatic duct fistulation technique (Corring *et al.*, 1972)

Hee *et al.* (1985) use another surgical procedure: they cannulate an isolated segment of duodenum ("pouch") which receives the pancreatic duct (see Figure 2).
Sometimes one of these two techniques is used with small modifications (Zebrowska *et al.*, 1983 and Pierzynowski *et al.*, 1988).
Both techniques have their advantages and disadvantages: the pouch technique allows the animals to be used for pancreatic juice collection during several months to over a year. Pancreatic duct fistulas remain functional for a few weeks maximum.
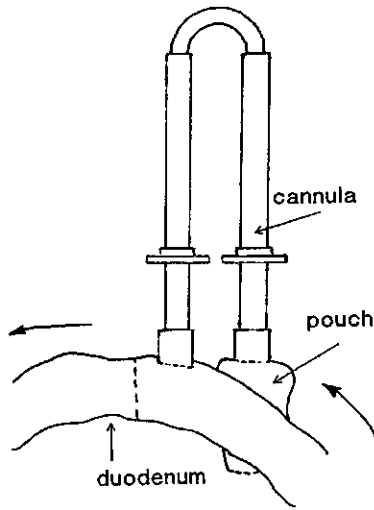
Figure 2.   Pancreatic cannulation with the pouch technique (Hee *et al.*, 1985)

With the pancreatic duct fistulation technique "clean" and non activated pancreatic juice can be collected while the pouch technique can cause contamination of the juice by duodenal pouch contents (mucus, dead cells) as well as activation of the juice by duodenal enterokinase. The pouch technique involves the cutting of the gut at two sites which could affect gut motility. Both surgery techniques can cause leakage of pancreatic juice into the abdomen leading to peritonitis and even death of the animal.
There has not yet been a direct comparison between the two different fistulation techniques with regard to animal health, secretion rates and juice characteristics.

Methods for Assessing Pancreatic Enzyme Activity

The activity of pancreatic enzymes can be measured in pancreatic tissue (slaughtered animals), in pancreatic juice (fistulated animals), in digesta (slaughtered or cannulated

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animals), in blood plasma or serum (Frobish *et al.*, 1971) and in faeces (Nitsan and Liener, 1976).

Only small enzyme-molecules (e.g., lipase) can be found in the blood (cell leaking or absorption). Small amounts of digestive enzymes (not broken down, not absorbed) can be found in faeces.

The mode of action of enzymes can be represented as follows:

enzyme +   → [enzyme-substrate]$_{complex}$ →   enzyme +
substrate   ←   product(s)

This reaction can be followed spectrophotometrically (absorbance change due to increase in colored product or decrease in colored substrate) or titrimetrically (when substrate or product possesses acidic properties).
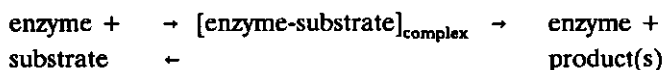
Enzyme activity is usually expressed as units of activity (μmol/min):

One unit of activity is the amount of enzyme that forms one μmol product per minute or breaks down one μmol substrate per minute under standardized assay conditions (pH, temperature, buffer composition) (Bergmeyer and Gawehn, 1978).

The measured activity depends on the assay method, the pH and the temperature in the assay and on the substrate used (Bell Davidson Emslie-Smith, 1972a). To ensure comparability of test results it is necessary to use standardized units in expressing enzyme activities.

Enzyme activity can be calculated as units per kg live weight, per gram of pancreatic tissue (dry or wet), per ml pancreatic juice, per mg of enzyme (specific activity) or per mg of protein present in the pancreatic juice. The enzyme activity can also be expressed as total activity (per pancreas or per 24 hours).

Using fistulation techniques, enzyme activity can be expressed per ml of pancreatic juice or as total activity per 24 hours.

Evaluation of Methods to Analyze Pancreatic Enzyme Activities

Literature data on enzyme activities cannot be compared directly because of the many different assay procedures that are being used with regard to substrate, temperature, pH. Further difficulties in interpreting pancreatic enzyme assay results are caused by activation

of enzyme precursors (trypsinogen, chymotrypsinogen), the standardization of the time interval between last feed intake and slaughter (when tissue samples are obtained) or pancreatic juice sampling and the storage of the samples until analysis.

A number of factors can affect the stability of enzymes during storage: extreme pH-values, heat, freezing and thawing, micro-organisms etc. (Bergmeyer and Gawehn, 1978).

Freezing and thawing of samples has according to Lindemann *et al.* (1986) no influence on the activity of trypsin, chymotrypsin, amylase or the gastric proteases.

Gorrill and Friend (1970) kept their samples of pancreatic tissue and ileal digesta at -10 °C after adding glycerol to the digesta samples (1:1) and found no activity decrease for trypsin and chymotrypsin. Hee *et al.* (1988a) stored their pancreatic juice samples at 5 °C and analyzed them within 12 hours of collection. During this period enzyme activities (amylase, lipase, trypsin, chymotrypsin) did not decrease.

Low (1982) analyzed digesta samples for trypsin, chymotrypsin and peptidases within one month of collection. He found that storage at -20 °C for less than three months did not influence enzyme activities in the samples.

Howard and Yudkin (1963) analyzed pancreatic tissue homogenates after storage for one night in a refrigerator. Enzyme activities (trypsin, amylase, proteinase) had not diminished. Magee and Hong (1959) found that amylase and protease activities in pancreatic juice decreased when the pancreatic juice was kept at room temperature. Amylase and trypsin activities decreased quickly in contaminated pancreatic juice.

Legg and Spencer (1975) studied the stability of pancreatic enzymes in human duodenal fluid to storage temperature and pH. They found that the activity of amylase, trypsin and lipase remained relatively constant over a 4 week period when stored at -20 ^{0}C. Storage at room temperature caused a considerable decline in enzyme activities, while storage at 4 ^{0}C gave intermediate results. At pH < 5, enzyme activities diminished substantially within 4 weeks of storage at -20 ^{0}C.

Corring (personal communication, 1988) recommended storage of pancreatic juice at -80 ^{0}C to prevent loss of enzyme activities.

Gorrill and Thomas (1967) and Reboud *et al.* (1962) described enzyme analysis quite extensively, with special reference to activation influences (see Figures 3 and 4).

It is clear that biochemical reactions in stored biological samples can influence enzyme activities depending on storage conditions.

The effects of different storage conditions on enzyme activities in pancreatic juice should be investigated in more detail. Storage conditions as well as assay methods should be

standardized to facilitate comparison of research data.

Regulation of Pancreatic Secretion, a Feedback Mechanism

As early as the 1920s, Elman and McCaughan (1937) established the fatal effect of total loss of pancreatic secretions in dogs: after 2 or 3 days of total pancreatic juice deprivation by means of a pancreatic duct catheter, the animal lost appetite, followed by vomiting and death on day 5 to 8 after surgery.

Corring (1973, 1974) fitted pigs weighing ca 45 kg with pancreatic fistulas and duodenal cannulas. He found that pancreatic secretion is regulated by a short-term negative feed back mechanism: the pancreatic secretion increased within 2 hours of pancreatic juice withdrawal from the animal (volume fivefold, total protein secretion 1.5- to twofold and enzyme activities twofolds compared to the values before juice withdrawal). Infusion of a salts mixture ($NaHCO_3$ and $NaCl$, in amounts equal to pancreatic juice composition) or of deproteinized pancreatic juice in the duodenum did not lower pancreatic juice secretion. This indicates that the active substance in juice secretion regulation can be found in the protein fraction of the juice. Infusion of an activated trypsin solution (even with pH < 7) or of the collected pancreatic juice did lower juice secretion.

From these findings Corring (1974) concluded that the (proteolytic) enzymes are responsible for the feed back, possibly through an inhibitory action on the synthesis and release of secretin and cholecystokinin-pancreozymin (CCK-PZ).

After perfusion (intravenously, i.v.) with secretin during half an hour the amount of pancreatic juice increased, while the amount of secreted protein remained constant. From this it can be concluded that secretin can regulate the secreted amount of pancreatic juice without affecting the amount of protein secreted.
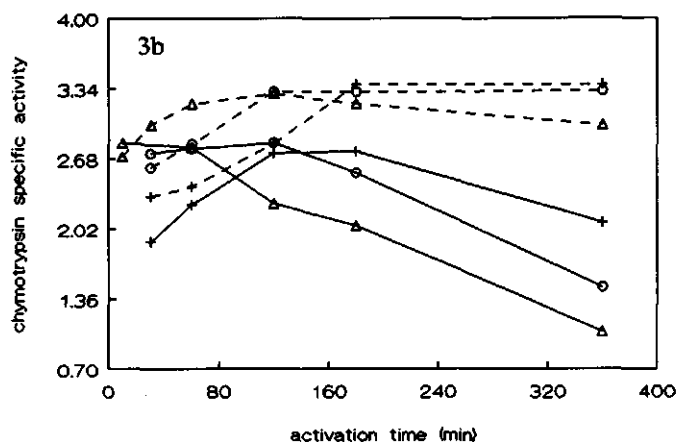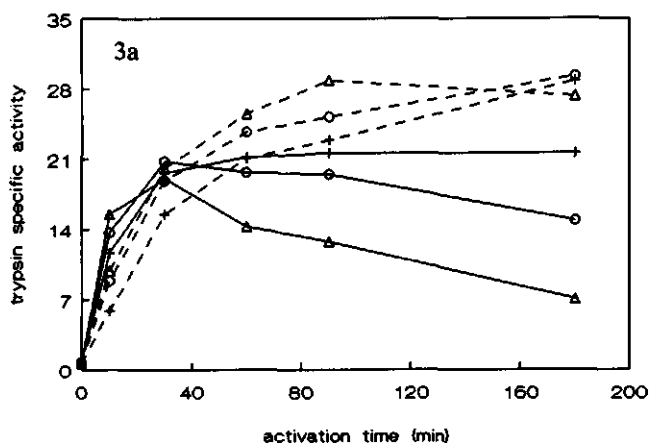
Figure 3a. Effect of pancreatic juice protein concentration and $CaCl_2$ on activation of trypsinogen by enteropeptidase at 37°C:
—————— no $CaCl_2$; - - - - - - 0.05 M $CaCl_2$ final concentration; + 0.13 mg protein/ml; O 0.27 mg protein/ml; ▲ 0.54 mg protein/ml.
One trypsin unit on ordinate equals hydrolysis of 1 μmole TAME per minute and per mg pancreatic juice protein (Gorrill and Thomas, 1967).

Figure 3b. Effect of pancreatic juice protein concentration and $CaCl_2$ on activation of chymotrypsinogen by enteropeptidase at 37°C:
—————— no $CaCl_2$; - - - - - - 0.05 M $CaCl_2$ final concentration; + 0.13 mg protein/ml; O 0.27 mg protein/ml; ▲ 0.54 mg protein/ml.
One chymotrypsin unit on ordinate equals hydrolysis of 1 μmole BTEE in reaction mixture of 26% methanol (v/v) per minute and per mg pancreatic juice protein (Gorrill and Thomas, 1967).

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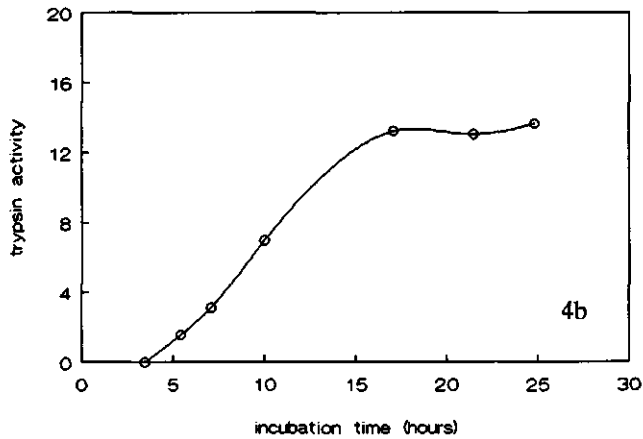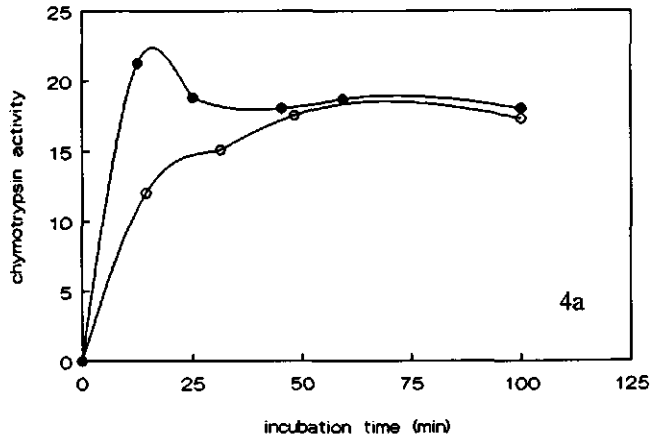
Figure 4a.   Activation of chymotrypsinogen in rat pancreatic tissue. Activity measured with ATEE as a substrate. ○ incubation with trypsin at 0°C; ● incubation with trypsin at 35 °C; (Reboud *et al*., 1962).

Figure 4b.   Activation of trypsinogen in porcine pancreatic juice. Incubation with trypsin at 0°C; (Reboud *et al*., 1962).

After glucagon injection (1 mg, i.v.) the amount of protein and the volume of juice decreased, even when the collected juice was not reintroduced to the duodenum. This could be due to inhibition of secretin by glucagon (competition between secretin and glucagon acting on the same receptor).

Nervous (nervus vagus) and hormonal (secretin and cholecystokinin-pancreozymin) mechanisms control pancreatic secretion. The release of these hormones is controlled by the secretion of the pancreas itself (mainly trypsin).

Effects of Age on Pancreatic Secretion

Corring *et al.* (1978) studied the development of the pancreas and pancreatic enzymes in young piglets. They found that the development of the pancreas until 4 weeks of age is due to hyperplasia and afterwards (from 4 to 8 weeks of age) to both hyperplasia and hypertrophy.

In the rat and the chicken onset of amylase synthesis and development could be stimulated by corticosteroids (Koldovsky and Kahn, 1964 and Yalovsky *et al.*, 1969, both cited by Aumaître, 1972).

This was confirmed in the piglet by Baintner and Németh (1982) who treated suckling piglets with triiodothyronine and prednisolone between two and three weeks of age. At three weeks of age, the animals were killed and sampled. Amylase activity in the pancreas and trypsin activity in the intestinal contents were higher in treated piglets than in untreated controls. Chymosin activity in gastric contents was lower in treated animals.

Chapple *et al.* (1990a,b,c) studied the influence of corticosteroids on carbohydrase development in suckling piglets. They found that the amylase activity in pancreatic tissue can be substantially stimulated by glucocorticoid (hydrocortisone) injections (25 mg/kg body weight), administered every other day for the last two weeks of suckling (Chapple *et al.* 1990a). ACTH injections had no effect on pancreatic tissue enzyme activity. In a further study, Chapple *et al.* (1990b) found no effect of hydrocortisone administration on small intestinal disaccharidase production in 14 to 26 day old piglets. In 6 day old piglets, Chapple *et al.* (1990c) also found an positive effect of hydrocortisone injection on amylase activity in pancreatic tissue. Lactase activity was enhanced by hydrocortisone treatment and by ACTH injection. Chapple *et al.* (1990a,b,c) concluded that glucocorticoid levels can stimulate digestive enzyme development in suckling piglets.

Secretion Rate

Kvastnitskii (1951) performed extensive studies on physiology of digestion in pigs. One of the factors he investigated was the pancreatic secretion in fistulated piglets from 20 days of

age.

He found in piglets from 20 to 30 days of age a secretion of 150 to 350 ml per 24 hours. This secretion rate is similar to the values found by Pekas *et al.* (1960) in piglets between 4 and 5 weeks of age (secretion of .0 to 11.0 ml per hour, pH between 7.8 and 8.2).

In pigs aged 200 to 250 days, Kvastnitskii (1951) found a pancreatic secretion rate of 8000 to 9000 ml per 24 hours.

Harada *et al.* (1988) measured pancreatic secretion in suckling piglets under anaesthetized conditions in response to various stimulants. Their results indicate that piglets from 3 days of age are capable of pancreatic juice secretion stimulated by exogenous secretin and vagal stimulation and by means of endogenous secretin through duodenal acidification.

Pekas *et al.* (1966) used fistulated piglets to measure pancreatic juice secretion. The spontaneous secretion in piglets from 4 weeks of age onwards was very variable. This was probably due to Pavlov reflexes.

Pierzynowski *et al.* (1988) fistulated piglets at 3 days to 9 weeks of age. They found no influence of suckling on pancreatic juice output in unweaned piglets. After weaning, the feeding of solid food increased postprandial secretion (see Figure 5).

Weström *et al.* (1989) assessed the levels of pancreatic juice secretion in piglets before and after weaning. They found that during the first four weeks of life (before weaning) pancreas secretion remained low (ca .5 ml per hour per kg body weight). After weaning (at 5 weeks of age) the secretion increased to 2 ml per hour per kg live weight by 12 weeks of age.

Figure 6 summarizes data on pancreatic juice secretion in response to age in pigs.

Enzymatic Activity

Suckling piglets are fully adapted to the ingestion and digestion of sow's milk. The secretion of lactase and lipase is well developed at an early age. Pancreatic proteolytic enzyme secretion is not fully developed in suckling piglets. The digestion of milk proteins takes place primarily in the stomach through the clotting of milk and the action of chymosin and lactate. Immunoglobulins should not be hydrolyzed, they are to be absorbed from the gut lumen in a complete form. Therefore, colostrum contains a trypsin inhibitor to prevent the breakdown of these proteins.

Kvastnitskii (1951) assessed the enzymatic activity of pancreatic juice. He found a decrease in proteolytic and lipolytic activity of pancreatic juice between 20 and 200 days of age. During this period, amylase activity remained relatively constant. The digestive capacity of the stomach increased considerably with age, so that Kvastnitskii (1951) concluded that the

fully developed digestive capacity of the young piglet's pancreas compensated for the insufficiently developed capacity of the stomach. Unfortunately, Kvastnitskii does not give detailed information on sampling techniques or enzyme analyses.

In slaughter experiments with suckling piglets which had access to creep feed, Corring *et al.* (1978) found that between 4 and 8 weeks of age the total enzymatic activity per pancreas increased 4- to 20-fold.

Protein concentration in pancreatic juice increased remarkably in response to CCK-8 injection after one week of age in anaesthetized piglets. The ratio of amylase to protein (mU/μg) was 80 mU/μg at 3 days old; it increased gradually from 6 to 21 days of age and afterwards it increased abruptly to 300 mU/μg at 28 days of age (Harada *et al.*, 1988). These findings agree with the results of Hudman *et al.* (1957), who found very low amylase activity per gram of dry pancreas in one day old piglets. The amylase activity increased sharply until day 28. After day 28 amylase activity stayed relatively constant. Pancreatic amylase activity per piglet (per total pancreas) increased until day 35. Between day 14 and day 21 this increase was relatively small; during this period the pancreatic weight increased sharply while the amylase activity per gram of pancreas remained relatively constant.

According to Pekas *et al.* (1966), the activities of protease, amylase and lipase in the pancreatic juice of piglets aged 4 to 7 weeks varied widely and with no apparent association between them.

Gorrill and Friend (1970) studied enzyme activities in pancreases from piglets between 3 and 5 weeks of age. They did not find a distinct relationship between proteolytic enzyme activity in pancreatic tissue and in the ileal digesta. Trypsin activity increased much more than chymotrypsin activity between 3 and 5 weeks of age.

Enzyme activity and especially changes herein are determined by developmental stage at birth and by feed composition (Bailey *et al.*, 1956). Therefore it can be expected that there are considerable variation between animals with respect to enzymatic development.

Weström *et al.* (1987) studied the development of pancreatic enzymes in porcine pancreatic tissue from the fetal period to adulthood. They found an quantitative change in enzyme activities as well as a qualitative change: enzyme activities in activated pancreatic tissue increased during the fetal period, after birth a decrease in enzyme activities occurred during ca one week and afterwards enzyme activities increased further, especially between 10 to 14 weeks and 6 months of age. It was also shown that after birth 'new' types of proteinases developed and 'fetal' types disappeared during the first 14 weeks of life.

Creep feeding of piglets before weaning can play an important role in the development of

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pancreatic enzymes with age:

Creep feeding of piglets caused increased pancreatic amylase activity. At 10 weeks of age there was no measurable effect of creep feeding on pancreatic amylase activity (Shields *et al.*, 1980).

After weaning at 4 weeks of age (Lindemann *et al.*, 1986) a depression occurred in amylase, lipase, trypsin and chymotrypsin activity during the first week after weaning. There was however no effect on gastric proteases.

Hartman *et al.* (1961) found no influence of weaning (at 1 week of age) on amylase activity in piglets' pancreatic tissue between 1 and 8 weeks of age. After weaning, lipase and proteinase activities in pancreatic tissue declined during the first week after weaning. Hartman *et al.* (1961) found no significant difference in enzyme activities of gastrointestinal chyme between adult pigs and 1 to 2 month old piglets.

In a pig of 50 kg ca 10 g of enzymatic (endogenous) protein is secreted with the pancreatic juice every day (Corring and Jung, 1972).
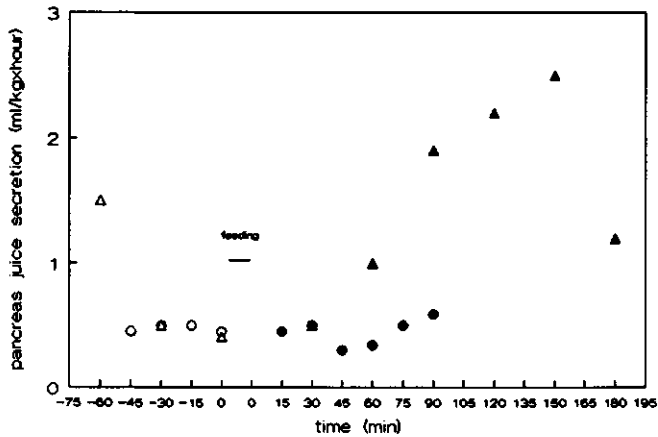
Figure 5.   Typical pancreatic juice secretion obtained before (unfilled symbols) an after (filled symbols) feeding in experiments on the same pig before (△,▲) and after (○,●) weaning. (Pierzynowski *et al.*, 1988).

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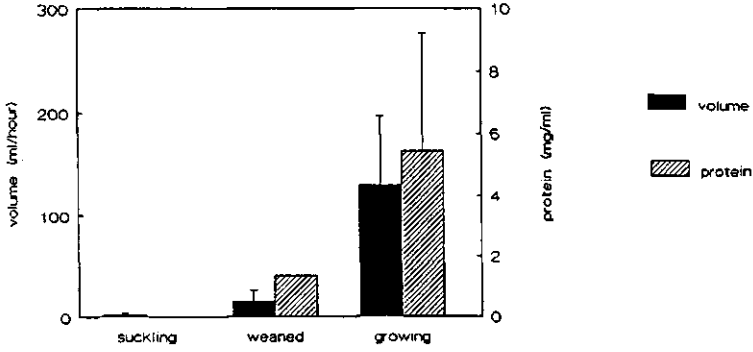
Figure 6a.   Pancreatic secretion in pigs of different ages.
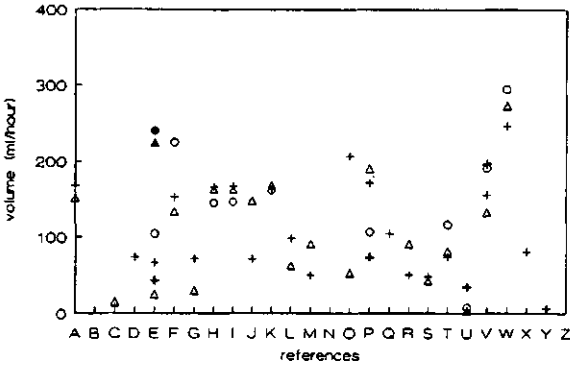
Figure 6b.   Pancreatic secretion (volume, ml/h).
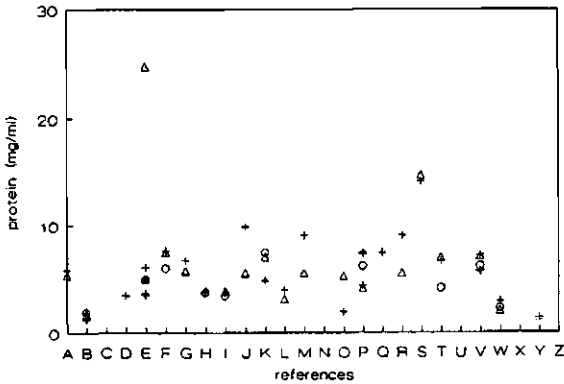
Figure 6c.   Pancreatic secretion (protein, mg/ml).

