580 Beretning fra Statens Husdyrbrugsforsøg

Editors: A. Just, H. Jørgensen and J. A. Fernández

København 1985

Proceedings of the 3rd International Seminar on

Digestive Physiology in the Pig

Copenhagen 16th – 18th May 1985

Indlæg ved det 3. internationale seminar om

Fordøjelsesfysiologi hos svin

København 16. – 18. maj 1985

I kommission hos Landhusholdningsselskabets forlag,
Rolighedsvej 26, 1958 København V.

Trykt i Frederiksberg Bogtrykkeri 1985

FORORD

De store fremskridt, der i de seneste årtier er sket inden for mange grene af videnskaben, har fået stor betydning, bl.a. for husdyrenes ernæring.

Tilgængeligheden af denne enorme mængde viden og teknik har muliggjort etablering af helt nye forskningsområder samt udvidet allerede eksisterende betragteligt. Som en følge af denne udvikling er der et voksende behov for møder, hvis formål er at udveksle og diskutere resultater og begreber inden for et afgrænset område som f.eks. fordøjelsesfysiologien hos svin.

I 1979 arrangerede Dr. R. Braude og hans medarbejdere i Shinfield det første internationale seminar vedrørende fordøjelsesfysiologi hos svin. Proceedings fra dette seminar er publiceret i Technical Bulletin No. 3: "Current Concepts of Digestion and Absorption in the Pig" (Eds. A. G. Low and I.Partridge).

Tre år senere fandt det andet seminar sted i Versailles under ledelse af Dr. A. Rerat og hans stab. Proceedings fra dette seminar blev publiceret i "Les Colloques de l'INRA" No. 12 med titlen "Digestive Physiology in the Pig" (Eds. J. P. Laplace, T. Corring and A. Rerat).

I fortsættelse af de tidligere seminarer vil det tredie blive afholdt i København i dagene 16. - 18. maj 1985. I alt 110 forskere fra mange lande forventes at deltage, og deres bidrag forventes at omfatte ca. 85 rapporter.

Skønt en så lang og omfattende række af undersøgelser klart vil tilfredsstille et af hovedformålene med seminaret, nemlig at fremlægge den nyeste viden inden for fordøjelsesfysiologien, er der næppe tilstrækkelig tid til detaillerede diskussioner af de enkelte arbejder, hvis seminaret afholdes på traditionel vis.

I et forsøg på at klare dette problem blev det besluttet, at alle bidrag - på nær introduktionsindlæggene inden for hver session - skulle præsenteres som posters, og at proceedings skulle udsendes mindst 4 uger før mødets afholdelse, hvilket skulle gøre det muligt for deltagerne at blive fortrolig med indholdet heraf, inden mødets afholdelse.

Seminaret afholdes på Landbrugsorganisationernes konferencecenter "Kollekolle", hvor deltagerne vil bo, arbejde og slappe af sammen, hvilket skulle give en yderligere mulighed for en effektiv og gavnlig meningsudveksling.

Seminaret vil bestå af fem sessioner, som hver vil omfatte et 45 min. introduktionsindlæg over emnet, fulgt af en 10 min. vejledning af diskussionslederen, derpå 1-1½ time til et studium af posters, og herefter 1-1½ time til en afsluttende diskussion.

Sessionerne vil inkludere emner vedrørende udvikling af fordøjelseskanalen og dens funktioner i de unge dyr, perspektiver med svinet som en mulig model for humane studier, nye ideer til fremtidige forsøg og forsøgsmetodik i relation til resultaternes gyldighed.

I proceedings bibeholdes sessions-rækkefølgen, som fastlagt ved inddelingen af seminaret, omfattende følgende emner: den neuro-endokrine kontrol af fordøjelsesprocesserne, fodersammensætningens indflydelse på udskillelsen af fordøjelsessekreter til fordøjelseskanalen, træstoffraktionens betydning for fordøjelighed, absorption og stofskifteprocesser, indflydelsen af fordøjelseskanalens mikroflora på fordøjelsesprocesserne samt resultater vedrørende fordøjelighed og absorption af næringsstoffer og resultaternes praktiske anvendelse.

Vi ønsker at takke forfatterne for deres udmærkede bidrag, der er en forudsætning for seminaret.

En hjertelig tak for værdifuld støtte rettes til Danske Korn- og Foderstof- Im- og Eksportørers Fællesorganisation (DAKOFO), Danske Slagterier, Statens jordbrugs- og veterinærvidenskabelige Forskningsråd, NOVO Industri A/S, Eurolysine, Galenica A/S og De danske Mejeriers Fællesorganisation.

København, februar 1985

A. Just, H. Jørgensen og
J. A. Fernández

FOREWORD

The last few decades have seen tremendous advances in a number of disciplines, which have had a great impact on, amongst others, the field of animal nutrition.

Naturally, the availability of such a vast amount of new knowledge- and techniques has opened quite a few new research fields and considerably widened old ones. As a consequence of this development, there is a growing need for meetings, whose objective is confined to the exchange and discussion of new results and concepts in specialized areas such as the digestive physiology of the pig.

In 1979, Dr. R. Braude and his co-workers at Shinfield, arranged the first international seminar on digestive physiology in the pig. The proceedings of this seminar can be found in the Technical Bulletin No. 3: "Current Concepts of Digestion and Absorption in the Pig" (Eds. A.G. Low and I. Partridge).

Three years later, the second seminar took place at Versailles, arranged by Dr. A. Rerat and his staff. The seminar proceedings were published in "Les Colloques de l'INRA" No. 12 entitled "Digestive Physiology in the Pig" (Eds. J.P.Laplace, T. Corring and A. Rerat).

Following up the previous seminars, the third will take place in Copenhagen, May 16-18, 1985. About 110 scientists from many countries are expected to participate and their contributions will comprise approximately 85 reports.

Although such a large and wide variety of studies will clearly satisfy one of the main objectives of the seminar, namely to review the newest findings concerning digestive physiology, there is scarcely enough time for detailed discussions of individual reports, should the seminar be carried out in a traditional manner.

Therefore, in an attempt to circumvent this problem, it was decided that all contributions, except for those which introduce each session, should be presented as posters, the proceedings being circulated several weeks prior to the meeting, thus allowing the participants to get acquainted with the contents before the beginning of the meeting. Furthermore, the seminar will be held at "Kollekolle", a conference center built by the Danish Agricultural Organizations, where participants will live, work and relax together,

thus providing additional opportunity for effective and productive exchange of views.

The seminar will comprise five sessions, each one including a 45 min. introduction to the subject followed by a 10 min. guidance by the discussion leader, then about 1-1½ hour poster studies and finally 1-1½ hour discussion.

The sessions are intended to include topics concerning developments of the digestive tract and its function in the young animal, perspectives of the pig as a potential model for human studies, new ideas for future reseach and research methodology in relation to the validity of the results.

The proceedings follow the sequence of sessions into which the seminar has been divided and include the following topics: neuro-endocrine control of digestion; the secretory response of the digestive tract to the diet; the influence of dietary fibre on digestion, absorption and metabolism; the influence of the gut microflora on digestion processes; finally, experimental results concerning digestion and absorption of nutrients and their practical applications.

The organizers wish to thank the authors for their excellent papers. Without their contribution the seminar could not have been possible.

We are especially grateful for the valuable support given by the Danish Grain and Feed Trade Association, the Association of Danish Slaughterhouses, the Danish Agricultural and Veterinary Research Council, NOVO Industry Inc., Eurolysine, Galenica Inc. and the Danish Dairy Federation.

Copenhagen February 1985

A. Just, H. Jørgensen and J. A. Fernández

CONTENTS

SESSION 2

THE SECRETORY RESPONSE OF THE DIGESTIVE TRACT TO THE DIET

Discussion leader: T. Corring

SESSION 3

THE ROLE OF DIETARY FIBRE IN DIGESTION, ABSORPTION AND METABOLISM

Discussion leader: R. A. Argenzio

Introductory lecture:

Posters:

SESSION 4

THE INFLUENCE OF THE GUT MICROFLORA ON THE DIGESTIVE PROCESSES

Discussion leader: T. Zebrowska

Introductory lecture:

Posters:

SESSION 5

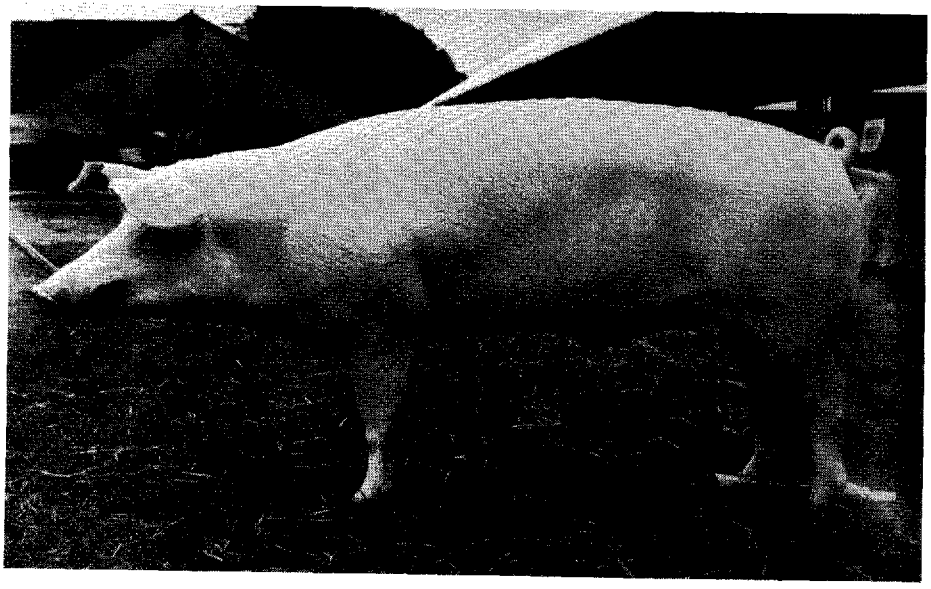
DIGESTION AND ABSORPTION OF NUTRIENTS: RESULTS AND THEIR PRACTICAL APPLICATIONS

Discussion leader: S. H. M. Metz

SESSION 1

THE NEURO-ENDOCRINE CONTROL OF THE DIGESTIVE PROCESSES

Discussion leader: J.P. Laplace



THE NEURO-ENDOCRINE CONTROL OF THE DIGESTIVE PROCESSES

J. J. HOLST

Institute of Medical Physiology C
The Panum Institute, University of Copenhagen
DK-2200 Copenhagen N, Denmark

INTRODUCTION

One of the most conspicuous advances in the field of gastrointestinal neuro-endocrinology has been the uncovering of the role of the regulatory peptides in the control of digestive processes. Not only have recent technical advances made it possible to define more precisely the role of established hormones like gastrin, cholecystokinin and secretin in endocrine control, but it is now evident that regulatory peptides are also essential transmitters in neural and so-called paracrine control. Studies in pigs have been fundamental for this development. Be it that the first endocrine mechanism to be discovered, the control of pancreatic bicarbonate secretion by secretin, was observed in dogs; but it is typical that the first gastrointestinal hormones to be isolated, gastrin, secretin and cholecystokinin were all isolated from pig tissue; indeed, the vast majority of the regulatory peptides that are known today have been isolated from pig tissue. Minor species differences are characteristic of the structure of the regulatory peptides; in order not to violate the principle of species homology, many workers presently perform physiological experiments using pig models. In addition, it has been the consensus that pigs are very convenient laboratory animals, easy to handle (particularly true for young pigs), and cheap. Thus, a considerable body of information about gastrointestinal neuro-endocrine mechanisms is contained in the literature. Pertinent features of this shall be reviewed here.

REGULATORY PEPTIDES

1) Identification of regulatory peptides.

The first gasstrointestinal peptides were isolated on the basis of

their biological activity: it was known that a particular extract contained a peptide with a certain biological activity. By application of various methods of protein purification, chromatography, etc., and a bioassay for this activity, preparations of increasing purity were obtained. The peptides secretin, cholecystokinin and gastrin were isolated this way (Jorpes and Mutt, 1973); other more recent examples include Gastric Inhibitory Polypeptide, which was isolated on the basis of its inhibitory effect on gastric acid production; Vasoactive Intestinal Polypeptide, VIP, which was isolated on the basis of its vasodilatory effects, and motilin, a motility enhancing peptide, all of which were isolated from pig gut extracts (See Table 1 for list of regulatory peptides and references).

However, the group of regulatory peptides has expanded greatly in the last years, because other techniques of peptide identification have supplemented this original physiological approach. Some peptides were discovered by simple chemical isolation. Thus, Pancreatic Polypeptide, PP, a peptide with important effects on pancreatic secretion and gall bladder secretion/emptying, was isolated from pancreatic extracts during the commercial purification of insulin. Later research has established that pancreatic polypeptide is produced in the islets of Langerhans and that it is a hormone, which is released in response to ingestion of meals by a mechanism which depends on efferent activity in the vagus nerves (see Schwartz, 1983).

Many regulatory peptides are amidated in their carboxyterminus. This feature, which is often essential for their biological activity, inspired Tatemoto and Mutt (1978) to devise a method for isolation of amidated peptides. Thus, they isolated from porcine intestinal extracts the peptides PHI and PYY (P for peptide, second and third letter indicating in one-letter code the N-terminal and C-terminal amino-acids), both important peptides as discussed below.

Some of the gastrointestinal regulatory peptides were originally discovered with the aid of immunochemical analyses for peptides originally isolated from the central nervous system. This is true for somatostatin, the hypothalamic growth hormone inhibiting factor, and neurotensin, a central nervous system neurotransmitter. Interestingly, in the gut, the latter functions as a hormone. The dual brain-gut localization of substance P was realized already when it was discovered in the thirties.

Other peptides were identified by immunochemical methods developed for amphibian peptides. Most of the latter have been isolated from frog skin, where these peptides are produced in holocrine glands, possibly functioning as defense poisons. This is true for Gastrin Releasing Peptide, GRP, which was discovered with assays against bombesin, a peptide isolated from the skin of the frog, Bombina Bombina.

An unexpected source of new peptides was found when modern methods of gene technology were applied in the elucidation of the structure of regulatory peptide precursors. Typically, these precursors consist of 100-200 amino acids, as compared to 8-36 of the regulatory peptides. It now appears that the excess part of the precursor may often contain new, unexpected peptides with biological activity. Thus, the enteroglucagon precursor was shown to contain in addition to the main enteroglucagon peptide, glicentin, another two peptides of 37 and 34 amino acids, named GLP-1 and GLP-2 because of their structural relationship with glucagon (GLP for glucagon-like peptides), both of which are secreted from the gut when the release of enteroglucagon is stimulated (Ørskov, 1985). Similarly, the abovementioned peptide, PHI, was found to be an additional product of the VIP-gene.

2) Peptide families.

Another characteristic feature of the regulary is their grouping in so-called families (see Table 1). The relationship may consist of identical sequence stretches (cf. cholecystokinin and gastrin) or significant homologies in sequence. The largest family is the secretin/glucagon family, which also comprises the peptides PHI/VIP, GIP, the GLPs, and enteroglucagon. Undoubtedly, these peptides reflect differentiations of an original, ancestral peptide (Rehfeld, 1981).

3) Heterogeneity.

Differential processing and proteolysis of the peptide precursor explain another characteristic of the regulatory peptides, their pronounced molecular heterogeneity (Table 1). Thus, the gastrin precursor may be cleaved to produce gastrins comprising from 4 to approximately 50 amino acids, they have the C-terminal tetrapeptide, in which the biological activity resides, in common. Furthermore, the larger forms of gastrin occur with or without sulfation of the tyrosine residue in position 7, a fact which doubles the number of variant forms of gastrin (by some named "microheterogeneity").

Tabel 1. List of regulatory peptides in the gastrointestinal tract with indication of name, abbreviation, number of amino acids, other products of the propeptide, localization, main function, and a key reference.

Name	Number of amino acids	Other peptide products of precursor (propeptide)	Localization nerves/endocrine cells	Endocrine function proven	Main activity	Key reference
Gastrin	17	Big gastrin (G-34),component I,G-14,G-4 (5-6)	+/+	+	Gastric acid secretion mucosal growth	Boel et al.,1983 Walsh and Lam,1980, Yoo et al.,1982
Cholecystokinen (CCK)	33	CCK-8,CCK-12 (?),CCK-39 CCK-58,CCK-4	+/+	+	Neurotransmission,pancreatic enzyme secretion,gall bladder motility	Deschenes et al., 1984
Gastrin releasing polypeptide (GRP)	27	(Mammalian Bombesin) Neuromedin C (C-terminal decapeptide)	+/		Gastrin secretion	Spindell et al., 1984
Substance P	11	Substance K, neuropeptide K neuropeptide P	+/+		Neurotransmission smooth muscle activ.	Nawa, 1984 Tatemoto, 1984
Neurotensin	13	Precursor structure unknown	+/+		Neurotransmission *gastric inhibition*	Goedert, 1984
Motilin	22	Precursor structure unknown	/+		Small intestinal motility	Christophides & Bloom, 1981
Somatostatin	14	Somatostatin 1-28 Somatostatin 1-12 (1-28)	+/+	+	Paracrine function General inhibitory activity	Reichlin, 1983
TRH	3	Structure of intestinal TRH largely unknown	?		?	Richter et al., 1984
Methionine-Enkephalin	5	Heptapeptide Leu-enkephalin, octapeptide	+/		Neurotransmission small intestinal motility	Rossier et al., 1984

Endorphin	31	-MSH, ∝-MSH, CLIP, ACTH β-lipotropin	/+		Gastrointestinal function unknown	Krieger, 1984
Corticotropin Releasing Factor (CRF)	41	(Mammalian sauvagine)	/+		Gastrointestinal function unknown	Nieuwenhuijzen, Kruseman et al., 1982; Vale et al., 1983
Glicentin	69	Oxyntomodulin, glucagon GRPP, GLP-1, GLP-2	/+		Intestinal growth gastric inhibition	Holst, 1983; Holst, 1985a
GIP (Gastric inhibitory polypeptide)	42	Precursor structure unknown "GIP 8000"	/+	+	Gastric inhibition insulin secretion	Brown, 1982
Secretin	27	Precursor structure unknown	/+	+	Pancreatic bicarbonate secretion	Schaffalitzky de Muckadell, 1980
Vasoactive Intestinal Polypeptide (VIP)	27	PHI (peptide histidine-isoleucineamide)	+/		Neurotransmission, electrolyte secretion, smooth muscle relaxation	Itoh et al., 1983 Said, 1984
Growth hormone releasing factor (GRF)	42		?		Gastrointestinal activity unknown	Guillemin, 1983
Pancreatic Polypeptide (PP)	36	Icosapeptide	/		Inhibition of pancreatic and bile secretion	Schwartz, 1983
PYY (peptide Tyr-Tyr)	36	Precursor structure unknown	/+		Pancreatic inhibition	Tatemoto, 1982a
NPY (neuropeptide Y (Tyr))	36	Precursor structure unknown	+/		Co-exists with norepinephrine, facilitates effects of NE	Tatemoto, 1982b

4) Morphology.

The gastrointestinal regulatory peptides are produced in endocrine cells, in paracrine cells and in peptidergic nerves. The endocrine cells occur in two forms: the open cells, which reach the lumen of the gland or viscus. At the luminal cell surface microvilli protrude; basally, the secretory granules are found. The secretin, GIP and enteroglucagon cells are typical open cells. The closed cells do not establish contact with the gland lumen, and apparently therefore receive regulatory signals by the blood route or by innervation. Such cells are typically found in the mucosa of the stomach, for the pig stomach, particularly in the cardiac gland region; some of these cells are apparently true glucagon-producing A-cells, like those of the endocrine pancreas (Holst, 1985a).

Paracrine cells are believed to control neighbour cell function by local secretion of the transmitter. The concept of paracrine regulation was supported by the discovery of "endocrine cells" with cytoplasmic processes, which in a dendrite-like fashion reach other cells (Larsson, 1980). In this way, a single cell may contact large numbers of near-by cells. The somatostatin cells of the intestinal mucosa are particularly abundantly supplied with these processes. Surprisingly, such processes are rare in porcine gastrointestinal somatostatin cells.

As indicated in Table 1, a large number of the regulatory peptides are found in nerve fibres innervating the digestive organs. This group includes peptides, which are also known to be secreted by endocrine cells (e.g. cholecystokinin). These so-called peptidergic nerve fibres are thin and characterized by varicosities in which the peptides are stored; presumably, the peptide is released diffusely from the varicosities upon stimulation. Some fibres are afferent with cell bodies in either the spinal ganglia or the vagal nuclei (e.g. substans P containing fibres); other fibres are efferent with cell bodies in local ganglia (like VIP).

The peptidergic neurotransmitters are frequently found to co-exist with traditional transmitters; this seems to be the rule rather than the exception (Lundberg & Hokfelt, 1983). Co-existence has been demonstrated between two peptides, peptide and amine, and peptide and acetylcholine. Upon stimulation such nerve fibres, both transmitters are released from varicosities or nerve endings. The two transmitters may

serve specialized functions, or support or potentiate the action of the other. In addition, the amines or acetylcholine typically have a rapid onset and a short duration of activity, whereas the peptides are comparatively slow transmitters, particularly suitable for sustained action. Prominent examples of co-existence include VIP and acetylcholine, and NPY and noradrenaline.

HORMONAL REGULATION

The endocrine pancreas. The secretion of the glucoregulatory hormones, insulin and glucagon, is likely to play a prominent role in digestive function and growth but will not be discussed in detail here. The other two hormones of the endocrine pancreas, somatostatin and pancreatic polypeptide, however, are directly involved in digestive control. In pigs, the islets of Langerhans are difficult to isolate and few studies are therefore available. However, a number of studies with an isolated perfused pancreas have been published. In many respects the results with this model are comparable to those obtained with other models, so that most results are likely to be applicable to pig physiology as well.

It seems that somatostatin may act both as a hormone and as a local paracrine transmitter. Somatostatin is known to inhibit the secretion of the remaining cells of the islets, also in the pigs (Fahrenkrug et al., 1979), and it is likely that the D-cells, in which the peptide is produced, tonically inhibit insulin and glucagon secretion. The somatostatin secretion is, in turn, influenced by the autonomic nerve supply; both sympathetic and parasympathetic nerves are inhibitory, and activity in these nerves may therefore increase insulin and glucagon secretion via suppression of somatostatin secretion (Holst et al., 1983a). As a hormone somatostatin may influence gastrointestinal blood flow and absorption of protein, carbohydrates and lipids (Schusdziarra, 1980). Furthermore, somatostatin is capable of inhibiting practically all secretory processes of the digestive tract, endocrine as well as exocrine. This is true for gastric acid secretion as well as gastrin secretion; for pancreatic enzyme secretion; for secretion of enteric succus and electrolytes as well as gastrointestinal hormones and neurotransmitters. Somatostatin is so potent that it can be used clinically in the treatment of bleeding ulcers, pancreatitis and severe diarrhoea, because of its inhibi-

tory properties. In this connection, it is of interest that stable, orally active long-acting analogs have been synthetized (Bauer et al., 1982; Veber et al., 1984).

Somatostatin is also produced and released by endocrine cells (and nerve fibres) in the gastrointestinal mucosa. Possibly, part of somatostatin's physiological role here is paracrine control; on the other hand, these cells, which are open, may be influenced by luminal stimuli and triggered to secrete somatostatin to the blood. Acid introduced in the proximal intestine releases somatostatin in amounts, which may influence acid secretion. Thus, somatostatin may take part in a negative feed back control system for acid secretion. In humans, it appears that the concentration of somatostatin in peripheral venous plasma is mainly derived from the gastrointestinal mucosa and that it varies with the gastric emptying of acid (Webb et al., 1985).

The intestinal cells produce mainly somatostatin-28 as opposed to somatostatin-14, the main product of the endocrine pancreas (see Table 1) (Baldissera & Holst, 1985). The two forms have related biological effects, and the biological significance of this differential processing of the precursor in the two tissues is not known.

Gastrin release in pigs has been studied by several authors (e.g. Christiansen et al., 1978; Cranwell & Hansky, 1980). The main secretory product is gastrin 1-17, but, due to a longer halflife in the circulation, gastrin 1-34 is found in equal concentrations in the peripheral circulation. Since both stimulate acid secretion, they are both physiologically important acid secretagogues (Walsh & Lam, 1980). Gastrin secretion in the pig is controlled in a tight interplay between endocrine, paracrine and neuronal control mechanisms. The endocrine control is mediated by hormones like GIP, which inhibits gastrin secretion possibly by stimulating local release of somatostatin. Gastrin cells are open, and may be susceptible to luminal stimuli; thus luminal application of peptone broth may stimulate gastrin secretion in the isolated perfused antrum. It has been said that one of the main functions of gastrin is its growth promoting activity in the gastrointestinal tract; most of the studies concerning this were performed in murine species, however. (Johnson, 1977).

Secretin release has been studied in intact pigs as well as in isolated perfused preparations of the pig duodenum (Fahrenkrug et al.,

1977; Schaffalitzky de Muckadell et al., 1977). Secretin is secreted by open cells of the upper jejunal mucosa, and the most important stimulus for its release is HCl. No other stimulus, including nutrients, mixed food preparations, or bile will cause secretin release except when mixed with acid to a pH, which would in itself be stimulatory for secretin release. The autonomic nervous system does not seem to influence secretin secretion in pigs (Holst et al., 1979). Determined with the present day radioimmunoassays, secretin shows remarkably little molecular heterogeneity. Furthermore, the precursor structure is unknown. In peripheral plasma from pigs, secretin concentrations are low, around 1 pmol/l (like in man). The pig pancreas is extremely sensitive to secretin; a plasma concentration of 3 pmol/l is sufficient to stimulate pancreatic fluid secretion significantly and maximal effect is obtained at 20-50 pmol/l (Jensen et al., 1978). The pH threshold for secretin release is 2, but it has been shown that introduction of just a few ml of 0.1 M HCl in the duodenal bulb is sufficient to cause secretin release and pancreatic secretion (Holst et al., 1979). Thus there is little doubt that in the pig pancreatic bicarbonate secretion is indeed related to emptying of gastric acid into the duodenum with secretin as the transmitting link. Secretin is thought by some to function also as a entergastrone: an intestinal hormonal inhibitor of acid secretion (Chey et al., 1981); but whether or not this is true for the pig is not known. In the concentration range obtained in arterial plasma under physiological conditions, secretin does not influence the secretions of the endocrine pig pancreas (Holst et al., 1980b).

Cholecystokinin secretion has also been studied in intact as well as in isolated organ preparations. Cholecystokinin in the pig shows remarkable molecular heterogeneity, and it is questionable whether the originally isolated molecule, cholecystokinin (CCK)-33, is the hormone proper, or should be regarded as a prohormone (Rehfeld, 1981; Cantor et al., 1984). Newly released CCK (from isolated pig duodenum) consists mainly of the cholecystokinin octapeptide, which is also, according to some investigators, the predominating peptide in plasma. However, all of the many forms (see Fig. 1) which possess the C-terminal octapeptide also have CCK bioactivity. An intermediary form of 22 amino acids has recently been found to occur in both tissue and plasma and may, together with the octapeptide, be regarded as the hormone pro-

per (Walsh et al., 1982). In pigs, HCl in rather large amounts (50 ml 0.1 M) increases greatly the release of CCK, whereas in anaesthetized animals other stimuli like lipids are ineffective; probably, this is due to restricted absorption of lipids in anaesthetized animals. In humans, mixed meals and lipids are effective stimuli for CCK-release, and it has been shown that if reproduced by infusions of CCK octapeptide, these responses are sufficient to significantly influence pancreatic enzyme secretion (Walsh et al., 1982). Electrical stimulation of the vagus nerves cause a slow release of CCK; the responsible transmitter may be GRP, which is a potent stimulus for CCK-secretion (Cantor et al., 1984). In the pig's pancreas, both CCK-8, -33 and -39 are potent stimuli to the pancreatic enzyme secretion. Thus, it is likely that a hormonal regulation of pancreatic enzyme secretion by CCK is an essential mechanism in pigs (Jensen et al., 1981a; 1981b).

Gastric Inhibitory Polypeptide, GIP, is produced by open cells of the upper intestinal mucosa of the pig, and is found in mainly two forms, one corresponding to the originally isolated peptide of 42 amino acids and another somewhat larger form (Krarup and Holst, 1984). GIP is secreted in response to luminal stimuli, both lipids and carbohydrates, and it has been shown that only actively absorbed carbohydrates release GIP (Sirinek et al., 1983). GIP secretion is not influenced by any of the division of the autonomic nervous system (Lauritsen & Holst, unpublished). GIP was isolated on the basis of its inhibitory effect on acid secretion in denervated gastric pouches in dogs (Brown, 1982). The inhibitory effect, however, is neither seen in animals nor in man with intact innervation of the stomach. Likewise, in the pig, activity in the vagal nerves abolishes the inhibition of gastrin secretion caused by GIP (Holst et al., 1983b). Thus, the physiological importance of GIP as an enterogastrone is yet unsettled. GIP, a member of the glucagon family of peptides, is a potent secretagogue for insulin secretion, and is believed by many to be the most important incretin, i.e. the intestinal endocrine factor responsible for the increased secretion of insulin in response to glucose, when the latter is given by the oral versus the intravenous route. Some authors therefore coined a new meaning to the acronym, GIP: Glucose dependent Insulin-releasing Peptide. GIP is probably not the only incretin, but is likely to be of major significance for the control of insulin secretion also in the pig (Jensen et al., 1981c).

Motilin is a peptide of 23 amino acids, which is found in endocrine cells of the upper intestinal mucosa, and in the circulation (Christofides and Bloom, 1981). Motilin is released by luminal acid in pigs (Modlin et al., 1978). Most studies, however, have been made in dogs and man. It is released in response to meals. It is also released by electrical stimulation of the vagus nerves, and both this effect and spontaneous oscillations of motilin concentrations in plasma are inhibited by atropine, suggesting involvement of classical cholinergic pathways (Lee et al., 1981). Infusions of motilin cause accelerated gastric emptying. The role of motilin in the generation of the migratory motility complexes (MMC) in man and dogs has attracted considerable interest. The evidence today favours a role for motilin in the generation of the MMCs in the upper intestine; however, motilin may not initiate the MMC (Lee et al., 1983; Sarna et al., 1983). Motilin also shows molecular heterogeneity but little is known about the chemistry of the components other than the 23-amino acid peptide itself.

Endocrine cells of the lower intestine produce and secrete three hormonal peptides, whose roles in metabolism are not yet well established. Neurotensin, a putative neurotransmitter in the central nervous system, is produced in the socalled N-cells, and released by lipids and carbohydrates (Hammar & Leeman, 1981; Goedert, 1984). After its release the peptide is rapidly cleaved to smaller forms. PYY is one of the members of the PP family, of 36 amino acids and 50% homology with pancreatic polypeptide (Tatemoto, 1982a). Its spectrum of activity is far from established. It has recently been found to be stored together with the third peptide, enteroglucagon, in the granules of the so-called L-cell (Sundler et al., 1984). Enteroglucagon covers (at least) two peptides, glicentin and oxyntomodulin (both isolated from pig extracts); both contain the entire glucagon sequence plus extensions at either both termini or only the C-terminus (Holst, 1983). In addition, the enteroglucagon precursor gives off the peptides, GLP-1 and GLP-2 (see Table 1), and it has recently been established that both enteroglucagon and GLPs are released from the pig's ileum, when stimulated with glucose or the neuropeptide GRP (Table 1). (Holst & Ørskov, unpublished studies). Enteroglucagon may act by way of its glucagon sequence on liver and pancreatic endocrine function (Holst, 1985a), but for all of the three peptides interest has focused on their activity on the gastric acid production; all have been reported to

inhibit acid secretion. PYY has also been reported to inhibit pancreatic exocrine secretion and may be identical to pancreotone, a supposedly hormonal inhibitor of pancreatic secretion, contained in intestinal extracts, but never chemically defined.

There is a report on gross intestinal villous hypertrophy in a patient with what appeared to be an enteroglucagon-producing tumour (Gleeson et al., 1971). Furthermore, in numerous studies a remarkable correlation between intestinal epithelial growth and plasma concentrations of enteroglucagon have been found under clinical or experimental conditions with greatly varying rates of epithelial renewal and growth. Possibly, one of the main effects of enteroglucagon, therefore, is to control intestinal trophism (Bloom and Polak, 1982).

PARACRINE REGULATION

The concept of paracrine regulation has been alluded to several times above. It has also been pointed out that the most suggestive evidence for paracrine control stems from the peculiar morphology of cells suspected of paracrine secretion. However, it must be said that unambiguous proof for paracrine control is not available for any system

The gastric secretions, both endocrine and exocrine, and the endocrine pancreas are the key targets for paracrine control. Numerous somatostatin producing cells are found in close relation to the parietal cells in the gastric mucosa of most species, including pigs (Holst et al., 1983c). In view of the exceedingly potent inhibitory activity of somatostatin on parietal cell secretion, a paracrine regulation is highly likely. The somatostatin secretion, in turn, may be regulated by hormones like glucagon, GIP and gastrin, all of them known to stimulate somatostatin secretion. Electrical stimulation of the vagus nerves strongly inhibits somatostatin-secretion (whereas sympathetic nerve stimulation has little effect), and this mechanism may thus contribute to the enhancing effect of vagal activity on acid secretion (Holst et al., 1981; Holst et al., 1983b). Somatostatin cells are also found in close relation to the gastrin cells in the pig antrum (Holst et al., 1983c). The secretion of gastrin and somatostatin shows a characteristic inverse relationship; acid in the antral lumen stimulates somatostatin secretion and inhibits gastrin secretion; vagal activity enhances gastrin secretion and inhibits somatostatin secretion (Holst

et al., 1983b). That the two are indeed functionally linked in a feedback manner, is indicated by experiments with antisera against somatostatin; if administered to the perfused antrum gastrin secretion may be enhanced. Likewise, administration of a somatostatin-antagonist, D-Ala_5, D-$Tryp_8$-somatostatin may abolish the inhibition of gastrin secretion caused by luminal acid (Holst et al., 1984a). Although the coupling between gastrin and somatostatin secretion is probably not complete, it is highly likely that gastrin secretion is indeed subjected to paracrine control as described.

Also somatostatin in the pancreatic islets is believed to regulate insulin and glucagon secretion in a paracrine manner. In various preparations of pancreatic islets, antiserum against somatostatin has had an enhancing effect on insulin and/or glucagon secretion (Honey et al., 1981). In the pig pancreas there is generally an inverse relationship between somatostatin secretion and insulin and glucagon secretion; again the inverse coupling may be broken, for example during stimulation with arginine, which will stimulate the secretion of all four islet peptides (Holst et al., 1980a).

It may be that the somatostatin cells in the intestinal mucosa have similar paracrine functions and that other cells may have similar functions; but presently there is little experimental evidence to support this.

NEURAL REGULATION

There is no doubt that neural regulation is important for the coordinated functions of the digestive organs, also in pigs. From the vast surgical experience in man it is clear that parasympathetic denervation, vagotomy, has limited effects on the overall functions of the gastrointestinal tract, but this is due to an abundancy of regulatory mechanisms; if one mechanism fails, others take over. In addition, the nervous system of the gut as well as the stomach and the pancreas may function in spite of extrinsic denervation; in fact, complete reflex arches, like those involved in the propagation of the peristaltic wave, remain functioning in spite of extrinsic denervation. Because of this, the name "little brain" has been coined to the system (Gershon, 1981). The name is apt, because - like in the central nervous system -

a large number of different cell types may be identified in the ganglia of the enteric nervous system; it has blood-enteric-nervous-system barrier, and a large number of neurotransmitters participate in the transmission of signals. Among these, one finds many peptides, including VIP, enkephalins, substance P, cholecystokinin, Gastrin Releasing Polypeptide, somatostatin and NPY. For some of the peptides their regulatory functions in pigs have been worked out in detail.

The neuropeptide VIP (and the associated gene product, PHI) is found in numerous fibres in all organs of the porcine gastrointestinal tract (Fahrenkrug, 1979). Like in other animals it is likely that VIP is an important neurotransmitter in the salivary glands (Lundberg, 1981). VIP-nerves are abundant in all layers of the stomach wall, and are likely to be responsive for the socalled adaptive relaxation of this organ. VIP-nerves are found in close relationship to blood vessels in all organs, and is likely to be involved in regulation of blood flow. The VIP-nerves synapse with vagal fibres, and blood flow increases may be triggered this way, but it is possible that local reflexes may also initiate a VIP-mediated increases in blood flow (Fahrenkrug, 1979). VIP-nerve fibres are particularly abundant around sphincters (Alumets et al., 1979), and there is quite compelling evidence that VIP controls relaxation of the lower esophageal sphincter in pigs (Aggestrup & Jensen, 1982). The mentioned effects are all derived from the effect of VIP (and PHI) on smooth muscles. VIP-nerves also control secretory processes. VIP-nerves are abundant in the submucosa of the intestine, and VIP potently enhances intestinal secretion. Intravenous infusion of VIP in pigs to levels corresponding to those observed in humans with VIP producing tumours cause sprouting diarrhoea within hours (Modlin et al., 1978b). The pig pancreas is heavily innervated with VIP/PHI containing nerve fibres, and it is very likely that the remarkable effect of electrical stimulation of the pancreatic vagal nerve supply in the secretion of fluid and bicarbonate, is mediated by neuronally released VIP (Holst et al., 1984b).

Another abundant neuronal peptide is Gastrin Releasing Peptide, GRP, which is found in nerve fibres in the gastric mucosa and in the pancreas. GRP nerves are probably responsible for the socalled "cephalic phase" of gastrin secretion; thus, "desensitization" with large doses of GRP, which renders the gastrin cells completely unresponsive to usually effective doses of GRP, also abolishes the enhancing effect

of electrical stimulation of the vagal nerve supply to the antrum (Holst et al., 1984a, Knuhtsen et al., 1984). In the pancreas, GRP-nerves are likely to be involved, together with acetylcholine, in the enhancing effect of parasympathic nerve activity on enzyme secretion (Knuhtsen et al., 1985). On the whole, there is little doubt, that neural regulation of pancreatic secretion in pigs is of much greater significance than previously anticipated (Holst, 1985b).

The neuropeptide NPY was recently isolated from porcine nervous tissue (Tatemoto, 1982b) and found to coexist with noradrenaline in sympathetic nerve fibres in many regions of the intestinal tract (Ekblad et al., 1984). NPY has been found to potentiate the effect of noradrenaline in many systems, and is likely to explain part of the many inhibitory effects of activity in the sympathetic nervous system.

REFERENCES

AGGESTRUP, S., & JENSEN, S. L. 1982. Regul. Pept. 4:155-162.

ALUMETS, J., FAHRENKRUG, J., HÅKANSON, R., SCHAFFALITZKY DE MUCKADELL, O. B., SUNDLER, F., UDDMAN, R. 1979. Nature 280:155-156.

BALDISSERA, F. G.A., & HOLST, J. J. 1985. Regul. Pept. in press.

BAUER, W., BRINER, U., DOEPFNER, R., HALLER., HUGUENIN, P., MARBACH, P., PETCHER, T. J., & PLESS, J. 1982. Life Sciences 31: 1133-1138.

BLOOM, S. R., & POLAK, J. M. 1982. In: (ROBINSON, J. W. L., RIECKEN, E. O., & DOWLING, R. H. eds.): Mechanism of intestinal adaptation. pp. 189-199. MTPress, Lancaster.

BOEL, E., VUUST, J., NORRIS, F., WIND, A., REHFELD, J. F., & MARCKER, K. 1983. Proc. Natl. Acad. Sci. USA 80: 2866-2869.

BROWN, J. C. 1982. Gastric Inhibitory Polypeptide. Monographs on endocrinology. Vol. 24. Springer-Verlag, Berlin.

CANTOR, P., HOLST, J. J., & REHFELD, J. F. 1984. Dig. Dis. Sci. 29 (Suppl.) : A-14.

CHEY, W. Y., KIM, M. S., LEE, K. Y., & CHANG, T.-M. 1981. Amer. J. Physiol. 240: G239-G244.

CHRISTIANSEN, L. A., LINDKÆR JENSEN, S. L., REHFELD, J. F., STADIL, F., & CHRISTENSEN, K. C. 1978. Scand. J. Gastroenterol. 13: 719-725.

CHRISTOFIDES,N. D., & BLOOM, S. R. 1981. In: BLOOM, S. R., & POLAK, J. M. eds. Gut Hormones. pp. 273-279. Churchill Livingstone, Edinburgh.

CRANWELL, P. D., & HANSKY, J. 1980. Res. Vet. Sci. 29: 85-88.

DESCHENES, R. J., LORENZ, L. J., HAUN, R. S., ROOS, B. A., COLLIER, K. J., & DIXON, J. E. 1984. Proc. Natl. Acad. Sci. USA 81: 726-730.

EKBLAD, E., WAHLESTEDT, C., EKELUND, M., HÅKANSON, R., & SUNDLER, F. 1984. Front. Horm. Res. 12: 85-90.

FAHRENKRUG, J. 1979. Digestion 19: 149-169.

FAHRENKRUG, J., SCHAFFALITZKY DE MUCKADELL, O. B. & HOLST, J. J. 1977. Scand. J. Gastroenterol. 12: 273-279.

FAHRENKRUG, J. F., SCHAFFALITZKY DE MUCKADELL, O. B., HOLST, J. J. & LINDKÆR JENSEN, S. 1979. Amer. J. Physiol. 237: E535-E541.

GERSHON, M. D. 1981. Ann. Rev. Neurosci. 4: 227-272.

GLEESON, M. H., BLOOM, S. R., POLAK, J. M., HENRY, K., & DOWLING, R.H. 1971. Gut 12: 773-778.

GOEDERT, M. 1984. Trends Neurosci. Issue of January, pp. 3-5

GUILLEMIN, R. 1983. Clin. Res. 31: 338-341.

HAMMER, R. A., & LEEMAN, S. 1981. In: (BLOOM, S. R., & POLAK, J. M., eds.). Gut hormones. pp. 290-299. Churchill Livingstone, Edinburgh

HOLST, J. J. 1983. Gastroenterology. 84: 1602-1613.

HOLST, J. J. 1985a. Enteroglucagon. In: (MUTT, V., ed.). Adv. Metabol. Dis. 11: In press.

HOLST, J. J. 1985b. Neural regulation of pancreatic exocrine function. In: BROOKS, DI MAGNO, GARDNER, GO, LEBENTHAL, & SCHEELE, eds. The exocrine pancreas, basic and clinical aspects. Raven Press, New York.

HOLST, J. J., SCHAFFALITZKY DE MUCKADELL, O. B. & FAHRENKRUG, J. 1979. Acta Physiol. Scand. 105: 33-51.

HOLST, J. J., JENSEN, S. L., & SCHWARTZ, T. W. 1980a. Acta Physiol. Scand. 109: 9-13.

HOLST, J. J., JENSEN, S. L., SCHAFFALITZKY DE MUCKADELL, O. B. & FAHRENKRUG, J. 1980b. Front. Hormone Res. 7: 119-131.

HOLST, J. J., LAURITSEN, K. B., LINDKÆR JENSEN, S., SCHAFFALITZKY DE MUCKADELL, O. B., & VAGN NIELSEN, O.V. 1981a. J. Physiol. 318: 327-337.

HOLST, J. J., SOTTIMANO, C., OLESEN, M., JENSEN, S. L., NIELSEN, O. V. 1981b. Peptides 2 (Suppl. 2): 215-221.

HOLST, J. J., JENSEN, S. L., KNUHTSEN, S. & NIELSEN, O. V. 1983a. Am. J. Physiol. 245: E542-E548.

HOLST, J. J., JENSEN, S. L., KNUHTSEN, S., NIELSEN, O. V. & REHFELD, J. F. 1983b. Amer. J. Physiol. 244: G515-G522.

HOLST, J. J., KNUHTSEN, S., JENSEN, S. L., FAHRENKRUG, J., LARSSON, L.-I., & NIELSEN, O. V. 1983c. Scand. J. Gastroenterol. 18 (Suppl. 82): 85-99.

HOLST, J. J., KNUHTSEN, S., JENSEN, S. L., NIELSEN, O. V., & POULSEN, S. S. 1984a. Dig. Dis. Sci. 29 (Suppl.): A-36.

HOLST, J. J., FAHRENKRUG, J., JENSEN, S. L., NIELSEN, O. V. & POULSEN, S. S. 1984b. Regul. Pept. 8: 245-259.

HONEY, R. N., ARIMURA, A., & WEIR, G. C. 1981. Endocrinology 109: 1071 1074.

ITOH, N., OBATA, K., YANAIHARA, N., OKAMOTO, H. 1983. Nature 304: 547-549.

JENSEN, S. L., FAHRENKRUG, J., HOLST, J. J., KUHL, C., NIELSEN, O. V. REHFELD, J. F., & SCHAFFALITZKY DE MUCKADELL, O. B. 1978. Amer. J. Physiol. 61: E781-E789.

JENSEN, S. L., HOLST, J. J., NIELSEN, O. V., & REHFELD, J. F. 1981a. Digestion 22: 305-309.

JENSEN, S. L., REHFELD, J. F., HOLST, J. J., FAHRENKRUG, J. F., NIELSEN, O. V. & SCHAFFALITZKY DE MUCKADELL, O. B. 1981b. Acta Physiol. Scand. 111: 225-231.

JENSEN, S. L., HOLST, J. J., MOODY, A. J., NIELSEN, O. V., & LAURITSEN, K. B. 1981c. Acta Physiol. Scand. 111: 233-238.

JOHNSON, L. R. 1977. Ann. Rev. Physiol. 39: 135-158.

JORPES, J. E. & MUTT, V. 1973. Handb. Exp. Pharmacol. Vol. 34, Springer Verlag, Berlin.

KNUHTSEN, S., HOLST, J. J., KNIGGE, U., OLESEN, M., & NIELSEN, O. V. 1984. Gastroenterology. 87: 372-378.

KNUHTSEN, S., HOLST, J. J., JENSEN, S. L., KNIGGE, U. & NIELSEN, O. V. 1985. Amer. J. Physiol. In press.

KRARUP, T. & HOLST, J. J. 1984. Regul. Pept. 9: 35-46.

KRIEGER, D. T. 1983. Clin. Res. 31: 342-453.

LARSSON, L.-I. 1980. Am. J. Physiol. 239: G237-G246.

LEE, K. Y., CHANG, T. & CHEY, W. Y. 1981. Life Sci. 29: 1093-1097.

LEE, K. Y., CHANG, T.-M., & CHEY, W. Y. 1983. Amer. J. Physiol. 245: G547-G553.

LUNDBERG, J. & HOKFELT, T. 1983. Trends. Neurosci. 6: 235-333.

LUNDBERG, J. M. 1981. Acta Physiol. Scand. Suppl. 496: 1-57.

MODLIN, I. M., MITZNEGG, P., & BLOOM, S. R. 1978a. Gut 19: 399-402.

MODLIN, I. M., BLOOM, S. R., & MITCHELL, S. J. 1978b. Gastroenterology 75: 1051-1054.

NAWA, H., HIROSE, T., TAKASHIMA, H., INAYAMA, S., & NAKANISHI, S. 1983. Nature 306: 32-36.

NIEUWENHUIJZEN KRUSEMAN, A. C., LINTON, E. A., LOWRY, P. A., REES, L. H., & BESSER, G. M. 1982. Lancet II: 1245-1246.

REHFELD, J. F. 1981. Am. J. Physiol. 240: G255-G266.

REHFELD, J. F., HOLST, J. J. & JENSEN, S. L. 1981. Regul. Pept. 3: 15-28.

REICHLIN, S. 1983. New Engl. J. Med. 309: 1495-1501.

RICHTER, K., KAWASHIMA, E., EGGER, R., & KREIL, G. 1984. EMBO j. 3: 617-621.

ROSSIER, J., LISTON, D., PATEY, G., CHAMINADE, M., FOUTZ, A. S., CUPO, A., GIRAUD, P., ROISIN, M., HENRY, J. P., VERBANCK, P., & VANDERHAEGEN, J.-J. 1984. Cold Spring Harbour Symp. 48: 393-404.

SAID, S. I. 1984. Peptides 5: 143-150.

SARNA, S., CHEY, W. Y., CONDON, R. E., DODDS, W. J., MYERS, T., & CHANG, T.-M. 1983. Amer. J. Physiol. 245: G277-G284.

SCHAFFALITZKY DE MUCKADELL, O. B. 1980. Scand. J. Gastroenterol. Suppl. 61: 1-20.

SCHWARTZ, T. W. 1983. Gastroenterology 84: 5-20.

SCHUSDZIARRA, V. 1980. Horm. Metab. Res. 12: 563-577.

SCHAFFALITZKY DE MUCKADELL, O. B., FAHRENKRUG, J. & HOLST, J. J. 1977. Scand. J. Gastroenterol. 12: 267-272.

SIRINEK, K. R., LEVINE, B. A., O'DORISIO, T. M., & CATALAND, S. 1983. Proc. Soc. Exp. Biol. Med. 173: 379-385.

SPINDELL, E., CHIN, W. W., PRICE, J., REES, L. H., BESSER, G. M., & HABENER, J. F. 1984. Proc. Natl. Acad. Sci. USA. 81: 5699-5703.

SUNDLER, F., BOTTCHER, G., ALUMETS, J., EKBLAD, E., HÅKANSON, R., & SCHWARTZ, T. W. 1984. Dig. Dis. Sci. 29 (Suppl.): A-84.

TATEMOTO, K. 1982a. Proc. Natl. Acad. Sci. USA. 79: 2514-2518.

TATEMOTO, K. 1982b. Proc. Natl. Acad. Sci. USA.79: 5485-5489.

TATEMOTO, K. & MUTT, V. 1978. Proc. Natl. Acad. Sci. USA 78: 1415-1419.

TATEMOTO, K. 1984. Dig. Dis. Sci. 29 (Suppl.): A-87.

VALE, W., RIVIER, C., BROWN, M., SPIESS, J., KOOB, G., SWANSON, L., BILEZIKIJAN, L., BLOO, F., & RIVIER, J. 1983. Rec. Prog. Horm. Res. 39: 245-270.

VEBER, D., et al. 1984. Life Sciences 34: 1371-1378.

WALSH, J. H., LAMERS, C. B., & VALENZUELA, J. E. 1982. Gastroenterology 82: 438-444.

WALSH, J. H. & LAM, S. K. 1980. Clinics Gastroenterol. 9: 562-592.

WEBB, S., LEVY, I., WASS, J. A. H., LLORENS, A., PENMAN, E., CASAMITJANA, R., WU, P., GAYA, J., MARTINEZ, M. J., & RIVERA, F. 1985. J. Clin. Endocrinol. Metab. In press.

YOO, O. J., POWELL, C. T., & AGARWAL, K. L. 1982. Proc. Natl. Acad. Sci. USA. 79: 1049-1053.

ØRSKOV, A. C., KNUHTSEN, S., BALDISSERA, F., POULSEN, S. S. & HOLST, J. J. 1985. Acta Physiol. Scand. In press.

INFLUENCE OF BILIARY AND PANCREATIC SECRETIONS ON ENDOLUMINAL pH AND DUODENAL MOTILITY IN THE PIG.

ABELLO J., CORRING T., LAPLACE J.P.
Laboratoire de Physiologie de la Nutrition
INRA - F 78350 Jouy-en-Josas (France)

SUMMARY

The consequences on the intra duodenal pH of the continuous extracorporeal derivation of bile or of pancreatic secretion, were studied in fasted or fed pigs, whose duodenal pH and motility were recorded during 8 hr periods. The role of bile and pancreatic secretion in the neutralisation of duodenal contents was established in fasting pigs, while the acid pH in fed animals was almost unaffected by the deprivation of these two secretions.

INTRODUCTION

The intraduodenal pH is an important factor determining the extent of enzyme hydrolysis and nutrient absorption. Several components are responsible for the pH value : gastric acid secretion, buffer ability of feed, H^+ absorption by the mucosa, bicarbonate secretion from intestinal, biliary and pancreatic sources. The neutralisation ability of pancreatic juice has been established in man (DUTTA, RUSSEL and IBER, 1979). That of bile remains to evidence. Moreover, the coordination of motor and secretory events is important (e. g. bicarbonate release when gastric acid is emptied). A secretory component of myoelectric migrating complex (MMC) is well known in fasted man and dog as well (LAPLACE, 1984). That results in a cyclic variation of duodenal pH, acid during the phase II and close to neutrality during phase I and III of MMC. Such a pH pattern has been assigned in the pig to the intermittent gastric emptying of acid digesta (BUENO and FIORAMONTI, 1982). However we recently evidenced (see LAPLACE, 1984), in the pig, the existence of a secretory component of MMC, i.e. a cyclic variation of biliary and pancreatic flow to the duodenum.Thus the aim of the present work was to evaluate the role of bile and pancreatic secretion in determining the duodenal pH value in fasted and fed pigs, as well as to assess their respective contribution to the cyclic variation of pH along the MMC cycle.

MATERIALS AND METHODS

Ten Large White pigs (51 kg mean live weight) were submitted to the chronic fistulation of either the common bile duct (5 pigs) or the pancreatic duct (5 pigs). In addition they were all fitted with a permanent tubing in the duodenal bulb for the continuous return of the secretions (JUSTE, CORRING and LE COZ, 1983), and with a duodenal T shaped cannula (around 20 cm below the pylorus) allowing to record the pH using an INGOLD combined glass microelectrode. The intraduodenal pH and the duodenal electromyogram (EMG) recorded from chronic electrodes (LAPLACE, 1978) located approximately at 5, 10, 20 and 25 cm distal to the pylorus were registered for 8 hr-periods. Recordings were performed in fasted or fed pigs, with both secretions returned or without either bile or pancreatic juice restitution, depending on the pig. Each animal was tested twice in each situation resulting from this factorial combination. The pH was described by the percentage of the total time during which it varied within one of the four ranges : $pH > 6$; $6 > pH > 4$; $4 > pH > 2$; $pH < 2$. A mean of the pH values was calculated separately for each phase of MMC.

RESULTS AND DISCUSSION

In fasted pigs, with secretions normally flowing into the duodenum a $pH > 6$ was recorded for 70 p. cent of the 8 hr-period. Lower pH occurred for short periods : 19 p.cent ($6 > pH > 4$), 8 p.cent ($4 > pH > 2$) or less than 1 p.cent ($pH < 2$) of the time. Total suppression of bile restitution resulted in a shortened time of $pH > 6$ (- 30 per cent). Meanwhile, there was a significant increase of time with acid pH either $6 > pH > 4$ (+ 31 per cent) or $4 > pH > 2$ (+ 23 per cent). In the same way, the total deprivation of pancreatic secretion strongly reduced the time of $pH > 6$ (- 53 per cent) to the benefit of the acid periods which were increased by + 30 per cent ($6 > pH > 4$) and + 22 per cent ($4 > pH > 2$). These observations in fasted pigs point out (Figure 1) the important role of pancreatic secretion in the neutralisation of duodenal juice, and that of bile as well.

Feeding pigs with 800g standard diet resulted in a significant acidification over the 8 postprandial hours with a 36 per cent ($6>pH>4$)

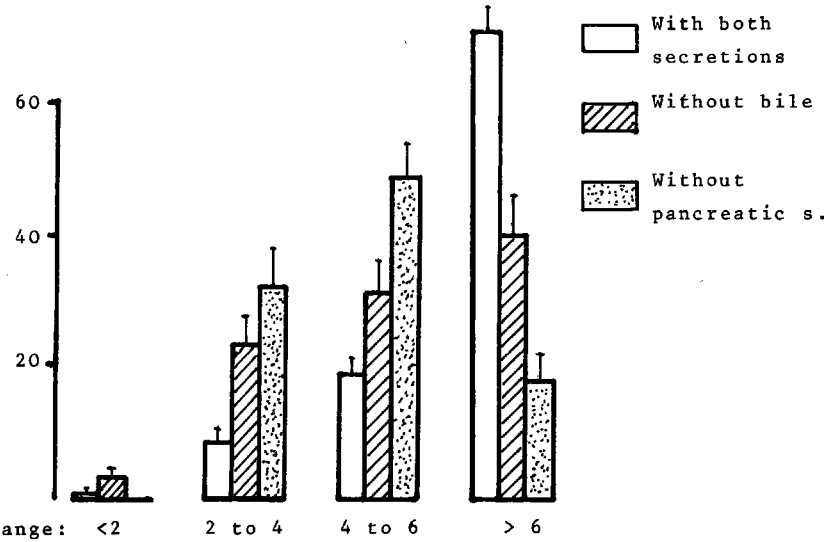


Figure 1. Relative importance of bile and pancreatic secretion in the neutralisation of duodenal juice in fasted pigs (as per cent of recording duration).

and a 6 p.cent increase (4 > pH > 2), while there was a decrease (-43 per cent) of the time with a pH > 6. But in these fed animals, neither the deprivation of bile, nor that of pancreatic secretion, induced significant variations of the pH pattern.

Depriving pigs of bile or pancreatic secretion did not significantly affect the duration of fasting MMC (60 to 70 mn) nor that of their constitutive phases. The cyclic pH pattern, which parallels the MMC cycle, persisted. However the mean values of pH calculated for each phase of MMC (table 1) showed a significant acidification, whatever the MMC phase considered, when bile was not returned and even more when pancreatic secretion was not returned to the pig.

Therefore bile and/or pancreatic secretions are not directly responsible for the sudden relative alcalinisation occurring during phase III and persisting over phase I. Nevertheless they play a significant role in the neutralisation of duodenal contents i.e. of acid emptied by the stomach. That is true in fasting pigs only. In fed pigs the pH is much more acid for hours after the meal : bile and pancreatic secretions are uneffective in the latter conditions,

Table 1. Influence of the suppression of bile or pancreatic secretion restitution on the mean value of the pH during the 3 consecutive phases of MMC in fasting pigs.

MMC phase	I	II		III
		1st half	2nd half	
With bile and pancreatic s.	7.17	6.11	5.81	6.62
	np 112	np 95	np 95	np 113
	nv 1834	nv 2042	nv 1946	nv 419
Without bile	6.17	4.86	4.97	5.40
	np 74	np 66	np 66	np 74
	nv 568	1293	nv 1264	nv 233
Without pancreatic s.	5.56	4.32	4.11	5.14
	np 45	np 38	np 38	np 46
	nv 465	nv 1028	nv 1009	nv 142

np = number of MMC phases considered

nv = number of pH values (measured every min)

probably due to the large volume of acid digesta emptied by the stomach. That points out the importance of the secretions to determine the duodenal pH value in fasted animals. But food overpasses the neutralisation abilities of the digestive secretions.

REFERENCES

BUENO, L., FIORAMONTI, J. 1982. In "Motility of the digestive tract". (Ed. WIENBECK M.), Raven Press, New York p. 169-173.

DUTTA, S.K., RUSSEL, R.M. & IBER, F.L. 1979. Dig. Dis. Sci., 24: 775-780.

JUSTE, C., CORRING, T. & LE COZ, Y. 1983. Lab. anim. Sci. 33 : 199-202.

LAPLACE, J.P. 1978. Ann. Zootech. 27 : 377-408.

LAPLACE, J.P. 1984. Reprod. Nutr. Develop. 24 : 707-765.

PLASMA AMINO ACID LEVELS AND ENDOCRINE RESPONSE IN THE PIG FOLLOWING INTAKE OF DIFFERENT DIETARY PROTEINS

C.A. BARTH, K.E. SCHOLZ, H. HAGEMEISTER
Institut für Physiologie und Biochemie der Ernährung
Bundesanstalt für Milchforschung, Hermann-Weigmann-Straße 1,
D-2300 Kiel, Germany

SUMMARY

It was the purpose of this study to describe postprandial venous plasma changes of amino acids and hormones following consumption of casein or soy protein. There was a greater postprandial rise of arginine and lower rise of methionine concentration following soy as compared to casein. The difference persisted for 7 hours but was not apparent in the fasted state. No differences of serum insulin or triiodothyronine concentrations were observed.
It is concluded that in the pig different dietary proteins provoke differences of postprandial amino acid levels but not of insulin or triiodothyronine.

INTRODUCTION

The biological value of dietary protein is traditionally expressed in terms of nitrogen balance. In a more general sense it could and should include a wide array of biochemical parameters related to general health and physiology, as for example serum lipids, organ functions, bioavailibility of essential nutrients, tissue protein turnover, amino acid kinetics, hormonal responses etc.
In this connection we have compared the circadian changes of amino acids and several hormones following a 4 weeks consumption of an animal and a plant protein.

MATERIAL AND METHODS

6 adult female Göttingen minipigs (body weight 25.3-34.9 kg) were fed a semisynthetic diet composed of 22 weight % protein, 49 % cornstarch, 15 % fat, 6 % cellulose, 8 % mineral-vitamin mixture and 1 % cholesterol, assuring a daily intake of 0.29 MJ/kg body weight of energy. The body weight of the animals changed less than 3.5 % of the

initial value.
Diets were fed for 5 weeks (twice a day 50 % of the daily ration). A two weeks experimental period ensued. During this period postprandial blood samples were taken on the 1th and 8th day following the morning meal (8:00 A.M.) as indicated in the legends.
24 hrs. and 48 hrs. later fasting samples were taken. During this time the animals had no food. Then the ordinary feeding schedule resumed and on the 8th day of the experimental period the same sampling scheme was repeated. Sampling was done by aspirating blood through a permanent venous catheter (V. jugularis).
Amino acids were determined in a sulfosalicylic-acid supernatant of heparinized plasma and hormones were measured in serum by radio-immuno-assay.

RESULTS AND DISCUSSION

There was no difference of fasting plasma amino acid levels due to the kind of protein consumed (Tab. 1). However, highly significant differences were observed for postprandial plasma levels of serine, valine, methionine, isoleucine, tyrosine, phenylalanine, ornithine and arginine.
The most marked differences were the 59 % higher arginine and 39 % lower methionine plasma levels following soy as compared to casein. These differences persisted for 7 hrs. after the meal and were concordant with the amino acid composition of the proteins consumed. They most probably reflect the different amino acid composition of casein and soy, as did the differences of valine and tyrosine.
The unexpected rise of isoleucine and phenylalanine following soy may be due to metabolic transformations by the intestine and/or liver; however, portal plasma levels and blood flow have to be determined before final conclusions can be drawn. There were no significant differences of urea and ammonium ion concentrations between the dietary groups.
It is noteworthy that casein caused higher venous methionine concentrations. Dietary methionine is known to prevent hepatic lipid accumulation and raise serum lipid levels (Hevia et al., 1980). Probably this explains why dietary casein causes higher rates of hepatic very low density lipoprotein secretion in rats (Sugano et

Table 1: Fasting and postprandial venous plasma amino acid concentrations in groups of minipigs consuming either soy protein isolate (n=6) or casein (n=6). Values in boxes were highly significantly different from the corresponding soy values ($p<0.001$). *$p < 0.01$. Averages of 12 blood samples. Tryptophan not included. Fasting for 24 hours.

	CASEIN					SOJA				
	Fast	Time after Feeding				Fast	Time after Feeding			
Amino acid	ing	25'	155'	310'	430'	ing	25'	155'	310'	430'
					/umol/l					
Tau	53	87	52	40	36	40	99	41	44	42
Asp	7	8	8	7	6	8	11	7	9	8
Thr	186	295	359	273	256	195	338	278	249	235
Ser	135	189*	185	134	132	147	262	198	167	159
Glu	288	383	375	334	333	271	431	330	314	295
Gly	607	597	581	542	558	575	654	594	596	594
Ala	256	412	382	291	304	276	453	404	315	300
Cit	58	60	102	89	84	66	67	86	97	91
Val	388	533	578*	479	460	397	525	431	418	409
Cys	59	68	55	54	60	72	77	57	56	64
Met	31	84*	81	55*	46	28	60	38	32	31
Ileu	185	270*	274	179	168	193	343	245	216	204
Leu	242	431	390	281	261	264	460	305	268	255
Tyr	87	244	262*	189	175*	92	242	176	149	136
Phe	78	135*	131	94	88	86	178	127	112	105
Orn	58	108*	132	105	89	66	138	137	132	109
Lys	178	424	368	272	231	199	441	301	261	225
His	89	141	147	114	105	99	159	122	120	112
Arg	112	206	171	133*	119*	127	365	243	217	185
Σ	2997	4675	4633	3665	3511	3201	5303	4120	3772	3559

al., 1982a; Pfeuffer and Barth, 1984) and by this mechanism hyperlipidemia in rodents (Pfeuffer and Barth, 1985).