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Legend to Figure 6b and 6c.

	+	△	O	+	▲	●
A	high amount of TI	low amount of TI				
B	0% CP	30% CP	10% CP	40% CP		
C	milk protein 10 kg LW	soy protein 10 kg LW				
D	16% CP					
E	juice reintroduced	juice reintroduced	juice reintroduced	juice reintroduced	juice not reintroduced	juice not reintroduced
F	low fiber	high fiber alphafloc	high fiber straw			
G	NaCl-infusion	glucose-infusion				
H	15% CP 2% CFat	15% CP 10% CFat	0% CP 2% CFat			
I	idem	idem	idem			
J	no WB	40% WB				
K	0% CP	16% CP SBM	16% CP 'supro 620'			
L	untoasted soy	toasted soy				
M	St/Su/C	B/SBM				
O	B/W/FM	W/WB/C	W/WF/C	WF/C		
Q	review					
R	St/C	B/SBM				
S	SBM	canola meal				
T	control	high fat	high starch			
U	suckling, 2.5 kg LW	suckling, 8 kg LW	weaned, 8 kg LW	weaned, 13 kg LW		
V	St	10% cellulose	10% strawmeal	7.5% pectin		
W	one meal	two meals	three meals			
Y	weaned, 15 kg LW					

TI = trypsin inhibitor, CP = crude protein, CFat = crude fat, C = casein, FM = fish meal, SBM = soybean meal, Su = sucrose, St = starch, B = barley, W = wheat, WF = wheat flour, WB = wheat bran.

A=Ozimek and Sauer (1985), B=Corring and Saucier (1972), C=Pekas et al. (1966), D=Corring et al. (1972), E=Corring (1974), F=Maenhout (1988), G=Simoes Nunes (1982), H=Hee et al. (1988a), I=Hee et al. (1985), J=Langlois et al. (1987), K=Ozimek et al. (1984), L=Schumann et al. (1983), M=Zebrowska et al. (1983), O=Partridge et al. (1982), P=Zebrowska (1985), Q=Corring (1979), R=Zebrowska et al. (1984), S=Imbeah et al. (1988), T=Corring and Chayvialle (1987), U=Piersynowski et al. (1988), V=Mosenthin et al. (1988), W=Hee et al. (1988b), X=Wass (1965), Y=Ihse and Lilja (1979), Z=Harada et al. (1988)

Conclusions

From the data summarized herein it is clear that pancreatic secretion (volume, protein and enzyme activities) develops with increasing age of the animal. Alterations in feeding regimen, feed intake and diet composition (e.g., at weaning) interacts with this age-dependency.

Pancreatic fistulation is difficult to perform in small animals, so that not many data are available on pancreatic juice secretion in young piglets. Furthermore, enzyme development also depends on creep feed intake, weaning age and composition of weaner diet. Therefore it is difficult to obtain a clear picture of pancreatic secretion in young piglets. More research in this field is needed.

Data on pancreatic secretion in older pigs (sows and boars) are not known.

After Kvastnitskiis experiments described in his thesis (1951), no further long-term studies have been made on the changes in pancreatic juice secretion with age.

Effects of Feed Intake and Feeding Frequency on Pancreatic Secretion

Secretion Rate

Corring *et al.* (1972) studied the effect of a meal on pancreatic secretion. They used fistulated animals of ca 40 kg.

They found a strong increase in pancreatic protein excretion after a meal; protein secretion stayed high for 5 to 7 hours, showing a peak about 3 hours after starting the meal. This phenomenon can be explained by the negative feed back mechanism discussed in a previous paragraph: during the postprandial period, secreted enzymes form complexes with the ingested substrate so that they are no longer available for the down-regulation of pancreatic secretion. As soon as the substrate is hydrolyzed the enzyme is liberated from the enzyme-substrate/product-complex and down-regulation of secretion is resumed.

In suckling piglets, this phenomenon is not found (Pierzynowski *et al.*, 1988) since feed intake during suckling occurs almost continuously (ca 24 meals a day).

Pancreatic juice secretion depends on the frequency of feeding: Hee *et al.* (1988b) used four barrows to investigate this phenomenon. They found an increase in daily secreted juice volume of .5 liter for each additional meal (feeding 1 to 3 times daily).

Enzymatic Activity

Corring and Jung (1972) noted that with a fixed diet the amino acid composition of the pancreatic juice of pigs is constant.

From this it can be concluded that the enzymes ratio in pancreatic juice does not change during the day when a fixed diet is fed to adapted pigs. Changes in enzyme activities during the day occur only in terms of total amounts of enzymes, the relative proportion of e.g., trypsin remains the same.

The amount of secreted protein per 24 hours is not related to the feeding frequency (number of meals per day): when feed intake was 900 gram per day (1 x 900 or 2 x 450, protein in feed = 16.86 %) 5 to 7 grams of protein was secreted in the pancreatic juice.

Hee et al. (1988b) found with increasing feeding frequency (one, two or three meals daily) that the amylase secretion increased two-fold with each additional meal. The lipase secretion was highest when the pigs were fed once daily. The secretion of protein, trypsin and chymotrypsin was not affected.

Adaptation of Enzyme Activities to Diet Composition

Digestive enzymes adapt to the amount of substrate in the feed (Corring, 1980).

When changing from milk to starch rich feed (at 35 days of age) a sharp increase in amylase activity occurs in piglets 7 days after the diet change (Aumaître and Rérat, 1966, unpublished data, cited by Aumaître, 1972).

The adaptation to a new diet takes 5 to 7 days in rats and growing pigs. After this period a new, stable enzyme status has been established (Ben Abdeljlil and Desnuelle, 1964; Corring, 1980).

Chymotrypsin activity starts changing 48 hours after a diet change and reaches a new stable level at 5 days after the diet change (Corring and Saucier, 1972).

The adaptation to diet composition (5 to 7 days) takes much longer than the feedback regulation (2 to 3 hours) discussed earlier. The feedback mechanism responds directly to the amount of enzymes present in free form in the gutlumen. This 'short term' feed back mechanism ensures an appropriate flow of enzymes into the gut lumen when feed is given to adapted animals.

The relatively slow reaction of pancreatic secretion in response to feed composition could be explained by other physiological changes occurring after a diet change, such as gastric

emptying rate and gut motility.

The mechanism of nutritional regulation of pancreatic function (biosynthesis as well as secretion of enzymes) is not completely clear yet. In 1977, Corring reviewed the adaptation of pancreatic enzymes to dietary components. The hydrolysis products of dietary components stimulate enzymatic secretion through release of CCK-PZ, secretin, insulin and/or other gastro-intestinal hormones or peptides.

To elucidate the nutritional regulation of pancreatic secretion, Corring and Chayvialle (1987) measured hormones and peptides in the blood of pigs fed on different diets (control vs high-starch or high-fat) to check the influence of these substances on pancreatic enzyme secretion. Pancreatic enzyme secretion (amylase and lipase) clearly adapted to the amount of substrate in the feed (starch and fat respectively). With all three diets a postprandial rise in plasma cholecystokinin, somatostatin and pancreatic polypeptide was found in the blood. Plasma secretin levels did not alter after feed intake.

Corring and Chayvialle (1987) found that plasma levels of secretin and cholecystokinin were not affected by an increase in fat or starch intake. Thus, these two hormones do not seem to play an important role in the nutritional regulation of the lipase or amylase secretion by the pancreas.

Corring (1980) discussed the physiological significance of the adaptation of digestive enzymes to the diet: Enzyme secretion is sufficient to digest much larger amounts of substrate than are normally ingested, when enzyme activities measured *in vitro* are compared with feed components intake *in vivo*. However, the digestion of a substance *in vivo* could necessitate a higher enzyme concentration over a certain period, because rate of hydrolysis depends on enzyme concentration. Rate of hydrolysis is important in the digestive tract of animals where substrates are transported along the gut.

The ability of enzyme secretion to adapt to diet composition eliminates the need for a continuously high secretion rate, which would cause unnecessary losses of (unabsorbed) endogenous protein.

Corring (1980) also refers to experiments with rats performed by Lê-Thanh Uyên (1969, cited by Corring, 1980) in which prolonged protein malnutrition caused an increase in the secretion of proteins (enzymes) with pancreatic juice. This hypersecretion is possibly a source of protein substrate for the animal.

The physiological backgrounds of pancreatic adaptation mechanisms are not fully understood yet.

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Effects of Diet Composition on Pancreatic Secretion

Secretion Rate

Pekas *et al.* (1966) found a twofold pancreatic juice secretion in piglets (age at surgery: 45 days) fed on (heat treated) soya protein compared to piglets fed on milk protein. This could be caused by (heat stable) trypsin inhibitors in the soya feed.

Zebrowska *et al.* (1983) performed experiments with growing pigs (ca 35 kg) on two diets: diet A (purified): casein, wheat starch, sucrose, soya oil and diet B (cereals): barley meal, soybean meal. With diet B they found higher pancreatic juice secretion (2182 versus 1204 g per 24 hours), but no difference in enzyme activities.

The same effect of feed composition was found by Partridge *et al.* (1982). They fed two diets to growing pigs: diet A (purified): starch, sucrose, casein, maize oil, cellulose and diet B (cereals): barley, wheatings, fish meal. Diet A caused a pancreatic flow of 1273 ml per 24 hours, diet B a flow of 4962 ml per 24 hours.

Corring (1980) found with cereal diets in pigs of ca 45 kg a pancreatic flow of 2500 ml per 24 hours.

Table 1.   Effect of diet composition on pancreatic juice flow (Zebrowska, 1985)

	diet I	II	III	IV	
wheat	88.7	44.4	44.4	-	%
wheat bran	-	44.3	-	-	%
wheat flour	-	-	44.3	85.7	%
casein	7.0	3.5	6.0	6.0	%
wheat starch	-	3.5	1.0	-	%
vit/min-mix	4.3	4.3	4.3	4.3	%
cellulose	-	-	-	4.0	%
flow rate pancreatic juice	4108	4560	2556	1757	ml/24h

Zebrowska (1985) found with pigs of ca 34 kg an effect of diet on pancreatic flow (see Table 1). Secretion of pancreatic juice was higher in pigs fed diets containing wheat or wheat + wheat bran than in pigs fed wheat flour or wheat flour + wheat.

In Table 2 some data on pancreatic juice secretion in growing pigs in response to diet composition are summarized.

Enzyme Activity

The pancreatic enzyme levels depend on feed composition (protein, fat, carbohydrates, fiber) (Grossman *et al.*, 1943; Lindemann *et al.*, 1986).

Wheat bran caused in rats an elevation of the amylase and trypsin activities in the pancreas (Schneeman *et al.*, 1982).

In experiments with rats (Howard and Yudkin, 1963) it was shown that amylase and trypsin activities in pancreatic tissue were influenced independently from one another by changes in the quantity or quality of carbohydrates or proteins in the feed.

Protein in the feed. Snook and Meyer (1964) measured the enzymatic activity of the digesta in relation to the protein content of the diet. They suggested that the protease activity is enhanced through an increase in the synthesis and secretion of digestive enzymes and a decrease in the rate of enzyme breakdown in the digestive tract.

According to Pekas *et al.* (1964) the soy protein digestion depends on (pancreatic) enzymes; this dependency decreases with increasing age of the animals.

In ca 7 weeks old piglets, protease, amylase and lipase secretion was about 5 times higher when (heat treated) soya protein was fed compared to milk protein (Pekas et al., 1966).

The biosynthesis of proteolytic enzymes in the pancreas increases when untreated soya (containing trypsin inhibitor) is fed (Corring, 1980).

Schumann *et al.* (1983) investigated the pancreatic secretion in growing pigs (ca 35 kg) in relation to the quality of the protein source in the diet. They found a higher pancreatic juice secretion in pigs fed a diet with untoasted soybean meal (solvent extracted) compared to pigs fed a diet with toasted soybean meal. The protein secretion over 24 hours was elevated with the untoasted soya-diet, as was the trypsin, chymotrypsin, amylase and to a lesser extent the lipase activity. Schumann *et al.* (1983) explained these effects by the presence of a trypsin inhibitor in untoasted soya, which blocks the trypsin-mediated feed back to the pancreas.

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Table 2.   Pancreatic secretion in pigs adapted to different diets per 24 hours.

Reference	Feed intake (kg diet)	Vol. (l)	Prot. (g)	Live weight	Type of cannula
Corring et al. 1972	0.9 barley-soya	1.7	6.1	42 kg	duct
Corring 1980	1.0 barley-soya	2.5	18.6	45	duct
Zebrowska et al.	1.5 starch-casein	1.2	10.9	35	pouch
1981	1.5 barley-soya	2.2	12.1	35	pouch
Partridge et al.	1.6 barley-fish meal	5.0	9.8	48	duct
1982	1.6 starch-casein	1.3	6.7	48	duct
Schumann et al.	0.9 raw soybean meal	2.4	9.4	35	duct
1983	0.9 toasted soybean meal	1.5	4.7	35	duct
Zebrowska et al.	1.5 barley-soya	2.2	12.1	40	pouch
1983	1.5 starch-casein	1.2	10.9	40	pouch
	1.8 starch-soya	3.8	14.4	35-50	pouch
Ozimek et al.	1.7 protein-free	3.9	19.0	35	pouch
1984	1.7 soybean meal	4.0	28.6	35	pouch
	1.7 isolated soy protein	3.9	28.9	35	pouch
Ozimek et al.	1.7 raw soy-product	4.0	23.6	40	pouch
1985	1.7 autoclaved soy	3.6	19.4	40	pouch
Hee et al.	1.8 high fat	3.9	15.3	35-100	pouch
1985	1.8 control	4.0	15.0	35-100	pouch
	1.8 protein-free	3.5	12.2	35-100	pouch
Zebrowska et al.	1.4 wheat	4.1	17.9	34	pouch
1985	1.4 wheat/wheat bran	4.6	19.0	34	pouch
	1.4 wheat/wheat flour	2.6	15.8	34	pouch
	1.4 wheat flour	1.8	13.0	34	pouch
Langlois et al.	1.6 no wheat bran	1.7	14.6	38	duct
1987	1.6 40% wheat bran	3.6	19.7	38	duct
Hee et al.	1.8 control	4.0	15.1	35	pouch
1988a	1.8 high fat	3.9	15.3	35	pouch
	1.8 protein-free	3.5	13.1	35	pouch
Imbeah et al.	1.6 soybean meal	1.2	16.4	47	pouch
1988	1.6 canola meal	1.0	15.4	47	pouch
Mosenthin et al.	1.8 starch	3.7	26.9	60	pouch
1988	1.8 cellulose	3.2	22.8	59	pouch
	1.8 straw meal	4.6	28.5	69	pouch
	1.8 pectin	4.7	27.0	72	pouch

According to Pond *et al.* (1971) the difference in performance in favor of piglets fed diets with ISP (Isolated Soybean Protein) compared to animals fed on FPC (Fish Protein Concentrate) can not be explained by differences in pancreatic enzyme activities. Enzymes

of the stomach or gut wall could also play a role in this respect.

In conclusion, protein degrading enzymes respond to the amount of protein as well as the type of protein in the feed. Trypsin inhibitors in the feed can induce hypersecretion of the pancreas by blocking the feedback mechanism controlling secretion.

Fat in the feed. The specific lipase activity in pancreatic juice of pigs increases by 700 % when the triglyceride intake is increased from 30 to 220 g (Corring, 1980). It is not unlikely that lipase synthesis is more stimulated by unsaturated fatty acids than by saturated fatty acids. Furthermore the ratio between carbohydrates, proteins and fats in the diet could influence lipase activity, as could the relative amounts of carbohydrates and fats in terms of energy (Corring, 1980).

Ozimek *et al.* (1985) also investigated the effect of fat quantity and quality on lipase secretion in the pig. When raising the amount of fresh canola oil in the diet from 0 to 15 %, lipase secretion increased from 2.96×10^3 U per 24 hours (when no canola oil was added) to 10.17×10^3 U per 24 hours. The inclusion of 15 % peroxidized canola oil resulted in an 6 to 9 fold increase in lipase activity.

Forman and Schneeman (1980) studied the effect of pectin and fat on the exocrine pancreas in rats. They found an increase in the activity of lipase and a decrease in the activities of amylase and to a lesser extent of trypsin and chymotrypsin when the amount of maize oil in the diet was increased from 5 to 25 %. An effect of pectin was found only on enzyme activities in the small intestine.

The amount of fat in the feed as well as the quality (degree of saturation, chain length) affects lipase secretion in pigs.

Starch in the feed. Increasing the amount of starch in the diet from 160 to 600 g resulted in an increase of the specific amylase activity (Corring, 1980).

Effects of different types of starch (maize, potato, wheat, raw vs cooked) are not known.

Conclusions. From these data it is clear that pancreatic enzyme secretion adapts specifically to the substrates available from the ingested food.

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Effects of Environment and Stress on Pancreatic Secretion

Enzymatic Activity

Szabó *et al.* (1976) found that total amylase and lipase content in pancreatic homogenate were influenced by environmental temperature: concentrations were low at high temperatures (38 - 40 ^{0}C, relative humidity 50 %) and high at low temperatures (0 - 2 ^{0}C, relative humidity 90 %) compared to control temperature (25 - 27 ^{0}C, relative humidity 60 %). In these experiments feeding was ad lib, so that feed intake could have affected results.

Discussion

Snook (1974) assumes that in healthy, normally fed animals it is unlikely that changes in enzyme levels (induced by diet composition) have a significant influence on the physiological well-being of the animal.

Several workers stress the discrepancy between the excessive production of enzymes and the adaptation of enzyme activities to different diets (Low, 1982; Partridge *et al.*, 1982; Zebrowska *et al.*, 1983). The amount of digestive enzymes secreted by the pancreas is theoretically sufficient to hydrolyze 100 times the ingested amount of feed (substrate).

Not much is known about the dilution and breakdown of enzymes in the digestive tract. According to Snook (1965) probably more than 90 % of trypsin and chymotrypsin is inactivated by proteolytic enzymes of the gut wall and by micro organisms. It is possible that starch can reduce the hydrolysis of amylase because starch + amylase as a complex cannot be broken down by proteolytic enzymes.

Much research has been done on influences of diet composition on pancreatic enzyme secretion. These data should be combined with digestibility data to gain knowledge on the physiological significance of adaptation mechanisms. Other factors influencing pancreatic secretion should also be taken into account.

More work should be done on enzyme development in young piglets in relation to weaning and diet composition.

Another point of interest is the breakdown of secreted enzymes in the digestive tract ("half life time") (De Lange, pers. commun. 1988). This was investigated by Asche *et al.* (1987). They studied the molecular weight profiles of the soluble proteins in the digestive tract of

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piglets and compared them with the molecular weights of the digestive enzymes. These data suggested that most of the endogenous proteins were hydrolyzed rapidly.

Comparisons should be made between secreted amounts of enzymes and enzyme activities in (ileal) digesta and faeces.

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STORAGE OF PORCINE PANCREATIC JUICE: EFFECT ON ENZYME ACTIVITIES

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Summary

A total of 80 samples of porcine pancreatic juice were stored at three different temperatures (4 ^{0}C, -20 ^{0}C and -80 ^{0}C) for different periods of time (0 to 21 days).
Activities of pancreatic enzymes (trypsin, chymotrypsin and lipase) have been measured in the stored pancreatic juice.
The results show that enzyme activities are affected by storage temperature.
From one week after sampling, activities of trypsin, chymotrypsin and lipase in pancreatic juice samples stored at 4 ^{0}C differ from activities measured immediately after sampling (at day 0) ($p < .05$). In frozen pancreatic juice samples, enzyme activities remain fairly constant during three weeks of storage.

Introduction

Pancreatic enzyme secretion in pigs has received much attention from several animal research workers (Partridge *et al.*, 1982; Hee *et al.*, 1988; Pierzynowski *et al.*, 1988).
Fistulation techniques for pancreatic juice collection *in vivo* have been developed (Corring *et al.*, 1972; Hee *et al.*, 1985; Pierzynowski *et al.*, 1988). Thus secretion rates over extended periods of time and in response to different diets can be assessed.
Usually, the collected juice cannot be analyzed immediately: the samples must be stored after collection. Unfortunately, storage conditions are often not published when experimental results are given.
Corring (pers. comm., 1988) advises storage at -80 ^{0}C to prevent enzyme activity decline. Different workers use different methods to store their pancreatic juice samples from collection until analysis (see Table 1).

The purpose of this experiment was to determine the effect of different storage temperatures on enzyme activities in pancreatic juice.

Table 1.   Storage of pancreatic juice by different authors.

Reference (Author, Year)	Storage conditions Time	Temp.	Comment
Reboud *et al.*, 1962	months	-20 ^{0}C	rats, lyophilized juice
Partridge *et al.*, 1982	< 8 h	4 ^{0}C	pigs
Langlois *et al.*, 1987	?	-80 ^{0}C	pigs
Hee *et al.*, 1988	< 12 h	5 ^{0}C	pigs
Imbeah *et al.*, 1988	?	-30 ^{0}C	pigs
Pierzynowski *et al.*, 1988	?	-20 ^{0}C	pigs

Materials and Methods

Animals, Diets, Housing and Sampling

A castrated male pig (age 14 months), fitted with a pancreatic cannula according to the method of Hee *et al.* (1985) was used for collection of pancreatic juice from a small isolated segment of duodenum. The method of Hee *et al.* (1985) was slightly modified: the one-way valve at the re-entrant part of the cannula caused blockage of the cannula and was therefore removed.

The pig was fed a commercial pig diet (NE 10000 kJ/kg, 18.2 % CP, 1.21 % dig. Lysine, 0.73 % dig. Methionine/Cysteine) twice daily at 0800 and 1700 hours, 1000 g each meal. At five different days a sample of pancreatic juice was taken.

The samples were taken at 1300 hours, five hours after feeding, when pancreatic juice production was at its maximum in this pig (Wichers, 1988).

Each sampling took five to ten minutes and ca 30 ml of juice was collected during this time. An aliquot of each sample was analyzed for enzyme activities within five hours after sampling.

The remainder of each sample was divided in three subsamples: one subsample was stored at 4 ^{0}C, one subsample was stored at -20 ^{0}C and the third subsample was stored at -80 ^{0}C. The stored subsamples were divided into five portions (one portion for each day of analysis after storage) so that no repeated freezing and thawing took place. All stored subsamples were tested for activities of trypsin, chymotrypsin and lipase after the designated storage period (1, 4, 7, 14 or 21 days).

A total of 80 aliquots was analyzed for enzyme activities.

Analytical and Statistical Procedures

Enzyme analysis on day 0 was completed within five hours from sampling. Samples were kept on ice from sampling until analysis was completed.

Frozen samples were left to thaw at room temperature and put on ice immediately after thawing had been completed.

Trypsin activity was measured with Nα-p-toluolsulphonyl-L-argininmethylester (TAME) as a substrate (Bergmeyer, 1974).

Chymotrypsin activity was measured with N-benzoyl-L-tyrosinethylester (BTEE) as a substrate (Bergmeyer, 1974).

Lipase activity was measured using a method developed by Van Oort (not published, 1988) with 1-thio-2,3-tributyryl-glycerol (not commercially available) as a substrate: the substrate is hydrolyzed to free fatty acids and a glycerol-like substance with a free SH-group. DTNB (5,5-dithiobis(2)-nitro-benzoic-acid) is added to form a colored product on reaction with the previously formed SH-group.

A spectrophotometer (Perkin-Elmer, type 550) was used to measure the extinction changes. Extinction changes were recorded on paper to facilitate calculations.

Pancreatic juice was diluted 10 to 100-fold with a buffer solution (depending on the enzyme and on the amount of enzyme activity present in the sample).

All analyses were carried out at 25 ^{0}C, trypsin analysis at pH 8.1, chymotrypsin analysis at pH 7.8 and lipase analysis at pH 8.5.

No activation of zymogens was performed prior to analysis of trypsin and chymotrypsin.