Table 2: Fasting and postprandial changes of hormones. $\bar{x} \pm$ S.E.M.

Hormone	Fasting (24 h)	Time after Feeding (min) 25	155	310	430
		CASEIN			
Insulin (µU/ml)	7 ± 2.7	58 ± 7.6	17 ± 1.9	19 ± 3.0	10 ± 3.2
T_3 (ng/dl)	47 ± 10.8	82 ± 9.6	74 ± 10.4	83 ± 8.4	90 ± 14.9
		SOJA			
Insulin (µU/ml)	7 ± 2.2	63 ± 18.0	22 ± 3.2	16 ± 4.4	14 ± 2.0
T_3 (ng/dl)	48 ± 8.5	79 ± 9.1	66 ± 11.4	75 ± 6.2	90 ± 10.1

Insulin and triiodothyronine concentrations were measured in order to correlate these with the simultanous plasma amino acid pattern. However, no significant differences of postprandial hormone levels were observed if casein- and soy-fed animals were compared (Tab. 2). We conclude that the diet-induced changes of amino acids do not cause differences of the endocrine response in the pig.

It is interesting that the pig - other than the rat (Sugano et al., 1982b) - does not seem to respond with higher insulin levels following dietary casein. This may be relevant for the fact that the pig - as man and monkey - does not show casein-induced hypercholesterolemia whereas rats are sensitive to casein.

REFERENCES

HEVIA, P., ULMAN, E.A., KARI, F.W. & VISEK, W.J. 1980. J. Nutr. 110: 1231-1239.

PFEUFFER, M. & BARTH, C.A. 1984. Ern. Umschau 31:246.

PFEUFFER, M. & BARTH, C.A. 1985. (submitted for publication).

SUGANO, M., KAZUNARI, T. & IDE, T. 1982a. J. Nutr. 112:855-862.

SUGANO, M., ISHIWAKI, N., NAGATA, Y. & IMAIZUMI, K. 1982b. Br. J. Nutr. 48:211-221.

STUDIES OF ZINC ABSORPTION IN THE 14 DAY OLD PIG USING ISOLATED INTESTINAL BRUSH BORDER MICROVILLI: COMPETITION BY OTHER METAL IONS

P. BLAKEBOROUGH, D.N. SALTER
National Institute for Research in Dairying
Shinfield, Reading RG2 9AT, U.K.

SUMMARY

The effect of Fe^{2+}, Fe^{3+}, Cu^{2+} and Cd^{2+} on the uptake of ^{65}Zn by brush border membrane microvilli isolated from the intestines of 14 day old pigs was investigated using a rapid filtration assay. Uptake of zinc by a saturable carrier mechanism operating at low zinc concentrations was inhibited in a competitive manner by Cd^{2+}, but not by Fe^{2+}, Fe^{3+} or Cu^{2+}. Zinc uptake at higher concentrations by a non-saturable mechanism, presumably involving passive processes, was however inhibited by Fe^{2+}, Fe^{3+} and Cu^{2+}. Results suggest that the saturable zinc uptake mechanism is specific to zinc and metals close to it in the periodic table, whereas the non-saturable mechanism is shared with other divalent and trivalent metals.

INTRODUCTION

The brush border membrane of the intestine controls the initial stage of the absorption of nutrients; that of transfer from the intestinal lumen into the enterocytes. Preparations of microvilli from this membrane have been of great value in understanding the mechanism of nutrient absorption (1).

Zinc uptake by rat brush border membrane microvilli is a biphasic process (2). At low zinc concentrations the metal is taken up by a saturable, carrier-mediated mechanism, whereas at higher concentrations uptake is not saturable and is probably due to passive processes. We have confirmed these results using pig intestinal microvilli (4) and here examine the effect of other trace elements on zinc uptake.

MATERIALS AND METHODS

$^{65}ZnCl_2$ (20μCi/μmole) was obtained from Amersham International

plc, Amersham, U.K. Brush border membrane microvilli were prepared from the frozen intestines of 14 day old milk formula-fed pigs as described in (3). Experiments to study uptake of ^{65}Zn were conducted in a final volume of 0.5ml buffer containing 20mM Tris/HCl, pH 7.5, 1-100μM ^{65}Zn and 0, 20 or 100μM $FeCl_3$, $FeCl_2$, $CuCl_2$ or $CdCl_2$. The reactions were initiated by adding the microvilli (50μg protein) and continued for 1min at 25°C. Reactions were terminated by adding 5ml ice-cold 20mM Tris/HCl, pH 7.5, followed by rapid filtration through a cellulose nitrate membrane of porosity 0.45μ. The filters were washed with buffer, dried in air, and transferred to vials. ^{65}Zn was estimated using a γ-scintillation spectrometer (Intertech CG 4000).

RESULTS

Fig. 1 shows the results obtained, plotted as ^{65}Zn uptake against zinc concentration. The effects on ^{65}Zn uptake when Fe^{3+}, Fe^{2+} or Cu^{2+} were used as the competing ions were similar. There was no inhibition of ^{65}Zn uptake when the zinc concentration was low (1-10μM). However, inhibition of ^{65}Zn uptake was observed at higher zinc concentrations (50-100μM). Lineweaver-Burk analysis gave K_M values for ^{65}Zn uptake which were similar (range: 40.8 - 44.4μM) for all these cations and independent of competing cation concentration. Both concentrations of Cd^{2+} significantly inhibited ^{65}Zn uptake at all zinc concentrations (Fig. 2). K_M values for ^{65}Zn uptake were 47, 68.6 and 68.2 μM respectively, for 0, 20 and 100μM Cd^{2+}.

DISCUSSION

Much information about the initial stage of zinc absorption by the pig has been deduced from studies using brush border membrane microvilli (4). Zinc uptake is optimal at pH 7-8 and large amounts of zinc can be taken up by 2 processes independent of an external source of energy. Up to 10μM zinc, uptake is due to a saturable, carrier-mediated process with a K_M of approx. 42.5μM. At higher concentrations of zinc a non-saturable process is also

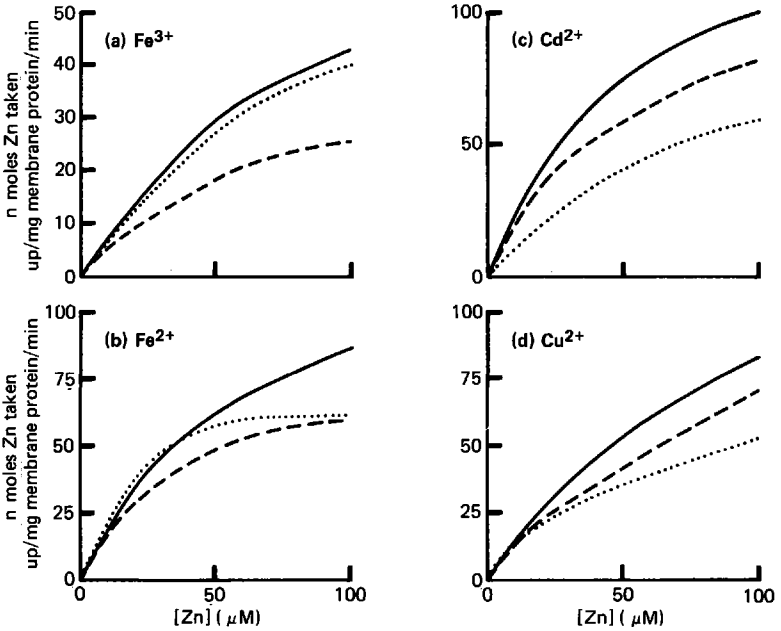


Fig. 1 The effect of trace metals in zinc uptake by microvilli

Microvilli were incubated for 1 min at 25°C with 1–100 μM ^{65}Zn and 0 (——); 20 (— —); or 100 (·····) μM metal ion (as indicated in the figure). Microvilli were collected by rapid filtration and assayed for ^{65}Zn. Results are the means of 3 determinations.

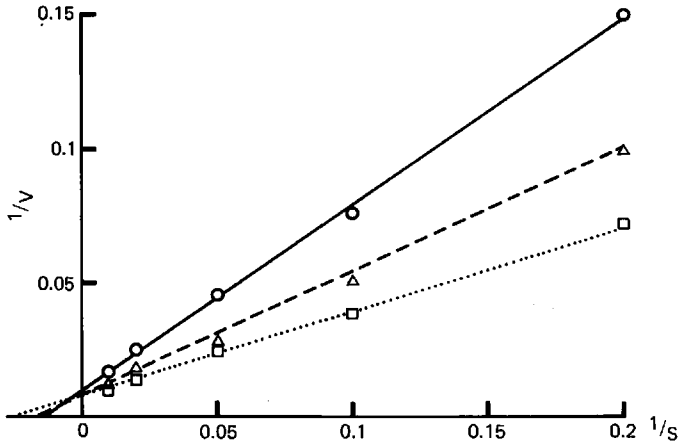


Fig. 2 Lineweaver-Burk plot of effect of Cd^{2+} on zinc uptake by microvilli.

The results shown in Fig 1(c) were re-plotted to produce a Lineweaver-Burk curve. (O—O) -0μMCd^{2+}; (△- -△) -20μMCd^{2+}; (□···□) -100μMCd^{2+}. Linear regression analysis was used to construct the best fitting straight lines through the experimental points

observed which at 100μM provides up to 50% of the uptake. In the region 1-10μM zinc it is possible to examine the saturable mechanism of zinc uptake with minimal contribution from the non-saturable one. Zinc uptake is linear up to approx. 2min, justifying the use of a 1min reaction time in initial velocity studies.

The saturable mechanism of zinc uptake is specific to the metal and others close to it in the periodic table. Of the metals tested only Cd^{2+} inhibited zinc uptake in the region 1-10μM zinc. Lineweaver-Burk analysis showed that inhibition by Cd^{2+} was competitive. Cd^{2+} is therefore able to share the absorptive mechanism of zinc at least as far as the enterocyte cytosol. The non-saturable mechanism of zinc uptake is a non-specific process as indicated by the fact that all 4 trace metals inhibited zinc uptake at 50-100μM zinc. It appears to be a process of passive diffusion or binding, shared by divalent and trivalent cations. In this respect the present results provide a biochemical basis for the reported metabolic interaction that occurs between zinc and copper, higher zinc concentrations resulting in alleviation of the symptoms of copper toxicity (5).

The concentration of zinc in the intestinal contents of pigs fed according to the estimated requirement level in the diet of 50mg/Kg DM (5) is likely to be in the higher range studied here, and therefore subject to competition by other metal ions. The specific saturable mechanism for zinc uptake will however be functioning optimally in aiding zinc homeostasis unaffected by competing nutrients. In piglets given milk or replacement formulas, in which the concentration of zinc is lower (1-5mg/l) the saturable mechanism will predominate.

REFERENCES

(1) HOPFER, V. 1977. Am. J. Physiol. 233: E445-E449.

(2) MENARD, M.P. & COUSINS, R.J. 1983. J. Nutr. 113: 1434-1442.

(3) BOOTH, A.G. & KENNY, A.J. 1974. Biochem. J. 142: 575-581.

(4) BLAKEBOROUGH, P. & SALTER, D.N. 1983. 15th FEBS Meeting. Abstracts p.139.

(5) AFRC The Nutrient Requirements of Pigs, Commonwealth Agricultural Bureaux (1983) p.275, 287.

REGULATION OF PANCREATIC SECRETION IN THE PIG BY NEGATIVE FEEDBACK AND PLASMA GASTROINTESTINAL HORMONES

T. CORRING, J.A. CHAYVIALLE*, C. SIMOES NUNES, J. ABELLO
Laboratoire de Physiologie de la Nutrition, INRA-CNRZ
78350 Jouy-en-Josas, France and *INSERM, Unité de Recherches
Physiopathologie, Hôpital E. Herriot, 69834 Lyon Cédex 2, France

SUMMARY

The aim of the investigation was to study the level of circulating gastro-intestinal hormones in pigs when pancreatic secretion was re-introduced or removed from the duodenal lumen. Permanent fistulae were fitted into the pancreatic duct and duodenum of 16 pigs, and catethers were introduced into the portal vein and a jugular vein as well. Plasma hormone content was determined at different times during two periods of 30 and 60 min each, when the juice was returned to the pigs; these two periods included a 120 min-interval when the juice was not reintroduced into the intestinal lumen.

When the pancreatic juice was not returned to the pigs, plasma secretin content rose significantly in the portal blood (+ 52.5% at min 30 and +27% at min 120) and in the peripheral blood (+ 34.6 % at min 30; + 18.9 % at min 120) compared to the mean values recorded during intraduodenal re-introduction of the secretion. In parallel, the volume of juice secreted and protein output increased significantly but their concentration did not. The mean plasma levels of cholecystokinin were not affected by the removal of the pancreatic secretion except at min 30, in the portal blood, when its value was 33 % higher than the mean during the periods of restitution. The mean plasma levels of gastrin, somatostatin, V.I.P.and P.P. in the portal and peripheric blood were unaltered. These results suggest that in pigs, secretin may have an important role in the regulation of pancreatic secretion by negative feedback.

INTRODUCTION

Regulation of pancreatic secretion by negative feedback was shown in the rat (GREEN and LYMAN, 1972), in the pig (CORRING, 1974) and in the hamster (ANDREN-SANDBERG and IHSE, 1983). It would not exist in the dog and is controversed in man. Its mechanism is still

unknown and involvement of gastro-intestinal hormones has been suggested as well in the rat by GREEN and LYMAN (1972), as in the pig by CORRING (1974) or in the hamster by ANDREN-SANDBERG and IHSE (1983). The reported study was aimed to determine the plasma levels of some gastro-intestinal hormones in the pig, when collected pancreatic juice was either returned into the duodenal lumen or removed from it.

MATERIAL AND METHODS

Sixteen castrated Large White pigs of 45 $\pm$ 5 kg body weight, were fitted with permanent fistulae into the pancreatic duct and the duodenum respectively (CORRING et al., 1972). Furthermore, two catheters were introduced into the portal vein and one jugular vein respectively.

Animals were fed a standard growth diet (16 % proteins). 800g of the diet diluted in 1600 ml H_2O, were given at each meal (9 a.m. and 5 p.m.).

Experimental scheme :

The experimentation was performed in the fasted animal and consisted in the following assay : a first period during which the pancreatic juice was returned on into the duodenum (30 min), a second period during which the juice was removed and replaced by a saline solution pH 8.4 (120 minutes) and a third period during which the pancreatic juice was returned into the duodenum (60 min). Pancreatic juice was continuously collected and sampled every 30 min. Portal and jugular blood samplings were done at the beginning and the end of the first period, every 15 min during the first hour and every 30 min during the second hour of the juice removal period, at the 15th, 30th and 60th min of the third period.

Analysis :

The volume of pancreatic juice secreted was measured every 30 min and total proteins were determined in all juice samples. The following gastro-intestinal hormones were determined in portal and jugular plasma by radioimmunoassay : secretin, cholecystokinin, somatostatin, V.I.P., P.P., gastrin.

RESULTS

35 assays were done in the 16 pigs. Values obtained during the juice removal period are expressed in percentage of the corresponding mean value determined during the two periods of juice reintroduction into the duodenum.

Pancreatic juice :

Within the 30 min following removal of pancreatic secretion from the intestine, the amount of total proteins secreted by the exocrine pancreas significantly increased ($P < 0.01$) and was 60 min later 280% of the mean value determined during the juice restitution periods. A sharp decrease was observed between the 60th and 90th min and total proteins output significantly increased again within the last 30 min of the juice removal period (227%). Increases in pancreatic total proteins were entirely due to increases in the volume of pancreatic secretion which showed two peaks arising within the first hour and from the 90th to the 120th min of the juice removal period (290 % and 306% $P < 0.01$, respectively). The content of total protein in the juice was not affected when pancreatic secretion was removed from the intestine.

Gastro-intestinal hormones :

The plasma level of secretin in the portal vein increased immediately after the beginning of the removal of the pancreatic secretion from the duodenum and was 30 min later 152.5% higher than the corresponding value determined during the restitution periods. A second peak value was observed at the 120th min of the second experimental period. A similar pattern was observed in the jugular blood.

The removal of the pancreatic juice did not lead to a change in the plasma level of cholecystokinin, except 30 min later in the portal vein when the level was 32 % higher than the corresponding value obtained during the restitution periods.

As for the other studied gastrointestinal hormones, neither plasma level of somatostatin, nor that of somatostatin, nor that of gastrin or V.I.P., or P.P. were affected by the removal of pancreatic secretion from the intestine.

DISCUSSION

When the pancreatic juice was removed from the duodenum of the pig, the output of pancreatic total proteins significantly increased and this was mainly due to the large increase in the volume of the pancreatic secretion. In contrast, GREEN and LYMAN (1972) observed a high increase in the content of total proteins in the pancreatic juice and a slight increase in the volume. Furthermore, our results showed a marked effect of the restitution of the juice following the removal period. The mean rate (total proteins and volume) was lower during this second restitution period than during the first one. It looks like the negative feedback effect related to the pancreatic juice flowing into the duodenum was greater than the opposite effect, i.e., cessation of inhibition of the pancreatic secretion due to the removal of the juice from the intestine.

In experimental conditions, only an increase in the plasma level of secretin was noted when the pancreatic juice was removed from the intestine and replaced by a saline solution, pH 8.4. Furthermore, the pattern of pancreatic juice volume and changes in plasma levels of secretin were very similar. We can suggest that in the pig, the protein part of the pancreatic juice (CORRING, 1974) flowing into the duodenum, exerts a negative feedback effect upon the secretion by inhibiting the release of secretin by intestinal mucosa into blood. Concerning the effect of pancreatic juice removal on plasma level of cholecystokinin, we cannot definitively conclude. Our results have to be considered with caution since the used R.I.A. dosage does not allow to detect a short molecular form of the hormone smaller than the C-terminal nonapeptide. It appears essential to study the active molecular forms of CCK circulating in the pig.

REFERENCES

ANDREN-SANDBERG, A. & IHSE,I. 1983. Scand. J. Gastroenterol. 28 : 697-706.

CORRING T., AUMAITRE,A. & RERAT,A. 1972. Ann. Biol. anim. Bioch. Biophys. 12 : 109-124.

CORRING, T. 1974. Ann. Biol. anim. Bioch. Biophys. 14 : 487-498.

GREEN, G.M. & LYMAN, R.L., 1972. Proc.Soc. Exp. Biol.Med. 140 :6-12.

STOMACH EMPTYING OF MILK DIETS IN PIGS-A MATHEMATICAL MODEL ALLOWING DESCRIPTION AND COMPARISON OF THE EMPTYING PATTERNS.

J.A. DECUYPERE, R.M. DENDOOVEN AND H.K. HENDERICKX.
University of Ghent, Faculty of Agricultural Sciences, Laboratory of Nutrition and Hygiene, Proefhoevestraat, 10, B-9230 Melle , Belgium.

SUMMARY

The effect of doubling the nutrient intake in pigs fed a liquid cow's milk diet was investigated.

This was done, either by doubling the volume given at once, either by doubling the dry matter content.

There were two experiments in which the cumulative outflow of fresh contents, dry matter, crude protein and total carbohydrates, relative to the intake, was studied.

This was done using pigs equipped with a simple duodenal cannula and an occluding balloon.

The emptying pattern was biphasic. A rapid emptying, followed by a period during which the emptying was inhibited, was seen during the first emptying phaze, the so called initial emptying. Thereafter the emptying was resumed again during the second phaze: the final emptying.

The individual emptying patterns were described very accurately by an adapted mathematical function with four parameters : $y = c \times (1-2^{-(a \times t)^b}) + dt^3$. The parameters c, b and a describe the initial emptying which is intrinsically exponential while d is descriptive for the final emptying.

The clotting of the milk was complete after about 15 min. The clotting certainly was responsible for the biphasic appearence of the emptying pattern.

Doubling the nutrient intake, either by doubling the volume given, either by doubling the dry matter content of the milk, did not change the initial emptying pattern to a great extend, except in the first experiment, where doubling the volume increased the relative amount of dry matter and nutrients emptied.

The relative amounts of dry matter and nutrients emptied during the final emptying phaze on the other hand were inversily related to the amount of nutrients given.

The mathematical model used is compared with other models used for describing cumulative emptying patterns and discussed in the light of the physiological events regulating the gastric emptying.

INTRODUCTION

Weaning at ages of less than one week requires liquid feeding regimens and successfull artificial rearing mostly is obtained if very frequent meals are given, otherwise digestive disturbances may occur. Gastric emptying pattern and clotting behaviour of the milk replacer are thought to be important for preventing those conditions.

Present experiments were set up to study gastric emptying of liquid milk diets in function of the amount of nutrients given at once. This was done either by doubling the volume given, either by doubling the dry matter content of the milk. Both factors are thought to be

important in the regulation of stomach emptying and in the clotting behaviour of the milk (EMMONS and LISTER, 1976). In order to compare the different emptying patterns obtained, the mathematical model described by ELASHOFF et al. (1982) was adapted.

MATERIAL AND METHODS

Experiment 1

Two female pigs (age + 6 weeks) were used. A simple duodenal canula was placed approximately 15 cm after the pylorus. When not in experiment the pigs were fed a dry cow's milk based diet, coded MA, ad libitum. The composition of the diet is given in table 1.

Three treatments were used : in treatment 1 a liquid milk was prepared by blending 1 part MA powder with 4 parts of water. The quantity given was 500 g (MA20-500). In treatment 2 liquid milk of the same composition was used but the quantity given was halved : (MA20-250). In treatment 3 the dry matter content of the milk was doubled by mixing 2 parts of MA powder with 3 parts of water and 500 g was offered (MA40-500).

If a sampling was planned the next day the pigs were fasted overnight. There were 3 replications per treatment and per pig. For sampling a two-way Foley balloon catheter was introduced 10 cm distally via the duodenal canula and the balloon was distended by 3 to 6 ml water untill the intestinal lumen was occluded.

The sampling of the gastro-duodenal outflow started at the moment the milk was offered and lasted 3 hours. The contents were collected per 5 min. during the first 30 minutes, per 15 min. during the 90 min. that followed and per 30 min. for the last hour.

Experiment 2.

One female pig (age 11 w) was used. The treatments used and the other experimental conditions were identical as in experiment 1 except for the amounts given, which were adapted weekly to the liveweight of the animal ; the amounts given were fixed at 1.25 and 2.5 % of the weight. The different treatments then were MA20-1.25, MA20-2.5 and MA40-2.5. There were 6 replications per treatment.

The cumulative outflow of fresh contents and nutrients was calculated and expressed as percentage of the intake for obtaining individual outflow curves. A mathematical function was deduced from the power-exponential function described by ELASHOFF et al. (1982) ($y=1-2^{-(t/t1/2)^b}$). A parameter (c) was inroduced allowing the curve to reach an asymptotic value instead of 1 and the second part of the emptying-curve was described using the factor t^3. The modified equation is : $y = c \times (1-2^{-(a \times t)^b})+d \times t^3$. The parameter a equals 1/t1/2, while b determines the shape and c the asymptotic value of the first part of the curve. The value of d describes eventual increases at later times in the emptying process. Curve fitting was done using a program written in BASIC by the authors for a personal computer.

Table 1. Composition of the MA diet.

Ingredients (kg)	
Full-cream-milk powder (spray dried)	50
Skim-milk powder (spray dried)	50
Vitamins and minerals	0.340

RESULTS

General observations

The nature and the amount of the duodenal outflow clearly indicated that there were three different fazes in the emptying process. Duodenal outflow started nearly at the same moment the pigs took their milk and the material emptied in this first faze had nearly the same appearence and consistency of the milk ingested. Thereafter clotting was obvious as emptied material clearly separated in a yellow coloured whey fraction and clot fragments. In the same time the amount emptied gradually diminished.

After this second faze of emptying, fine granular material appeared in the effluent, the quantity of which gradually increased up to approximately one third at the end of the observation period.

Curve fitting.

The mathematical model described the emptying pattern very closely The mean R^2 value for all the curves obtained in the two experiments was 0.99 (range 0.94 to 0.99).

The two first fazes, the initial rapid outflow of unchanged milk and the slower emptying of clot fragments and whey are described by the first part of the function (power-exponential), whereby parameter c quantitates the amount emptied (%) during these two fazes, while 1/a or t1/2 is the time (min.) taken to empty half of this quantity. Parameter b indicates if the initial emptying pattern, before t1/2 has been reached is faster (b<1), equal (b=1) or slower (b>1) than exponential. After t1/2 the situation is inverted as illustrated in fig 1.

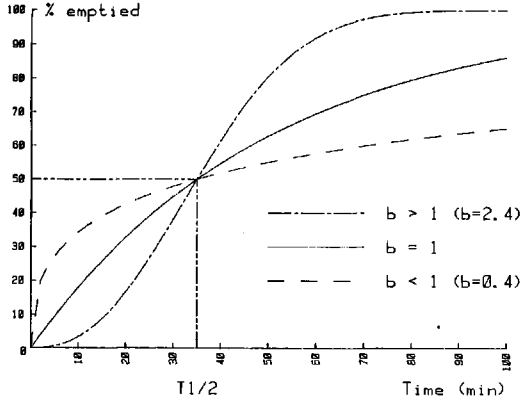


Fig. 1: Initial emptying curves with varying b values.

These two first fazes are called "initial emptying pattern" in the further discussion. The extend to which the clot is further hydrolyzed and emptied is quantitated by the parameter d of the second part of the function and is called "final emptying pattern" further.

Gastric emptying patterns

Experiment 1

For none of the observations there was a difference between fresh contents and nutrients emptied for the parameters 1/a and b. Parameter c was always higher for the fresh contents ($p>0.01$) than for dry matter, protein and carbohydrates. Parameter d also was higher for fresh contents than for the other factors studied. Therefore, in order to

compare the treatments, the mean value of the parameters for fresh contents, dry matter and nutrients was calculated, except for c and d for which the values for fresh contents were excluded. Those results are presented in table 2A.
The treatments had no effect on the amount of fresh material emptied, neither were there any significant differences for the t1/2 values. The amount of nutrients emptied and the emptying pattern of the nutrients however clearly were influenced by the treatments.
The most marked differences were observed between treatments MA20-250 and MA20-500 by which both nutrient intake and the volume ingested was doubled. This resulted in a change of the initial emptying pattern from faster than exponential (b=0.85) to slower than exponential (b=1.34). The proportional amount of nutrients emptied however was much higher after doubling the volume (72% versus 45%). Doubling the nutrient intake without changing the volume ingested (MA20-500 versus MA40-500) further flattened the slope of the initial emptying but to a lesser extend (1.44 versus 1.34). The final emptying pattern described by parameter d, was also clearly influenced by the treatments. By increasing the nutrient intake the final emptying was postponed resulting in a smooth slope of the curve, the effect being most pronounced if the volume together with the nutrient intake were doubled.
Experiment 2.
The results of experiment 2 are presented in table 2B.

Table 2A. Gastric emptying pattern: experiment 1 : pig 1 and 2.

treatment	MA20-250	MA20-500	MA40-500
parameter 1/a or t1/2 (min.)			
mean parameter (2)	26.5(1.2)(1)	17.2(0.6)	22.9(0.7)
parameter b			
mean parameter (2)	0.85(0.10)x(4)	1.34(0.15)y	1.44(0.06)y
parameter c (%)			
fresh contents	122(15)	122(4)	101(8)
mean parameter (3)	45(6)x	72(4)y	42(3)x
parameter d x 10-6			
fresh contents	4.7(1.9)	1.6(0.6)	3.0(0.7)
mean parameter (3)	2.4(0.5)x	1.5(0.3)xy	1.0(0.1)y

Table 2B. Gastric emptying pattern : experiment 2 ; pig 3.

treatment	MA20-1.25	MA20-2.5	MA40-2.5
parameter 1/a or t1/2 (min).			
mean parameter (2)	18.0(1.5)x(4)	29.0(1.9)y	34.5(3.3)y
parameter b			
mean parameter (2)	1.37(0.12)	1.31(0.08)	1.17(0.06)
parameter c (%)			
fresh contents	151(12)	142(4)	199(40)
mean parameter (3)	58(3)	59(3)	51(5)
parameter d x 10-6			
fresh contents	7.7(1.7)	3.0(1.0)	0.6(0.4)
mean parameter (5)	3.6(0.4)x	1.7(0.3)y	0.8(0.2)z

(1) mean (standard error); (2) mean parameter for fresh contents, dry matter, crude protein and total carbohydrates. (3) mean parameter for dry matter, crude protein and total carbohydrates.(4) xyz : mean values in the same row with different superscripts differ at P>0.05 ; values without superscript don't differ.

As between nutrient differences were quite comparable with the differences obtained in the first experiment, the mean values of the parameters were calculated as in the first experiment.

Neither volume or nutrient density had a clear effect on the initial emptying pattern in this experiment, except that t1/2 increased with increasing amounts of nutrients given.
Parameters b and c on the other hand did not differ between the treatments.

Doubling the nutrient intake had a linear effect on parameter d wich was halved by each treatment, indicating that the final emptying was postponed.

DISCUSSION

The present experiments clearly indicate that the outflow patterns were biphasic and comparable with the outflow patterns described in the work of LAPLACE and TOMASSONE (1970). By those authors the best curve fitting was obtained by a 3rd power polynomial function. This function was thought to be also usefull in interpreting the results of the present experiments. However curve fitting indicated that, although the model described the mean outflow pattern very well, systematic errors were introduced in more than half of the observations.

For discussing the results obtained, comparable work in pigs is rather scarce. BRAUDE et al. (1970) in their experiment used pigs of 4 to 5 weeks of age and a feeding regimen comparable with treatment MA20-250 in the first of the present experiments. Clotting occured between 15 and 30 minutes as in the present experiments. Emptying however was nearly complete in 2 hours.

Both present experiments clearly indicate that the outflow relative to the intake of fresh contents is completely different from nutrient outflow, especially for parameters c which is much higher for fresh contents. Those high outflow rates have to be attributed to the preferential and rapid emptying of the liquid and the digestive secretions of the stomach, pancreas and bile, as saliva secretion is known to be low in liquid meals.
Also parameter d differ clearly between fresh content and nutrient outflow for which d is much lesser in most treatments. As in the initial emptying this difference has to be attributed to the digestive secretions. Besides this there seems to be an important treatment effect, although the variability don't allow proper statistical evaluation. In both experiments, the highest d value for fresh contents is computed for the smallest amount of food given, indicating that the smaller amount of milk given, the more rapid further emptying is resumed.

For neither parameter of the function studied and in none of the 2 experiments there was a difference between dry matter, protein and total carbohydrate, although in all treatments total carbohydrate emptied faster than protein, a phenomenon which is well documented for water-soluble carbohydrates.

It is temptative to compare the values of b with the starting index described in the earlier work concerning gastric emptying (HUNT and MACDONALD 1954) as both quantitate the deviation of a given emptying pattern from a pure exponential one.
Two main factors were thought to be determining for the starting index. First, the individual characteristics of the stomach. This is also true for the b value (ELASHOFF et al. 1982). The second factor is the volume ingested. Volumes greater or smaller than a critical

amount resulted in negative, respectively positive starting indices (HUNT and MACDONALD 1954). This critical amount was depending on the so called "receptive capacity" of the gastro-intestinal tract, a resultant of the propulsive forces generated by the stomach and the inhibitive forces originating from the small intestine. This concept was confirmed in more recent experimental work in pigs (LAPLACE and TOMASSONE 1970; LAPLACE et al. 1981) in which the third power polynomial function was used for the description of the emptying pattern. The intercept of this function, being negative or positive, as the starting index, was taken as a measure of this receptivity which was thought to be greatly influenced by the prealable fasting period of the animals and depending on the intervals between and the volume of the meals. Nevertheless, the intercept was negative (LAPLACE and TOMASSONE 1970) in one and positive in another (LAPLACE et al 1981) experiment, what is suggestive that other determinants, as individual differences, could also be important.
This could be the reason for the difference of the b value for treatment MA-250 and MA-1.25 in experiment 1 and 2 respectively, as the volumes given relative to liveweight did not differ to a great extend (1.5 and 1.25%).
Doubling the volume given in experiment 1 resulted in a b value of about 1.3 indicating that the initial emptying was delayed. Nevertheless the relative amounts of fresh contents emptied during the initial faze were identical for both treatments the amounts of nutrients emptied was much higher after doubling the volume, indicating that the emptying, nevertheless somewhat delayed, proceeded at a much faster rate thereafter, as can be deduced from the t1/2 values. The increase of the initial emptying rate after ingestion of increasing volumes is a phenomenon which repeatedly has been described (HUNT and MACDONALD 1954).
However, this phenomenon could not be reproduced in the second experiment, the b values being both 1.3 and the relative amounts emptied identical both for fresh material as for the nutrients. The half time however was markedly longer. This discrepancy most probably is due to the fact that in the first experiment some critical gastric volume (LAPLACE 1982) was bypassed while not in experiment 2.

The influence of doubling the nutrient intake is more clearly demonstrated by comparing treatments MA-20 and MA-40 as the same volume was given. Doubling the nutrient intake slowed down the intial emptying (t1/2) in both experiments, the differences did not reach significance however, neither did parameter b differ in both experiments. The relative amount of dry matter and nutrients emptied was clearly lower in the first, but only slightly lower in the second experiment. This phenomenon is also described in man (HUNT and STUBBS 1975) and most probably results from stronger inhibitive forces generated in the small intestine.

Again it is temptative to relate parameter d to the "third emptying phaze" as described by HUNT and MACDONALD (1954) and to the third power coefficient of the polynomial function of LAPLACE and TOMASSONE (1970), all of which indicate that the final emptying proceeds at a faster rate than expected by extrapolation of the foregoing emptying pattern. The "third emptying phaze" described in the work of HUNT and MACDONALD (1954) was not quantitatively described however. The magnitude of the coefficient of the third power in the polynomial function, as described by LAPLACE and TOMASSONE (1970) depends on the quadratic part of the function and has a significant value even if no increase in the final emptying rate is observed, while

factor d in the present function is a really quantification of the enhancement of the emptying process and is almost independent from the values of the first part of the function, since if t is large, the final emptying is described by c+dt^3.

In the present experiments factor d describing the final emptying of dry matter and nutrients seems to be closely related to the nutrient intake, whereby, especially in the second experiment, the relationship was inversely linear. The delay of the final emptying after doubling the nutrient intake most likely is due to the fact that stronger inhibitive forces are generated in the small intestine, although it is also known that the consistency and hardness of the clot and hence the resistence to liquification, is proportional to the dry matter content of the milk (EMMONS and LISTER 1976).

REFERENCES

BRAUDE, R., NEWPORT, M.J. & PORTER, J.W.G. 1970. Brit. J. Nutr. 24 : 827-842.

ELASHOFF, J.D., REEDY, T.J. & MEYER, J.H. 1982. Gastroenterology 83 : 1306-1312.

EMMONS, D.B. & LISTER, E.E. 1976. Can. J. Anim. Sci. 56: 317-325.

HUNT, J.H. & MACDONALD, I. 1954. J. Physiol. 126: 459-474.

HUNT, J.M. & STUBBS, D.F. 1975. J. Physiol. 245: 209-225.

LAPLACE, J.P. & TOMASSONE, R. 1970. Ann. Zootechn. 19: 303-332.

LAPLACE, J.P., PONS, O., CUBER, J.C., KABORE, C. & VILLIERS, P.A. 1981. Ann. Zootechn. 30: 209-248.

LAPLACE, J.P. 1982. In : Digestive physiology in the pig. (Eds. J.P. LAPLACE, T. CORRING & A. RERAT) Colloques de l'INRA n°12. INRA, Paris p. 29-44.

EFFECT OF THE PHYSICAL FORM OF DIETARY GUAR GUM ON NUTRIENT ABSORPTION IN THE PIG

L.M.J. HEPPELL & A.L. RAINBIRD
National Institute for Research in Dairying
Shinfield, Reading, Berkshire, England*

SUMMARY

The effects of the physical form (powder or granulated) of dietary guar gum on the absorption of glucose and α-amino nitrogen from the small intestine and on plasma insulin levels were studied in growing pigs.

Powdered guar gum caused a significant reduction in plasma insulin levels and the rate of α-amino nitrogen absorption. However two granulated preparations had no effect on α-amino nitrogen absorption although one of them caused a significant decrease in plasma insulin levels.

INTRODUCTION

Previous studies in both man (Jenkins et al., 1978) and pigs (Sambrook & Rainbird, 1985) have shown that the addition of finely powdered guar gum to the diet markedly reduced post-prandial hyperglycaemia and hyperinsulinaemia. Recently, two granulated guar gum preparations have become available; these are designed not to hydrate and impart viscosity until they reach the stomach. Powdered guar gum by contrast increases the viscosity of a meal or drink before consumption and this leads to palatability problems. Such problems could seriously limit the potential value of guar gum in the management of diabetes and hyperlipidaemia in man.

The aim of the study was to determine whether granulated guar gum preparations were as effective as the powdered form in decreasing the rate of absorption from the small intestine and reducing post-prandial plasma insulin levels.

*Address from 1/4/85: The Animal & Grassland Research Institute, Shinfield, Reading, Berkshire, England.

MATERIALS AND METHODS

Six male Large White x Landrace pigs (≈30kg) were prepared with an indwelliong vena cava catheter. Three guar gum formulations, one powdered (Meyprogat 150; Meyhall Chemical UK Ltd, Wirral, Merseyside, England) and two granulated (Meyprotin [granules covered with protein]; Meyhall Chemical UK Ltd, and Lejguar; Britannia Pharmaceuticals, Reigate, Surrey) were used. These were added to a semi purified diet (Table 1) and water (mixed in the ratio 1:2.5 w/v). The air-dry diet was fed at a level of 3.5% of body weight per day. Blood samples were collected over seven hours after feeding and were analysed for glucose, α-amino nitrogen and insulin.

Table 1. Composition of diet (g/kg)

Maize starch	550.9
Soyabean oil	80.0
Megalac 95 (tallow)	80.0
Casein	180.0
Solka Floc	60.0
Trace mineral mix	10.0
Vitamin mix	2.0
Dicalcium phosphate	31.0
Choline hydrochloride	1.1
Sodium chloride	5.0
Vitamin B_{12}	30μg
Guar gum (when added)	40g

RESULTS AND DISCUSSION

See Table 2.

Table 2 shows that only the powdered guar gum was effective in decreasing post-prandial α-amino nitrogen levels. Plasma glucose peaks were unusually small and decreases could not be detected. However, plasma insulin levels were markedly decreased following consumption of powdered guar gum which is consistent with a reduced rate of glucose absorption. The granulated guar gums were less effective and only one caused a reduction in plasma insulin levels.

Table 2. Mean peak post-prandial plasma glucose, ∝-amino nitrogen and insulin concentrations after pigs received meals without or with different types of guar gum.

Type of Guar Gum	Plasma glucose (mmol/l)	Plasma ∝-amino nitrogen (mg/l)	Plasma insulin (munits/l)
Control (no guar gum)	6.45	129.8	64.9
Meyprogat 150	6.16	105.1***	40.0**
Meyprotin	5.92	129.2	47.9*
Lejguar	6.36	128.6	54.0
SED	0.448	5.34	6.29

* P<0.05; ** P<0.01; *** P<0.001; significantly different from the control.

We conclude that the different preparations of guar gum vary in their ability to reduce the rate of absorption of nutrients. These effects are probably related to the greater ability of the powdered than of granulated guar gum to increase the viscosity of the meal and stomach contents (Heppell & Rainbird, unpublished observations).

REFERENCES

JENKINS, D.J.A., WOLEVER, T.M.S., LEEDS, A.R., GASSULL, M.A., HAISMAN, P., DILAWARI, J., GOFF, D.V., METZ, G.L. & ALBERTI, K.G.M.M. (1978). Brit. Med. J. i, 1392-1394.

SAMBROOK, I.E. & RAINBIRD, A.L. (1985). Br. J. Nutr. 54, in the press.

ALR gratefully acknowledges receipt of an MRC/AFRC Special Training Fellowship in Human Nutrition.

The authors wish to thank Mr R.M.W. Hopkins of Meyhall Chemical Co (UK) Ltd for the gift of guar gum.

EFFECTS OF WHEAT STRAW MEAL ON PORTAL PLASMA CONCENTRATIONS OF GLUCOSE, α-AMINO-N AND IMMUNOREACTIVE INSULIN IN THE GROWING PIG

K. MALMLÖF[1], C. SIMOES NUNES[2], J. ÖRBERG[3]

Departments: of Animal Nutrition and Management[1] and of Animal Physiology[3], Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden

INRA, Laboratoire de Physiologie de la Nutrition[2], Centre de Recherches Zootechniques, 78350 Jouy-en-Josas, France

SUMMARY

Effects of a low-(LF) and a high-fibre (HF) diet on portal plasma glucose, α-amino-N and insulin concentrations were studied in 3 growing pigs (35-65 kg). The diets were fed in isonitrogenous amounts at 800 h and 1600 h. Blood samples were drawn hourly throughout a 16 h period (2 x 8 h postprandial periods). In the beginning of each period samples were taken every 30 min. In comparison with the LF diet the HF diet produced a higher mean plasma concentration of glucose ($p < 0.05$) but a lower mean plasma concentration of insulin ($p < 0.05$) during the 16 h studied. Portal plasma concentrations of α-amino-N were not specifically affected by the HF diet. Irrespective of dietary treatment the maximum plasma insulin responses to the 1600 h meal were only 55-65 % ($p < 0.005$) of the corresponding values following the 800 h meal.

INTRODUCTION

The growth promoting effect of insulin is widely known and has also been demonstrated in the pig (Romsos et al., 1971). In some experiments fibre inclusion into pig diets have been reported to increase protein deposition in growing pigs (Sherry et al., 1981; Malmlöf and Håkansson, 1984). This is a little bit surprising, since in studies with human subjects insulin levels often are suppressed by diets rich in fibre (Albrink et al., 1979). The aim of this study was therefore to elucidate the effect of a high-fibre diet on portal plasma insulin concentrations in the pig. In this connexion it was also of interest to study the effects of the high-fibre diet on portal plasma glucose and amino acid (α-amino-N) concentrations.

MATERIALS AND METHODS

This experiment was performed parallel to the one previously presented in this report (Effects of wheat straw meal on portal plasma concentrations of urea and ammonia-N in the growing pig, (Malmlöf and Simoes Nunes). In this work animals, experimentals and diet composition of the low- (LF) and high-fibre (HF) diets were thus the same. Chemical analyses of plasma samples were performed on a Technicon autoanalyzer. Glucose was analysed by a glucose oxidase method (Glox®, Kabi Diagnostica, Stockholm, Sweden). α-amino-N was analysed according to Method No. 01 (Technicon, Scandinavia, Stockholm, Sweden). The concentration of immunoreactive insulin in plasma was measured essentially as described by Herbert et al. (1965). In our assay we used ^{125}I-insulin (Phadeseph®, Pharmacia, Uppsala, Sweden). A purified porcine insulin was used as standard (Novo Res. Institute, Bagsvaerd, Denmark). The lower detection limit of insulin was 0.05 ng/ml plasma. Potential differences in mean levels of parameters produced by the two diets were evaluated by a three-way analysis of variance, including diet, the individual animal and time of sampling as the prime sources of variation. Within dietary treatment potential differences in maximum values of parameters following the 800 h and 1600 h meals were tested with a statistical model containing the individual animal and time of sampling as variation factors.

RESULTS AND DISCUSSION

As can be seen from Fig. 1 and Tab. 2 the two diets had a similar influence both on portal plasma α-amino-N dynamics and on the mean plasma α-amino-N level during the 16 hours studied. However, the HF diet evoked a somewhat higher ($p < 0.05$) mean plasma level of glucose than did the LF diet. This was probably due to the fact that, in connexion with the LF diet, the maximum portal glycaemia developed in response to the 1600 h meal was significantly ($p < 0.01$) lower than that evoked by the 800 h meal. No such diurnal variation in glycaemic response to a meal could be noticed when the animals received the HF diet. Surprisingly, this was however the case with regards to portal plasma insulin concentration. As a matter of fact, in association with both dietary treatments the

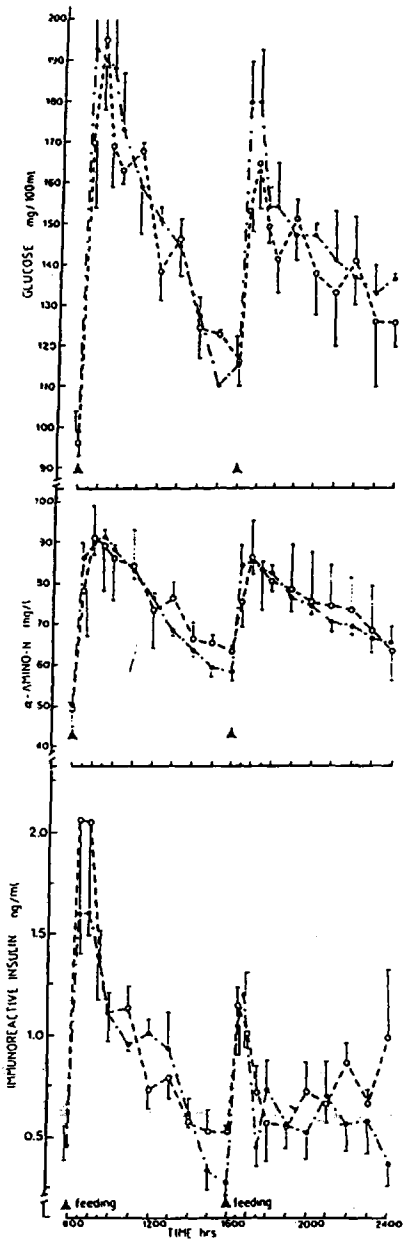


Figure 1. Portal plasma glucose, α-amino-N and immunoreactive insulin concentrations during 16 h (2 x 8 h postprandial periods) in the same animals feed low-(LF) and high-(HF) fibre diets (Mean ± SEM; n = 3)

○---○ LF ●—·—● HF

1600 h meal gave rise to maximum plasma insulin concentrations which were only 55-65 % ($p < 0.005$) of the corresponding values following the 800 h meal.

Table 2. Mean portal plasma concentrations of glucose, α-amino-N and immunoreactive insulin during 16 h (2 x 8 h post-prandial periods) in pigs given low-(LF) and high-fibre (HF) diets (LS-MEANS; n = 3)

Diet	Glucose mg/100 ml	α-amino-N mg/l	Immunoreactive insulin ng/ml
LF	144.4[a]	75.0[a]	0.91[a]
HF	150.5[b]	74.6[a]	0.79[b]

a, b, Values within columns with different superscrips differ significantly ($p < 0.05$).

Despite the higher mean plasma glucose level associated with the HF diet, this diet produced on average a lower plasma level of insulin. This observation is in agreement with Albrink et al. (1979), who also found a depressing effect of high-fibre diets on post-prandial insulin concentrations in humans. However, in that experiment no specific effect of the fibre diet on blood glucose levels was reported.

In conclusion our results demonstrate that some aspect of insulin metabolism was affected by the admixture or wheat straw meal into the diet. However, in order to answer the question if it was insulin release, insulin turn over or both that were affected further experiments must be performed.

REFERENCES

ALBRINK, M.J., NEWMAN, T. & DAVIDSON, P.C. 1979. Am. J. clin. Nutr. 32:1486-1491.

HERBERT, V., LAU, K-S., GOTTLIEB, C.W. & BLEICHER, S.J. 1965. J. clin. Endocr. Metab. 25:1375-1384.

MALMLÖF, K. & HÅKANSSON, J. 1984. Swed. J. agric. Res. 14:53-57.

ROMSOS, D.R., LEVILLE, G. & ALLEE, G.L. 1971. Comp. Biochem. Physiol. 40 A:557-568.

SHERRY, P.A., HARRISON, P.C. & FAHEY, G.C. Jr. 1981. J. Anim. Sci. 53:1309-1315.

A TRANSIENT HYPERSENSITIVITY TO DIETARY ANTIGENS IN THE EARLY WEANED PIG: A FACTOR IN THE AETIOLOGY OF POST WEANING DIARRHOEA (PWD)

B.G. MILLER[a], A. PHILLIPS[b], T.J. NEWBY[a], C.R. STOKES[a], F.J. BOURNE[a]
[a]Department of Veterinary Medicine, University of Bristol, Langford House, Langford, Bristol BS18 7DU
[b]Queen Elizabeth Hospital for Children, Hackney Road, London E2 8PS

SUMMARY

Three week weaned pigs fed ad libitum onto an experimental diet (23% protein DM) in which the sole protein source was full fat soya induced severe PWD. The diarrhoea was associated with a proliferation of E. Coli, crypt hyperplasia, villus atrophy, xylose malabsorption and in increase in intra epithelial lymphocytes. Animals which had been weaned for 5-7 days onto the experimental diet showed a positive skin test to the subcutaneously injected soya into their ear. Adequate prior feeding of the soya before weaning prevented both the xylose malabsorption and the diarrhoea.

INTRODUCTION

The aetiology of PWD is both complex and multifactorial. Profuse enterotoxigenic E. Coli (ETEC) may be found throughout the large and small intestine in cases of PWD, yet the same strains maybe isolated from clinically healthy pigs. The converse is also true in that weaned pigs may develop a mild 'nutritional' diarrhoea in the absence of any ETEC. In fact Kenworthy and Allen (1966) described the characteristic lesion of PWD as villus atrophy and crypt hyperplasia associated with malabsorption which may or may not be associated with ETEC. We have suggested (Miller et al 1983) that weaning itself induces these changes and that this predisposes to PWD.

The immune system of the gastrointestinal tract is presented with a unique dilemma. On the one hand it must respond with vigour to prevent potential pathogens from replicating and colonising the gut whilst on the other it must not over-respond to harmless dietary components. Failure to mount the appropriate response would result in either catastrophic infection disease or damaging allergic reactions. Protection from damaging gut hypersensitivity reactions

may be afforded by the active development of immune exclusion, elimination and tolerance. We have shown that prior to the development of this 'protected state' individuals may pass through a period of sensitivity. Such a transient hypersensitivity to dietary antigen following weaning may be one of the factors which predisposes to PWD.

MATERIALS AND METHODS

The animals used in these experiments were enzootic pneumonia free Large Whites from the University Pig Unit. Piglets were weaned at three weeks and fed ad libitum an antibiotic free experimental diet (23% Crude Protein) in which the sole protein source was a full fat Soya (Trusoy: British Soya Products).

Experiment 1. A litter of pigs (n= 8) were abruptly weaned at three weeks of age and xylose absorption measured on day 0, 2, 5 and 11 d post weaning. A second litter (n= 6) were also weaned at three weeks, however prior to weaning from 7 d they were cycled in two groups being kept off the sow for 24 h and then returned for 24 h (ie one on, one off). Whilst off the sow they were allowed ad libitum access to a suspension in water of Trusoy. This procedure encouraged substantial consumption of the Trusoy prior to weaning. Xylose absorption was measured on this second litter at 0 and 5 days post weaning. All animals were daily assessed for diarrhoea, and rectally swabbed on day 5.

Experiment 2. 6 litters of pigs (n= 33) were abruptly weaned at three weeks of age and skin tested respectively at 0, 1, 2, 5, 7 and 13 d post weaning (NB injected with a sterile Trusoy extract 24 h previously). The animals which were sacrificed 13 d post weaning were starved upon the onset of diarrhoea and the feed gradually reintroduced. This procedure facilitated survival of the piglets to 13 d.

RESULTS AND DISCUSSION

We have previously suggested (Miller et al 1983) that a transient hypersensitivity to dietary antigens post weaning may facilitate the onset of PWD. We reported that prior feeding of the weaning diet can significantly affect the onset and severity of PWD and that weaning onto a minimally antigenic diet can prevent PWD.

The data reported here further substantiates this hypothesis.

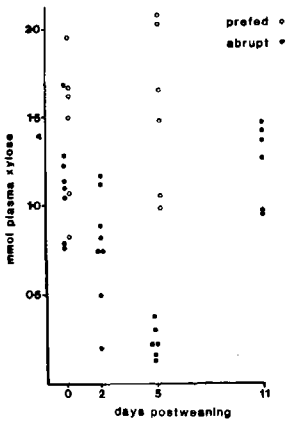


FIG 1

Experiment 1: Postweaning the pigs which had received no prior feeding showed xylose malabsorption (fig 1) which was maximal by day 5 but recovered by day 13. All pigs were positive for haemolytic E. coli and developed severe diarrhoea (NB Two pigs died prior to day13). In contrast the pigs which had prior feeding of Trusoy showed no diarrhoea, no haemolytic E. coli and no xylose malabsorption. These animals presumably had developed immune tolerance to the Trusoy by the time they were weaned.

Experiment 2: As in experiment one pigs developed a severe PWD 5 days post weaning. At both 25% and 75% along the small intestine pigs showed crypt hyperplasia and villous atrophy (fig 2). The crypt hyperplasia occurring before the villous atrophy on day 2 at 25% along the small intestine and on days 1 and 2 at the 75% level. Rotavirus was found on days 2 and 5 and as suggested by Lecce (1982) clearly could be a contributing factor causing villus damage. However we feel that this cannot explain all of the morphological changes since Rotavirus was found solely at the 25% level yet we observed maximal changes in the distal small intestine. Also it is hard to imagine why prior feeding of antigen before weaning could prevent a Rotaviral induced PWD.

Under both light and scanning electron microscopy adhering bacteria were found on days 5 and 7 post weaning but were absent by day 13. Increased nos. of both goblet cells and intra epithelial lymphocytes (IELs) were present from day 5. Animals showed negative skin tests against the Trusoy extract whether assessed by increased ear thickness or histology on days 0, 1, 2 and 13. However animals fed the soya diet for either 5 or 7 days showed swelling (fig 3) and cellular infiltration at the site of injection.

These changes can also be induced by oral immunisation with soluble protein antigen in unweaned pigs (Stokes et al 1981) and are

similar to those reported in mice undergoing cell mediated immune reactions (Mowat and Ferguson 1981). The transient positive skin tests at days 5 and 7 to the dietary antigen and the increased nos. of IELs and goblet cells further suggest that the primary gut damage in the post weaned pig is due to a delayed hypersensitivity reaction. Such a reaction would produce a marked increase in the rate of crypt cell division resulting in crypt hyperplasia and would markedly influence the functional properties of the epithelial cells migrating up the villus. This could explain why diarrhoea in the absence of ETEC can occur and why the postweaned pig exhibits both maldigestion and malabsorption of both fluid and nutrients. As to why this should induce a proliferation of ETEC is not clear but it may be related to an altered nutrient balance in the large intestine which in the absence of protective milk antibody results in disease. It does however explain why the post weaned pig is so sensitive to enterotoxin and therefore is devastated by an ETEC.

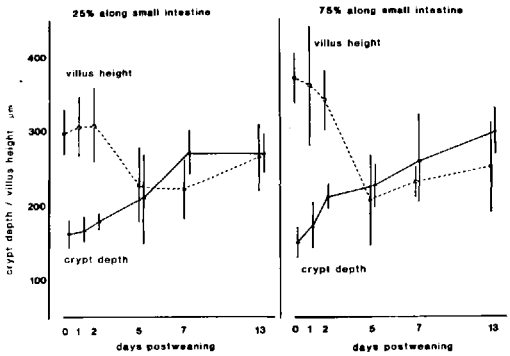


FIG 2

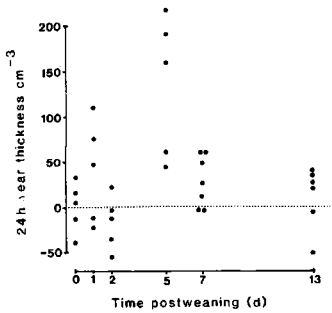


FIG 3

REFERENCES

KENWORTHY, R. & ALLEN, W.D. (1966) J. Comp. Path. 76 31-44.

LEECE, J.G., CLARE, D.A., BALSBAUGH, R.K. & COLLIER, D.V. (1982) Proc. Amer. Sci. Anim Sci 193.

MILLER, B., NEWBY, T.J., STOKES, C.R., HAMPSON, D. & BOURNE, F.J. Ann Rech Vet. (1983) 14(4) 487-492.

MOWAT, A.M. & FERGUSON, A. (1981) Clin. Exp. Immunol. 43, 574-582.

STOKES, C.R., NEWBY, T.J., & BOURNE, F.J. (1981) Curr. Top. Vet. Med. Anim Sci 12. 224-239.

EFFECT OF GASTRIC CANNULATION ON GASTROINTESTINAL MOTILITY IN THE PIG

by A.L. RAINBIRD and J.W. SISSONS
National Institute for Research in Dairying,
Shinfield, Reading, Berks, England*

SUMMARY

The effect of gastric cannulation on antral, pyloric and duodenal motility was studied in three pigs prepared with recording electrodes implanted at these sites.

Recordings of myoelectric activity of the stomach and duodenum suggested that the methods used for gastric cannulation and emptying and replacement of digesta did not disturb normal motor function, except when all of the digesta was removed from the stomach during sampling : replacement of digesta restored normal activity.

INTRODUCTION

Gastric cannulation has frequently been used to study the effect of dietary changes on gastric emptying patterns in pigs. A simple vinyl rubber cannula placed midway on the greater curvature of the stomach in pigs fitted with chronically implanted electrodes on the gastric antrum has been shown to cause no disturbance of the myolectric activity of that region (Cuber et al., 1980). However when the stomach was rapidly emptied via the cannula, the spike bursts which followed each other without interuption at the antrum after feeding, almost totally disappeared. When the animal was given a replacement meal the spike bursts reappeared in a normal fed pattern. In our previous studies of gastric emptying in pigs (Low and Rainbird, 1983) the animals were not refed following procedures for the removal of contents from the stomach. Instead after sampling, the digesta was returned to the animal through the gastric cannula.

*Address from 1/4/85: The Animal and Grassland Research Institute, Shinfield, Reading, Berkshire, England.

The aim of the present study was to examine the effect of gastric cannulation and emptying and replacement of digesta via the cannula on gastrointestinal motility.

MATERIALS AND METHODS

Three male Large White x Landrace pigs of 30kg initial liveweight were used. The animals were surgically prepared with a simple gastric cannula and recording electrodes. The cannula, made from "Kemetal" (ICI Ltd, London), had a barrel with a diameter of 29mm and a basal disc of 70mm diameter. Each group of electrodes consisted of three multistranded stainless steel wires coated with Teflon (Cooner Sales Company Inc., Chatsworth, California). They were attached to the serosal surface of the antrum at 5-10cm from the pylorus, pylorus, and proximal duodenum between 5-15cm distal to the pylorus.

The animals received a high fat semi-purified diet (Sambrook and Rainbird, 1985). Each meal consisted of 600g of air dry diet and 1500ml water. The animals were fed twice daily : the morning meal was given following a period of RSA on the duodenum on recording days (at approx 0900 - 1000h) and at 1700h.

Electromyographic recordings were made for 6 hours a day on 5 days with a Devices Model 7 polygraph (Welwyn Garden City, Herts, England). The stomach was emptied as described by Low and Rainbird (1983) on three of the five days.

RESULTS AND DISCUSSION

The introduction of the cannula did not disturb the electrical activity of the antrum, pylorus or duodenum, which is consistent with the findings of Cuber et al. (1980). The mean frequency (number/minute) of spike bursts at the antrum was 4.6 during fasting and in the first hour after feeding. These values are similar to those recorded in non-cannulated animals of 4.6 and 4.9 respectively (Rainbird, 1983).

The cyclic MMC pattern on the proximal duodenum was replaced by a period of continuous ISA after feeding similar to that observed by other workers (Ruckebusch and Bueno, 1976). The mean duration of this period of ISA was 193 minutes, which is comparable to values found previously in non-cannulated animals fed the same diet at NIRD (Rainbird, 1983).

Although when the animals were fed a normal post-prandial pattern of electrical activity was observed, when the stomach was emptied the pylorus became quiescent i.e. no spike bursts were seen. However when digesta was returned to the stomach via the cannula, a normal 'fed' pattern of spike bursts reappeared. In addition, the following period of ISA was extended, as seen after feeding.

We conclude that gastric cannulation did not lead to major changes in patterns of electrical activity of the gut. In contrast, emptying of the stomach through the gastric cannula disrupted the electrical activity of the pylorus, but when digesta was returned via the cannula a normal 'fed' pattern of electrical activity resumed.

REFERENCES

CUBER, J.-C., LAPLACE, J.-P. & VILLIERS, P.A. 1980. Reprod. Nutr. Dev. 20, 1161-1172.

LOW, A.G. & RAINBIRD, A.L. 1983. Proc. Nutr. Soc. 42, 23A.

RAINBIRD, A.L. 1983. Studies on the physiological action of dietary fibre in the stomach and small intestines of growing pigs. Ph.D. Thesis. University of Reading.

RUCKEBUSCH, Y. & BUENO, L. 1976. Br. J. Nutr. 35, 397-405.

SAMBROOK, I.E. & RAINBIRD, A.L. 1985 Br. J. Nutr. 54, In the press.

COMPARATIVE INTESTINAL TRANSPORT OF SMALL PEPTIDES AND FREE AMINO ACIDS AND INFLUENCE ON INSULIN AND GLUCAGON PRODUCTION IN THE CONSCIOUS PIG.

A.A. RERAT, C. SIMOES NUNES, J.C. CUBER
Laboratoire de Physiologie de la Nutrition
I.N.R.A.-C.N.R.Z., 78350 Jouy-en-Josas, France.

SUMMARY

The quantitative kinetics of appearance of amino acids (a.a.) in the pig portal vein was studied after duodenal perfusion with an enzymatic hydrolysate of milk proteins (oligopeptides) or a solution of free a.a. of the same composition. The amounts of a.a. appearing in the portal vein were higher after perfusion of the hydrolysate than after that of the free a.a. independently of the length of time after the perfusion. Thus, nitrogen infused in the small intestine as small peptides was absorbed more rapidly than as free a.a. The production of insulin and glucagon was stimulated by the perfusions, especially during the first hour; the stimulation of glucagon was larger after perfusion of the hydrolysate than after that of the a.a. solution.

INTRODUCTION

Like free amino acids, oligopeptides may be conveyed by the enterocyte. Their transport is then combined with a hydrolysis in the cell and their a.a. are taken up by the portal blood. At present, it appears that the intestinal transport systems of oligopeptides are independent of those used by the free amino acids, thus reducing the competition for the sites of absorption and rendering the latter more efficient. Only few in vivo experiments have been made and the methods of estimation were either the rate of disappearance of nitrogenous matters from perfused intestinal loops or the rate of appearance of alpha-amino nitrogen in the peripheral blood. However, under these conditions the spatio-temporal compensatory capacities of the gut cannot be expressed and the systemic blood variations are highly buffered by the hepatic uptake of amino acids. We therefore carried out a study in the conscious pig with the aim of quantifying the appearance of alpha-amino nitrogen in the portal blood after introduction into the duodenum of a mixture of small peptides or an a.a. solution

of the same composition (RERAT et al., 1984a, 1985a), and in order to measure the concomitant production of insulin and glucagon.

MATERIAL AND METHODS

Measurement of the nutritional exchanges (E) between the arterial blood and the gut lumen during the post-prandial time (dt), is based on the porto-arterial differences in nutrient concentrations (Cp-Ca) quantified by a simultaneous measurement of the portal blood flow rate (D) according to the formula: $E = (Cp-Ca)\ D\ Dt$ (RERAT et al.1984 b). Six Large White pigs (mean body weight 57.7 $\pm$ 0.99 kg) were fitted with two catheters placed in the portal vein and carotid artery (measurement of Cp-Ca), an electromagnetic flow probe around the portal vein (measurement of D) and a permanent duodenal catheter for perfusion of the nutrient mixtures into the proximal small intestine. After a period of recovery of 8 d., each animal was subjected to four trials at 3-4 d. intervals, starting after 18h-fasting and lasting for 5 h. Each trial was characterized by the nature of the product perfused during 30 min. into the duodenum (peptide mixture vs. a.a. solution of the same composition). The peptide mixture (RERAT et al., 1985a) was composed of a mixture of mild hydrolysates obtained at 38°C by the action of chymotrypsine and trypsine in a membrane enzymatic reactor on whey proteins (1/3) and casein (1/3) and a mixture of non phosphorylated peptides extracted from the enzymatic casein hydrolysate (1/3). Carotid and portal blood was sampled (2 ml per route) simultaneously at regular intervals from the beginning of the perfusion until the fifth hour for determination of the levels of alpha-amino nitrogen (trinitrobenzensulfone in leucine equivalents), insulin and glucagon. The portal blood flow rate (D) was recorded continuously by the electromagnetic method.