Enzyme activities were also calculated as a percentage of the activity on day 0 (reference value).

Data were subjected to analyses of variance according to the following model:

$$Y_{ijk} = \mu + \text{SUBSAMPLE}_{i(k)} + \text{DAY}_j + \text{DAY*TEMP}_{(j*k)} + e_{ijk}$$

In which:

Y_{ijk}	- dependent variable
μ	- overall mean
$\text{SUBSAMPLE}_{i(k)}$	- subsample nested within temperature

$$\text{(TEMP= } 4\,^0\text{C: i = 1, 2, 3, 4, 5)}$$
$$\text{(TEMP=-20 }^0\text{C: i = 6, 7, 8, 9, 10)}$$
$$\text{(TEMP=-80 }^0\text{C: i = 11, 12, 13, 14, 15)}$$

DAY_j - days after sampling, storage period
$$(j = 0, 1, 4, 7, 14, 21)$$
$DAY*TEMP_{j*k}$ - interaction between storage period and temperature
e_{ijk} - error term

The effects of DAY, SUBSAMPLE and DAY*TEMP were tested against the error term. All tests were performed using SAS-GLM (SAS 1985).
Post hoc analyses of the interaction between DAY and TEMP were performed using Student's T tests (SAS-GLM: SAS, 1985).

Results

Activities of trypsin, chymotrypsin and lipase are presented in Tables 2, 3 and 4, respectively. Relative activities (percentages of activity on day 0) of trypsin, chymotrypsin and lipase are shown in Figures 1, 2 and 3, respectively.
No differences in enzyme activity were found ($p > .05$) between storage at -20 ^0C and at -80 ^0C. When stored frozen, enzyme activities did not change over the three weeks storage period ($p > .05$).
Between day 0 and day 1, trypsin activity showed a small increase which was however not statistically significant.
After day 0, trypsin activities in frozen samples ranged from 119 to 144 % of the activity found on day 0, chymotrypsin activities in frozen samples ranged from 88 to 107 % and lipase activities in frozen samples from 46 to 108 %.
All enzyme activities decreased in samples stored at 4 ^0C.
The enzyme activities of samples stored at 4 ^0C differed from the initial enzyme activity ($p < .05$) from 14, 4 and 7 days of storage onwards for trypsin, chymotrypsin and lipase respectively.
After three weeks of storage at 4 ^0C enzyme activities were reduced to 31, 39 and 5 % of the activities on day 0 for trypsin, chymotrypsin and lipase respectively.

Table 2.   Trypsin activities in pancreatic juice stored at different temperatures from 0 to 21 days (Least Square Means [x]), units per ml.

TEMP	DAY 0[y]	1	4	7	14	21
4 ^{0}C	47.7[aA]	62.5[aA]	51.5[aA]	35.0[abA]	21.9[bcA]	14.7[cA]
-20 ^{0}C	47.7[aA]	60.5[aA]	62.2[aA]	61.1[aB]	57.2[aB]	64.3[aB]
-80 ^{0}C	47.7[bcA]	58.0[abA]	59.6[abA]	68.6[aB]	64.6[abB]	56.7[abB]

[x]   Least Square Means within a row (abc) or column (AB) that do not have a common superscript differ (T test, p < .05). $R^2 = 0.91$, RSD = 14.48
[y]   On day 0, samples had not been stored prior to analysis, all analyses performed with samples on ice.

Table 3.   Chymotrypsin activities in pancreatic juice stored at different temperatures from 0 to 21 days (Least Square Means [x]), units per ml.

TEMP	DAY 0[y]	1	4	7	14	21
4 ^{0}C	31.9[aA]	30.0[abA]	25.2[bcB]	21.4[cdB]	16.5[deB]	12.5[eB]
-20 ^{0}C	31.9[aA]	33.6[aA]	32.9[aA]	29.5[aA]	30.9[aA]	29.4[aA]
-80 ^{0}C	31.9[abA]	34.2[aA]	32.1[abA]	30.6[abA]	30.0[abA]	28.0[bA]

[x]   Least Square Means within a row (abcde) or column (AB) that do not have a common superscript differ (T test, p < .05). $R^2 = 0.90$, RSD = 4.09
[y]   On day 0, samples had not been stored prior to analysis, all analyses performed with samples on ice.

Table 4.   Lipase activities in pancreatic juice stored at different temperatures from 0 to 21 days. (Least Square Means [x]), units per ml.

TEMP	DAY 0[y]	1	4	7	14	21
4 ^{0}C	21.3[aA]	16.9[abA]	11.5[abcA]	6.0[bcA]	2.0[cA]	1.0[cC]
-20 ^{0}C	21.3[abA]	22.9[aA]	15.1[abA]	12.3[abA]	9.9[bA]	12.0[abBC]
-80 ^{0}C	21.3[abA]	19.0[abA]	15.8[abA]	13.9[abA]	10.3[bA]	22.1[aAB]

[x]   Least Square Means within a row (abc) or column (ABC) that do not have a common superscript differ (T test, p < .05). $R^2 = 0.53$, RSD = 9.05
[y]   On day 0, samples had not been stored prior to analysis, all analyses performed with samples on ice.
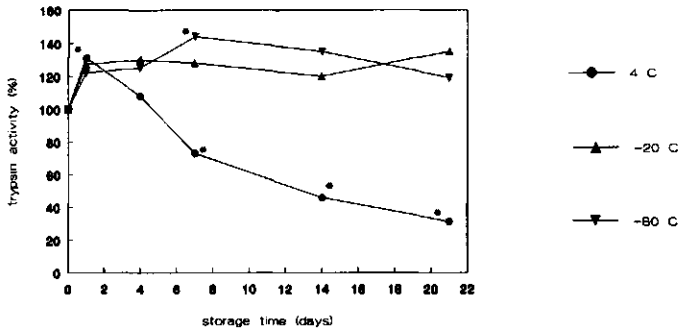
Figure 1.　Trypsin activity as a percentage of activity on day 0. (Least Square Means, Standard Error of LSM = 7.8). * = Relative activity differs from 100 % (p < .05).
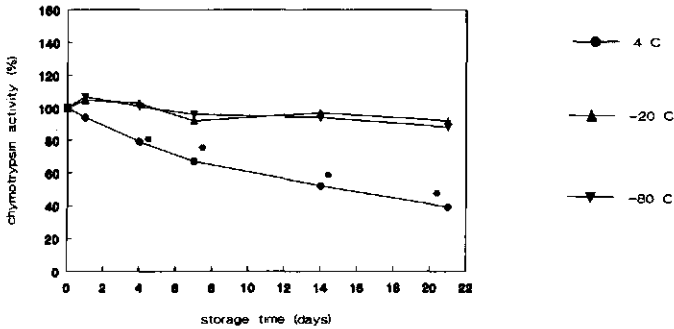
Figure 2.　Chymotrypsin activity as a percentage of activity on day 0. (Least Square Means, Standard Error of LSM = 6.5). * = Relative activity differs from 100 % (p < .0.5).
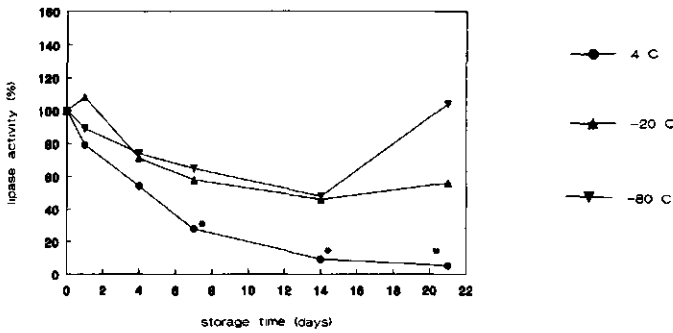
Figure 3.　Lipase activity as a percentage of activity on day 0. (Least Square Means, Standard Error of LSM = 20.7). * = Relative activity differs from 100 % (p < .05).

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Discussion

Lipase activity showed considerable variation between measurements. Therefore, it is advisable to analyze more samples when lipase activity is to be measured.

We did not activate the proteolytic enzymes prior to analysis.

The fact that considerably high levels of these enzymes were found in unactivated pancreatic juice samples indicated that activation of zymogens was not necessary. Hee *et al.* (1988) and Sauer (pers. comm. 1988) had to use an activation procedure to be able to measure proteolytic enzyme activities. The fact that we did not use a one-way valve in our cannula to prevent backflow of chyme into the pouch may have caused activation of trypsinogen to trypsin by enterokinase. Enterokinase from the duodenal wall could have entered the pouch or enterokinase could be secreted by the pouch wall. This enterokinase probably activated trypsinogen after which trypsin activated trypsinogen and chymotrypsinogen (chain reaction).

The decline of enzyme activities in pancreatic juice stored at 4 ^{0}C is probably due to auto-degradation of enzymes or to degradation of enzymes by other enzymes or by microorganisms (contamination of juice). In frozen pancreatic juice, enzymes and microorganisms are less active.

From the data presented here it is clear that enzyme activities in pancreatic juice are affected by storage temperature.

From the present experiment it can be concluded that pancreatic juice can be stored frozen for at least three weeks without deleterious effects on the activities of trypsin, chymotrypsin and lipase.

These results are in agreement with the data reported by Legg and Spencer (1975) who investigated the stability of pancreatic enzymes in human duodenal fluid when stored at different temperatures.

When stored at 4 ^{0}C, pancreatic juice can be stored for 7, 1 and 4 days before assessment of trypsin, chymotrypsin and lipase activity, respectively.

The small increase in trypsin activity between day 0 and day 1 of pancreatic juice samples might be caused by activation of remaining trypsinogen by trypsin present in the pancreatic juice samples. However, if this is the case, then it is not clear why this trypsinogen was not activated in the first place.

More research is needed on changes in enzyme activity during storage of pancreatic juice and other samples (e.g. pancreatic tissue, chyme).

Changes in enzyme activity of pancreatic juice within one day from sampling ('short-term changes') should also be investigated.

It is important to find optimal storage conditions for biological samples in which enzyme activities are to be determined.

Reboud *et al.* (1962) stored their lyophilized pancreatic juice samples for months at -20 °C. Lyophilization (freeze-drying) of pancreatic juice might be a method to prevent enzyme activity changes during long-term storage. This possibility should be studied in more detail. When analyzing pancreatic enzyme activities, activation of proteolytic enzymes should be performed to check if maximal activity is obtained before analysis.

Optimalization of storage conditions for pancreatic juice would improve reliability and comparability of literature data.

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Chapter V

GASTRIC PROTEIN BREAKDOWN AND PANCREATIC ENZYME ACTIVITIES IN RESPONSE TO TWO DIFFERENT DIETARY PROTEIN SOURCES IN NEWLY WEANED PIGLETS.

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Summary

Seventy piglets were weaned at 25 days of age and fed diets based on either skim-milk powder (SMP) or soybean protein concentrate (SOY). At 0, 3, 6 and 10 days after weaning, piglets were anaesthetized, their pancreas removed, and digesta collected from different sections of the digestive tract. The ratio of trichloro acetic acid (TCA) precipitable protein to total (crude) protein (pp/cp) in gastric digesta was higher with SOY feed than with SMP feed. In the jejunum no difference was found, hence, the degree of protein breakdown in jejunal chyme did not differ between protein sources. Trypsin activities in jejunal chyme and in pancreatic tissue increased after weaning. Chymotrypsin activity in pancreatic tissue tended to decrease after weaning and did not reach 'weaning-levels' for at least ten days. Pancreatic trypsin developed more rapidly than chymotrypsin after weaning. Chymotrypsin activities in jejunal digesta was higher for the SMP fed piglets than for the SOY fed piglets. Protease activities in the jejunum at day 6 after weaning were clearly affected by feed intake after weaning. The ratio between trypsin and chymotrypsin activity in pancreatic tissue and jejunal chyme was higher for SOY fed piglets than for SMP fed piglets. It was concluded that the stomach plays an important role in the digestion of milk protein and that the development of pancreatic proteases after weaning (synthesis, secretion, breakdown) depends on feed intake and on dietary protein source.

Introduction

Newly weaned piglets often show depressed feed intake and growth when fed vegetable proteins compared to proteins from animal sources. As the piglets get older, the digestion of vegetable protein increases, while the digestion of milk protein is high immediately after weaning and hardly increases with advancing age of the piglets (Decuypere et al., 1981, Wilson & Leibholz, 1981a,c, Owsley et al., 1986a). Immediately following weaning, the digestive system of the piglet has to adapt to the new feeding regimen with respect to pH, enzyme secretion and gut motility.

It is well known that soy protein for example, can cause digestive problems in young piglets, due to the presence of antinutritional factors, oligosaccharides and/or antigens. Also, the type of protein itself may cause digestive disturbances, especially in young piglets (Giesting, 1986, Sissons, 1989).

After weaning, pancreatic enzyme activities may decline dramatically (Lindemann et al.,

1986, Owsley et al., 1986b). The development of pancreatic proteases after weaning may thus depend on the protein source in the diet as well as on the amount of feed ingested. This experiment was undertaken to study the effects of skim-milk powder and soy protein concentrate on feed intake, protein breakdown, digesta pH and trypsin and chymotrypsin activity in digesta and pancreatic tissue of newly weaned piglets.

Materials and Methods

Animals, housing, feeding
Ten litters of seven piglets each (Great York*(Great York*Dutch Landrace)) were weaned at 25 days of age. No creep feed was provided during the suckling period. After weaning, the piglets were housed individually in wooden crates (60*70 cm) with bedding. Visual contact between piglets was not possible. Within litters, piglets were allocated to one of two dry, pelleted diets. Weaning weights were similar within litters, between diets. The composition of the diets is given in Table 1. Diets were formulated to contain equal amounts of protein, energy, fiber, fat and lactose. Skim-milk powder and soy protein concentrate were the sole sources of protein in the SMP and the SOY diet, respectively. The piglets were fed twice daily (morning and afternoon) at an 8 hour interval. From weaning onwards, the morning feed was given one hour before the designated time of sampling and the evening feed was given 8 hours later. Daily feed allowance was restricted to 5% of liveweight.

Sampling
At weaning (day 25), one piglet from each litter was taken from the sow, anaesthetized through inhalation anaesthesia (N_2O, O_2, halothane, no premedication) and sampled. At 3, 6 and 10 days after weaning (day 28, 31 and 35, respectively) two piglets from each litter (one on the SMP diet and one on the SOY diet) were anaesthetized one hour after the morning feeding. The piglets were weighed, and exsanguinated from the jugular veins and artery. An off midline incision was made to expose the digestive tract. The stomach was secured *in situ* and removed from the abdominal cavity. The small intestine was divided in 'duodenum' (first two meters of the small intestine from pylorus), 'ileum' (last two meters of small intestine up to the ileocecal valve) and 'jejunum' (remaining middle section of the small intestine) and removed from the abdominal cavity. The different parts were emptied into aluminium foil trays. The contents were weighed, mixed and the pH measured. One

gram of digesta (gastric and jejunal) were mixed with two ml of trichloro acetic acid solution (TCA, 30 g/l) to precipitate the non-absorbable fraction of the nitrogenous material (Ternouth *et al.*, 1974, Souffrant *et al.*, 1991). These digesta samples were kept in a refrigerator (0-4 °C) until analysis. The remainder of the digesta was frozen immediately at - 20 °C.

Table 1.　　Composition of the diets (%).

	SMP	SOY
Ingredients:		
maize/wheat starch	29.50	28.90
skim-milk powder	46.00	-
soy protein concentrate (63% CP)[1]	-	25.40
dextrose	13.44	11.06
lactose	-	22.50
soya oil	2.00	2.20
cellulose	5.00	4.10
ground limestone	.75	1.45
mono Ca phosphate	.50	2.25
salt	.30	.30
$KHCO_3$	.30	.20
$NaHCO_3$	.10	.20
L-lysine-HCl	-	.14
DL-methionine	.11	.22
L-threonine	-	.08
vit/min premix	1.00	1.00
Contents (calculated from ingredient composition):		
dry matter	93.80	93.95
crude protein	16.21	16.44
digestible crude protein	15.43	14.72
ether extract	2.51	2.44
crude fiber	4.95	4.95
crude ash	5.46	5.79
starch	25.71	25.71
net energy (MJ/kg)	10.21	10.26
Analyzed contents:		
dry matter	89.71	88.98
crude protein	16.89	16.74

[1]　　Soycomil-P: Loders Croklaan, Wormerveer, The Netherlands. Trypsin Inhibitor Activity: less than 3 mg inhibited trypsin per gram (95% reduction compared to untoasted soybean meal). Urease: less than .1 mg N/g*minute at 30 °C. Antigens: not present.

The pancreas was excised, freed from connective tissue, weighed and frozen immediately at -20 °C. The whole procedure took approximately 20 minutes per piglet.

The frozen digesta and pancreas of each piglet was freeze dried and ground within two weeks of sampling. Samples for enzyme analysis were stored at -20 °C under N_2 gas to prevent enzyme activity loss.

Analyses

Gastric and jejunal digesta were analyzed for TCA-precipitable protein (Ternouth *et al.*, 1974) and crude protein content. Jejunal digesta and pancreatic tissue samples were analyzed for trypsin and chymotrypsin activity (Bergmeyer, 1974). Zymogens in pancreatic tissue samples were activated with porcine enterokinase (Sigma Chemical) before enzyme analyses. Trypsin and chymotrypsin activities were measured at pH 8.1 and 7.8, respectively, using TAME (Nα-toluolsulphonyl-L-arginine-methylester, Merck) and BTEE (N-benzoyl-L-tyrosine-ethylester, Fluka Biochemika) as respective substrates (Bergmeyer, 1974). Extinction changes were measured at 247 and 256 nm for trypsin and chymotrypsin respectively, using a spectrophotometer (Beckman DU-64).

An analysis of variance was performed separately for each day of slaughter using SAS-GLM (SAS, 1990) according to the following model: $Y_{ij} = \mu + PS_i + b_1{}^*FI + e_{ij}$

In which:
	Y_{ij}	= dependent variable
	μ	= overall mean
	PS_i	= dietary protein source (i = 1, 2)
	$b_1{}^*FI$	= feed intake, overall covariable
	e_{ij}	= error term

The effect of feed intake within protein source (interaction between protein source and feed intake) was found to be non-significant for any of the parameters and was therefore not included from the model. To determine the effect of protein source on feed intake, the following model was used: $Y_{ij} = \mu + PS_i + e_{ij}$

In which:
	Y_{ij}	= dependent variable (feed intake)
	μ	= overall mean
	PS_i	= dietary protein source (i = 1, 2)
	e_{ij}	= error term

To reveal overall changes and trends during the post weaning period, the following model was used: $Y_{ij} = \mu + SD_i + e_{ij}$

In which: Y_{ij} = dependent variable
               μ = overall mean
               SD_i = day of sampling (i = 0, 3, 6, 10)
               e_{ij} = error term

Results

Feed intake, average daily gain, pancreatic weight and digesta pH
At weaning, the piglets were 25 days of age and weighed 7.15 kg (sd = 0.98, n = 70). No diarrhoea or other signs of illness were observed throughout the experiment.
Feed intake and average daily gain are presented in Table 2. During the first three days postweaning, no significant differences in feed intake or growth were found between protein sources. Variation in feed intake and growth was considerable during this period. Feed intake affected average daily gain during the whole post-weaning period (10 days).
From 3 days after weaning, differences in average daily gain between protein sources (p<0.05) appeared. Piglets fed on SMP had a higher average daily gain from 3 days after weaning onwards compared to piglets on SOY. Feed intake affected average daily gain during the whole post-weaning period (10 days).
The weight of the pancreas (absolute, and relative to liveweight) increased after weaning (Figure 1). Feed intake and protein source did not affect the weight of the pancreas (Table 3).
No effects of feed intake or protein source on digesta pH were detected (p>0.05), except for the pH in the ileum which was higher for the SOY fed piglets. Changes in gastrointestinal pH are depicted in Figure 2. The pH in the gastrointestinal tract gradually increased from the proximal to the distal section of the small intestine.

Table 2.   Feed intake (FI, gram per day) and average daily gain (ADG, gram per day) per post-weaning period (LSMeans, b=coefficient of regression).

	protein source			feed intake		
	SMP	SOY	p	b	p	rootMSE:
FI (g/d):						
day 0-3	173	178	.8843	-	-	83
day 0-6	215	234	.5825	-	-	78
day 0-10	256	234	.3561	-	-	52
ADG (g/d):						
day 0-3	5	47	.5551	.02	.9571	155
day 0-6	157	63	.0072	.40	.0679	68
day 0-10	185	131	.0193	.74	.0025	46
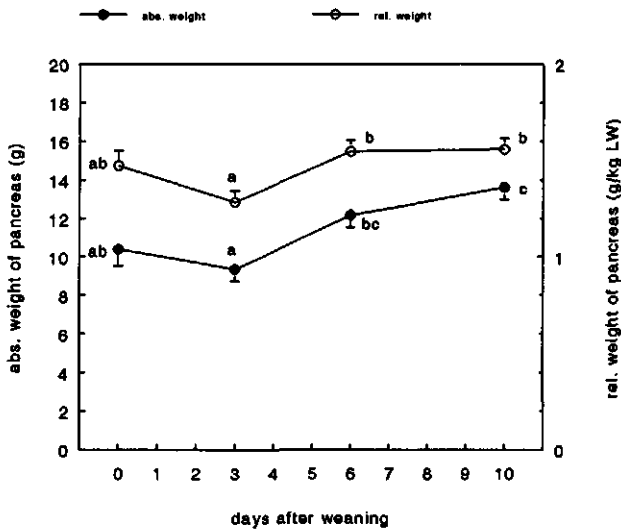
Figure 1.   Pancreatic weight (fresh tissue), absolute (g) and relative to liveweight (g/kg), LSMeans with StdErr of LSM.

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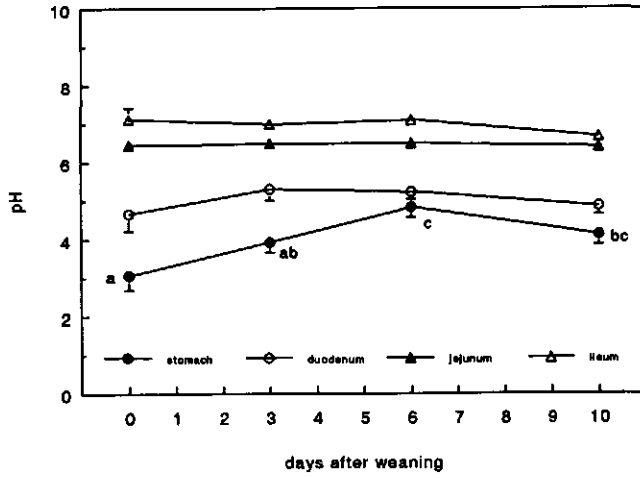
Figure 2.   Digesta pH in different sections of the digestive tract. LSMeans with StdErr of LSM.
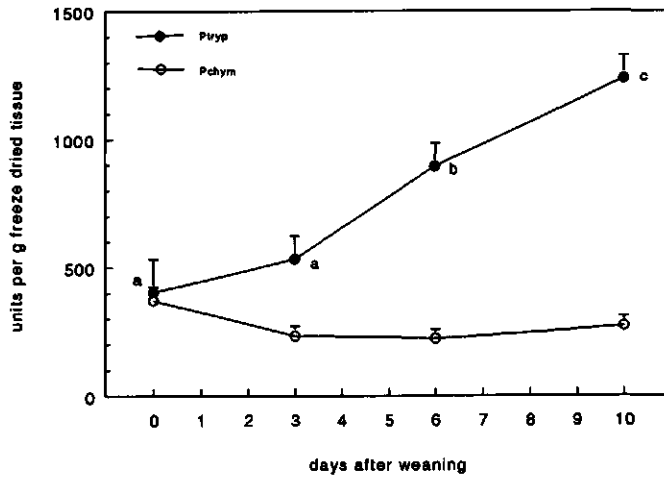
Figure 3.   Trypsin and chymotrypsin activities in pancreatic tissue (Units per gram freeze dried tissue). LSMeans with StdErr of LSM.

Protein breakdown in stomach and jejunum

The source of dietary protein clearly affected ($p < 0.01$) the degree of protein breakdown in the stomach (Table 4). The ratio precipitable protein / crude protein (pp/cp) was lower for the SMP-diet, indicating a greater degree of protein breakdown. In the diets, this ratio was 0.79 and 0.82 for the SMP and the SOY diet, respectively. Feed intake did not affect the pp/cp ratio in the stomach. The ratio precipitable protein / crude protein in the jejunal digesta, was not affected by protein source nor feed intake.