RESULTS AND DISCUSSION

The nature of the perfusion had no influence on the intensity and pattern of the blood flow rate. For all experiments and animals (n = 24), the mean portal flow rate over 5 h amounted to 2310 $\pm$ 99 ml/min (i.e. 38.4 $\pm$ 1.4 ml/kg/min); the post-perfusion variations showed the same trends as the post-prandial ones (RERAT et al., 1984b) The

absorption was very rapid in all cases since more than 1/3 of the aminoacids (a.a. in leucine equivalent) was recovered in the portal vein at the end of the first hour after the perfusion. However, the amounts of a.a. appearing in the portal vein were the largest after perfusion with the hydrolysate; the differences occurred very early and significantly (68 % during the first hour after perfusion of 55g; 87 % during the period of 30 to 120 min after perfusion of 110g) and persisted all the longer as the perfused quantity was high. During the perfusions, the amounts of a.a. absorbed exceeded those perfused. This excess (17 to 41 g a.a) was greater than the recycled endogenous nitrogen found with natural meals (RERAT et al., 1977).

Production of insulin and glucagon was rather low since it did not reach half the amounts produced after ingestion of 14 % protein diets (RERAT et al., 1985b). However, they were both stimulated by the perfusion, since the portal blood levels of insulin increased 4-5 times, 30 min after the perfusion and the portal levels of glucagon 3-5 times, 60 min after the perfusion. These values returned to the initial levels more or less rapidly according to the type of perfusion. The amounts of insulin produced were the largest during the first hour (about 55 - 70 µg/h) representing 30-45 % of the total production measured within five hours. No significant difference according to the nature or magnitude of the perfusion was observed. The quantities of glucagon produced during the first post-perfusion hour (1.5 - 6.0 µg/h) represented 27 - 49 % of the total amount secreted within five hours. The glucagon production was more than two times higher after perfusion of the hydrolysate than after that of a.a., however, the differences were only significant during the first hour.

REFERENCES

RERAT, A., VAISSADE, P., VAUGELADE, P., ROBIN, P., ROBIN, D. & JUNG, J. 1977. Vth Intern.Symp. on aminoacids, Budapest,D3: 1-8.

RERAT,A., LACROIX, M., SIMOES NUNES, C., VAUGELADE, P. & VAISSADE, P. 1984a. Bull. Acad. Nat. Med. 168: 385-391.

RERAT, A., VAISSADE, P. & VAUGELADE, P. 1984b. Brit.J.Nutr.51:517-525

RERAT, A., SIMOES NUNES, C., LACROIX, M., VAUGELADE, P. & VAISSADE P. 1984. C.R. Acad. Sci., in press

RERAT, A., CHAYVIALLE, A., KANDE, J., VAISSADE, P., VAUGELADE, P. & BOURRIER, T. 1985. Canadian J.Phys.Pharmacol., in press.

EFFECT OF WEANING ON THE STRUCTURE AND FUNCTION OF PIGLET SMALL INTESTINE

M.W.SMITH*, B.G. MILLER+, P.S. JAMES*, F.J. BOURNE+
*AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT
and +Department of Veterinary Medicine, University of Bristol,
Langford, Bristol BS18 7DU.

SUMMARY

Weaning piglets onto a soya bean diet causes marked changes in villus height and crypt depth in the small intestine. Acceleration of enterocyte development under these conditions is accompanied by inhibition of the capacity of enterocytes to digest and absorb nutrients.

INTRODUCTION

Weaning piglets onto a solid diet causes both structural and functional changes to take place in the small intestine. Initial villus shortening is followed by an increase in crypt cell proliferation, lactase and sucrase activities fall and diarrhoea often develops. The suggestion is that these effects result from hypersensitivity reactions taking place in intestinal tissue in response to dietary antigens. The recent application of quantitative cytochemistry and autoradiography to sections of intestinal tissue allows one to determine digestive and absorptive profiles for enterocyte development along the villus and thereby identify different ways in which enterocytes adapt to changed environmental circumstances (Smith, 1985). The present work uses these techniques to determine the effects of weaning upon enterocyte development.

MATERIALS AND METHODS

Piglets taken from sows 21, 22 and 23 days after birth were fed a diet containing 23.4% crude protein (Protisoya), 10% fat, 46.5% carbohydrate and 6% fibre for 5 days before being killed for experiment. Other piglets left on the sow were killed at corresponding times to act as controls. Pieces of mid intestine were removed and fixed for measurement of intestinal structure, frozen for cyto-

chemical determination of disaccharidase activities or placed in an uptake apparatus for autoradiographic determination of amino acid transport.

RESULTS AND DISCUSSION

Villus height and crypt depth showed no significant age-dependent variation 26 to 28 days after birth for tissue taken from unweaned and weaned piglets. Weaning onto a soya bean diet for 5 days, however, did cause a pronounced fall in villus height and increase in crypt depth (villus heights of 496 ± 13 and 222 ± 26 μm; crypt depths of 158 ± 22 and 283 ± 11 μm for tissue taken from unweaned and weaned pigs respectively; means ± SEM). Further experiments were undertaken to determine the effect of weaning on disaccharidase development. The results obtained are summarized in Fig.1.

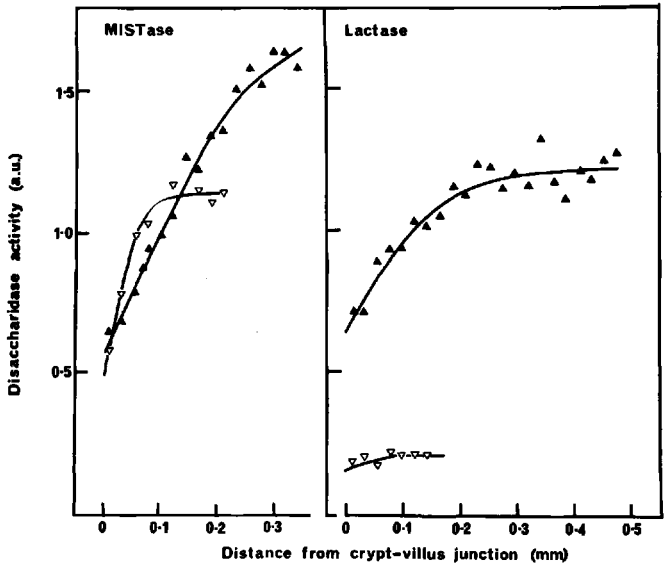


Fig.1. Effect of weaning on MISTase and lactase development. Lactase and MISTase (maltase, isomaltase, sucrase and trehalase) activities were determined cytochemically in intestines taken from weaned (▽) and unweaned (▲) piglets.

MISTase activity increases rapidly in brush border membranes of enterocytes migrating over the lower part of the villus. The final activity reached is higher in unweaned piglets, mainly because enzyme development takes place over a longer distance. Lactase activity also increases rapidly as enterocytes leave the crypt-villus junction and weaning causes a large inhibition in activity. The greater susceptibility of lactase to inhibition at weaning is similar to that recorded during the onset of graft-vs-host disease in neonatal mice (Lund et al., 1985). Further experiments were undertaken to determine the capacity of enterocytes to absorb 1mM lysine and alanine. The results of these experiments are shown in Fig.2.

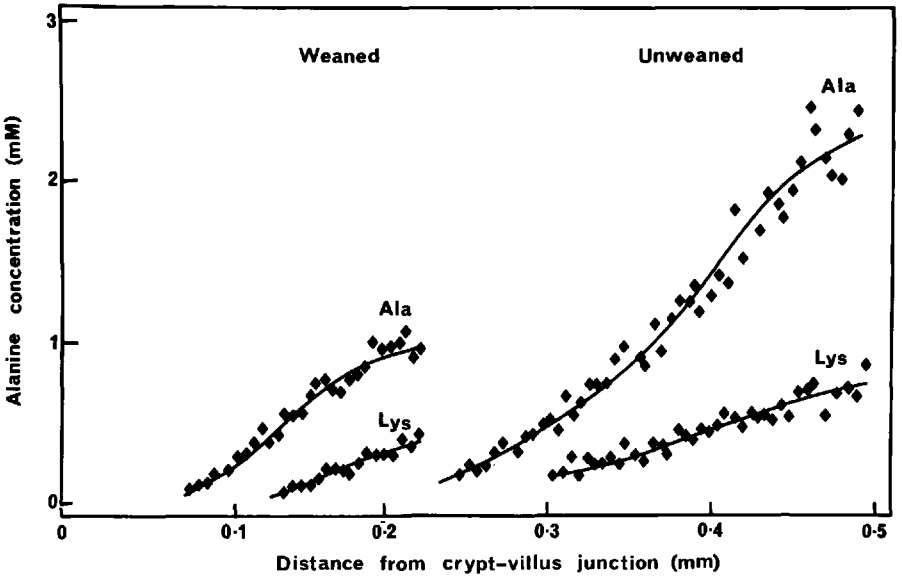


Fig.2. Developmental profiles for amino acid transport. [^{3}H] Alanine (Ala) and lysine (Lys) uptake was determined autoradiographically in intestines taken from weaned and unweaned piglets.

The ability of enterocytes to transport amino acids occurs late in development and lysine absorption remains less than for alanine. The effect of weaning is to shorten the time needed to express absorptive properties and reduce the maximal capacity of individual

enterocytes to transport amino acids. Similar changes in alanine transport occur after weaning 15 day old piglets onto a normal commercial pig feed (Smith, 1984). Logistic curves were fitted to data shown in Figs. 1 and 2 to assess, quantitatively, changes taking place in enterocyte development. Some of the results obtained from this analysis are given in Table 1.

Table 1. Logistic constants describing how weaning affects enterocyte development.

Treatment	Measured parameter	Logistic constants	
		m	c
Unweaned	Alanine transport	384 ± 11	2.5 ± 0.2
	Lysine transport	409 ± 23	1.9 ± 0.3
	MISTase activity	69	1.7
	Lactase activity	7	1.2
Weaned	Alanine transport	137 ± 4	0.9 ± 0.1
	Lysine transport	179 ± 7	0.7 ± 0.1
	MISTase activity	10	1.1
	Lactase activity	NV	0.2

Values for m give the distances (μm) from the crypt-villus junction where increase in enzyme activity or amino acid transport takes place at maximal rate. Values for c give the maximal values for enzyme activity (o.d.) or transport (mM). NV: Negative value for m in the intestinal crypt.

Weaning reduces significantly the value of m for all measured parameters. There are also smaller differences in m between alanine and lysine transport and MISTase and lactase activity. Values for c are in all cases reduced by weaning, this effect being greater for lactase and least for MISTase development. The net result of these opposite effects on enterocyte development is likely to determine the efficiency with which intestines adapt to changes in their immediate environment.

REFERENCES

LUND, E.K., PICKERING, M.G., SMITH, M.W. & FERGUSON, A. 1985. Gastroenterology, in the press.

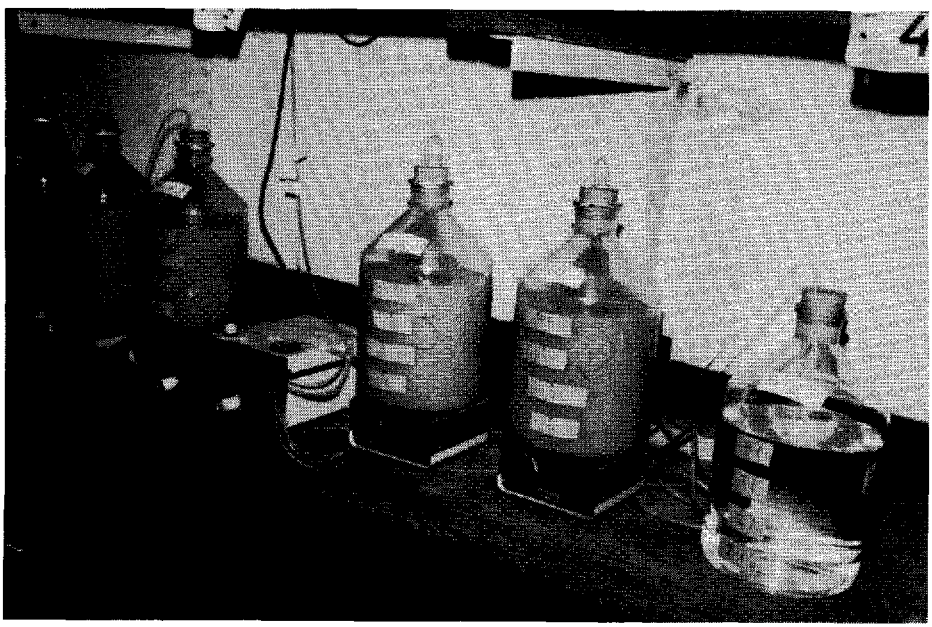
SMITH, M.W. 1984. J agric.Sci., Camb. 102: 625-633.

SMITH, M.W. 1985. Ann. Rev. Physiol. 47: 247-260.

SESSION 2

THE SECRETORY RESPONSE OF THE DIGESTIVE TRACT TO THE DIET

Discussion leader: T. Corring


4

THE SECRETORY RESPONSE OF THE DIGESTIVE TRACT TO THE DIET

ERIK SKADHAUGE
Department of Veterinary Physiology and Biochemistry
The Royal Veterinary and Agricultural University
Bülowsvej 13, DK-1870 Copenhagen V, Denmark

INTRODUCTION

This survey presents a brief outline of normal absorption and secretion of salt and water in the pig intestine, and the interaction with uptake of nutrients. The pathophysiological mechanisms involved in secretory and malabsorption diarrhoea is reveiwed. A short survey of intestinal adaptation is included.

Due to the importance of the pig as a domestic animal and its utility as a model for human experimental physiology a vast number of experiments has been performed on this species. Comprehensive surveys on the digestion and nutrient absorption have recently been presented by Kidder & Manners (1978) and by Low (1980).

Responsible for both absorption and secretion in the gastrointestinal tract are the events in the transporting cells lining the gut wall. The unidirectional transport of ions can be measured with isotopes in the direction of secretion (from plasma to gut lumen) and absorption (from lumen to plasma). The ion transport leads to an electrical potential difference across the gut wall which, together with diffusion, allows transport across the cell walls or between the cells through the lateral intercellular spaces. The main driving force responsible for active transport across the epithelial cells is the Na pump. The concept of active transport was first formulated by Hans H. Ussing and the cellular transport described in the Koefoed-Johnsen-Ussing model. Due to its importance, also for the absorption of nutrients, a short outline of the Na transport model, and the glucose-amino acid interaction with Na transport, will be presented.

The Ussing model assumes that the apical membrane is Na-permeable, and the basolateral membrane K-permeable (Figure 1). The Na pump is localized to the basolateral membrane. During expenditure of metabolic energy this pump transports Na out of the cell in exchange for K which is moved into the cell. The pump keeps the intracellular concentration of K high, of Na low. It is stimulated by the Na,K-sensitive ATPase. The inflow of Na across the apical membrane

The Ussing/Crane model

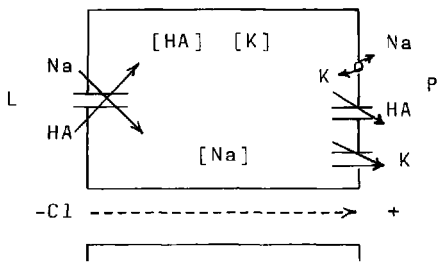


Figure 1.
L = lumen, P = plasma, HA = hexose-amino acid, ⎯⎯ = permeability, ↶ = Na pump.

is passive in response to the combined driving forces of "down-hill" movement along a concentration and an electrical gradient since the intracellular potential is negative. The reason for this is that more Na is moved out of the cell by the Na pump than K in. The net Na transport from lumen to plasma creates a negative potential inside the gut lumen which allows a diffusion across the lateral intercellular spaces or, in combination with Na, across the apical membrane. Chloride and K diffuse across the basolateral membrane.

Proteins and carbohydrates are in the chyme hydrolyzed only to th dipeptide and dihexose level. The final hydrolysis to hexose and amino acid takes place by enzymes localized to the apical membrane (at the brush border). Hexoses and amino acids are then coupled to the Na transporter and moved "up-hill" into the cells by the "down-hill" movement of Na. This is the so-called Na gradient mechanism originally formulated by R.K. Crane. Hexoses and amino acids are assumed to diffuse "down-hill" from the basolateral side of the cell into the blood stream.

The water movement across the gut wall takes place by two mechanisms. The first is general osmosis i.e. water movement "down-hill" an osmotic difference between chyme and plasma, if present. As the chyme generally is iso-osmotic or slightly hyperosmotic to plasma the general osmotic forces across the gut wall are small and the majority of water movement takes place in linkage with the solute flow. This so-called solute-linked water flow is caused by a local hypertonicity build up in or between the cells. In addition the villi may develop a countercurrent system which leads to slight hypertonicity of the top of the villi i.e. in the regions of presumed highest rate of solute and nutrient transport. The solute-linked water flow and local hypertonicity explains why net volume flow can take place in the direction from gut lumen to plasma even when the gut contents are 100-200 mOsm hyperosmotic to plasma (Skadhauge 1973).

NORMAL ABSORPTION OF SALT AND WATER

The intestinal tract is the largest transcellular compartment in the body. The action of the enzymes of the gastrointestinal juices, and the microbial fermentation, require a certain water content of the chyme. The ionic and osmotic composition must be controlled in order to ensure an optimal milieu for the digestive and absorptive processes. In addition the flow rate along the gut, and the retention in the various parts, must be well adjusted. Failure to maintain this delicate balance due to rapid changes of the diet, including weaning, leads to increased sensitivity to pathogenic agents, in worst cases to diarrhoea and loss of the animal, in less severe cases to relative malabsorption and retarded growth.

The basic condition for normal digestive function is therefore normal electrolyte and water transport along the length of the gut. The following sections will deal with this subject in the pig under normal and pathological conditions (diarrhoea).

ABSORPTION IN THE SMALL INTESTINE

Studies aiming at a detailed description of normal flow and absorption of salt and water in the small intestine are few, but available evidence (Low 1980, Low et al. 1978, Partridge 1978) shows that the volume flow along duodenum and jejunum is 2-4-fold higher than the intake of water in adult pigs (30-40 kg). Only after passage of the ileum is the volume/day equal to the rate of oral intake. The fecal excretion is reduced to 20-1 % depending on the type of feed. As the concentrations of electrolytes in chyme of the oral small intestine is close to that of plasma (Hamilton & Roe 1977) it can be concluded that the anal part of the small intestine (ileum) absorbs the large amount of secretions coming into the gut at a rate of at least 3-fold the oral intake. The colon absorbs an amount of salt and water roughly equal to the rate of intake, or a daily amount equal to nearly half of the content of the extracellular fluid.

The absorption of glucose, NaCl, and water in the jejunum was studied by an in vivo intraluminal perfusion technique (Argenzio 1980). This segment was found to secrete net solute (largely NaCl) and water in the absence of glucose in the perfusion medium, even with 150 mM Na on the luminal side. With rising luminal glucose concentration (0-80 mM) net water transport changed from secretion to absorption

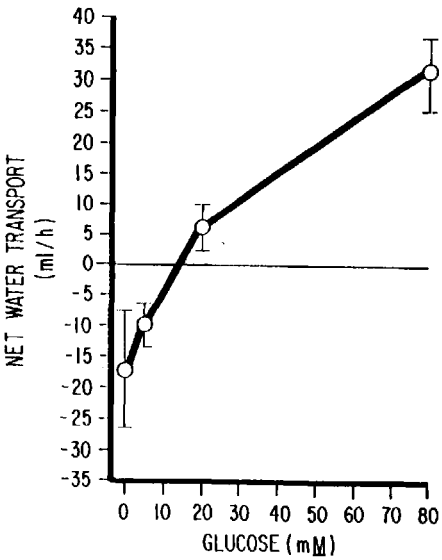


Figure 2.

(Figure 2). The glucose transport followed Michaelis-Menten kinetics; both secretion and absorption was strictly iso-osmotic to plasma since a linear relationship between net solute and water transport was observed. The slope was 0.31 equal to 310 mOsm of the transported fluid. As the glucose concentration must be reduced along the ileum (Sambrook 1979) it can be inferred that the absorption of NaCl can proceed in ileum even at low glucose concentration. In vitro studies of mid-ileum show a marked stimulation of Na absorption by luminal glucose (Jesus & Smith 1974a). These authors followed also the Na transport of mid-ileum during the first 10 days of the life of the pig. Their study (Jesus & Smith 1974b) showed a marked reduction of net Na absorption after suckling, but measurements after 12 hours fasting (the piglets were denied access to the sow) showed a doubling of the net Na transport per cm^2 serosal area over 10 days. Since the short circuit current (SCC) apparently was reduced to the half, transport of other ions must change as well.

The intestinal absorption has also been studied in a slaughter study in which 4 week old piglets were weaned to a liquid diet containing PEG as an unabsorbable water marker (Hamilton & Roe 1977). The osmoality and pH and the concentration of PEG, NaCl and K were measured in 12 segments from stomach to descending colon. Bearing in mind that the weaning most likely has changed the transport rates (see later) this study shows little net transport of Na and water across the wall of the small intestine as the concentrations of Na and PEG remained fairly constant throughout the small intestine. The concentration of Cl fell from over 100 mM to less than 40 mM, and the pH increased, indicating a $Cl\text{-}HCO_3$ exchange. Total osmolality was nearly 600 mOsm in proximal jejunum and fell to nearly 400 mOsm in the colon. The major water and Na absorption took in this study place

in colon. Other studies (Harpur & Popkin 1965) and (Case et al. 1981) have shown osmolalities of normal chyme in the small intestine of 4-500 mOsm indicating that the balance between break-down of nutrients, which increases the osmolality, and the power of solute-linked water absorption allows volume transport from lumen to plasma from fluids hyperosmotic to plasma.

ABSORPTION IN COLON

The colonic transport has been much better elucidated in the pig. In general the role of the hind gut in mammalian nutrition, homeostasis, and disease has been studied intensively in recent years and several informative reviews are available (Kaspar & Goebell 1982, Just 1983, Rérat 1978, Wrong et al. 1981). In the pig, a monogastric omnivore, the large intestine is more developed than in carnivores. It is, however, without the large volume and the many infoldings which characterize monogastric herbivores and allow these animals to have a prolonged and selective retention of the nutritionally most valuable parts of the contents (Engelhardt et al. 1983). Compared to dog, horse and cow (see Bayley 1978) the pig has a relative volume of total gastrointestinal tract in colon, which is double as large as in dog and cow, but two thirds of that of the horse.

The large intestine in pigs is not only the site of electrolyte and water absorption, an important breakdown of carbohydrates to SCFA and absorption takes place (Argenzio & Southworth 1974, Clemens et al. 1975) but not of amino acids (Just 1983, Just et al. 1981, Rérat 1978, Rudolph et al. 1983). Because of the large acid production by microbial fermentation it is not surprising that electrolyte studies have shown a pronounced ability to secrete HCO_3; in fact, the large bowel has even been denoted a "supplementary rumen" (Argenzio & Stevens 1984). The SCFA production and absorption may account for up to three quarters of basal metabolic rate (Argenzio & Stevens 1984). A large part of the SCFA is metabolized already in the epithelium (Imoto & Namioka 1978a, Argenzio & Southworth 1974).

An important in vivo perfusion study has been carried out in pigs weighing 46 kg on the average (Argenzio & Whipp 1979). Sodium, Cl, HCO_3 and acetate transport was studied from perfusion fluids having ionic contents resembling those of the normal contents of colon. The absorbate was near iso-osmotic to plasma and the main electrolyte content was Na-acetate. The colon had a daily capacity to absorb 8.6 li-

ters of water, 1.9 mols Na, 2.9 mols acetate and to secrete 1.0 mol HCO_3. (This capacity is approximately the double of the production rate estimated by Imoto & Namioka (1978b)). The osmolality of the absorbate was 370 mOsm; the Cl absorption was independent of the Na absorption, but Cl absorption was equal to HCO_3 secretion. It will therefore appear that the normal large intestine of the pig has the capacity to absorb not only NaCl and water but a substantial amount of nutrients. Even with the rapid perfusion rate applied in this study the concentration of acetate fell to the half along the perfused segment. Under natural conditions the acetate concentration remains approximately 100 mM throughout the length of the gut indicating a continued rate of production. The total concentration of SCFA is the double (Clemens et al. 1975). Perfusion with Cl as the major anion also resulted in a large absorption of Na and water (Cromp et al. 1980). Anaesthesia (halothane) had little effect on the estimates of solute transport by pigs colon (Argenzio & Lebo 1980).

A number of in vitro studies measured colonic electrolyte function in the newborn pig. Bentley & Smith (1975) investigated isolated pieces of mucosa from the spiral colon in the Ussing chamber of one day old pigs. Unidirectional measurements of Na and Cl were made and the short circuit current (SCC) measured. The net Na transport was considerably larger than the SCC but the difference was only partly accounted for by Cl absorption. The K transport was near zero. The ionic concentrations of colonic contents were measured in one day old and in adult pigs. These analyses showed a more than 50 % reduction of Na concentration and near 4-doubling of K and fairly constant Cl concentration along the colon. This indicates an approximate 80 % absorption of NaCl from the large intestine, whereas the increase in K concentration is passive due to the water absorption accompanying the NaCl and acetate absorption.

The colonic Na transport undergoes important changes during the first 10 days of post-natal life, a period of very fast growth rate of the entire gut. At birth the average transmural electrical potential difference (PD) was measured to 43 mV lumen negative and a net Na absorption was measured. Both PD and Na transport was stimulated by amino acid (methionine) in the luminal bathing solution (Hénin & Smith 1976). This transport and the stimulating effect disappeared gradually over the 10 days being nearly absent already after day 4. Concomitantly, the Na transport increased by a factor of at

least 2 and became increasingly inhibitable with the Na-channel blocking agent amiloride in the mucosal solution (Cremaschi et al. 1979). This indicates an important change of the Na transport which switches from a type of that of the small intestine to one characteristic of the colon in other species. Sodium transport at 10 days of life depends on Na permeability of a channel of the apical membrane and not, as at birth, on a Na-non-electrolyte co-transport. This amiloride-sensitive Na channel is in other epithelia (see Skadhauge et al. 1985) important for the control of net rate of Na absorption. Its permeability is mainly modulated by aldosterone. Due to the rapid growth of the young pig, and the limited Na content of sows milk, piglets will need to conserve Na. A high aldosterone concentration of around 2000 pg/ml plasma has been reported on the six' day (Ferguson et al. 1979). Injection of canrenoate, an aldosterone antagonist, inhibited the amiloride-sensitive augmentation of Na transport reported above (Ferguson et al. 1979).

In larger pigs (20-30 kg) the colonic absorption of Na and water was doubled by dietary NaCl restriction (Argenzio & Lebo 1982). In slightly larger pigs fed a high-NaCl diet injections of DOCA reduced fecal Na output to 20 % of the control value (Gregorian et al. 1973). These studies indicate that the output from the gut of Na-(Cl) is under mineralocorticoid regulation in the pig as in other monogastric omnivores. The large intestine is, however, also under cyclic nucleotide regulation as the net absorption of Na was reduced by theophylline, a phosphodiesterase inhibitor (Argenzio & Lebo 1982, Argenzio & Whipp 1983a).

PATHOPHYSIOLOGY OF SECRETORY DIARRHOEA

From a physiological viewpoint 4 distinct types of intestinal malfunction leading to diarrhoea are found in the pig: Infection with toxic bacteria, particularly E.coli, viral enteritis with rota- or coronavirus (transmissible gastroenteritis), swine dysenteria (infection with treponema hyodysenteria), and finally, the complex syndrome of post-weaning diarrhoea which involves antigenic reactions leading to structural and enzymatic changes,and osmotic diarrhoea.

This section describes first the basic mechanisms of secretory diarrhoea, and the possible compensation by oral replacement solutions, and deals then in turn with the pathogenic mechanisms mentioned above.

The pathophysiology of secretory diarrhoea has been studied exten-

sively in the last 2 decades and the mechanism of the oral glucose-electrolyte treatment has been elucidated. Briefly, the non-hemorrhagic diarrhoea induced by toxins of E.coli or V. cholerae is caused by secretion of NaCl and water in the oral end of the small intestine and the effect of oral replacement fluid is compensating augmentation of the absorption further anally in the small intestine.

According to the model of Field (see Field et al. 1980) the Cl transport from plasma to lumen in the crypts of Lieberkühn is augmented by the pathogenic agents. The toxins bind to the apical surface of the enterocytes and induce increased intracellular concentration of either cAMP or cGMP. The former is characteristic of cholera toxin, the latter of the heat-stable E.coli toxin. These cellular mediators will, added to the bathing fluid of in vitro experiments, induce Cl secretion. The enhanced Cl transport leads also to Na and water secretion, and the diarrhoea causes a severe fecal loss of extracellular fluid. The glucose-stimulated NaCl and water absorption from the top of the villi remains, however, intact. There is so far no direct evidence for the location of Cl and water secretion to the crypts in the small intestine but in the colon of rats, in which no villi are present, the prostaglandin-induced secretion has by microcollection of fluid been demonstrated to come from the crypts (Welsh et al. 1982).

The oral replacement fluid contains, as formulated by WHO for the use in man (Field et al. 1980), around 100 mM glucose, NaCl to replace the continued loss with feces, and some KCl and HCO_3. In pigs, as in other monogastric mammals, the majority of carbohydrate of ingested food is absorbed almost quantitatively in the small intestine (Keys & DeBarthe 1974). A large part of ileum will therefore have a very low glucose concentration. This concentration is enlarged by the oral replacement fluid. When the NaCl-gradient mechanism is stimulated water is also absorbed and the diarrhoea is counteracted. As pointed out in the previous section the Na transport of the pig small intestine is very sensitive to the presence of glucose; a high glucose concentration should therefore be important in cases of secretory diarrhoea in the pig. The purpose of the addition of $KHCO_3$ to the WHO formula is to replace the fecal loss of these ions. The augmented flow of NaCl through the large intestine leads to an augmented Na-K and Cl-HCO_3 exchange which gives a loss of K and HCO_3. For the E.coli infection in the pig it should be noted that this pathophysio-

logical mechanism is further augmented by a direct action of the toxin on the colon.

E.COLI DIARRHOEA

In a series of papers Hamilton et al. (1977, 1978a, 1978b) have investigated the effects of heat-stable and heat-labile E.coli enterotoxins, choleratoxin and theophylline on the small intestine of weanling pigs. The reason for the use of theophylline in these and other studies is, as noted, that it inhibits the phosphodiesterase and therefore augments the concentration of cyclic nucleotides. In the papers of Hamilton et al. ligated loops of jejunum were used in animals kept under anaesthesia throughout the experiment. PEG served as a marker of net volume movement. Most experiments were of 20 minutes duration. A marked secretion of water and NaCl was observed. Unidirectional fluxes of Cl and Na were carried out, but changing specific activities may limit the interpretation of the results. The toxins and theophylline showed additive effects, and glucose augmented absorption from control loops and impaired the secretion. The mucosal concentration of cAMP was not changed significantly (Hamilton et al. 1978c); the heat-stable toxin affects however cGMP.

The passive permeability properties of duodenum and middle and distal jejunum were also measured after exposure to heat-stable coli toxin (Presnell et al. 1979). The osmotic permeability coefficient (by the authors denoted filtration coefficient) was estimated by adding mannitol to a total osmolality of 600-700 mOsm to a solution containing 160 mM NaCl. Interpretation of the results is therefore limited by the likely presence of solute-linked water flow in the control loops, but presumably absence in the toxin-treated loops. No change in apparent osmotic permeability was actually observed with mannitol as osmotic agent, but the osmotic permeability was slightly impaired by osmotic agents with reflection coefficients below unity (reflection coefficient for erythritol = 0.9, for urea = 0.7). The reflection coefficient was not affected by the toxin indicating that the toxin does not damage the intestinal wall as such, but affects the active NaCl transport and the accompanying water flow.

The effects of heat-stable E.coli toxin and theophylline were studied both in vivo and in vitro on pig jejunum and ileum (Argenzio et al. 1984). The effect of theophylline was as described in other species, a net secretion of Cl was induced. Heat-stable toxin induced

a rise in the mucosal concentration of cGMP and an increased secretion of HCO_3 both in jejunum and ileum. In addition a net absorption of NaCl was in both organs changed to secretion. The SCC as studied in vitro was, in agreement with these findings, augmented by heat stable toxin and theophylline in an additive way.

In contrast to the findings in some other species the proximal colon of the pig is also influenced by the action of heat-stable E. coli enterotoxin (and by theophylline and choleratoxin as well). Argenzio & Whipp (1981) found by loop experiments in vivo a large reduction of water absorption, with net Na absorption reduced to near zero and increased secretion of HCO_3. There was a clear dose-response curve for heat-stable toxin. Similar effects were brought about by choleratoxin and theophylline but there was a difference. Colitoxin selectively elevated mucosal cGMP. choleratoxin cAMP, whereas theophylline augmented both. The lumen-negative transepithelial PD was augmented during the cause of action of the toxins. The mucosa was also mounted in vitro, and after theophylline PD and SCC were observed to rise in parallel. This shows, that the change of PD is due to movement of charge, presumably an ion secretion. In a subsequent study of the proximal spiral colon in vitro Argenzio & Whipp (1983b). found an only moderate SCC equal to the small difference between large unidirectional fluxes of Na and Cl. An electroneutral coupled NaCl transport does therefore seem to be of importance. The effect of heat-stable toxin was apparently not that of changing the Cl flux in the serosa-mucosa direction as in other species (Field et al. 1980) but to reduce Na transport om the serosa-mucosa direction.

Of great interest in these studies is the fact that colitoxin inhibits salt and water absorption in <u>colon</u> as such. The diarrhoea is therefore also of primarily colonic origin. As mentioned above HCO_3 is secreted in great quantity due to the direct colonic action of the toxin and not only as a part of a compensatory mechanism. As coronavirus produces acid secretion there is a pathophysiological basis for the clinical observation that feces pH is high during infection with choleratoxin but low when transmissible gastroenteritis is the pathogenic agent (Bertschinger 1984, Bergeland & Henry 1982).

Recent advances in pathophysiological insight and in treatment of enterotoxigenic diarrhoea has been summarized by Powell (1984).

VIRAL DIARRHOEA

Transmissible gastroenteritis (TGE) is caused by a coronavirus infection. The intestinal malfunction leads to diarrhoea. Its pathophysiology has been studied in some detail in relation to salt and water transport in the small and large intestine. In vivo perfusion of the proximal jejunum, mid-jejunum, ileum and colon 40 hours after the infection in 3 week old piglets showed only impaired NaCl and water transport in the first segment (Butler et al. 1974). The proximal jejunum was therefore analysed further in vitro by the Ussing-chamber technique (McClung et al. 1976). These authors observed Na and Cl secretion in all tissues. The secretion was changed to absorption of Na by luminal glucose (3o mM). Chloride was less affected. The control rates of net Na and Cl transport were not affected by TEG, but the glucose response was abolished. Addition of ouabain reduced the SCC due to impaired Na transport, but theophylline increased SCC reflecting augmented Cl secretion. In a further study the time course of the infection was studied. It was progressing the first 40 hours, with complete recovery after 6 days (Kerzner et al. 1977). This study, and a following study in the ileum (Shepherd et al. 1979), characterized the reaction of several enzymes of the enterocytes to the infection. The lactase, sucrase and Na,K-ATPase (in units/g protein) were strongly reduced, whereas the concentration of thymidine kinase was quadrapled. This can readily be interpreted to indicate that the absorption defect is caused by the villus atrophy characteristic of TGE with a preponderance of undamaged crypt cells. These lack the Na-glucose cotransport system in their apical membrane as well as the sessile enzymes causing the final hydrolysis to amino acid and hexose. The latter study (Shepherd et al. 1979) demonstrated the same glucose stimulation of ileal SCC (presumably Na transport) as in jejunum. This demonstrates directly the physiological rationale for the effect of oral glucose also in this disease, albeit with a reduced response.

Since TGE may lead to a loss of glucose from the small intestine, due to the impaired absorption of carbohydrate, Argenzio et al. (1984) addressed the question of the nature of compensation in colon. This usually allows 3 week old piglets to survive the infection but leads regularly to death in younger animals (3 day old piglets were studied). Argenzio et al. slaughtered the animals 2 days after infection in the

younger, 4 days in the older group. PEG was used as a water marker. As to be expected control animals had a lower PEG concentration in stomach and small intestine than given in the milk. With the PEG concentration of stomach content as a baseline ileum of control pigs had a 5-fold higher concentration, colon 9-fold indicating 80 and near 90 per cent absorption of water, respectively. At 3 weeks of age these values were higher. In the infected piglets no water absorption was apparent along the entire gut in the 3 day old group, but a pronounced colonic compensation (around 5-fold increase in marker concentration) was observed in the 3 week old pigs. The 3 day old pigs had a 6-fold higher carbohydrate concentration in colon during infection, in the 3 week groups it was reduced to 3/4, but with a slightly increased SCFA concentration; this was higher than in the 3 day old group. The main reason for the improved colonic compensation in the older animals is therefore the development of microbial fermentation. This reduces the osmotic load imposed by undigested carbohydrate (due to the infection of the small intestine). The SCFA' which are absorbed lead also to a high rate of Na absorption which augments the water absorption.

The summary, the TGE diarrhoea is osmotic in origin due to malabsorption caused by the villus atrophy of the small intestine.

ROTAVIRUS DIARRHOEA

Two days after infection with rotavirus 6 day old piglets were prepared for in vivo infusion experiments of jejunum and ileum (Graham et al. 1984). In addition stool analysis was made. The infectio resulted in a major change of net absorption of water, Na and 3-O-methylglucose into secretion both in jejunum and ileum. In addition the levels of sucrase and lactase of the mucosa were strongly reduced Over 3 days after infection fecal osmolality increased from 250 mOsm to 350 mOsm with the difference accounted for two thirds by lactose and one third by Na. In a previous study Telch et al.(1981) demonstrated impaired glucose absorption both in vivo and in vitro.

The pathogenecity of the rotavirus diarrhoea is just as for TGE: impaired absorption and enzyme digestion in the small intestine leads to an overload of colon giving an osmotic diarrhoea made worse by a reduced absorption of Na, acetate and water in colon. The authors suggest that virus enteritis should be treated with oral solutions with less glucose than in the WHO formula so that no more carbohy-

drate is given than is actually absorbed. Some clinical studies point to greater advantage of a glucose-amino acid solution (Bywater & Woode 1980),and it is understandable that simple withholding of food may reduce the scour.

SWINE DYSENTERIA (TREPONEMA HYODYSENTERIE)

In this disease the morphological findings point to a primary affection of the colon with unaffected small intestine. These pathological events have been amply confirmed by physiological experiments. Net solute and water absorption from the entire small intestine, studied by in vivo perfusion, was identical in control and infected pigs (Argenzio 1980) whereas colonic water and electrolyte (Na, K, Cl, HCO_3) absorption, studied by in vivo installation by the loop technique (Argenzio et al. 1980),was abolished in infected animals. Unidirectional Na and Cl fluxes showed no change of serosa-mucosa movement, but severe reduction of fluxes in the mucosa-serosa direction. In agreement with this the cellular concentration of cAMP was little affected (Schmall et al. 1983). The severe loss of electrolytes and water occurring in the disease is therefore caused exclusively by failing colonic absorption. It might be borne in mind also in this context that an amount of Na equal to nearly half the content of the extracellular volume is daily absorbed in the colon. With a primary colonic affection the efficacy of the oral glucose-electrolyte treatment is therefore in this disease due to small intestinal compensation of a loss occurring further anally.

POSTWEANING DIARRHOEA

The large and complex problems of adjustment of the pig intestine to weaning are outside the scope of this survey but a few notes concerning the adaptation in general and the pathophysiology of diarrhoea in particular are relevant.

As will be pointed out in the section on adaptation any major variation in amount and type of food ingested will lead to morphological changes of the intestine, to different rates of flow and enzymatic composition of secretory juices, and to changed physiological transport parameters. A change from sows milk to other nutrients will therefore eo ipso, regardless of age of the piglet, lead to transient and permanent changes of the variables mentioned above. To this comes the specific effects of the start of the weaning pro-

cess in relation to the age of the piglet. Many experiments are needed to distinguish clearly between the effect of the weaning process itself (which depends heavily on the time schedule of feeding change-over and the type and amount of food), and the time after birth when the weaning process is instituted. To this comes the problems of effects of race, and differences in general maintenance of the sows and piglets; among these factors is environmental temperature (Pouteaux et al. 1982, Dauncey 1983). All the parameters finally influence the chance of pathogenic agents to develope to a stage which causes diarrhoea in the already diarrhoea-prone weaning piglet.

One major hypothesis concerning the ethiology of postweaning diarrhoea has been advanced by Miller et al. (1984a, b). These authors conclude that pigs have a transient immune hypersensitivity to dietary antigenes which leads to villus atrophy, crypt cell hyperplasia and malabsorption. This causes increased susceptibility to E.coli enteritis and consequently diarrhoea (Miller et al. 1984c). The malabsorption is likely to have a central position in the pathogenesis of the diarrhoea which may occur even in the absence of pathogenic microorganisms. The general reduction of absorption in the small intestine, as demonstrated by Hamilton & Roe (1977), and the lack of enzymatic adaptation to the ingested foods, particularly carbohydrates, may lead to an osmotic overload of the colon. The structural and enzymatic changes in the postweaning period were recently confirmed by Hampson & Kidder (1984). As Miller's group these authors point to the antigenicity of the diet and substantiate this concept with the finding of smaller structural changes and less reduction of brush-border enzyme activity in a group weaned on to a less antigenic diet (Hampson et al. 1983). These effects were demonstrated very clearly in a study by Etheridge et al. (1984). They collected feces from three groups of piglets weaned at four weeks of age to two diets the first consisted of a corn-soybean meal starter diet, the second a steamed rolled oat groats-casein diet; a third group remained with the sow. Feces was collected daily and analysed for minerals (Na, K, Cl, Ca, P) and some organic substances (lactose, glucose and SCFA). Total osmotic excretion was measured by dissolving the dried feces into a known quantity of water and measuring osmolality by freezing point depression. The three diets resulted in very different pattern (Table 1). Osmolality was as noted not determined on fresh feces but from the authors' account of water content of the feces, approximate

Table 1. Osmotic composition of feces.

Food	Corn-soybean	Oats-casein	Sow's milk
Organic molecules	16 %	12 %	8 %
Minerals	31 %	39 %	71 %
Unknown "osmolality"	53 %	49 %	21 %
Approximate osmolality	750 mOsm	440 mOsm	250 mOsm

estimates of total osmolality can be given. These are included in the table.

The table shows that the postweaning diets present an osmotic stress to the colon as they lead to an increased output of organic molecules of low molecular weight and an osmolality which will force water movement in the serosa-mucosa direction. The large amount of unknown "osmolality" in feces is probably also due to organic molecules. The conclusion is that the decreased absorption of nutrients, particularly carbohydrates, in the small intestine leads to unnatural fermentation and loss of nutrients and water from the large intestine.

As this pathogenicity of diarrhoea is similar to that of viral diarrhoea it is not surprising that restriction of feed intake has significantly reduced the incidence and severity of postweaning diarrhoea (Ball & Aherne 1982).

ADAPTATION OF INTESTINAL ABSORPTION

The word adaptation can be used in several contexts in relation to intestinal transport: 1. The change of various parameters as functions of time after a change in diet or other external or internal disturbances. 2. The difference between "before and after" when a new steady state has been achieved. 3. The causal relations between observed changes in transport functions and the releasing mechanisms.

Adaptation of intestinal absorption of nutrients and of salt and water is difficult to characterize even for one clinical or experimental situation. The problems in describing adaptation to external and internal changes are manyfold larger. Dietary, hormonal, pathological, and environmental factors can all change one or several absorption patterns on various segments of the gut.

The description of a transport pattern for a given intestinal segment is difficult because a complete kinetic characterization requires

many experiments. Consider for example a carrier-mediated transport which, following Michaelis-Menten kinetics, changes in such a way from state A to state B that the affinity decreases considerably (K_m is increased), but the maximal transport rate (V_{max}) is increased. In this case transport measurements at low concentration of the substance will show reduced rate of absorption from A to B whereas measurements at high concentration will show increased absorption!

The description of adaptive changes is also difficult because it is significant which intestinal parameters the change are related to. Absorption may be related to the intact organism (either kg body weight, surface area, basal metabolic rate, etc.), or calculated per cm along the intestine, per cm^2 serosal or mucosal surface area, or per mg protein or DNA in the mucosa. These differences will often prevent meaningful comparison between experiments. Finally comes the difficulty in determining whether adaptation is best characterized by local rates of absorption at naturally occurring concentrations in the chyme, by maximal absorption capacity (as determined in vivo or in vitro) or by fractional absorption related to localized segments of the gut.

To these more fundamental problems comes technical difficulties and the inherent limitation in the various techniques available to the nutritionist or intestinal physiologist. There is always a difference between in vivo and in vitro experiments (Smith 1980). This is at least partly due to the absence of normal blood flow in vitro (see Mailman 1982) and - for fast diffusing molecules - caused by different unstirred layers (see Section I in Skadhauge & Heintze 1983).

A large body of knowledge of intestinal adaptation is available to day but largely at the descriptive level. The time course of events, the change in structure and transport function for transpositions of gut segments, changes in food intake, reaction to hormones, etc. are known in detail. The requirements for composition of chyme and pancreatico-biliary secretion to maintain normal structure and function are well known (Robinson et al. 1981). Knowledge about what causes these changes is, however, virtually non-existant. For the important adapative regulation of sugar and amino acid transport there is little knowledge about the regulation at a subcellular level. The central question of whether increased transport depends on inducing more carriers in existing cells or producing new cells with a higher density of carriers is not known (Karasow & Diamond 1983).

The same ignorance applies to the effects of aldosterone on Na transport (Skadhauge 1983, Clauss et al. 1984).

In the case of the pig an important maturation takes place over the first 8 weeks of life. This involves gastric digestion, acid secretion and proteolytic enzyme production (Cranwell 1984), as well as plasma gastrin concentration (Bunn et al. 1981), development of digestive enzymes (Aumaitre 1971, Hartman et al. 1961, Manners 1976) and the change in size of the organs (McCance 1974). The villus height and crypt depth and the amino acid-Na transport interaction are all affected by the energy intake (Dauncey et al. 1983). Early weaning may lead to morphological changes similar to an acute inflammatory response (Kenworthy 1976).

Important information concerning correlation of structure and function in food utilization can be obtained from detailed physiological studies of the adaptative changes induced by selective breeding ans crossing of races. The classical papers of Hjalmar Clausen (1953, 1956) report a cross-breeding experiment with wild pigs and Danish landrace. The comparison showed that when the food intake for each kg live weight gain fell by 46 % the total length of the gut increased by 45 %. Ludvigsen (1982) discovered that Danish landrace pigs digest crude fiber better with age than pigs of other breeds. The weight of colon is about 20 % higher than in the other breeds. As suggested by Manners (1976) these differences call for detailed physiological studies to reveal the mechanisms involved.

REFERENCES

ARGENZIO, R.A. 1980. Am.J.Vet.Res. 41:2000-2006.

ARGENZIO, R.A. & LEBO, D. 1980. Am.J.Vet.Res. 41:39-45.

ARGENZIO, R.A. & LEBO, D. 1982. Can.J.Physiol.Pharmacol. 60:929-935.

ARGENZIO, R.A., LIACOS, J., BERSCHNEIDER, H.M., WHIPP, S.C. & ROBERTSON, D.C. 1984. Can.J.Comp.Med. 48:14-22.

ARGENZIO, R.A., MOON, H.W., KEMENY, L.J. & WHIPP, S.C. Gastroenterology 86:1501-1509.

ARGENZIO, R.A., WHIPP, S.C. & GLOCK, R.D. 1980. J.Infect.Dis. 142: 676-684.

ARGENZIO, R.A. & SOUTHWORTH, M. 1974. Am.J.Physiol. 228:454-460.

ARGENZIO, R.A. & STEVENS, C.E. 1984. Proc.Nutr.Soc. 43:13-23.

ARGENZIO, R.A. & WHIPP, S.C. 1978. J.Physiol. 295:365-381.

ARGENZIO, R.A. & WHIPP, S.C. 1981. J.Physiol. 320:469-487.

ARGENZIO, R.A. & WHIPP, S.C. 1983a. Am.J.Vet.Res. 44:1480-1487.

ARGENZIO, R.A. & WHIPP, S.C. 1983b. Can.J.Physiol.Pharmacol. 61:1138-1148.

AUMAITRE, A. 1971. Ann.Zootech. 20:551-575.

BALL, R.O. & AHERNE, F.X. 1982. Can.J.Anim.Sci. 62:907-913.

BAYLEY, H.S. 1978. J.Anim.Sci. 46:1800-1802.

BENTLEY, P.J. & SMITH, M.W. 1975. J.Physiol. 249:103-117.

BERGELAND, M.E. & HENRY, S.C. 1982. In "The Veterinary Clinics of North America". (Ed. L.G. Biehl). Vol. 4, No. 2, p. 389-399.

BERTSCHINGER, H.U. 1984. Z.fortschr.Veterinärmed. April:29-32.

BUNN, C.M., HANSKY, J., KELLY, A. & TITCHEN, D.A. 1981. Res.Vet.Sci. 30:376-378.

BUTLER, D.G., GALL, D.G., KELLY, M.H. & HAMILTON, J.R. 1974. J.Clin. Invest. 53:1335-1342.

BYWATER, R.J. & WOODE, G.N. 1980. Vet.Rec. 106:75-78.

CASE, G.L., LEWIS, L.D., PHILLIPS, R.W. & CLEEK, J.L. 1981. Am.J.clin Nutr. 34:1868-1878.

CLAUSEN, H. 1953. The Improvement of Pigs. The George Scott Robertson Memorial Lecture. Publ. M. Boyd, Belfast. p. 1-24.

CLAUSEN, H. 1956. Lantwirtschaftlich angewandte Wissenschaft. p. 5-42

CLAUSS, W., ARNASON, S.S., MUNCK, B.G. & SKADHAUGE, E. 1984. Pflügers Arch. 401:354-360.

CLEMENS, E.T., STEVENS, C.E. & SOUTHWORTH, M. 1975. J.Nutr. 105:759-768.

CRANWELL, P.D. 1984. Proc.Aust.Soc.Anim.Soc. 15:145-157.

CREMASCHI, D., FERGUSON, D.R., HENIN, S., JAMES, P.S., MEYER, G. & Smith, M.W. 1979. J.Physiol. 292:481-494.

CRUMP, M.H., ARGENZIO, R.A. & WHIPP, S.C. 1980. Am.J.Vet.Res. 11:1565 1568.

DAUNCEY, M.J., INGRAM, D.L., JAMES, P.S. & SMITH, M.W. 1983. J.Physiol. 341:441-452.

ENGELHARDT, W., RECHKEMMER, G., LUCIANO, L. & REALE, E. 1983. In: "New Trends in Pathophysiology and Therapy of the Large Bowel". (eds. L.Barbara, M. Miglioli & S.F. Phillips). Elsevier Science Publ. 1-17.

ETHERIDGE, R.D., SEERLEY, R.W. & HUBER, T.L. 1984. J.Anim.Sci. 58: 1403-1411.

FERGUSON, D.R., JAMES, P.S., PATERSON, J.Y.F., SAUNDERS, J.C. & SMITH M.W. 1979. J.Physiol. 292:495-504.

FIELD, M., FORDTRAN, J.S. & SCHULTZ, S.G. (eds.) 1980. "Secretory Diarrhoea" American Physiological Society, Bethesda, MD.

GRAHAM, D.Y., SACKMAN, J.W. & ESTES, M.K. 1984. Digestive Diseases and Sciences. 29:1028-1035.

GRIGORIAN, M.E., GREKIN, R.J., MITCHELL, J., DYBUS, S.P., BUITEWEG,

J.J. & BOHR, D.F. 1983. Proc.Soc.Exp.Biol.Med. 174: 265-269.

HAMILTON, D.L. & ROE, W.E. 1977. Can.J.comp.Med. 41:241-250.

HAMILTON, D.L., ROE, W.E. & NIELSEN, N.O. 1977. Can.J.comp.Med. 41: 306-317.

HAMILTON, D.L., FORSYTH, G.W., ROE, W.E. & NIELSEN, N.O. 1978b. Can. J.comp.Med. 42:316-321.

HAMILTON, D.L., JOHNSON, M.R., FORSYTH, G.W., ROE, W.E. & NIELSEN, N.O. 1978c. Can.J.comp.Med. 42:327-331.

HAMILTON, D.L., JOHNSON, M.R., ROE, W.E. & NIELSEN, N.O. 1978a. Can. J.comp.Med. 42:89-96.

HAMPSON, D.J. & KIDDER, D.E. 1984. Proc.Nutr. 43: 18A.

HARPUR, R.P. & POPKIN, J.S. 1965. Can.J.Biochem. 43:1157-1169.

HARTMAN, P.A., HAYS, V.W., BAKER, R.O., NEAGLE, L.H. & CATRON, D.V. 1961. J.Anim.Sci. 20:114-123.

HENIN, S. & SMITH, M.W. 1976. J.Physiol. 262:169-187.

IMOTO, S. & NAMIOKA, S. 1978a. J.Anim.Sci. 47:479-487.

IMOTO, S. & NAMIOKA, S. 1978b. J.Anim.Sci. 47:467-478.

JAMES, P.S. & SMITH, M.W. 1976. J.Physiol. 262:151-168.

JESUS, H. & SMITH, M.W. 1974a. J.Physiol. 243:225-242.

JESUS, H. & SMITH, M.W. 1874b. J.Physiol. 243:211-224.

JUST, A. 1983. "The Role of the Large Intestine in the Digestion of Nutrients and Amino Acid Utilization in Monogastrics". IVth Int. Symp. Protein metabolism and nutrition, Clermont-Ferrand (France), (ed. INRA publ.)

JUST, A., JØRGENSEN, H. & FERNANDEZ, J.A. 1981. Br.J.Nutr. 46:209-219.

KARASOV, W.H. & DIAMOND, J.M. 1983. Am. J. Physiol. G443-G462.

KASPER, H. & GOEBELL, H. eds. 1982. Colon and Nutrition. Falk Symposium 32. MTP Press.

KENWORTHY, R. 1976. Res.Vet.Sci. 21:69-75.

KEYS, J.E. & DeBARTHE, J.V. 1974, J.Anim.Sci. 39:57-62.

KIDDER, D.E. & MANNERS, M.J. 1978, Digestion in the Pig. Scientechnica Bristol.

KERZNER, B., KELLY, M.H., GALL, D.G., BUTLER, D.G. & HAMILTON, J.R. 1977. Gastroenterology 72:457-461.

LOW, G.A. 1980. J.Sci.Food Agric. 31:1087-1130.

LOW, G.A., PARTRIDGE, I.G. & SAMBROOK, I.E. 1978. Br.J.Nutr. 39:515-526.

LUDVIGSEN, J.B. 1982. Ann. Meeting EAAP, Leningrad. p. 1-10.

MAILMAN, D. 1982. Ann.Rev.Physiol. 44:43-55.

MANNERS, J.M. 1976. Proc.Nutr.Soc. 35:49-55.

McCANCE, R.A. 1974. J.Anat. 117:475-479.

McCLUNG, H.J., BUTLER, D.G., KERZNER, B., GALL, D.G. & HAMILTON, J.R. 1976. Gastroenterology 70:1091-1095.

MILLER, B.G., NEWBY, T.J., STOKES, C.R. & BURNE, F.J. 1984a. Res.Vet. Sci. 36:187-193.

MILLER, B.G., NEWBY, T.J., STOKES, C.R., HAMPSON, D.J. & BROWN, P.J. 1984b.Am.J.Vet.Res. 45:1730-1733.

MILLER, B.G., PHILLIPS, T.J., STOKES, C.R. & BOURNE, F.J. 1984. Proc. Nutr.Soc. 45:116A.

PARTRIDGE, I.G. 1978. Br.J.Nutr. 39:527-537.

POUTEAUX, V.A., CHRISTISON, G.I. & RHODES, C.S. 1982. Can.J.Anim.Sci. 62:1199-1209.

POWELL, D.W. 1984. Pharmac.Ther. 23:407-416.

PRESNELL, K.R., ROE, W.E., NIELSEN, N.O. & HAMILTON, D.L. 1979. Can. J.comp.Med. 43:44-49.

RERAT, A. 1978. J.Anim.Sci. 46:1808-1837.

ROBINSON, J.W.L., DOWLING, R.H. & RIECKEN, E.-O. 1982. Mechanisms of Intestinal Adaptation. Falk Symposium 30.MTP Press.

RUDOLPH, B.C., BOGGS, L.S. KNABE, D.A., TANKSLEY, T.D. & ANDERSON, S. A. 1983. J.Anim.Sci. 57:373-386.

SAMBROOK, I.E. 1979. Br.J.Nutr. 42:267-277.

SCHMALL, L.M., ARGENZIO, R.A. & WHIPP, S.C. 1983. Am.J.Vet.Res. 44: 1309-1316.

SHEPHERD, R.W., GALL, D.G., BUTLER, D.G & HAMILTON, J.R. 1979. Gastro-enterology 76:20-24.

SKADHAUGE, E. 1973. Renal and Cloacal Salt and Water Transport in the Fowl (Gallus domesticus). Dan.Med.Bull. 20, suppl. I.

SKADHAUGE, E. & HEINTZE, K. 1983. Intestinal Absorption and Secretion. Falk Symposium 36. MTP Press.

SKADHAUGE, E., CLAUSS, W., ARNASON, S.S. & THOMAS, D.H. 1985. Proc. 1st Int.Comp.Physiol.Biochem.Congr. Springer Verlag.

SMITH, M.W. 1980. In "Current Concepts of Digestion and Absorption in Pigs", Techn. Bull. 3, Nat. Inst. Res. Dairying, Reading. p. 157-172.

TELCH, J., SHEPHERD, R.W., BUTLER, D.G., PERDUE, M., HAMILTON, J.R. & GALL, D.G. 1981. Clin.Sci. 61:29-34.

WELSH, M.J., SMITH, P.L., FROMM, M. & FRIZZELL, R.A. 1982. Science 218:1219-1221.

WRONG, O.M., EDMONDS, C.J. & CHADWICK, V.S. 1981. The Large Intestine. Its Role in Mammalian Nutrition and Homeostasis. MTP press.

ROLE OF ARACHIDONIC ACID PATHWAYS IN COLONIC ION TRANSPORT AND MUCOSAL PROTECTION

R.A. ARGENZIO AND J.A. LIACOS
School of Veterinary Medicine
North Carolina State University
Raleigh, NC 27606 USA

SUMMARY

The objective of the present study was to test the hypothesis that endogenous metabolites of the cyclooxygenase pathway of arachidonic acid metabolism provide a cytoprotective response to mucosal injury. Using an in vivo preparation of the porcine colon, the response to bile salt-induced mucosal injury was studied in the presence or absence of cyclooxygenase or phospholipase blockade. Net water movement, transmural potential difference (PD), and mannitol clearance from blood to lumen were quantified as indices of damage in each of the above conditions. Results showed that: a) bile salt-induced colonic secretion was dose-dependent but was unaffected by any of the enzyme inhibitors; and b) bile salt-induced increases in colonic permeability were dose dependent, and were increased in the presence of phospholipase and cyclooxygenase blockade. These results suggest that metabolites of the cyclooxygenase pathway, viz. prostaglandins, provide a measure of cytoprotection in response to an acute mucosal injury, and may be important endogenous mediators of mucosal protection and repair. In addition, the diarrhea associated with bile salt malabsorption does not appear to involve activation of phospholipase or metabolites of the arachidonic acid cascade.

INTRODUCTION

The role of arachidonic acid metabolites in colonic cytoprotection or in diarrheogenic and inflammatory processes of the colon is an unresolved and controversial issue. Several studies have shown that prostaglandins (PGs) of the E type activate adenyl cyclase, and thus, may be diarrheogenic. Indeed, PG levels are raised in injured colonic mucosa associated with diarrhea, and they have also been implicated in mediating inflammatory processes of the colon. On the other hand, both exogenous and endogenous PGs protect the stomach and upper small bowel against a variety of injurious agents, which would otherwise cause severe tissue destruction. Thus, they have been termed "cytoprotective" agents.

The present study examines the role of the two major pathways of arachidonic acid metabolism in colonic secretion and mucosal integrity by selective blockade of one or both pathways during acute mucosal injury induced by bile salts.

MATERIALS AND METHODS

Experimental animals were crossbred pigs weighing 15-20 kg. Each animal was surgically prepared with a small cecal cannula 7-10 days prior to study. On the day of the experiment, the large intestine was thoroughly cleansed by infusing warmed saline into the cecal cannula. The pig was then anesthetized with pentobarbital Na and connected to a respirator. The carotid artery and jugular vein were catheterized to allow measurements of arterial pressure, blood gases, transmural potential difference (PD) and for infusion and sampling of isotopes and drugs. The spiral colon was then exposed, and two or three 10 cm loops were constructed, which allowed recirculation of experimental solutions from heated reservoirs. The colon was then replaced and the incision closed.

After a 10 min equilibration period, zero time samples were simultaneously withdrawn from the reservoirs and artery, and 50 μCi of ^{14}C-mannitol was injected into the vein. Arterial samples were then taken at intervals to determine the plasma ^{14}C activity. Samples were taken from the reservoirs at 30 min intervals for determination of polyethylene glycol and ^{14}C.

RESULTS AND DISCUSSION

An initial series of experiments compared the effect of 3mM deoxycholic acid (DCA) under control or intraluminal (0.5 mM) indomethacin (cyclooxygenase inhibitor) conditions. These results, shown in Table 1, indicate that the net secretory response induced with DCA was unaffected by indomethacin, and control values were reestablished within 30-60 min after the DCA was removed.

In contrast, both the mannitol clearance and ΔPD were significantly greater in the presence of indomethacin during the DCA exposure, and restoration of normal values appeared sooner in control loops.

Time control experiments over a 2 hr period in the absence of DCA revealed no significant differences in any of the measured parameters. Thus, the effect of intraluminal indomethacin cannot be ascribed to a nonspecific damaging effect of this agent on the mucosa.

Table 1. Effect of Indomethacin on mucosal injury induced with 3 mM deoxycholate (DCA).

Parameter	PERIOD (min)					
(n=6 ± SE)	Control	DCA	RECOVERY			
	0-30	30-60	75-105	105-135	135-165	165-195
NET H_2O Movement (µl/cm/30 min)†						
CONTROL	-300	+ 600	0	-200	-250	-275
	±200	± 200	± 50	±105	±100	±100
INDOMETHACIN	+ 75	+1000	+100	-220	-100	-350
	±100	± 200	± 75	± 50	± 75	± 60
Mannitol Clearance (µl/cm/30 min)						
CONTROL	10	170	50	10	70	160
	± 4	± 60	± 20	± 10	± 30	± 40
INDOMETHACIN	20	340*	100*	100*	100	160
	± 6	± 40	± 30	± 40	± 20	± 30
ΔPD (mV)						
CONTROL	+0.5	-2.1	+4.0	+4.0	+4.0	+4.3
	±0.5	±2.5	±1.0	±1.0	±1.0	±1.0
INDOMETHACIN	0.0	-9.0*	-2.0*	-1.5*	-1.0*	0.0*
	±0.5	±3.0	±2.5	±1.0	±1.0	±1.0

†Positive values = net secretion, negative = net absorption.
*$P<0.05$ compared to control.

A second series of experiments was conducted with 3 groups of pigs, which received either an intravenous buffer control, i.v. indomethacin (10 mg/kg) or i.v. mepacrine, (10 mg/kg), an agent which inhibits release of arachidonic acid. Each pig was prepared with 3 loops, which received 0, 3, or 6 mM DCA, and then all 3 loops were given 6 mM DCA at the end of the 2 hr recovery period. This latter response was combined for all 3 loops inasmuch as no significant effect of the preceding treatment was present (Table 2).

Net water secretion was again observed in loops receiving DCA, which was clearly dose-dependent but unaffected by the inhibitor used. Mannitol clearance increased in pigs receiving indomethacin or mepacrine in the presence of either 3 or 6 mM DCA. The response was dose-dependent and appeared to increase with time. Changes in the PD in general reflected the mannitol clearance, and were greater in pigs given indomethacin or mepacrine.

Table 2. Effect of indomethacin and mepacrine on mucosal injury induced by 3 or 6 mM deoxycholate (DCA)

Parameter and Animal Treatment Group	Loop Treatment			
	Control	3 mM	6 mM	6 mM
(n=6 ± SE)				
Net H_2O Movement (µl/cm/30 min)†				
CONTROL	-130	+390	+1650	+1700
	± 70	±130	± 180	± 100
INDOMETHACIN	+200	+600	+1730	+1580
	±500	± 60	± 190	± 130
MEPACRINE	- 20	+650	+1500	+1460
	±130	±140	± 210	± 110
Mannitol Clearance (µl/cm/30 min)				
CONTROL	9	80	200	300
	± 6	± 10	± 30	± 30
INDOMETHACIN	18	170*	360*	480*
	± 11	± 30	± 60	± 50
MEPACRINE	22	160*	390*	490*
	± 7	± 30	± 50	± 50
ΔPD (mV)				
CONTROL	-0.3	-0.8	-1.2	-1.5
	±0.3	±0.2	±0.2	±0.5
INDOMETHACIN	-0.2	-2.0*	-3.0*	-2.6*
	±0.2	±0.3	±0.5	±0.6
MEPACRINE	-0.3	-5.0*	-4.2*	-5.7*
	±0.3	±1.0	±0.9	±1.2

†Positive values = net secretion, negative = net absorption.
*$P<0.05$ compared to control.

Thus, the results of these inhibitor studies strongly implicate endogenous metabolites of the cyclooxygenase pathway in some degree of protection against acute mucosal injury. Although the underlying mechanism of this protection is unknown, the present results suggest that additional studies in both acute and chronic colonic injury are warranted. Such studies may shed light upon basic mechanisms of epithelial injury and repair as well as defining specific therapeutic agents to be used in colonic disease.

SOME QUALITATIVE AND QUANTITATIVE ASPECTS OF DIGESTION AND ABSORPTION OF ESSENTIAL FATTY ACIDS (EFA) IN THE GROWING PIG

K. CHRISTENSEN
Royal Veterinary and Agricultural University
Department of Animal Physiology
Rolighedsvej 25, DK-1958 Copenhagen V, Denmark

SUMMARY

1. The dietary concentrations of linoleic and linolenic acid were underestimated when the fat was extracted with diethylether (EE) compared to extraction with EE following a hydrochloric acid hydrolysis (HCl-EE). For a purified fat-poor diet the underestimation was 585% for linoleic and 684% for linolenic acid. 2. EFA were excreted via the bile in amounts related to the dietary intake. Even in EFA deficiency EFA were excreted. 3. On a virtually fat-free diet about 8 g fat was excreted in the bile daily, but only 1.6 g appeared in faeces. 4. Of EFA only linoleic acid was detected in faeces. 5. Addition of 90 g soyabean oil/kg diet depressed microbial activity in the gut, whereby more linoleic acid escaped hydrogenation.

INTRODUCTION

EFA comprise linoleic (18:2n6) and linolenic (18:3n3) acid and their n6- and n3-polyunsaturated fatty acids (PUFA), which may be synthesized from them in the organism.

It is common practise to express the dietary concentration of EFA in percent of dietary energy. Dietary energy may be expressed as, a gross energy (GE) either determined by bomb calorimetry or more frequently calculated on basis of the chemical composition or b in some cases as metabolizable energy or c socalled available energy calculated from the chemical composition by multiplication with some arbitrary factors. Sometimes d it is even not stated what it is.

Routinely, dietary fat and fatty acids have been extracted with EE as prescribed by official procedures, although it is well established that HCl-EE generally extracts more fat and fatty acids. This may affect the calculation of dietary energy. To what extent will the extraction procedure influence the amount of fat and EFA and thereby the dietary concentration of EFA?

Lipids are mainly excreted from the organism via the bile. To what extent are EFA excreted via the bile, and is the excretion dependent on the EFA status of the organism? Is the excretion of EFA in faeces related to the dietary intake?

The present paper describes some preliminary experiments focusing on the problems in determining the intake and the digested and absorbed amounts of EFA.

MATERIALS AND METHODS

Two basic diets without and with addition of 4.3% soyabean oil were used for examination of the effect of the fat extraction procedure on the dietary concentrations of linoleic and linolenic acid. Diets A and B were purified diets without (A) and with (B) soyabean oil consisting of (A, B in %): Maize starch (17.50, 8.26) potato meal (20.00, 21.00), tapioca meal (30.00, 31.50), casein (20.00, 21.50), cellulose (8.00, 8.60), vitamin-mineral mixture (4.50, 4.84) and soyabean oil (0, 4.30). Diets C and D were conventional diets without (C) and with (D) soyabean oil consisting of (C, D in %): Barley (74.30, 65.90), soyabean meal (20.00, 24.72), meat and bone meal (3.00, 2.17), vitamin-mineral mixture (2.70, 2.91) and soyabean oil (0, 4.30). Fat was extracted with EE and HCl-EE. GE was determined both by bomb calorimetry and by calculation multiplying the amount of fat, carbohydrates and protein with 39.8, 17.6 and 23.8 kJ/g, respectively. Four samples per diet were used.

Three female littermates of Danish Landrace were used for studying the excretion of EFA via the bile. The pigs were fed diet A without (Pig 1) and with soyabean oil corresponding to 1.2 (Pig 2) and 2.3 (Pig 3) % of gross energy (GE%) from 15 to 50 kg live weight. Then they were slaughtered, and the gallbladder with its content of bile was removed for extraction of fat. Analyses of fat and fatty acids were as described by Kruse et al. (1977).

Ten pigs of Danish Landrace receiving one of three dietary concentrations of linoleic acid corresponding to 0.04, 0.4 and 9.5 GE% were used for studies of the apparent digestibility of linoleic and linolenic acid. Details about these experiments are described (Christensen, 1985).

RESULTS AND DISCUSSION

EE extracted more fat from the purified diets (A+B), whereas HCl+ EE extracted more fat from the conventional diets (C+D). For all diets, however, HCl-EE extracted more fatty acids and more EFA than EE, especially in the case of diet A. The dietary concentrations of linoleic and linolenic acid expressed in GE% were similar whether determined by bomb calorimetry or calculated from the chemical composition. However, as shown in Table 1 extraction with EE consistently underestimated the concentrations of EFA, especially in the case of diet A, the difference being 585% for linoleic and 684% for linolenic acid. The reproducibility of the determination expressed as the coefficient of variation (CV%) varied more than found previously (Christensen, 1985).

Table 1. Concentrations of linoleic and linolenic acid (% of gross energy) in purified and conventional diets after extraction with EE or HCl-EE

	Purified diets				Conventional diets			
Diet	A		B		C		D	
Soyabean oil	−		+		−		+	
	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
				linoleic acid				
EE	0.027	16.7	5.10	2.9	1.78	1.4	6.83	3.2
HCl-EE	0.185	3.0	5.29	2.0	2.91	1.1	7.89	5.9
				linolenic acid				
EE	0.0044	0.2	0.75	2.7	0.23	1.4	0.96	3.6
HCl-EE	0.0345	9.1	0.81	2.0	0.33	3.4	1.10	6.4

The excretion of linoleic acid in the bile was found to be dependent on the EFA status (20:3n9/20:4n6) of the organism (blood plasma) (Table 2). None of n6-PUFA were detected in the bile although present in plasma, whereas 20:5n3, 22:5n3 and 22:6n3 were present both in bile and plasma. It is remarkable that EFA were excreted even in the EFA deficient state. Of the biliary EFA only small amounts of linoleic acid were present in faeces, indicating reabsorption and/or

hydrogenation. Thus a conventional digestibility trial cannot estimate the digested amounts of EFA. At a dietary linoleate concentration of 0.04 GE% no 16:1 and 18:1 were detectable in faeces, but linoleic acid was present indicating that this fatty acid had been incorporated into microbes thereby avoiding hydrogenation. At linoleate intakes of 9.5 GE%, however, greater amounts of 18:2 escaped hydrogenation, apparently due to a reduced microbial activity in the gut. The methane production in pigs receiving 90 g soyabean oil/kg

Table 2. Daily excretion of fat and fatty acids in the bile of pigs receiving 0.2, 1.2 or 2.3 % of gross energy as linoleate[1]

GE% linoleate	0.2	1.2	2.3
20:3n9/20:4n6 of bile	1.51	0.32	0.15
20:3n9/20:4n6 of plasma	1.61	0.19	0.01
Total lipid, g	8.04	7.32	10.92
Total fatty acids, g	4.00	3.68	5.35
18:2n6, mg	249	452	860
18:3n3, mg	12	15	35
n3-PUFA, mg	72	93	163

1) Anticipating 1.2 l bile daily (Sambrook, 1978)

diet was 40% lower than in pigs receiving the basal diet without added oil (Christensen, 1985).

Although, about 8 g total lipid was excreted via the bile on a virtually fat-free diet, only 1.6 g was excreted in faeces. Most of this may have been reabsorbed, as fat is not generally regarded as an energy source for microbes.

Obviously, more knowledge about excretion and reabsorption of fat and fatty acids in relation to the dietary intake and EFA status of the organism is needed to understand the regulation of EFA metabolism.

REFERENCES

CHRISTENSEN, K. 1985. 577. Ber. fra Statens Husdyrbrugsforsøg. 158pp.

KRUSE, P.E., DANIELSEN, V., NIELSEN, H.E. & CHRISTENSEN, K. 1977. Acta Agric. Scand. 27:289-296.

SAMBROOK, I.E. 1978. Proc. Nutr. Soc. 37:87A..

SHORT-TERM EFFECTS OF RAW SOYBEAN DIET INGESTION UPON THE EXOCRINE PANCREATIC SECRETION IN THE PIG

T. CORRING, A.M. GUEUGNEAU, J.A. CHAYVIALLE*
Laboratoire de Physiologie de la Nutrition, INRA-CNRZ
78350 Jouy-en-Josas, France and * INSERM, Unité de Recherches de Physiopathologie, Hôpital E. Herriot, 69834 Lyon Cédex 2, France.

SUMMARY

The aim of the investigation was to study the short-term effects of raw soybean diet ingestion upon the exocrine pancreatic secretion in the pig. Permanent fistulae were fitted into the pancreatic duct and duodenum of 6 pigs, and a catheter was introduced into a carotid artery. The animals were adapted to a heated soybean diet for 15 days then successively submitted to a first 4-day experimental period during which they were fed on the same diet and to a second 8-day experimental period during which they received a raw soybean diet. Ingestion of the raw soybean diet immediately induced an overall increase in volume of pancreatic juice secreted and in V.I.P. and secretin plasma levels as well. Concentration and total protein output were not significantly affected; nor was CCK plasma level modified except within the first 3 days of the second experimental period, when it increased. We suggest that feedback regulation of the pancreatic secretion is involved in the response of the exocrine pancreas to raw soybean diet ingestion.

INTRODUCTION

Raw soybean meal is known to cause growth inhibition and to produce pancreatic enlargment and pancreatic enzyme hypersecretion in rats and chicks, although it does not produce pancreatic enlargment in larger animal species that have been tested (dogs, pigs, calves or monkeys). Most of the studies have been performed on the pancreatic tissue and this investigation was aimed to study in the pig the short-term effects of raw soybean diet ingestion upon the exocrine pancreatic secretion in the fistulated pig.

MATERIAL AND METHODS

A heated soybean diet (16 p.100 proteins) and a raw soybean diet (16 p.100 proteins) were used and provided, according to the experimental scheme, in 2 meals per day (9 a.m. and 4 p.m.) of 800g each diluted in 1600 ml H_2O. Six castrated Large White pigs have been adapted to the heated soybean diet for 8 days. At 38.8 $\pm$ 0.9 kg body weight, they were fitted with permanent fistulae into the pancreatic duct and duodenum (CORRING et al., 1972) and a catheter was introduced into a carotid artery. After a 7-day recovery period study during which they were fed on the heated soybean diet, all the pigs were successively submitted to a first 4-day experimental period (heated soybean diet) and to a second 8-day experimental period during which they received the raw soybean diet.

Pancreatic juice was continuously collected and reintroduced into the duodenum after measurement of volume and sampling for analysis. Arterial blood samplings were done at 12.00 and 4 p.m.

Analysis :

The proteins end enzyme activities of chymotrypsin, trypsin, lipase and amylase were determined in all juice samples. Secretin, cholecystokinin, gastrin, somatostatin, V.I.P. and P.P. were measured in plasma by radio immunoassay.

RESULTS

All the data obtained during the period of raw soybean diet ingestion are expressed in percentage of the corresponding mean value determined during the 4-day period of heated soybean diet ingestion.

Pancreatic secretion :

Volume of pancreatic secretion significantly increased on the first day of raw soybean diet ingestion and was higher, throughout the second experimental period, than mean volume recorded when the pigs ingested the heated soybean diet. The overall increase was about 21 p.100. Content of total proteins in pancreatic juice was not significantly affected but showed a tendancy to decrease. Total protein output increased (about 10 p.100) but not significantly. No change was observed in enzyme activity, except for trypsin specific activity which increased on day 3 and was higher to the end of the

second experimental period.

Gastrointestinal hormones :

Plasma levels of somatostatin, gastrin and P.P. were not modified by raw soybean diet ingestion. In contrast, plasma levels of secretin and V.I.P. strongly and immediately increased and were higher, throughout the second experimental period, than corresponding values obtained within the first experimental one (secretin : + 75 p.100 ; V.I.P: +100 p.100). Cholecystokinin plasma level showed a significant increase (+ 60 p.100) within the first 3 days of the raw soybean ingestion period.

DISCUSSION

Ingestion of raw soybean diet by the pig did not significantly affect the total protein output and concentration in the pancreatic secretion. In constrast, volume of juice was higher than during the heated soybean diet ingestion period.

SCHUMANN et al. (1983) obtained similar results in studying the pancreatic secretion of pigs during 24 hours and on day 6 of a raw soybean ingestion period. Variations observed in plasma levels of V.I.P. and secretin explained the increase in the volume of the pancreatic secretion. In the rat (BRAND and MORGAN, 1981), ingestion of a raw soybean diet evoked a release of cholecystokinin and an increase in the content of the total proteins in pancreas. We suggest that feed back regulation of pancreatic secretion could be probably involved in the response of pancreatic exocrine secretion to ingestion of raw soybean diet in the pig.

REFERENCES

BRAND, S.J. & MORGAN, R.G.H. 1981. J. Physiol. 329 : 325-343.

CORRING, T., AUMAITRE, A. & RERAT, A. 1972. Ann. Biol. anim. Biochim. Biophys. 12 : 109-124.

SCHUMANN, B., SOUFFRANT, W.B., MATKOWITZ, R. & GEBHARDT, G. 1983. Wiss. K. Marx Univ. Leipzig, Math. Nat. R. 32 : 570-575.

THE DEVELOPMENT OF THE STOMACH IN THE PIG: THE EFFECT OF AGE AND WEANING. I STOMACH SIZE, MUSCLE AND ZONES OF MUCOSA.

P.D. CRANWELL
School of Agriculture, La Trobe University,
Bundoora, Victoria 3083, Australia

SUMMARY

In a study on stomach development 176 Large White X Landrace pigs from twenty nine litters were used. The results indicate that pigs given access to solid food before weaning and weaned on to solid food (C pigs) have more stomach tissue per unit body-weight than pigs fed entirely on sows' milk (M pigs). Differences between the proportions of the various zones of mucosa and muscle in the M and C pigs were not significant.

INTRODUCTION

The aims of the experiments were to study the development of the size of the stomach and the proportions of the four zones of mucosa; viz. cardiac, pars oesophagea, fundic and pyloric (antral); and to determine what effects introduction of solid food and weaning have on this development.

MATERIALS AND METHODS

The stomach weight data came from seventy nine pigs (twenty four litters), 1 - 50 d and 1 - 17.2 kg body-weight reared entirely by the sow (milk-fed M), and ninety seven pigs (twenty nine litters), 18 - 115 d, 4.6 - 38.5 kg body-weight reared by the sow for 21 - 39 d but allowed access to solid food at 12 - 14 d and entirely dependent on solid food after weaning (creep-fed, C). The data on gastric muscle and mucosa came from fifteen M pigs and twenty C pigs (seven litters), 10 - 55 d, 3 - 21 kg body-weight. All pigs were killed with an overdose of sodium pentabarbitone. The stomach was immediately removed, opened along the greater curvature, emptied of contents, rinsed in physiological saline, blotted dry and weighed. The regions of the mucosa were identified (Fig. 1), dissected free of the underlying muscle tissue and weighed.

RESULTS AND DISCUSSION

There were significant correlations between stomach weight and body-weight for both M and C pigs. The regression equations were: for M pigs $Y = 4.56X + 1.65$, $r^2 0.90$, $P<0.001$, and for C pigs $Y = 6.56X - 6.28$, $r^2 0.95$, $P<0.001$, where X is body-weight (kg) and Y is stomach weight (g). The data from thirty six M pigs, 1 - 14 d (Table 1) were included in the estimate of both regression equations as there were no treatment differences until the pigs were 14 d.

The stomach weight: body-weight values in pigs were divided into four age-groups and are presented in Table 1. The values for M pigs in age-group 2 were significantly lower than those in age-group 1. The values for C pigs in age-group 3 were all from animals which had been weaned on to solid food. They had significantly more stomach tissue per unit body weight than the C pigs in the previous age-group and the M pigs in the age-group 3.

The proportions of the zones of mucosa as a percentage of total mucosal weight and the proportion of muscle as a percentage of total stomach weight are presented in Table 2. The differences between treatments for each zone of mucosa and for muscle were not significant. The stomach weight: body-weight values for these pigs were: M pigs 4.3± 0.14 g/kg and for C pigs 5.7± 0.20 g/kg and the difference between treatments was significant (df 37; $P<0.001$). The results indicate that although C pigs had more stomach tissue per unit body weight than M pigs the proportion for each zone of mucosa and for muscle were similar. There were significant correlations between body-weight and weight of each zone of mucosa and muscle for both M pigs and C pigs (Fig. 2, Table 3).

The difference in the rates of gastric development in M and C pigs could be due to a number of factors which include differences in the physical and chemical nature of the diets, changes in the patterns of feeding and gastric emptying and the effect of weaning. For instance, it is known that pigs reared by the sow suck twenty or more times per 24 h and that during each sucking they receive about 40 - 50 ml milk (Elsley, 1970). Weaned pigs have been observed to eat less often (nine to ten times per 24 h) and ingest more dry matter per feed (80 g per meal; Auffray & Marcilloux, 1980). Gastric distension has been shown to be of significant importance in the release of gastrin in the pig (Stadaas & Schrumpf, 1974) and

Table 1. Stomach weight: body-weight (g/kg) in M and c pigs

Age-group		1		2		3		4	
Age (d)		1 - 14		15 - 28		29 - 56		57 - 115	
Body-wt (kg)		1.0 - 5.6		4.4 - 11.6		6.2 - 25.0		18.0 - 38.5	
Stomach wt (g)		5 - 27		18 - 54		36 - 182		125 - 298	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Stomach wt: body-wt (g/kg)	M	5.3	0.14(36)§ ***	4.3 ††	0.10(17)	4.7 †††	0.15(26)	-	-
	C	-	-	4.9	0.17(21) ***	6.3	0.12(53)	6.4	0.21(23)

Differences between age-groups were significant: ***P<0.001
Differences between treatments were significant: ††P<0.01, †††P<0.001
§ Number of pigs in parentheses

Table 2. Proportions of different zones of mucosa and muscle in the stomachs of M and C Pigs

	% Total Mucosal Wt					
	M pigs			C pigs		
Zone of mucosa	Mean	SE	C.V.%	Mean	SE	C.V.%
Cardiac	19.8	0.7	12.7	21.0	0.9	20.8
Pars oesophagea	4.8	0.5	37.8	5.0	0.3	27.0
Fundic	55.9	1.4	9.6	54.8	1.1	9.5
Pyloric (antrum)	19.5	1.0	20.0	19.2	0.6	15.8
	% Total Stomach Wt					
Muscle	44.4	1.6	13.9	43.8	0.9	9.9

Table 3. Correlations between body-weight (kg) and gastric mucosal and gastric muscle weights (g) in M and C pigs

Relationship Body-wt (kg) v.	Treatment	Regression Equation	r^2	P
Cardiac mucosal wt (g)	M	Y = 0.38X + 0.05	0.87	<0.001
	C	Y = 0.67X - 0.65	0.89	<0.001
Pars oesophageal wt (g)	M	Y = 0.06X + 0.21	0.62	<0.01
	C	Y = 0.17X - 0.26	0.75	<0.001
Fundic mucosal wt (g)	M	Y = 1.02X + 0.53	0.93	<0.001
	C	Y = 2.35X - 7.52	0.84	<0.001
Antral mucosal wt (g)	M	Y = 0.30X + 0.53	0.71	<0.01
	C	Y = 0.64X - 1.07	0.85	<0.001
Stomach muscle wt (g)	M	Y = 1.42X + 3.12	0.90	<0.001
	C	Y = 2.70X - 2.12	0.93	<0.001

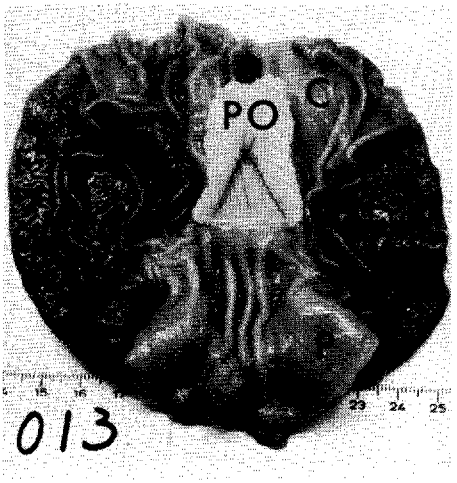


Fig. 1. The zones of the gastric mucosa from Pig 013, 39 d, 12.5 kg body-weight, stomach weight 77 g. PO, pars oesophagea; C, cardiac; F, Fundic; P, pyloric (antrum). (Scale divisions in mm).

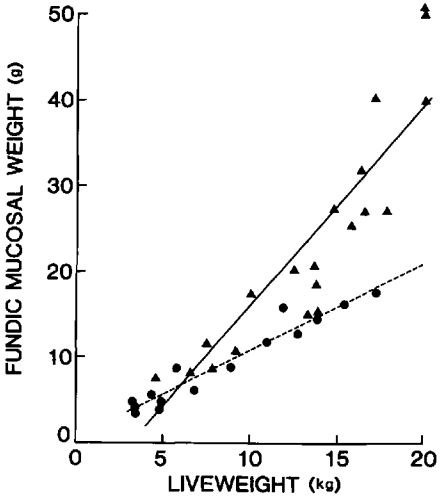


Fig. 2. Linear regression of fundic mucosal weight (g) v. liveweight (kg) in M pigs (-●-) and C pigs (-▲-). The regression equations are in Table 3.

Cranwell & Hansky, (1980) have found that the postprandial gastrin response to intake of food is greater in pigs fed solid food than in those suckled by the sow. Gastrin is a trophic hormone for the stomach in adult animals (Johnson, 1981) and could be acting in this way in young pigs.

REFERENCES

AUFFRAY, P. & MARCILLOUX, J.C. 1980. Reprod. Nutr. Develop. 20: 1625-1632.

CRANWELL, P.D. & HANSKY, J. 1980. Res. vet. Sci. 29: 85-88.

ELSLEY, F.W.H. 1970. In "Lactation" (Ed. I.R. Falconer) Butterworths, London, p. 393-411

JOHNSON, L.R. 1981 In "Physiology of the Gastrointestinal Tract" (Ed. L.R. Johnson) Raven Press, New York, p. 169-196.

STADAAS, J. & SCHRUMPF, E. 1974. Scand. J. Gastroenterol. 9: 781-785.

THE DEVELOPMENT OF THE STOMACH IN THE PIG: THE EFFECT OF AGE AND WEANING. II ACID AND PROTEOLYTIC ENZYME SECRETORY CAPACITY.

P.D. CRANWELL
School of Agriculture, La Trobe University,
Bundoora, Victoria 3083, Australia.

SUMMARY

Seventy Large White X Landrace pigs (thirty five littermate pairs) from twelve litters were used in gastric secretion studies under anaesthesia. The results indicate that gastric acid and proteolytic enzyme secretory capacity develops more rapidly in pigs given access to solid food before weaning and weaned on to solid food (C pigs) than pigs fed entirely on sows' milk (M pigs).

INTRODUCTION

The aims of the study were to determine the effects of age, introduction of solid food and weaning on gastric acid and proteolytic enzyme secretion.

MATERIALS AND METHODS

Pigs and their treatment

Twelve litters of Large White X Landrace pigs were used in the experiment (six groups of two). During the day following farrowing the pigs of each litter were paired according to sex and size and cross-fostered, i.e. one pig from each pair was allocated to each sow. One litter from each group was reared entirely by the sow (milk fed, M) whereas the other litter was reared by the sow for 21 d, but was allowed access to solid food by 14 d and was entirely dependent on solid food after weaning (creep fed, C). The solid food used throughout the experiment was Pig Creep Starter Crumbles + Mecadox (Barastoc Products, Victoria, Australia) and contained 210 g crude protein (nitrogen x 6.25)/kg.

Gastric perfusion experiments

Gastric perfusion experiments were carried out in thirty five litter-mate pairs of pigs (<24 h - 42 d old) which were alloted to four age-groups (Tables 1 & 2). The procedure followed was similar

to that described by Tudor *et al.* (1977) except that in all pigs other than those in age-group 1 (Tables 1 & 2) the secretagogue, betazole hydrochloride (Histalog, Eli Lilly, Indianapolis, USA), was infused intravenously at a dose rate of 3 mg/kg per h for 2 h. The acid secretory response to Histalog in youg pigs is maximal at this dose rate (Cranwell & Stuart, 1983).

Analytical Procedures

Acidity of the gastric perfusate was measured by potentiometric titration with 2.5 mmol - NaOH to pH 7.00 under carbon dioxide-free conditions. The highest consecutive 30 min outputs were doubled to express maximum hourly output, i.e. mmol H^+/h. Proteolytic enzyme concentration was determined by the method described by Fourie *et al.* (1974) with the exception that the gastric perfusate was not further diluted. Again the highest consecutive 30 min outputs were doubled to express maximum hourly output i.e. units/h. A unit (u) is defined as being equivalent to ΔA_{280} of 0.001 per min at pH 2.0 at 37°, measured as trichloroacetic acid-soluble products using haemoglobin as substrate.

RESULTS AND DISCUSSION

There were significant correlations between maximal acid output (mmol H^+/h) and liveweight (kg) for both M and C pigs (Fig. 1). The slope of the regression line for C pigs was significantly different from that for M Pigs ($P<0.05$). There were also significant correlations between maximal proteolytic enzyme output ($\log_{10}$ units/h) and liveweight (kg) for both M and C pigs (Fig. 2). The slope of the regression line for C pigs was significantly different from that for M pigs ($P<0.05$).

The results of this study indicate that the stomach of the newborn pig is capable of secreting HCl and proteolytic enzymes but its secretory capacity is significantly lower than that of older pigs (Tables 1 & 2). It was evident that the pattern of development of secretory capacity for acid differed from that for proteolytic enzymes. Whereas the increase in maximal acid output with liveweight was linear the increase in maximal proteolytic enzyme output with liveweight was logarithmic (Figs. 1 & 2). Also acid output per unit liveweight remained relatively constant from 18 - 42 d in both M and C pigs but

Table 1. Maximal acid output in response to Histalog infusion$^{\Theta}$ (3mg/kg/h, IV) or Histalog injection^{+} (3mg/kg/15min, IM) in M and C pigs

Age-group		1^{+}			2$^{\Theta}$			3$^{\Theta}$			4$^{\Theta}$	
Age (d)		<1			4 - 14			18 - 22			25 - 42	
Livewt (kg)		1.0 - 1.6			1.8 - 4.3			4.0 - 7.3			6.2 - 12.9	
No of pigs/ treatment		8			14			8			15	
		Mean	SE		Mean	SE		Mean	SE		Mean	SE
Maximal acid output (mmol H^{+}/h)	M	0.56	0.07	***	2.84	0.34		4.15 †	0.59	*	6.95 †††	0.87
	C	-	-		-	-	***	6.32	0.65	**	10.59	0.94
Per unit livewt (mmol H^{+}/kg/h)	M	0.41	0.05	***	0.92	0.05	*	0.67 ††	0.10		0.68 †††	0.07
	C	-	-		-	-		1.07	0.08		1.13	0.09

Differences between age groups were significant: *P<0.05, **P<0.01, ***P<0.001.
Differences between treatments were significant: †P<0.05, ††P<0.01, †††P<0.001.

Table 2. Maximal proteolytic enzyme output in response to Histalog infusion$^{\Theta}$ (3mg/kg/h, IV) or Histalog injection^{+} (3mg/kg/15min IM) in M and C pigs

Age-group		1^{+}			2$^{\Theta}$			3$^{\Theta}$			4$^{\Theta}$	
Age (d)		1			4 - 14			18 - 22			25 - 42	
Livewt (kg)		1.0 - 1.6			1.8 - 4.3			4.0 - 7.3			6.2 - 12.9	
No of pigs/ treatment		8			14			9			15	
		Mean	SE		Mean	SE		Mean	SE		Mean	SE
Maximal proteolytic enzyme output (k units/h)	M	0.4	0.08	***	1.85	0.28	**	4.44 ††	0.92	**	18.41 †††	3.18
	C	-	-		-	-	***	12.42	1.89	***	45.40	6.04
Per unit livewt (k units/kg/h)	M	0.31	0.06	**	0.60	0.06		0.74 †††	0.14	**	1.74 †††	0.23
	C	-	-		-	-	***	2.18	0.33	**	4.88	0.64

Differences between age groups were significant: **P<0.01, ***P<0.001.
Differences between treatments were significant: ††P<0.01, †††P<0.001.

proteolytic enzyme output per unit liveweight increased over the 6 week period of the experiment with a very rapid increase occurring after 3 - 4 weeks (Tables 1 & 2).

The evidence presented here indicates that pigs given access to solid food before weaning and weaned on to solid food have greater acid and proteolytic enzyme secretory capacities than pigs fed entirely on sows' milk. The factors responsible for the observed

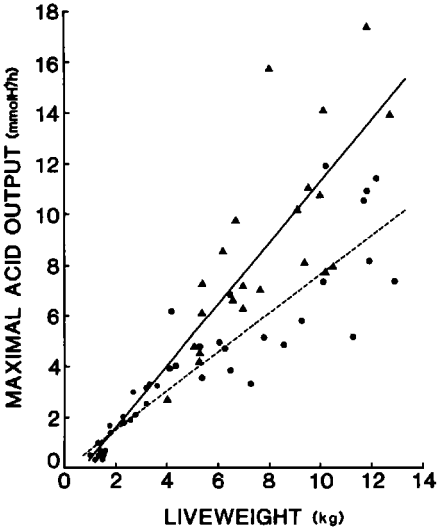


Fig. 1. Linear regression of maximal acid output (mmol/h) v. liveweight (kg) in M pigs (●) and C pigs (▲). The regression equations were: M pigs (----), $Y = 0.77X - 0.06$, $r^2 0.81$, $P<0.001$, C pigs (——), $Y = 1.21X - 0.83$, $r^2 0.79$ $P<0.001$.

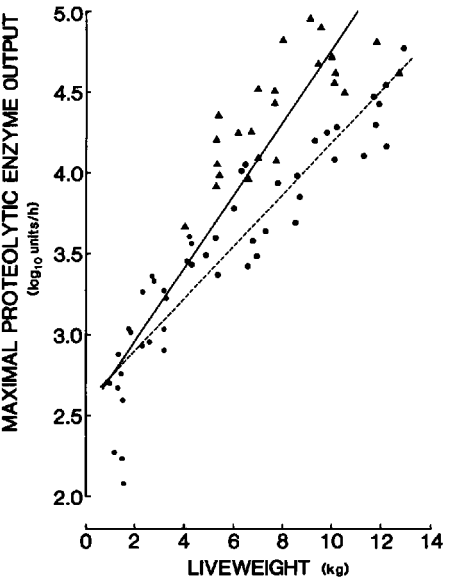


Fig. 2. Linear regression of maximal proteolytic enzyme output ($\log_{10}$u/h) v. liveweight (kg) in M pigs (●) and C pigs (▲). The regression equations were: M pigs (----), $\log_{10}Y = 0.16X + 2.58$, $r^2 0.85$, $P<0.001$, C pigs (——) $\log_{10}Y = 0.23X + 2.50$, $r^2 0.85$, $P<0.001$.

differences in rates of gastric development were discussed in paper I and could well be mediated by hormones. Candidate hormones include gastrin, ACTH, corticosteroids, thyroxine and epidermal growth factor (Henning, 1981; Johnson, 1981).

REFERENCES

CRANWELL, P.D. & STUART, S. 1983. Proc. Aust. Physiol. Pharmacol. Soc. 14: 39P

FOURIE, J., ARNOT, R.S., CARTER, J., HICKMAN, R. & TERBLANCHE, J. 1974. S. Afr. med. J. 48: 1873-1875.

HENNING, S.J. 1981. Am. J. Physiol. 241: G199-G214.

JOHNSON, L.R. 1981. In "Physiology of the Gastrointestinal Tract" (Ed. L.R. Johnson) Raven Press, New York, p. 169-196.

TUDOR, E. McI., SCHOFIELD, G.C. & TITCHEN, D.A. 1977. Ann. Rech. Vet. 8: 450-459.

PRODUCTION OF GASTRIC PROTEASES DURING THE ONTOGENY OF PIGS. SOME PROPERTIES OF PIG CHYMOSIN.

B. FOLTMANN
Institute of Biochemical Genetics
Ø. Farimagsgade 2A, 1353 Copenhagen K, Denmark

SUMMARY

During their first week of life, piglets produce appreciable amounts of chymosin, but virtually no pepsin. Pig chymosin is structurally related to calf chymosin, but has a higher milk-clotting activity toward porcine milk than toward bovine milk. It is suggested that absence of pepsin and production of chymosin is a prerequisite for postnatal uptake of immunoglobulins.

INTRODUCTION

The gastric proteases all belong to the superfamily of aspartic proteases (group EC 3.4.23). The gastric juice of adult mammals contains predominantly two types of pepsins: pepsin A (EC 3.4.23.1) and pepsin C, also called gastricsin (EC 3.4.23.3); the two types show differences in immunochemical reations, pH optimum, specificity and stability toward denaturation at pH 7. It is estimated that their primary structures show about 50% of identity only. It has long been know that young calves produce a specific milk-clotting protease, chymosin (EC 3.4.23.4), in English previously called rennin; but it has now been observed that chymosin apparently is characteristic for newborn mammals with postnatal uptake of immunoglobulins. For a general review, see Foltmann (1981).

This communication describes the development of chymosin and pepsin A in piglets, and some properties of pig chymosin are summarized.

EXPERIMENTAL PROCEDURES

Extraction of gastric mucosa took place in a Potter-Elvehjem homogenizer (5 ml of water per g of wet weight tissue); suspended tissue was removed by centrifugation at 12,000 g for 15 min at 2°C, see further Foltmann et al. (1981).

Electrophoreses were carried out in gels of agar or agarose. Detection took place by digestion of hemoglobin or by clotting of casein (Foltmann et al. 1985).

Immunochemical methods: Antisera were raised in rabbits. Quantitative determinations were carried out with monospecific antisera and rocket immunoelectrophoresis. The homogeneity of the purified enzymes was tested by crossed immunoelectrophoresis against anti-(total mucosal extract). Tests for partial immunochemical identity were carried out as tandem crossed immunoelectrophoresis against monospecific antisera. For details about the methods see Axelsen (1983).

Purification of pig chymosin: A pool of crude extracts of gastric mucosa was adjusted to pH 6. Prochymosin was adsorbed at DEAE-cellulose, suspended in the extract. Subsequent purification was carried by chromatography on DEAE-cellulose at pH 6.0 to 5.4, gelfiltration on Sephadex G-100, activation at pH 2, and repeated ion exchange chromatography. The method was analogous to that described for calf chymosin (Foltmann, 1966).

Assays for enzymic activity: The milk-clotting activity against bovine milk was tested with skim-milk powder reconstituted in 0.01 M $CaCl_2$. The activity against porcine milk was tested with fresh milk, skimmed and with pH adjusted to 6.3. The general proteolytic activity was tested by digestion of acid denatured hemoglobin and precipitation with trichloro acetic acid (Foltmann, 1966).

RESULTS AND DISCUSSION

Enzymic activity. Pig chymosin has optimum for general proteolytic activity at pH 3.5. This corresponds to the optimum for calf chymosin. With hemoglobin as substrate the activity of pig chymosin at pH 3.5 is 5 % only of that of calf chymosin. The activity of pig chymosin at pH 3.5 corresponds to 2 % of the activity of pig pepsin A at pH 2. Determinations of milk-clotting activities show that the activity of pig chymosin is about 6 times larger against porcine milk than against bovine milk. Conversely, calf chymosin is more active against bovine milk than against porcine milk. Thus the results suggest that an evolutionary adaptation has occured between the structure of the caseins and the specificities of the chymosins.

Immunochemical reactions and primary structures. Pepsin A, -C, and chymosin from one species show no immunochemical cross-reactions. But chymosins from calf and piglet show partial immunochemical identity; pepsin A from cow and pig likewise show partial immunochemical identity. Corresponding to this pepsin A from cow and pig have about 80 % of homology in their primary structures, whereas the overall homology among pepsin A, -C, and chymosin from cattle is about 40 % only. Preliminary results indicate a similar relationship among the gastric proteases from pig.

Table 1. Aligment of the N-terminal amino acid sequence of piglet chymosin (PC), calf chymosin (CC), pig pepsin (PP), and cattle pepsin (CP).

PC:	Gly	Glu	Val	Ala	Ser	Glx	Pro	Leu	Thr	Asn	Tyr	Leu	Asp	Thr	Gln	Tyr
CC:	Gly	Glu	Val	Ala	Ser	Val	Pro	Leu	Thr	Asn	Tyr	Leu	Asp	Ser	Gln	Tyr
PP:			Ile	Gly	Asp	Glu	Pro	Leu	Glu	Asn	Tyr	Leu	Asp	Thr	Glu	Tyr
CP:			Val	Ser	Glu	Gln	Pro	Leu	Gln	Asn	Tyr	Leu	Asp	Thr	Glu	Tyr

In order to illustrate the structural homology the N-terminal amino acid sequences of chymosin and pepsin A from pig and cattle are aligned in Fig 1. It is seen that the peptide chains of the chymosins

are two residues longer than that of the pepsins, and among the first 14 residues pig and calf chymosin differ at two positions only.

Development. Extracts of piglet gastric mucosa were tested by rocket immunoelectrophoresis against anti-(pig chymosin) and anti-(pig pepsin A). During the first week of life 1 - 4 mg of chymosin was observed per g of mucosa. The production of chymosin declines rapidly after the first week. Pepsin A is virtually absent during the first week of life, and a rapid increase in the production of pepsin A takes place after about 3 weeks. Pepsin C was observed by zymograms only; the qualitative results indicate that the production of pepsin C develops earlier than that of pepsin A. As measured by milk-clotting, the total enzymic activity passes through a minimum from 10 to 20 days of life.

Pepsin A will cleave immunoglobulins in the $F(ab)_2$ and the Fc fragments. It is therefore suggested that neonatal proteases with great milk-clotting and weak general proteolytic activities are essential for mammals with postnatal transfer of immunoglobulins from mother to young.

The physiological significance of the clot-formation has not been investigated. It should be recalled that a mere acid precipitation of casein produces a flocculous precipitate, whereas by enzymic action a rubberlike clot is formed. It appears likely that the newborn mammal has the advantage of taking liquid food (milk) that solidify in the stomach and thereby provide the mechanical stimulus that initiate the subsequent reactions in the digestive tract.

REFERENCES

AXELSEN, N.H. (ed.) 1983. Handbook of Immunoprecipitation in Gel Techniques. Scand.J.Immunol. 17: suppl. no. 10.

FOLTMANN, B. 1966. C.R.Trav.Lab.Carlsberg 35: 143-231.

FOLTMANN, B. 1981. Essays in Biochemistry 17: 52-84.

FOLTMANN, B., JENSEN, A.L., LØNBLAD, P., SMIDT, E. & AXELSEN, N.H. 1981. Comp.Biochem.Physiol. 68 B: 9-13.

FOLTMANN, B., SZECSI, P. & TARASOVA, N.I. 1985. Anal. Biochem. (in press).

DIGESTION, TRANSIT TIME AND BILIARY SECRETION FOLLOWING DIFFERENT DIETARY PROTEINS IN THE ADULT PIG*)

H. HAGEMEISTER, K. SCHOLZ, E. KINDER, C.A. BARTH
Institut für Physiologie und Biochemie der Ernährung
Bundesanstalt für Milchforschung, Kiel, Germany

W. DROCHNER
Institut für Tierernährung der Tierärztlichen Hochschule
Hannover, Germany

SUMMARY

Digesta of the ileum were quantitatively collected in 3 Göttingen strain minipigs fitted with T-shaped cannulas. When rations containing soy isolate as a sole nitrogen source were fed there was a higher flow rate of digesta compared to casein. Nevertheless there was a higher precaecal protein digestibility of soy isolate within the first 18 hrs postprandial. No significant differences of bile secretion have been found but there was a higher content of phospholipids in the bile following soy isolate feeding.

INTRODUCTION

We know from the literature that soy isolate, as compared to casein, leads to a higher fecal excretion of bile acids in pigs (Kim et al., 1980). We have observed, using the slaughter techniques, that pigs fed a semisynthetic ration containing soy isolate as protein source had higher intestinal chymus contents than pigs fed casein (Scholz et al., 1985). In soy protein fed animals the average chymus wet weight was 44 %, the dry weight 41 % and the nitrogen 22 % higher. Their intestinal bile acid content was also 80 % higher (4.5 mmol for the soy isolate group compared to 2.4 mmol for the casein group). In order to understand the underlying mechanism it was of interest to measure in fistulated pigs the transit time of chymus to the end of the small intestine and the precaecal digestion of the protein. Additionally the bile secretion and the bile composition has been measured.

*) Supported by the VO (EG) No. 271-12.3

MATERIAL AND METHODS

Animals: We used in repeated trials 3 male adult Göttingen minipigs (35 kg) fitted with "T-shaped" cannulas at the ileum, 0.1 m proximal to the ileo-caecal junction. Diets: "Cas 20" and "Soy 20" contained acid-precipitated casein (Biogen-Casein, Bayerische Milchversorgungs GmbH, Nürnberg) or soy isolate (Purina No. 610, Ralston Purina Company) respectively (20.8 %), lard (7.1 %) margarine (7.1 %) maize starch (51.9 %) cellulose (5.7 %) and mineral-vitamin mixture (7.5 %). The animals were fed according to the maintenance requirement of energy and fasted on the evening before and for 33 hour after beginning of digesta collection. Digesta collection procedure: Shortly before the morning feeding (6:00 A.M.) the intestine was closed by a inflatable catheter distal to the cannula for 33 hrs. The digesta were frozen immediately (at latest after 15 min) in liquid nitrogen, stored at -20^{o}C and freeze-dried. Analysis: All samples were analyzed for dry matter, nitrogen and unsoluble markers, i.e. polyethylene powder (Dijkstra and Kommiak, 1970) and cobalt spinell (unsoluble Co-Al_2O_3 compound). Precaecal digestibility was calculated on the basis of a 100 % recovery of polyethylene powder and cobalt spinell. In a further trial bile secretion was studied in 2 groups of female adult minipigs fed with a ration of Cas 20 or Soy 20, respectively plus 1 % cholesterol for two weeks. 1 1/2 hours after the morning feeding, bile was collected from the ductus choledochus in anesthesia in 3 consecutive periods of 20 minutes each.

RESULTS AND DISCUSSION

The above mentioned 40 % higher weight of chymus in the small intestine in pigs fed soy isolate, may be causative for the higher flow rate of digesta as observed in these experiments (Tab. 1). Soy induced a 14 to 20 % higher recovery of the unsoluble markers after 18 hrs (Tab. 1). However, this difference disappeared when calculated for a period of 33 hours postprandially.

The question arises whether the higher chymus flow is caused by a higher bile secretion and/or osmolarity of the bile juice. As demonstrated in table 2 measurements of the bile secretion within the first 20 minutes showed a tendency to a 25 % higher flow rate for soy

fed animals which disappeared completely in the consecutive two 20 min periods not shown. Probably a higher secretion of pancreatic juice following soy as observed in mice by Roy et al. (1981) is of greater importance in this respect.

Table 1: Recovery of markers and protein at the ileum in a period of 18 hrs and 33 hrs postprandial following different dietary proteins

period	marker/protein	Casein (n=13)	Soy isolate (n=8)	p
		% of supply		
18 hrs	polyethylene	39.0 ± 3.3	46.6 ± 3.5	<0.05
	cobalt spinell	37.1 ± 1.8	42.2 ± 2.0	<0.10
	protein	13.1 ± 1.4	9.7 ± 1.3	NS*
33 hrs	polyethylene	65.5 ± 3.0	66.7 ± 2.6	NS
	cobalt spinell	61.2 ± 3.3	59.5 ± 3.2	NS
	protein	19.0 ± 1.6	15.0 ± 2.1	NS

Values are $\bar{x}$ ±SEM, *NS = not significant

Table 2: Bile volume and biliary lipids following different dietary proteins

	Casein (n=6)	Soy isolate (n=6)	p
Bile volume (ml/20 min)	6.7 ± 1.3	8.4 ± 1.2	NS*
Bile acids (mmol x l^{-1})	88.4 ± 0.3	88.8 ± 2.9	NS
Phospholipids (mmol x l^{-1})	3.8 ± 0.3	6.2 ± 1.0	<0.05
Cholesterol (mmol x l^{-1})	1.7 ± 0.2	1.8 ± 0.2	NS

Values are $\bar{x}$ ± SEM, *NS = not significant

The higher flow rate of digesta (marker) following soy isolate feeding compared to casein feeding does not lead to lower digestibility of protein. On the contrary, as demonstrated by the recovery of the protein 18 and 33 hours postprandially (Tab. 1), there was a tendency of lower protein recovery at distal part of the ileum. Fig. 1 shows that there was - in relation to the marker - a higher precaecal digestibility of soy isolate compared to casein within the

first three of 6 consecutive 5 hrs collection periods.

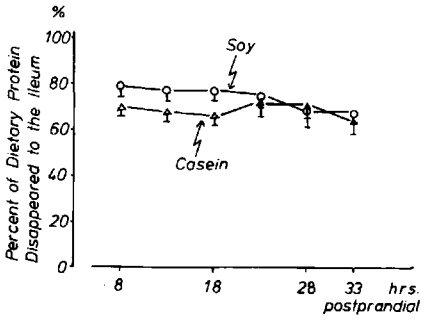


Figure 1: Protein digestibility to the ileum calculated by relating the recovery of protein to the marker polyethylene. $\bar{x} \pm$ SEM.

These findings are in complete agreement with preliminary results of experiments with pigs fitted with reentrant cannulas (Drochner et al., unpublished).

One can conclude, that soy isolate feeding compared to casein leads to a higher chymus volume and chymus flow which is not accompanied by a lower digestibility of nitrogen in the small intestine. Although more chymus was found in the proximal small intestine following soy protein, there must be a simultanous higher reabsorption of nitrogen in the termial ileum. Probably the higher total intestinal content reflects a slower digestion and absorption of soy protein compared to casein. This could be an explanation for varying results of precaecal protein digestibility of soy protein dependent on the extent of protein supply (Leibholz, 1981). We have to find out these mechanisms in future experiments.

REFERENCES

KIM, D.N., LEE, K.T., REINER, J.M. & THOMAS, W.A. 1980. Exp. Molec. Pathol. 33:25-35.

SCHOLZ, K.-E., KINDER, E., HAGEMEISTER, H. & BARTH, C.A. 1985. (submitted).

DIJKSTRA, N.D. & KOMMINK, A. 1970. Versl. Landbouw Onderz. No. 738, 23.

ROY, D.M. & SCHNEEMAN, B.O. 1981. J. Nutr. 111:878-885.

LEIBHOLZ, J. 1981. Brit. J. Nutr. 46:59-69.

QUANTITATIVE BILE ACID ABSORPTION FROM THE BOWEL IN PIG. EFFECT OF A REENTRANT BILIARY FISTULA. CONTRIBUTION OF THE DISTAL INTESTINE.

Catherine JUSTE, Véronique LEGRAND-DEFRETIN, T. CORRING, A. RERAT
Laboratoire de Physiologie de la Nutrition
INRA-CNRZ, 78350 Jouy-en-Josas, France.

SUMMARY

Total bile acid absorption from the gut into the portal vein, was investigated all along the light/dark cycle in 10 pigs : 5 pigs with an intact biliary tract, and 5 pigs fitted with a reentrant bile fistula allowing 2 levels of reinfusing the collected bile – into the upper duodenum or into the lower ileum.

The reported results allowed :

- first, to further define the physiological dynamics of the enterohepatic circulation in the intact animal,
- second, to evaluate for the first time, the effect of a chronic choledocal fistula reentrant into the upper duodenum on the dynamics of the enterohepatic circulation of total bile acids,
- third, to quantify the in vivo contribution of the distal bowel, to the overall intestinal bile acid absorption.

INTRODUCTION

Intestinal absorption of bile acids in the intact animal or in man, which is an essential step of the enterohepatic circulation, remains poorly investigated. Furthermore, the extent of bile acid absorption across various parts of the bowel under physiological conditions has not been defined.

These studies were undertaken :

- first, to provide quantitative data on the absorption of total bile acids from the entire bowel, in the normally fed, unaesthetized pig with an intact biliary tract.
- second, to quantitate the contribution of various segments of the bowel to the overall intestinal absorption. The present report is concerned with the absorption across the distal part of the ileum and the large bowel only.

MATERIAL AND METHODS

Ten Large White pigs, weighing 54 ± 3 kg at the beginning of the experiment were used. One week before surgery, they began to receive the experimental diet, provided in 2 equal daily meals of 800 g each, at 9 h 15 and 17h 15. They were then divided in 2 groups :

- 5 pigs, whose biliary tract was kept intact, were fitted with a carotid catheter, a portal catheter, and a flow probe around the portal vein.
- The 5 remaining pigs were also fitted with a choledocal catheter with two possibilities of reintroducing the collected bile : into the upper duodenum, or into the lower ileum, 90 cm above the ileocaecal valve.

After full recovery from operation, appearance of total bile acids (determined in sera with 3α-hydroxysteroid dehydrogenase) in the portal vein was measured on 20-min intervals and over a 24-hour period study,

- .in the pigs with an intact biliary tract,
- .in the bile fistulated pigs, when the secretion was reinfused into the upper duodenum,
- .in the bile fistulated pigs, when the secretion was recycled into the lower ileum.

In the bile fistulated pigs, bile acid secretion rate in bile was concomitantly measured.

RESULTS AND DISCUSSION

In the pigs with an intact biliary tract, total bile acid concentration measured over an entire light/dark cycle, averaged 83.9±2.0μM in portal venous blood, and 15.6 ± 0.6 μM in arterial blood. These values are in good agreement with previous results obtained from rat (CRONHOLM and SJÖVALL, 1967; BARNES et al., 1976), but are 2.5 to 4-fold higher than in man (ANGELIN et al., 1982). Nevertheless, the present finding of systemic arterial concentrations about 1/6 those in portal blood strongly agrees with the ratio usually found in man or in cebus monkey. The total amount of bile acids absorbed per 24 hr by the entire bowel was 216.9 ± 20.4 mmol. This study is the first quantitation of *in vivo* bile acid absorption.

Another new finding of the reported study was that the 24-hour kinetics of portal concentration and intestinal absorption rate, and to a lesser extent of arterial concentration, exhibited a similar pattern. This allows to conclude that the portal bile acid circulation is a highly reliable reflection of the dynamics of the intestinal absorption, whereas systemic arterial circulation of bile acids provides a less convenient figure of the absorptive events.

A bile fistula, reentrant into the upper duodenum, as practized in this study, did not affect the mean 24-hour values previously reported for portal and arterial concentrations of total bile acids (87.5 $\pm$ 2.4 µM and 19.1 $\pm$ 0.8 µM respectively) or for their absorption from the intestine (226.4 $\pm$ 23.7 mmol/24 hr). Nor was the general pattern of the respective daily kinetics affected by the fistula. Over the 24-hour period study, absorption represented 95.3 $\pm$ 12.5 % of the secretion concomitantly measured, and the daily secretion kinetics resembled those of the absorption. The latter results allow to apprecia te for the first time, the convenience of such a fistula in studying the enterohepatic circulation of total bile acids in pig.

When the bile secretion was recycled into the lower ileum, the mean 24-hour concentration of bile acids in portal and arterial blood decreased by 82.6 and 79.9 % respectively. This was accompagnied by a 77.0 and 77.3 % decrease in the total amount of bile acids absorbed from — and secreted into the intestine respectively. This means that, under the reported conditions, the last meter of the ileum plus the large bowel contribute together to reabsorb only about 23 % of the bile acids daily engaged into the enterohepatic circulation. This leads to reassess the importance (shaped by *in vitro* studies) of the distal ileum in the physiological bile acid absorption.

REFERENCES

CRONHOLM, T. & SJÖVALL, J. 1967. Eur. J. Biochem. 2 : 375-383.

BARNES, S., BILLING, B.H. & MORRIS, J.S., 1976. Proc. Soc. Exp.Biol. Med. 152 : 292-297.

ANGELIN B., BJÖRKHEM, I., EINARSSON, K. & EWERTH, S. 1982. J. Clin. Invest. 70 : 724-731.

SOYABEAN PRODUCTS STIMULATING NET EXCESS OF SECRETION IN THE LGT MODEL

M.J.A. NABUURS, A. HOOGENDOORN
Central Veterinary Institute
P.O. Box 65, 8200 AB Lelystad, The Netherlands

SUMMARY

The ligated gut test (LGT) in pigs is the standard method to examine *E. coli* strains for enterotoxin production. Because solid pig food has been associated with diarrhoea, we decided to examine soyabean products (the main protein source in food for piglets), in the same manner as *E. coli* strains.

After injection of different soya products into ligated segments of the small intestine fluid accumulation was observed, indicating a net excess of secretion. The factor in soya products responsible for this effect was found to be highly thermo-stable, as it resisted heating at 120°C during an hour. No indications for a possible allergic phenomenon accounting for the fluid accumulation were found.

From the results of this study it can be concluded that soyabean products can give similar results in the LGT as enterotoxigenic *E. coli* strains.

INTRODUCTION

Several factors may be involved in the etiology of diarrhoea in pigs. Enterotoxigenic strains of *E. coli* are among the most important infectious agents. To examine strains of *E. coli* for enterotoxin production, the ligated gut test (LGT) has been developed. In this test the net excess of secretion in a ligated gut segment of a young pig is measured after injection of an *E. coli* culture.

Because solid pig food has been associated with diarrhoea (Wittig, 1964; Bertschinger *et al.* 1979), we decided to examine food components in the same manner as *E. coli* strains, i.e. by testing them in an LGT. Soyabean products are among the major protein sources in pig food and were therefore tested first. Here we report some preliminary results.

MATERIALS AND METHODS

Soyabean peptone (SP) (Oxoid, U.K.) was used as a 4% solution in distilled water. It had an osmolarity of approximately 150 mmol. Soyabean flour (SF) was commercially obtained. Five grams were added to 100 mls of distilled water and the mixture was incubated overnight at room temperature with constant agitation. The supernatant was used for the test (osmolarity approximately 200 mmol).

All tests were done with pigs from a minimal disease herd.

In experiments 1 to 4 piglets were weaned one day before the test at an age of four weeks. Until weaning they had no access to feed other than sows milk. Piglets in experiment 5 were fed a

commercially available soyabean containing feed (content 24% defatted soyabean commercial designation 44) after weaning. In this last experiment piglets were weaned 0, 3, 7 or 11 days prior to surgery, two pigs in each case.

From 24 hours until 6 hours prior to surgery piglets received only water, after that nothing was given. The piglets were premedicated with azaperone (1 mg/kg intramuscularly) and anaesthetized with metomidate (2 mg/kg intravenously). In experiment 1 to 4 the first ligated intestinal segment was prepared 60 cm caudally from the stomach. The segments were approximately 20 cm long; between two segments 5 cm was left unused. The segments were injected as indicated under results. In experiment 5 piglets were injected with SP in ligated segments of approximately 25 cm in the first, middle and last third of the small intestine. The segments in all experiments were injected with a volume of 10 ml of test fluid or physiological saline (negative control, osmolarity 290 mmol) or with 2 ml of an O149 K91 K88 *E. coli* strain (positive control). This strain had been isolated from a piglet that died of diarrhoea; it was freshly cultured for 24 hours. Piglets were killed 20 hours after surgery and the length and content volume of each segment were determined. The LGT quotient (Q) was calculated by dividing the volume (in mls) by the length (in cms). All segments were tested for the presence of known enterotoxigenic *E. coli*.

RESULTS

Table 1 shows the results of experiments 1 and 2. The proximal part of the small intestine is clearly more sensitive to the action of the different agents than the distal part. Both the SP and the SF preparations gave Q-values considerably higher than those observed after injection of physiological saline, but not as high as those of the *E. coli* culture. In exp. 2 heat-treated soyabean products were used since in commercial animal food heat treatment is always applied in view of the anti-nutritional properties of soyabeans. Again the Q-values of loops injected with the SP or SF preparations were markedly higher than those of the negative controls, but usually lower than those of the *E. coli* injected loops.

Table 1 *Q-values of experiments 1 and 2*

Segment number	Injected with	Experiment 1		Experiment 2	
		pig 1	pig 2	pig 1	pig 2
1	SP	3.3	2.3	2.5	6.5
2	*E. coli*	3.5	3.5	5.0	5.0
3	SF	3.0	1.5	2.0	2.5
4	SP	1.0	1.5	1.2	1.0
5	*E. coli*	3.0	2.5	2.7	6.0
6	phys. saline	0.3	0.1	0.5	0.1
7	SF	1.0	1.2	1.0	1.5
8	SP	0.2	0.1	0.5	2.5
9	*E. coli*	1.3	0.1	0.3	0.5
10	SF	0.3	0.1	0.7	0.5

In the third experiment SP and SF were previously autoclaved at 120°C for one hour. In one pig a pattern of Q-values was found as in the animals of experiments 1 and 2. The other pig appeared to be a low responder in that all values, including those for *E. coli*, were relatively low (Table 2). Nevertheless all Q-values of SP or SF injected segments were higher than observed in the negative control segment.

In view of the variability in response between individual pigs, heated and unheated SP preparations were tested in the same animal in exp. no. 4. No consistent differences were found in the Q-values obtained with the two preparations. In general the sensitivity of pig no. 2 in exp. 4 appeared to be less than that of pig no. 1 (Table 2).

Table 2 *Q-values of experiments 3 and 4*

Segment number	Experiment 3 injected with	pig 1	pig 2	Experiment 4 injected with	pig 1	pig 2
1	SP	3.3	0.9	phys. saline	0.1	0.1
2	*E. coli*	6.0	0.9	SP	4.2	1.0
3	SF	3.0	1.3	SP 120°C	3.8	1.0
4	SP	2.0	0.8	SP	1.3	0.9
5	*E. coli*	5.0	3.0	SP 120°C	1.3	1.6
6	phys. saline	0.5	0.2	*E. coli*	5.5	3.8
7	SF	2.0	1.3	SP	0.6	1.3
8	SP	0.7	0.3	SP 120°C	0.1	0.1

As soyabean products can cause food allergy in calves (Kilshaw and Sisson, 1979), piglets in experiment 5 were fed a commercially available soyabean containing food. The results are shown in table 3.

Table 3 *Influence on LGT of prior exposure to soyabean containing feed*

Place loop in small intestine	Number of days piglets had free access to soya containing feed after weaning 11[1)]	7	7	3	3	0	0
First third part	+[2)]	+	+	+	+	+	+
Middle third part	–[3)]	–	–	–	+	+	+
Last third part	–	–	–	+	+	+	+

1) Each column represents one piglet.

2) + means a $Q > 1.0$.

3) – means a $Q < 0.2$.

From this table it is clear that the LGT results did not show a higher number of positive responses in animals which had received soya before the test than in animals which did not. Enterotoxigenic *E. coli* were not found in segments other than those injected with the organism.

DISCUSSION

A Q-value of $\geqslant$ 1.0 in the LGT is generally taken as proof for the enterotoxigenicity of an *E. coli* strain. Such values were repeatedly seen in this study after injection of the soyabean products. The mechanism by which these products disturb the secretion/absorption balance is unknown. Based on the results of experiment 5 it appears unlikely that allergic phenomena play a role. Anti-nutritional factors also do not appear to be involved as they are heat-labile and no difference in Q-values was found before and after heat treatment. Toxic substances harmful to resorptive epithelial cells might result in loss of function and thus also in a net excretion excess. Involvement of such products cannot be excluded, but no histological evidence to support this has been found: the damage to the epithelium was comparable to that found in the *E. coli* injected loops (unpublished results). Of *E. coli* enterotoxins it is known that they exert their effect through a secretion increase and it is tempting to speculate that the effect of the soyabean products might also be caused by an increased active secretion.

In view of the fact that soyabeans are widely employed in pig food and because of the possible relationship of our findings with diarrhoea in piglets, further studies are under way to investigate the action mechanism as well as the practical relevance of the findings. In addition, gnotobiotic instead of SPF-animals are tried in order to minimize the variation in the response of individual piglets. The first results indicate that more reproducible results can be realized in this manner.

REFERENCES

BERTSCHINGER H.U., EGGENBERGER E., JUCKER H., PFIRTER H.P.: 1979, Vet. Microbiol., 3: 281.

KILSHAW P.J., SISSON J.W.: 1979, Res. Vet. Sci., 27: 361.

WITTIG W.: 1964, Archiv. Exp. Vet. Med., 18: 1151.

EFFECT OF DIARRHOEA ON CARBOHYDRATE AND WATER ABSORPTION IN THE NEONATAL PIG

M.J. NEWPORT
National Institute for Research in Dairying
Shinfield, Reading, RG2 9AT, United Kingdom

SUMMARY

Absorption of glucose, lactose and water was estimated from perfused loops in the jejunum, ileum or colon of pigs between 4 and 10d age. Compared with healthy controls, diarrhoea had little effect on absorption or intestinal morphology. There were some indications of an enhanced potential for absorption in the ileum and colon of pigs with diarrhoea, suggesting an attempt to compensate for an increased substrate load from the proximal intestine. The perfusion technique did not indicate net secretion of water in pigs with diarrhoea. Perfusion flow rates in our experiments may have been less than those in vivo, leading to an overestimate of water balance.

Perfusions were carried out under anaesthesia for 4h, with collection periods of 30 mins. This technique may be useful in other physiological or nutritional studies in neonatal pigs.

INTRODUCTION

Diarrhoea is a common problem in young pigs and may be ascribed to one, or a combination of, factors. Causative agents include Escherichia coli, Clostridium, Salmonella, rotavirus, coronaviruses, food allergies and indigestible dietary ingredients. Under some conditions diarrhoea will lead to malabsorption, and always by definition, to an increased loss of water. Under our experimental conditions diarrhoea is often seen in pigs weaned at 2d age, although it is not usually fatal, and may be transient. None of the causative agents listed above has been consistently established as a cause of our outbreaks (Newport, Turvey & Brooker, 1982), a situation found in about 25% of cases in human infants in a recent survey (Ellis, Watson et al., 1984).

We have studied the effects of this diarrhoea of obscure aetiology, seen in our pigs, on the absorption of glucose and water from the intestine using a chronic perfusion procedure in anaesthetized pigs between 4 and 10 days of age. A histological examination of the intestine was also made. This procedure may be a useful technique in studying absorption under conditions similar to those in the human intestine.