Enzyme activities in pancreas and jejunum

Protease activities in pancreatic tissue and jejunal digesta are shown in Table 4. Trypsin activity in pancreatic tissue (units per gram freeze dried tissue) increased after weaning (Figure 3). No effects of feed intake or protein sources on pancreatic protease activities (U/g freeze dried material) were found ($p > 0.05$). At ten days after weaning, the chymotrypsin activities in pancreatic tissue for the SMP groups were similar to the chymotrypsin activities at the day of weaning.

Trypsin activities in jejunal digesta (units per gram freeze dried digesta) increased markedly after weaning. At ten days after weaning, trypsin activity in the jejunal digesta was 7 to 8 times as high as the activity at the day of weaning. Chymotrypsin activity in jejunal digesta also increased after weaning, but at a much slower rate than trypsin activity. At 6 and 10 days after weaning, piglets fed the SMP diet had higher chymotrypsin activities in the jejunal digesta than piglets fed the SOY diet ($p < 0.05$).

At 6 days after weaning, jejunal protease activities (per gram freeze dried material and total units) were affected by feed intake. This was not found on day 3 or day 10 after weaning. The total amount of trypsin and chymotrypsin in pancreas and jejunum at day 6 was positively influenced by feed intake.

The ratio between trypsin and chymotrypsin activity in pancreatic tissue and in jejunal digesta are given in Table 4. At 6 and 10 days after weaning, the ratio between trypsin and chymotrypsin activity in jejunal digesta was higher for the SOY fed piglets. This effect was not found in the pancreas. Feed intake did not affect the tryp/chym ratio.

Table 3. Liveweights (g), pancreas weights (g), digesta weights (g) and pH in the digestive tract (LSMeans, b=coefficient of regression). LW=liveweight, PW=pancreas weight, relPW=pancreas weight relative to liveweight (g/kg).

protein source	at weaning: day 0	after weaning: day 3 SMP	SOY	p	feed intake: b	p
LW (g)	7016	7303	7333	.9501	2.63	.3876
PW (g)	10.4	9.25	9.47	.7678	-.00	.6893
relPW (g/kg)	1.47	1.28	1.29	.8626	-.00	.1309
digesta weights:						
stomach (g)	80	132	170	.2936	.23	.3155
duodenum (g)	3	4	5	.5079	.01	.3083
jejunum (g)	41	126	139	.7345	.30	.2067
ileum (g)	3	17	19	.7561	-.00	.8537
total (g)	127	279	333	.4294	.54	.2230
digesta pH:						
stomach	3.07	3.83	4.03	.7472	.01	.1192
duodenum	4.67	5.41	5.23	.5785	-.01	.0441
jejunum	6.45	6.48	6.52	.7640	.00	.6769
ileum	7.13	7.05	6.97	.7129	.00	.6736

protein source		day 6 SMP	SOY	p	b	p
LW (g)	-	8048	7570	.2754	10.82	.0014
PW (g)	-	13.20	11.12	.1154	0.03	.0022
relPW (g/kg)	-	1.63	1.47	.2669	0.00	.1175
digesta weights:						
stomach (g)	-	222	241	.4997	.26	.1649
duodenum (g)	-	6	7	.3640	-.02	.0850
jejunum (g)	-	187	142	.0435	-.04	.7664
ileum (g)	-	21	15	.2671	.05	.1567
total (g)	-	435	404	.3905	.26	.2930
digesta pH:						
stomach	-	4.81	4.86	.8832	.00	.4121
duodenum	-	5.26	5.19	.8548	.00	.5493
jejunum	-	6.44	6.56	.3949	.00	.3324
ileum	-	6.90	7.30	.0071	.00	.2872

protein source		day 10 SMP	SOY	P	b	p
LW (g)	-	8744	8665	.8525	17.66	.0005
PW (g)	-	13.80	13.42	.7025	.04	.0011
relPW (g/kg)	-	1.58	1.54	.7208	.00	.1939
digesta weights:						
stomach (g)	-	237	253	.7431	1.01	.0472
duodenum (g)	-	3	13	.0063	.06	.0605
jejunum (g)	-	172	206	.4612	.69	.1402
ileum (g)	-	13	17	.4756	.05	.4269
total (g)	-	425	489	.2812	1.81	.0053
digesta pH:						
stomach	-	4.15	4.09	.9393	.00	.6865
duodenum	-	4.44	5.07	.3579	.00	.5443
jejunum	-	6.36	6.45	.5896	-.00	.6939
ileum	-	6.95	6.46	.4869	-.00	.5677

Table 4. Ratio precipitable protein to crude protein (pp/cp) and enzyme activities in the digestive tract (LSMeans). (P=pancreas tissue; J=jejunum digesta; tryp=trypsin activity, units per gram freeze dried material; chym=chymotrypsin activity, units per gram freeze dried material; tot=total activity in pancreas or jejunum).

	at weaning: day 0	after weaning: day 3			feed intake:	
protein source	–	SMP	SOY	p	b	p
ratio pp/cp:						
stomach	.67	.58	.99	.0025	-.00	.4367
jejunum	.49	.95	.85	.6158	-.00	.7725
enzyme activities:						
Ptryp (U/g)	404	612	452	.3178	.06	.9540
Pchym (U/g)	371	271	194	.4876	-.33	.6415
Jtryp (U/g)	55	243	194	.5474	-.71	.1744
Jchym (U/g)	51	92	60	.0998	-.19	.1279
Ptryptot (Units)	1028	1237	919	.3382	-.27	.8956
Pchymtot (Units)	943	561	385	.4601	-.85	.5679
Jtryptot (Units)	244	1521	1478	.9230	-.16	.9547
Jchymtot (Units)	192	502	419	.5091	.07	.9275
total trypsin (P+J)	1358	2758	2398	.4483	-.43	.8853
total chym. (P+J)	1246	1062	805	.2245	-.78	.5532
Ptryp/Pchym	1.17	2.74	3.18	.4838	-.00	.8509
Jtryp/Jchym	1.77	2.83	3.69	.3681	-.00	.7525

		day 6				
protein source		SMP	SOY	p	b	p
ratio pp/cp:						
stomach	–	.56	.86	.0011	– .00	.1815
jejunum	–	.46	.67	.0966	– .00	.8864
enzyme activities:						
Ptryp (U/g)	–	808	979	.4805	.29	.8548
Pchym (U/g)	–	236	208	.6207	– .02	.9534
Jtryp (U/g)	–	345	378	.4474	.90	.0051
Jchym (U/g)	–	94	73	.0291	.13	.0462
Ptryptot (Units)	–	2130	2382	.6883	4.74	.2664
Pchymtot (Units)	–	667	499	.3392	1.06	.3660
Jtryptot (Units)	–	5018	3741	.0887	20.21	.0005
Jchymtot (Units)	–	1441	688	.0032	4.43	.0078
total trypsin (P+J)	–	7148	6123	.3413	24.95	.0023
total chym. (P+J)	–	2108	1187	.0106	5.49	.0201
Ptryp/Pchym	–	4.16	4.90	.5599	.00	.7337
Jtryp/Jchym	–	3.82	5.27	.0255	.00	.2915

Table 4. (continued)

	at weaning:	after weaning: day 10			feed intake:	
protein source	-	SMP	SOY	p	b	p
ratio pp/cp:						
stomach	-	.50	.91	.0047	-.00	.2516
jejunum	-	.69	.62	.7944	.00	.1042
enzyme activities:						
Ptryp (U/g)	-	1247	1225	.9145	-1.71	.4164
Pchym (U/g)	-	308	234	.1785	.06	.9106
Jtryp (U/g)	-	399	424	.5859	.14	.7607
Jchym (U/g)	-	90	59	.0246	.06	.6567
Ptryptot (Units)	-	3664	3280	.5658	4.13	.5332
Pchymtot (Units)	-	926	633	.0985	2.39	.1683
Jtryptot (Units)	-	4998	6040	.3841	21.72	.0780
Jchymtot (Units)	-	1154	846	.2435	3.56	.1773
total trypsin (P+J)	-	8661	9320	.6019	25.86	.0509
total chym. (P+J)	-	2080	1479	.0723	5.95	.0727
Ptryp/Pchym	-	4.37	5.69	.1428	-.00	.6346
Jtryp/Jchym	-	4.55	7.72	.0002	.00	.6800

Discussion

Feed intake, average daily gain, pancreatic weight and digesta pH

The high variation in feed intake as noticed in the present experiment is a phenomenon often observed in practical piglet feeding and also known from literature (Armstrong and Clawson, 1980 and Cranwell and Moughan, 1989). The higher average daily gain on the SMP diet, also, is in accordance with findings from literature (Walker *et al.*, 1986).

The relative weight of the pancreas increased especially between day 3 and day 6. This positive allometry is in accordance with data from Owsley *et al.* (1986b) and Lindemann *et al.* (1986).

The pH of digesta increased along the digestive tract due to the addition of more alkaline substances to the digesta (pancreatic juice, bile, gut wall secretions). After weaning, the pH in the stomach was high (3.8-4.9) compared to the optimal pH for pepsin action (pH 2-4, Beynon and Bond, 1989), while pH in the small intestine was low (4.4-7.3) compared to the optimum pH for trypsin and chymotrypsin action (pH 7.8-8.1, Beynon and Bond, 1989). However, the pH was measured in the total, mixed chyme of each segment, so it is possible that at some sites in the stomach (e.g. close to the gastric wall or at the pyloric site, as reported by Kamphues (1987)) pH was lower than the values reported in Table 3.

The pH values agree with the data of Newport and Keal (1982), Schnabel *et al.* (1982) and Wilson and Leibholz (1981b).

Protein breakdown in stomach and jejunum

The TCA-precipitable protein fraction in digesta can be regarded as not directly absorbable (Souffrant, 1991), since it consists of the larger nitrogen containing molecules such as non-digested feed protein and enzymes. The ratio between TCA-precipitable protein and crude protein (pp/cp ratio) is an indication for the degree of dietary protein breakdown, especially in the stomach. In the stomach a relatively small proportion of the N compounds consists of secreted enzymes and mucus.

A clear difference was found in gastric precipitable protein /crude protein ratio (pp/cp) between SMP fed and SOY fed piglets, indicating a difference in degree of protein breakdown between the two diets. This difference probably cannot be attributed to antinutritional factors or antigens, since levels of these are very low in the soy protein concentrate used in this study (Table 1). It may be hypothesized that the different gastric pp/cp ratio may have consequencies for further breakdown and thus absorption of hydrolyzed protein in various parts of the gastrointestinal tract. It appears that the stomach could play an important role in the difference in protein digestibility between soy and milk protein. The clotting capacity of milk proteins may play a major role in this respect. As a result, the emptying of the stomach might also differ between the two protein sources as was found by Zebrowska *et al.* (1983) and by Laplace *et al.* (1984). Unfortunately, gastric emptying could not be measured in this experiment. The pp/cp ratio could be a useful parameter in the study of the digestion (breakdown) of protein in the stomach where absorption is probably negligible or zero (Bayley, 1978). Feed intake had no effect on the pp/cp ratio in the stomach. The amount of feed consumed at the last meal before slaughter might have had an influence on the degree of protein breakdown. The amount of gastric digesta at slaughter (as an indicator of the last meal size) was affected by feed intake at day 10 (Table 3).

At the jejunal level, no effect of protein source on pp/cp ratio was found. This is in agreement with results from Newport and Keal (1982), who weaned piglets at 2 days of age onto liquid diets containing either skim-milk powder, soybean isolate or soybean concentrate.

Enzyme activities in pancreas and jejunum

Although trypsin and chymotrypsin activities in pancreatic tissue and jejunal digesta (Units per gram freeze dried material) were variable between piglets, a systematic increase in

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trypsin activity in pancreas and jejunum after weaning was found. In contrast with data from Lindemann *et al.* (1986) and Owsley *et al.* (1986b), no post-weaning decline in trypsin activity was found, which could be due to differences in breed or age of piglets or feed intake. At day 6, a significant positive effect of feed intake on jejunal protease activities (per gram freeze dried digesta and total units) was found. Therefore, it can probably be concluded that the decline in enzyme activities found by Lindemann *et al.* (1986) and Owsley *et al.* (1986b) is related to low feed intake after weaning rather than to weaning itself. Chymotrypsin activity per gram freeze dried pancreatic tissue did diminish after weaning, whereas chymotrypsin activity per gram freeze dried jejunal chyme increased. Total chymotrypsin activity in the pancreas and in the jejunum showed the same development after weaning. From these data it appears that chymotrypsin synthesis cannot keep up with chymotrypsin secretion or that the rate of hydrolysis of chymotrypsin in the digestive tract changes after weaning.

No effect of dietary protein source on trypsin or chymotrypsin in pancreatic tissue was found. Newport and Keal (1982) did find higher trypsin and chymotrypsin activities in pancreatic tissues of soy protein concentrate fed piglets. These conflicting results are probably due to differences in weaning age (2 days vs 25 days) and post weaning diets (liquid vs dry diets, dietary crude protein content 24 vs 16%, other type of soy protein concentrate).

Variation in feed intake during the first three days after weaning was not related to enzyme activities at day 3. Most regression coefficients on feed intake were negative at this time, indicating limitations of the enzyme system during the early post-weaning period. This was also suggested by Lindemann *et al.* (1986).

At day 6, feed intake between weaning and sampling clearly had positively affected jejunal trypsin and chymotrypsin activities. The suggestion that post-weaning feed intake could be a major factor in pancreatic enzyme synthesis and secretion has previously been made by Owsley *et al.* (1986b). At day 10, the effect of feed intake on enzyme activities was still positive, but not significant anymore.

The fact that, at day 6, not only total protease activities in jejunal digesta but also protease activities per gram freeze dried jejunal digesta increased with increasing post-weaning feed intake suggests that, at this time, pancreatic protease synthesis and secretion is not yet fully adapted to the changed feed intake after weaning. This is also illustrated by the increase of jejunal trypsin activity (units per gram freezedried digesta) between day 0 and day 6. After day 6, this activity remains fairly constant at approximately 400 units per gram freeze dried digesta.

With SMP fed piglets, jejunal chymotrypsin activity was higher than with SOY fed piglets.

This effect has also been reported by Owsley *et al.* (1986b) and by Newport and Keal (1982).

The difference in jejunal trypsin/chymotrypsin ratio between SMP and SOY at day 6 and 10 indicates an effect of dietary protein source on specific protease (either trypsin or chymotrypsin) synthesis and secretion and/or a diet-dependent breakdown of pancreatic proteases in the gastrointestinal tract.

From the changes in trypsin/chymotrypsin ratio (t/c ratio) in pancreatic tissue and jejunal digesta it is concluded that trypsin and chymotrypsin synthesis, secretion and/or breakdown is regulated independently as previously been suggested by Efird *et al.* (1982) in pigs and by Snook and Meyer (1964) in rats. Independent regulation of synthesis/secretion might also be expected from the specificity of the two enzymes: trypsin preferably cleaves peptic bonds next to the alkaline amino acids arginine or lysine. Chymotrypsin is less specific and cleaves peptic bonds involving phenylalanine, tryptophane, tyrosine (aromatic amino acids) or leucine (Wood *et al.*, 1974).

Arginine, particularly is more abundant in the soy protein concentrate used in this experiment compared to the skim-milk powder (7.2 vs 3.0 g/100 g of amino acids, respectively). From this it is expected that the t/c ratio will be higher in piglets fed the SOY diet.

From this experiment it can be concluded that the stomach probably plays a major role in the digestion of milk protein. According to Pekas *et al.* (1964), soy protein digestion depends more on pancreatic digestion. However, SOY fed piglets did not show higher pancreatic enzyme activities in pancreatic tissue or jejunal chyme than SMP fed piglets. Therefore, it can be concluded either that SMP and SOY are equally well utilized by newly weaned piglets or that differences in protein digestibility between milk proteins and soya proteins are caused by differences in gastric protein digestion, absorption and/or by differences in endogenous nitrogen secretion. The latter has been found to be higher with soy proteins compared to milk proteins (Makkink *et al.*, unpublished results). The pancreas is probably not the major source of this increase in endogenous nitrogen, since the activity of two major nitrogen containing components (trypsin and chymotrypsin) did not differ between the two protein sources. The first three days after weaning comprise a difficult period for the young pig: during this time, pancreatic enzyme activities in pancreatic tissue and jejunal digesta do not respond to dietary protein source or feed intake.

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Implications

Early weaned piglets encounter several problems during the process of adaptation to post weaning solid feeds. Major digestive processes such as feed intake patterns, enzyme synthesis and secretion, motility and absorption need to be modified to meet the changing demands imposed on the piglet's digestive tract. It is unlikely, therefore, that only one small part in this intricate system could be the sole source of postweaning problems in the pig. Especially the often low and irregular feed intake of newly weaned piglets may hamper the development and adaptation of the digestive capacity in these animals. Improving the piglet's feed intake (total amount and frequency of meals) during the early post-weaning period could stimulate the development and adaptation of the digestive system. However, the first three post-weaning days will probably remain strenuous for the early weaned piglet.

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EFFECT OF DIETARY PROTEIN SOURCE ON FEED INTAKE, GROWTH, PANCREATIC ENZYME ACTIVITIES AND JEJUNAL MORPHOLOGY IN NEWLY WEANED PIGLETS.

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Summary

Seventy piglets with no access to creep feed were weaned at 28 days of age and fed one of four diets based on either skim milk powder (SMP), soya protein concentrate (SPC), soyabean meal (SBM) or fish meal (FM). At 0, 3, 6 and 10 days after weaning, piglets were euthanized and the pancreas and digesta from stomach and small intestine were collected, freeze-dried and analyzed for dry matter (DM) nitrogen (N) and trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) activity. Small intestinal tissue samples were taken to judge gut wall morphology. Results indicated that dietary protein source affected post-weaning feed intake, pancreatic weight, gastric pH and gastric protein breakdown, and pancreatic and jejunal trypsin and chymotrypsin activity. Post-weaning feed intake appeared to be an important factor in digestive development of newly weaned piglets.

Introduction

In pigs, digestive disorders are frequently encountered in the early post-weaning period. The adaptation of the gastrointestinal tract with regard to motility, secretion, digestion and absorption is often impaired, resulting in poor feed intake, growth (Okai *et al.*, 1976), and health status. These problems are at least partly related to the transition from sows milk to solid post-weaning diets containing different protein and energy sources.

After birth, the protein digestive capacity of young piglets is adapted to the digestion of milk proteins. Proteins of plant origin are digested to a lesser extent than milk proteins resulting in poor performance when fed to newly weaned piglets (Wilson & Leibholz, 1981a, 1981c).

This experiment was designed to study the development of pancreatic enzymes of newly weaned piglets in relation to dietary protein source and post-weaning feed intake.

Materials and Methods

Seventy piglets with no access to creep feed during the suckling period were weaned at 28 days of age. Ten piglets were anaesthetized immediately after weaning (day 0), weighed and exsanguinated from the jugular veins and artery. The gastrointestinal

tract was divided in four segments: stomach, duodenum (= first 2 meters from pylorus), ileum (=last 2 meters of the small intestine) and jejunum (=remaining part of the small intestine). Digesta were collected quantitatively from stomach and small intestinal segments. In fresh gastric digesta trichloro-acetic acid (TCA) precipitable protein was measured according to Ternouth *et al.* (1974). Digesta samples were weighed, pH measured and samples were frozen and stored at -20 °C until freeze-drying. The pancreas was excised, freed from adhering tissues, frozen and stored at -20 °C until freeze-drying. After freeze-drying, stomach and jejunum digesta samples were ground (1 mm) and analyzed for dry matter and crude protein content.

The TCA-precipitable protein fraction of the digesta comprises proteins and large peptides which cannot be absorbed by the intestinal wall without further hydrolysis (Souffrant, 1991).

The ratio between TCA-precipitable protein (pp) and crude protein (cp) in gastric digesta was therefore calculated to determine the degree of gastric protein breakdown.

Trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) activities were measured in jejunal digesta and in pancreatic tissue after freeze-drying (Bergmeyer, 1974, Van Baak *et al.*, 1991).

The remaining sixty piglets were fed one of four experimental diets based on either skimmilk powder (SMP), soya protein concentrate (SPC), soyabean meal (SBM) or fish meal (FM). Diet compositions are given in Table 1. At 3, 6 and 10 days after weaning, 5 piglets per diet were anaesthetized one hour after feeding 100 grams of the diet. Samples were collected, processed and analyzed as described above (20 piglets per day). At 6 days after weaning, duplicate tissue samples (approximately 0.5cm x 0.5cm) were taken from the proximal and distal jejunum to assess villus lengths and crypt depths. These tissue samples were stored in 3 ml cryotubes (Sanbio BV Biological Products, P.O. Box 540, NL-5400 AM, Uden), frozen immediately in liquid nitrogen and kept at -70 °C until further analysis. Sections were cut using a cryostat (2800 Frigocut N, Reichert-Jung), stained with toluedine-blue and the length of ten villi and the depth of ten crypts was measured in each sample.

Analysis of variance was performed using the GLM-procedure of SAS (SAS, 1990) for each day of sampling separately using the following model:

$$Y_{ij} = \mu + D_i + b_1{}^*FI + e_{ij}$$

in which Y_{ij} = dependent variable μ = overall mean, D_i = effect of diet (i = 1, 2, 3, 4), $b_1{}^*FI$ = effect of feed intake (overall co-variable) and e_{ij} = error term.

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Chapter VI

Table 1.　　Composition of experimental diets

diet:	SMP	SPC	SBM	FM
ingredient (%)				
skimmilk powder[1]	47.00	-	-	-
soya protein concentrate[2]	-	25.40	-	-
soyabean meal[3]	-	-	34.40	-
fish meal[4]	-	-	-	21.30
maize/wheat-starch	29.50	28.90	20.78	34.17
dextrose	13.34	10.96	10.96	10.96
lactose	-	22.50	22.50	22.50
soya oil	2.00	2.20	2.75	2.00
cellulose	5.00	4.10	2.85	5.00
ground limestone	0.75	1.45	1.45	0.75
mono Ca phosphate	0.50	2.25	2.10	0.25
NaCl	0.30	0.30	0.30	0.30
$KHCO_3$	0.30	0.20	-	1.10
$NaHCO_3$	0.10	0.20	0.40	0.40
L-lysine HCl	-	0.14	0.16	-
DL-methionine	0.11	0.22	0.21	0.10
L-threonine	-	0.08	0.04	0.07
vitamin and mineral premix	1.00	1.00	1.00	1.00
Cr_2O_3	0.10	0.10	0.10	0.10
calculated contents (%)				
dry matter	93.80	93.95	93.30	93.88
crude protein (N*6.25)	16.21	16.44	16.38	16.22
ether extract	2.51	2.44	3.18	3.54
crude fiber	4.95	4.95	5.02	4.95
inorganic matter	5.46	5.79	5.99	5.05
net energy (MJ/kg)	10.20	10.25	10.21	10.49
analyzed contents (%)				
dry matter	91.21	92.41	92.70	92.53
crude protein (N*6.25)	16.39	16.97	14.55	14.84
buffering capacity[5]	7.2	6.4	6.4	7.7
pellet hardness[6]	14.25	18.00	3.88	4.38

[1] Crude protein 35.1 %. Trypsin Inhibitor Activity <0.5 mg inhibited trypsin per gram product. Antigens 2 titre log2. Protein Dispersability Index 0.93.
[2] Soycomil P, Loders Croklaan BV, P.O. Box 4, 1520 AA Wormerveer, The Netherlands. Crude protein 63.9 %. Trypsin Inhibitor Activity 1.3 mg inhibited trypsin per gram product. Antigens less than 1 titre log2. Protein Dispersability Index 0.04.
[3] Crude protein 39.2 %. Trypsin Inhibitor Activity 1.6 mg inhibited trypsin per gram product. Antigens 5 titre log2. Protein Dispersability Index 0.12.
[4] Crude protein 69.2 %. Trypsin Inhibitor Activity 1.1 mg inhibited trypsin per gram product. Antigens 2 titre log2. Protein Dispersability Index 0.10.
[5] ml 1 N HCl needed to reach pH 4.00 in a suspension of 20 g feed in 100 ml demineralized water.
[6] Determined by Kahl Pellet Tester (Amandus Kahl Nachf. Maschinenfabrik, 2057 Reinbek, Hamburg). Pellet hardness expressed in kgf.