MATERIALS AND METHODS

Pigs were weaned at 2d and given at hourly intervals a liquid milk substitute based on dried skim-milk and soyabean oil. The pigs were observed daily for diarrhoea. Pigs between 4 and 10d of age with diarrhoea were studied using a perfusion technique within 24h, and where possible healthy pigs of the same age were also examined.

Pigs were maintained under anaesthesia with metomidate HCl/suicalm for 4h. Loops (25cm) were prepared in either the jejunum, ileum or colon and perfused with solutions containing 10mM glucose or lactose, salts and PEG. Perfusion rates were 1.0 and 0.5ml/min for small intestine sites and colon respectively. Perfusates were collected over 30 min periods, after an initial 30 min equilibration period. Body temperature was maintained at 37^{o} by infra-red lamps placed below the bench. Pigs were killed at the end of the experiment, and tissue removed for histological examination.

RESULTS AND DISCUSSION

Diarrhoea had no significant effects ($P>0.05$) on glucose, lactose or water absorption from intestinal loops, apart from increased water absorption ($P<0.05$) in the colon when diarrhoea was apparent (Table 1). There was a large range of values within each group.

Table 1. Absorption of glucose, water and lactose from the intestine of healthy (H) and diarrhoeic (D) piglets (means ± SE)

	No. pigs		Glucose (μM/25cm/h)		Water† (ml/25cm/h)		Lactose (μM/25cm/h)	
	H	D	H	D	H	D	H	D
Jejunum	8	6	284±59	161±29	8.4±5.8	5.4±2.4	126±34	118±28‡
Ileum	6	6	126±15	281±88	5.8±2.5	6.8±2.4	18±9	116±46†
Colon	4	5	153±116	59±32	-2.5±2.1	12.7±3.8		

† from glucose perfusions. ‡ 4 values only.

The absorption data in Table 1 indicate relative potential for absorption as they are derived from perfusions at constant substrate concentration and flow rate. In vivo substrate concentrations and flow rates may be different, and the load for absorption may be increased in diarrhoea. The trends in potential absorption along the intestine (Table 1) lead to the speculation that diarrhoea may enhance the potential for absorption in the lower intestine in an attempt to compensate for greater substrate flow from the proximal region.

Villus lengths in the mid-jejunum and ileum were slightly, but significantly reduced ($P<0.05$ or $P<0.01$), in pigs with diarrhoea (Table 2).

Table 2. Villus lengths (μM) in the small intestine of healthy (H) and diarrhoeic (D) piglets (means ± SE)

	H	D
No. pigs	14	12
Jejunum (proximal)	544 ± 26	511 ± 25
Jejunum (mid)	667 ± 34**	523 ± 36
Ileum	650 ± 35*	539 ± 36

* $P<0.05$, ** $P<0.01$ (H vs. D)

Thus, under our conditions, diarrhoea did not lead to water or glucose malabsorption (Butler, Gall et al., 1974; Keljo, Perdue et al., 1981) or villus atrophy (Davidson, Gall et al., 1977) as has been reported with experimentally-induced virus infections. Perhaps the lack of effect on glucose absorption is not surprising as viruses could not be established as a likely cause of diarrhoea, and there was also little effect on villus morphology. The further, but unlikely, possibility that the efficiency of absorption is reduced during the pre-clinical phase of diarrhoea could not be examined by our experimental design. Our results showed a net absorption of water (Table 1) in all sites in the intestine of pigs with diarrhoea. Flow rates through the intestine of intact pigs with diarrhoea may be greater than under our perfusion conditions, resulting in a net secretions of water. The possibility that intestinal flow rate is greater in pigs with diarrhoea is being examined in further experiments.

We suggest that the perfusion technique described in this paper may be useful in other physiological and nutritional studies in neonatal pigs.

REFERENCES

BUTLER, D.G., GALL, D.G., KELLY, M.H. & HAMILTON, J.R. 1974. J. Clin. Invest. 53 : 1335-1342.

DAVIDSON, G.P., GALL, D.G., PETRIC, M., BUTLER, D.G. & HAMILTON, J.R. 1977. J. Clin. Invest. 60 : 1402-1409.

ELLIS, M.E., WATSON, B., MANDAL, B.K., DUNBAR, E.M., CRASKE, J., CURRY, A., ROBERTS, J. & LOMAX, J. 1984. Arch. Dis Childh. 59 : 848-855.

KELJO, D., PERDUE, M., TELCH, J., SHEPHERD, R., BUTLER, D., GALL, G. & HAMILTON, R. 1981. Pediat. Res. 15(4) Pt II : 536, abs 573.

NEWPORT, M.J., TURVEY, A. & BROOKER, B.E. 1982. Res. Vet. Sci. 32 : 48-51.

PROTEIN DIGESTION AND METABOLISM IN NEONATAL PIGS OF MILKS CONTAINING DIFFERENT PROPORTIONS OF CASEIN AND WHEY PROTEINS

BY
M.J. NEWPORT and M.J. HENSCHEL
National Institute for Research in Dairying
Shinfield, Reading, RG2 9AT, United Kingdom

SUMMARY

Pigs were weaned at 2d age and given milk substitutes containing casein:whey proteins in the ratio of 20:80, 50:50 or 0:100. The diets were given either 24 or 6-times per day until the pigs were killed at 16d age. Performance and N retention were similar for all treatments, but tended to be lower with milks containing only whey protein. Blood plasma urea N was lower ($P < 0.05$) in diets containing equal proportions of casein and whey proteins. The amount of digesta in the stomach from the casein-predominant diet was not affected by feeding frequency, but feeding 6-times/day increased the amount of digesta in the stomach from diets enriched with whey protein. Increases in the proportion of non-protein N suggested that whey proteins may be more amenable to hydrolysis in the stomach.

The relevance of these results to protein nutrition and digestion in human infants is discussed.

INTRODUCTION

Cow's milk contains predominantly casein protein, human milk mainly whey proteins, and sow's milk approximately equal proportions of both types (Gurr, 1981). The proportion of these milk proteins may be an important consideration in the formulation of milk substitutes designed to be close to the natural milk of the species.

Recent studies in neonatal pigs have shown that performance and protein metabolism may be improved using a milk containing approximately equal proportions of casein and whey proteins derived from cow's milk (Newport & Henschel, 1984 and 1985).

The effects on nitrogen retention and plasma urea N were similar to those reported from clinical trials (Berger, Scott et al. 1979; Raiha, Heinonen et al. 1976), indicating that the neonatal pig may be a useful model for protein nutrition in the human infant.

Further studies are now reported in which the effects of casein:whey protein ratios are compared in pigs fed 24 or 6-times per day, the less frequent interval being more relevant to the human situation. The amounts and composition of digesta in the stomach were also studied, previous studies in pigs having indicated that whey proteins may increase the rate of gastric emptying.

MATERIALS AND METHODS

Milk substitutes were prepared containing ratios of casein:whey proteins of 80:20, 50:50 or 0:100. Diets (approx 20% DM) were isonitrogenous and isoenergetic, and prepared from skim milk, dried whey protein and soyabean oil. Ten pigs/treatment were weaned at 2d age and given 250ml/kg/day either at hourly intervals or six-times per day. They were killed at 16d age, at 1h after a feed. N retention was estimated from a four-day collection period.

RESULTS AND DISCUSSION

Performance and N retention were not significantly affected ($P > 0.05$) by either the proportions of casein and whey proteins, or by the frequency of feeding (Table 1). These results differ from Newport & Henschel (1985) where a casein:whey protein ratio of 60:40 gave a small, but significant ($P < 0.05$) improvement in performance compared with a ratio of 80:20. In the present experiment mean values for pigs given the diet containing only whey protein indicated poorer performance, a trend consistent with our previous experiment.

Table 1. Effects of feeding frequency and ratio of casein:whey proteins on performance, % N retention and blood plasma urea N.

No. feeds/day	24			6			SEM
Casein:whey proteins	80:20	50:50	0:100	80:20	50:50	0:100	(40df)*
Wt. gain (g/d)	135	128	116	117	124	103	9.6
Feed (g DM)/gain(g)	1.02	1.05	1.10	1.01	1.00	1.21	0.070
% N retention	86.7	85.1	84.4	86.3	84.7	83.7	1.56
Plasma urea N (mg %)†	4.45	2.43	5.22	5.47	2.73	4.66	0.912

* Five missing values. † effects of protein ratios significant ($P < 0.05$).

Casein:whey protein ratios of 40:60 or 60:40 have given small increases in % N retention compared with a ratio of 80:20 in pigs (Newport & Henschel, 1984 & 1985) and infants (Berger, Scott et al., 1979). An increase was not found in the present experiment, but the reduction at the ratio 0:100 is consistent with our previous results (Newport & Henschel, 1985).

The effects of protein ratios on blood plasma urea N were significant ($P < 0.05$) and are consistent with both our previous experiments, suggesting some improvement in protein utilization of milks containing equal proportions of casein and whey proteins. Similar effects were reported from the studies on infants.

It has previously been considered that human milk contains a ratio of casein:whey proteins of 40:60. Therefore studies on infants have not investigated further enrichment with whey proteins. More recent analysis suggests that this ratio in human milk is 20:80 (Hambraeus et al.,1978) and therefore further enrichment of current formulae might be appropriate. Previous studies with our pig model would support this view (Newport & Henschel, 1985). In addition, the pig studies have indicated some adverse effects of total replacement of casein by whey. These may be related to changes in amino acid balance (Newport & Henschel, 1985) or in reduced coagulation and retention time in the stomach (Table 2).

Table 2. Weight, pH and composition of digesta in the stomach.

No. feeds/day	24			6			SEM	Sig of:	
Casein:whey proteins	80:20	50:50	0:100	80:20	50:50	0:100	(40df)	P	F
Weight, g	127.2	75.7	43.3	101.8	126.3	61.1	11.71	***	NS
pH	4.22	4.06	3.99	4.96	4.58	5.01	0.19	NS	*
% dry matter	24.6	21.2	15.5	26.7	22.6	17.5	1.43	***	NS
total N, g	1.49	0.62	0.18	1.29	1.30	0.45	0.149	***	*
NPN as % TN	8.0	11.1	18.2	5.1	9.2	18.9	1.87	***	NS

P, protein ratios. F, feeding frequency.

Clearly whey proteins reduced the amount of digesta and its dry matter content. It is interesting that frequency of feeding had a marked effect on the amount of digesta from whey-enriched diets but not on the amount from the casein-predominant diet. The increasing proportions of non-protein N suggest that whey proteins may be more easily hydrolysed than casein, at least in the initial stages of digestion.

We conclude that the nutritional advantage of enriching milk substitutes with whey proteins to simulate sow's milk is small, and unlikely to be of practical significance in pig production. The same criteria do not apply in human nutrition, particularly when considering premature infants.

REFERENCES

BERGER, H.M., SCOTT, P.H., KENWARD, C., SCOTT P. & WHARTON, B.A. 197 Arch. Dis. Child. 1979. 54 : 98-104.

GURR, M.I. 1981. J. Dairy Res. 48 : 519-554.

HAMBRAEUS L., LONNERDAL B., FORSUM, E. & GEBRE-MEDHIN, M. 1978. Acta Paed. Scand. 67 : 561-565.

NEWPORT, M.J. & HENSCHEL, M.J. 1984. Pediat. Res. 18 : 658-662.

NEWPORT, M.J. & HENSCHEL, M.J. 1985. J. Pediat. Gastroent. Nutr. In press.

RAIHA, N.C.R., HENINONEN, K. RASSIN, D.K. & GAULL, G.E. 1976. Pediatrics 57 : 659-674.

CONTRIBUTION OF GASTRIC LIPOLYSIS TO THE DIGESTION OF FAT IN THE NEONATAL PIG

M.J. NEWPORT and GAIL L. HOWARTH
National Institute for Research in Dairying
Shinfield, Reading, RG2 9AT, United Kingdom

SUMMARY

In 16d-old pigs given a milk substitute, 22% of the fatty acids ingested as triglycerides were hydrolysed to free fatty acids in the stomach, and 26% of the triglycerides ingested were present as di- or mono-glycerides. Gastric lipolysis was due to a lipase secreted by stomach tissue. Tongue and salivary glands contained negligible lipase activity, but pancreatic lipase activity was several-fold greater than in other tissues. Lipase activity/g. tissue was not significantly different in pigs killed within 24h of birth. The extent of gastric lipolysis was similar to that reported for infants, but unlike infants could not be ascribed to lingual lipase.

INTRODUCTION

Previous studies of lipase development in pigs have been concerned only with pancreatic tissue (Aumaitre, 1972; Corring et al., 1978) and did not consider the possibility of gastric hydrolysis which is known to be important in several species including man (Hamosh, 1984). In infants, lingual lipase secreted from the tongue can make a considerable contribution to lipolytic activity. We have studied gastric hydrolysis of lipids in neonatal pigs mainly to evaluate the potential for animal model studies relevant to infant nutrition. These findings may have some relevance to early weaning under practical conditions.

It would be dangerous to assume that the pig is an appropriate model for lipid digestion in infants particularly as the apparent digestibility of lipid in milk formulas is usually poorer in infants (Fomon et al., 1970) than in pigs (Braude & Newport, 1973). Also newborn infants have very low levels of pancreatic

lipase (Zoppi et al., 1972) and bile secretion (Watkins et al., 1973). However it is possible that the gastric phase of lipolysis may be similar, and the superior intestinal phase of lipid digestion in the pig account for the improvement in the overall digestibility compared with the infant. Also, if similar mechanisms for digestion could be established then more detailed studies would be possible in an animal model.

MATERIALS AND METHODS

Pigs were weaned at 2d age and given a milk substitute at hourly intervals containing (g/kg) 730 dried skim-milk, 270 soyabean oil. They were killed at 16d age. Digesta were removed from the stomach, lipids extracted, and separated into glyceride classes and free fatty acids by TLC. Lipid fractions were quantified by analysis of their fatty acids. Stomach tissue, pancreas, tongue and salivary glands were homogenized and analysed for lipase activity using an emulsion containing ^{3}H-glycerol trioleate (Nilsson-Ehle & Schotz, 1976). Lipase was also analysed in tissues from pigs killed within 24h of birth.

RESULTS AND DISCUSSION

Some hydrolysis of milk lipids occurred in the stomach (Table 1).

Table 1. Lipid hydrolysis products in digesta from the stomach of artificially-reared pigs. (mean values ± SE for 15 pigs)

Molar %'s			
Triglyceride	Diglyceride	Monoglyceride	Free fatty acids (% total fatty acids)
73.9 ± 2.97	16.7 ± 2.49	9.4 ± 2.40	22.1 ± 4.08

It is apparent that gastric lipolysis in the pig is dependent on a lipase secreted by stomach tissue (Table 2), unlike rats and infants where gastric lipolysis is nearly all due to secretion from lingual glands in the tongue (Hamosh, 1984).

Table 2. Lipase activity at pH 6.0 (nMoles fatty acids hydrolysed/min at 37°)

(means ± SE)

	No. pigs	Tongue gland	Salivary	Stomach	Pancreas	Pancreas (at pH 8.0)
per g. wet wt:						
Weaned	11	1.6±0.80	2.6±0.99	47.0±14.2	8794±1460	7329±1907
Suckled (newborn)	6	3.9±1.42	4.4±2.54	33.2±7.96	10300±2924	7131±2216
per whole organ:						
Weaned	11	7.5±3.77	13.6±4.87	1488±471.9	45493±6579	37697±8811
Suckled (newborn)	6	25.1±9.13	7.4±3.25	230±57.2	7733±1696	5297±1120

Pig gastric lipase had an optimum pH at 6.2. Surprisingly, a second peak at about pH 8.0 was found. This could be an intracellular lipase liberated during sample preparation, or possibly pancreatic lipase from reflux of intestinal digesta. The latter is possible as bile-stained digesta was found in some pigs. Pancreatic lipase activity was very high, confirming previous reports (Aumaitre, 1972; Corring et al., 1978).

Our results suggest that the extent of pre-intestinal lipolysis is similar to that in infants (Hamosh et al.,1978) but the enzyme responsible is gastric rather than lingual lipase.

REFERENCES

AUMAITRE, A. 1972. Wld. Rev. Anim. Prod. 8 : 54-67.
BRAUDE, R., & NEWPORT, M.J. 1973. Br. J. Nutr. 29 : 447-455.
CORRING, T., AUMAITRE, A. & DURAND, G. 1978. Nutr. Metab. 22 : 231-243.
FOMON, S.J., ZIEGLER, E.E., THOMAS, L.M., JENSEN, R.L. & FILER, L.J. 1970. 23 : 1299-1313.
HAMOSH, M. 1984. In "Lipases". (Eds. B. Borgstrom and H.L. Brockman). Elsevier, Amsterdam. p.49-81.
HAMOSH, M., SIVASUBRAMANIAN, K.N. & SALZMANN-MANN, C. 1978. J. Pediatrics. 93 : 674-679.
NILSSON-EHLE, P. & SCHOTZ, M.C. 1976. J. Lipid Res. 17 : 536-541.
WATKINS, J.B., SZCZEPANIK, P., GOULD, J., KLEIN, P. & LESTER, R. 1973. Gastroenterology 64 : 817 abs 134.
ZOPPI, G., ANDREOTTI, G., PAJNO-FERRARA, F., NJAI, D.M. & GABURRO, D. 1972. Pediat. Res. 6 : 880-886.

THE RESPONSE OF THE SECRETION AND ACTIVITY OF PANCREATIC ENZYMES TO THE QUALITY AND QUANTITY OF FAT

L. OZIMEK, W.C. SAUER AND G. OZIMEK
Department of Animal Science, The University of Alberta, Edmonton, Alberta, Canada

SUMMARY

The level of fat (0 versus 15%) as well as the quality of fat (fresh versus peroxidized fat) resulted in pronounced adaptive responses by the pancreas in studies with pigs that were surgically modified to allow for continuous collection of pancreatic juice. The inclusion of 15% fresh canola in the diet resulted in a 5-fold increase in total lipase activity; the inclusion of 15% peroxidized canola oil resulted in a 8-10 fold increase in total lipase activity.

INTRODUCTION

Certain natural food and feed constituents, such as fat, are subject to changes during storage and processing. Oxidation of unsaturated fatty acids leads to the formation of hydroperoxides and their secondary products. Interaction of these compounds with protein lead to damage of specific amino acids, imbalance in the rate of release of amino acid(s) during digestion, changes in the activities of the digestive enzymes and loss of protein solubility. In vitro studies showed an inhibitory effect of linoleic acid hydroperoxides and the secondary degradation products on the activity of RN-ase, trypsin, pepsin and lipase (Matsushita, 1975). The present studies were carried out to determine the effect of level of fat (0 versus 15) and quality (fresh versus peroxidized fat) on the secretion of the digestive enzymes of pigs surgically modified to allow for continuous collection of pancreatic juice.

MATERIALS AND METHODS

Four barrows with average initial weight of 35 kg, were fitted with re-entrant cannulas for collection of pancreatic juice according to procedures described by Hee et al. (1985). Following a 3 week recuperation period, the pigs were fed 4 cornstarch-based diets that contained 15% protein from isolated soy protein. The pigs were fed equal amounts of the diets, 900 g each meal, at 08.00 and 20.00 h.. Diet 1 contained no canola oil (FF); diet 2, 15% fresh canola oil (CO-F); diet 3, 15% canola oil that was heated under vacuum at 180°C for 12 h (CO-12) and diet 4, 15% canola oil heated under vacuum at 180°C for 24 h (CO-14).

Heating of canola oil lowers it quality by inducing peroxide formation. The canola oil was included at the expense of cornstarch. The remainder of the diet was made up of 10% dextrose, 5% cellulose and a 3.4% vitamin-mineral premix. The experimental period lasted 14 days. Samples of pancreatic juice were taken every 2 h during two 24 h collections, from 08.00 h on day 8 until 08.00 h on day 9 and from 08.00 h on day 10 to 08.00 h on day 11 of the experimental period. A distillation method for the determination of malonaldehyde (to indicate the rancidity of the diet) was used (Tarladgis et al., 1960). The contents were 0.4, 3.2, 13.8 and 16.7 mg per kg for the FF, CO-F, CO-12 and CO-24 diets, respectively. Enzyme activities were measured according to procedures outlined by Bergmeyer (1974). Samples of pancreatic juice, prior to the assay for chymotrypsin and trypsin, were activated according to Glazer and Steer (1977). Volume activity is expressed as activity units/liter (U/L). One unit of activity is defined as the hydrolysis of 1 µmol substrate in 1 minute. Total enzyme activities were calculated as follows : volume activity (U/L) x volume of pancreatic juice (L) = U.

RESULTS AND DISCUSSION

Flow of pancreatic juice

The average daily flow varied from 2.8 for diet CO-F to 3.7 l for diet CO-12 (Table 1). In general, minimum and maximum flow of pancreatic juice occurred from 4-6 and 8-12 h after feeding, respectively.

Chymotrypsin

The total chymotrypsin activity was higher for diets CO-12 and CO-24 than for diet CO-F (Table 1). Maximum volume activity (U/L) was observed 4-6 h after each meal, coinciding with the time when flow of pancreatic juice was minimum. Vice versa, minimum volume activity, 8-12 h, coincided with maximum flow. A similar pattern was observed for the other enzymes.

Trypsin

There were no differences ($P > .05$) between the total trypsin activities among the 4 diets (Table 1).

α-amylase

Total α-amylase activity was higher in diet FF (which contained 15% more cornstarch) than in the other diets (Table 1). The present results show that total pancreatic lipase activity is very sensitive to changes in the dietary content of carbohydrate.

Lipase

There were differences ($P < .05$) in the total lipase activity among the diets

(Table 1). The present studies show the enormous capacity of the pancreas to adapt to fat, not only to the amount, but also to the quality.

Endogenous protein secretion

The protein secretion was higher ($P < .05$) for diet CO-24 than for diet FF, which can be attributed to the combined increases in total enzyme activities (Table 1). Total enzyme activities during day (08.00 h - 20.00 h) and night (20.00 h - 08.00 h).

There were no differences ($P > .05$) for total enzyme activities, protein secretion and flow of pancreatic juice between day and night.

Table 1. Comparison of total exocrine pancreatic secretion during 24 h.

	Diet			
	FF	CO-F	CO-12	CO-24
Volume of pancreatic juice, l	3.0 ± .7[1)]	2.8 ± .7	3.7 ± .1	3.1 ± 1.2
Protein secretion, g	17.3 ± 4.8[a]	19.9 ± 5.7[ab]	22.0 ± 1.0[ab]	22.5 ± 3.9[b]
α-amylase activity, U x 10^3	35.6 ± 10.1[c]	17.7 ± 3.2[a]	14.6 ± 4.6[a]	26.2 ± 3.1[b]
Chymotrypsin activity, U x 10^3	117.0 ± 13.2[ab]	107.4 ± 8.4[a]	138.0 ± 4.7[b]	128.3 ± 37.4[ab]
Trypsin activity, U x 10^3	176.1 ± 68.0	218.3 ± 26.0	228.4 ± 28.0	241.6 ± 106.0
Lipase activity, U x 10^3	2.96 ± 1.07[a]	10.17 ± 1.35[b]	25.00 ± 2.58[c]	18.11 ± 5.55[c]

1) Means ± standard deviation

a,b,c Means within the same row with different superscripts differ ($P < .05$).

REFERENCES

BERGMEYER, H.U., 1974. Methods of enzymatic analysis. Academic Press, Inc., New York, pp. 885-889, 819-823, 1009-1012 and 1021-1024.

GLAZER, G. & STEER, M.L., 1977. Anal. Biochem., 77 : 130-140.

HEE, J., SAUER, W.C., BERZINS, R. & OZIMEK, L., Can. J. Anim. Sci., 65 : xx-xx (in press).

MATSUSHITA, S., 1975. J. Agr. Food Chem., 23 : 150-154.

TARLADGIS, B.G., WATTS, B.M., YAUNATHON, M.T. & DUGAN, L., 1960. JAOCS, 37 : 44-47.

THE INFLUENCE OF DIFFERENTLY PROCESSED SOYBEAN MEALS ON THE EXOCRINE PANCREATIC SECRETION OF GROWING PIGS

TERESA ŻEBROWSKA
Institute of Animal Physiology and Nutrition, 05-110 Jabłonna, Poland
T.D. TANKSLEY, JR., D.A. KNABE
Texas A and M University, Department of Animal Science, College Station, Texas 77843, USA

SUMMARY

The influence of raw and heat treated soybean meals was studied on the volume and composition of pancreatic juice in pigs. The daily output of pancreatic juice was 2908, 1707 and 1821 ml when Raw, Normal and Ruminant soybean meals were fed, respectively. There were no differences in the total protein content, total and specific activity of trypsin, chymotrypsin, carboxypeptidases A and B and amylase in pancreatic juice.

INTRODUCTION

The raw soybean meal stimulates the rat and chick pancreas to secrete excessive amounts of enzymes and develops a hypertrophy of the pancreas (Liener and Kakade, 1980). In pigs, the raw soybean meal lowers the activities of trypsin and chymotrypsin in the pancreatic tissue and does not develop enlargement of the gland (Yen et al., 1977). However, the effect of raw soybean meal on the volume and enzyme output in the pancreatic juice of pigs was never measured directly. This experiment was designed to study the effect of raw and heat-treated soybean meals on the volume and enzyme composition of pancreatic juice in the growing pig.

MATERIALS AND METHODS

Five castrated male crossbred pigs weighing approximately 35 kg were equipped with cannulas allowing quantitative collection of the pancreatic juice. The pigs were fed on corn-starch-based diets formulated to contain 12% protein from the Raw, Normal or Ruminant soybean meals. The Normal meal represented meals that were processed under conditions employed by most commercial plants. The Ruminant meal was processed under conditions used to produce rumen by-pass meals. The diets were given at 6.00 and 18.00 hours in equal portions at the level of 40 g/kg live weight in the following order: Normal, Raw and Ruminant for 3 pigs and Ruminant, Normal and Raw for 2 pigs. On the 6th day of feeding on each experimental

diet the pancreatic juice was collected continuously for 4 consecutive 6 h periods into bottles kept in ice-water bath. The juice was measured and sampled every hour and returned to the duodenum by means of a peristaltic pump.

Total N, protein and trypsin, chymotrypsin, carboxypeptidases A and B, and α-amylase activity were estimated as described by Żebrowska et al. (1983). The trypsin inhibitor activity was estimated according to Hammerstrand et al. (1981).

RESULTS AND DISCUSSION

The content of trypsin inhibitor in the Normal, Ruminant and Raw soybean meals was 3.95, 1.77 and 27.46 mg/g, respectively. Pigs fed on the Raw diet secreted over 60% more pancreatic juice than with the Normal and Ruminant diets (Table 1).

Table 1. Mean flow and composition of pancreatic juice in 24 h

	Diets		
	Normal	Ruminant	Raw
Pancreatic juice, ml	1707[a]	1821[a]	2908[b]
Protein, g	11.0	11.3	13.5
Trypsin, units x 10^{-3}	149.8	143.1	176.1
Chymotrypsin, units x 10^{-3}	82.4	81.4	83.0
Carboxypeptidase A, units x 10^{-3}	9.1	9.4	11.2
Carboxypeptidase B, units x 10^{-3}	24.6	26.9	28.9
Amylase, units x 10^{-3}	550.0	593.8	728.4

a, b - $P \leq 0.01$

The difference in the volume of juice could have been due to the level of trypsin inhibitor or other compounds destroyed or changed during the heat treatment of soybean flakes. The total volume of the juice (1.76-2.91 l/24 h) was greater than that reported by Partridge et al. (1982) and Żebrowska et al. (1983) for the corn-starch-casein diets but smaller than that for pigs given barley-based diets. The mean hourly volumes of juice (Fig. 1) were quite variable and changing with time being highest 0-1 and 5-8 h and lowest 2-3 and 11-12 h after feeding. The juice secreted during the first 6 h after feeding had more total protein and higher enzyme activities ($P \leq 0.01$) than that collected during 7 to 12 h after feeding.

In humans pancreatic response to meals is highly dependent on gastric emptying. With many types of meal there is a good reciprocity between the duration of gastric emptying and that of pancreatic secretion. It is possible that the Raw diet produced more gastric

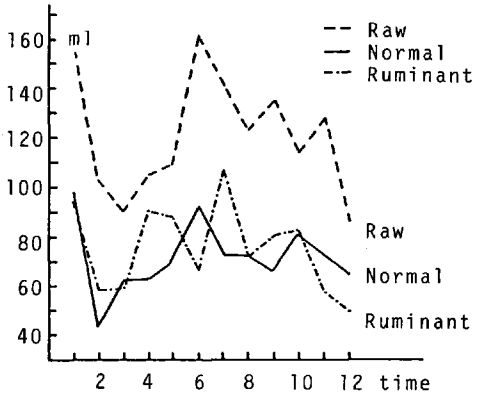


Fig. 1. Hourly pattern of pancreatic juice flow according to diet composition

juice and emptied from the stomach at a different rate than the Normal and Ruminant diets and these resulted in a larger volume of pancreatic juice.

Total protein content and total activity of trypsin and amylase were about 20% higher with the Raw than with the Normal and Ruminant diets, but the difference was not significant. The specific activity for each of the enzymes was similar for the three diets. This was in contrast with the findings on rats and chickens that the raw soybean meal increased markedly the pancreatic enzyme secretion. Yen et al. (1974) found that in growing pigs a raw soybean diet decreased the trypsin and chymotrypsin activities in the pancreatic tissue and in the intestinal contents and did not cause hypertrophy of the pancreas. It is possible, therefore, that the pig reacts in a different way to the raw soybean meal than the chick and the rat.

REFERENCES

LIENER, I.E. and KAKADE, M.L. 1980. Protease inhibitors. In: I.E. Liener (Ed.). Toxic Constituents of Plant Foodstuffs. Academic Press, New York.

HAMMERSTRAND, G.E., BLACK, L.T. & GLOVER, J.D. 1981. Cereal Chem. 58:42

PARTRIDGE, I.G., LOW, A.G., SAMBROOK, I.E. & CORRING, T. 1982. Br. J. Nutr. 48: 137-145.

YEN, J.T., JENSEN, A.H. & SIMON, J. 1977. J. Nutr. 107: 156-165.

ŻEBROWSKA, T., LOW, A.G. & ŻEBROWSKA, H. 1983. Br. J. Nutr. 49: 401-410.

THE INFLUENCE OF THE LEVEL AND SOURCE OF FIBRE IN THE DIET ON THE EXOCRINE PANCREATIC SECRETION IN GROWING PIGS

TERESA ŻEBROWSKA
Institute of Animal Physiology and Nutrition
05-110 Jabłonna, Poland

SUMMARY

The exocrine pancreatic secretion was studied in pigs fed on diets based on wheat, wheat bran, wheat flour and cellulose. Average daily volume of pancreatic juice and the content of K, Na, Cl^- and HCO_3^- were greater ($P \leqslant 0.01$) with wheat or wheat + wheat bran diets than with wheat + wheat flour or wheat flour + cellulose diets. Composition of the diets had no effect on the total activity of enzymes.

INTRODUCTION

Previous studies (Partridge et al., 1982; Żebrowska et al., 1983) have shown marked differences in the volume and composition of pancreatic juice secreted by pigs fed semipurified or cereal based diets. These differences could be due to the type of dietary fibre (natural fibre of cereals vs cellulose powder). Various types of fibre have been found to influence the output of pancreatic juice in rats (Sommer and Kasper, 1980).
The objective of the present study was to measure the volume and composition of pancreatic juice in pigs fed on diets containing different types and levels of crude fibre.

MATERIAL AND METHODS

Six male Polish Landrace pigs Line 21, initially of about 34 kg, were used. They were each fitted with a re-entrant cannula which allowed to collect the pancreatic juice quantitatively and to re-introduce it into the duodenum. The diets (Table 1) were made of wheat, wheat bran and wheat flour and supplemented with casein to a similar N content. The diets were given to the pigs at 8.00 and 20.00 h in equal portions at the level of 4% live weight/d. The diets were mixed with water immediately before feeding. After 5 days adaptation to the test diets 48 h collection of pancreatic

juice were carried out.

Table 1. Composition of diets (g/kg)

	Diets			
	I	II	III	IV
Wheat	887	444	444	-
Wheat bran	-	443	-	-
Wheat flour	-	-	443	857
Casein	70	35	60	60
Wheat starch	-	35	10	-
Mineral-vitamin mixture	43	43	43	43
Cellulose	-	-	-	40
Crude protein, %	15.03	15.54	14.95	14.52
Crude fibre, %	4.08	6.37	2.05	3.90

The flow of juice was measured hourly and sampled, and the remaining amount was returned to the duodenum. Total N, protein, Na, K, Cl^- and HCO_3^- contents and trypsin, chymotrypsin, carboxypeptidases A and B and amylase activities were estimated as described by Partridge et al. (1982).

RESULTS AND DISCUSSION

Average daily volumes of pancreatic juice with diets I and II were greater ($P \leqslant 0.01$) than with diets III and IV (Table 2). Replacement of half of the wheat in diet II by wheat bran increased only slightly the volume of juice and the electrolytes contents as compared to diet I. Replacement of 50% wheat by wheat flour (diet III) decreased significantly ($P \leqslant 0.01$) the volume of juice and the protein, Na, K, Cl^- and HCO_3^- contents. The pigs given diet IV produced 2.6 times less pancreatic juice than those on diet II. The contents of protein, K, Na, Cl^- and HCO_3^- were also less ($P \leqslant 0.01$) than with diets I and II. The type of diet had no influence on the total activity of pancreatic proteases, peptidases and amylase. This is in accordance with the findings of Partridge et al. (1982) and Żebrowska et al. (1983) that pigs fed on diets of similar protein content secreted pancreatic juice of a similar total enzyme activity.

Table 2. Mean output of pancreatic juice and some of its enzymic and mineral components in 24 h

	Diets			
	I	II	III	IV
Pancreatic juice, ml	4108[a]	4560[a]	2556[b]	1757[b]
Protein, g	17.9[a]	19.0[a]	15.8[b]	13.0[c]
Trypsin, units x 10^{-3}	184.3	188.2	214.0	193.4
Chymotrypsin, units x 10^{-3}	117.2	112.2	114.0	90.6
Carboxypeptidase A, units x 10^{-3}	5.0	5.2	5.6	7.1
Carboxypeptidase B, units x 10^{-3}	21.9	22.6	28.5	30.1
Amylase, unit x 10^{-3}	843.3	873.8	855.5	542.7
K, g	1.34[a]	1.43[a]	0.85[b]	0.52[b]
Na, g	15.3[a]	16.6[a]	10.1[b]	6.5[b]
Cl^-, mmol	85.7[a]	90.2[a]	72.0[b]	56.7[b]
HCO_3^-, mmol	604.0[a]	646.0[a]	349.0[b]	238.0[b]

a, b, c - $P \leq 0.01$

From the results presented it is evident that the diet composition can have a marked effect on the daily secretion of pancreatic juice. Since the diets used were similar in the type of starch and the content of crude protein and differed in the content and type of crude fibre, the latter seems to be responsible for the changes in the volume and composition of pancreatic juice. Average values for the daily secretion of HCO_3^- were 2.5 times greater for diet I and II than for diet IV. This means that the total buffering capacity of juice were markedly greater with diet I and II than with diet IV. These results therefore may suggest also that there was a much greater secretion of gastric acid with diets I and II than with diet IV which resulted in a higher production of secretin, the major stimulant for electrolyte and water secretion in monogastrics.

REFERENCES

PARTRIDGE, I.G., LOW, A.G. & SAMBROOK, I.E. 1982. Br.J.Nutr. 48: 137-145.

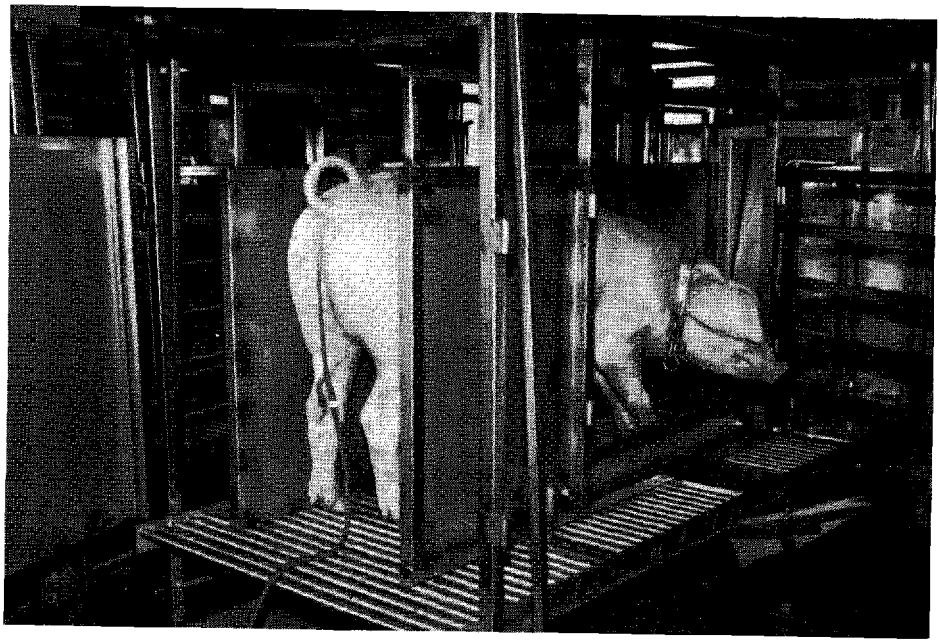
SOMMER, H. & KASPER, H. 1980. Hepato-gastroenterol. 27; 477-481.

ŻEBROWSKA, T., LOW, A.G. & ŻEBROWSKA, H. 1983. Br.J.Nutr. 49: 401-410.

SESSION 3

THE ROLE OF DIETARY FIBRE IN DIGESTION, ABSORPTION AND METABOLISM

Discussion leader: R. A. Argenzio



THE ROLE OF DIETARY FIBRE IN DIGESTION ABSORPTION AND METABOLISM

A.G. LOW
National Institute for Research in Dairying, Shinfield, Reading, Berkshire, RG2 9AT, England*

SUMMARY

Dietary fibre is composed of a very heterogenous mixture of substances, mainly associated with plant cell walls, which may be defined as non-starch polysaccharides and lignin. The complexity of its physical and chemical properties make accurate and reproducible detailed analysis very difficult. Raising the dietary fibre content of the diet tends to increase voluntary feed intake. Gastric, pancreatic and small intestinal secretions are generally higher when additional dietary fibre is given: in particular nitrogenous secretions increase and this is a factor determining the apparent digestibility of proteins. Transit is often faster when dietary fibre is added to low fibre diets but little effect can be seen in cereal-based diets. In general the rate of nutrient absorption is decreased by additional dietary fibre: in addition the amount of energy absorbed in the small intestine is usually reduced, to a greater extent than for lipids and nitrogenous compounds. Fermentation of dietary fibre in the large intestine increases with age. The products (mainly volatile fatty acids) are absorbed and metabolized but reliable quantitative estimates of their contribution to energy supply are lacking.

INTRODUCTION

In recent years there has been renewed interest in the role of dietary fibre in pig nutrition. This arises from the increasing pressure placed upon pig producers to find alternative and cheaper types of feedstuffs, in view of the widespread demands that grains and protein concentrates should only be eaten directly by man.

* Address from 1 April 1985: The Animal and Grassland Research Institute, Shinfield, Reading, Berkshire, RG2 9AT, England.

During the last twenty years or so it has become clear from epidemiological studies of human populations that there is an apparent inverse relationship between the consumption of a diet that is rich in foods which contain cell walls (e.g. high extraction cereals, fruit, vegetables) and the incidence of such diseases as diabetes, diverticular disease, large bowel cancer, coronary heart disease, gallstones and obesity. This evidence is often described in terms of the "dietary fibre hypothesis". This hypothesis also includes the statement that a diet providing a low intake of cell walls is a causative factor in the aetiology of the disease, in some cases, while in others it provides conditions under which other factors may be more active (Southgate, 1982).

Interest in the first of these issues has led recently to an increase in research on the role that dietary fibre can play in pig nutrition; the second issue has led to the recognition that the digestive system and metabolism of pigs are strikingly similar to those of man and that the responses of pigs and man to some types of dietary fibre are similar (Leeds, Kang, Low and Sambrook, 1980). Thus the pig appears to be a good model for man in this context. This review will include discussion of the role of dietary fibre both in agricultural aspects of pig nutrition and of studies in pigs which may help to explain the mode of action of dietary fibre in man.

DEFINITIONS

Much has been written on this topic and it is clear that there is no complete definition of the term dietary fibre. Indeed the definition depends upon the viewpoint of the person attempting to state what dietary fibre is. However, two definitions appears to be useful: (a) "the sum of the polysaccharides and lignin which are not digested by the endogenous secretions of the digestive tract" (Trowell, Southgate, Wolever, Leeds, Gassull and Jenkins,

1976) and (b) "non starch polysaccharides and lignin" (Southgate, 1982). The first is a conceptual definition embracing chemical, physical and physiological aspects of fibre which cannot yet be measured fully, while the second describes an entity that can be measured using existing methods, though not without difficulty. It is important to recognize that these definitions include not only the constituents of plant cell walls, but also non-starch polysaccharide storage gums, extracted from plants such as guar gum, which do not fulfill a function within the cell wall, and algal polysaccarides such as carrageenans.

CHEMICAL AND PHYSICAL PROPERTIES

Apart from lignin, which is an aromatic polymer of phenolic alcohols, dietary fibre consists of a very wide range of polymers of pentoses (especially arabinose and xylose) and hexoses (especially glucose, fructose, galactose). The principal structural group include the β-glucans (cellulose and β-(1-3, 1-4)-glucans and the heteroglycans (pectic substances, hemicelluloses, storage and exudate gums and algal polysaccharides such as agar, caraqeenans and alginates). In addition the oligosaccharides raffinose and stachyose, which are found in legumes, are components of dietary fibre. It has also been proposed that certain types of processed starch which contain ester, ether or phosphate derivatives ('resistant starch') and which provide steric blocks to host amylase activity should also be regarded as dietary fibre. A detailed account of the chemistry of dietary fibre was presented by Southgate (1976).

The ability of dietary fibre to hold water in varying amounts has often been used as an explanation for differing effects of dietary fibre on faecal output. The water holding capacity of dietary fibre *in vitro* varies enormously depending on source, maturity of the plant, whether or not it has been processed, particle size, the pH and electrolyte composition of the surrounding fluid and so on. Nevertheless, Stephen and Cummings (1979) found that those dietary fibres which hold the least water

in vitro (e.g. bran) produced the largest increases in faecal output, while gums such as pectin which hold large amounts of water *in vitro* produced almost no change in faecal output. Increases in faecal water output following dietary fibre supplementation are also the result of larger bacterial numbers (Stephen and Cummings, 1980); bacteria typically contain 80% water. It is generally found that coarsely ground dietary fibre leads to a greater water-holding capacity than when it is finely ground and that it induces a greater faecal output. Dietary fibres also tend to have ion exchange properties for most monovalent cations (McConnell, Eastwood and Mitchell, 1974) and calcium (James, Branch and Southgate, 1978). In addition pH-dependent adsorption onto dietary fibre is known to occur, for example in the case of bile acids and seems to be associated with suppression of ionization within the dietary fibre (Eastwood and Hamilton, 1968). Thus dietary fibre includes a complex variety of chemical structures together with a wide spectrum of physical properties, which give rise to diverse physiological and nutritional effects. There is inadequate detailed understanding of the relationship between the structure and function of dietary fibre and this is a barrier to progress in its use both in animal nutrition and medicine. It is important to recognize the complexity and diversity of the mechanisms involved: it is evident that many types of dietary fibre can influence the intake, digestion, absorption and metabolism of all the major nutrient types.

ANALYTICAL METHODS

At a practical level dietary fibre is defined by the analytical method used for its measurement. A wide range of methods is available but comparisons between them show major differences, because of the differing susceptibility of the complex mixture of substances to different extraction procedures.

Crude Fibre This method was developed over 150 years ago as a means of measuring the indigestible fraction of animal feedstuffs.

The sample is treated sequentially with petroleum ether, hot sulphuric acid, boiling water and alkali. The resultant insoluble residue contains mainly cellulose and lignin but recovery is rarely complete (Van Soest and McQueen, 1973). Nevertheless this remains a standard method in many countries.

Neutral Detergent Fibre This method, described by Van Soest and Wine (1967) involves digestion of the sample in neutral detergent solution. After filtration the residue is dried and weighed. Although lignin and cellulose are usually fully recovered, hemicellulose recovery may be incomplete because its water-soluble components are largely lost during filtration.

Acid Detergent Fibre This method compliments the previous procedure and was developed by Van Soest (1963). Digestion of the sample in acid detergent solution is followed by filtration, and the residue is then dried and weighed, to provide a measure of the cellulose and lignin contents of the sample. Other components of dietary fibre are largely excluded.

Non Starch Polysaccharide Methods Initial removal of starch (by enzymic hydrolysis) is followed by separation into cellulose, non-cellulose polysaccharides and lignin, acid hydrolysis and finally colorimetric (Southgate, 1969) or gas liquid chromatographic (Englyst, Wiggins and Cummings, 1982) measurement of the individual sugar constituents of each fraction. A rapid enzymic procedure has recently been described by Asp, Johansson, Hallmer and Siljestrom (1983): following this hydrolysis samples can be analysed in as much detail as required.

Although the crude fibre, acid and neutral detergent fibre methods of analysis continue to have value in practical pig nutrition because of the good inverse correlation between the values obtained and the digestible or metabolizable energy content of the diet, they do not provide sufficient information for elucidating the detailed effects of dietary fibre. The importance of accurate and detailed chemical and physical characterization of dietary fibre in experimental work cannot be over-emphasised. In addition agreement on a standard method of analysis would

transform our ability to interpret results from different research centres. It is apparent from a number of comparative studies that different methods of analysis give very different results for components of dietary fibre; for example, Millard and Chesson (1984) prepared dietary fibre extracts from swede (Brassica napus) as fed and from the ileal digesta of pigs by six different published methods. The individual components of both the insoluble and soluble fractions were then analysed by a single method to give remarkably different results in many instances; examples of the range of values in the insoluble fraction (g/kg feed) are: arabinose 4.5 - 0.2, xylose 8.4 - 2.1, galactose 7.0 - 0.0, phenolics 4.4 - 2.0. The lowest values in each case were obtained using the acid detergent fibre method and the high values from recently developed extraction methods. A comprehensive review of analytical procedures for dietary fibre has been edited by James and Theander (1981).

DIETARY FIBRE AND FEED INTAKE

It is well established that as the dietary fibre content of the diet increases, so the voluntary feed intake of pigs increases: the ARC (1967) concluded that for every 1% increase in dietary fibre content up to a total of 100g/kg diet (measured as crude fibre) a 3% increase in diet intake occurred. At the same time the growth rate falls, because increased appetite does not compensate fully for the reduced dietary energy concentration as the dietary fibre content increased particularly in young pigs (Owen and Ridgman, 1968). Furthermore the carcass weight as a percentage of liveweight falls as the dietary fibre content of the diet is increased, because of the larger weight of gut contents and the heavier gut tissue. Behind such general statements lies much uncertainty about the responses of pigs to specific types of dietary fibre: no thorough comparison of the palatability of different types of dietary fibre is available. When the dietary fibre content of the diet was increased beyond 100g crude fibre/kg diet, as in the studies by Baker, Becker, Jensen and Harrison (1968) on growing pigs given 0-400g cellulose/kg diet, then the

voluntary feed intake fell from 2.63 to 1.50kg/day, and daily gain fell from 760 to 250g/day.

Zoiopoulos, English and Topps (1982) found that the voluntary feed intake of sows was 7.79kg/d (85.0 MJ digestible energy) when given a diet containing 400g/kg of oat husks; corresponding values of 5.80kg/d (60.4 MJ digestible energy) were observed when the diet contained 300g/kg barley straw. At present it is not known what attribute of these two sources of dietary fibre led to the differences in feed intake. Information on the responses to different types of dietary fibre is of particular importance when attempts are made to control the feed intake of breeding animals, in which excessive gains can occur under ad libitum systems. In the same way, knowledge of the effects of specific attributes of different dietary fibres on intake and satiety would be of great value in clinical nutrition.

An interesting hypothesis to explain how moderate intakes of dietary fibre may increase voluntary feed intake has been proposed by Bergner (1981). The bacterial flora of the large intestine hydrolyses undigested proteins and transforms the amino acids so released to tyramine and tryptamine, amine derivatives of tyrosine and tryphophan, respectively. These can saturate the satiety centre of the hypothalamins and reduce feed intake. However, by lowering the pH in the gut lumen, by increased production of volatile fatty acids from diets with a higher dietary fibre content, this effect could be reduced, because the amine-producing bacteria are only active at a relatively high pH.

EFFECTS OF DIETARY FIBRE ON DIGESTIVE SECRETIONS

The effects of dietary fibre on digestive secretions in pigs appear to be considerable. For example, mean volumes (litres) of saliva and gastric juice, bile and pancreatic juice for low and high-dietary fibre diets (based on (a) starch, casein and cellulose or (b) barley and either fishmeal or soyabean meal) secreted per 24h in 40kg pigs were respectively: 4.0, 8.0 (saliva and gastric juice), 1.2, 1.7 (bile), 1.2, 2.2 (pancreatic juice)

(Zebrowska, Low and Zebrowska, 1983; Sambrook, 1981): the neutral detergent fibre contents of the diets were 50g/kg and 180g/kg respectively, while crude fibre concentrations were similar, emphasising the large hemicellulose content of the barley-based diet. While these results should not be taken to indicate a definite link between dietary fibre and secretion, by far the largest difference between the diets was in their dietary fibre content. The reasons for such apparent effects remain to be elucidated.

Certain types of dietary fibre increase the viscosity of meals and of the gut contents; one example is guar gum, which has been found to increase nitrogen secretion in isolated loops of jejunum in conscious growing pigs from 35 to 67 mg/m/h (equivalent to an increase from 15 to 25g/d if secretion occurs at a uniform rate throughout the small intestine (Low & Rainbird, 1984). The increased nitrogen contains both protein and DNA; the latter is likely to be a constituent of mucosal cells, the exfoliation of which increased following guar gum addition to diets in rats (Johnson, Gee and Mahoney, 1984). Furthermore intestinal protein synthesis in rats was increased when they consumed 99g dietary fibre/kg from a cereal based diet rather than a semi-synthetic diet containing 40g cellulose/kg (Southon, Livesey, Gee and Johnson, 1985). A similar effect has been observed on the flow of nitrogen and amino acids passing through the ileum of pigs receiving protein-free diets with supplementary cellulose by Sauer, Stothers and Parker (1977) and Taverner, Hume and Farrell (1981). Cellulose supplementation of diets has also been shown to increase the output of faecal nitrogen, to a greater extent than oat hulls, while methylcellulose had little effect (Whiting and Bezeau, 1957). These results have important implications for interpretation of data on the apparent digestibility of nitrogen: it seems possible that as both insoluble and soluble types of dietary fibre can enhance endogenous nitrogen secretion, so the apparent digestibility of nitrogen may be a function not only of

the inherent digestibility of the dietary protein, but also of the type and amount of dietary fibre with which it is incorporated in the diet. Further information on this topic could have important implications in relation to the efficiency of protein digestion.

Dietary fibre may also influence the movement of water in the gut: for example the rate of gastric emptying of water is reduced by guar gum (Rainbird and Low, 1983) while additional dietary cellulose greatly increases the volume of digesta in the ileum and faeces of pigs (Partridge, 1978). Similar effects of dietary fibre on gut water volume and faecal output have been observed by other authors, but so far it has not been possible to identify the features of the fibre concerned. It has rarely been possible to demonstrate whether inhibited water absorption or increased secretion is occuring, but Rainbird, Low and Zebrowska (1984) found that guar gum decreased the net absorption of water in isolated loops of jejunum in pigs.

EFFECTS OF DIETARY FIBRE ON GUT MOTILITY AND TRANSIT

So far there is very little information on the effects of dietary fibre on motor activity in either the small or large intestines of pigs. Sissons, Rainbird and Thurston (1984) showed that gastric motility appeared to be unaffected by addition of guar gum to the diet, but duodenal motility was reduced when assessed in terms of the duration of periods of irregular spike activity (Sissons and Rainbird; unpublished results). Changes in motility of this kind can be expected to modify the time course of glucose absorption (Rayner, Gregory and Goodall, 1984).

Bran has been shown to stimulate propulsive colonic motility in pigs (Fioramonti and Bueno, 1980); this appears to be the result of poorly understood direct mechanical factors rather than of fermentation products, including VFA (Bardon and Fioramonti 1983). This is an area of gastrointestinal physiology that is developing rapidly and it is likely that studies with pigs can contribute to basic understanding of the effects of dietary fibre on the digestive processes.

Although the effects of dietary fibre on transit of dietary components are doubtless mediated in part by motility phenomena, this topic has hardly been explored in any species. Studies in man on overall transit time have often been made on the assumption that this is a physiologically significant measure of gut function, but this has not been clearly demonstrated. It is now thought that transit may influence rather than be the result of events related to diet type in the large intestine (in which dietary residues spend most of their total transit time). For example Cummings, Southgate, Branch, Houston, Jenkins and James (1978) found that faeces weight varied from 65 to 194g/d in 16 normal subjects all eating the same amount of dietary fibre, while transit time ranged from 31 to 117h; the length of transit time varied inversely with faecal weight. Further evidence that transit time can alter faecal output comes from studies in man by Stephen (1980) who found that pharmacological slowing of transit was accompanied by a fall in faecal output, and vice versa; in the same studies it was also found that there is an inverse relationship between the extent of bacterial degradation of dietary fibre and transit time. Thus in the case of man substantial individual differences in transit time of the same diet are one aspect of the function of the large intestine, which can also be modified by such factors as the fibre source and the physical form of the diet. It is evident that there is also considerable variation in the digestibility of dietary fibre in different pigs and it seems likely that here too individual transit times are found, but as in man the reasons for this remain unknown.

The results of some recent studies on the effect of dietary fibre on overall transit time in pigs are summarised below.

Table 1. Effects of dietary fibre on overall mean transit time in pigs (Data from: Kuan, Stanogias and Dunkin (1983) (1), Ehle Jeraci, Robertson and Van Soest (1982) (2), Fioramonti and Bueno (1980) (3), Bardon and Fioramonti (1983) (4), and Canguilhem and Labie (1977) (5).

Source and level in diet (g/kg)		Diet type	Initial liveweight	Time (h)	Reference
Lucerne leaf meal	50	Semi-purified	44kg	43.7	1
" " "	100	"	"	41.6	
" " "	150	"	"	29.7	
" " "	200	"	"	28.4	
Coarse bran	312	"	70kg	51.6	2
Fine bran	472	"	"	49.7	
Lucerne meal	308	"	"	36.0	
Solka-floc	150	"	"	71.0	
No added fibre	-	Milk powder	50kg	120.0	3
Bran	170	"	"	66.0	
No added fibre	-	Milk	90kg	98.6	4
Bran (100g/d)	-	"	"	64.3	
No added fibre	-	Cereal	30kg	49.0	5
Bran(100 or 200g/d)	-	"	"	52.0	
No added fibre	-	Milk replacer	"	107.0	
Bran (100g/d)	-	"	"	79.0	

It is evident that additions of various types of dietary fibre can have marked effects upon transit time of diets, especially if these are essentially free of fibre as in the case of those based on milk or a milk replacer. On the other hand, bran addition to a cereal diet (with a high content of dietary fibre) had no effect (Canguilhem and Labie, 1977); similar observations have been made by E.A. Latymer (paper appears elsewhere in this publication) who found that bran, lactulose and pectin supplements to a cereal diet in pigs did not significantly alter transit times either in various regions of the gut, or overall, though in some regions the water phase moved faster than the solid phase (except for pectin which did not lead to phase separation). It may be that there is a minimum transit time in growing pigs, irrespective of the dietary fibre content of the diet.

Very fast transit times are often observed in piglets immediately after weaning, with concurrent (but not necessarily related) problems of diarrhoea and loss of appetite. This may be linked with the change from an all-milk diet to a diet with cereals, and thus containing dietary fibre; Schnabel, Bolduan and Guldenpenning (1983) supplemented a weaner diet with bran and found that mean transit time was greatly reduced, but without undesirable side-effects. The piglets were said to be constipated by weaning, presumably because of low feed intake, which was not recorded.

A number of technical problems in measuring transit time remain to be resolved including: (a) should the value used be 50% of the time for recovery of the whole of the marker, or some other value, or the time when peak concentratin of the marker occurs? (b) what marker should be used?

DIETARY FIBRE AND NUTRIENT ABSORPTION

a. Within the small intestine

It has been shown that the rate of glucose absorption from the small intestine is reduced by a wide variety of types of dietary fibre in man, the largest effects being seen with those sources which increase the viscosity of the meal and gut contents (Jenkins, Wolever, Leeds, Gassull, Haisman, Dilawari, Goff, Metz and Alberti, 1978). Similar effects have also been found for guar gum in pigs (Leeds, Kang, Low and Sambrook, 1980) and these have been shown to be at least in part due to reduced absorption from isolated loops of jejunum (Rainbird, Low and Zebrowska, 1984). The absorption of amino acids in pigs is also known to be delayed by guar gum (A.G. Low; unpublished). Other indications that dietary fibre may influence the rate of nutrient absorption came from studies by Rerat, Vaissade and Vaugelade (1979) who measured glucose and amino acid absorption into the hepatic portal vein during an 8 hour period: 40 and 54% of ingested amino acids and 40

and 45% of ingested glucose were recovered from barley and wheat, respectively. Barley has a higher dietary fibre content than wheat, and this is due almost entirely to more soluble non-cellulosic compounds (Englyst, Anderson and Cummings, 1983): it is therefore possible that the effects found in the case of guar gum may apply to whole foods.

b. Combined Studies of Ileal and Faecal Digestibilities

The effects of dietary fibre on nutrient absorption as measured in digesta from the terminal ileum and in faeces have been quite widely studied. The results of one of the most comprehensive studies, by Just, Fernandez and Jorgensen (1983), in which a series of semi-purified diets including fibre from oats, barley and carboxymethyl cellulose were compared in 60-90kg pigs fitted with simple ileal cannulas are shown below.

Table 2. Effects of crude fibre on apparent digestibility of nutrients in 60-90kg pigs (data from Just, Fernandez and Jorgensen, 1983)

Crude Fibre	: diet (g/kg)	33	57	84	109	137	161
	ileum	0.0	0.0	0.0	0.0	0.0	0.0
	faeces	0.55	0.60	0.68	0.63	0.51	0.59
Nitrogen	: diet (g/kg)	35.7	35.8	36.0	35.0	35.4	35.7
	ileum	0.78	0.84	0.82	0.78	0.76	0.75
	faeces	0.93	0.91	0.87	0.84	0.81	0.77
Stoldt Fat	: diet (g/kg)	72	72	72	72	77	78
	ileum	0.75	0.81	0.81	0.79	0.81	0.79
	faeces	0.85	0.83	0.80	0.76	0.80	0.78
Gross Energy	: diet (MJ/kg)	19.36	19.19	19.25	19.09	19.27	19.20
	ileum	0.75	0.72	0.69	0.65	0.56	0.52
	faeces	0.92	0.90	0.87	0.84	0.79	0.78
Lysine	: diet	(not given in paper)					
	ileum	0.92	0.93	0.91	0.90	0.87	0.86
	faeces	0.95	0.93	0.90	0.87	0.84	0.80

These results typify those found by other groups i.e. the major effects were that fibre additions markedly depressed ileal apparent energy digestibility but effects on other components of the diet were small or non-existent; on the other hand faecal apparent digestibility of nitrogen, lysine and energy fell sharply, while the values for Stoldt fat declined to a lesser extent and those for crude fibre remained approximately constant. The proportions of nutrients disappearing during passage through the large intestine increased for those included in gross energy, but fell for all other components, while possible net synthesis of Stoldt fat and lysine occurred, presumably as a result of microbial metabolism.

Values for the apparent digestibility of components of dietary fibre in pigs in the ileum and faeces of four pigs given a variety of diets are shown below:

Table 3. Apparent digestibilty of components of dietary fibre in pigs (data from Keys and Debarthe (1974))

		Source of Dietary Fibre			
		Alfalfa	Grain Sorghum	Texas Kleingrass	Coastal Bermuda Grass
Dry Matter	: diet (g/kg)	912.5	928.6	926.6	928.9
	ileum	0.39	0.41	0.38	0.47
	faeces	0.68	0.70	0.66	0.75
Cell Walls	: diet (g/kg)	275.9	281.3	305.6	328.7
	ileum	-0.05	-0.03	-0.06	0.39
	faeces	0.32	0.31	0.22	0.50
Cellulose	: diet	174.6	143.5	148.9	144.9
	ileum	-0.09	-0.08	-0.07	0.33
	faeces	0.38	0.33	0.21	0.48
Hemicellulose	diet	64.5	119.9	127.9	161.9
	ileum	0.10	0.04	0.05	0.47
	faeces	0.33	0.32	0.25	0.54

The apparent digestibility of each of the three fractions of dietary fibre measured was often similar both in ileal digesta and

faeces; the apparent digestibility values for coastal bermuda grass in the ileal digesta are associated with a very high variance and may be the result of imperfect marking or sampling of the digesta from the ileal cannulas. The amounts of dietary fibre digested in the small intestine appeared to be very small. However, Millard and Chesson (1984) measured a wide variety of components of ileal digesta collected from two pigs which had received a diet based on swede (Brassica napus) and observed the following apparent digestibility values: rhamnose 0.31, fucose 0.0, arabinose 0.42, xylose 0.03, mannose 0.02, galactose 0.48, cellulose 0.19, uronic acid 0.48 (as polysaccharide residues), frutrose 0.99, glucose 0.98 (as free sugars), phenolics 0.74, soluble protein 0.0, nitrogen 0.71. These values are remarkable for indicating extensive disappearance of cellulose in the small intestine, which was found to contain cellulolytic species of bacteria when pigs were fed swede but not bran.

The effects of dietary fibre on apparant absorption of minerals have been little studied in pigs, but Partridge (1978) provided a detailed picture of the effects of cellulose included at two levels in a semi-purified diet as follows:

Table 4. Effects of cellulose on apparent absorption of minerals in growing pigs (data from Partridge, 1978)

	Dietary cellulose g/kg			
	30		90	
	Ileal Digesta	Faeces	Ileal Digesta	Faeces
Digesta/Faeces	0.84	0.99	0.76	0.97
Organic matter	0.94	0.99	0.90	0.95
Water	0.81	0.99	0.72	0.98
Sodium	0.46	0.99	0.15	0.98
Potassium	0.90	0.97	0.88	0.86
Calcium	0.43	0.74	0.44	0.63
Phosphorus	0.64	0.81	0.69	0.74
Magnesium	-0.01	0.73	0.03	0.62
Zinc	0.10	0.60	0.24	0.37

The principal effect of additional cellulose was to depress net apparent absorption of sodium anterior to the terminal ileum; however there was substantially more water present in the digesta following consumption of the high cellulose diet and the increased amounts of sodium present were probably necessary to maintain toxicity of the ileal digesta.

A recent series of studies on the effects of cellulose, pectin and dried sugar beet pulp on nitrogen and amino acid apparent digestibility show that reductions in the apparant digestibility of dry matter are more marked than for nitrogen, while effects on amino acids are small, as table 5 shows:

Table 5. Effects of cellulose, pectin and sugar beet pulp on apparent digestibility in ileal digesta and faeces of pigs (data from Dierick, Varvaeke, Decuypere and Henderickx (1983))
(DM = dry matter, N = nitrogen, EAA = essential amino acids, NEAA = non-essential amino acids)

Dietary Fibre Source	Ileal Digesta				Faeces			
	DM	N	EAA	NEAA	DM	N	EAA	NEAA
Fibre-free (exp. 1)	0.91	0.88	0.90	0.89	0.95	0.94	0.93	0.93
Cellulose (75g/kg)	0.84	0.86	0.90	0.88	0.84	0.90	0.91	0.92
Pectin (50g/kg)	0.80	0.76	0.81	0.75	0.93	0.92	0.91	0.92
Fibre-free (exp. 2)	0.92	0.89	0.94	0.93	0.95	0.95	0.96	0.96
Cellulose (50g/kg)	0.85	0.89	0.93	0.92	0.93	0.95	0.96	0.96
" (100g/kg)	0.79	0.88	0.93	0.92	0.89	0.93	0.95	0.96
" (150g/kg)	0.74	0.84	0.94	0.93	0.84	0.91	0.93	0.93
Dried Sugar Beet Pulp (50g/kg)	0.87	0.90	0.96	0.95	0.94	0.95	0.96	0.96
" (100g/kg)	0.81	0.86	0.92	0.89	0.93	0.94	0.94	0.94
" (150g/kg)	0.72	0.81	0.90	0.88	0.92	0.92	0.94	0.94

(c) Faecal Digestibility Studies

A considerable literature exists on the effects of dietary fibre on overall digestibility of nutrients but it is difficult to draw specific conclusions from these studies because of the great variety of types of dietary fibre, feeding levels, dietary levels and analytical methods used.

As far as nitrogenous components of the diet are concerned, depressed digestibility has been shown following cellulose, lucerne leaf and alkali-treated straw supplementation of diets while oat feed, oat hulls, barley hulls, corn cobs led to only small effects. Pectin depressed nitrogen apparent digestibility in the experiments of Mosenthin and Henkel (1983) but urine nitrogen output fell so nitrogen balance was unaffected (urea nitrogen excretion was diverted from urine to the large intestine).

The digestion of starches is generally depressed by additional dietary fibre, but the digestion of dietary fibre itself is highly variable, and may increase with age and physiological state:

Cunningham, Friend and Nicholson (1962) found that cellulose digestibility increased from 0.05 to 0.18 as pigs fed at a growing pig level matured, while a value of 0.29 was achieved under maintenance feeding conditions. Further examination of the factors involved in such changes appears to be of importance in developing methods of feeding pigs with diets containing high levels of dietary fibre. One factor which appears to influence the digestibility of cellulose is the lignin content of the diet: delignification can lead to cellulose digestibility values of over 0.80 (Woodman and Evans, 1947).

There is some evidence that phosphorus absorption can be decreased by oat hulls (Moser, Peo, Moser and Lewis, 1982) and zinc absorption by wheat bran (Newton, Hale and Plank, 1983), though the mechanisms involved remain unclear.

The main results of the only comprehensive comparison of effects of dietary fibre, expressed as crude fibre, on overall nutrient digestibility in pigs of different ages is shown below:

Table 6. Effects of crude fibre content of the diet on apparent nutrient digestibility in pigs of various weights (data from Fernandez, Just and Jorgensen, 1979)

	Weight of Pigs	Crude Fibre (g/kg) 50	100	170
Nitrogen	20	0.81	0.71	0.55
	90	0.86	0.80	0.73
	225	0.85	0.80	0.72
Stoldt Fat	20	0.62	0.53	0.55
	90	0.64	0.61	0.65
	225	0.65	0.61	0.63
Crude Fibre	20	0.42	0.30	0.26
	90	0.49	0.44	0.32
	225	0.39	0.47	0.45
Gross Energy	20	0.81	0.70	0.56
	90	0.83	0.75	0.61
	225	0.82	0.76	0.66

This study indicates that the depressive effects of crude fibre (from barley, maize, wheat bran and oat hull meal) is much reduced as pigs grow beyond 20kg live weight, and also that improvements with age were greatest in pigs fed at the highest level of crude fibre. These authors also demonstrated significant increases in the apparent digestibility of most components of diets based on either manioc, barley, soyabean meal or fishmeal in 200-250kg sows compared with 50-70kg growing pigs, but the magnitude differed

according to the diet.

An important conclusion to be drawn from studies in which high levels of dietary fibre have been given to pigs is that stated amounts of energy, protein and minerals, in particular, which are needed in the diet for a given level of response may need to be revised upwards. At the same time the increased intakes implied may be difficult to attain given the appetite which limits the productivity of contemporary pigs. Thus attention towards increasing appetite, in conjunction with the use of high levels of dietary fibre would appear to be merited.

FERMENTATION IN THE LARGE INTESTINE

Once dietary fibre leaves the small intestine its role as an agent which influences the digestion and absorption of other nutrients changes to that of being a nutrient in its own right, as a result of bacterial fermentation. A detailed review of the microbiological aspects is not presented here as it is covered in the accompanying review by B. Ratcliffe. There is considerable time for fermentation in the large intestine (20-40 hours at least, in pigs) and at the same time extensive water absorption, which is influenced by fibre, occurs: for example in 40kg pigs 3152g of water were absorbed from a cereal-based diet and 986g from a low-fibre semi-purified diet (Low, Partridge and Sambrook, 1978). Increasing the level of dietary fibre also leads to significant increases in the weight of the large intestinal tissue (Kass, Van Soest, Pond, Lewis and McDowell, 1980); though the reason for this is not understood, it may be hypothesised that the need for additional musculature to contain and to propel larger volumes of digesta in a factor.

By far the most important products of the bacterial fermentation from a nutritional point of view are volatile fatty acids (VFA) which are generally present at concentrations in the range 150-200 mM compared with 5-40 mM in the stomach and small intestine. They may be formed from any component of dietary fibre except for lignin as well as other undigested diet residues. Acetic acid tends to predominate with smaller amounts of propionic and butyric acids; the proportions mainly vary according to the

type of dietary fibre, and the site within the large intestine. In general as the dietary fibre content of the diet increases, so the proportion of acetic acid rises, as shown for alfalfa by Kass, Van Soest, Pond, Lewis and McDowell (1980). VFA are readily absorbed from the gut and enter the central pathways of intermediary metabolism both in the gut tissue and throughout the body.

The rates of VFA production have been measured by a variety of methods in order to estimate their contribution to the energy supply for the pig. In an early study, Friend, Nicholson and Cunningham (1964) compared portal-venous VFA concentration differences in 30kg pigs and concluded that 15-28% of maintenance energy requirements might be met by VFA. However, these values make no allowance for hepatic production of VFA (Imoto and Namioka, 1978b) and are thus open to doubt. More recently Kass (1980) regressed the amounts of VFA produced in the caecum and colon of pigs slaughtered 2, 4, 8 or 12h after feeding diets containing 0, 200, 400 or 600g alfalfa meal/kg. Assuming all VFA were absorbed, 6.9, 11.3, 12.5 and 12.0% of maintenance energy could be provided for 48kg pigs and 4.8, 11.4, 14.0 and 12.9% in 89kg pigs given the diets containing 0, 200, 400, 600g alfalfa meal/kg respectively.

Kennelly, Aherne and Sauer (1981) estimated VFA production in the caecum by the continuous isotope dilution method and calculated that caecal production of VFA could provide 19.7% of maintenance energy requirements from a barley-soya diet, and 10.1, 15.5 or 11.1% when diets containing 0, 273, 520g alfalfa/kg were fed. However, the problem with this method is that although correction can be made for VFA interconversions, the isotope is not contained within the caecum and thus sampling takes place from a pool of varying and uncertain size. Imoto and Namioka (1978b) demonstrated that in addition to extensive hepatic production of VFA, there was extensive metabolism of VFA in the wall of the large intestine.

In vitro incubations of the contents of the large intestine have also provided estimates of the contribution of VFA to pig maintenance energy requirements. Values of 5.5 and 3.9% for caecal VFA (Farrell and Johnson, 1972), and values for VFA from large intestinal contents as a whole range from 5.0-25% (Gargallo

and Zimmerman, 1981; Imoto and Namioka, 1978a; Argenzio, 1982). Each of these values is based on assumed steady state conditions and must be related to the entire volume of caecal and colonic contents, which vary continuously in vivo and thus cannot be measured accurately.

The problems involved in measurement of the contribution that VFA may make to the energy requirements of the pig are such that no confident statement can be made and further work is clearly needed.

VOLATILE FATTY ACID METABOLISM IN PIGS

Although some absorbed volatile fatty acids are metabolized in the gut wall, substantial amounts also enter the blood. A tracer dose of U-^{14}C-acetate introduced into the caecum of 22-28kg pigs was rapidly absorbed and peak levels were found in the blood within 30 minutes; from this time until the end of sampling 5 hours later, ^{14}C was found in all major classes of lipid (including free cholesterol and cholesterol esters), plasma proteins and other water-soluble compounds (Latymer and Woodley, 1984). In a second study, two 70-78kg pigs, which also received U-^{14}C-acetate into the caecum, were killed following a 96 hour collection period, and the percentage recovery of the dose was as follows: small intestine wall and contents 0.8, large intestine contents 0.1, large intestine wall 1.5, liver 0.6, kidney 0.1, blood 0.1, carcass 23.6, urine 2.7, faeces 5.2; losses of, 65.3% assumed to be $^{14}CO_2$, were calculated by difference (Latymer and Low, 1984).

The nutritive value of acetate has been estimated to be 56-59% in terms of % use of metabolizable energy, i.e. approximately 8.8kJ were deposited per gram of supplementary acetate in growing pigs under thermoneutral conditions (Jentsch, Schiemann and Hoffman, 1968; Imoto and Namioka, 1983).

These data indicate that absorbed acetate is metabolized with an efficiency aproximately three quarters of that of glucose when measured under conditions of growth. Energetic efficiency is generally higher in maintenance than in growth but comparative values for acetate do not exist. In addition, data on the

metabolism of propionate and butyrate are not available. Some of the energy arising from fermentation of fibre in the gut is lost to the animal because it is either in the form of heat from the fermentation reaction, or methane; it has been calculated that the latter represents 14-17% of the apparently digested energy arising from fermentation (Agricultural Research Council, 1981).

EFFECTS OF DIETARY FIBRE ON THE WHOLE BODY

The overall effects of dietary fibre on energy metabolism have been succinctly summarised by Just 1982a, Just 1982b, and Just, Fernandez and Jorgensen (1983). In a series of studies they found that the efficiency of use of metabolizable energy fell by a mean 0.7% units for every increase of 10g crude fibre/kg diet. This decrease corresponded with an increase in the proportion of dietary energy disappearing in the large intestine: the linear relationship between the percentage of energy which disappeared in the large intestine (X) and the net energy value of the diet (Y) (expressed as a percentage of metabolizable energy in the diet) was $Y = 74.5 - 0.49X$.

A recent review of the effects of dietary fibre on pig performance and body composition, sow productivity and some possible ways of improving its nutritive value has been made by Low (1985). Laplace and Lebas (1981a and b) have prepared very detailed reviews on the effects of dietary fibre in a variety of animal species.

REFERENCES

AGRICULTURAL RESEARCH COUNCIL 1967 In "The Nutrient Requirements of Farm Livestock". Agricultural Research Council, London. p. 56-63.

AGRICULTURAL RESEARCH COUNCIL 1981 In "The Nutrient Requirements of Pigs". Commonwealth Agricultural Bureaux, Farnham Royal. p. 41-44.

ARGENZIO, R.A. 1982. Les Colloques de I'INRA. 12 : 207-215.

ASP, N.G., JOHANSSON, C.G., HALLMER, H. & SILJESTROM, M. 1983. J. Agric. Food Chem. 31 : 476-482.

BAKER, D.H., BECKER, D.E., JENSEN, A.H. & HARMON, B.G. 1968. J. Anim. Sci. 27 : 1332-1335.

BARDON, H. & FIORAMONTI, J. 1983. Br. J. Nutr. 50 : 685-690.

BERGNER, H. 1981. Pig News Inf. 2 : 135-140.

CANGUILHEM, R. & LABIE, C. 1977. Rev. Med. Vet. 128 : 1669-1681.

CUMMINGS, J.H., SOUTHGATE, D.A.T., BRANCH, W.J., HOUSTON, H., JENKINS, D.J.A. & JAMES, W.P.T. Lancet 1 : 5-8.
CUNNINGHAM, H.M., FRIEND, D.W. & NICHOLSON, J.W.G. 1962. Can. J. Anim. Sci. 42 : 167-175.
DIERICK, N., VEERVAEKE, I., DECUYPERE, J. & HENDERICKX, H.K. 1983. Rev. Agric (Bruxelles) 36 : 1691-1712.
EASTWOOD, M.A. & HAMILTON, D. 1968. Biochim. Biophys. Acta. 152 : 168-173.
EHLE, F.R., JERACI, J.L. ROBERTSON, J.B. & VAN SOEST, P.J. 1982. J. Anim. Sci. 55 : 1071-1080.
ENGLYST, H., ANDERDON, V. & CUMMINGS, J.H. 1983. J. Sci. Food Agric. 34 : 1434-1440.
ENGLYST, H., WIGGINS, H.S. & CUMMINGS, J.H. 1982. Analyst (London). 107 : 307-318.
FARRELL, D.J. & JOHNSON, K.A. 1972. Anim. Prod. 16 : 43-47.
FERNANDEZ, J.A., JUST, A. & JORGENSEN, H. 1979. EAAP 30th Ann. Mtg. Harrogate, England. Paper P5.7.
FIORAMONTI, J. & BUENO, L. 1980 Br. J. Nutr. 43 : 155-162.
FRIEND, D.W., NICHOLSON, J.W.G. & CUNNINGHAM, H.M. 1964. Can. J. Anim. Sci. 44 : 303-308.
GARGALLO, J. & ZIMMERMAN, D.R. 1981. J. Anim. Sci. 53 : 1286-1291.
IMOTO, S. & NAMIOKA, S. 1978a J. Anim. Sci. 47 : 467-478.
IMOTO, S. & NAMIOKA, S. 1978b J. Anim. Sci. 47 : 479-487.
IMOTO, S. & NAMIOKA, S. 1983. J. Anim. Sci. 56 : 858-866.
JAMES, W.P.T., BRANCH, W.J. & SOUTHGATE, D.A.T. 1978. Lancet i : 638-639.
JAMES, W.P.T. & THEANDER, O. (Editors) 1981. "The Analysis of Dietary Fiber in Food". Marcel Dekker, New York.
JENKINS, D.J.A. WOLEVER, T.M.S., LEEDS, A.R., GASSULL, M.A., HAISMAN, P., DILAWARI, J., GOFF, D.V., METZ, G.L. & ALBERTI, K.G.M.M. 1978. Br. Med. J. 1 : 1392-1394.
JENTSCH, W., SCHIEMANN, R. & HOFFMANN, L. 1968. Arch. Tierernähr. 18 : 352-357.
JOHNSON, I.T., GEE, J.M. & MAHONEY, R.R. 1984. Br. J. Nutr. 52 : 477-487.
JUST, A. 1982a Livest. Prod. Sci. 9 : 569-580.
JUST, A. 1982b Livest. Prod. Sci. 9 : 717-729.
JUST, A., FERNANDEZ, J.A. & JORGENSEN, H. 1983. Livest. Prod. Sci. 10 : 171-186.
KASS, M.L., VAN SOEST, P.J., POND, W.G., LEWIS B. & McDOWELL, R.E. 1980. J. Anim. Sci. 50 : 175-191.
KENNELLY, J.J. AHERNE, F.X. & SAUER, W.C. 1981. Can. J. Anim. Sci. 61 : 349-362.
KEYS, J.E. & DEBARTHE, J.V. 1974. J. Anim. Sci. 39 : 53-57.
KUAN, K.K., STANOGIAS, G. & DUNKIN, A.C. 1983. Anim. Prod. 36 : 201-209.
LAPLACE, J.P. & LEBAS, F. 1981a. Wld. Rev. Nutr. Diet. 37 : 84-176.
LAPLACE, J.P. & LEBAS, F. 1981b. Wld. Rev. Nutr. Diet. 37 : 177-228.
LATYMER, E.A. & LOW, A.G. 1984. Proc. Nutr. Soc. 43 : 12A.
LATYMER, E.A. & WOODLEY, S.C. 1984. Proc. Nutr. Soc. 43 : 22A.
LEEDS, A.R., KANG, S.S., LOW, A.G. & SAMBROOK, I.E. 1980. Proc. Nutr. Soc. 39 : 44A.
LOW, A.G. 1985 In "Recent Advances in Animal Nutrition - 1985" (Ed. W. Harresign) Butterworths, London (in press).
LOW, A.G., PARTRIDGE, I.G. & SAMBROOK, I.E. 1978. Br. J. Nutr. 39 : 515-526.

LOW, A.G. & RAINBIRD, A.L. 1984. Br. J. Nutr. 52 : 499-505.
MACCONNELL, A.A., EASTWOOD, M.A. & MITCHELL, W.D. 1974. J. Sci. Food Agric. 25 : 1457-1464.
MILLARD, P. & CHESSON, A. 1984. Br. J. Nutr. 52 : 583-594.
MOSENTHIN, R. & HENKEL, H. 1983. Les Colloques de I'INRA. 16 : 447-450.
MOSER, R.L., PEO, E.R., MOSER, B.D. & LEWIS, A.J. 1982. J. Anim. Sci. 54 : 800-805.
NEWTON, G.L., HALE, O.M. & PLANK, C.O. 1983. Can. J. Anim. Sci. 63 : 399-408.
OWEN, J.B. & RIDGMAN, W.J. 1968. Anim. Prod. 10 : 85-91.
PARTRIDGE, I.G. 1978. Br. J. Nutr. 39 : 539-545.
RAINBIRD, A.L. & LOW, A.G. 1983. Proc. Nutr. Soc. 42 : 88A.
RAINBIRD, A.L., LOW, A.G. & ZEBROWSKA, T. 1984. Br. J. Nutr. 52 : 489-498.
RAYNER, V., GREGORY, P.C. & GOODALL, E.D. 1984. Gut 25 : A1310-A1311.
RERAT, A., VAISSADE, P. & VAUGELADE, P. 1979. Ann. Biol. Anim. Biophys. 1979. 19 : 737-747.
SAMBROOK, I.E. 1981. J. Sci. Food Agric. 32 : 781-791.
SAUER, W.C., STOTHERS, S.C. & PARKER, R.J. 1977. Can. J. Anim. Sci. 57 : 775-784.
SCHNABEL, E., BOLDUAN, G. & GULDENPENNING, A. 1983. Arch. Tierernähr. 33 : 371-378.
SISSONS, J.W., RAINBIRD, A.L. & THURSTON, S.M. 1984. Gut 25 : A1318-A1319.
SOUTHGATE, D.A.T. 1969. J. Sci. Food Agric. 20 : 331-335.
SOUTHGATE, D.A.T. 1976. In "Fiber in Human Nutrition" (Eds. G.A. Spiller & R.J. Amen). Plenum Press, New York p. 31-72.
SOUTHGATE, D.A.T. 1982. In "Dietary Fibre in Health and Disease" (Eds. G.V. Vahouny & D. Kritchevsky). Plenum Press, New York p. 1-7.
SOUTHON, S., LIVESEY, G., GEE, J.M. & JOHNSON, I.T. 1985. Br. J. Nutr. 53 : 87-95.
STEPHEN, A.M. & CUMMINGS, J.H. 1979. Gut 20 : 722-729.
STEPHEN, A.M. 1980. "Dietary Fibre and Human Colonic Function" PhD Thesis, Univ. Cambridge, Cambridge.
STEPHEN, A.M. & CUMMINGS, J.H. 1980. Nature. 284 : 283-284.
TAVERNER, M.R., HUME, I.D. & FARRELL, D.J. 1981. Br. J. Nutr. 46 : 149-158.
TROWELL, H., SOUTHGATE, D.A.T., WOLEVER, T.M.S., LEEDS, A.R., GASSULL, M.A. & JENKINS, D.J.A. 1976. Lancet 1. 967.
VAN SOEST, P.J. 1963. J. Ass. Off. Agric. Chem. 46 : 829-835.
VAN SOEST, P.J. & McQUEEN, R.W. 1973. Proc. Nutr. Soc. 32 : 123-130.
VAN SOEST, P.J. & WINE, R.H. 1967. J. Ass. Off. Analyt. Chem. 50 : 50-55.
WHITING, F. & BEZEAU, L.M. 1957. Can. J. Anim. Sci. 37 : 106-113.
WOODMAN, H.E. & EVANS, R.E. 1947. J. Agric. Sci. (Camb.) 37 : 202-210.
ZEBROWSKA, T., LOW, A.G. & ZEBROWSKA, H. 1983. Br. J. Nutr. 49 : 401-410.
ZOIOPOULOS, P.E., ENGLISH, P.R. & TOPPS, J.H. 1982. Anim. Prod. 35 : 25-33.

INFLUENCE OF FIBRE ON THE ^{15}N-LABELLING OF AMINO ACIDS IN THE DIGESTIVE TRACT OF ^{15}N-LABELLED PIGS

H. BERGNER, O. SIMON, I. PARTRIDGE[x], URSULA BERGNER
Institute of Animal Nutrition, Department of Animal Production and Veterinary Medicine, Humboldt-University Berlin, Invalidenstr. 42, GDR-1040 Berlin

SUMMARY

Supplements of fermentable fibre (partly hydrolysed straw meal) to the diets of pigs depressed the apparent digestibility of the various amino acids gradually but at a different degree. If the incorporation of ^{15}N into faecal amino acids was measured during a labelling period with ^{15}N ammonium salts the atom-% ^{15}N excess of the amino acids correlated highly with the addition of the fibre source. This observation was not made 10 days after the end of a labelling period. The results are discussed in connection with the microbial activities in the large intestine.

INTRODUCTION

The aim of the investigation was to study the digestibility of amino acids in pigs in relation to the dietary fibre content as well as the ^{15}N-labelling of the same amino acids in ^{15}N-labelled pigs.

MATERIALS AND METHODS

Experiment 1

Four male castrated pigs (55-65 kg) either received a fish meal diet, a fish meal diet + partly hydrolysed straw meal, a horse bean diet and a horse bean diet + partly hydrolysed straw meal. The supplement of straw meal was 20 % of DM of the ration. The content of crude fibre of the rations was 3.0 %, 10.0 %, 5.3 % and 12.1 %, respectively (Bergner et al., 1984).

During a 10-day ^{15}N-labelling period 385 mg ^{15}N-excess (^{15}N') per $kg^{0.75}$ were applied in a mixture of ammonium acetate and ammonium chloride orally. The pigs were housed individually in metabolic cages. Faeces were collected during the labelling period and the

[x]Present adress: Nat. Inst. Res. in Dairying, Shinfield, Reading, Berks RG 2 9 AT (UK)

contents of ^{14}N-amino acids and of ^{15}N-amino acids were estimated in faeces.

Experiment 2

Three male castrated pigs (39-43 kg), which were fitted with T-cannulas at the terminal ileum, received after a ^{15}N-labelling period (6 days with 150 mg $^{15}N'/kg^{0.75}$. d) a wheat-fish meal diet supplemented with different amounts of partly hydrolysed straw meal. Ileal and faecal amino acid digestibilities were estimated using an indicator method with Cr_2O_3.

RESULTS

Experiment 1

The apparent amino acid digestibility and the ^{15}N-labelling of amino acids in faeces are given in Table 1.

Table 1. Amino acid digestibility (%) and atom-% ^{15}N-excess of amino acids (included in parenthesis)

Crude fibre (% of DM)	3.0	5.3	10.0	12.1
Lys	91.1 (0.08)	83.0 (0.13)	83.7 (0.12)	71.4 (0.27)
His	92.3 (0.06)	91.0 (0.09)	86.4 (0.10)	82.4 (0.21)
Arg	93.2 (0.07)	93.3 (0.12)	87.6 (0.13)	85.5 (0.29)
Asp	90.3 (0.11)	84.8 (0.20)	81.7 (0.23)	75.2 (0.31)
Thr	90.6 (0.06)	83.2 (0.17)	82.7 (0.11)	73.1 (0.21)
Ser	91.4 (0.06)	89.4 (0.15)	85.0 (0.12)	82.7 (0.23)
Glu	96.6 (0.10)	95.7 (0.21)	91.9 (0.25)	89.7 (0.36)
Pro	99.1 (0.06)	95.1 (0.05)	90.8 (0.04)	89.8 (0.05)
Gly	92.2 (0.09)	87.7 (0.15)	86.1 (0.15)	79.0 (0.19)
Ala	91.1 (0.11)	82.7 (0.17)	83.6 (0.15)	71.3 (0.21)
Val	93.9 (0.09)	90.8 (0.15)	88.9 (0.16)	84.4 (0.32)
Ile	90.8 (0.10)	88.3 (0.15)	83.8 (0.14)	77.6 (0.26)
Leu	92.0 (0.08)	89.3 (0.16)	85.4 (0.13)	82.1 (0.19)
Tyr	91.9 (0.05)	89.2 (0.13)	87.4 (0.11)	82.1 (0.17)
Phe	91.5 (0.04)	90.1 (0.07)	85.0 (0.07)	80.9 (0.14)
Nitrogen	92.6 (0.11)	89.1 (0.32)	85.6 (0.33)	79.4 (0.50)

Experiment 2

The ^{15}N-labelling of amino acids in the ileal digesta and in faeces is shown in Table 2.

Table 2. Atom-% ^{15}N-excess of amino acids in the ileal digesta and in faeces (10 days after the end of the ^{15}N-labelling period)

Crude fibre (% of DM)	4.0		6.9		13.2	
	Digesta	Faeces	Digesta	Faeces	Digesta	Faeces
Asp	0.09	0.16	0.09	0.10	0.09	0.12
Thr	0.08	0.17	0.04	0.14	0.09	0.06
Ser	0.25	0.23	0.08	0.11	0.10	0.16
Glu	0.23	0.15	-	0.13	0.12	0.13
Pro	0.13	0.09	0.05	0.08	0.08	0.13
Gly	0.23	0.17	0.10	0.13	0.12	0.13
Ala	0.28	0.31	0.08	0.11	0.13	0.12
Val	0.29	0.29	0.13	0.12	0.14	0.14
Ile	0.32	0.27	0.13	0.18	0.11	0.16
Leu	0.27	0.15	0.17	0.17	0.17	0.15
Tyr	0.20	-	0.06	0.12	0.07	0.09
Phe	-	-	0.07	0.08	0.05	0.10
N	0.10	0.14	0.08	0.11	0.13	0.12

The excretion of ^{15}N-amino acids in faeces in dependence of DM-intake is presented in Table 3.

Table 3. Excretion of ^{15}N-amino acids in faeces (μg ^{15}N-amino acid/100 g DM-intake)

Crude fibre (% of DM)	4.0	6.9	13.2		4.0	6.9	13.2
Asp	0.23	0.22	0.40	Ala	0.28	0.15	0.26
Thr	0.11	0.14	0.09	Val	0.21	0.14	0.26
Ser	0.14	0.10	0.23	Ile	0.15	0.16	0.22
Glu	0.23	0.30	0.49	Leu	0.14	0.26	0.36
Pro	0.05	0.06	0.16	Tyr	-	0.07	0.09
Gly	0.15	0.16	0.23	Phe	-	0.07	0.14

DISCUSSION

In both experiments the apparent digestibility of all amino acids correlated negatively with the dietary crude fibre content. The digestibilities of Lys, Ala, Thr, Asp and Ile were most affected by the crude fibre content (digestibilities 70-80 % at 12 to 13 % crude fibre in the DM). On the other hand, the digestibility of Pro and Glu was only little influenced (~90 % at the highest crude fibre contents).

In experiment 1 the incorporation of ^{15}N into the various amino acids (Table 1) increased generally if a fermentable fibre source was supplemented to the diet. Highest atom-% ^{15}N excess values were measured for Glu, Asp and Ala and lowest values were estimated for Pro, Phe and Tyr. It should be mentioned that in this experiment the ^{15}N-incorporation was measured during the labelling period and therefore, the ^{15}N-incorporation into the amino acids could be strongly influenced by the microbial utilisation of highly labelled ^{15}N originating from urea.

In experiment 2 the ^{15}N-excess of the amino acids in ileal digesta and faeces was measured 10 days after the end of the labelling period. In this case ^{15}N for microbial amino acid and protein synthesis in the large intestine was mainly derived from endogenous proteins. No correlation between ^{15}N-labelling of amino acids in ileal digesta or in faeces and dietary crude fibre content was observed (Table 2). However, the total amount of faecal excretion increased with increasing crude fibre content of the diet for the majority of the ^{15}N-amino acids (Table 3).

It was concludet that, the depressing effect of partly hydrolysed straw meal on the apparent digestibility of amino acids is caused by the microbial de novo synthesis of amino acids in the large intestine as well as by the physical properties of this fibre source.

REFERENCES

BERGNER, H., BERGNER, URSULA & ADAM, K. 1984. Arch. Tierernähr. 34: 441-455

ILEAL AMINO ACID DIGESTIBILITY MEASUREMENT IN PIGS FED HIGH FIBER DIETS : ILEO-RECTAL ANASTOMOSIS VERSUS ILEO-COLIC POST-VALVE FISTULATION

DARCY-VRILLON B., LAPLACE J.P.
Laboratoire de Physiologie de la Nutrition
INRA - Centre de Recherches Zootechniques
F-78350 Jouy-en-Josas, France

SUMMARY

In an attempt to develop a routine technique to collect ileal digesta, for dietary protein evaluation, ileo-rectal anastomosis was tested against ileo-colic post valve fistulation as a reference. It appeared that both techniques supplied similar results with standard or wheat bran enriched diets, whereas significantly different nitrogen and amino acid digestibilities were recorded with a beet pulp diet. Explanatory hypotheses are considered for that particular case. Further experiments are needed in various conditions to completely assess the validity of the ileo-rectal anastomosis technique for routine tests.

INTRODUCTION

Ileal digestibility is a better tool than faecal digestibility for dietary protein evaluation. However, the accuracy of ileal digestion coefficients depends on the method used for digesta collection. The ileo-colic post-valve fistulation, said I.C.P.V., (DARCY, LAPLACE and VILLIERS, 1980) has been improving the methodology by overcoming the main defects of previous techniques such as ileo-ileal or ileo-caecal reentrant fistulations (DARCY, LAPLACE, 1980). Nevertheless I.C.P.V. which can be considered as a reference is too tedious and time-consuming for routine measurements. To perform routine tests on raw materials, in order to formulate diets on the basis of their available amino acid content, a simplified procedure is required. The ileo-rectal anastomosis (I.R.A.) might be an appropriate technique to obtain ileal digesta excreted and collected just like faeces (LAPLACE, DARCY-VRILLON, PICARD, 1985). The aim of this preliminary study is to test I.R.A. against I.C.P.V. in the case of high fiber diets.

MATERIALS AND METHODS

Five pigs (51.8 ± 1.2 kg live weight) were housed in metabolic crates. Two of them were submitted to I.C.P.V. fistulation according to DARCY, LAPLACE and VILLIERS (1980). The others were prepared by I.R.A., bypassing the caeco-colic area which was isolated and fitted with a cannula for distal outflow (LAPLACE, DARCY-VRILLON, PICARD, 1985). All pigs received 3 isonitrogenous (16 p. cent crude protein) diets : 1) Standard : barley 60, maize 15, soya bean meal 15, lucerne meal 6.5 per cent ; 2) wheat bran diet : wheat bran 45.4, purified maize starch 41.3, casein 9.3 per cent; 3) beet pulp diet : beet pulp 32.0, purified maize starch 50.7, casein 13.3. The last two diets had the same crude fiber content (5.3 per cent). The total cell wall content (N.D.F., VAN SOEST, 1963) was 18.4, 22.0, 12.5 for standard, bran and pulp diets respectively. In addition to the normal dietary supply of minerals and vitamins, the I.R.A. pigs received additional minerals and vitamins to compensate for their increased losses.

The I.C.P.V. data are based on 22 days from the 2 pigs fed once a day (mean intake 765 ± 30 g dry matter). The I.R.A. data are based on 26 days from the 3 pigs fed twice a day except for some tests on beet pulp (one meal per day only, due to the bad appetibility of pulps). Whatever the case, the mean daily intake by I.R.A. pigs was 1613 ± 45 g. Each diet was tested (after habituation) during 3 consecutive days at least. Digesta collected were analysed for their dry matter and total nitrogen content day by day, and for their amino acid content on 3 days-pooled samples. Amino acid analysis was performed after acid hydrolysis (24 hrs, 48 hrs and oxidation for sulfur amino acids).

RESULTS AND DISCUSSION

As shown in table 1, both I.C.P.V. and I.R.A. supply similar ileal apparent digestibility values for dry matter and nitrogen in the case of standard diet, and for nitrogen only in the case of wheat bran diet. The I.R.A. gives a much lower digestion coefficient of dry matter than does I.C.P.V. in the latter case. As for beet pulp diet, both dry matter and nitrogen digestibilities are much lower when estimated by I.R.A., whatever the number of daily meals. Therefore it

Table 1. Digestion coefficients of 3 different diets according to the technique used for ileal digesta collection (* P < 0.05).

Diet		Standard			Wheat Bran			Beet Pulp		
TECHNIQUE		I.R.A.		I.C.P.V.	I.R.A.		I.C.P.V.	I.R.A.		I.C.P.V.
INDIVIDUAL AMINO ACIDS	ASX	71.6	-	71.0	79.6	-	83.1	79.5	*	87.6
	THR	68.8	-	66.1	77.9	-	82.5	76.9	*	85.8
	SER	75.0	-	74.6	80.4	-	85.9	71.3	*	83.0
	GLX	81.5	-	81.7	88.2	-	91.5	85.8	*	91.8
	PRO	74.6	-	76.3	89.1	-	92.5	64.7	-	93.2
	GLY	66.6	-	63.8	68.2	-	78.0	48.9	*	77.0
	ALA	71.1	-	68.2	69.0	-	77.4	69.7	*	83.5
	VAL	72.7	-	70.5	82.8	-	87.6	81.9	*	89.0
	ILE	74.4	-	72.9	82.6	-	87.2	81.2	*	88.6
	LEU	76.9	-	75.8	85.5	-	89.1	88.4	*	93.1
	TYR	77.8	-	76.0	89.0	-	91.0	88.6	*	93.2
	PHE	77.5	-	76.1	86.8	-	89.8	89.6	-	93.5
	LYS	74.8	-	72.4	86.3	-	89.9	87.5	*	93.1
	HIS	79.1	-	77.9	88.8	-	92.2	86.7	*	92.5
	ARG	84.4	-	83.4	86.6	-	91.2	85.0	*	92.4
	CYS	74.3	-	74.6	66.0	-	76.6	52.2	*	71.0
	MET	83.3	-	84.4	87.7	-	90.9	92.6	*	95.0
Sum 17 AA		76.2	-	75.3	84.1	-	88.3	81.0	*	90.2
Total nitrogen		70.0	-	66.5	78.1	-	81.9	74.1	*	87.2
Dry Matter		59.8	-	62.8	62.2	*	76.4	67.7	*	81.3

appears that, depending on the diet, I.R.A. does not always give values similar to the I.C.P.V. ones.The digestion coefficients for the individual amino acids, do not differ according to the collection technique when pigs are fed the standard or the wheat bran diets. Though non significant, the deviation between I.C.P.V. and I.R.A. values is greater for wheat bran. In the case of beet pulp, I.R.A. technique provides significantly lower values in almost all cases. The amino acid digestibility pattern (hierarchy of the individual coefficients) is roughly the same, in all cases, even for beet pulp with wide significant differences between techniques. Moreover, the common pattern corresponds to that previously described on an average (DARCY, LAPLACE, DUEE, 1982).

Two mechanisms could account for the differences recorded between I.R.A. and I.C.P.V. A lack of complete dry matter recovery, with bran diet for example, might result in abnormally high coefficients in I.C.P.V. pigs. Such an hypothesis can not be discarded, though the bran and standard digesta had the same dry matter contents (12 per cent). As regards the beet pulp diet, which always results in more liquid digesta (8 per cent dry matter), the preserved ileocolic sphincter function could account for a real improvement of digestibility due to a relative slowing down of the passage of such fluid contents. Thus it should be concluded that I.R.A. may allow ileal digesta collection and consequent digestibility measurements. However further work is needed to completely assess the validity of the I.R.A. technique for routine tests, in various conditions.

REFERENCES

DARCY, B., LAPLACE, J.P. 1980. Ann. Zootech. 29 : 137-145.

DARCY, B., LAPLACE, J.P. & DUEE P.H., 1982. Ann. Zootech. 31 : 279-300.

DARCY B., LAPLACE, J.P. & VILLIERS, P.A. 1980. Ann. Zootech. 29: 147-177.

LAPLACE J.P., DARCY-VRILLON B. & PICARD, M. 1985. In "Journées Rech. Porcine en France". (Eds INRA-I.T.P.), vol. 17, Paris, p. 353-370.

VAN SOEST, P.J. 1963. J. Ass. Official Analyt. Chem. 46 : 829-835.

MEASUREMENT OF STARCH AND NON-STARCH POLYSACCHARIDES AND THEIR BREAKDOWN IN THE SMALL INTESTINE OF MAN

H.N. ENGLYST, J.H. CUMMINGS
MRC Dunn Clinical Nutrition Centre
Addenbrooke's Hospital, Trumpington Street,
Cambridge, CB2 1QE, U.K.

SUMMARY

An accurate method has been developed for the measurement of starch and non-starch polysaccharides (NSP) in food and digesta and has been used to study polysaccharide breakdown in the small bowel of man. NSP is totally recovered from the ileum and a starch fraction measured as resistant starch largely resists digestion in the small intestine.

INTRODUCTION

The physiological significance of a polysaccharide is dependent on the extent and site of its breakdown in the digestive system. Polysaccharides hydrolysed and absorbed in the small intestine are available for metabolism as monosaccharides. Polysaccharides which escape digestion in the small bowel are available for fermentation by bacteria in the large intestine. Products of fermentation, the volatile fatty acids, are absorbed and metabolised. Faecal bulk is increased by the stimulation of microbial growth, more rapid transit and in the case of non-digestible fibre by the physical presence and water holding capacity of the material.

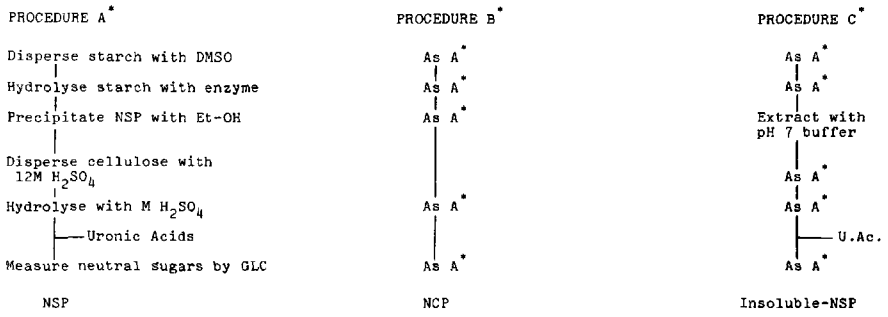
The fate of a particular polysaccharide in the digestive system is dependent on its chemical and physical properties. We have developed an analytical system for accurate measurement of plant carbohydrate (1-3) in the diet and in digesta and have applied it to a number of food products and the investigation of polysaccharide breakdown in man using ileostomy patients as a model.

METHOD

Procedure for measurement of total NSP and its main components

The technique measures total NSP and its components (fig.).

Analysis for Non-Starch Polysaccharides (NSP)



Calculation of NSP:

Total NSP	=	sum of sugars procedure A*
Cellulose	=	glucose procedure A* - glucose procedure B*
NCP	=	total NSP - cellulose
Insoluble NSP	=	sum of sugars procedure C*
Soluble NSP	=	sum of sugars procedure A* - sum of sugars procedure C*

Determination of total NSP: starch is dispersed with DMSO, hydrolysed with α-amylase and pullulanase and NSP precipitated with ethanol. The starch-free residue is then dispersed with 12M H_2SO_4 and hydrolysed with M H_2SO_4. Total NSP is calculated as the sum of released neutral sugars measured as alditol acetates by GLC and uronic acids measured colorimetrically.

Separation of total NSP into cellulose and NCP: if dispersion with 12M H_2SO_4 is omitted in the procedure for total NSP only NCP is hydrolysed when treated with M H_2SO_4. A value for cellulose can then be obtained as the difference between total NSP glucose and NCP glucose in the two procedures.

Separation into Soluble and Insoluble NSP: if the precipitation with ethanol in the procedure for total NSP is replaced by a 1 h extraction with buffer at pH 7 a value is obtained for insoluble NSP. A value for soluble NSP is then calculated as the difference between total NSP and insoluble NSP.

Measurement of Resistant Starch (RS): RS is not a part of NSP but if required RS can be measured as previously described (1) or as the difference in glucose obtained by procedure A when starch is dispersed with DMSO and when it is dispersed by acetate buffer.

Studies in man using ileostomy patients as a model

A series of test meals including various cereal products were given to subjects with an ileostomy following a 24 h period on a plant polysaccharide-free diet. Ileostomists have had their large intestine removed and the terminal ileum formed into a stoma on the anterior abdominal wall. Effluent was collected at 2 hourly intervals and analysed for its carbohydrate content. Transit time of the test meal was monitored by measurement of the NSP-xylose content of the effluent.

When analysing intestinal contents fucose and galactose originating from mucus are measured together with NSP from plant material. Fucose does not normally interfere since it is largely absent from plant foods, but a correction for endogenous galactose must be made and may be carried out using the fucose:galactose ratio measured on the plant polysaccharide-free diet.

RESULTS AND DISCUSSION

The Table shows values for NSP and RS in a number of food products. White flour contains 2.6 g NSP per 100 g of which 60% is measured as soluble NCP. Wheat bran contains 41.67 g of NSP per 100 g material of which only 8% is measured as soluble NCP. Since the extent of polysaccharide fermentation is linked to its solubility different physiological significances may be expected from NSP originating from wheat endosperm and wheat bran. Oats and barley are characterised by a high content of β-glucan, measured as soluble NCP-glucose. NSP content and composition is similar for the two potatoes but in the cooked and cooled potatoes some starch has retrograded to a fraction resistant to hydrolysis with α-amylase in vitro. This fraction has previously been described as resistant starch (RS) (1).

In a series of investigations (in press) using ileostomy patients as a model we have shown that NSP is virtually completely recovered from the small intestine of man. Resistant starch, to a large extent survives breakdown. These techniques are now being applied to carbohydrate digestion in healthy volunteers.

When the pattern of polysaccharide breakdown in the gut is established by in vivo investigations it may be possible from a detailed in vitro analysis to predict the fate and physiological

significance of food carbohydrates.

Non-Starch Polysaccharides (NSP) and Resistant Starch (RS) in some foods

Sample		Total*	Constituent Sugars*					
			Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic Acid
White Wheat Flour	Cellulose	0.16					0.16	
	Soluble NCP	1.58	0.55	0.67	0.05	0.16	0.15	-
	Insoluble NCP	0.86	0.27	0.43	0.02	-	0.14	-
	Total NSP	2.60	0.82	1.10	0.07	0.16	0.45	-
	Resistant Starch	0.19					0.19	
Wheat Bran, Allinsons	Cellulose	7.98					7.98	
	Soluble NCP	3.22	1.00	1.58	0.01	0.14	0.22	0.27
	Insoluble NCP	30.37	8.79	17.18	0.12	0.57	2.81	0.90
	Total NSP	41.57	9.79	18.76	0.13	0.71	11.01	1.17
	Resistant Starch	-					-	
Porage Oats, Scots	Cellulose	0.28					0.28	
	Soluble NCP	3.98	0.23	0.18	0.01	0.11	3.37	0.08
	Insoluble NCP	2.96	0.74	1.06	0.09	0.08	0.84	0.15
	Total NSP	7.22	0.97	1.24	0.10	0.19	4.49	0.23
	Resistant Starch	-					-	
Pearl Barley	Cellulose	1.44					1.44	
	Soluble NCP	3.89	0.39	0.39	0.06	0.07	2.94	0.04
	Insoluble NCP	6.50	1.77	3.03	0.20	0.06	1.33	0.11
	Total NSP	11.83	2.16	3.42	0.26	0.13	5.71	0.15
	Resistant Starch	-					-	
Whole Rye Flour	Cellulose	1.40					1.40	
	Soluble NCP	4.61	1.41	2.14	0.15	0.10	0.73	0.08
	Insoluble NCP	7.68	2.22	3.62	0.18	0.21	1.33	0.12
	Total NSP	13.69	3.63	5.76	0.33	0.31	3.46	0.20
	Resistant Starch	0.21					0.21	
Potatoes (raw)	Cellulose	1.77					1.77	
	Soluble NCP	2.63	0.26	0.01	t	1.25	0.09	1.02
	Insoluble NCP	0.61	0.08	0.09	0.06	0.35	-	0.03
	Total NSP	5.01	0.34	0.10	0.06	0.60	1.86	1.05
	Resistant Starch	-					-	
Potatoes (cooked and cooled)	Cellulose	1.63					1.63	
	Soluble NCP	2.52	0.23	0.01	0.03	1.19	0.19	0.87
	Insoluble NCP	0.57	0.08	0.08	0.06	0.32	-	0.03
	Total NSP	4.72	0.31	0.09	0.09	1.51	1.82	0.90
	Resistant Starch	2.08					2.08	

* g/100g dry matter.

ACKNOWLEDGEMENTS

This work has been supported by grants from the National Association of British and Irish Millers and by the Danish Medical, Technical, Agricultural and Veterinary Research Councils.

REFERENCES

1. ENGLYST, H., WIGGINS, H.S. & CUMMINGS, J.H. 1982. Analyst 107: 307-318.
2. ENGLYST, H.N. & CUMMINGS, J.H. 1984. Analyst 109: 937-942.
3. ENGLYST, H.N., ANDERSON, V. & CUMMINGS, J.H. 1983. J. Sci. Fd. Agric. 34: 1434-1440.

THE USE OF MULTIPLY CANNULATED PIGS TO EXAMINE THE EFFECT OF DIETARY FIBRE SUPPLEMENTS ON BILE ACID METABOLISM IN THE PORCINE HINDGUT

K. FADDEN[1,2], R.W. OWEN[1], E. LATYMER[3], A.G. LOW[3], M.J. HILL[1], A.N. MASON[2]

[1]PHLS, Centre for Applied Microbiology and Research, Bacterial Metabolism Research Laboratory, Porton Down, Salisbury, Wiltshire. SP4 0JG U.K. [2]Dept. Chemistry and Biochemistry, [3]Liverpool Polytechnic, Byrom St. Liverpool. L3 3AF U.K. [3]National Institute for Research in Dairying, Shinfield, Reading. RG2 9AT U.K.

SUMMARY

Using the multi-cannulated pig as a model for the human, this study was conducted to determine the effect of fibre on the metabolism of steroids along the intestine. Wheat bran, pectin and lactulose had little effect on steroid metabolism, and although dilution of faecal bile acids was observed, this effect was not statistically significant.

INTRODUCTION

A major obstacle to the study of the human intestinal ecosystem is its inaccessibility. Consequently, studies pertaining to the large intestine of man have been mediated via model systems. Conventional swine fitted with simple gut cannulas permits convenient access to the large bowel. This model has been used to examine the effect of fibre on factors which may influence steroid metabolism in the large intestine with regard to the aetiology of colorectal cancer (CRC) in man.

Evidence from population and case-control studies suggests that the incidence of CRC is related to an elevated faecal concentration of secondary bile acids and/or an increased ratio of lithocholic acid (LCA):deoxycholic acid (DCA) (1). Therefore, reduction of secondary bile acid concentration and/or repression of bacterial 7α-dehydroxylase in the intestinal lumen may be important prophylactically in terms of CRC. A negative correlation exists between high dietary fibre consumption and the incidence of CRC (2). It has been postulated that this 'protective' effect of fibre could be due to repression of 7α-dehydroxylation arising from increased

lumenal acidity after the bacterial fermentation of fibre (3). Alternatively, fibre may act as a bulking agent thereby reducing the concentration of secondary bile acids in digesta.

As the pig has a similar digestive physiology to man and will consume an omnivorous diet, a collaborative study was undertaken with the National Institute for Research in Dairying, Reading, to investigate the effect of fibre upon steroid metabolism, microbial flora, volatile fatty acid production, transit time and pH within the large intestine. This paper reports on the effect of fibre upon bile acid metabolism.

MATERIALS AND METHODS

Four Landrace x Large White boars were fitted with simple gut cannulas in the terminal ileum, caecum and mid-colon. Four dietary regimes were used, i) control diet (C), ii) control + 10% wheat bran (WB), iii) control + 5% pectin (P), and iv) control + 5% lactulose (L). Pigs were fed each of the four diets for a two week period in a Latin Square arrangement. Digesta samples plus faeces were collected on the last four days of each dietary regime. Four day collections were freeze-dried and pooled. 500mg of each pooled sample was extracted using the Soxhlet method and fractionated using DEAP-LH-20 column chromatography (4). Free bile acids were quantified by gas-liquid chromatography.

RESULTS AND DISCUSSION

Table 1. Mean percentage conversion of chenodeoxycholic acid (CDCA) to lithocholic acid (LCA).

Diet	Sampling Sites: Ileum	Caecum	Colon	Faeces
C	3	59	77	82
WB	5	50	80	84
P	1	42	76	80
L	1	46	77	74

Table 1 shows that irrespective of diet 7α-dehydroxylation was minimal in the ileum, but markedly increased in the caecum, and maximum conversion of CDCA to LCA was generally attained by the mid-

colon. Similar observations were reported by Hill (5) in colectomy and hemicolectomy patients. Analysis of variance (ANOVA) using a Latin Square design revealed that the fibre supplements did not significantly affect 7α-dehydroxylation at any of the three cannulation sites or faeces. However, the fibre supplements did not significantly increase lumenal acidity and inhibition of 7α-dehydroxylation would have been suprising.

Table 2. Mean total free bile acid (FBA) concentration (mg/g wet wt)

Diet	Sampling Sites: Ileum	Caecum	Colon	Faeces
C	1.18	0.42	0.79	0.88
WB	1.22	0.35	0.64	0.68
P	0.85	0.49	0.95	0.68
L	0.84	0.58	0.74	0.75

Table 2 shows that with the exception of P the FBA concentration was highest in the terminal ileum and lowest in the caecum, with similar concentrations occuring in the colon and faeces. Significant differences (ANOVA) in FBA concentration could not be detected between the control diet and the fibre supplemented diets. It is conceivable that the difference in fibre content between the control and test diets was insufficient to effect significant changes in steroid metabolism, steroid concentration or pH. Therefore, use of a lower residue control diet may be advantageous, but, then sample collection from cannulation sites becomes more difficult. Nevertheless, this study has shown that the multi-cannulated pig is an excellent model for studying steroid metabolism within the large intestine.

REFERENCES

OWEN, R.W., DODO, M., THOMPSON, M.H., HILL, M.J. 1984. Biochem. Soc. Trans. 12:861.

BURKITT, D.P. 1978. Am. J. Clin. Nutr. 31 (Suppl. 10):658.

FADDEN, K., OWEN, R.W., HILL, M.J., MASON, A.N. 1984. Biochem. Soc. Trans. 12:860.

OWEN, R.W., THOMPSON, M.H., HILL, M.J. 1984. J. Steroid Biochem. 21:593-600.

HILL, M.J. 1982. In Dietary Fiber in Health and Disease. Eds. Vahouny, G.V., Kritchevsky, D. Plenum Press. N.Y. and London.

THE EFFECT OF WHEAT BRAN, WHOLE CROP PEAS, AND BEET PULP ON THE DIGESTIBILITY OF DIETARY COMPONENTS IN A CEREAL-BASED PIG FEED

H. GRAHAM, K. HESSELMAN, P. ÅMAN*
Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences
S-750 07 Uppsala, Sweden

SUMMARY

Pigs cannulated at duodenum and terminal ileum were fed a basal cereal-based diet and three high fibre diets containing 66.6 % of the basal diet and 33.3 % of wheat bran, whole crop peas or sugar beet pulp. Chromic oxide was used as a marker and apparent duodenal-, ileal- and faecal digestibilities were determined for dry matter, crude protein, crude fat, starch, Klason lignin, and soluble-, insoluble- and total non-starch polysaccharides. The effects of different types of fibre on the digestibilities of dietary components in pig feed are discussed.

INTRODUCTION

In recent years there has been a growing interest in the role of fibre in pig nutrition. Two reasons for this are the development of new methods for fibre analysis and of advanced animal models. Wheat bran, whole crop peas and beet pulp are three different types of dietary fibres. In this investigation the effect of these fibres on the digestion of dietary components in a cereal-based diet was studied in pigs cannulated at duodenum and terminal ileum.

MATERIALS AND METHODS

The basal diet contained 26.6 % oats, 26.6 % barley, 26.6 % wheat, 6.0 % peas, 6.0 % soybeans, 5.0 % fish meal, 0.5 % calcium oxide, 1.4 % dicalcium phosphate, 0.3 % sodium chloride, and 1.0 % vitamin supplement. Three high fibre diets were prepared by mixing 66.6 % of the basal diet with 33.3 % of wheat bran, whole crop peas or sugar beet pulp.

Three pigs, all fitted with replaceable T-cannula (Björnhag and Jonsson, 1984) close to the ileo-caecal junction were used. Two of the pigs also had a second T-cannula in the duodenum just distal to the pancreatic and bile ducts. The pigs weighed approximately 45 kg

at the beginning of the experiment and were fed twice daily (8.00 and 16.00) with 4.0 % of liveweight per day of the basal and whole crop peas diets or 3.6 % of liveweight per day of the bran and sugar beet diets. Each pig was fed all experimental diets. Chromic oxide (5 g kg^{-1}) was included in the diets as a digestibility marker. After a seven day adaptation period, faeces (grab samples) were collected on days 8-10, ileal samples on day 11 and duodenal samples on day 13. Digesta was collected for a 12 hours period after the morning feeding. All samples were immediately frozen, freeze-dried and ground on a Wiley mill to pass a 1 mm screen.

Dry matter, ash, crude protein (Nx6.25), crude fat after acid hydrolysis, starch, non-starch polysaccharide (NSP) residues and Klason lignin were analysed according to our standard methods (Åman and Hesselman, 1984). Chromic oxide was determined by atomic absorption. Starch was degraded using the termamyl-amyloglucosidase system and the insoluble fraction isolated by centrifugation (Åman and Hesselman, 1984). The buffer-soluble polymers were precipitated with 80 % ethanol and isolated by centrifugation. The insoluble fraction was analysed for NSP residues and Klason lignin and the buffer-soluble polymers for NSP residues.

RESULTS AND DISCUSSION

The basal diet contained 15.1 % NSP (Table 1) and the composition of the sugar residues indicated that ß-glucans were the predominant soluble NSP and ß-glucans, arabinoxylans and cellulose the dominating insoluble NSP. The commercial wheat bran contained 36.3 % NSP with insoluble cellulose and arabinoxylans as chief constituents, and the whole crop peas 34.7 % NSP with insoluble cellulose, xylans and pectins as predominant constituents. The beet pulp contained more NSP (71.5 %) than the other products and a high content of uronic acid and arabinose residues showed that pectins were main constituents.

Most duodenal digestibilities were negative due to digestive secretions and possibly errors associated with the marker. The highest ileal- and faecal digestibilities of dry matter were obtained for the low fibre basal diet (Table 2). At the ileum the beet pulp had the lowest dry matter digestibility, reflecting the high NSP content, while in faeces the wheat bran diet had the lowest digestibility, indicating the resistance of wheat bran fibre

Table 1. Chemical composition of the cereal-based basal diet and the three high fibre products (% DM).

Constituent	Basal diet	Wheat bran	Whole crop peas	Beet pulp
Ash	6.1	6.0	6.0	3.4
Protein (Nx6.25)	17.7	14.7	18.5	11.4
Crude fat (HCl)	3.4	5.7	1.9	1.6
Starch	47.1	16.1	20.3	1.0
Soluble non-starch polysaccharides	3.3	2.6	2.5	5.6
Insoluble non-starch polysaccharides	11.8	33.7	32.2	65.9
Klason lignin	2.5	6.7	5.3	2.4

Table 2. Apparent ileal- and faecal digestibilities of dry matter (DM), crude protein (Nx6.25, CP), crude fat (HCl, CF), starch, soluble- (SNSP), insoluble- (INSP) and total (TNSP) non-starch polysaccharides and Klason lignin (KL).

Sample	DM	CP	CF	Starch	SNSP	INSP	TNSP	KL
Basal diet								
Ileal	65.3	68.6	64.2	94.9	30.3	18.6	21.1	14.7
Faecal	78.7	78.3	57.9	98.9	92.2	35.1	47.6	-21.0
Wheat bran diet								
Ileal	51.9	67.1	64.7	94.7	26.8	7.5	9.8	-44.9
Faecal	64.1	75.6	53.4	99.1	82.9	11.6	28.8	-82.1
Whole crop peas diet								
Ileal	62.3	72.9	65.3	92.9	40.2	27.8	29.9	28.7
Faecal	69.5	71.2	48.4	99.2	86.9	39.1	47.2	-18.1
Beet pulp diet								
Ileal	49.6	58.0	49.6	95.8	-87.2	54.1	38.0	20.1
Faecal	74.1	74.2	36.7	99.4	83.8	72.1	73.4	-64.2

Table 3. Apparent ileal- and faecal digestibilities of major non-starch polysaccharide residues.

Sample	Arabinose residues	Xylose residues	Galactose residues	Glucose residues	Uronic acid residues
Basal diet					
Ileal	20.3	10.4	-	27.4	13.9
Faecal	61.1	24.2	-	45.6	57.7
Wheat bran diet					
Ileal	7.6	-2.8	-	14.7	14.6
Faecal	30.6	26.9	-	12.3	28.4
Whole crop peas diet					
Ileal	19.2	15.3	-	36.1	16.8
Faecal	63.7	17.6	-	44.3	59.4
Beet pulp diet					
Ileal	45.9	21.7	33.3	35.8	42.4
Faecal	89.4	21.3	84.7	62.4	92.1

to microbial degradation. Added wheat bran had only a small influence on the ileal- and faecal digestibilities of crude protein and crude fat, while whole crop peas increased the ileal digestibility of crude protein, possibly reflecting the good quality of pea proteins. Beet pulp decreased the ileal digestibilities of both crude protein and crude fat. The low faecal digestibilities of crude fat for the whole crop pea and beet pulp diets are notable (Mason, 1983). Except for the whole crop pea diet, ileal digestibilities for starch were around 95 % in all diets. Less available starch in whole crop peas may explain the lower digestibility in this diet. Small amounts of starch were found in faeces.

Soluble- and insoluble NSP were partly digested before terminal ileum, indicating microbial activity in the upper gastro-intestinal tract (Table 2). The highly negative duodenal- and ileal digestibilities of soluble NSP and the high ileal digestibility of insoluble NSP in beet pulp clearly show a solubilization of insoluble NSP. The high content of soluble NSP in the small intestine of the pigs when fed this diet will have a great influence on the chemical and physical properties of the digesta (Fleming & Wasilewski, 1984). The faecal digestibilities were high for soluble NSP and variable for insoluble NSP. Except for the wheat bran diet, the ileal digestibilities of Klason lignin were significant. However, the negative faecal digestibilities of Klason lignin indicate a formation in the lower gut of compounds which will be determined as Klason lignin.

With the exception of the xylose residues in the wheat bran diet, all NSP residues were digested to a significant amount before terminal ileum (Table 3). A considerable fermentation of most NSP residues in the lower gut was also obvious. The pattern of digestion of NSP residues was indicative of the composition of the cell wall components in the feeds (Maillard & Chesson, 1984).

REFERENCES

BJÖRNHAG, G. & JONSSON, E. 1984. Livest. Prod. Sci. 11: 179-184

FLEMING, S.E. & WASILEWSKI, M.M. 1984. Nutr. Rep. Int. 30: 825-834.

MASON, V.C. 1983. Int. J. Vit. Nutr. Res., Suppl. 25: 27-38.

MAILLARD, P. & CHESSON, A. 1984. Br. J. Nutr. 52: 583-594.

ÅMAN, P. & HESSELMAN, K. 1984. Swedish J. Agric. Res. 14: 135-139.

THE EFFECT OF CRUDE FIBRE CONTENT ON THE DIGESTIBILITY AND THE RATE OF PASSAGE IN THE SMALL AND LARGE INTESTINE OF PIGS

L.A. DEN HARTOG[1], P.J. BOON[1], J. HUISMAN[2], P. VAN LEEUWEN[2] AND E.J. VAN WEERDEN[2]

1) Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM WAGENINGEN, The Netherlands
2) ILOB/IGMB, P.O. Box 15, 6700 AA WAGENINGEN, The Netherlands

SUMMARY

The effect of crude fibre content (diet 1, 5.2%; diet 2, 9.2% crude fibre) on the rate of passage (with Cr-EDTA as marker) and digestibility in the small and large intestine was investigated. Additional crude fibre in diet 2 originated from alfalfa and oat huskmeal. The apparent ileal and faecal digestibilities were higher for diet 1 than for diet 2. The rate of passage of the marker when diet 2 was fed (9.2% c.f.) was decreased in the small intestine and increased in the large intestine as compared to diet 1 (5.2% c.f.).

INTRODUCTION

The digestibility of nutrients depends on the composition of the diet. An increase in the content of crude fibre in the diet reduced nutrient digestibility (Kass et al., 1980; Muller and Kirchgessner, 1982). The rate of passage of digesta may also be dependent on diet composition. Fibre might increase the rate of passage (Murray, 1976). Because the digestibility may be related to the rate of passage an experiment was carried out to investigate the effect of crude fibre content on the ileal and faecal digestibility and rate of passage in the small and large intestine.

MATERIAL AND METHODS

The rate of passage of chromium in ileal digesta and nutrient digestibility. Six barrows (40 kg initial weight) were fitted with ileocecal re-entrant canulas. The pigs were fed two diets (diet 1 with 5.2% crude fibre (c.f.) diet 2 with 9.2% c.f., see Table 1) according to a change over design. The diets were milled on a 2.5 mm screen. The pigs were fed once daily, at 08.00 a.m., at a level, twice their maintenance requirement. The diets were fed to the pigs on the basis of the same supply of net energy and digestible protein. As a result the pigs fed diet 2 received 15% more diet. Each rate of passage determination (24 hours a day) at the terminal ileum (Cr.EDTA as marker) was repeated on 3 consecutive days for each pig and for each diet. Ileal digesta were determined by analysis of ileal digesta collected quantitatively during 4 other days (24 hours/day).

The rate of passage of chromium in the large intestine.

Eight barrows (40 kg initial weight) were fitted with a single T-canula approximately 10 cm before the ileo-caecal sphincter. Test diets, feeding time and feeding regimen were identical to those used during the ileal determinations.
In order to determine the rate of passage in the large intestine two markers, Cr-EDTA (liquid phase) and $BaSO_4$ (solid phase) were infused into the distal ileum. The markers were infused at 4 hours after feeding, the time that passage of ileal digesta was at a maximum. Faeces were collected during 72 hours. Every 3 hours during the period from 08.00 a.m. to 23.00 p.m. and after 9 hours during the period from 23.00 p.m. to 08.00 a.m.. Each rate of passage determined (72 hours) was repeated 6 times for each pig.

Faecal digestibility of nutrients.

Four non-canulated pigs were fed the same test diets according to the same feeding regimen and feeding time as was previously described. Faecal digestibility coefficients were determined by analysis of faeces collected quantitatively during 5 days.

RESULTS AND DISCUSSION

The apparent faecal digestibilities of dry matter, organic matter, crude protein, crude fat, crude fibre and NFE were higher ($P < 0.05$) for the diet low in fibre (Table 2). With the exception of crude fat and crude fibre, the apparent ileal digestibilities of the same parameters were also higher ($P < 0.05$) for the low fibre diet (Table 2). For both diets, except for crude fat, of which there was a net appearance in the large intestine, there was a significant ($P < 0.05$) disappearance of the nutrients that were measured. The higher net appearance of crude fat for the high fibre diet can be explained by the increased fermentation in the hindgut. Crude fibre was mainly digested in the large intestine.
Figures 1 and 2 present the rate of passage of organic matter at the distal ileum for each hour, and the cumulative Cr excretion as a percentage of the intake. From these results it can be concluded that the pattern of passage of organic matter was similar for both diets while the high fibre diet had a lower passage rate. The retention time of the marker, based on 85% recovery, was 6.3 and 7.1 h ($P < 0.05$) for the low and high fibre diet, respectively.
Figure 3 presents the average Cr excretion in the faeces. The excretion curves were identical for the solid and liquid phasemarkers. The peak of excretion of the markers were found to be 24 and 20 h after infusion for the low and high fibre diet, respectively. Following infusion, up to 35h after, the cumulative excretion of the markers was higher for the high fibre diet ($P < 0.05$).