Initially, the effect of feed intake within diet was also tested, but this was found to be not significant for all variables except for chymotrypsin activity three days after weaning and was therefore eliminated from the model.

Since the overall effect of feed intake on chymotrypsin activity on day 3 was not significant, the effects of diet and of feed intake within diet on chymotrypsin activities at day 3 were analyzed according to the following model:

$$Y_{ij} \quad = \quad \mu + D_i + b_{1i}*FI + e_{ij}$$

in which Y_{ij} = dependent variable (chymotrypsin activity at day 3), μ = overall mean, D_i = effect of diet (i = 1, 2, 3, 4), $b_{1i}*FI$ = effect of feed intake within diet and e_{ij} = error term.

To evaluate the development of gastrointestinal tissue weights after weaning the following model was used:

$$Y_{ij} \quad = \quad \mu + W_i + e_{ij}$$

in which Y_{ij} = dependent variable (gastric or intestinal tissue weight), W_i = day after weaning (i = 0, 3, 6, 10) and e_{ij} = error term.

Results

Feed intake and growth

At weaning piglets were 28 days of age and had a mean live weight of 7.1 kg (SD=1.3, n=70).

Feed intake during the first three days post-weaning was affected by dietary protein source (Table 2). Piglets on SBM and FM consumed more feed than piglets on the SMP diet. Ten piglets out of 60 (5 on SMP, 3 on SPC and 2 on SBM) consumed less than 50 grams of feed during the first three days after weaning. From three days after weaning, feed intake was similar for all diets. Average daily gain was not affected by dietary protein source (Table 2). During the first three days after weaning, growth was very variable between piglets and feed intake strongly affected average daily gain. From three days after weaning, no effect of feed intake on growth was found (Table 2).

Tissue weights

Dietary protein source did not influence the weight of the stomach and the small intestine (absolute and relative to live weight) after weaning (Table 3a). The relative weight of the small intestinal tissue decreased during three days after weaning (Figure 1). The relative weight of gastric tissue increased gradually after weaning (Figure 1).
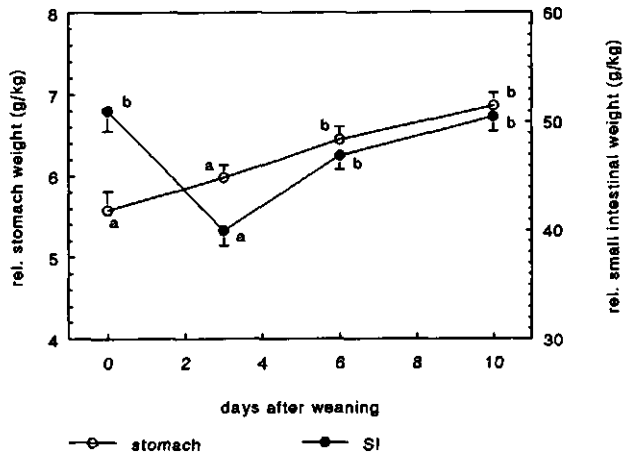
Figure 1.   Development of relative gastrointestinal tissue weights (gram per kg live weight) after weaning (Least Square Means + Standard Error of LSM). Data points within a tissue carrying different letters are significantly different (p<0.05).

Pancreatic weight was clearly affected by dietary protein source. At day 3, the weight of the pancreas (g per kg live weight) was higher for piglets fed SPC and FM than for piglets fed SBM. At day 6, the pancreatic weight (g) was high for piglets fed SPC and SMP and low for piglets fed FM. At day 10, the relative weight of the pancreas was high for piglets fed SPC and low for piglets on the FM diet (Table 3a). At days 6 and 10 post-weaning, small intestinal tissue weight was positively affected by preceding feed intake. At day 6 the same was found for the weight of the pancreas (absolute and relative).

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Table 2. Effect of diet on average daily feed intake (FI) (Least Square Means) and growth (ADG) (LSMeans (StdErr of LSM)). b = linear regression coefficient. p = probability. LSMeans within a column carrying different superscripts are significantly different (p<0.05)

FI (g/d) number of piglets	day 0 - 3 60	day 3 - 6 40	day 6 - 10 20
SMP	56[a]	286	354
SPC	80[ab]	318	406
SBM	95[b]	314	376
FM	112[b]	339	453
StdErr	14	23	39

ADG (g/d) number of piglets	day 0 - 3 60	day 3 - 6 40	day 6 - 10 20
SMP	26.0 (27.6)	224.3 (45.0)	268.2 (45.7)
SPC	- 6.3 (26.5)	292.8 (44.3)	256.7 (44.6)
SBM	- 0.4 (26.6)	215.4 (44.3)	172.5 (44.2)
FM	25.7 (27.3)	273.0 (47.5)	217.4 (52.5)
effect of feed intake:			
linear regression coefficient	2.56	0.27	0.43
p (reg.coeff. = 0)	0.0001	0.3962	0.1598

Digesta pH

At days 3 and 6 after weaning, digesta pH was not affected by dietary protein source. At day 3, jejunum pH was positively related to feed intake (Table 3b). At day 10, piglets fed the soya diets had lower gastric pH than piglets fed the FM diet.

Gastric pp/cp ratio

The pp/cp ratio in the stomach digesta was lower for piglets fed the FM diet than for piglets on SPC, indicating a higher degree of gastric protein breakdown in gastric digesta present at the time of slaughter with piglets fed the FM diet (Table 3b).

Gut wall morphology

All piglets killed at day 6 post-weaning had abnormally shaped villi (tongue-shaped, leaf-shaped, Kik *et al.*, 1990).
Jejunal villus lengths (proximal and distal) and distal crypt depths at day 6 after weaning were positively related to feed intake (Table 3b).

Enzyme activities

Enzyme activities are presented in Table 4a (trypsin) and in Table 4b (chymotrypsin). Four out of 20 piglets sampled at day 3 post-weaning had consumed less than 10 grams of feed daily and these piglets were analyzed separately because these piglets ('non-eaters') had higher enzyme activities in pancreatic tissue and higher chymotrypsin activities per gram jejunal digesta than piglets that did consume appreciable amounts of feed after weaning ('eaters') (Table 4a and 4b). The ratio between trypsin and chymotrypsin activity in pancreas and jejunum was higher for 'eaters' than for 'non-eaters' (Table 4a).

At day 3, trypsin activity in the jejunum was affected by protein source (Table 4a). The highest trypsin activity was found with piglets fed SMP and the lowest activity was noticed in piglets fed SBM and FM.

A significant effect of feed intake within diet on chymotrypsin activities at day 3 post-weaning was found (Table 4b). Chymotrypsin activity in pancreatic tissue was positively related to feed intake only for piglets on the FM diet. Pancreatic chymotrypsin activity and total chymotrypsin activity (pancreas + jejunum) were higher for the FM fed piglets than for the soya fed piglets. Jejunal chymotrypsin activity was higher for piglets fed SMP than for piglets fed FM.

At day 6, enzyme activities in pancreatic tissue and jejunal chyme were lowest for piglets fed the FM diet compared to the other diets. Total enzyme activities (pancreas + jejunum) were highest for the SPC fed piglets. Trypsin activity per gram freeze-dried jejunal digesta was lower in piglets fed FM than in piglets fed SMP (Table 4a). Chymotrypsin activity per gram freeze-dried jejunal chyme was lower in piglets fed FM and SPC than in piglets fed SMP. Total enzyme activities in the pancreas were highest for SPC fed piglets and lowest for FM fed piglets (Table 4a and 4b). Total trypsin + chymotrypsin activities in pancreas + jejunum at day 6 were positively related to post-weaning feed intake. This was mainly due to the strong relation between feed intake and pancreatic enzyme activities (total and per gram pancreatic tissue).

Table 3a.   Effect of diet and feed intake on organ weights (g and g/kg LW) and digesta weights (g) of newly weaned piglets (LSMeans (StdErr), b=coefficient of regression)

day 0:	stomach:	small intestine:	pancreas
tissue weight (g)	38.2 (3.1)	345.5 (21.4)	10.37 (0.84)
tissue weight (g/kg)	5.6 (0.2)	51.0 (1.9)	1.53 (0.08)
digesta weight (g)	113.9 (26.6)	124.8 (28.9)	–

	diet:					feed intake:	
	SMP	SPC	SBM	FM	p	b	p
day 3:							
organ weights:							
stomach (g)	42.7	41.8	43.3	43.2	0.9938	0.023	0.6522
stomach (g/kg)	6.2	5.9	5.5	6.3	0.2393	-0.001	0.6845
small intestine (g)	301	264	313	280	0.5600	0.336	0.3001
" (g/kg)	39.7	38.4	39.9	41.8	0.8598	0.003	0.9252
pancreas (g)	11.39	11.46	10.02	10.73	0.6080	-0.006	0.5970
" (g/kg LW)	1.53[ab]	1.66[b]	1.27[a]	1.59[b]	0.0194	-0.003	0.0119
digesta weights:							
stomach (g)	177.1	136.6	130.2	129.9	0.6722	1.354	0.0017
small intestine (g)	95	71	124	79	0.2260	0.797	0.0033
day 6:							
organ weights:							
stomach (g)	54.5	54.4	48.7	47.2	0.3203	0.057	0.0628
stomach (g/kg)	6.6	6.2	6.7	6.3	0.5402	-0.003	0.2918
small intestine (g)	370	405	362	355	0.4816	0.657	0.0050
" (g/kg)	44.7	46.1	49.8	47.0	0.4835	0.009	0.6534
pancreas (g)	13.47[bc]	15.41[c]	11.68[ab]	10.91[a]	0.0026	0.038	0.0001
" (g/kg LW)	1.63	1.76	1.64	1.41	0.1954	0.002	0.0189
digesta weights:							
stomach (g)	125.0	204.7	202.1	191.8	0.2869	-0.127	0.6490
small intestine (g)	158	258	214	168	0.2398	-0.049	0.8774
day 10:							
organ weights:							
stomach (g)	62.9	57.3	64.7	60.3	0.8318	0.073	0.2154
stomach (g/kg)	6.7	6.5	7.4	7.0	0.3218	-0.002	0.6047
small intestine (g)	481	438	459	421	0.6692	0.924	0.0062
" (g/kg)	51.6	49.9	51.7	48.6	0.7757	0.037	0.0873
pancreas (g)	15.62	17.59	14.52	13.17	0.1389	0.019	0.1386
" (g/kg LW)	1.67[abc]	1.98[c]	1.65[ab]	1.54[a]	0.0439	-0.000	0.8674
digesta weights:							
stomach (g)	198.7	166.4	273.5	202.0	0.2966	0.737	0.0721
small intestine (g)	208	185	188	130	0.6318	-0.169	0.6529

Table 3b. Effect of diet and feed intake on digesta pH, ratio precipitable protein / crude protein in the stomach, and gut wall morphology of newly weaned piglets (LSMeans (StdErr), b=coefficient of regression)

day 0:		small intestine:		
	stomach:	duodenum	jejunum	ileum
pH	3.80 (0.46)	5.82(0.18)	6.85(0.15)	7.47(0.12)
pp/cp stomach	0.64 (0.04)	-	-	-

	diet:					feed intake:	
	SMP	SPC	SBM	FM	p	b	p
day 3:							
digesta pH:							
stomach	6.38	4.99	4.64	6.40	0.1821	0.012	0.1722
duodenum	6.49	6.10	5.90	6.50	0.1516	-0.000	0.9484
jejunum	7.10	7.57	6.78	7.28	0.0743	-0.007	0.0098
ileum	7.37	7.83	7.65	7.78	0.6212	-0.003	0.2388
pp/cp stomach	0.19	0.28	0.18	0.19	0.5874	-0.000	0.2328
day 6:							
digesta pH:							
stomach	4.29	5.18	5.12	6.11	0.2550	-0.001	0.8299
duodenum	5.98	5.76	5.94	6.22	0.8252	-0.002	0.5083
jejunum	7.20	7.20	7.36	7.30	0.8835	-0.000	0.9626
ileum	7.71	7.52	7.84	7.87	0.3606	0.001	0.4907
pp/cp stomach	0.17	0.35	0.25	0.29	0.1825	0.000	0.5441
villus length (μm):							
proximal	314	301	286	296	0.7462	0.400	0.0172
distal	300	280	300	269	0.5551	0.436	0.0131
crypt depth (μm):							
proximal	132	139	129	137	0.5149	0.043	0.3536
distal	134	130	144	132	0.6271	0.148	0.0431
day 10:							
digesta pH:							
stomach	5.36[ab]	3.89[a]	4.94[a]	6.63[b]	0.0167	0.004	0.4883
duodenum	5.94	5.91	6.08	6.37	0.6338	-0.001	0.5715
jejunum	7.48	7.75	7.70	7.02	0.1031	-0.000	0.8342
ileum	8.10	7.96	7.89	8.12	0.7991	-0.000	0.8005
pp/cp stomach	0.40[ab]	0.57[b]	0.42[ab]	0.27[a]	0.0266	0.000	0.3648

Table 4a.   Effect of feed intake on trypsin activities in digesta and pancreas of newly weaned piglets (b=coefficient of regression)

day 0:				LSM	StdErr
Ptryp (trypsin activity per gram freeze-dried pancreatic tissue)				923	136
Jtryp (trypsin activity per gram freeze-dried jejunal digesta)				180	41
Ptryptot (total trypsin activity in pancreas)				2193	483
Jtryptot (total trypsin activity in jejunum)				979	272
tottryp (total trypsin activity in pancreas + jejunum)				3172	670
Pt/c (Ptryp/Pchym)				1.33	0.23
Jt/c (Jtryp/Jchym)				1.59	0.27

day 3:	'eaters'	'non-eaters'	p
Ptryp	1225	3029	0.0002
Jtryp	268	150	0.2980
Ptryptot	2963	9239	0.0001
Jtryptot	1547	370	0.0739
tottryp	4510	9608	0.0025
Pt/c	2.85	1.08	0.0192
Jt/c	4.02	0.84	0.0009

	diet:					feed intake:	
	SMP	SPC	SBM	FM	p	b	p
day 3:							
'eaters'							
Ptryp	2025	1068	904	1287	0.1572	12.64	0.0408
Jtryp	602^c	360^b	154^a	152^a	0.0014	0.57	0.6089
Ptryptot	5266	2348	1974	3325	0.1923	31.78	0.0973
Jtryptot	4149^b	1524^a	1011^a	954^a	0.0005	5.76	0.3710
tottryp	9415^b	3871^a	2985^a	4279^a	0.0153	37.54	0.0735
Pt/c	3.80	3.71	2.29	2.22	0.1952	0.01	0.2601
Jt/c	5.99	4.64	3.13	3.45	0.0666	0.01	0.3079
day 6:							
Ptryp	2526	2209	2041	1718	0.5375	8.45	0.0212
Jtryp	790^b	649^{ab}	534^{ab}	382^a	0.0389	1.47	0.0751
Ptryptot	7287^b	7803^b	5033^{ab}	3832^a	0.0477	40.40	0.0003
Jtryptot	4285	6269	4265	3784	0.1346	19.38	0.0077
tottryp	11571^{bc}	14072^c	9298^{ab}	7615^a	0.0010	59.79	0.0001
Pt/c	5.94	4.44	4.52	6.36	0.2082	0.00	0.4585
Jt/c	5.18	7.61	5.52	7.98	0.0536	0.01	0.4571
day 10:							
Ptryp	3407	2939	2669	4846	0.0985	14.20	0.0237
Jtryp	837	823	734	544	0.1261	-0.99	0.2283
Ptryptot	11327	11665	8912	14435	0.3896	60.10	0.0086
Jtryptot	8430	7787	7503	3746	0.3285	2.48	0.8810
tottryp	19757	19452	16415	18180	0.7094	62.58	0.0114
Pt/c	6.77	5.44	6.70	6.15	0.6862	0.01	0.4115
Jt/c	7.00	8.29	6.48	6.42	0.2180	-0.00	0.8065

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Table 4b. Effect of feed intake on chymotrypsin activities in digesta and pancreas of newly weaned piglets (b=coefficient of regression)

day 0:			LSM	StdErr
Pchym (chymotrypsin activity per gram freeze-dried pancreatic tissue)			796	134
Jchym (chymotrypsin activity per gram freeze-dried jejunal digesta)			105	12
Pchymtot (total chymotrypsin activity in pancreas)			1899	421
Jchymtot (total chymotrypsin activity in jejunum)			560	85
totchym (total chymotrypsin activity in pancreas + jejunum)			2459	478

day 3:	'eaters'	'non-eaters'	p
Pchym	467	2868	0.0001
Jchym	63	150	0.0008
Pchymtot	1138	8992	0.0001
Jchymtot	369	199	0.1520
totchym	1507	9191	0.0001

diet: effect of:	SMP feed intake within diet		SPC feed intake within diet		SBM feed intake within diet		FM feed intake within diet		diet
	b	p	b	p	b	p	b	p	p
day 3:									
'eaters'									
Pchym	-11.85	0.2422	-0.07	0.9777	-8.20	0.0742	10.94	0.0008	0.0148[1]
Jchym	1.98	0.2423	-0.48	0.2496	0.26	0.7037	0.15	0.6609	0.2751
Pchymtot	-55.26	0.1150	-3.24	0.6802	-22.62	0.1243	32.62	0.0015	0.0181[2]
Jchymtot	2.49	0.7572	-3.97	0.0746	9.15	0.0263	2.54	0.1613	0.0354[3]
totchym	-52.78	0.1571	-7.21	0.4069	-13.48	0.3693	35.16	0.0015	0.0216[4]

	diet: SMP	SPC	SBM	FM	p	feed intake: b	p
day 6:							
Pchym	432	532	442	269	0.1079	1.52	0.0229
Jchym	154[b]	95[a]	104[ab]	51[a]	0.0087	0.12	0.4580
Pchymtot	1230[ab]	1913[b]	1088[a]	574[a]	0.0085	7.84	0.0011
Jchymtot	810	850	846	508	0.3374	2.24	0.0990
totchym	2040[bc]	2763[c]	1934[b]	1082[a]	0.0028	10.08	0.0003
day 10:							
Pchym	536	556	399	793	0.0756	1.75	0.0644
Jchym	124	102	114	84	0.3237	-0.11	0.4038
Pchymtot	1803	2190	1285	2368	0.1755	8.25	0.0212
Jchymtot	1195	868	1159	580	0.1283	0.48	0.7719
totchym	2999	3058	2443	2947	0.5082	8.73	0.0127

[1] FM>SPC   [3] SMP>FM
[2] FM>SPC, FM>SBM   [4] FM>SPC, FM>SBM

Discussion

Feed intake and growth

The low feed intake of piglets on the SMP diet may be related to the physical form of the feed. Feeds containing large amounts of skimmilk powder are difficult to pellet and will therefore result in hard pellets which are not readily accepted by young piglets (Jensen, 1966, Liptrap & Hogberg, 1991). Pellets of the SMP diet were harder than pellets of the SBM and FM diet (Table 1) and feed intake on the SMP diet was lower during the first three post-weaning days. Feed intake on the SPC diet was intermediate although pellets of this diet were very resistant to crumbling.

Piglets fed the SMP diet consumed less feed during the first three post-weaning days than piglets fed the FM diet (Table 2) and at the same time had higher gastric digesta weights (Table 3a). This may indicate that gastric emptying was faster in piglets fed FM as was also found by Sève & Laplace (1975) with early weaned piglets fed solid diets containing milk and fish proteins. This could have resulted in a more regular feed intake for piglets fed the FM diet.

The differences in feed intake between diets did not result in differences in average daily gain during the first three post-weaning days. Piglets fed the diets based on animal proteins (SMP and FM) seemed to gain some liveweight during the first three days post-weaning allthoug the differences between diets were not significant. Average daily gain agreed with results of Bark *et al.* (1986), who studied feed intake during the first week post-weaning in piglets weaned at 21 days of age.

Tissue weights

The relative weight of the empty stomach increased gradually after weaning, while the relative weight of the small intestinal tissue decreased from weaning until three days after weaning. The same trends were found by Kelly *et al.* (1991) with piglets weaned at 14 days of age. The initial post-weaning decrease in relative small intestinal weight may be related to the decline in feed intake in these animals compared to intake during the suckling period. It could also indicate post-weaning gut wall damage, because it was found that all piglets at day 6 had abnormally shaped jejunal villi. The development of the gut wall (morphology, growth, secretion and absorption) seems to be disturbed in the early post-weaning period.

The (relative) pancreatic weight of SPC and SMP fed piglets increased from weaning until day 10. This is in accordance with the results of Kelly *et al.* (1991) with piglets weaned at 14 days of age and fed a diet containing skim-milk powder, fish meal and soyabean meal. Piglets on SBM and FM diets had the lowest relative pancreatic weight at day 3 and day 6, respectively. These differences are difficult to explain,

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especially because they are not related to the content of antinutritional factors in the diets.

Digesta pH

The increase in stomach pH after weaning is in accordance with data from Efird *et al.* (1982) and Wilson & Leibholz (1981b). This result can be explained by post-weaning feed intake pattern and/or the buffering capacity of the post-weaning diets. A high pH in the stomach could lead to bacterial proliferation (Banwart, 1981) and to disturbance of normal pepsin function (Kidder & Manners, 1978).

At day 10, piglets on the soya diets had lower pH in gastric contents than piglets on the animal protein sources. This could be explained by the buffering capacity of the diets, which was lower for the soya diets (Table 1).

Gastric pp/cp ratio

The changes in gastric pp/cp ratio after weaning indicate that the degree of gastric protein breakdown is higher for solid diets than for sows milk. This could be explained by the predominance of chymosin compared to pepsin activity in suckling piglets. Before weaning, coagulation of milk proteins (clot formation) is more important than protein hydrolysis (Cranwell & Moughan, 1989). Between day 3 and 10 an increase in gastric pp/cp ratio was found, indicating changes in gastric protein breakdown and/or changes in gastric emptying patterns.

At day 10, gastric pp/cp ratio was lower for the FM fed piglets than for the SPC fed piglets, indicating a higher degree of gastric protein breakdown with the FM diet.

It is clear that the extent of gastric protein hydrolysis depends on age (or time after weaning) and dietary composition (protein source). This was also found by Leibholz (1986) who reported that piglets aged 28 days had higher stomach pH and less gastric protein breakdown than older piglets.

Gut wall morphology

The gut wall damage as reflected by the abnormally shaped villi at 6 days post-weaning is a common finding in literature (Hampson, 1986, Deprez *et al.*, 1987, Cera *et al.*, 1988).

Villus lengths were comparable to results from Deprez *et al.* (1987) of piglets at day 6 after weaning onto a dry diet and from Hampson (1986) with piglets at day 5 after weaning and from Miller *et al.* (1986) with piglets at one week after weaning.

Crypt depths were slightly smaller than data reported by Hampson (1986), Miller *et al.* (1986), Deprez *et al.* (1987) and by Kelly *et al.* (1991).

The positive relation of villus lengths and (distal) crypt depths with feed intake as

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found in this experiment is not known from literature. This relation could indicate a stimulatory effect of feed intake on development of the gut wall.

It was proposed by Gall & Chung (1982) in rabbits and by Cera *et al.* (1988) in pigs that low feed intake during the early post-weaning period may be a contributing factor to reduced villus height. However, Kelly *et al.* (1991) also found reductions in villus height in piglets fed through gastric intubation to maintain continuous nutrient supply.

Enzyme activities

The development of trypsin and chymotrypsin activities in pancreas and jejunum after weaning strongly depended on dietary protein source and post-weaning feed intake. At day 3, 'non-eaters' apparently stored large amounts of trypsin and chymotrypsin in their pancreatic tissue without substantial secretion into the gut. Intestinal substrate availability seems to be involved in the stimulation of pancreatic trypsin and chymotrypsin secretion as was suggested by DiMagno *et al.* (1973), Niederau *et al.* (1986) and Valette *et al.* (1992).

This mechanism is stimulated through the digestive endproducts of intestinal protein digestion (Grendell & Rothman, 1981, Valette *et al.*, 1992). DiMagno *et al.* (1973) found that essential amino acids infused into the duodenum or jejunum of humans stimulated pancreatic enzyme secretion. They postulated, that the products of protein digestion after absorption inhibit pancreatic enzyme secretion through glucagon release. The study by Niederau *et al.* (1986) showed that arginine and lysine (the sites of tryptic cleavage) specifically caused the release of trypsinogen in pancreatic tissue homogenate whereas phenylalanine and tryptophan (sites of chymotryptic cleavage) caused release of trypsinogen and chymotrypsinogen.