Figure 1. Cumulative passage of chromium (% of intake)

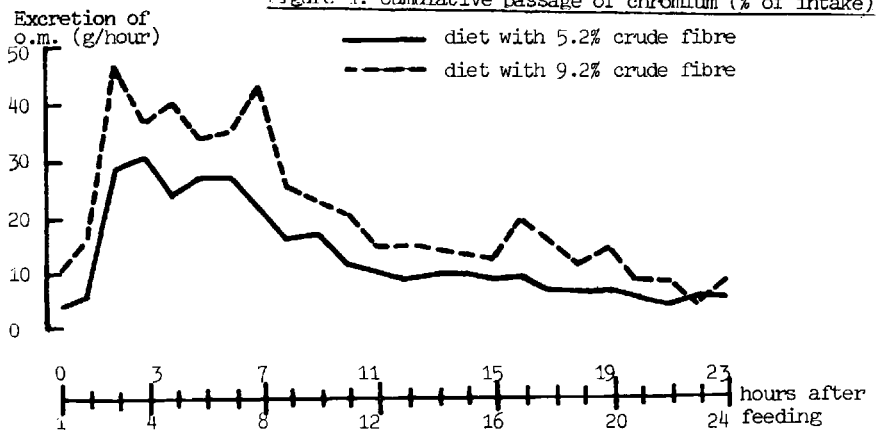


Figure 2. Rate of passage of organic matter at the distal ileum

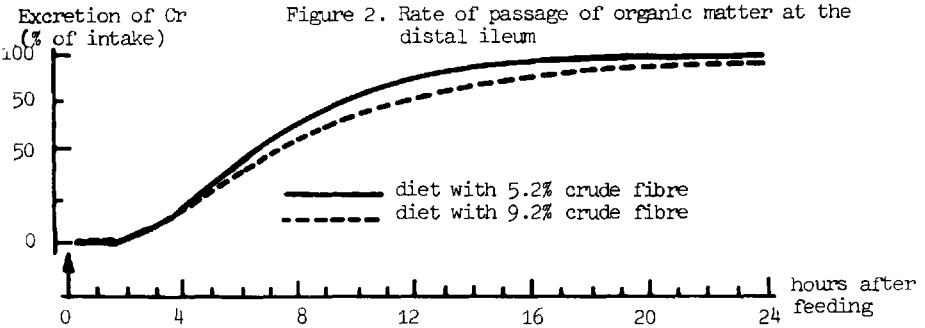


Figure 3. Excretion of chromium with faeces

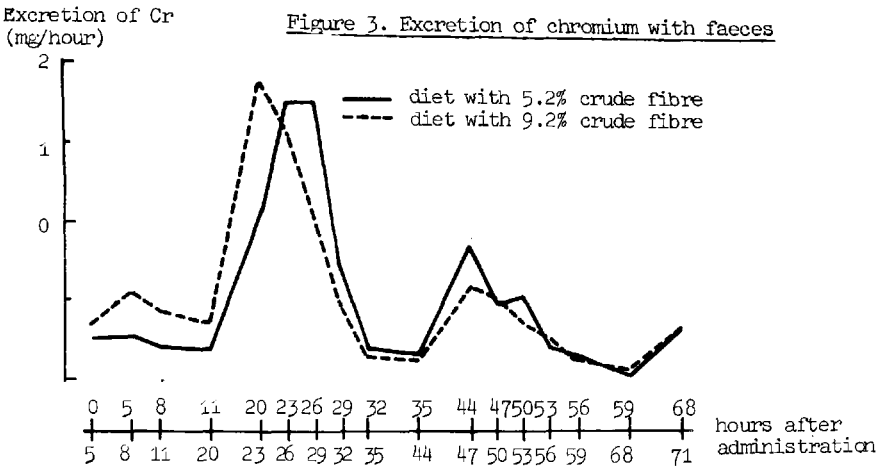


Table 1 Formulation and analysis of the diets (in % of product)

	diet 1	diet 2
Cereal grains (maize, wheat, barley and oat)	71.4	57.1
Soybean meal solvent extracted	18.0	14.4
Citrus pulp	2.0	1.6
Pollards	1.3	1.0
Oat huskmeal	1.0	10.5
Alfalfa meal	2.5	11.7
Vitamins, minerals, lysine, methionine	3.8	3.7
Analysis: crude protein	17.7	16.4
crude fibre	5.2	9.2
ADF	5.7	10.2
NDF	13.2	20.5
Lignine	1.0	1.1
Net energy (MJ/kg)(calculated)	9.2	8.1

Table 2. Digestibility coefficients

	Ileum		Faeces	
	5.2% c.f. diet	9.2% c.f. diet	5.2% c.f. diet	9.2% c.f. diet
Dry matter	$67.4^{a} \pm 1.3$	$58.6^{b} \pm 2.5$	$83.7^{a} \pm 0.1$	$74.7^{b} \pm 1.2$
Organic matter	$70.7^{a} \pm 1.1$	$62.0^{b} \pm 2.4$	$85.9^{a} \pm 0.2$	$76.5^{b} \pm 1.2$
Crude protein	$72.9^{a} \pm 3.1$	$68.3^{b} \pm 2.4$	$85.9^{a} \pm 0.9$	$78.5^{b} \pm 2.4$
Crude fat	61.6 ± 7.0	58.8 ± 6.6	$60.4^{a} \pm 1.9$	$49.6^{b} \pm 6.7$
Crude fibre	10.5 ± 4.1	5.2 ± 6.4	$41.6^{a} \pm 1.1$	$28.2^{b} \pm 3.5$
Nitrogen Free extract	$75.8^{a} \pm 0.8$	$69.8^{b} \pm 2.1$	$91.3^{a} \pm 0.1$	$85.3^{b} \pm 0.5$

Data with a different superscript within ileal or faecal data differ significantly ($P < 0.05$)

REFERENCES

KASS, M.L., SOEST, P.J. VAN & POND, W.G., 1980. J.Anim.Sci. 50: 175-191

MULLER, H.L. & KIRCHGESSNER, M., 1982. 9th Symposium on Energy Metabolism, Lillehammer

MURRAY, A.G., 1976. M.Sc.Thesis. University of Aberdeen, Faculty of Sciencen U.K.

THE EFFECT OF VARIOUS CARBOHYDRATE SOURCES ON THE DIGESTIBILITY OF MINERALS IN THE SMALL AND LARGE INTESTINE OF PIGS.

L.A. DEN HARTOG[1], J. HUISMAN[2], H. BOER[1] and G.H.A. VAN SCHAIJK[1]
[1] Department of Animal Nutrition, Agricultural University
Haagsteeg 4, 6708 PM Wageningen, The Netherlands.
[2] ILOB, IGMB, Postbox 15, 6700 AA Wageningen, The Netherlands.

SUMMARY

The effect of inclusion of different carbohydrate sources in pig diets (pectin, cellulose or strawmeal) on the apparent digestibility of Na, K, Mg, Ca and P was studied at the distal ileum and over the whole digestive tract. The amount of Na that passed the distal ileum was 3.6 to 4.2 times higher than the Na intake. The highest ileal secretion was found when the diet with pectin was fed. The Ca excretion was increased ($p < 0.05$) when the cellulose diet was fed. There was a tendency that when the pectin diet was fed the apparent ileal absorption of K, Mg, Ca and P was slightly decreased. Determined over the whole digestive tract there were no tendencies.

INTRODUCTION

Pig diets in the Netherlands usually contain many byproducts with a high amount of crude fibre. In humans, it was found that an increase in fibre content resulted in a reduction of mineral availability (Van Dokkum, 1984). Partridge (1978) observed a reduced apparent digestibility of Ca, P, Mg and K when high cellulose diets were fed. Therefore an experiment was carried out to study the effect of different carbohydrate sources (pectin, cellulose and straw meal) on the digestibility of minerals in the small and large intestine of pigs.

MATERIAL AND METHODS

Ten crossbred barrows with average initial weight of 40 kg fitted with ileocecal re-entrant canules were used. The experiment comprised four treatments, tested in a cross over design:

1 100% control diet (see tables 1 and 2)
2 95% control diet + 5% pectin
3 95% control diet + 5% cellulose
4 95% control diet + 5% straw meal*

* milled through 1 mm mesh size.

Table 1. **Formulation (%) of the control diet.**

barley	36.9
maize	45.7
soybean meal, solvent extracted	4.4
meat meal tankage	11.0
$CaCO_3$	0.1
NaCL	0.3
mineral/vitamin premix	1.6

The diet contained 20 ppm virginiamycine and 125 ppm Cu.

Table 2. **Chemical analysis (%) of the control diet and the carbohydrate sources.**

	Control diet	Pectin	Cellulose	Strawmeal
dry matter	88.8	91.5	92.4	93.4
ash	5.5	1.4	0.2	9.4
organic matter	83.3	90.0	92.2	84.0
crude protein	17.2	2.2	0.6	3.3
crude fat	4.2	0.1	0.15	0.8
crude fibre	3.1	0.4	57.3	41.0
NFE	58.9	87.4	34.2	38.9
NDF	11.6	0.15	92.2	75.7
ADF	4.2	0.03	86.7	47.4
lignin	0.5	0.0	0.3	7.8
pectin	1.1	56.0	--	--
Na	0.18	0.16	0.29	0.06
K	0.66	0.29	0.29	1.20
Mg	0.17	0.05	0.16	0.90
Ca	0.83	0.22	0.08	0.03
P	0.75	0.07	--	0.14

Table 3. Apparent ileal and faecal digestibility coefficients* of some minerals.

	Ileal				Faecal			
Minerals	control	pectin	cellulose	strawmeal	control	pectin	cellulose	strawmeal
Na	-361.6 ± 45.5	-422.4 ± 62.6	-356.9 ± 32.3	-356.4 ± 42.2	74.9 ± 5.4	70.5 ± 6.2	68.8 ± 3.4	71.3 ± 4.8
K	66.3 ± 11.1	55.9 ± 11.6	65.7 ± 5.6	67.6 ± 12.0	76.6 ± 5.1	79.1 ± 3.0	78.6 ± 2.7	77.9 ± 3.3
Mg	23.5 ± 9.8	18.0 ± 4.1	18.3 ± 10.4	21.4 ± 3.9	36.8 ± 6.0	42.2 ± 5.1	41.5 ± 4.7	42.3 ± 4.9
Ca	33.1 ± 9.0	26.4 ± 3.7	27.5 ± 3.5	33.0 ± 3.2	a 43.0 ± 5.1	ab 39.3 ± 4.5	a 34.7 ± 4.0	ab 38.5 ± 5.0
P	27.9 ± 7.3	25.0 ± 8.0	28.1 ± 6.0	25.5 ± 7.3	41.7 ± 3.4	46.0 ± 3.0	42.1 ± 2.7	44.1 ± 3.9

*Means and standard deviation.

Data with a different superscript within ileal digesta and faeces in the same sow differ significantly ($p < 0.05$).

After surgery, the pigs were allowed to recover during three weeks. Thereafter, a ten days period for adaptation to the test diets followed. Faeces were collected quantitatively during five days and ileal digesta quantitatively during four days (24 hours/day) following the adaptation period. After these collection periods the pigs were changed over to another treatment followed by and adaptation period of 14 days. A similar collection procedure followed as was described previously. The pigs were housed individually in balance cages, and were fed twice daily (wet feed, ratio feed : water = 1 : 2.5). The feeding level was 2.4 x net energy required for maintenance (293 kJ/$G^{3/4}$).

RESULTS AND DISCUSSION

The apparent digestibility coefficients of the minerals Na, K, Mg, Ca and P determined at the distal ileum and over the whole digestive tract are presented in table 3. These coefficients are calculated from the difference between the mineral intake and excretion at the distal ileum or in faeces, respectively, and expressed as a percentage of intake. The amount of Na that passed the distal ileum was 3.6 to 4.2 higher than the Na intake. Partridge (1978) also found that a large amount of Na passed at the distal ileum. The largest excretion was found when a high cellulose diet was fed. In the present study there was no effect of cellulose on Na excretion. Pectin decreased the ileal digestibility coefficient of Na.

Inclusion of 5% cellulose significantly decreased ($P < 0.05$) the apparent Ca digestibility determined over the whole digestive tract. When the pectin diet was fed there was a tendency that the digestibility coefficients of K, Mg, Ca and P determined at the distal ileum were slightly decreased. However, determined over the whole digestive tract there were no tendencies. The amount of ileal digesta per kg feed intake that passed at the distal ileum was 2.87, 2.94, 3.00 and 3.19 kg for the control, pectin, cellulose and straw meal diet respectively ($p > 0.05$).

REFERENCES

DOKKUM, W. VAN 1984, Doctoral thesis. University of Amsterdam, 137 pp.
PARTRIDGE, I.G. 1978. Br. J. Nutr. 39 : 539-545.

We like to thank the LEB Funding Agency (LEB-fonds) for financial support towards these studies.

THE EFFECT OF VARIOUS CARBOHYDRATE SOURCES ON THE ILEAL AND FAECAL DIGESTIBILITY OF PROTEIN AND AMINO ACIDS IN PIGS

J. HUISMAN[1], L.A. DEN HARTOG[2], H. BOER[2], E.J. VAN WEERDEN[1] AND W.J.G. THIELEN[2]
1) ILOB/IGMB, Institute for Animal Nutrition Research - TNO
P.O. Box 15, 6700 AA WAGENINGEN, The Netherlands
2) Department of Animal Nutrition; Agricultural University
Haagsteeg 4, 6708 PM WAGENINGEN, The Netherlands

SUMMARY

The effect of inclusion of different carbohydrate sources (pectin, cellulose or straw meal) in pig diets on the ileal and faecal digestibility of protein and amino acids was determined. The inclusion of 5% pectin or 5% cellulose did not significantly effect the ileal and faecal digestibility ($P > 0.05$). The inclusion of 5% straw meal significantly decreased ($P < 0.05$) the ileal digestibility of protein and nine of the amino acids. With the exception of glycine, the faecal digestibility of protein and amino acids was not significantly ($P > 0.05$) effected by the inclusion of 5% straw meal.

INTRODUCTION

In The Netherlands formulation of pig diets is based on the supply of faecal digestible amino acids from 1984 onwards. Amino acid digestibilities determined at the distal ileum are a better measure than the faecal digestibility. Amino acids disappearing in the hind gut do not contribute to protein synthesis in pigs (Zebrowska, 1975). Therefore studies were carried out to investigate the ileal and faecal digestibilities of protein and amino acids of different feedstuffs and/or complete diets. Milling-byproducts form a substantial part of pig diets in The Netherlands. In general these products are rich in crude fibre. The crude fibre content of the diet will rise when these products are included in the pig feed. Diet composition and especially crude fibre content can influence the apparent ileal and faecal digestibility of amino acids (Sauer et al., 1980). In the present study the effect of inclusion of different carbohydrate sources (pectin, cellulose and straw meal)in the diet on the ileal and faecal digestibility of protein and amino acids was investigated.

METERIALS AND METHODS

Ten crossbred barrows with average initial weight of 40 kg fitted with ileo-caecal re-entrant cannulae were used.

The experiment comprised four treatments, tested in a cross-over design.

1 100% control diet (see tables 1 and 2)
2 95% control diet + 5% pectin
3 95% control diet + 5% cellulose
4 95% control diet + 5% straw meal*

* milled through 1 mm mesh size

After surgery, the pigs were allowed to recover during three weeks. Thereafter, a ten days' period for adaptation to the test-diets followed. Faeces were collected quantitatively during five days, ileal digesta quantitatively during four days (24 hours/day) following the adaptation period. After these collection periods the pigs were changed-over to another treatment, followed by an adaptation period of 14 days. A similar collection procedure followed, as was described, previously. The pigs were housed individually in balance cages and were fed twice daily (wet feed, ratio feed : water = 1 : 2.5). The feeding level was 2.4 net energy required for maintenance (293 kJ/$g^{\frac{3}{4}}$).

RESULTS AND DISCUSSION

The ileal and faecal digestibility coefficients of organic matter, crude protein and amino acids are shown in Table 3. The following results were obtained

Organic matter: The ileal digestibility was significantly reduced in the cellulose diet. The faecal digestibility was significantly reduced in the cellulose and straw meal diet.

Crude protein: The ileal and faecal digestibility coefficients in the diets containing pectin, cellulose and straw meal, were lower than in the control diet. However, the difference was only significant for the straw meal diet.

Amino acids: The ileal digestibility of the diets containing pectin and cellulose differed slightly from the control diet. All amino acids, with the exception of arginine, were of lower digestibility in the straw meal diet than in the control diet. The differences were significant for the following amino acids: isoleucine, lysine, phenylalanine, threonine, valine, tyrosine, alanine, aspartic acid and glutamic acid.

The faecal digestibilities of all amino acids of the three test diets were lower

than of the control diet. However, only for glycine in the straw meal diet was the difference significant.

Table 1. Formulation (%) of the control diet

Barley	36.9
Maize	45.7
Soybean meal, solvent extracted	4.4
Meat meal tankage	11.0
$CaCO_3$	0.1
NaCl	0.3
Mineral/vitamin premix	1.6

The diet contained 20 ppm virginiamycine and 125 ppm Cu.

Table 2. Chemical analysis (%) of the control diet and the carbohydrate sources

	Control diet	Pectin	Cellulose	Straw meal
Dry matter	88.8	91.5	92.4	93.4
Ash	5.5	1.4	0.2	9.4
Organic matter	83.3	90.0	92.2	84.0
Crude protein	17.2	2.2	0.6	3.3
Crude fat	4.2	0.1	0.15	0.8
Crude fibre	3.1	0.4	57.3	41.0
NFE	58.9	87.4	34.2	38.9
NDF	11.6	0.15	92.2	75.7
ADF	4.2	0.03	86.7	47.4
Lignin	0.5	0.0	0.3	7.8
Pectin	1.1	56.0	-	-

Table 3. The apparent ileal and faecal digestibility of organic matter, protein and amino acids (%)

	ileal				faecal			
Diets	Control diet	Pectin diet	Cellulose diet	Straw diet	Control diet	Pectin diet	Cellulose diet	Straw diet
Organic matter	74.8^{a}	72.8^{ab}	70.1^{b}	72.8^{ab}	87.5^{d}	87.3^{d}	81.2^{e}	83.7^{e}
Protein	71.4^{a}	69.8^{ab}	69.5^{ab}	67.3^{b}	84.7	82.8	81.1	82.5
Arginine	81.2	82.9	82.8	82.0	89.4	88.2	87.9	88.0
Histidine	75.1	71.6	74.6	71.4	87.4	85.4	85.8	85.5
Isoleucine	74.9^{a}	73.8^{a}	74.6^{a}	70.4^{b}	83.0	80.7	80.0	80.3
Leucine	78.9	77.8	78.7	76.0	87.0	85.0	85.0	85.1
Lysine	73.4^{a}	71.4^{a}	73.0^{a}	68.3^{b}	83.2	80.8	80.1	80.1
Methionine	80.8	79.5	80.2	77.3	85.1	82.2	81.6	82.1
Phenylalanine	80.5^{a}	78.3^{ab}	80.1^{a}	76.7^{b}	86.6	84.8	84.7	85.0
Threonine	66.2^{a}	64.2^{ab}	64.1^{ab}	62.0^{b}	82.4	80.0	79.2	79.7
Tryptophan	66.5	65.0	65.5	63.2	85.9	83.3	84.0	83.5
Valine	73.3^{a}	71.9^{ab}	72.7^{a}	69.7^{b}	84.3	81.9	81.6	81.7
Alanine	76.6^{a}	75.3^{ab}	75.8^{a}	72.6^{b}	87.0	85.1	84.7	84.6
Aspartic acid	64.2^{a}	62.0^{ab}	62.1^{ab}	58.9^{b}	83.8	81.7	80.9	80.6
Cystine	63.7	58.0	56.4	56.2	78.9^{ab}	78.6^{ab}	75.0^{a}	79.5
Glutamic acid	81.2^{a}	79.9^{ab}	80.5^{ab}	78.2^{b}	90.2	88.8	88.5	88.5
Glycine	70.9	68.0	68.7	68.1	89.3^{a}	87.9^{ab}	87.5^{ab}	87.0
Proline	77.5	74.4	76.7	76.0	90.4	88.8	89.4	90.1
Serine	72.0	70.4	70.4	69.1	86.1	84.5	83.9	84.0
Tyrosine	71.1^{a}	70.4^{a}	68.6^{a}	65.4^{b}	83.2	80.1	80.5	80.8

Data with different superscript between treatments within ileum or faecal coefficients are significantly different ($P < 0.05$).

REFERENCES

SAUER, W.C., JUST, A., JØRGENSON, H.H., FEKADU, M. & EGGUM, B.O. 1980. Acta Agric Scand. 30: 449-458.

ZEBROWSKA, T. 1975. Rocz. Nauk. rol. B-97-1, 117-123.

THE INFLUENCE OF DIFFERENTLY TREATED STRAW ON THE ILEAL AND FAECAL DIGESTIBILITY OF NUTRIENTS AND THE UTILIZATION OF DIGESTED PROTEIN AND ENERGY

A, JUST , H. JØRGENSEN AND J. A. FERNÁNDEZ
National Institute of Animal Science
Rolighedsvej 25, 1958 Copenhagen V, Denmark

SUMMARY

Inclusion of 15% ground barley straw in balanced diets to growing pigs depressed the ileal and faecal digestibility of all nutrients except for the ileal digestibility of linoleic acid, which was improved. Untreated straw had the lowest and NaOH-treated straw the highest digestibilities. Addition of straw increased the concentration of VFA and lactic acid in faeces expressed per kg dry matter intake. The utilization of digested crude protein was increased by addition of straw to the diets in the order untreated , NH_3-treated and NaOH-treated straw, whereas the utilization of energy was decreased in the same order.

INTRODUCTION

Numerous investigations have shown that diet composition influence the ileal and faecal digestibility as well as the proportion of dietary nutrients disappearing from the caecum-colon (Sauer et al., 1980; Zebrowska, 1982, Just, 1983). In addition Just et al. (1981) showed the value of nitrogenous substances absorbed from the caecum-colon for protein synthesis was almost nil, and Just et al. (1983) found the value of energy disappearing from the caecum-colon to be only half of that of energy absorbed from the small intestine. The purpose of the present investigation was to elucidate in more detail the influence of differently treated barley straw on the ileal and faecal digestibility of dietary nutrients and its influence on protein and energy utilization.

MATERIALS AND METHODS

The diets were composed of barley, wheat, soya bean meal, meat and bone meal, and 15% of either untreated, NH_3-treated or NaOH-

treated barley straw and added minerals and vitamins according to the Danish standards. The chemical composition of dietary dry matter is given in Table 1.

Table 1. Chemical composition of diet dry matter.

Diets [1]	Basal diet	Diets indcluding 15% straw:		
		Untreated	NH_3-treated	NaOH-treated
Crude protein	20.2	19.2	19.6	19.0
Crude fat	3.2	3.1	3.1	3.1
Crude fibre	4.6	11.0	10.9	10.1
NFE substances	65.7	60.5	60.2	60.7
Soluble carbohydrate	53.4	44.9	44.5	45.3

[1] All diets were added 0.5% chromic oxide as marker.

Simple T-cannulae were inserted at the terminal ileum 3-5 cm anterior to the ileo-caecal valve (visual) in 16 female pigs (4 litters each of 4 pigs) at about 40 kg live weight. Three digestibility experiments were performed with each pig during the growth period from 50-80 kg. Each experiment consisted of a five day preparation period and a seven day collection period. In the initial four days of the collection period faeces were collected quantitatively twice daily and ileal digesta was collected from 7 to 9 a.m. and from 11 a.m. to 1 p.m., from 9 to 11 a.m. and from 1 to 3 p.m., from 8 to 10 a.m. and from 12 a.m. to 2 p.m. on day 5, 6 and 7, respectively. Urine was collected by using balloon catheters. Daily feed intake amounted to approximately 90% of the Danish standard and the pigs were fed identical meals three times daily exactly 8 hours apart.

At the same time a combined balance-slaughter experiment comprising 60 pigs was performed with the same diets to study the utilization of apparent digestible crude protein, energy and minerals. An identical factorial design was used for the digestibility experiments with cannulated pigs and for the combined N-balance-slaughter investigations. The design and most results of the balance-slaughter investigation as well as more details of the digestibility experiments with cannulated pigs will be published elsewhere.

RESULTS AND DISCUSSION

The ileal and faecal digestibilities of some nutrients and the utilization of digested crude protein and energy are given in Table 2

Table 2. The influence of differently treated barley straw on the ileal (I) and faecal (F) nutrient digestibility and the utilization of digested crude protein and energy.

Diets	Basal diet		Diets including 15% straw: Untreated		NH_3-treated		NaOH-treated		Significance of straw	
	I	F	I	F	I	F	I	F	I	F
Crude protein	78	84	71	73	76	75	74	74	*	***
Lysine	87	84	81	76	86	77	84	74	*	***
Threonine	76	81	68	73	74	74	73	71	NS	***
Crude fat	59	61	51	53	58	59	59	57	NS	**
Stearic acid	69	-184	48	-32	67	-44	73	-65	*	***
Linoleic acid	75	97	83	95	80	96	78	96	**	***
Crude fibre	-10	39	6	12	1	18	- 9	24	*	***
NFE substances	73	92	70	83	68	84	66	85	***	***
Soluble carbohydr.	91	100	93	99	94	100	91	99	NS	***
Energy	68	84	61	69	61	71	58	72	***	***
	Concentration of VFA and lactic acid, mmol/kg diet dry matter intake									
VFA	60	83	63	177	39	174	35	158	NS	***
Lactic acid	72	55	125	119	129	365	152	240	*	***
	Deposited percent of digested									
Crude protein		40		43		42		45		*
Energy		31		29		29		27		*

Addition of 15% straw to the diets generally depressed the ileal and faecal digestibility, but in contrast to the results of Just (1983) and Just et al. (1983) the proportion of the nutrients disappearing from the caecum-colon was not increased but rather decreased especially by untreated straw. Among the diets including straw, untreated straw gave the lowest and NaOH-treated straw the highest digestibilities. Inclusion of untreated straw in the diet depressed the ileal digestibility of stearic acid, but the faecal digestibility was although negative improved by adding all three kinds of straw. The ileal digestibility of linoleic acid was improved by all kinds of straw, but the faecal digestibility was slightly depressed by untreated straw.

Untreated straw increased the concentration of VFA expressed per kg diet dry matter intake in ileal digesta and all kinds of straw increased the concentration of lactic acid in ileal digesta and in faeces. Thus, the results indicate that addition of straw to diets increases the rate of fermentation/microbial activity in the digestive tract.

The addition of straw to the diets apparently improved the utilization of digested crude protein, but the utilization of digested energy was depressed, which is in accordance with Just (1983) and Just et al. (1983). The negative influence of straw in the diets decreased in the order: untreated, NH_3-treated and NaOH-treated. The collective influence of untreated, NH_3-treated and NaOH-treated straw on the digestibility and the utilization were for crude protein -6.6%, -6.3% and -0.9% and for energy -23%, -21% and -25%, respectively.

REFERENCES

JUST, A. 1983. 4th Int. Symp. Protein Metabolism and Nutrition, Clermont-Ferrand. Les Colloques de l'INRA, 16. EAAP Publ. 31: 289-309.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A. 1981. Brit. J.Nutr. 46: 209-219.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A. 1983. Livest. Prod. Sci. 10:171-186.

SAUER, W. C., JUST, A., JØRGENSEN, H., FEKADU, M. & EGGUM, B. O. 1980. Acta Agric. Scand. 30:449-459.

ZEBROWSKA, T. 1982. In: Digestive Physiology in the pig. (Eds. J. P. Laplace, T. Corring, A. Rerat). Les Colloques de l'INRA. 12:225-236.

THE EFFECT OF DIETARY FIBRE ON THE RATE OF PASSAGE THROUGH DIFFERENT SECTIONS OF THE GUT IN PIGS

EVA A. LATYMER[a], A.G. LOW[a] & SUSAN C. WOODLEY[b]
National Institute for Research in Dairying
Shinfield, Reading RG2 9AT, England

SUMMARY

The aim of the work was to measure the effect of dietary additions of guar gum, cellulose (Solka Floc), bran, lactulose and pectin on transit time of digesta to the ileum, caecum, colon and rectum of cannulated pigs in vivo. The liquid phase tended to move faster than the solid phase of digesta in the small intestine, except when pectin (50g/kg diet) or guar gum (60g/kg) were added to the diet. The phase separation tended to decrease as the volume of gut contents reduced during transit through the colon. Both phases arrived more or less together in faeces.

INTRODUCTION

The nutritive value of diets rich in dietary fibre for pig production is variable and difficult to predict. The age of the pig, the source and the physical and chemical nature of the fibre and the level of feeding all affect this value. Dietary fibre often influences the overall transit time of the diet and it has been suggested that this is a physiologically important measure of its effect, both as a nutrient source in pig production and in clinical nutrition. We have found the pig to be a suitable and convenient model for detailed study of the effect of different dietary fibres in relation to human nutrition and in particular, their possible role in the aetiology of diverticulitis, colonic cancer, diabetes and ischaemic heart disease. It is known that inclusion of different levels or qualities of dietary fibre in the diet will result, apart from other effects, in changes in the pattern and the time available for metabolic and absorptive processes along the digestive tract, which deserve more detailed identification.

From 1 April 1985: [a]Animal and Grassland Research Institute, Shinfield, Reading, England, [b]Food Research Institute, Shinfield, Reading, England.

Cannulated pigs allowed frequent sampling along the intestine during tests on a series of diets over a long period under "normal" conditions *in vivo*, while the animals still remained in good health and grew normally. The gastrointestinal cannulated pig, as a model system, has been previously used by Fadden et al. (1984) who showed that the function of the anaerobic flora of the large intestine was unaffected by the presence of several cannulas. Also, Close et al. (1984) showed that the presence of a cannula in the caecum of similar construction to that used in the present experiments, did not significantly affect the overall energy metabolism of pigs.

MATERIALS AND METHODS

For all experiments Large White x Landrace boars of initial live weight around 25kg were surgically fitted with simple cannulas in the terminal ileum, caecum and ascending colon (ca. mid-length of colon). A removable plug which closely fitted into the barrel was used to keep each cannula free of gut contents and to minimise the possibility of the outside environment entering the gut in case the screw cap was not completely airtight. Before any measurements on the rate of passage were carried out, the animals were left to recover from surgery for 14 days.

The pigs were fed twice a day on a basal diet of barley and soya, with a daily allowance (d.a.) of 45g/kg body weight, or the basal diet substituted with either purified cellulose (Solka Floc; 150g/kg) in a preliminary test on 3 pigs (trial I), or guar gum (20, 40 or 60g/kg) during a second test (incomplete latin square design) on the same 3 pigs (trial II). In a further exploratory test on 2 pigs (trial III) and in an experiment on 4 pigs (using a latin square design) (trial IV) the basal diet was fed at 35g/kg body weight. In trials III and IV the basal diet was supplemented with bran (100g/kg) with a d.a. of 39g/kg body weight and lactulose or pectin (50g/kg) with a d.a. of 37g/kg body weight. The diets were fed wet in all trials, using 2.5l water/kg diet.

The transit time measurements started 5d after the change of diets. The solid phase of digesta was marked with ^{103}Ruthenium Phenanthroline and the liquids with ^{51}Chromium complexed to EDTA

according to Faichney (1975). During trials I and II the markers were mixed into homogenized intestinal contents and injected through the cannula into the terminal ileum. In trials III and IV the markers were mixed into the morning feed. After administration of the markers, samples were taken for 51h from all cannulas every 3h, and the total faeces output was collected every 3h.

Besides the time of first appearance of the marker (FATT) and the time of arrival of the peak of radioactivity (APTT) at the four sites a third measure of the transit time (TT) was the sum of ^{103}Ru and ^{51}Cr counts in digesta during a given time period as a percentage of the sum of counts/g of samples taken during the whole collection period (PC).

RESULTS AND DISCUSSION

The substitution of Solka Floc in the control diet (trial I) did not significantly modify FATT, APTT or PC. However, there was a tendency for TT to speed up, when expressed as the PC, in the ileum but to slow it down in the colon. The substitution of guar gum similarly did not produce any significant TT differences, but when given at levels of 20 or 40g/kg diet, there was a tendency for APTT to be later and for PC of the solid phase to be smaller in the terminal ileum, whilst when fed at 60g/kg level, the transit times of both liquids and solids were similar.

Percentage of total ^{103}Ru and ^{51}Cr counts/g digesta (PC) over 51h, collected during intermediate periods in solid (S as ^{103}Ru) and liquid (L as ^{51}Cr) phases of samples.

Site	Time after feeding marker (h)	Basal		Wheat bran 100g/kg		Lactulose 50g/kg		Pectin 50g/kg		SE*
		S	L	S	L	S	L	S	L	
Ileum	3	39.5	51.1	46.8	74.0	32.5	54.4	33.1	34.7	4.04
Caecum	9	52.0	69.8	48.8	63.8	56.3	73.8	54.6	62.3	3.51
Ascending colon	21	40.0	47.8	41.0	54.8	42.9	52.8	50.0	52.5	2.41
Faeces	36	36.8	40.1	34.0	39.5	21.8	23.6	29.9	32.0	1.50

* SE of differences between marker means for a given diet.

In trial III the only significant difference ($P < 0.05$) was found in the colon where the pectin-supplemented diet had the most rapid FATT. However, in test IV using similar diets the PC at the terminal ileum was significantly smaller which means that TT was slower for the pectin-containing than for the other diets. In the lower parts of the gut there were no significant dietary differences for any parameter. In trials III and IV there were highly significant differences ($P < 0.001$) in TT when expressed as the PC of the solids and of liquids in all sites within the gut and for all diets except that containing 50g/kg pectin when the difference was not significant. In all treatments this difference decreased in faeces, and was not significant in trial III but it was significant ($P < 0.01$) in trial IV.

The independent progress of both the solid and liquid phases of digesta through the digestive tract seen here has been previously noticed in ruminants by Faichney (1975). The high viscosity induced by guar gum which keeps the digesta in a uniformly suspended state was recently measured by Heppell, Rainbird & Low (personal communication). The lack of separation of the two phases of digesta after feeding the diet with 50g/kg pectin or 60g/kg guar gum may be attributed to the colloidal nature of the solution so that liquid and suspended solids moved more or less together.

These results show that the passage of liquids and solids may differ during transit through the gut. Such an effect would clearly not be detected if only faeces are collected or if only one marker is used, which is frequently the case when transit time measurements are made.

REFERENCES

CLOSE, W.H., HEAVENS, R.P., STEPHENS, D.B. & SAMBROOK, I.E. 1984. Proc. Nutr. Soc. 43: 66A.

FADDEN, K., OWEN, R.W., HILL, M.J., LATYMER, E.A., LOW, A.G. & MASON, A.N. 1984. Biochem. Soc. Trans. In press.

FAICHNEY, G.J. 1975. In "Digestion within the gastrointestinal tract of ruminants". (Eds. I.W. McDonald and A.C.I. Warner) University of New England Press. p.277-291.

FAECAL AMINO ACID DIGESTIBILITY AND GROWTH PERFORMANCE OF PIGS ON FIBROUS DIETS

N.P. LENIS

Institute for Livestock Feeding and Nutrition Research (I.V.V.O.)
P.O. Box 160, 8200 AD Lelystad, The Netherlands.

SUMMARY

In two growing and digestibility/N-balance experiments with pigs the determined faecal digestible lysine levels in the diets were found to be much lower than the levels calculated via faecal N-digestibility of the dietary components, particularly on fibrous diets, which had a negative effect on growth performance.

INTRODUCTION

From literature and from digestibility experiments with less digestible feedstuffs at the IVVO it was found, that as the protein quality of diets decreased, apparent faecal lysine digestibility in pigs decreased to a greater extent than did N-digestibility (Slump et al., 1977, Lenis, 1980). In order to find out whether N-digestibility indeed does not provide a good estimate of the digestibility of the individual amino acids, especially for less digestible diets and to get more information about faecal amino acid digestibility and growth performance on fibrous diets, two growing and digestibility/N-balance experiments were carried out at the IVVO at the end of the seventies. Applying apparent faecal N-digestibility to the individual amino acids (lysine) should result in lower growth performance on less digestible diets.

MATERIALS AND METHODS

In exp.1 two types of diets each with 2 levels of digestible lysine (0,67 and 0,53% resp.), calculated by means of applying tabulated N-digestibility values of the dietary components to lysine, were tested. The diets B and D contained 12.1% crude fibre coming from a.o. 20% grassmeal, 20% coconut expeller and 8% wheat bran. Cereals and soybeanmeal were the main components in the diets A and C. All diets contained 10-20% maize glutenfeed. The (calculated) net-energy content of the diets was the same, as 5% soybean oil was

included in the fibre-rich diets. The design (calculated contents in the diets) of both experiments is given in table 1. In exp.1 growth performance on diets A and C should be better than on diets B and D respectively.

In exp.2 only one type of diet (fibre-rich, almost equal to the diets B and D in exp. 1) was tested in order to prevent differences in net-energy content between diets. Diets B and D were supplemented with 10 kg casein/1000 kg feed. This design only could give some information about the problem under study, if the levels of digestible lysine were just around the requirement of the animals. If the way of calculating digestible lysine via N-digestibility was correct, supplementing diet C with casein (treatment D) should not result in better growth performance. This was not expected to happen. In both experiments diets were composed on the basis of chemical analyses (Weende, amino acids) in the dietary ingredients.

Both growing experiments were carried out with crossbred boars in the weight range of 25/30 - 100 kg. Exp.1 was completed with 73 grouphoused (4-6 per pen) and groupfed animals; in exp.2 36 boars were used, housed in a pen(4) and individually fed. Feed was offered as a slurry according to the Dutch Standards for energy. Parallel to both experiments digestibility- and N-balance experiments were performed with the same type of animals: in exp.1. measurements were made three times during the growing period (on average at 41, 64 and 92 kg liveweight) with 12 animals (3 animals/ treatment); in exp.2 four animals were used in a Latin square design (measurements at on average 50, 67, 90 and 104 kg). Always after a pre-period of 7-11 days faeces and urine were collected quantitatively during 10 days.

Table 1. Some calculated contents in the diets used in experiment 1 and 2 (g/kg)

	Experiment 1				Experiment 2			
Diet	A	B	C	D	A	B	C	D
Lys	7.7	8.6	6.2	7.1	7.8	8.5	8.5	9.2
Dig. lys	6.7	6.7	5.3	5.3	5.8	6.5	6.4	7.1
XP	175	195	159	180	176	185	185	194
Dxp	148	147	132	132	133	141	141	149
XF	49	121	48	121	85	85	85	85
NE_f(MJ/kg)	9.0	9.0	9.0	9.0	9.2	9.2	9.2	9.2

RESULTS AND DISCUSSION

The digestibility/balance experiments could be carried out without hardly any problem. The determined lysine and crude fibre contents and the average results of the digestibility/balance experiments 1 and 2 are given in table 2.

Table 2. Average results of the digestibility/balance experiments

	Experiment 1				Experiment 2			
Diet	A	B	C	D	A	B	C	D
Lys	7.5	8.5	6.5	7.3	7.8	8.5	8.5	9.2
XF (g/kg)	4.9	12.0	5.2	11.5	8.5	8.3	8.3	8.4
Dig.coef.T	83.3	72.7	81.1	71.8	73.3	71.3	73.9	73.2
Dig.coef.XP	82.0	72.1	81.5	72.2	72.5	71.0	73.2	73.2
Dig.coef.XL	80.5	83.7	80.3	85.0	76.8	77.4	79.9	78.9
Dig.coef.XF	50.0	44.3	43.5	42.5	51.0	46.6	51.2	51.3
Dig.coef.XX	89.6	82.0	87.4	81.1	80.9	78.4	80.8	79.9
Dig.coef.GE	83.6	73.4	81.9	72.3	74.3	72.1	74.9	74.6
Dig.coef.lys	78.3	65.2	76.0	64.1	68.4	68.6	70.0	71.5
Dig.lys(g/kg)	5.9	5.5	4.9	4.7	5.3	5.8	6.0	6.6
N ret.(g/day)	24.1	20.5	20.8	19.8	24.2	25.6	26.6	27.1
NE_f (MJ/kg)	8.7	8.5	8.6	8.6	8.7	8.6	8.9	8.8

The standard deviations in the digestibility/balance experiments are not given in table 2 because lack of space. They were quite normal, in general on the fibrous diets a little bit higher. In both experiments the determined lysine and crude fibre contents were close to the calculated contents. Lysine digestibility was always below N-digestibility, on the fibrous diets B and D in exp.1 more than on the diets A and C. In all the diets of exp.1 the determined digestible lysine content was much lower than the digestible lysine content, calculated via tabulated N-digestibility values of the dietary components. This was also observed in exp.2, but less pronounced. In exp.1 the higher N-retention on diet A, compared to diet B, is in agreement with the determined higher digestible lysine content of diet A, although it partly may be attributed to the higher net-energy content of diet A as well.

In the growing experiments there were some problems with the feed intake of the animals: in exp.1 in group B and especially in group D, in exp.2 the animals had to be fed below the standards up

to 65 kg liveweight. The average results of the growing experiments are given in table 3. In several cases the analyses of variance

Table 3. Average results of the growing experiments.

	Experiment 1				Experiment 2			
Diet	A	B	C	D	A	B	C	D
Number of animals	19	19	18	17	9	9	9	9
Initial weight (kg)	25.7	26.2	25.9	25.7	28.8	28.8	28.9	29.
Final weight (kg)	104.7	104.7	102.0	102.4	93.8	97.8	99.6	101.
Feed intake (kg/day)	2.18	2.18	2.20	2.11	1.96	1.98	2.03	2.
Daily gain (g/day)	805	745	724	685	685	740	764	783
Feed/kg gain (kg)	2.71	2.92	3.04	3.08	2.87	2.68	2.65	2.
Slaughter loss (%)	23.7	25.8	25.3	26.5				
Backfat (mm)	18.1	15.1	17.7	15.2	15.1	15.5	16.3	15.
Meat (%)	57.9	60.0	56.7	57.4	58.7	60.5	58.9	58.
Fat (%)	33.9	30.9	34.8	33.6	33.2	31.0	32.3	32.

showed very significant differences between treatments. In exp.1 t much better growth performance on diet A, compared to diet B, is agreement with the results of the digestibility/balance experimen Comparing C and D is more difficult because of the lower feed inta of the animals on diet D, especially at the end of the growing perio In exp.2 the differences in growth performance of the animals on die B and D, compared to A and C respectively, can be attributed to t supplements of casein to the diets B and D. The differences between and D were only significant ($P<0.05$) in the first half of the growi period, not in the second half. However the levels of digestible l sine in the diets C and D of exp.2 possibly have n't been high enou compared to the requirement of the animals, which makes it difficu to attribute the observed differences to an incorrect way of calcula ing lysine digestibility.

It can be concluded, particularly from exp.1, that applying fa cal N-digestibility to lysine results in determined faecal digestib lysine levels, being much lower than calculated via N-digestibilit especially on fibrous diets, which leads to lower growth performance

REFERENCES

Lenis, N.P. 1980. Report no. 133 IVVO, Lelystad

Slump, P. et al., 1977. Z.Tierphysiol., Tierernährg. u. Futtermi telkde. 39:257-272.

EFFECTS OF WHEAT STRAW MEAL ON PORTAL PLASMA CONCENTRATIONS OF UREA AND AMMONIA-N IN THE GROWING PIG

K. MALMLÖF, C. SIMOES NUNES
Department of Animal Nutrition and Management,
Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden
INRA, Laboratoire de Physiologie de la Nutrition,
Centre de Recherches Zootechniques, 78350 Jouy-en-Josas, France

SUMMARY

Effects of a low-(LF) and a high-fibre (HF) diet on portal plasma urea and ammonia-N concentrations were studied in 3 growing pigs (35-65 kg). The diets were fed in isonitrogenous amounts at 800 h and 1600 h. Blood samples were drawn hourly throughout a 16 h period (2 x 8 h postprandial periods). In the beginning of each period samples were taken every 30 min. In comparison with the LF diet the HF diet produced lower mean plasma concentrations of urea ($p < 0.005$) and ammonia-N ($p < 0.0005$) during the 16 h studied. After an initial rise surprisingly high plasma urea concentrations were registered throughout the 8 h postprandial period following the 1600 h meal. Despite differences in plasma urea levels this phenomenon was observed in association with both dietary treatments.

INTRODUCTION

In a previous study with growing pigs Malmlöf and Håkansson (1984) observed an increase in faecal N output and a decrease in urinary N excretion in response to a high-fibre diet. An increase in the fibre content of a diet certainly raises the amount of fermentable organic matter entering the caecum-colon. Under such conditions, a thriving caeco-colonic microflora may incorporate more endogenous N, i.e. blood urea, into bacterial cells (Mosenthin & Henkel, 1978, Bergner, 1982). If this apprehension is correct, then portal concentrations of urea and ammonia-N probably would be lowered. The aim of this study was to see if this assumption could be verified.

MATERIALS AND METHODS

The effects of a low-fibre diet LF (84 % barley; 12 % fish meal; 4 % minerals and vitamins) and a high-fibre diet HF (LF + 17 % wheat straw meal) on urea and ammonia-N concentrations in portal plasma were studied in three female Sw. Landrace x Yorkshire pigs (35-65 kg). Chemical composition of the diets is given i Table 1.

Table 1. Chemical composition of diets (g/kg)

Diet	Crude protein*	Crude fibre**	NDF***
LF	172	37	135
HF	147	105	264

*Kjeldahl-N x 6.25 **According to Swedish standards
***Robertson & Van Soest (1981)

The diets were fed in isonitrogenous amounts, according to a function of the metabolic weight of the animals (15.4 g crude protein/ $W^{0.75}$). Permanent blood cannulas were introduced in the portal vein according to Rerat et al. (1980). In general blood samples were drawn once an hour throughout two successive 8 h postprandial periods following the feedings at 800 h and 1600 h. However, during the first two hours of each postprandial period sampling occurred every 30 min. Upon sampling the blood was collected in heparinized tubes, immediately centrifuged, plasma decanted and frozen at -20^{o} C until analysed.

The effect of the two diets was studied an equal number of times in the same individual animal. In association with each dietary treatment a total of 7 sampling protocoles, including 3 replications in 2 animals, were completed. Chemical analyses of plasma were performed on a Technicon autoanalyzer. Urea was analysed by Clinical method No. 1 (Technicon, N.Y. USA) and ammonia-N essentially as described by Imler et al. (1972). Differences in mean levels of the parameters were evaluated by a three-way analysis of variance, including diet, the individual animal and time of sampling as the prime sources of variation.

RESULTS AND DISCUSSION

When the animals received the HF diet a significantly ($p < 0.005$) lower mean portal plasma concentration of urea was observed, during the 16 h studied (Table 2). As suspected, the portal urea concentrations were thus suppressed by the admixture of wheat straw meal into the diet. Figure 1 indicates that this suppressed was consistent throughout the whole period studied. This is in accordance with the results by Malmlöf and Håkansson (1984) who recorded a depression in urinary N excretion level throughout a 24 h period in pigs on a high-fibre diet.

Table 2. Mean portal plasma concentrations (mg/l) of urea and ammonia-N during 16 h (2 x 8 h postprandial periods) in pigs given low-(LF) and high-fibre (HF) diets (LS-MEANS; n = 3)

Diet	Urea	Ammonia-N
LF	159.9^{a}	6.2^{c}
HF	149.2^{b}	5.8^{d}

a, b, c, d, figures within columns with different superscrips differ significantly: a = b ($p < 0.005$), c = d ($p < 0.0005$)

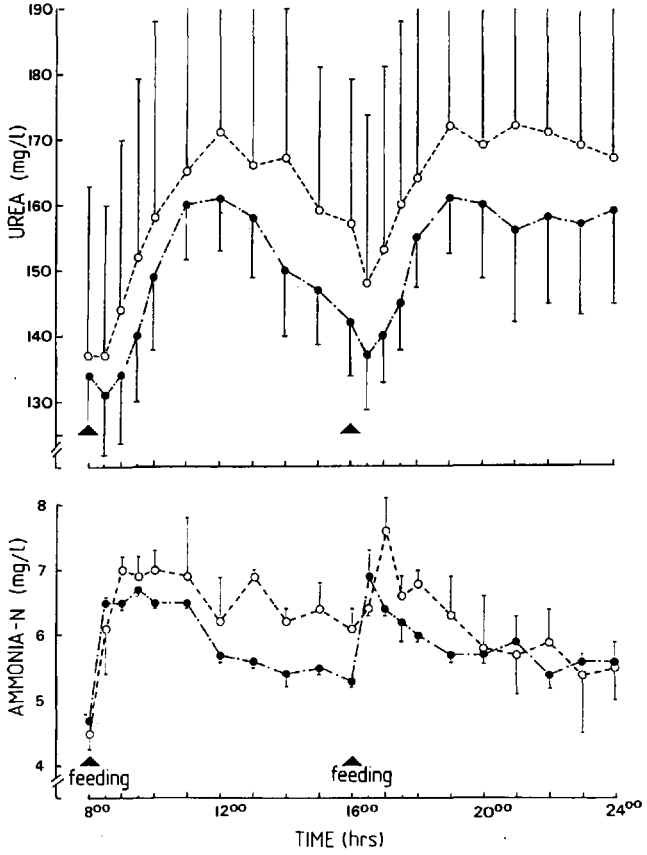


Figure 1. Portal plasma urea and ammonia-N concentrations (mg/l) during 16 h (2 x 8 h postprandial periods) in the same animals feed low-(LF) and high-(HF) fibre diets (Mean ± SEM; n = 3) o--o LF ●—●—● HF

Irrespective of type of diet the 800 h meal produced steadily increasing plasma urea concentrations with a peak at about 4 h postprandially. Thereafter plasma urea values gradually declined. In the postprandial period following the 1600 h feeding, no similar decline in plasma urea concentrations could be noticed. This is rather surprising since, at this time of day, the two previous meals would have provided the caeco-colonic microflora sufficient amounts of fermentable organic matter for optimal growth and blood urea utilization. This could be expected especially when the animals received the HF diet. However, it is possible that in the same period arterial concentrations of urea also were elevated, thus masking an important influx of urea from the blood into the digestive tract. The generally rapid postprandial rise in portal plasma ammonia-N observed (Fig. 1) indicates that in the pig, blood urea is not the sole source of portal ammonia in the early postprandial state. However, it is possible that at a later stage of the postprandial period urea becomes a more important source of portal ammonia and that the lower mean portal ammonia-N concentration registered in association with the HF diet anyhow reflects an increase in blood urea utilization by the caeco-colonic microflora.

In conclusion our results support thus to some extent the concept that the increase in faecal N often seen in pigs on high-fibre diets is a result of an increased blood urea utilization by the caeco-colonic microflora (Mosenthin and Henkel, 1978, Bergner, 1982). However, simultaneously drawn portal and arterial blood would provide more unambiguous information of the movements of urea and ammonia across the intestinal wall. Therefore, such studies are planned.

REFERENCES

BERGNER, H. 1982. In Physiologie digestive chez le porc. Les colloques de l'INRA,12,pp. 237-245. (J.P. Laplace, T. Corring & A. Rerat, editors).

IMLER, M., FRICK, A., STAHL, A., PETER, B. & STAHL, J. 1972. Clin. Chim. Acta, 37:245-261.

MALMLÖF, K. & HÅKANSSON, J. 1984. Swed. J. agric. Res. 14:53-57.

MOSENTHIN, R. & HENKEL, H. 1978. Z. Tierphysiol. Tierern. u. Futtermittelkde. 40:122-123.

RERAT, A., VAUGELADE, P. & VILLIERS, P. 1980. In Current concepts of digestion and absorption in pigs. Tech. Bull. 3,pp. 177-214. (S.G. Low & I.G. Partridge, editors). Reading: NIRD.

ROBERTSON, J.B. & VAN SOEST, P.J. 1981. In The analysis of dietary fiber in food.pp. 123-158 (W.P.T. James & O. Theander, editors), Marcel Dekker, New York.

EFFECT OF DIETARY COMPOSITION ON THE CONTRIBUTION OF LARGE INTESTINE TO TOTAL DIGESTION IN THE GROWING PIG

S.H.M. METZ, R.A. DEKKER, H. EVERTS
Institute for Livestock Feeding and Nutrition Research,
P.O. Box 160, 8200 AD Lelystad, the Netherlands.

SUMMARY

Results are presented indicating a large contribution of large intestine to total digestion in growing pigs, at a high feeding level, consuming mixed feeds of commercial feedstuffs.

INTRODUCTION

Increasing the proportion of fibrous byproducts in pig feeds in the Netherlands has recently led to a series of investigations on digestibility and energy utilization. Important experimental factors in these investigations were origin and type of the dietary carbohydrates (v.d. Honing et al., 1982). For understanding the physiological basis of the results, the effects of origin and type of carbohydrates on the contribution of large intestine (=LI) to total digestion and on the type of fermentation in LI are essential. This paper deals with the results of 2 experiments on the contribution of LI to total digestion in growing pigs at a high level of feed intake.

MATERIALS AND METHODS

Ileal and faecal digestion were measured in growing females, housed individually in pens in order to enable the pigs to move freely. At a live weight between 35 and 45 kg, pigs were cannulated in the ileum, about 30 cm from the ileal-coecal junction, using a T-shape cannula with inner diameter of 19 mm. During the experiments the animals were fed twice a day, equal meals at 6.30 a.m. and 3.30 p.m. Five diets were used, 1 cereal diet and 4 fibrous byproduct diets. All diets were mixtures of commercial feedstuffs, supplemented with minerals+vitamins. The diets were fed as a slurry, feed to water ratio was 1 : 2.5. Level of feed intake was about 0.98 MJ $NE_f/kg^{\frac{3}{4}}$ liveweight. Further experimental details are given per experiment.

Experiment 1: This experiment was performed with 4 pigs, grow-

ing from 45 to 115 kg. Two diets were used: a cereal diet (A) and a byproduct diet (B) (feedstuff composition: see Metz and Dekker, 1985). The carbohydrate fractions per kg DM amounted for diets A and B respectively to: starch + sugars 535 and 246 g, crude fibre (CF) 35 and 95 g and NDF 145 and 368 g. The experiment included 3 periods of 4 weeks. Within each period simultaneously some pigs were fed the cereal diet, others the byproduct diet. Change-over of feed occurred during the first 3 days of each period. Within a period both ileal and faecal digestion were measured in the 2nd and in the 4th week, while in the 3rd week only faecal digestion was measured. Ileal digesta was collected 3 days a week, per day all over 24 hours 8x 30 minutes with 2.5 hours interval. Ileal and faecal digestion were measured by partial collection, using a marker (Cr-mordanted NDR; 4 g/kg feed). The marker method appeared reliable for measuring faecal digestion (Everts, unpublished results).

Experiment 2: This experiment was performed with 4 cannulated pigs and 2 intact (=non-cannulated) pigs, growing from 50 to 140 kg. Four diets were used: a cereal diet with the same feedstuff composition as in exp. 1 (diet A*) and 3 byproduct diets. All byproduct diets had an equal proportion (55%) of tapiocameal, solv. extr. soybean meal, some cane molasses, animal fat and min.+vit. The complementary part was dried potato pulp, dried beet pulp, dried citruspulp and some dried potato protein for diet C; coconut expeller, linseed expeller and solv. extr. soybeanmeal for diet D; hominy feed, rice bran and wheat middlings for diet E. The carbohydrate fractions per kg DM amounted for diets A*, C, D and E respectively to: starch + sugars 522, 324, 303 and 411 g; CF 44, 104, 83 and 66g; NDF 149, 193, 205 and 179 g. This experiment included 4 periods and had a Latin Square design for the cannulated pigs. The intact animals received the same diets, randomly distributed over the periods, in order to compare faecal digestion in intact and in cannulated pigs. Further methodology was the same as in exp. 1, except that ileal digesta was collected between 7 a.m. and 11 p.m., daily 4x during 80 minutes, with 4 hours interval.

RESULTS

Table 1 compares digestibility coefficients (DC) in cannu-

Table 1. Faecal DC in intact (I) and cannulated (C) pigs (n=16)

	I	C	C - I mean	C - I SD
dry matter (DM)	76.2	76.6	0.4	1.4
org. matter (OM)	79.4	79.8	0.4	1.2
N	70.0	70.4	0.4	2.2

lated and intact animals for 4 diets together. The results indicate that faecal DC for DM, OM, and N is not affected by cannulation. Also for each diet separately and for the separate dietary components within each diet no effect of cannulation upon DC has been observed (not shown in the Table). Collection of digesta does not alter faecal DC from cannulated pigs (Table 2). So, ileal and faecal DC can be measured at the same time in the same animals.

Large significant differences in ileal DC are observed between the diets for all components except for starch which is allmost completely digested before the terminal ileum (Table 3). Smaller significant differences between diets are observed for faecal DC of DM and OM. These variations between diets in ileal and faecal digestion are obviously connected with composition and origin of the dietary carbohydrates: DM, OM and N are better digested, both at the ileal and at the faecal level, from the cereal diets than from all the other diets. NDF is hardly digested at the end of the ileum; faecal DC for NDF is lowest for diets A, A* and E. LI has contributed substantially to digestion of OM in all diets (Table 4), especially in diets B, C and D (more than 30% of total OM-digestion). Contribution of LI to digestion has always been much lower for N than for OM. For all diets starch digestion was approximately completed at the terminal ileum while digestion of NDF and pectins occurred largely in LI (not in Table 4). The precision of

Table 2. Effect of collecting ileal digesta on faecal DC (n=11)

	- collection	+ collection	difference mean	difference SD
DM	77.1	77.1	0.0	1.5
OM	80.2	80.3	-0.1	1.3
N	71.6	70.6	1.0	3.9

Table 3. Ileal and faecal digestibility coefficients.

	Experiment 1		Experiment 2			
diet	A	B	A*	C	D	E
Ileal DC DM	69.1	41.1	68.1	47.3	46.3	56.7
OM	71.9	45.2	70.0	52.5	50.7	60.5
N	71.5	54.6	68.1	60.1	57.2	64.5
Starch	96.5	96.9	96.4	97.0	97.4	96.4
Faecal DC DM	81.9	72.7	80.2	77.7	74.8	73.2
OM	83.6	74.8	82.4	81.0	78.3	77.2
N	81.1	65.1	77.1	64.5	70.9	68.5
NDF	43.6	67.8	39.5	63.6	55.6	36.8

estimation of the contribution of LI to total digestion was adequate precise for DM and OM, but not for N. The results shown in Table 4 indicate that feeding commercial diets to growing pigs at a high level of feed intake may lead to large contributions of LI to total digestion. This may have consequences for energy evaluation because utilization of digestible energy from fermentative processes in the LI is supposed to be considerably lower than utilization of energy digested in the small intestine by the enzymes of the animal (ARC, 1981). This aspect deserves considerable attention in the near future.

Table 4. Contribution of large intestine to total digestion (%)

	OM		N	
	mean	SD	mean	SD
diet A	14.1	3.3	11.8	4.6
B	39.4	3.9	15.9	8.9
A*	14.5	0.8	11.4	4.2
C	35.5	1.7	8.0	5.5
D	35.4	1.5	17.9	5.0
E	21.5	1.3	5.7	2.6

REFERENCES

Honing, Y. van der, 1982. In: Energy metabolism of farm animals (Eds. A. Ekern and F. Sundstol) EAAP-Publ. no 29, p. 202-205.

Agricultural Research Council (1981). The nutrient requirement of pigs. CAB, Slough. England.

Metz, S. H. M. and Dekker, R. A. 1985. In: Digestive Physiology in the Pig. (Eds. A. Just, H. Jørgensen and J. A. Fernández). Report No. 580. Nat.Inst.Anim.Sci. Copenhagen, pp. 369-372.

EFFECTS OF DIETARY SUBSTITUTION OF COMPLEX POLYSACCHARIDES ON GASTRIC EMPTYING, DIGESTA TRANSIT TIME TO TERMINAL ILEUM AND RECTUM, AND NUTRIENT UTILIZATION IN THE GROWING PIG.

ZOË V. POTKINS[+], T. L. J. LAWRENCE[+], J. R. THOMLINSON[‡]
[+]Dept. Animal Husbandry, University of Liverpool, Veterinary Field Station, Neston, L64 7TE, England and
[‡]Dept. Veterinary Pathology, The University, Liverpool, L69 3BX. England.

SUMMARY

Studies on nutrient utilization (1), on digesta transit time to the rectum (2) and to the terminal ileum (3) and on gastric emptying (4) were made with diets in which bran (B), oatfeed (OF), guar gum (GG) or pectin (P) had been substituted for barley (which in (4) was coarsely or finely ground) in a control diet. Comparisons in (2) were made using logarithmic, and in (3) and (4) semi-logarithmic, transformed data. Fine barley, GG or P significantly accelerated gastric emptying. No significant effects were found on transit time to the terminal ileum but 300 g B per kg diet reduced transit time to the rectum ($p < 0.05$). OF (225 g/kg diet) reduced apparent digestibility (dig.) of dry matter (DM) at the terminal ileum ($p < 0.05$). At the rectum dig. of DM and DM and gross energy decreased significantly ($p < 0.01$ and $p < 0.001$ respectively) with increasing levels of B and OF respectively and 50 g GG per kg diet significantly reduced dig. ($p < 0.05$) and retention of nitrogen (N) ($p < 0.01$). N retention was also reduced with diets containing 300 g OF ($p < 0.05$).

INTRODUCTION

Foodstuffs containing high proportions of complex polysaccharides may induce chemical and physical changes in, and affect the transit

time of digesta. These effects could modify the environment of the gut and therefore affect nutrient utilization, bacterial proliferation, water absorption and diahorrea. These experiments were designed to investigate some of these effects by substituting complex polysaccharides in barley based diets.

MATERIALS AND METHODS

All diets were barley-based (850 g/kg in control diet). In studies to the rectum substitution levels for barley of bran (B) and oatfeed (OF), and guar gum (GG) and pectin (P), were between 0 and 300 g/kg diet and 0 and 50 g/kg diet, respectively. In studies to the terminal ileum and in studies on gastric emptying, substitution levels were 225 and 50 g/kg diet for B and OF, and GG and P, respectively. At the terminal ileum (using 7 pigs with simple cannulae) and at the rectum (using 60 pigs) transit time and apparent digestibility (dig.) of dry matter (DM), nitrogen (N), gross energy (GE) and modified acid detergent fibre (MADF) were determined. N balance studies (using 60 pigs) were made at the same time. Ten pigs with gastric cannulae were used to study gastric emptying for which barley was ground through either a 1.56 mm (fine) or a 4.68 mm (coarse) screen. Gradient slopes were used to compare transit times by transforming data to a logarithmic base for studies to the rectum and to a semi-logarithmic base for studies to the terminal ileum and on gastric emptying

RESULTS AND DISCUSSION

Statistically significant differences found at the rectum are given in Table 1. A linear relationship was found between the level

of B or OF in the diet and the dig. of DM, and between the level of OF and the dig. of GE. At 300 g/kg diet B decreased transit time (m) to the rectum and OF decreased N retention. The effects of increasing fibre in the diet on the dig. of DM and GE are in agreement with those reported by Kennelly and Aherne (1980). At a level of 50 g per kg diet GG reduced N dig. and N retention.

Table 1. Statistically significant data from studies to the rectum.

		Bran or Oatfeed (g/kg diet)					
Bran		0	75	150	225	300	SED
MADF in diet (g/kg)		55	60	66	71	77	
N in diet (g/kg)		28	29	30	30	31	
Apparent digestibility	DM	0.792	0.775	0.753	0.746	0.733**	0.009
m		5.19	4.32	6.31	5.09	8.30*	1.12
Oatfeed							
MADF in diet (g/kg)		58	80	103	125	148	
N in diet (g/kg)		28	28	27	26	25	
Apparent digestibility	DM	0.768	0.741	0.703	0.643	0.614***	0.012
	GE	0.759	0.744	0.709	0.646	0.617***	0.013
N retention ($g/day/W^{0.75}$)		0.96	1.07	1.04	1.02	0.880*	0.052
			Guar Gum (g/kg diet)		Pectin (g/kg diet)		
		0	10	50	10	50	
MADF in diet (g/kg)		58	57	55	57	55	
N in diet (g/kg)		28	28	28	28	27	
Apparent digestibility N		0.783	0.771	0.734	0.776	0.753*	0.015
N retention/N intake		0.466	0.463	0.415	0.448	0.432**	0.015

At the terminal ileum the dig. of the DM of the diet containing OF was significantly lower than that of the control diet ($p < 0.05$), the coefficients being 0.562 and 0.440 respectively. No significant differences were found in the other variables. Murray, Fuller and Pirie (1977) also found no difference in the rate of passage of diets containing complex polysaccharides to the terminal ileum but

reported a significant decrease in N dig..