Valette *et al.* (1992) found in experiments with pancreas-cannulated growing pigs fed diets based on either casein or rapeseed that dietary protein source influences pancreatic enzyme secretion.

Skimmilk powder appeared to be the strongest stimulant of trypsin synthesis and secretion during the first three days post-weaning. At day 6, high pancreatic and jejunal trypsin activities were found with the SPC fed piglets, while at day 10, the effect of dietary protein source on trypsin activities had disappeared. Feed intake was positively related to pancreatic trypsin activity and therefore might affect trypsin synthesis.

The interaction between dietary protein source and feed intake during the first three post-weaning days with respect to (especially pancreatic) chymotrypsin activities is striking. Only piglets on FM (= piglets consuming on average more than 100 grams feed per day during the first three post-weaning days) showed a positive relation

between feed intake and pancreatic chymotrypsin activity.

It is evident, that piglets on the FM diet had the highest feed intake during the first three days post-weaning. This could imply a more regular development of feed intake of newly weaned piglets on the FM diet. It could also imply a better development of the enzyme system compared to piglets on the other three diets. At day 6, lowest chymotrypsin activities were found with the FM diet and highest with the SMP and SPC diets. By day 10 the differences between diets had disappeared, while feed intake was still related to total pancreatic chymotrypsin activity.

The trypsin/chymotrypsin ratio in pancreas and jejunum was lower for 'non-eaters' than for 'eaters'. This is in accordance with the findings from Corring *et al.* (1978), Efird *et al.* (1982), Owsley *et al.* (1986) and Lindemann *et al.* (1986) who state that chymotrypsin is the predominant pancreatic protease during the suckling period, while trypsin increases specifically after weaning. Our results indicate that the shift from chymotrypsin to trypsin is related to post-weaning solid feed intake rather than to weaning itself.

Conclusions

From the results of this experiment it can be concluded that post-weaning feed intake is affected by type of post-weaning diet. Dietary protein source also influenced pancreatic tissue weight and trypsin and chymotrypsin activities in pancreatic tissue and jejunal chyme.

In piglets fed SPC or FM, low pancreatic tissue weight coincided with low jejunal enzyme activities.

Piglets fed the SMP diet had low feed intakes but higher gastric digesta weights compared to piglets fed the FM diet. This suggests a higher rate of gastric emptying when FM was fed. A faster gastric emptying combined with a higher gastric pH leads to a more regular supply of more alkaline digesta to the duodenum. From this it can be expected that the pancreas is less challenged to secrete bicarbonate into the gut lumen. This hypothesis is supported by the finding that pancreatic tissue weight was also lower for piglets fed the FM diet. The low enzyme activities in the jejunum of piglets fed FM could reflect a lower need for pancreatic enzymes when gastric emptying occurs more gradually. Dietary buffering capacity, gastric protein hydrolysis and gastric emptying seem to be important factors in the digestion of different protein sources by newly weaned piglets.

Higher feed intake was positively related to villus length at day 6 after weaning. This finding merits further study as villus atrophy is an important consequence of weaning and might account for considerable endogenous nitrogen losses in newly weaned piglets.

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From the results presented herein it can de derived that feed intake as well as dietary composition are important factors for the development of the digestive organs of newly weaned piglets. Dietary fish meal had a stimulatory effect on the development of post-weaning feed intake, probably related to modifications of gastric emptying patterns related to intestinal protein digestion through pancreatic secretions.

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A NOTE ON THE RESPONSE OF PANCREATIC JUICE SECRETION
TO DIFFERENT STIMULANTS
IN YOUNG PIGLETS

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Introduction

Pancreatic enzyme activities are often reported to decline after weaning (Lindemann *et al.*, 1986, Owsley *et al.*, 1986). It is suggested (Jones, 1986) that this decline may induces disturbed (protein) digestion in newly weaned piglets. This might account for the post-weaning lag often observed in pigs. The usually low feed intake after weaning could also be responsible for the low digestive enzyme activities.

The regulation of pancreatic enzyme secretion, especially during the adaptation phase just after weaning, is insufficiently clear yet. Pancreatic secretion is known to be stimulated by the presence of (hydrolyzed) dietary components in the small intestine. This stimulation is mediated through hormones and peptides produced by the intestinal wall (Solomon, 1987).

When pancreatic enzyme secretion is low, this could be due either to low responsiveness of the pancreas towards stimulatory hormones (secretin, CCK) or to diminished release of these hormones.

The purpose of this study was to investigate the response of pancreatic secretion in young piglets to exogenous stimulation by either intravenous hormone injection or an intraduodenal infusion of a hydrolyzed casein solution of pH 2.0.

A method was developed and two trials were performed to study these responses in anaesthetized piglets before and after weaning.

Materials and Methods

trial 1

Five piglets from one litter were weaned at 4½ weeks of age (no access to creep feed during the suckling period), housed individually and fed one of two experimental diets (Table 1) based on either skim milk powder (SMP) or soyabean meal (SBM). The piglets received the diets at a daily amount of 4% of their live weight, divided in two portions. At 8.00 am, 200 gram was fed and the rest at 4.30 pm. At 8½ weeks of age, the piglets were anaesthetized (O_2, N_2O, halothane) one hour after the morning feeding without premedication. The external jugular vein and the pancreatic duct were catheterized (catheter diameter 2 mm). After surgery, the piglets were kept under anaesthesia while collections of pancreatic juice were made. After two hours of 'basal secretion collection', an intravenous injection of secretin (Sekretolin, Hoechst, Frankfurt am Main, Germany) was given (0.1 clinical unit ($\approx$ 8 pmol) per kg live weight). The dose injected was within the range of doses used by Harada *et al.*

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(1988). After secretin injection, stimulated pancreatic juice secretion was collected for another half hour. Hereafter, the piglets were euthanized (T61, intravenously) and the pancreas was removed.

Table 1.   Composition of experimental diets

| | trial 1 | | trial 2 | |
	SMP	SBM	SMP	SBM
ingredient				
soybean meal (47% CP)	-	34.40	-	34.40
skimmed milk powder (35% CP)	45.50	-	47.00	-
maize starch	29.60	39.84	29.50	20.88
dextrose	15.00	15.00	13.44	10.96
lactose	-	-	-	22.50
sunflower/soy oil	2.00	2.00	2.00	2.75
cellulose	5.00	2.85	5.00	2.85
vit/min premix	1.00	1.00	1.00	1.00
calculated contents				
crude protein	16.08	16.37	16.21	16.38
crude fat	2.49	2.44	2.51	3.18
crude fibre	4.95	5.02	4.95	5.02
inorganic matter	5.33	5.88	5.46	5.99
net energy (kcal/kg)	2555	2477	2440	2443

trial 2
Eight piglets from one litter with no access to creep feed during the suckling period were used in this trial. At 23 days of age, two suckling piglets were anaesthetized and catheterized as described for trial 1. An additional catheter was placed in the duodenum. Pancreatic juice was collected for two hours. Hereafter, 25 ml of a hydrolysed casein (casein enzymatic hydrolysate, Sigma Chemical Co, St Louis, USA) solution (5 gram casein per 100 ml, pH 2.0) was infused into the duodenum and another two hours of pancreatic juice collection followed. Afterwards, the pancreas was removed and the piglets euthanized (T61, intravenously).
The remaining six piglets were weaned at 26 days of age, individually housed and fed one of two diets based on either skimmed milk powder (SMP) or soybean meal (SBM). At 32 days of age, the piglets (three on SMP and three on SBM) were anaesthetized, catheterized, sampled and euthanized as described above.

Trypsin and chymotrypsin activities were assessed in pancreatic juice and freeze dried pancreatic tissue according to Bergmeyer (1974). Trypsinogen and chymotrypsinogen were activated by enterokinase (Sigma Chemical Company, St. Louis, USA) before analysis.

Results

No health problems were encountered between weaning and sampling of the piglets.

<u>trial 1</u>
In trial 1, all catheterizations and collections of pancreatic juice were successful. Live weights, pancreatic weight and pancreatic juice volume secretion are presented in Table 2. No effect of diet could be detected for any of the parameters measured. The basal pancreatic juice secretion was 0.36 ml per hour and was not affected by diet. Pancreatic juice secretion increased more than 20-fold after stimulation with intravenous secretin.

Table 2.   Live weights, pancreatic weights and juice secretion. Trial 1.

age (days) diet number of piglets	56-60 SMP 2	SBM 3	$\sqrt{MSE}$
live weight (kg)	15.0	13.3	1.25
pancreas weight (g)	27.8	23.5	4.71
pancreas weight (g/kg LW)	· 1.85	1.76	0.33
basal secretion (ml/h)	0.36	0.36	0.19
stimulated secretion (ml/h)	8.40	10.0	2.87

<u>trial 2</u>
In trial 2, collections could not be made in one piglet (at day 23) due to surgical problems. Live weights, pancreatic weight and pancreatic juice secretion (volume and enzyme activities) are presented in Table 3.
No significant effects of diet or age could be demonstrated.
Intraduodenal infusion of an acid solution of hydrolyzed casein resulted in a trhree-to five-fold increase in pancreatic juice secretion (Table 3). Enzyme activities in

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pancreatic juice (units per ml) decreased upon stimulation with hydrolyzed casein of pH 2.0 (Table 3). Enzyme secretion (units per hour) was not significantly changed after stimulation.

Table 3.   Live weights, pancreatic weights, juice secretion and enzyme activities. Trial 2. Enzyme activities in pancreatic tissue are expressed as units per gram freeze-dried tissue.

	23	32		√MSE
age (days)	23	32		
diet	–	SMP	SBM	√MSE
number of piglets	2	3	3	
live weight (kg)	7.1	8.5	8.7	1.66
pancreas weight (g)	5.9	12.2	9.2	2.57
pancreas weight (g/kg LW)	0.82	1.47	1.07	0.36
basal secretion (ml/h)	0.25	0.23	0.24	0.17
trypsin act. (U/ml)	401	1237	1801	789
trypsin act. (U/h)	100	280	553	484
chymotrypsin act. (U/ml)	62	538	345	292
chymotrypsin act. (U/h)	15	128	87	102
stimulated secretion (ml/h)	0.85	1.21	0.77	0.61
trypsin act. (U/ml)	204	389	793	282
trypsin act. (U/h)	173	447	503	317
chymotrypsin act. (U/ml)	52	107	104	72
chymotrypsin act. (U/h)	44	118	57	61
trypsin act. (U/g pancreas)	1918	3200	3998	998
chymotrypsin act. (U/g pancreas)	1134	968	815	385

Discussion

The basal pancreatic juice secretion in these trials is approximately ten-fold lower than volumes found by Pierzynowski *et al.* (1988). After secretin stimulation pancreatic juice secretion was also lower than reported by Pierzynowski *et al.* (1988). These differences may be due to the fact that the dose of secretin used in our study was five times lower than the dose used by Pierzynowski *et al.* (1988). Another possible explanation lies in the fact that our piglets were anaesthetized which may have decreased the responsiveness of the pancreas.

Despite the relatively low dose of secretin, all piglets clearly responded to the

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injection with an increase in pancreatic juice secretion. From trial 1 it can be concluded that 8 weeks old piglets are capable of increasing the pancreatic secretion in response to intravenous secretin injection. From trial 2 it appears that even before weaning the pancreas responds to an intraduodenal load of hydrolysed casein of low pH. Upon duodenal infusion of the test-solution (trial 2), a more diluted pancreatic juice is secreted after weaning: the volume is increased three- to five-fold, while trypsin secretion (Units/ml) is decreased two- to three-fold and chymotrypsin secretion (Units/ml) is decreased three- to five-fold. Enzyme secretion in units per hour hardly changes after duodenal administration of a hydrolyzed casein solution of pH 2.0.

These trials show that young piglets are capable of changing their pancreatic secretion in response to stimulants. It appears that a decline in pancreatic secretion as found by Lindemann *et al.* (1986) and by Owsley *et al.* (1986) is not due to an inability of the pancreas to respond to stimulants.

Anaesthetized piglets fitted with a pancreatic duct catheter can provide a useful model to study the response of pancreatic secretion to various stimulants (intravenously, intragastric or intraduodenally).

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Chapter VIII

GENERAL DISCUSSION

Introduction

The main objective of the present investigations was to study the development of protein digestive capacity in young weaned piglets. Protein is the most expensive feed ingredient and especially young piglets have difficulties digesting non-milk proteins (Wilson and Leibholz, 1981). As pointed out in Chapter I, the digestion of non-milk proteins requires a physiological adaptation of the digestive functions of the newly weaned piglet. This adaptation may be more difficult when the dietary protein source is less similar to sow milk protein.

To investigate the development of pancreatic secretion after weaning it would have been useful to fit piglets with a pancreatic cannula. This kind of surgery had been applied in older swine, however, it did not prove succesful in young piglets. Therefore, this technique was abandoned and slaughter experiments were designed.

For practical and statistical reasons, the experiments described in this thesis have been performed with individually housed piglets. It was shown with fattening pigs (De Haer, 1992) that housing system affects the feed intake patterns and growth of the animals. In individual housing systems less competition will occur, but on the other hand the pigs may be less encouraged to start eating by observing their 'room-mates' eat (De Haer, 1992). The impact of these influencing factors on the performance of young piglets during the early post-weaning period is unknown. Individual housing probably aggravates post-weaning social stress in young piglets, thereby increasing activity and lowering piglet performance as compared to group housing.

In Chapter I protein digestion in piglets is described and it is concluded that differences in protein digestibility occur between protein sources. These differences are related to the age of the piglets. Young piglets have greater difficulties digesting plant proteins than older piglets. In other words, the differences in digestibility diminish with advancing age of the piglets (e.g., Wilson and Leibholz, 1981).

From this finding, several research questions and issues were formulated (Chapter I). Different aspects of protein digestive capacity of newly weaned piglets were investigated and described in this thesis. In this chapter these aspects will be considered and discussed in an integrated approach combining the results obtained from the experiments described in the previous chapters.

1.   Is the difference in apparent (ileal and faecal) nitrogen digestibility between vegetable protein and milk protein in young piglets caused by the different protein sources (dietary factors) or related to endogenous nitrogen losses (interaction between dietary and animal factors)?

Digestibility of protein is usually measured as 'apparent faecal' N (nitrogen) digestibility, calculated as follows:

$$Ndig = \frac{N_{feed} - N_{faeces}}{N_{feed}}$$

N_{feed} = N in feed (g/d)

N_{faeces} = N in faeces (g/d)

Thus, also 'apparent ileal' N digestibility can be measured. This is done by using cannulated pigs and replacing N_{faeces} by $N_{ileal\ chyme}$. Faeces or ileal chyme can be collected quantitatively (24 hours a day) to obtain the amount of N excreted per day. Another possibility is to include an indigestible marker in the feed (Cr_2O_3, Co-EDTA or others) which can be used to calculate daily N excretion when faeces or chyme is not collected quantitatively.

Zebrowska (1973), Just *et al.* (1981) and Darcy *et al.* (1983) showed that proteins digested by the microflora in the caecum or colon are of no benefit to the pig because they do not contribute to body protein synthesis. Nitrogen in the large intestine may be primarily absorbed as urea or utilized by intestinal bacteria for microbial protein synthesis. Dietary factors may affect the degree of urea formation and absorption and the synthesis of microbial protein.

Because of the confounding action of bacteria in the large intestine with regard to apparent nitrogen digestibility, it is recommended to determine nitrogen digestibility in pigs at the terminal ileum.

Nitrogen collected at the terminal ileum consists not only of exogenous (dietary) nitrogen but also of endogenous nitrogen originating from the pig such as digestive enzymes, mucus and sloughed off gut wall cells. Also bacteria are present at the terminal ileum that will utilize nitrogen from both endogenous and exogenous sources. These confounding aspects result in 'apparent' N digestibility as opposed to 'true' or 'real' N digestibility when corrections are made for the amount of endogenous N present in ileal chyme or faeces.

To unravel the causes of poor digestibility of plant proteins by young piglets a distinction should be made between apparent and true ileal N digestibility.

The ^{15}N-isotope dilution technique was used to determine endogenous N losses at the terminal ileum of piglets fed diets based on different protein sources (Chapter II). The

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protein sources used in this study, skim milk powder, soyabean meal, isolated soya protein and fish meal, were chosen for their practical application in piglet feeds and for their range in apparent nitrogen digestibility (Chapter I).

The prerequisites of the ^{15}N-isotope dilution technique have been discussed by Schulze *et al.* (1993). An important aspect of the ^{15}N-isotope dilution technique is the choice of an appropriate substance to serve as a reference pool for endogenous N secretion. This is necessary because it is extremely difficult and in some cases until now impossible to obtain direct samples of each precursor pool separately. From the study of Schulze *et al.* (1993) it appears that the TCA-soluble fraction of the blood plasma seems to be the most suitable reference pool for the determination of endogenous N losses.

From the results presented in Chapter II it was concluded that the differences in endogenous N losses between piglets fed the different protein sources were the main cause of the differences in apparent N digestibilities. Small differences in true ileal protein digestibility were found between isolated soya protein and the other protein sources, but all four protein sources were highly digestible (true ileal digestibility coefficients > 0.9). Similar results were obtained by Walker *et al.* (1986) using four week old piglets fed diets based on casein or various soya products and determining endogenous N losses by feeding a hydrolyzed casein diet.

Skim milk powder resulted in low endogenous N losses (less than 800 mg per day), similar to endogenous N losses when nitrogen free diets are fed to young piglets (Wilson and Leibholz, 1981).

The protein sources used in this study which are also applied in practical piglet feeding all had high true ileal nitrogen digestibilities. It may be expected that other protein sources have lower true nitrogen digestibilities as was shown by Huisman (1990) with young piglets fed common beans (*Phaseolus vulgaris*) as the sole source of dietary protein. The experiment described in Chapter II was performed with piglets of ± 7 weeks of age, weaned at 3½ weeks of age. In other words, these piglets were probably fully adapted to the experimental diets. It can be expected that the differences between diets are more pronounced when adaptation is not fully established as in the case of newly weaned piglets. More research is needed to study the development of apparent and true digestibility of various feed components during adaptational phases, e.g., during the early post-weaning period. This may, however, prove difficult because of the surgery and subsequent recovery periods needed to perform this type of experiment.

In the future we need to identify the contribution of the different sources of endogenous nitrogen recovered from the ileum when non-milk proteins are fed. This may improve and extend our knowledge on protein digestion in young piglets with regard to the site where interactions between dietary components and the gastro-intestinal tract occur. It may also provide tools to improve the digestion of non-milk proteins by newly weaned

pigs when the specific reactions of the animal in response to specific dietary factors is known in more detail.

From Chapter II it is clear that dietary protein source affects endogenous nitrogen losses at the terminal ileum of young piglets. Differences in apparent nitrogen digestibility were mainly caused by differences in endogenous nitrogen losses when diets based on skim milk powder, isolated soya protein, soyabean meal or fish meal were fed. The specific sources of endogenous nitrogen could not be distinguished in this experiment. The pancreas might be an important secretory gland in this respect because of its key role in protein digestion.

2.   *The pancreas is important for the secretion of proteolytic enzymes. Some aspects of pancreatic secretion in pigs and the effects of dietary factors on protein and enzyme secretion (obtained from literature)*

The pancreas is an important secretory gland for the digestion of protein. Many investigations have been reported in literature on the impact of pancreatic enzyme secretion on (protein) digestion in pigs (e.g. Pekas *et al.*, 1964; Corring, 1977; Zebrowska *et al.*, 1983; Hee, 1984).

Several authors studying the enzyme development of newly weaned pigs have found a decrease in pancreatic enzyme activities after weaning (Hartman *et al.*, 1961; Lindemann *et al.*, 1986; Owsley *et al.*, 1986). From these findings it has been suggested (Jones, 1986) that (pancreatic) digestive enzyme capacity might be a limiting factor in protein digestion by newly weaned piglets. A lack of pancreatic proteolytic enzymes may hamper intestinal protein digestion and may thus lead to increased amounts of nutrients entering the ileum and large intestine. This may enhance fermentation, proliferation of pathogens and probably digestive disorders (diarrhoea). This hypothesis deserves further study because a shortage of pancreatic enzymes could be compensated for by adding enzymes to the piglet diet.

Because of the importance of the pancreas both as a source of endogenous nitrogen and as a digestive gland producing proteolytic enzymes, it was decided to study the effect of diet on the development of pancreatic secretion in newly weaned piglets.

In Chapter III a review is made of literature on pancreatic secretion in pigs. It is clear from literature that pancreatic enzyme secretion depends on the composition of the diet (amount and type of protein, fat and carbohydrate), on age of the pig and on stress factors (weaning).

As stressed in Chapter III, the pancreatic enzyme activities in small intestinal contents as measured in the laboratory are usually manyfold higher than the theoretically required

activity for digestion of standard pig feeds (Corring, 1982; Zebrowska *et al.*, 1983). The fact that digestive enzyme levels adapt to dietary changes illustrates that the overproduction is only apparent, not true. This apparent overproduction is probably due to the suboptimal conditions in the gut lumen with regard to temperature, pH, substrate availability and presence of interfering substances (e.g. protease inhibitors) which may decrease the actual 'in vivo' enzyme activity compared to the enzyme activity measured 'in vitro' (under optimal conditions). In Figure 1 the effect of buffer pH on trypsin and chymotrypsin activities is illustrated. A one unit deviation from the optimum pH value leads to a considerable reduction in enzyme activity.

The causes of the apparent overproduction of digestive enzymes should be studied in more detail. It is clear that part of the apparent overproduction is needed to account for suboptimal digesta pH. The different types of substrates presented to the digestive enzymes 'in vivo' probably also require more enzyme activity than needed for the hydrolysis of specific synthetic substrates employed in 'in vitro' enzyme analyses. The synthetic substrates (TAME for trypsin analysis and BTEE for chymotrypsin analysis) are selected for their affinity for the respective enzymes.
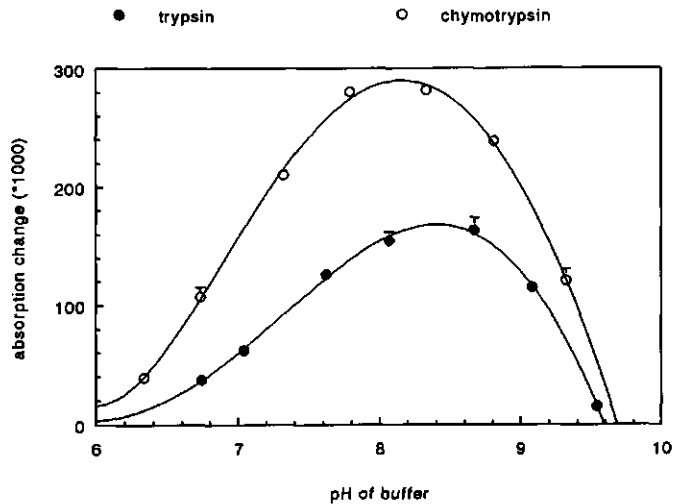

Figure 1.   Trypsin (Merck, 40 U/mg, porcine) and α-chymotrypsin (Merck, 350 U/mg, bovine) activity (ΔA) as affected by pH of the buffer solution (Makkink, 1993, unpublished results). Standard trypsin and chymotrypsin analyses are performed at pH 8.1 and 7.8, respectively.

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Not much is known about the kinetics of hydrolysis of pancreatic enzymes during the passage through the digestive tract. The timing of enzymatic digestion can also play an important role when considering the discrepancy between 'in vitro' measured enzyme activity in gut contents and apparently (calculated) required enzyme activity for digestion of ingested feed components. With regard to the timing of enzymatic hydrolysis various aspects of digestion are crucial, such as passage rate, mixing intensity, water content and pH of the chyme.

Furthermore, the literature search (Chapter III) revealed that no consistency exists with respect to methods of sample collection and analysis of pancreatic (proteolytic) enzymes. Enzyme activity depends on buffer composition, temperature, pH and substrate used. These analytical factors differ greatly among studies resulting in poor comparability of literature data.

An experiment was undertaken to study the effects of storage conditions (time and temperature) on pancreatic enzyme activities (Chapter IV). From the results of this and a follow-up experiment (Van Baak *et al.*, 1991) it was decided to freeze-dry samples for analysis of enzyme activities and store them at -20 °C before analysis to avoid reduction in enzyme activities. These measures, however, do not solve the problem of comparibility of results obtained by different methods of analysis. Absolute values for enzyme activities should be interpreted carefully but effects of specific treatments can be compared adequately between experiments.

From the literature review (Chapter III) it was derived that a decline in pancreatic enzyme activities often occurs after weaning. No detailed information is available on the relation between dietary protein source, post-weaning feed intake and proteolytic enzyme activities in young pigs. Therefore, two experiments were carried out to investigate these relationships.

3.   *Development of pancreatic trypsin and chymotrypsin activity after weaning in relation to dietary protein source*

In Chapter V and VI two experiments have been described dealing with the effect of dietary protein source on the development of protein digestive capacity in young piglets. In both experiments, pancreatic trypsin and chymotrypsin activities have been measured in both pancreatic tissue and small intestinal chyme. Pancreatic tissue samples were pretreated with enterokinase to activate the precursors of trypsin and chymotrypsin before measuring enzyme activities. Enzyme activities in tissue samples do not necessarily correlate with enzyme activities at the site of luminal digestion (Pekas *et al.*, 1964).

In the experiments described in Chapter V and VI positive correlations were found between pancreatic and jejunal trypsin and chymotrypsin activities (Table 1).

Table 1.   Correlations between pancreatic tissue enzyme activities and jejunal chyme enzyme activities (from experiments described in Chapter V and VI).

	Chapter V			Chapter VI		
	r	p	n	r	p	n
trypsin activity						
(units per gram)	0.487	0.0001	68	0.333	0.0052	69
trypsin activity						
(total units)	0.477	0.0001	67	0.307	0.0103	69
chymotrypsin activity						
(units per gram)	0.262	0.0306	68	0.273	0.0230	69
chymotrypsin activity						
(total units)	0.091	0.4660	67	-0.217	0.0738	69

From Table 1 it can be seen that correlations between pancreatic and jejunal activity were higher and more significant for trypsin than for chymotrypsin. This may indicate that the kinetics of intestinal (auto)hydrolysis of digestive enzymes are not the same for trypsin as for chymotrypsin. The kinetics of pancreatic enzymes in the gut lumen with respect to adsorption to digesta constituents, breakdown and loss of activity are not fully known.

The production (synthesis + secretion) of trypsin and chymotrypsin by the pancreas seems to be regulated independently of each other as was previously concluded by Snook and Meyer (1964) from experiments with rats and by Rothman (1976) from experiments with rabbits. It can be derived that this probably reflects the mode of pancreatic protease synthesis and secretion. The regulation of pancreatic secretion is mediated through the hydrolysis products of specific nutrients. Amino acids situated at specific cleavage sites are triggers for the release of the corresponding protease from isolated rat pancreatic tissue *in vitro* (Niederau *et al.*, 1986). It was shown by Niederau *et al.* (1986) that digestive end products directly act on rat pancreatic zymogen granules, causing selective release of specific digestive enzymes. Solomon (1987) summarized recent investigations on the effects of intra-intestinal amino acids, peptides and proteins on pancreatic secretion in different species. He concluded that intestinal (duodenum and jejunum) peptides and amino acids, but not intact proteins, stimulated pancreatic enzyme secretion. It is unclear which specific amino acids are the most potent stimulants of pancreatic secretion in different animal species.

Solomon (1987) suggested that the presence of pancreatic proteolytic enzymes in (the

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upper third of) the small intestine suppresses pancreatic enzyme secretion in rats, humans, cows, chickens, mice, hamsters and pigs, but not in dogs.

In the experiment described in Chapter V skim milk powder and soya protein concentrate were used as dietary protein sources. Another experiment (Chapter VI) comprised four diets based on skim milk powder, soya protein concentrate, soyabean meal and fish meal, respectively. The diets were balanced with respect to lactose since lactose has been reported to affect protein utilization in young piglets (Sewell and West, 1965).

In Chapters V and VI it was shown that dietary protein source during the early post-weaning period affects trypsin and chymotrypsin activities in pancreatic tissue and intestinal contents. This effect was most pronounced at day 6 after weaning (Chapter V) and at day 3 and 6 after weaning (Chapter VI). Dietary skim milk powder caused the highest jejunal enzyme activity in both experiments. In Chapter V this was the case for chymotrypsin while in Chapter VI this was more evident for trypsin activity than for chymotrypsin activity. These differences are difficult to explain because conflicting evidence arises from literature: Efird *et al.* (1982) found higher jejunal trypsin and chymotrypsin activities in piglets fed soya proteins compared to piglets fed milk proteins at one week after weaning (28 days of age), while Newport and Keal (1982) and Owsley *et al.* (1986) found lower jejunal chymotrypsin activity with piglets fed soya proteins than with piglets fed milk proteins.

Piglets fed fish meal had low trypsin activities in pancreatic tissue and jejunal digesta at day 3 and day 6 after weaning (Chapter VI). Chymotrypsin activities in pancreas and jejunum of piglets fed fish meal were high at day 3 and low at day 6 compared to the other protein sources (Chapter VI). Ternouth *et al.* (1975) found low amounts of trypsin and chymotrypsin secreted in calves fed diets based on fish protein concentrate compared to diets based on skim milk powder or soyabean flour.

Jenkins *et al.* (1980) measured the extent of hydrolysis of various proteins by different digestive enzymes separately *in vitro* and they found that skim milk is hydrolyzed to the largest extent by trypsin and chymotrypsin (68.1 and 51.8%, respectively). An isolated soyabean protein concentrate was hydrolyzed for 55.5 and 50.0% by trypsin and chymotrypsin, respectively. Fish protein concentrate was hydrolyzed for 46.2 and 21.2% by trypsin and chymotrypsin, respectively (Jenkins *et al.*, 1980). This would mean that to reach a fixed level of protein hydrolysis more trypsin and chymotrypsin is needed with fish and soya proteins than with skim milk protein. However, in our experiments (Chapters V and VI) we found lower jejunal enzyme activities with piglets fed soya proteins or fish meal (Table 2). From this we would expect a much higher protein digestibility when SMP was fed due to higher amounts of proteolytic enzymes present in the gut and due to higher susceptibility of milk proteins to hydrolysis by trypsin and

chymotrypsin. From Chapter II it can be seen that apparent ileal N digestibilities are indeed lower when non-milk proteins are fed (Table 2). These differences are, however, hardly reflected in true ileal N digestibility. The piglets used for the digestibility study (Chapter II) were 2 to 3 weeks older than the piglets used for protease measurements (Chapters V and VI). The differences in enzyme activities between newly weaned piglets fed different protein sources are not in agreement with the differences in (true) nitrogen digestibility found with older piglets (Chapter II). This may indicate that the effect of dietary protein source on jejunal protease activities diminishes or even reverses with increasing age of the piglets. This hypothesis is supported by the results from Chapter VI (Table 2) showing that at 3 days after weaning, the non-milk dietary proteins caused significantly lower trypsin activity in the jejunum while these differences had disappeared by day 6 after weaning.

From Chapters V and VI it is concluded that dietary protein source affects the activity of trypsin and chymotrypsin in pancreatic tissue and jejunal digesta. The differences in enzyme activities, however, cannot explain the differences in endogenous N losses as found in Chapter II.

It must be stressed here, that digestibility figures from the ^{15}N-isotope dilution experiment (Chapter II) were obtained with piglets of about 7 weeks old, who had been fed the experimental diets for approximately 3 weeks before sample collection. These piglets were therefore probably fully adapted to the diet composition and may be not directly comparable with the newly weaned piglets used in the slaughter experiments (Chapter V and VI). The differences between diets with respect to endogenous nitrogen losses as observed in the ^{15}N experiment (Chapter II) probably do not fully reflect the situation in newly weaned piglets.

During adaptation to a new diet, digestive responses (passage rate, pH, enzyme activities, gut wall morphology, apparent and true digestibilities) are probably more pronounced than in the case of fully adapted animals. In fully adapted piglets, the stressful event of weaning is vanquished and the digestive system has adjusted to the new diet.

If the pancreas would be an important source of endogenous nitrogen secretion, this should be reflected in the activity of the most important digestive enzymes secreted (trypsin and chymotrypsin). One would then expect to find lower trypsin and chymotrypsin activities in jejunal chyme of piglets fed diets based on skim-milk powder since lowest endogenous ileal nitrogen losses were observed when SMP was fed (Chapter II).

However, from Chapter V and VI it is concluded that feeding the SMP diet to newly weaned piglets resulted in higher intestinal activities of pancreatic enzymes. Therefore, it is concluded that another source of endogenous N secretion is probably responsible for the extra endogenous N losses when non-milk proteins are fed to young piglets.

Table 2.   Summary of results obtained from Chapter II (^{15}N experiment, 4 weeks after weaning) and Chapter VI (first 10 days after weaning). The results are expressed as a percentage of the values obtained with piglets fed skim milk powder (SMP=100%). SMP = skim milk powder, SI = soya protein isolate, SPC = soya protein concentrate, SBM = soyabean meal, FM = fish meal.

	SMP	SI	SPC	SBM	FM	remarks
Chapter II						
apparent ileal N digestibility	100	93	-	91	86	SMP>SBM,FM
true ileal N digestibility	100	106	-	98	96	SI>SMP
ileal endogenous N loss	100	251	-	181	198	SI>SMP
Chapter VI						
feed intake day 0-3	100	-	143	170	200	SBM,FM>SMP
feed intake day 3-6	100	-	111	110	119	NS
feed intake day 6-10	100	-	115	106	128	NS
relative pancreas weight at day 3	100	-	108	83	104	SPC,FM>SBM
relative pancreas weight at day 6	100	-	108	101	87	NS
relative pancreas weight at day 10	100	-	119	99	92	SPC>SBM,FM
gastric pH at day 3	100	-	78	73	100	NS
gastric pH at day 6	100	-	121	119	142	NS
gastric pH at day 10	100	-	73	92	124	FM>SPC,SBM
gastric pp/cp ratio day 3	100	-	147	95	100	NS
gastric pp/cp ratio day 6	100	-	206	147	171	NS
gastric pp/cp ratio day 10	100	-	143	105	68	SPC>FM
total jejunal trypsin activity day 3	100	-	37	24	23	SMP>rest
total jejunal chymotrypsin activity day 3	*	-	*	*	*	SMP>FM
total jejunal trypsin activity day 6	100	-	146	100	88	NS
total jejunal chymotrypsin activity day 6	100	-	105	104	63	NS
total jejunal trypsin activity day 10	100	-	92	89	44	NS
total jejunal chymotrypsin activity day 10	100	-	73	97	49	NS

*   calculation of percentages not possible due to interaction between protein source and feed intake.

4.   Development of pancreatic trypsin and chymotrypsin activity after weaning in relation to post-weaning feed intake

From the results of the experiments described in Chapters V and VI it also appears that feed intake is an important factor in the digestive development of newly weaned piglets. A positive relation was found between feed intake during the first six days post-weaning and trypsin and chymotrypsin activities in pancreatic tissue and jejunal digesta. Braude et al. (1970) reported a positive effect of increasing feed intake on jejunal proteolytic enzyme activity in young piglets fed cow's milk. Lindemann et al. (1986) and Owsley et al. (1986) suggested that the low intestinal digestive enzyme activities they observed with newly weaned piglets could be due to the depressed feed intake during the early post-weaning period.

Feed intake of newly weaned piglets is difficult to control experimentally because of the large variation in 'appetite' between individuals. Kelly *et al.* (1991a,b) used intragastric gavage feeding to obtain controlled and continuous feed intake during the early post-weaning period. From their studies on intestinal enzyme development they concluded that nutrient intake but also adaptational responses probably affect gut development. The experiments reported by Kelly *et al.* (1991a,b) are of interest since they describe the effects of feed intake on digestive adaptation during the early post-weaning period. However, they applied tube feeding to control feed intake and this may have interfered with 'normal' post-weaning development. To our knowledge, no experiments have been described in literature where feed consumption during the early post-weaning period was controlled in a more physiological manner.

In our experiments described in Chapters V and VI it was shown that feed intake is a major factor in the development of digestive capacity of young pigs. Piglets characterized as 'non-eaters' during the first three post-weaning days (Chapter VI) demonstrated their capacity of enzyme synthesis by the high trypsin and chymotrypsin activity in the pancreatic tissue. The enzyme secretion into the gut was probably lower for 'non-eaters' than for 'eaters', indicated by the low jejunal enzyme activities.

At day 6 after weaning, the positive relation between post-weaning feed intake and jejunal enzyme activities was most pronounced (Chapter V and VI). The changes in pancreatic response towards feed intake during the early post-weaning period demonstrate that three phases may be distinguished: during the first three days after weaning, the piglet is 'in trouble', as was also proposed by Le Dividich and Herpin (1992) who approached the post-weaning critical period from an energy-related point of view. The digestive system is trying to cope with the changes imposed upon it, feed intake is very variable and no clear response mechanisms can be identified. Between 3 and 6 days after weaning, the piglet clearly responds to changes in feed intake: higher feed consumption is related to higher jejunal (and pancreatic) enzyme activities and longer villi. Adaptation to the post-weaning situation is taking place. After day 6, the variation in feed intake diminishes and the relation between feed intake and enzyme activities disappears. The piglets have vanquished the nutritional stress related to weaning.

The influence of feed intake on the secretory capacity of the pancreas will probably also depend on the patterns of post-weaning feed intake. This aspect could not be statistically confirmed in our studies due to the variation in feed intake patterns between piglets and the relatively low number of animals used per experimental group. Studies from Kamphues (1987) and Rothert (1987) indicate that after a fasting/feed withdrawal period of one day piglets consumed approximately 50% of their 'normal' daily feed intake within the first 2 hours after re-alimentation. This temporarily increased feed intake resulted in higher gastric digesta weights, higher gastric pH and lower intestinal pH, possibly leading

to a reduction in enzymatic protein hydrolysis.

It must be stressed here that in our experiments no different levels of feed intake were imposed upon the piglets. Differences in feed intake are differences in voluntary feed intake and relations between feed intake and, e.g., enzyme activities may therefore reflect differences in 'adaptational status' of the piglets rather than causal effects of feed intake per se.

The actual secretion of trypsin and chymotrypsin into the gut lumen seems to be triggered by the presence of food (substrate) in the digestive tract (Chapter VI). From Chapter VI it can be concluded that feed intake and dietary protein source may interact with respect to pancreatic enzyme synthesis and secretion. A regular and gradual increase in feed intake after weaning is probably important for an undisturbed development of the pancreatic secretory capacity in young piglets.

All piglets sacrificed at 6 days after weaning had abnormal gut wall morphology (Chapter VI) with respect to villus shape. No gut wall samples were taken at weaning, but it may be expected from literature data (Chapter VI) that the gut wall damage was due to weaning. The decrease in relative small intestinal weight after weaning (Chapter VI) is in accordance with this finding.

The positive relation between post-weaning feed intake and jejunal villus lengths and crypt depths may indicate a stimulatory effect of feed intake on gut wall maturation (Chapter VI). Kelly *et al.* (1991b) also found a relationship between feed intake (controlled by tube feeding) and villus length and crypt depth at different sites along the small intestine. This finding merits further study, since villus atrophy is a common finding in weaned piglets and gut wall damage may contribute to increased endogenous nitrogen losses and to decreased absorptive capacity of the intestinal mucosa.

From the evidence presented in Chapters V and VI it is concluded that post-weaning feed intake has a greater influence on the development of post-weaning protein digestive capacity than dietary protein source *per se*.

Experimental evidence for the crucial role of feed intake (patterns and quantity) during the early post-weaning period is still lacking. Because voluntary feed intake in newly weaned piglets is generally low, irregular and very variable, it is difficult to study different feeding strategies with these animals. Forced feeding imposes an additional stress factor upon the newly weaned piglet, which will interfere with the already stressed state of the young pig.

The regulation of feed intake, especially in newly weaned piglets is insufficiently understood and requires further study.

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5.   *Gastric protein breakdown, digestive tract pH and development of protein digestive capacity*

The ratio between TCA-precipitable protein and crude protein (pp/cp ratio) measures the relative amount of non-hydrolyzed protein. The pp/cp ratio in gastric digesta at weaning was similar in the two experiments described (0.67 in Chapter V and 0.64 in Chapter VI). These piglets had only received sows milk (no solid feed) and the results indicate that the degree of gastric protein breakdown (reciprocal of pp/cp) was approximately 35 % when piglets were suckling the sow.

With regard to gastric protein breakdown after weaning, different results were obtained in the two experiments. In the first experiment (Chapter V) a clear effect of dietary protein source on gastric protein breakdown was found. About 45 % of the protein from skim milk powder was hydrolyzed in the stomach, while only 1 to 14 % of the protein from soya protein concentrate was broken down in the stomach. This intriguing result could not be reproduced in the second experiment (Chapter VI).

The degree of gastric protein breakdown at day 3 and day 6 after weaning was much higher in the second experiment (65 to 83 %, Chapter VI) than in the first experiment (1 to 44 %, Chapter V). Only at day 10 after weaning an effect of dietary protein source was found (Chapter VI): dietary fish meal resulted in a higher degree of gastric protein hydrolysis than soya protein concentrate. The method of analysis of TCA-precipitable protein was the same in both experiments and the pp/cp ratio's at weaning are similar for both experiments (as expected). The composition of the diets and the time between feeding and slaughter were similar for the two experiments described in Chapter V and VI. The differences in post-weaning gastric protein breakdown between the two experiments may be related to age of the piglets at weaning (25 days in Chapter V and 28 days in Chapter VI), gastric pH (higher in Chapter VI) and/or feed intake during the first three days post-weaning (175 g/d in Chapter V and 56 - 112 g/d in Chapter VI). The difference in weaning age between the two experiments was only three days and therefore it is unlikely that this would result in a large difference in gastric protein breakdown. From the low gastric pH (closer to the pH optimum of pepsin) found in the first experiment (Chapter V) compared to the second experiment (Chapter VI) a higher degree of protein breakdown would be expected in the first experiment (Chapter V). The higher initial feed intake in Chapter V, resulting in higher gastric digesta weight at day 3 might have inhibited gastric protein breakdown. It was not possible to study initial feed intake in Chapter V in more detail. It is possible that piglets had irregular feed intake patterns leading to disturbances in gastric protein breakdown, possibly related to gastric emptying.

The pH of the gastric digesta in both experiments was higher than the optimum pH value

for pepsin action. Gastric pH was higher than values reported by Hartman *et al.* (1961) but agreed with data from Efird *et al.* (1982). Maner *et al.* (1962) found lower intra-gastric pH with four weeks old piglets fed liquid diets based on casein than with piglets fed liquid diets based on isolated soya protein (3.2 vs 5.0) measured one hour after feeding. These contradictory results may be due to the form of the feed (liquid vs dry). Maner *et al.* (1962) found in addition a difference in total gastrointestinal tract passage rate between the two diets at four weeks of age (19 hours for the isolated soya protein diet vs 45 hours for the casein diet). This difference had disappeared at ten weeks of age. In Figure 1 the influence of buffer pH on trypsin and chymotrypsin activity is illustrated. In our experiments, the pH in the small intestine of newly weaned piglets (Chapter V and VI) was 1 to 3 units lower than the pH optimum for trypsin and chymotrypsin action. Therefore, trypsin and chymotrypsin activity 'in situ' (Chapter V) were probably only 12 to 25% of the activity measured 'in vitro'. In the experiment described in Chapter VI, jejunal pH ranged from 6.8 to 7.8 and therefore the 'effective' enzyme activity was probably 15 to 45% for trypsin and 40 to 85% for chymotrypsin (Figure 1). The pH in the intestinal digesta can be of major importance for the 'expression' of enzyme activity 'in vivo'.

6. Can a deficiency in pancreatic proteolytic enzyme synthesis/secretion be the cause of poor post-weaning piglet performance?

During the early post-weaning period piglet performance is often impaired. Many piglets display irregular feed intake: during the first few hours or days after weaning no solid feed is ingested, while afterwards hunger strikes and piglets tend to overeat. This phenomenon may be an important cause of post-weaning disorders. The gradual adaptation of the piglet's immature digestive system is impaired with respect to motility (Sissons, 1989), enzyme secretion (Jones, 1986) and pH regulation (Bolduan *et al.*, 1988). The research described in this thesis was (among other things) aimed at studying the possibility that a lack of pancreatic proteolytic enzyme synthesis and/or secretion could be a cause for depressed digestion and performance in newly weaned piglets. Studies by Lindemann *et al.* (1986) and Owsley *et al.* (1986) have shown depressed pancreatic enzyme activities in pancreatic tissue and intestinal chyme of piglets during the first few days after weaning.

Post-weaning feed intake is expected to be crucial in this respect because of the importance of substrate availability for the development of pancreatic secretion.

Newly weaned piglets are capable of synthetizing and secreting appreciable amount of pancreatic proteases in response to dietary stimuli (feed intake and dietary protein

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source). In Chapter VII it is shown that even before weaning piglets respond to hormonal stimulation with an increase in pancreatic secretion (volume and enzyme activities) (see also Harada *et al.*, 1986, 1988).

The relation between feed intake during the first three days post-weaning and enzyme activities was not significant (Chapter V). From Chapter VI it can be derived that when feed intake during the first three days post-weaning is zero or close to zero, trypsin and chymotrypsin activities in pancreatic tissue are high, indicating a distinct capacity to synthetize these enzymes immediately after weaning (Chapter VI).

This capacity also arises from results presented in Chapter VII. Especially trypsin secretion (units per hour) increased in young piglets when a hydrolyzed casein solution of pH 2.0 was infused into the duodenum. Harada *et al.* (1986) found a dramatic increase of pancreatic juice flow and protein output in young anaesthetized piglets after intraduodenal infusion of a HCl solution with pH less than 1.5. Above this pH value insufficient secretin was released to induce increased secretion. The two- to five-fold increase in juice volume secreted as reported in Chapter VII may be the result of both low intraduodenal pH and the presence of products of protein breakdown (hydrolyzed casein).

Harada *et al.* (1988) showed that 21-day-old piglets responded to an intravenous secretin injection with a four- to 15-fold increase in pancreatic juice secretion. From the evidence presented by Harada *et al.* (1986, 1988) and in Chapter VII it is clear that young piglets are capable of increasing their pancreatic secretion in response to intravenous and intraduodenal stimuli.

A schematic representation of digestive events occurring at weaning

Stress may be defined as tension or pressure caused by environmental changes and requiring adaptational action from the animal. Typical physiological and biochemical responses of the animal to a stressor include changes in gastric secretion and motility, increases in heart rate and blood pressure, and increases in the levels of plasma corticosterone, cortisol and catecholamines (Gué *et al.*, 1989). When the environmental change (e.g., weaning) comprises a severe strain to the animal, adaptation may be difficult or even impossible and acute stress may shift towards chronic stress, especially when coping strategies employed by the animal turn out to provide non-satisfactory results.

Although 'stress' is an ill-defined process, it is generally accepted (e.g. Stanton and Mueller, 1976; Worsaae and Schmidt, 1980) that early weaning is a stressful event comprising acute and chronic aspects: many factors in the environment of the young

animal undergo a sudden change, e.g., the piglets are removed from their dam, the piglets are often transferred to another pen, the piglets are presented with another type and acquisition of food (dry vs liquid, hard vs soft, cold vs warm, eating vs suckling). The young piglet has to cope with these changes appropriately. Generally stress invokes physiological responses involving hormonal, neural and behavioural mechanisms. Stress related hormones like ACTH (adreno-corticotropic hormone), corticosteroids and catecholamines (adrenalin and noradrenalin) play a key role in the response to various stressors (Gué et al., 1989; Dantzer and Mormède, 1983).

Weaning comprises stress to the young piglet and it may therefore be of interest to study the effect of stress-related hormones on digestive development of newly weaned piglets. This was done by Chapple et al. (1990a,b,c).

Chapple et al. (1990a,b,c) concluded from their studies on the effects of ACTH and glucocorticoids on newly weaned pig performance, that these hormones can stimulate the development of the pancreatic and intestinal enzyme system.

They found that glucocorticoid administration to nursing piglets can evoke premature elevation of the pancreatic and intestinal carbohydrase enzymes either through a direct effect on digestive capacity or indirectly through stimulation of feed intake.

Stress increases the need for energy and enhances general wakefulness (Uvnäs-Moberg, 1989).

From the field of animal ethology interesting mechanisms have been proposed (e.g. Selye, 1956; Dunn and Kramarcy, 1984) concerning adaptation to stress factors and coping strategies of animals.

It is proposed that analogous mechanisms may be applied to the digestive (nutritional) adaptation of piglets during the early post-weaning period. A schematic representation of possible coping mechanisms of the young pig in response to the nutritional aspects of the post-weaning situation is presented in Figure 2. The piglet may respond to its general state of stress by refraining from eating. This was seen in our experiments (Chapter VI) with 33% of the piglets fed the skim milk powder diet, 20% of the piglets fed the soya protein concentrate diet, 13% of the piglets fed the soyabean meal diet and none of the piglets fed the fish meal diet during the first three post-weaning days.

This 'coping strategy' can bring about various physiological consequences: the gastrointestinal tract adapts to the fasting state by changing its motility and secretion patterns (Sissons, 1989). The piglet may lose weight and find itself in a negative energy balance which will increase its susceptibility to infections and diseases. After fasting for some hours or days, the piglet will get hungry and start eating. This will upset its digestive system (Kamphues, 1987) and may lead to severe digestive disorders (diarrhoea).

The critical period of underfeeding during the first days after weaning was also recognized by Le Dividich and Herpin (1992), who focused on the negative energfy
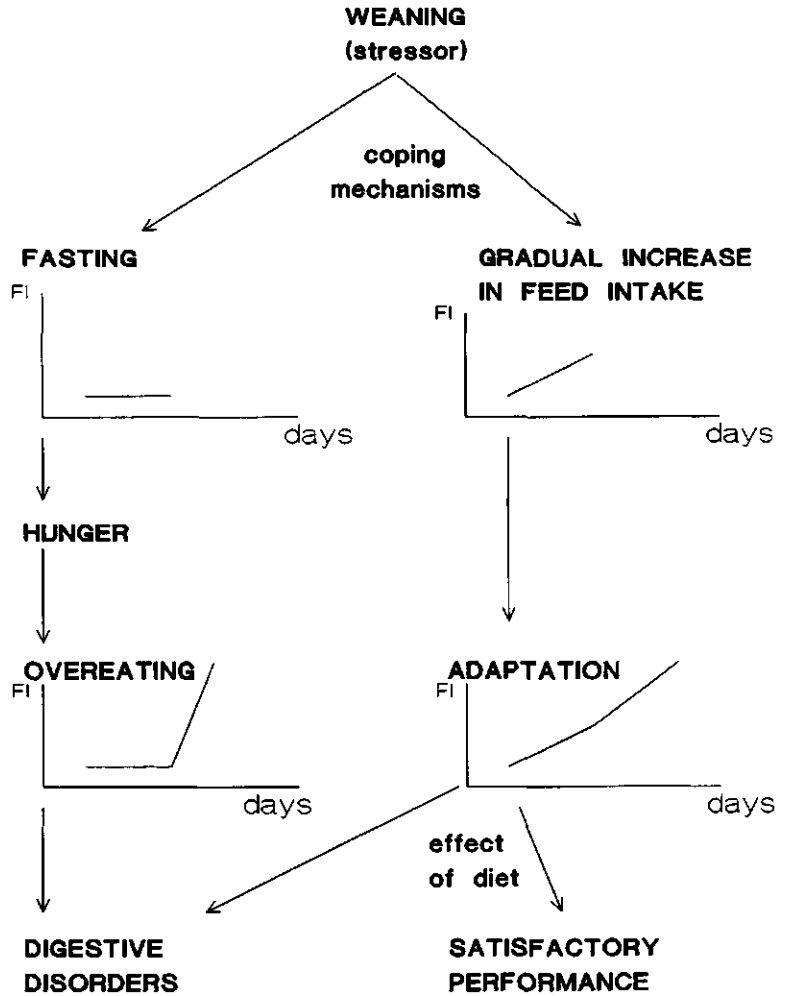
balance of newly weaned piglets.

Figure 2.   General mechanism of coping strategies in response to a nutritional stressor (weaning) in young piglets.

When early post-weaning feed intake is not impaired, the piglet gradually increases its feed intake after weaning and adapts its digestive system to the new feeding system and feed composition. This requires an adaptation of the different digestive organs (stomach, pancreas, small intestine) with respect to motility (gastric emptying, intestinal passage

rate), pH regulation (stomach pH, pancreatic and intestinal secretion), gut wall integrity (villus length and crypt depth) and digestive enzyme synthesis and secretion (stomach, pancreas, small intestine). As long as the piglet is not experiencing too severe stress, these adaptations may occur relatively smoothly. However, the demands imposed upon the piglet's digestive system may be too large when diets of a new composition and structure are fed. The familiarity of form and composition of the post-weaning diet may play an important role in this respect. An unfamiliar feed or feeding method may provoke inappropriate responses of the piglet's digestive system.

A complicating factor in the case of nutrition of newly weaned piglets is that the uptake of nutrients into the organism is disturbed resulting in loss of condition and increased susceptibility towards stress and disease.

Conclusions

In young piglets at four weeks after weaning, the differences in apparent digestibility between skim milk powder and soya products and fish meal are mainly due to differences in endogenous nitrogen losses and much less to differences in true nitrogen digestibility. The differences in endogenous nitrogen losses do not correspond with the differences in pancreatic protease activities in newly weaned piglets, indicating that the major source of endogenous nitrogen loss is not the pancreas.

Pancreatic enzyme activities as measured in jejunal digesta are not elevated in piglets losing high amounts of endogenous nitrogen at the terminal ileum. This indicates that pancreatic proteases are not the main source of endogenous nitrogen when diets based on skim milk powder, fish meal or soya proteins are fed. Other sources of endogenous nitrogen are probably more important and gut wall damage may be a critical factor in this respect.

From the experimental results presented in this thesis it is concluded that pancreatic enzyme production as such is not the 'bottle neck' in newly weaned piglet performance. Even before weaning, piglets are quite capable of adapting their enzyme secretion when an appropriate stimulus is given. The post-weaning decline in enzyme activities as found by Efird *et al.* (1982), Lindemann *et al.* (1986) and Owsley *et al.* (1986) is most likely related to poor feed intake after weaning.

In our studies we demonstrated that feed intake during the early post-weaning period is more important than dietary protein source for the development of the piglet's digestive system. The feed intake of newly weaned piglets may be stimulated by feeding diets of appropriate composition as was shown in our experiments for the piglets fed the fish meal diet.

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The detrimental effects of fasting during the first day(s) after weaning are possibly mediated through an increased gastrointestinal motility during fasting, leading to an increased digesta passage rate when the piglets start consuming the post-weaning diet. This phenomenon could hamper digestion and provoke bacterial growth, fermentation and gastrointestinal disorders (Sissons, 1989).

The dramatic lack of appetite in newly weaned piglets may be described to environmental, social or dietary stress factors corresponding with the process of weaning. The chemical and physical properties of the feed might be of importance when attempting to stimulate a gradual increase in feed intake of newly weaned piglets. In our studies fish meal resulted in increased feed intake of young piglets (Chapter VI) as was also found by Newport and Keal (1983).

Recommendations for further research

The identification and quantification of the various sources of endogenous nitrogen and the dietary factors influencing these sources should be an important issue in future research.

The regulation of feed intake in farm animals requires further study. Especially in newly weaned piglets, the effects of 'stress' on feed intake and digestive development should be examined in detail. Patterns of feed intake during the first week post-weaning should be studied in more detail.

The interrelationships of gastric and pancreatic functioning during digestive adaptation should be investigated in more detail: Research on isolated organs may provide knowledge on separate regulation mechanisms, but integrated responses to (nutritional) stimuli are of greater importance.

The regulation of exocrine pancreatic secretion merits further study, since contradictory results concerning the effects of weaning and diet composition are obtained from different studies. The different methods of analysis of (pancreatic) digestive enzymes should be compared and evaluated to detect artefact literature data arising from erroneous sample storage or analysis. The relation between enzyme activities measured using specific substrates and optimum assay conditions and enzyme activities actually expressed in the digestive tract is completely unclear and certainly merits further study. The differences in pancreatic enzyme secretion when different diets are fed are difficult to interpret because it is not completely known how much enzyme activity is needed to digest a certain amount of dietary protein. Therefore, one cannot decide conclusively if there is either an excess or a shortage of digestive enzymes secreted by the pancreas.

Implications

scientific

The digestion of protein is a complex process accomplished by the combined actions of the stomach, the pancreas and the small intestine. The regulation of pH in the digestive tract (stomach and small intestine) and the synthesis and secretion of proteolytic enzymes (stomach, pancreas and small intestine) control the digestion of dietary protein. An intact small intestinal wall is essential for the absorption of the products of protein digestion. From the research described in this thesis it is concluded that differences in endogenous nitrogen losses are the primary cause of differences in digestion between protein sources in young piglets. The origin of this endogenous nitrogen is insufficiently clear yet. The pancreas certainly plays an important role in the digestion of protein, but is not the major source of endogenous nitrogen losses in young piglets fed diets based on soya protein or fish meal. The importance of post-weaning feed intake (patterns) is clearly demonstrated in this study. Interactions between animal factors (adaptation after weaning, feed intake) and dietary factors (dietary protein source) were eminent in this study and stress the importance of an integrated approach in the study of digestive processes.

practical

The study of factors affecting protein digestion in farm animals is vital for improving the efficiency of animal production. Optimizing protein digestion will reduce the costs of animal production and the excretion of excessive amounts of minerals. The performance of piglets during the early post-weaning period is still unsatisfactory.

The research described in this thesis demonstrates that feed intake and dietary protein source are important factors in post-weaning pig performance. Pancreatic enzyme secretion *per se* is probably not a limiting factor in protein digestion, since young piglets are able to respond to stimuli with an increased secretion of digestive enzymes into the digestive tract. It is therefore not advisable to supplement weaner diets with exogenous proteolytic enzymes. Stimulation of endogenous enzyme secretion may, however, be insufficient in the newly weaned piglet. It is recommended to promote the piglet's indigenous capacity to synthetize and secrete digestive enzymes around weaning. This may be done by avoiding sudden changes at weaning. Minimizing the stress of weaning may help to stimulate feed intake during the early post-weaning period. Piglets should be encouraged to start feed consumption immediately after weaning. A post-weaning lag in feed intake lasting for more than a few hours should be prevented. The composition of the diet plays an important role herein.

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SUMMARY

Introduction

Over the past decades piglet weaning age has decreased from months to three to four weeks. With this early weaning digestive disorders in newly weaned piglets have become more apparent. Especially protein digestion depends on age of the pig: young piglets are less capable of digesting protein sources of vegetable origin. The difference in digestibility between animal and plant protein sources diminishes with advancing age of the piglets (Chapter I).

The causes of the low digestibility of certain protein sources in newly weaned piglets are not fully understood. Some authors have claimed that the secretion of (pancreatic) enzymes is insufficient in newly weaned piglets, causing depressed protein digestion and sometimes digestive disorders (diarrhoea) in young piglets.

The main objective of the present investigations was to study the development of protein digestive capacity in young weaned piglets.

Effect of dietary protein source on endogenous nitrogen losses in young pigs

To study the digestion of protein in detail, an experiment was carried out to determine the endogenous nitrogen losses in young piglets when different dietary protein sources were fed (Chapter II). It was concluded that differences in apparent nitrogen digestibility between skim milk powder, soya proteins and fish meal were mainly due to differences in endogenous nitrogen losses. The differences in true nitrogen digestibility were smaller than the differences in apparent nitrogen digestibility. Endogenous ileal nitrogen losses were 2 to 2½ times higher when non-milk protein were fed.

From this finding it was decided to investigate a possible source of endogenous nitrogen, the exocrine pancreas. The pancreas is an important gland secreting proteolytic enzymes and therefore a review was made of literature on pancreatic secretion in pigs (Chapter III). No consistency exists with respect to methods of sample collection and analysis of (pancreatic) enzymes. Therefore, we conducted an experiment to study the effect of storage conditions (time and temperature) on the activity of trypsin, chymotrypsin and lipase in pancreatic juice (Chapter IV). It was found that storage time and temperature affect enzyme activities and therefore it was decided to freeze-dry samples for enzyme analysis and store them frozen to minimize changes in enzyme activities.

Pancreatic secretion adapts to changes in dietary composition and often a decline in pancreatic enzyme secretion is found during the early post-weaning period. Research on pancreatic enzyme secretion in newly weaned piglets usually focuses on dietary composition and weaning age. Post-weaning feed intake is never taken into account,

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especially because piglets are usually not housed individually after weaning.

Because individual feed intake of newly weaned piglets is thought to be of major importance for the development of pancreatic secretion, this aspect was taken into account in two experiments.

Effect of dietary protein source and feed intake on pancreatic secretion in newly weaned piglets

Two experiments comprising 70 piglets each were carried out to study trypsin and chymotrypsin activities in pancreatic tissue and jejunal digesta of newly weaned piglets fed diets based on either skim milk powder, soya protein concentrate, soyabean meal or fish meal (Chapters V and VI). Feed intake of the piglets was recorded daily.

Trypsin and chymotrypsin activities in pancreas and jejunum were affected by dietary protein source, especially during the first week after weaning. Skim milk powder generally resulted in high jejunal protease activities. Fish meal caused low pancreatic and jejunal trypsin activity during the first post-weaning week and high pancreatic and jejunal chymotrypsin activities at day 3 after weaning. Pancreatic and jejunal chymotrypsin activities were low at day 6 after weaning when fish meal was fed.

The influence of dietary protein source on trypsin and chymotrypsin activities was most pronounced at day 3 after weaning and had disappeared at day 10.

The relation between feed intake and (jejunal) enzyme activities was more persistent: The effect of feed intake seemed to be greater than the effect of dietary protein source. It was concluded from Chapters V and VI that the first three days after weaning comprise the most difficult period for the young piglet. Many piglets consumed less than 10 grams feed per day and these 'non-eaters' had lower jejunal enzyme activities than piglets consuming more feed. Between 3 and 6 days after weaning, the relation between feed intake and enzyme activities became clear. The piglets were adapting to the new nutritional situation. By day 10, the differences in enzyme activities between diets had disappeared, the piglets had overcome the post-weaning nutritional difficulties.

Capacity of young piglets to respond to stimulation of pancreatic secretion

A final experiment was performed to check the ability of the young piglet to respond to pancreatic secretion stimulation (Chapter VII). It was shown that an increased pancreatic secretion was obtained when secretin was injected intravenously. Even before weaning, pancreatic secretion responded to intraduodenal infusion of a hydrolyzed casein

solution of pH 2. This finding confirmed the idea that pancreatic secretion as such is not the 'bottle-neck' in newly weaned piglet performance. The disturbed feed intake (quantity and pattern) often seen after weaning may, however, hamper smooth (protein) digestive capacity development after weaning in young piglets.

More (practical and scientific) attention for feed intake (patterns) during the early post-weaning period could provide tools for the improvement of post-weaning piglet performance.

Conclusions

Differences in apparent nitrogen digestibility between skim milk powder, soya products and fish meal in young piglets are due mainly to differences in endogenous nitrogen losses.

The exocrine pancreas is not the major source of endogenous nitrogen losses in young piglets.

Newly weaned piglets have the ability to respond to intravenous or intraduodenal stimulation by increasing their pancreatic secretion.

In young piglets, post-weaning feed intake is more important than dietary protein source for the development of pancreatic protease activities.

Dietary composition may influence post-weaning feed intake in young piglets.

Insufficient consistency with respect to sample storage and enzyme analysis hampers the interpretation of literature data.

SAMENVATTING

Inleiding

De speenleeftijd van biggen is over de laatste decennia teruggebracht van maanden tot drie à vier weken. Door dit vroege spenen zijn verterings-problemen bij pas-gespeende biggen duidelijker naar voren gekomen. Vooral de eiwitvertering hangt af van de leeftijd van de big: jonge biggen zijn minder goed in staat om plantaardige eiwitten te verteren dan oudere biggen. Het verschil in verteerbaarheid tussen dierlijke en plantaardige eiwitten neemt af met toenemende leeftijd van de big (Hoofdstuk I).
De oorzaken van de lage verteerbaarheid van bepaalde eiwitbronnen bij pas-gespeende biggen zijn nog niet volledig duidelijk. Sommige onderzoekers beweren dat de secretie van (pancreas-)enzymen onvoldoende is in pas-gespeende biggen, wat dan zou leiden tot verminderde eiwitvertering en soms verterings-storingen (diarree) bij jonge biggen.
De belangrijkste doelstelling van het onderhavige onderzoek was om de ontwikkeling van de eiwitverteringscapaciteit in jonge biggen nader te bestuderen.

Effect van eiwitbron in het voer op de ileale endogene stikstofverliezen in jonge biggen

Om de eiwitvertering gedetailleerd te bestuderen werd een experiment uitgevoerd om de endogene stikstof-verliezen vast te stellen bij jonge biggen, die voeders met verschillende eiwitbronnen kregen (Hoofdstuk II). Uit de resultaten van deze proef werd geconcludeerd, dat verschillen in schijnbare stikstof vertering tussen ondermelk poeder, soja eiwitten en vismeel vooral te wijten waren aan verschillen in endogene stikstof verliezen. De verschillen in werkelijke eiwitvertering waren kleiner dan de verschillen in schijnbare eiwitvertering. De endogene ileale stikstof verliezen waren 2 tot 2½ keer zo hoog bij voeders met soja of vismeel.
Hierna werd besloten een mogelijke bron van endogeen stikstof te onderzoeken, namelijk de exocrine pancreas. De pancreas is een belangrijke klier, die proteolytische enzymen uitscheidt en daarom werd een overzicht gemaakt van de literatuur met betrekking tot pancreas-sacretie bij varkens (Hoofdstuk III). Voor wat betreft de opslag en analyse van monsters voor enzymbepalingen is er weinig overeenstemming in de literatuur. Daarom werd een experiment opgezet om het effect vast te stellen van bewaar-omstandigheden (tijdsduur en temperatuur) op de activiteit van trypsine, chymotrypsine en lipase in pancreassap (Hoofdstuk IV). Er werd gevonden dat bewaartijd en -temperatuur invloed hebben op de enzymactiviteiten. Daarom werd besloten om monsters bestemd voor enzymanalyse te vriesdrogen en te bewaren bij -20 °C om verlies van activiteit te vermijden.
Pancreassecretie past zich aan aan veranderingen in voersamenstelling en vaak wordt

een teruggang in enzymactiviteiten na het spenen gezien. Onderzoek naar de enzymsecretie door de pancreas van pas-gespeende biggen is meestal gericht op voersamenstelling en speenleeftijd. De voeropname na het spenen wordt vrijwel nooit gemeten, waarschijnlijk omdat pas-gespeende biggen meestal niet individueel gehuisvest worden.

Omdat het vermoeden bestaat, dat de individuele voeropname van de biggen van groot belang voor de ontwikkeling van de pancreassecretie, is dit aspect meegenomen in twee proeven.

Effect van eiwitbron en voeropname op pancreassecretie bij pas-gespeende biggen

Twee experimenten, elk bestaande uit 70 biggen, werden uitgevoerd om de trypsine- en chymotrypsine-activiteit vast te stellen in pancreasweefsel en jejunumchymus van pas-gespeende biggen. Vier verschillende rantsoenen werden samengesteld, gebaseerd op ondermelkpoeder, soja-eiwit-concentraat, sojaschroot of vismeel (Hoofdstuk V en VI). De voeropname van de biggen werd dagelijks gemeten. Trypsine en chymotrypsine activiteiten in pancreas en jejunum werden beinvloed door eiwitbron, vooral gedurende de eerste week na spenen. Ondermelkpoeder leidde in het algemeen tot hoge protease activiteiten in het jejunum. Vismeel veroorzaakte een lage trypsine activiteit in pancreas en jejunum tijdens de eerste week na spenen en een hoge chymotrypsine activiteit in pancreas en jejunum op dag 3 na spenen. Chymotrypsine activiteit in pancreas en jejunum was laag op dag 6 na spenen als vismeel werd gevoerd.

Het effect van eiwitbron in het voer op de trypsine- en chymotrypsine-activiteiten was het duidelijkst op dag 3 na spenen en was verdwenen op dag 10.

De relatie tussen voeropname en enzymactiviteiten (in het jejunum) was sterker: het effect van voeropname leek groter te zijn dan het effect van eiwitbron. Uit Hoofdstuk V en VI werd geconcludeerd dat de eerste drie dagen na het spenen het moeilijkst zijn voor de jonge big. Veel biggen aten minder dan 10 gram per dag en deze 'niet-eters' hadden lagere enzym-activiteiten in het jejunum dan biggen die meer aten. Tussen dag 3 en dag 6 tekende zich de relatie tussen voeropname en enzym activiteiten duidelijk af. De biggen pasten zich aan aan de nieuwe voedings-omstandigheden. Op dag 10 waren de verschillen in enzymactiviteiten tussen voeders verdwenen, de biggen hadden de (voedings)problemen overwonnen.

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Het vermogen van jonge biggen om te reageren op stimulatie van pancreas-secretie

Een laatste experiment werd gedaan om het vermogen van de jonge big om te reageren op pancreas-stimulatie te testen (Hoofdstuk VII). Een toename van de pancreas-secretie werd gevonden na intraveneuze secretine-injectie. Zelfs vóór het spenen reageerde de secretie op een intra-duodenale infusie van gehydrolyseerd caseine met pH 2. Dit resultaat bevestigde het idee, dat de pancreas-secretie an sich niet de oorzaak is van de verminderde prestaties van pas-gespeende biggen. De verstoringen in voeropname (hoeveelheid en patroon), die vaak optreden na het spenen, kunnen de geleidelijke ontwikkeling van de (eiwit)verteringscapaciteit van jonge biggen belemmeren.
Meer (praktische en wetenschappelijke) aandacht voor voeropname(patronen) gedurende de eerste week na het spenen zou een bijdrage kunnen leveren aan de verbetering van de prestaties van pas-gespeende biggen.

Conclusies

De verschillen in schijnbare stikstof verteerbaarheid tussen ondermelkpoeder, soja produkten en vismeel zijn vooral te wijten aan verschillen in endogene stikstof-verliezen.

De exocriene pancreas is niet de belangrijkste bron van endogeen stikstof verlies in jonge biggen.

Pas-gespeende biggen zijn in staat om hun pancreas-secretie te verhogen in respons op intraveneuze of intraduodenale stimulatie.

Bij jonge biggen is de voeropname na het spenen belangrijker dan de eiwitbron in het voer voor de ontwikkeling van pancreas protease activiteiten.

Voersamenstelling kan de voeropname van pas-gespeende biggen beïnvloeden.

Onvoldoende overeenstemming met betrekking tot monster-bewaaromstandigheden en enzym-analyses bemoeilijkt de interpretatie van literatuur-gegevens.

LIST OF PUBLICATIONS

1. Storage of porcine pancreatic juice: effect on enzyme activities.

   C.A. Makkink, L.A.J. van der Westerlaken, L.A. den Hartog, M.J. van Baak, J. Huisman. *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde 63:267-272, 1990.*

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CURRICULUM VITAE

Caroline Annette Makkink werd op 3 september 1962, om 13.30 uur geboren te Arnhem. Zij behaalde in 1980 het VWO-diploma aan het Stedelijk Gymnasium te Arnhem. In september van datzelfde jaar begon ze met de studie Zoötechniek aan de toenmalige Landbouw Hogeschool te Wageningen. In maart 1988 studeerde zij af met Veehouderij (Gezondheids- en Ziekteleer) en Dierfysiologie als hoofdvakken en Veehouderij (Ethologie) als bijvak.

Vanaf 1 april 1988 was zij aangesteld als Assistent in Opleiding bij de vakgroep Veevoeding, waar het onderzoek beschreven in dit proefschrift werd verricht.

Van 1 oktober 1992 tot 1 februari 1993 was zij aangesteld als toegevoegd onderzoeker bij de vakgroep Veevoeding. In deze periode werd een verkennende studie met betrekking tot het fundamenteel dierfysiologisch onderzoek (onderdeel stofwisselings-fysiologie) in Nederland voltooid in opdracht van de Nationale Raad voor Landbouw-kundig Onderzoek.

Op 29 januari 1993 trouwde zij met Ben Rankenberg.

Per 1 maart 1993 is zij in tijdelijke dienst aangesteld als onderzoeker klimaat bij het Proefstation voor de Varkenshouderij te Rosmalen.