The gastric emptying rates are given in Table 2. The rate of emptying of the fine barley diet was faster than that of the coarse barley diet, significantly so in experiment 1. GG and P also significantly increased gastric emptying. These results do not support those of Rainbird and Low (1983 a and b) who, using semi-purified control diets, found no significant effect of GG on gastric emptying and a slower rate of emptying after 4 h for B.

Table 2. Gastric emptying rates

	Barley			Diet			
Experiment 1	Coarse	Fine	SED	Control	Bran	Oatfeed	SED
k (h^{-1})	-0.113	-0.189***	0.021	-0.155	-0.126	-0.173	0.033
Experiment 2				Control	Guar Gum	Pectin	SED
k (h^{-1})	-0.148	-0.174	0.012	-0.127	-0.174	-0.182*	0.021

REFERENCES

KENNELLY, J. J. & AHERNE, F.X. 1980. Can. J. Ani. Sci. 60:717-726.

MURRAY, A.G., FULLER, M.F. & PIRIE, A.R. 1977. Anim. Prod. 24:139 (Abstr.).

RAINBIRD, ANNA L. & LOW, A.G. 1983a. Proc. Nutr. Soc. 42:24A.

RAINBIRD, ANNA L. & LOW, A.G. 1983b. Proc. Nutr. Soc. 42:88A.

FAECAL BULKING ABILITY OF A CEREAL OR VEGETABLE BASED DIET FED TO PIGS IN RELATION TO BULKING EFFECTS IN MAN

J.A. ROBERTSON, A. CHESSON
Rowett Research Institute
Bucksburn, Aberdeen, AB2 9SB, U.K.

SUMMARY

Faecal bulking ability is an important characteristic of dietary fibre and studies have shown that cereal bran is a more effective bulking agent than vegetable fibre. Subject variation, fibre source and method of preparation have been shown to be important when establishing bulking ability. Taking these factors into account the faecal bulking response to dietary fibre in the pig was found to be similar to that in man.

INTRODUCTION

Faecal bulking agents are generally of plant origin and are usually categorised as dietary fibre. The response to fibre in the diet, however, is variable, with variation in stool weight being a consequence both of diet and individual response to the diet (Cummings et al., 1978). Diets rich in fat and protein have been shown to have little effect on stool weight whereas diets rich in carbohydrate increased stool weight. Though the laxative effect of carbohydrate was in part ascribed to the fermentation products of starch and sugar (saccharose) it was acknowledged that dietary fibre was the most effective faecal bulking agent in the diet (Williams & Olmsted, 1936).

Studies on faecal bulking ability in man have generally been confined to the use of fibre supplements to the normal diet, with the response to fibre measured as the change in faecal weight after conditioning to the supplement. These studies, examples of which are given in Table 1, (Cummings et al., 1978; Eastwood et al., 1983; Wrick et al., 1983) have shown that cereal bran maintains its bulking potential during gut transit but the water-holding capacity of vegetable fibre is destroyed during transit, due to fermentation of the fibre.

This investigation presents the results of faecal bulking estimation in pigs fed a cereal-based and a vegetable-based diet, compares the data to bulking effects reported in man and hence whether the pig might be a suitable model system to study the response to fibre in the diet in man.

MATERIALS AND METHODS

Four Large White x (Large White x Landrace) female pigs were used in the investigation. During faecal collection periods animals were restrained in cages (2.0 x 0.6m floor area) and maintained at an ambient temperature of 25°C. Feed was provided twice daily at 08.30h and 15.30h. Water was freely available to each animal.

Faecal collection was begun after 3 days acclimatization to the cages and continued for 7 days. Collections were made every 3h between 09.00h and 18.00h each day and faecal fresh weight determined for each pig during each 24h period.

Three diets were formulated to examine faecal bulking ability: a swede (Brassica napus cv. Danestone) based diet to provide 250g fibre/day; a wheat bran diet to provide 500g fibre/day; and a wheat bran/white fish meal/dry skim milk diet to provide 250g fibre/day. Each diet was supplemented with vitamins and minerals to a level sufficient to provide a maintenance diet for each animal. Feed was allocated to provide approximately 6MJ; 24% energy as protein in each swede feed, 30% energy as protein in bran feed and 31% energy as protein in the bran/fishmeal/-skim milk diet. Animals were introduced to the diet 10 days before faecal collection was begun. Faecal collections were made at 60kg and 90kg live weights for each pig.

Examples on the effect of fibre on faecal bulking in man have been taken from the literature and have been confined to studies where different fibre preparations were tested on the same subjects and where details of the fibre preparations were available.

RESULTS AND DISCUSSION

The effect of fibre supplementation on faecal fresh weight in man and the effect of fibre on faecal fresh weight in the pig is shown in Table 1. In man there was considerable variation in the effect of fibre in the diet between each study and between fibre sources, though within each study bran had a consistently greater effect than other fibre sources on faecal fresh weight.

The variation between studies was in part due to subject variation, but mainly due to differences in method to measure fibre intake. In study 1, fibre was measured as neutral detergent fibre, to represent cell wall material, and a pectin isolate was used with cabbage to compensate for pectin loss during neutral detergent fibre preparation. Studies 2 and 3, however, measured fibre intake from standard food composition tables and are more comparable than study 1. Fibre intake in pig estimated using food conposition tables and by analysis for cellulose,

component neutral sugar, uronic acids and lignin was found to be consistent with studies 2 and 3.

Table 1. Faecal bulking ability of dietary fibre estimated in man and in the pig

STUDY	DIET SUPPL.	FIBRE (g/day) Total	Supp.	FAECAL WEIGHT (g/day)	F.WEIGHT CHANGE (g/day)	F.WEIGHT CHANGE/20g FIBRE
	MAN					
	Bran (coarse)	13.6	12.6	157	54	86
1	Bran (fine)	12.8	11.8	128	25	42
	Cabbage*	16.3	15.3	110	8	10
	Bran (coarse)	32.0	13.0	183	63	97
	Potato (roller dry)	34.8	15.0	215	53	71
2	Potato (air dry)	33.4	15.0	207	37	49
	Potato (boiled)	34.0	15.0	231	27	36
	Bran	40.0	18.0	197	102	113
	Cabbage	40.3	18.3	143	55	60
3	Carrot	42.1	20.1	189	72	72
	Apple	41.9	21.9	203	62	57
	Guar	39.2	17.2	139	19	22
	PIG	Fibre(g/day)		Faecal Weight(g/day)		Faecal Weight /20g Fibre
	Bran 60 kg	500	-	2458	-	98
	Bran (1) 90kg	500	-	2160	-	86
	Bran (2) 90kg	250	-	1160	-	93
	Swede 60kg	250	-	812	-	65
	Swede 90kg	250	-	790	-	63

Study 1 = Wrick *et al.*, 1983; Study 2 = Eastwood *et al.*, 1983; Study 3 = Cummings *et al.*, 1978. * Cabbage + pectin; Bran(1) = Bran diet as for 60kg pig; Bran(2) = Bran/white fish meal/dry skim milk diet.

The use of pectin to supplement cabbage fibre in study 1 and the response to 20g cabbage fibre in that study compared to the response to cabbage fibre response in study 3 illustrates how fibre isolates may not always reflect the true response of the fibre source in the diet. This is also shown by the low bulking response to guar gum in study 3.

In each case, however, the addition of fibre to the diet resulted in a increase in faecal fresh weight. The increase was influenced by method o preparation as well as fibre source. For example, using fine bran instead of coars bran lowered the bulking response/20g fibre and different methods of preparin potato fibre affected the bulking response of this fibre source. The response t potato in each case, however, remained similar to that found for fruit and vegetabl fibre in study 3 and was less than the response to the equivalent amount of bran i the diet.

Dietary response to fibre using the pig, fed raw bran and raw swede, showe that fibre intake was extreme, at between 10 and 20 times the level generall accepted in man, and consequently faecal output was also much higher. If a linea dose response effect of fibre on faecal bulking is applied (Cummings et al., 1978 then it can be seen that response/20g fibre in the diet was similar to the respons noted for man. The response was independant of pig weight but as in man cereal fibr had a greater bulking ability than vegetable fibre. The response to fibre was als apparently unaffected by the increased presence of protein and fat used in th bran/fish meal/skim milk diet. This was also consistent with previous observation that fat and protein had little effect on stool weight (Cummings et al., 1979) an hence that the bulking ability noted was related primarily to the fibre content o the diet (Williams & Olmsted, 1936).

On the basis of faecal bulking ability of dietary fibre in the pi therefore, the pig would appear to be a suitable experimental model to use for ma when investigating this aspect of dietary fibre in human nutrition.

The results of the research, the contents of which are reported in th document, are the property of the Ministry of Agriculture, Fisheries and Food an are Crown Copyright.

REFERENCES

CUMMINGS, J.H., BRANCH, W., JENKINS, D.J.A., SOUTHGATE, D.A.T., HOUSTON, HELEN, & JAMES, W.P.T. 1980. Lancet 1: 5-8,

CUMMINGS, J.H., HILL, M.J. & JIRRAJ, T., HOUSTON, HELEN, BRANCH, W.J. & JENKINGS, D.J.A. 1979. Am.J.Clin.Nutr. 32: 2086-2093.

EASTWOOD, M.A., ROBERTSON, J.A. BRYDON, W.G. & MACDONALD, DOROTHY. 1983. Br.J.Nutr. 50: 539-547.

WILLIAMS, R.D. & OLMSTED, W.H.J. 1936. J.Nutr. 11: 433-438.

WRICK, K.L., ROBERTSON, J.B., VAN SOEST, P.J., LEWIS, B.A., RIVERS, J.M. ROE, D.A. & HACKLER, L.R. 1983. J.Nutr. 113: 1464-1479.

THE DIGESTIBILITY AND HYPOCHOLESTEROLAEMIC EFFECTS OF BARLEY, WHEAT, OATS AND RYE IN GROWING PIGS

I.E. SAMBROOK
Department of Human Nutrition, Food Research Institute (Reading), Shinfield, Reading, RG2 9AT, U.K.

SUMMARY

The study involved feeding whole, milled grain (barley, wheat, oats and rye) to growing pigs surgically fitted with indwelling vena cava catheters:-

1) to compare the hypocholesterolaemic effects of the four cereals.
2) to determine the nutritive value of simple cereal diets for pigs.

The results showed that:-

a) Plasma cholesterol levels were elevated only slightly but to different extents when 10g cholesterol/kg diet was added.
b) The pigs grew poorly when fed the simple cereal diets.
c) Nevertheless, there were striking differences between the diets in apparent digestibility of nitrogen and nitrogen retention.

INTRODUCTION

Elevated plasma cholesterol levels are thought to be associated with an increased incidence of atherosclerosis, - the lipid theory of heart disease (Kaunitz, 1977). A reduction in dietary cholesterol lowers plasma cholesterol levels and may thus lead to a lower risk of atherosclerosis and coronary heart disease (CHD). In addition, dietary fibre may protect against CHD by lowering plasma cholesterol levels (Trowell, 1972).

Previous work at NIRD with rabbits made hypercholesterolaemic by adding 10g cholesterol/kg diet, showed the marked hypocholesterolaemic effect of whole beans (Phaseolas vulgaris)(Finnigan, 1983). Similarly, hypercholesterolaemic pigs had their plasma cholesterol levels reduced by 40% in 7 days when baked beans (P. vulgaris) replaced 30% of the diet on a DM basis, (Sambrook, unpublished).

As wholefoods are of current interest in human nutrition, the present study examined the hypocholesterolaemic effects of whole

milled barley, wheat, oats and rye, (the major British cereal crops) using the pig as a model for man. In addition, since simple diets are of interest in pig production, the ability of the young pig to prosper on a single cereal diet was also studied.

MATERIALS AND METHODS

Twenty-four Landrace x Large White boars of 25-30kg initial liveweight were each surgically fitted with an indwelling vena cava catheter, inserted via the external jugular vein and exteriorised immediately behind the ear. Catheters were filled with sterile, heparinised saline (30 I.U./ml NaCl solution (9g/l)) and checked daily to ensure they remained functional for as long as possible.

Four diets consisting of milled whole barley (diet B), wheat (diet W), oats (diet O) or rye (diet R) with mineral and vitamin supplements (Table 1) were fed for 14 days. For the last 5 days there was a balance period with separate urine and faeces collection to measure nitrogen (N) retention and apparent digestibility of N for each diet. Each diet was then supplemented with 10g cholesterol/kg diet (diets BC, WC, OC and RC) and fed for a further 28 days. During this time, pre-feed plasma cholesterol concentrations were measured at 7 day intervals by an enzymatic method (Merkotest 14349 test kit).

Table 1. Formulation of diet

Ingredient	g/100kg
Cereal (barley, wheat, oats or rye)	98.05
Limestone	0.20
Dicalcium phosphate	0.50
Minerals and vitamins	1.25
	100.00
Cholesterol (diets BC, WC, OC and RC)	1.00

All diets were fed at 4% body weight per day, and mixed with water (400g/l) immediately before feeding which was in two equal feeds at 0900 and 1600. The chemical composition of the diets as fed is shown in Table 2.

Table 2. Chemical composition of air-dry diets.

Diet	%DM	% Ash	% TN (CP)	% ADF	% NDF	% Fat
Barley	88.67	3.65	1.68 (10.50)	4.73	14.99	3.78
Wheat	87.22	3.38	1.65 (10.31)	4.17	12.10	3.57
Oats	89.94	4.25	1.75 (10.94)	10.88	22.56	7.34
Rye	86.60	3.35	1.41 (8.81)	3.13	11.31	3.43

RESULTS AND DISCUSSION

From Table 3 it can be seen that the growth rate was similarly poor for all four diets.

Table 3. Daily gain, nitrogen (N) retention and apparent digestibilty of N in four cereal diets.

Diet	Daily gain g/d	N retention g/d	Apparent digestibility of N
Barley	250.0	5.47 a	68.92 ab
Wheat	232.2	3.14 a	72.89 ac
Oats	256.0	11.50 b	80.82 c
Rye	223.2	4.55 a	64.56 b
SED	52.13	1.426	3.587

Values with different subscripts are significantly different.

Least significant difference	NS	p<0.001	p<0.05

Pigs fed on barley or wheat diets supplemented with soya or fishmeal could be expected to give weight gains of around 600-700g/d, while animals fed purified diets with casein as the protein source have achieved gains of over 1000g/d. No comparable information is available for oats and rye diets. The poor growth on cereals alone reflected the shortage of essential amino acids, notably lysine and threonine, in these feeds. Also the relatively high content of fibrous material, which can contribute to the animal's energy needs, nevertheless reduced the energy intensity of the diet.

Despite the poor overall growth on all diets, there were some striking differences between the diets in N retention and apparent digestibility of N. Notably the oats diet was considerably better than the other three diets. The higher crude protein and total lipid contents (and hence energy content) in the oats may have contributed to the more efficient N utilization from this diet.

All the diets prevented the plasma cholesterol from reaching levels previously achieved in pigs fed purified diets (Sambrook, unpublished) but rye appeared the most and oats the least effective (Table 4).

Table 4. Plasma cholesterol concentrations (mg/100ml) before and during supplementation with 10g cholesterol/kg diet.

Diet	Pre-supplementation	Supplementation wk 1	wk 2	wk 3	wk 4
Barley	80.6	89.4 ab	86.8 ab	93.6 ab	87.9
Wheat	83.8	85.2 ab	98.0 ab	98.6 ab	104.5
Oats	75.2	100.9 a	112.2 a	128.3 a	97.4
Rye	67.7	72.4 b	86.4 b	85.8 b	79.7
SED	8.87	8.49	11.89	15.03	11.36

Values with different subscripts are significantly different

Least significant difference	NS	p<0.01	p<0.05	p<0.05	NS

The mechanisms by which dietary fibre may lower plasma cholesterol is not fully understood, but increased excretion of bile salts bound either by the fibre or by saponins present, is thought to be important. It has been suggested that the higher fat content in rolled oats may contribute to its hypocholesterolaemic effects by increasing the intake of polyunsaturated fats (Judd & Truswell, 1981). In this study, however, oats were the least effective in lowering plasma cholesterol, so some other factor such as protein composition or particle size of the cereal may have been more important in determining its effectiveness.

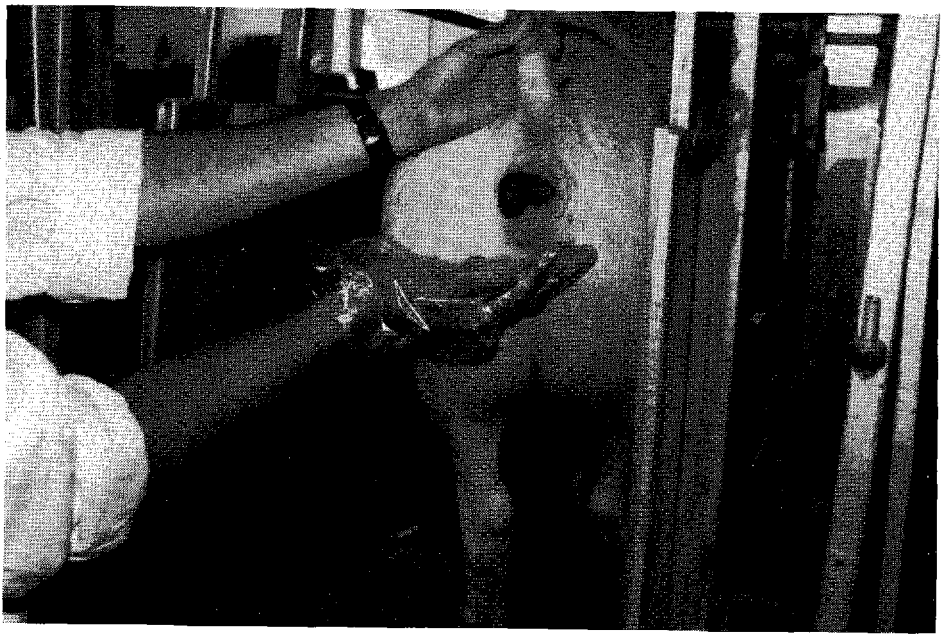
REFERENCES

FINNIGAN, T.J.A. 1983. J. Sci. Fd. Agric. 34 (3) 275-276.
JUDD, P.A. & TRUSWELL, A.S. 1981. Am. J. Clin. Nutr. 34 : 2061-2067.
KAUNITZ, H. 1977. Chem. Ind. 18 : 761-764.
TROWELL, H. 1972. Am. J. Clin. Nutr. 25 : 926-932.

SESSION 4

THE INFLUENCE OF THE GUT MICROFLORA ON THE DIGESTIVE PROCESSES

Discussion leader: T. Zebrowska



THE INFLUENCE OF THE GUT MICROFLORA ON THE DIGESTIVE PROCESSES

B. RATCLIFFE
National Institute for Research in Dairying,
Shinfield, Reading, Berkshire, RG2 9AT, England*

SUMMARY

There are apparent advantages and disadvantages obtained by the host from the microbial flora at all levels of the digestive tract. The microflora affects the growth of the pig, influences nutritional requirements and affects the mucosal epithelium of the alimentary canal. Bacterial activity modifies both endogenous and dietary substances in the gut and can contribute energy to the host via organic acids. Digestion of carbohydrates by microorganisms in the stomach of the young pig deprives it of small amounts of energy but, through lactic acid production, complements the limited secretion of hydrochloric acid and helps maintain the acidic, bactericidal barrier. Perhaps the single most beneficial function of the indigenous flora is the 'barrier' effect which, under normal circumstances helps to prevent the establishment of 'exotic' species in the gut and the invasion of pathogens. The similarity of the gut microflora in man and pigs is discussed and the potential use of the pig as a model in studies of the interactions between nutrition and gut microorganisms.

INTRODUCTION

Pasteur (1885) reflected on the part played by the gut microflora in the breakdown of dietary materials and the potential symbiotic involvement with the host. At that time, he concluded that life would not be possible without microorganisms. It is now well known and documented that, in fact, animals can survive in the absence of bacteria. A wide range of monogastric mammals can

*Address from 1st April 1985: Department of Human Nutrition, Food Research Institute Reading, Shinfield, Reading, Berkshire, RG2 9AT, England.

be maintained germ-free and certainly, in this respect, pigs present no particular problems. Organs and tissues which are not usually in intimate contact with bacteria are essentially the same in germ-free and conventional pigs (Gordon and Pesti, 1971). Germ-free pigs can perform normal bodily functions including adequate digestion and assimilation of ingested material and this indicates that obligatory symbiotic relationships have not evolved between pigs and bacteria.

The microflora in the gastro-intestinal tract can, however, be of great benefit to the pig; not only because of the potential synthesis and supply of micronutrients but also because of the ability of some bacteria to digest complex dietary components and so release simpler compounds which become available to the host.

The number of bacteria in the digestive tract is much greater than the number of mammalian cells making up the pig itself and it can be estimated that, in a pig of bacon weight, the associated bacterial mass may contribute 1-1.5 kg. It might be expected that, because of its size and complexity, the microflora would have widespread effects throughout the digestive system.

MICROBIAL ACTIVITY IN THE STOMACH

The microflora

At birth, the gastro-intestinal tract of the baby pig is sterile but, through intimate contact with its dam and the immediate surroundings, it quickly becomes contaminated with microorganisms. Many of these bacteria are unable to colonize the gut and simply pass through the system but the ingestion of milk provides a substrate for some organisms and they quickly become established in the stomach and intestines (Fuller, 1962; Barrow, 1978). Lactobacilli, streptococci, coliforms and *Candida spp.* have been detected in the stomachs of neonatal pigs (Smith, 1965; Tannock and Smith, 1970; Decuypere and Van der Heyde, 1972; Fuller *et al*, 1978; Ducluzeau and Raibaud, 1979). In the suckling pig, the predominant streptococci are *S. salivarius*, *S. faecium* and *S. faecalis s.s. liquefaciens* which are able to ferment lactose

(Barrow et al, 1977). But the predominant streptococci, after weaning, are strains which do not ferment lactose (Fuller, 1962).

After weaning, healthy, growing pigs have a more or less stable gastric flora with lactobacilli and streptococci as the major contributors (Fuller et al, 1960; Raibaud et al, 1961).

Microbial activity

It has been shown that bacteria in the monogastric stomach have α- and β- glucosidases and α- and β-galactosidases and so can compete with the host enzymes for simple carbohydrate substrates (Hawksworth et al, 1971). The most significant microbial activity in the stomach is the fermentation of sugars.

In suckling pigs, lactic acid, produced by bacterial glycolysis, is the major organic acid in the stomach (Friend et al, 1963). The lactose, present in the ingested milk, is digested by bacterial β-galactosidase and then passes via the Embden-Meyerhof pathway to pyruvate and finally to lactic acid. Baldwin (1970) has proposed two models of the process of bacterial fermentation in the gut and these are shown in Table 1. Free electrons are represented for simplicity and to avoid the necessity of entering into discussion of bacterial electron transport systems. In the third reaction, ADP and ATP are placed in parenthesis because the yield of ATP can vary depending on the microbial species and metabolic pathway. Reactions, similar to each of those in Table 1, occur to greater or lesser extents in all regions of the gut. Depending on which substrates are available and which microbial species are present, this determines the types and proportions of organic acids which are produced. Fermentation in the porcine stomach has been reviewed by Cranwell (1968) and Kidder and Manners (1978).

Cranwell et al (1976) found lactic acid to comprize 80-100 % of the total organic acids in the stomachs of young suckling pigs. Volatile fatty acids (VFA's) made up the remaining 0-20 %. Of the VFA's, acetic acid was the most predominant with only small amounts of propionic acid (<1 % of total organic acids) and traces of butyric acid.

Table 1. Two models of the fermentation processes in the alimentary canal. (Modified from Baldwin, 1970)

Carbohydrate fermentation-Model I

$1.5\ \text{Glucose} + 3\ \text{ADP} \rightarrow 3\ \text{pyruvate} + 6H^+ + 6e^- + 3\ \text{ATP}$

$1\ \text{Pyruvate} + 1\ \text{ADP} \rightarrow CO_2 + \text{acetate} + 2H^+ + 2e^- + 1\ \text{ATP}$

$2\ \text{Pyruvate} + 8H^+ + 8e^- [+ 2\ \text{ADP}] \rightarrow 2\ \text{propionate} [+ 2\text{ATP}]$

Overall:

$1.5\ \text{Glucose} + 6\ \text{ADP} \rightarrow CO_2 + \text{acetate} + 2\ \text{propionate} + 6\ \text{ATP}$

Carbohydrate fermentation-Model II

Organism A

$1.5\ \text{Glucose-P} + 4.5\ \text{ADP} \rightarrow 3\ \text{pyruvate} + 6H^+ + 6e^- + 4.5\ \text{ATP}$

$3\ \text{Pyruvate} + 6H^+ + 6e^- \rightarrow 3\ \text{lactate}$

Organisms B and C

$3\ \text{Lactate} + 3\ \text{ADP} \rightarrow 3\ \text{acetate} + 3\ CO_2 + 6H_2 + 3\ \text{ATP}$

$6H_2 + 1.5\ CO_2 + 1.5\ \text{ADP} \rightarrow 1.5\ CH_4 + 3\ H_2O + 1.5\ \text{ATP}$

Overall:

$1.5\ \text{Glucose-P} + 9\ \text{ADP} \rightarrow 3\ \text{acetate} + 1.5\ CO_2 + 1.5\ CH_4 + 3\ H_2O + 9\ \text{ATP}$

The fermentation of sugars to produce organic acids provides energy to the bacteria in the stomach and represents a small loss of energy to the pig. Lactic acid in the stomach is produced as both L(+) and D(-) isomers, in approximately equal quantities. Some mammals have a limited capacity to utilize D(-)-lactate (Brin, 1965; Dunlop and Hammond, 1965). However, the absorption and utilization of both isomers have been demonstrated in young pigs (Sissons et al, 1983; Christie and Cranwell, 1976) although significant quantities of the D(-) isomer were excreted in urine.

The proportions of the major end-products of fermentation differ in suckling pigs from those in older individuals. Friend et al (1963) found that after the provision of creep feed the level of lactic acid in the stomach decreased compared to when the piglets were consuming only milk.

Clemens and co-workers (1975) examined the levels of organic acids in the stomachs of older pigs (176 kg live weight) and lactic acid constituted about 50 % of the total. A maximum of 17 mmole of lactic acid was found after feeding and VFA's were found in similar quantities indicating that substantial fermentation occurred in the stomachs of mature pigs which were fed on a commercial, pelleted diet.

The low pH of the stomach plays an important role in the formation of milk clots in suckling pigs and in the denaturation of protein in general. In addition, gastric acidity protects the gut from invasion and proliferation of undesirable microorganisms since low pH is bactericidal for many species. Barrow et al (1977) suggested that lactic acid from bacterial fermentation was a major component in the regulation of gastric pH in young pigs. Hydrochloric acid production and pH of the stomach were determined in suckling pigs and littermates which had been weaned at 2 d of age and fed a milky substitute. Only 3 of the 20 suckling pigs and none of the early weaned pigs were secreting HCl by 10 d of age. There was a negative correlation between pH and lactic acid concentration and a positive correlation between pH and numbers of E.coli in the stomach. The secretion of HCl has been recorded for pigs of 1 d of age (Cranwell and Titchen, 1974; Cranwell et al, 1976) but some investigations failed to find evidence of HCl secretion in pigs at 7 d old (Kutas and Szabo, 1974). Cranwell and colleagues (1976) found HCl production from 1 d after birth but it did not occur in significant quantities until 24 d of age in some pigs.

The production of organic acids may act in an inhibitory way on HCl secretion since there is some evidence that the feed-back control is mediated by hydrogen ion concentration and not specifically HCl (Andersson, 1967). Organic acid may be inhibiting HCl secretion or supplementing a limited secretory capacity but the indigenous bacteria of the stomach nonetheless contribute significantly to the maintenance of low gastric pH in young pigs.

The gastric flora requires nitrogen for its own protein synthesis and, presumably, this is obtained from catabolism of dietary sources of nitrogen and endogenous losses within the stomach. Mason (1978) predicted that the involvement of the stomach microflora in protein digestion would be small but significant. There has been little work in this area to confirm this.

It has been suggested that fibre may be digested in the stomach (Kidder and Manners, 1978; Sambrook, 1980). Indeed, some large dietary particles appear to be retained in the stomach for

up to 60 hours (Clemens et al, 1975) and this could permit considerable fermentation to proceed. But there are technical problems in assessing small amounts of fibre digestion at this level in the gut and it may be more fruitful to consider the cellulolytic capabilities of the gastric flora in vitro. It seems unlikely that gastric digestion of fibre would have any marked nutritional significance for the pig, other than as a preliminary processing prior to fermentation in the more distal portions of the gut.

MICROBIAL ACTIVITY IN THE SMALL INTESTINE

The microflora

The small intestine contains high numbers of bacteria which are disproportionately distributed with a larger population in the lower portion than the upper. This may be related to the rates of passage of digesta through these sections (Savage, 1977). Streptococci, lactobacilli and coliforms become established in large numbers (10^{7-9} per g digesta in the lower ileum) after birth and remain throughout life. In the suckling pig, the predominant strains of streptococci can ferment lactose whereas after weaning S. equinus, which does not ferment lactose, becomes predominant (Fuller, 1962). Clostridium perfringens is present in large numbers prior to weaning but disappears thereafter as a result of its intolerance of high levels of linoleic and arachidonic acid (Fuller and Moore, 1967).

Bacteroides are the most numerous anaerobic genus throughout the small intestine and, after the first week of life, Veillonella also occur. In the lower ileum Sphaerophorus can be isolated and Peptostreptococcus are found after 180 d of age (Fuller and Lev, 1964; Fuller, 1966).

Fermentation of carbohydrate and dietary fibre

Acetic, formic, propionic, butyric and lactic acids have been found throughout the small intestines of young, growing pigs (Friend et al, 1963; Argenzio and Southworth, 1975). Lactate predominated and acetate constituted the greater proportion of VFA. It is difficult to quantify the production and absorption of these acids and the amount present in the digesta simply reflects

the difference between them. Some organic acid may be contributed directly by the digesta emptying from the stomach into the small intestine.

Although it has been shown that dietary fibre may be digested to some extent it is likely that this is limited because of the rapid passage of digesta through this portion of the alimentary canal (Keys and DeBarthe, 1974). Sugars, which are readily available, are the more likely substrates for fermentation.

Lipid metabolism

The microflora of the small intestine affects the metabolism of lipids in the host both directly, by the action of bacterial lipases on dietary and endogenous lipids, and indirectly by biohydrogenation of fatty acids, deconjugation of bile acids and modifications to cholesterol metabolism.

Little work has been done on the effects of the gut bacteria on digestion and absorption of lipids in the pig but evidence from other species suggests that the flora reduces the amount of dietary lipid available to the host (Yoshida et al, 1968; Boyd and Edwards, 1967). Rats receiving a diet containing 133g/kg lipid had a lipid digestibility of 0.68 whereas for germ-free rats the figure was 0.73 (Demarne et al, 1970). Work with pigs (90 kg) fed diets with or without antibiotic supplements, to suppress the effects of the flora, has shown similar improvements in fat digestibility in the absence of microbial influences. It was suggested that this was largely due to fat synthesis by the bacteria in the large intestine and, in fact, for the pigs treated with antibiotic there was a net absorption of fat from the hind-gut (Mason and Just, 1976).

Fatty acid absorption and, indeed, determination of fatty acid digestibility are considerably affected by the bacterial biohydrogenation of unsaturated fatty acids (Eyssen et al, 1973) which also occurs in the hind-gut. This results in an increase in the proportion of stearic acid and a decrease in the proportion of unsaturated fatty acids. There is some evidence to suggest that stearic acid is less well absorbed than the unsaturated fatty acids (Combe et al, 1976).

Bile acid metabolism is significantly altered by the gut bacteria (Eyssen, 1973). Free bile acids, produced from

cholesterol in the liver, are conjugated usually with taurine or glycine or possibly with sulphate or glucuronide. These primary bile acids are readily deconjugated by intestinal lactobacilli to form secondary bile acids (Gilliland and Speck, 1977). These can be less active in forming micelles, or less soluble, and tertiary bile acids, formed by an interaction between the gut flora and hepatic enzymes can be toxic. Eyssen et al (1975) examined the proportions of bile salts in the bile and faeces of germ-free and conventional piglets which were receiving a milk diet (Table 2). The results demonstrated that the gut bacteria produced changes in the proportions of bile acids even in the gall bladder via the cycling of secondary bile acids. This was confirmed by Wostmann et al (1979) who demonstrated, in a similar study, that in the germ-free piglet the cycling of bile acids was extremely efficient compared to conventional pigs which excreted more bile acids in faeces. This implies that the secondary bile acids are less well absorbed.

Table 2. Proportions of bile acids in the bile and faeces of germ-free (GF) and conventional (CV) pigs at 6 weeks of age. (From Eyssen et al, 1975).

		Bile Acids			
		chenodeoxy -cholic	hyocholic	hyodeoxy -cholic	litho-cholic
Bile	CV	0.12	0.47	0.41	trace
	GF	0.21	0.74	0.05	-
Faeces	CV	0.06	0.10	0.64	0.16
	GF	not different from bile			

Bile acid metabolism is intricately involved with the metabolism of cholesterol since this is used for the synthesis of bile salts. Evidence from germ-free rats leads to the conclusion that intestinal microorganisms increase the catabolism of cholesterol and interfere with its absorption (Van Eldere and Eyssen, 1984). But the mechanism for this is not understood and has been insufficiently investigated in pigs.

Nitrogen metabolism

Digestion and absorption of protein occurs rapidly in the small intestine as a result of the production of large quantities of endogenous enzymes and the large epithelial surface area. Bacteria in the gut have to compete with the enzymes of the host for nitrogen compounds in the diet. Urea diffuses from the tissues into the lumen and is a ready source of N to those bacteria equipped with urease. Microorganisms break down N compounds and produce ammonia and, if there is a source of fermentable energy, then the N may become incorporated into bacterial cells (Mason, 1974). The enzymic secretions of the host, mucin and sloughed epithelial cells form additional sources of N for microbial metabolism. The influence of the enteric bacteria on protein digestion is, however, likely to be small and the course of protein digestion in the small intestines of germ-free (GF) and conventional (CV) animals is similar (Salter, 1984) but this is not true for the large intestine.

Effects on endogenous enzymes

The microflora has the potential to digest many N compounds and the enzymes of the host are not spared. The ability of the flora to inactivate enzymes has been noted by Cheredkova and Nikitin (1970). Szabo (1979) compared the enzymic activity of germ-free and conventional piglets fed on a milk formula. Total proteolytic activity was studied along with the activity of 3 peptidases, α-amylase, lactase, cellobiase, saccharase, maltase and lipase. He found that enzyme activity in the pancreas was essentially the same in both groups. But the activity of the peptidases and disaccharidases associated with the intestinal mucosa was higher in GF than CV pigs. Also, the activity of peptidases and disaccharidases in the large intestine was found to be higher in GF pigs. This indicates that there may be direct or indirect effects of the flora on enzymes in the epithelial cells and also demonstrates bacterial inactivation of enzymes in the digestive tract. Interestingly, the CV pigs had elevated levels of lactase and lipase activity in the large intestine which was probably of microbial origin.

MICROBIAL ACTIVITY IN THE LARGE INTESTINE

The microflora

In mature pigs, the hind-gut contains vast numbers of microorganisms and they are concentrated in a greater density than in any other portion of the digestive tract (Decuypere and Van der Heyde, 1972). The flow of digesta is slow enough to permit microbial multiplication and extensive metabolic activity. The faecal flora tends to be very similar in composition to that of the colon but it is probably not representative of the caecum. Strict anaerobes dominate the flora which is present both in the lumen and attached to the intestinal epithelium (Harris and Kinyon, 1974; Allison et al, 1979).

Robinson et al (1981) have identified the most numerous Gram-negative species in the caecal flora as Bacteriodes ruminicola (35%), Selenomonas ruminantium (21%), Butyrivibrio fibrisolvens (6%) and Bacteroides uniformis (3%). The Gram-positive species were Lactobacillus acidophilus (7.6%), Peptostreptococcus productus (3%) and Eubacterium aerofaciens (2.5%). This contrasts with the faecal flora described by Salanitro and co-workers (1977) where the Gram-positive species, including Streptococcus and Eubacterium mainly, made up 90% of the flora. Similar results were obtained from the colon by Russell (1979).

Because of the technical difficulties in culturing and identifying the microbes of the large bowel, particularly those attached to the epithelium of the gut (Russell, 1979), it has not been possible to ascertain the roles of particular species of bacteria in the overall microbial activity of the hind-gut. But attempts have been made to look at specific metabolic attributes in vitro (Allison et al, 1979).

Digestion and fermentation

It is outside the aims and wishes of the author to attempt to review the processes of digestion and fermentation in the hind-gut in detail since this subject has been exhaustively reviewed in the recent past (Cranwell, 1968; Rerat, 1978; Kidder and Manners, 1978; Mason, 1980; ARC, 1981) and the specific aspects of nitrogen metabolism (Mason, 1978. 1984; Zebrowska, 1982) and VFA production

and absorption (Argenzio, 1982) have been discussed in depth. Nevertheless, several points need to be clarified or stressed.

In terms of the nutrition of the mature pig, the process of digestion, fermentation and the production of VFA in the large bowel is probably the single most significant contribution from the microflora. But it is not clear exactly how much energy is contributed. Certainly, in the young growing pig, there appears to be very little energy made available for live weight gain from the products of fermentation. But it has been estimated that as much as 30-40% of a sow's maintenance requirements could be met by fermentation products (Livingstone, 1983) although this has not been convincingly demonstrated in practice.

Organic acids are the end-products of a series of reactions in microbial metabolism at all levels in the gastro-intestinal tract. The substrate for this metabolism can be any of the components of the digesta entering the large intestine through the ileo-caecal valve. Undigested dietary material including fibre may be the major component, depending on diet composition, but significant quantities of endogenous material and dead and living bacterial cells will also be present. The energy yielding processes, somewhat simplified in Table 1, enable some bacteria to benefit from metabolic steps carried out by other bacteria including predigestion to glucose or pyruvate or the production of lactate which is an end-product for one type of microorganism but can then be metabolized by another.

Aspects of dietary fibre digestion and the uptake and utilization of VFA have been discussed by Low in the accompanying review. As long ago as 1942, Vartiovaara and Roine demonstrated the digestion of cellulose as a result of incubation with caecal contents *in vitro*. A Gram-positive, anaerobic coccus was isolated and it produced butyric and acetic acid *in vitro*. Cultures of this organism, when fed to pigs, improved the digestibility of cellulose from 0.5 to over 0.8 (Vartiovaara *et al*, 1944; Vartiovaara, 1947). This early work illustrates the potential for improving the digestive capacity of the large intestine but it gives little idea of how much energy becomes available to the pig for its own metabolic processes as a result. Evidence from work with growing pigs shows considerable digestion of fibre but little apparent benefit for the pig itself (Partridge *et al*, 1982).

Horszczaruk and Sljivovacki (1966, 1971) found that digestion of purified cellulose or lucerne meal, suspended in silk bags, in the large intestine, occurred mainly in the caecum rather than the colon. This could be related to the differences between the populations of bacteria in these sites, as discussed above. The age of pig was an important factor for fibre digestion since it occurred more extensively in 18 month old pigs than in those of 4 months of age. Also, digestibility of purified cellulose was better than for lucerne meal cellulose. These findings are in line with the current consensus which holds that fibre digestibility varies depending on botanical source (chemical nature of the fibre, degree of lignification etc.), degree of processing, the age of the pig and the time allowed for the digestive system (including the flora) to adapt to the diet.

The effects of the microflora on lipid metabolism are similar to those discussed for the small intestine but much more extensive. Cholesterol, dietary sterols, bile acids and lipids are considerably altered by microbial action. It has been shown that cholesterol can be hydrogenated to coprostanol and this can constitute 50 % of the total faecal sterols in rats (Kellogg, 1973). The extent and significance of such changes in pigs have not been fully elucidated.

As stated above, Mason and Just (1976) found marked effects of the gut flora on apparent digestibility of lipid and this was accompanied by considerable changes in the proportions of faecal fatty acids which was partly associated with the biohydrogenation of unsaturated fatty acids in the hind-gut. It is well to consider the scale of these changes when attempting to assess digestibilities of lipids from faecal samples.

Similarly, there are many difficulties in accurately assessing nitrogen digestibility. Proteins, peptides, amino acids and other N compounds from dietary, endogenous and bacterial sources are subject to bacterial action (Michel, 1966) and ammonia or amines are produced. Some of these products can be absorbed by the pig and the N derived from them excreted in the urine (Zebrowska, 1973) or possibly used for the synthesis of non-essential amino acids for incorporation into body tissues (Deguchi *et al*, 1978, 1980). It has been suggested that, in cases of marginal N intake,

such mechanisms may help to conserve body N by recycling endogenous N from the gut (Mason, 1984). Also, some of the N products of catabolism become incorporated into bacterial cells. One of the consequences of increased intake of fermentable substrates is a decrease in the apparent digestibility of N which is probably due, in part, to microbial multiplication and the increased output of bacterial N (Mason et al, 1976, 1982). Because of changes in the pattern of N excretion, the study of N digestibility in GF pigs does not appear to simplify the problems (Ratcliffe and Low, 1985).

INVOLVEMENT OF THE MICROFLORA IN ENTERIC DISEASE

The mature pig has a characteristic stable microflora which is unaffected by small dietary changes (Smith, 1965; Speck et al, 1970; Fuller, 1982). Strictly anaerobic bacteria are reported to be the most important in maintaining this stability (Savage, 1982). Microorganisms, many of which may be potential pathogens, constantly enter the system in the ingesta (food, water, faecal matter etc.). These microbes do not usually persist in the tract because of the competition offered by the members of the indigenous flora which are obviously well adapted to defend their particular niches. The results of studies with gnotobiotic animals has indicated that many of the bacteria interact to produce a synergistic resistance to invading organisms (Savage, 1977). So the microflora offers the pig a modicum of protection against enteric disease and this has become known as 'the barrier effect' or 'colonization resistance' (Van der Waaij et al, 1971). If the balance of the microflora is upset by environmental changes such as antibiotic treatment, drastic qualitative or quantitative alterations to feed intake or possibly stress, then the resulting changes in the microecology of the gut may permit a pathogenic organism to become established in sufficient number to cause disease.

It is well known, in research and farming practice, that the young pig is susceptible to enteric disorders, diarrhoea and early mortality at the time of weaning and these problems are more

prominent the earlier the pig is weaned. About the time of weaning, the piglet suffers from a decline in its passive immunity and its active immunity may not be completely developed. Also, dietary changes are imposed on the young pig, gradually in the case of the increasing intake of creep feed and then abruptly at weaning. This may also be accompanied by a change of housing. These factors alter the status quo of the immature flora which is developing and not well established and as a result the barrier effect may be diminished and pathogens may proliferate.

Swine dysentery has often been associated with the presence of large numbers of E.coli in the digestive tract (Sojka, 1965; Sinkovics, 1974). In an attempt to limit the numbers of intestinal coliforms and thereby reduce the incidence of disease, several workers have tried to enhance or supplement the barrier effect by feeding cultures of lactobacilli or streptococci (Muralidhara, 1974; Varga and Meszaros, 1974; Barrow et al, 1980; Underdahl et al, 1982). This is part of the principle of commercially-prepared 'probiotics' which may also be directed towards diminishing the effects of growth-depressing bacteria.

THE DEPRESSION OF GROWTH BY THE GUT FLORA

Jukes et al (1950) demonstrated that aureomycin incorporated in the diet of young pigs (approximately 15 kg live weight) produced marked improvements in average daily gain compared with control animals. The amount of antibiotic was too small to have a significant effect on any disease state so it was deduced that microorganisms in the gut had the capacity to depress growth. This seems to be because of effects on the efficiency of feed conversion and not on feed intake. Pigs, growing between 26 and 80 kg and receiving tylosin in the diet (20 mg/kg), had an average daily gain of 0.63 kg compared with 0.60 kg for controls ($P<0.001$) but average daily feed intake was 1.96 kg in both cases (Jones and Tarrant, 1982). This difference in efficiency between the two groups implies that gut bacteria interfere adversely in the processes of digestion and absorption.

Promotion of rapid growth using antibiotic or antibacterial agents has been the subject of many experiments over the last 30

years. But, despite this, the exact mode of action of such substances is still not understood.

Fuller and his colleagues have identified a strain of Streptococcus faecium which has been shown to depress growth in germ-free chickens and this effect was negated by dietary supplements of antibiotic (Fuller et al, 1979, 1983; Houghton et al, 1981).

It is unlikely that the same microorganism is involved in limiting weight gain in pigs. In fact, strains of S.faecium have been used as probiotic agents to help prevent colibacillosis and improve performance in pigs (Underdahl et al, 1982). There have been no documented attempts to identify and characterize the microorganisms which exert growth depressing effects in the pig.

Henderickx and Decuypere (1972) found that ammonia levels in the caeca of young pigs receiving supplements of virginiamycin or spiramycin were lowered to about half that of controls (Table 3).

Table 3. NH_3 (mmol/100g digesta) in the large intestines of young pigs with dietary supplements of viginiamycin (V) or spiramycin (S) compared with controls (C). (Henderickx and Decuypere, 1972)

Treatment	Caecum	Colon
C	3.11	2.06
V	1.50	1.47
S	1.55	0.99

Evidence such as this has led some authors to suggest that the effects of antibiotics on growth are mediated through effects on the particular bacteria involved in urea-ammonia metabolism in the gut (Francois and Michel, 1955; Visek, 1962, 1982). Part of the action of such agents may be to reduce the production of bacterial urease and so reduce the load of ammonia on the tissues of the pig. High levels of ammonia in the body are known to cause metabolic disturbances and it has been postulated that this may also limit growth in some way. Visek has been a proponent of this view and he has reviewed the subject in depth (1978, 1982). But the correlation between low intestinal ammonia and antibiotic treatment does not automatically imply a causative link with the promotion of growth.

Other possible mechanisms for the promotion of growth by

antibiotics include:
(a) Improved absorption of nutrients as a result of a reduction in gut wall thickness.
(b) Reduction of general microbial action in the gut, leading to improved lipid absorption, increased availability of essential nutrients and vitamins.
(c) Reduction of the level of microbial toxins which affect gut motility and may cause a low grade toxaemia in the pig.
(d) Damage to bacterial cell walls so reducing metabolic capabilities.
(e) Improved absorption as a result of increased intestinal alkaline phosphatase activity.

There is considerable scope for further work in this area and any combination of these mechanisms may be involved. These hypotheses have been discussed more fully by Hays (1969), Wallace (1970), Walton (1982), Henderickx et al (1980) and Radisson (1982).

PIG MODELS IN BIOMEDICAL RESEARCH

Pigs have increasing importance as animal models because of their many anatomical and physiological similarities to man (Glauser, 1966; Pond and Houpt, 1978; Dodds, 1982). Many branches of biomedical science, including immunology, haematology and dermatology, use the pig as a model. It is well fitted for use as a model in the study of nutrition and gastro-intestinal physiology. Its size and nature allows repeated collections of samples of blood or excreta and numerous surgical interventions and modifications have already been developed. Protein digestibilities of mixed diets have been shown to be similar for pigs and man (Forsum et al, 1981) and the release of gut hormones following a meal is qualitatively similar (Adrian and Bloom, 1981). The time taken for digesta to travel through the intestinal tract is very similar in the two species with transit times of about 2-4 hours for the small intestine and around 20-50 hours for the large intestine. There is little comparative evidence, however, to confirm that transit times are the same on similar diets.

With respect to the gut microflora, the pig is certainly better placed than many of the small mammalian species which have been studied historically. Rodentia and Lagomorpha habitually practise coprophagy and, in the germ-free state, suffer from enormously enlarged caeca (Coates, 1975) which causes difficulties in the interpretation of experimental results. Germ-free pigs do not show such marked abnormalities of the gut. In gnotobiotic isolators, pigs can be innoculated with one or more species of bacteria or even with a human microflora. Antibiotic treatment, for the suppression or elimination of gut fora effects, can be used when germ-free facilities are not available (Eggum et al, 1982; Mason et al, 1982).

Surgical modifications, using suitably sited multiple or re-entrant cannulas, allow the sampling of digesta and microflora in otherwise inaccessible sections of the gut (Low, 1980). Techniques for deriving and rearing gnotobiotic piglets have been reviewed by Miniats (1984) and these allow direct comparison of animals with and without enteric microorganisms.

In many cases, research with germ-free pigs may have been avoided because they have to be derived de novo for each experiment. Until recently, this has necessitated hysterectomy or hysterotomy and this can be both complicated and expensive with the additiional disadvantage that piglets may be obtained in a premature condition. A method has been developed for rearing germ-free piglets, obtained without operative surgery after normal parturition, (Ratcliffe and Fordham, 1984) based on a technique introduced by Ducluzeau et al, (1976). No surgical expertise is required and germ-free piglets can be maintained for long periods at relatively low cost. This should facilitate further study of the role of the gut microflora in digestion and metabolism for the pig per se and as a model for man.

There is increasing interest in the role of the gut microflora in diseases of man, particularly those which seem to have a nutritional interaction such as gastro-intestinal cancer. Microbial action produces nitrosamines and nitrosamides from nitrate or nitrite and secondary amines and these compounds are implicated as potent carcinogens or co-carcinogens (Drasar and Hill, 1974). Low pH in the caecum has been shown to have

potentially beneficial effects on bile salt-cholesterol metabolism (Eyssen et al, 1974) and is correlated with changes in urea-ammonia metabolism (Vince et al, 1978). The inclusion of suitable carbohydrate substrates, such as fibre or lactulose, in the diet promotes fermentation in the caecum and so lowers pH via the bacterial production of organic acids (Hill and Drasar, 1975).

These and other interactions are the subject of much current research but how useful is the pig to such studies? The flora in the porcine gut is similar to the human in a number of respects. Both have high levels of lactobacilli and coliforms (predominantly E.coli) in the ileum and large intestine. Of the strictly anaerobic microorganisms, representatives of the Gram-negative genera Bacteroides, Fusobacterium and Veillonella, and the Gram-positive genera Eubacterium, Propionibacterium, Bifidobacterium and Peptostreptococcus can commonly be found in the faeces of man and pig (Raibaud et al, 1982).

Unfortunately, bacteriologically, the stomach and proximal small intestine of the pig differ in a major respect from those of man. The stomach, duodenum and jejunem of pigs contain large numbers of bacteria, including lactobacilli and streptococci, some of which are intimately associated with or attached to the mucosal epithelium (Fuller et al, 1978; Barrow et al, 1980). In contrast, the stomach and upper small intestine of man is usually sterile. Occasionally, microorganisms have been found in the human duodenum or jejunem but usually at very low levels (Anderson et al, 1974). It has been suggested that the sterility of the human stomach is maintained by the low gastric pH (Hill, 1982) but it is not clear if man and pig differ in this respect because of the unreliable nature of the measurement of acidity which may show variation due to diet, time after eating and localized changes in pH within the stomach.

Despite these differences, the pig has proved to be a good model for studies on the medical aspects of dietary fibre (as discussed by Low in the accompanying review) and fermentation in the human gut (Fleming and Wasilewski, 1984).

CONCLUSIONS AND PROSPECTIVE VIEWS

It is not altogether clear whether or not the advantages of

harbouring a microflora outweigh the disadvantages. Pasteur (1885), as stated earlier, believed that life would become impossible without microorganisms whereas Metchnikoff (1907) took the opposite view and held that the gastro-intestinal flora was basically deleterious to the well-being of the host. The truth lies somewhere between these two tenets.

Energy gained from hind-gut fermentation must be balanced against dietary energy and nitrogen utilized by the bacteria and bound up in cells which escape digestion and are voided more or less intact in the faeces. Lipid metabolism is disturbed and the pattern and cycling of bile acids in the gut is altered by the presence of the microflora. The micronutrients synthesized by the bacteria must also be offset against dietary minerals and vitamins which are utilized or altered and so made unavailable to the pig. The promotion of growth using antibiotics demonstrates that, under certain conditions, the gut flora limits rapid growth, probably because of adverse effects on the efficiency of digestion and absorption. But antibiotic administration to animals receiving 'bulky' feeds may limit digestion and fermentation in the hind-gut and, by supposition, the energy available to the host. Disturbances to the ecological balance of the microflora are implicated in the aetiology of enteric disease in the young pig.

It seems likely that the net effect of the microflora on the nutrition and metabolism of the pig will depend on a number of factors including age, type of diet, bacterial status of the diet, cleanliness of housing, the presence of clinical or sub-clinical disease and the immunological status of the pig.

Manipulation of the gut microflora, its microecology or metabolism by altering dietary components, supplementing diets with specific antibacterial substances or using 'probiotic' agents has great potential for controlling enteric disease, increasing digestibility and utilization of feeds and possibly improving performance and so, the economics of pig production. Further information on the extent to which microorganisms are involved in the digestion and metabolism of all dietary constituents and on the individual strains which compose the microflora and their specific metabolic activites may facilitate the tailoring of diets and feeding systems to maximize the advantages of the flora and minimize the disadvantages.

As genetic engineering develops, it may become possible to alter the activities of the flora to complement nutritional requirements by supplying specifically engineered bacteria to the gut.

The main drawback to this optimistic thinking, is that bacteria which are modified genetically may spontaneously lose the acquired characteristic or mutate. Also, exotic species of bacteria, introduced to the gut, would have to survive the acid environment of the stomach in sufficient numbers to pass into the small and large intestines and, unless they had the necessary attachment factors for establishment on the mucosal epithelia, would be rapidly lost from the alimentary canal. The members of the indigenous flora compete strongly for their particular niche in the intestinal microenvironment and will not be readily dislodged by 'invading' microorganisms.

If these problems can be overcome, it could be feasible to produce pre-weaning feeds which contain bacteria with beneficial enzymic activity. This could allow the young pig to utilize feeds which are poorly digested at present. Additionally, it may be possible to promote fermentation, by seeding the gut with suitably engineered cellulolytic bacteria, and increase the energy available from this process for more mature animals.

REFERENCES

ADRIAN, T.E. & BLOOM, S.R. 1981. In "Nutrition in Health and Disease and International Development:Symposia from the XII International Congress of Nutrition". (Eds. A.E. Harper and G.K. Davis.) A.R. Liss, Inc:New York. p.873-882.

ALLISON, M.J., ROBINSON, I.M., BUCKLIN, J.A. & BOOTH, G.D. 1979. Appl.Environ.Microbiol. 37:1142-1151.

ANDERSON, C.M., CHALLACOMBE, D.N. & RICHARDSON, J.M. 1974. In "The Normal Microbial Flora of Man". (Eds. F.A. Skinner & J.G. Carr.) Academic Press:London p.197-203.

ANDERSSON, S. 1967. In "Handbook of Physiology". Section 6.II. (Ed. C.F. Code) Am.Phys.Soc:Washington DC. p.866-867.

ARC. 1981. "The Nutrient Requirements of Pigs. CAB:Slough.

ARGENZIO, R.A. 1982. In "Digestive Physiology in the Pig". (Eds. J.P. Laplace, T. Corring & A. Rerat) INRA:Paris. p.207-215.

ARGENZIO, R.A. & SOUTHWORTH, M. 1975. Am.J.Physiol. 228:454-460.

BALDWIN, R.L. 1970. Am.J.Clin.Nutr. 23:1508-1513.

BARROW, P.A. 1978. Ph.D. Thesis Univ. Reading.

BARROW, P.A., FULLER, R. & NEWPORT, M.J. 1977. Infect.Immun. 18:586-595.

BARROW, P.A., BROOKER, B.E., FULLER, R. & NEWPORT, M.J. 1980. J.appl.Bact. 48:147-154.

BOYD, F.M. & EDWARDS, H.M. 1967. Poult.Sci. 46:1481-1483.

BRIN, M. 1965. Ann.N.Y.Acad.Sci. 119:942-954.
COATES, M.E. 1975. Lab.Anim. 9:275-282.
COMBE, E., DEMARNE, Y., GUEGUEN, L., IVOREC-SZYLIT, O., MESLIN, J.C. & SACQUET, E. 1976. Wld.Rev.Nutr.Diet. 24:1-57.
CHEREDKOVA, A.N. & NIKITIN, Y.I. 1970. Uchan.Zap.Vitebsk.vet.Inst. 22:138.
CHRISTIE, A. & CRANWELL, P.D. 1976. Proc.Nutr.Soc. 35:27A.
CLEMENS, E.T., STEVENS, C.E. & SOUTHWORTH, M. 1975. J.Nutr. 105:759-768.
CRANWELL, P.D. 1968. Nutrition Absts.Revs. 38: 721-730.
CRANWELL, P.D. & TITCHEN, D.A. 1974. Res.Vet.Sci. 16:105-107.
CRANWELL, P.D., NOAKES, D.E. & HILL, K.J. 1976. Br.J.Nutr. 36:71-86.
DECUYPERE, J. & VAN DER HEYDE, H. 1972. Zbl.Bakt.Hyg., I.Abt.Orig. A221:492-510.
DEGUCHI, E., NIIYAMA, M., KAGOTA, K. & NAMIOKA, S. 1978. J.Nutr. 108:1572-1579.
DEGUCHI, E., NIIYAMA, M., KAGOTA, K. & NAMIOKA, S. 1980. Am.J.Vet.Res. 41:212-214.
DEMARNE, Y., SACQUET, E., FLANZY, J., GARNIER, H. & FRANCOIS, A.C. 1970. Ann.Biol.anim.Bioch.Biophys. 10:369-384.
DODDS, W.J. 1982. Fed.Proc. 41:247-256.
DRASAR, B.S. & HILL, M.J. 1974. In "Human Intestinal Flora". Academic Press:London.
DUCLUZEAU, R., & RAIBAUD, P. 1979. "Ecologie microbienne du tube digestif". INRA, Actualities Scient.Agron. 2.
DUCLUZEAU, R., RAIBAUD, P., LAUVERGEON, B., GOUET, PH., RIOU, Y., GRISCELLI, C. & GHNASSIA, J.C. 1976. Can.J.Microbiol. 22:563-566.
DUNLOP, R.H. & HAMMOND, P.H. 1965. Ann.N.Y.Acad.Sci. 119:1109-1130.
EGGUM, B.O., THORBEK, G., BEAMES, R.M. CHWALIBOG, A. & HENCKEL, S. 1982. Br.J.Nutr. 48:161-175.
EYSSEN, H. 1973. Proc.Nutr.Soc. 32:59-63.
EYSSEN, H., DE PAUW, G. & DE SOMER, P. 1973. In "Germfree Research-Biological Effects of Gnotobiotic Environments". (Ed. J.B. Heneghan) Academic Press:New York. p.277-283.
EYSSEN, H., SMETS, L. & BRASSINE, M. 1975. In "Abstracts of the Vth Int.Symp.Gnotobiology". Karolinska Institute:Stockholm.
FLEMING, S.E. & WASILEWSKI, M.M. 1984. Nutr.Rep.Int. 30:825-834.
FORSUM, E., GORANZON, H., RUNDGREN, M., THILEN, M. & HAMBRAEUS, L. 1981. Ann.Nutr.Metab. 25:137-150.
FRANCOIS, A.C. & MICHEL, M. 1955. Compt.Rend.Acad.Sci. 240:124-126.
FRIEND, D.W., CUNNINGHAM, H.M. & NICHOLSON, J.W.G. 1963. Can.J.Anim.Sci. 43:156-168.
FULLER, R. 1962. Ph.D.Thesis Univ.Reading.
FULLER, R. 1966. J.appl.Bact. 29:375-379.
FULLER, R. 1982. Advances Vet.Med. 33:7-15.
FULLER, R. & LEV, M. 1964. J.appl.Bact. 27:434-438.
FULLER, R. & MOORE, J.H. 1967. J.gen.Microbiol. 46:23-41.
FULLER, R., BARROW, P.A. & BROOKER, B.E. 1978. Appl.Environ.Microbiol. 35:582-591.
FULLER, R., COATES, M.E. & HARRISON, G.F. 1979. J.appl.Bact. 46:335-342.
FULLER, R., HOUGHTON, S.B. & COATES, M.E. 1983. Br.Poult.Sci. 24:111-114.
FULLER, R., NEWLAND, L.G.M., BRIGGS, C.A.E., BRAUDE, R. & MITCHELL, K.G. 1960. J.appl.Bact. 23:195-205.
GILLILAND, S.E. & SPECK, M.L. 1977. Appl.Environ.Microbiol. 33:15-18.

GLAUSER, E.M. 1966. Exp.Med.Surg. 24:181-190.
GORDON, H.A. & PESTI, L. 1971. Bacteriol.Rev. 35:390-429.
HARRIS, D.L. & KINYON, J.M. 1974. Am.J.Clin.Nutr. 27:1297-1304.
HAWKSWORTH, G., DRASAR, B.S. & HILL, M.J. 1971. J.Med.Microbiol. 4:451-459.
HAYS, V.W. 1969. Nat.Acad.Sci. Pub.1967.
HENDERICKX, H.K. & DECUYPERE, J.A. 1972. In "Germfree Research-Biological Effect of Gnotobiotic Environments". (Ed. J.B. Heneghan) Academic Press:New York. p.361-368.
HILL, M.J. 1982. Advances Vet.Med. 22:32-38.
HILL, M.J. & DRASAR, B.S. 1975. Gut. 16:318-323.
HORSZCZARUK, F. & SLJIVOVACKI, K. 1966. Roczn.Nauk. roln. 89B(1):67-71.
HORSZCZARUK, F. & SLJIVOVACKI, K. 1971. Rocz.Nauk. roln. 93B(1);143-147.
HOUGHTON, S.B., FULLER, R. & COATES, M.E. 1981. J.appl.Bact. 51:113-120.
JONES, P.W. & TARRANT, M.E. 1982. Anim.Proc. 34:115-121.
JUKES, T.H., STOKSTAD, E.L.R., TAYLOR, R.R., CUNHA, T.J., EDWARDS, H.M. & MEADOWS, G.B. 1950. Arch.Biochem. 26:324-325.
KELLOGG, T.F. 1973. Lipids. 8:658-659.
KEYS, J.E. & DEBARTHE, J.V. 1974. J.Anim.Sci. 39:53-56.
KIDDER, D.E. & MANNERS, M.J. 1978. In "Digestion in the Pig". Scientechnica:Bristol.
KUTAS, F. & SZABO, J. 1974. Acta Vet.Acad.Sci.Hung. 24:133-138.
LIVINGSTONE, R.M. 1983. Pig Farming. November p.70-73.
LOW, A.G. 1980. In "Current Concepts of Digestion and Absorption in Pigs". (Eds. A.G. Low and I.G. Partridge) NIRD:Reading. Tech.Bulletin 3. p.10-19.
MASON, V.C. 1974. Gasterenterology. 66:1271.
MASON, V.C. 1978. 3rd World Congress on Animal Feeding. p.197-210.
MASON, V.C. 1980. In "Current Concepts of Digestion and Absorption in Pigs. (Eds. A.G. Low and I.G. Partridge) NIRD:Reading. Tech. Bulletin 3. p.112-129.
MASON, V.C. 1984. Proc.Nutr.Soc. 43:45-53.
MASON, V.C. & JUST, A. 1976. Z.Tierernahrg.u.Futtermittelkde. 36:301-310.
MASON, V.C., KRAGELUND, Z. & EGGUM, B.O. 1982. Z.Tierphysiol.Tierernahrg.u.Futtermittelkde. 48:214-252.
METCHNIKOFF, E. 1907. "The Prolongation of Life". Heinemann:London.
MICHEL, M.C. 1966. Ann.Biol.anim.Biochim.Biophys. 6:33-46.
MINIATS, O.P. 1984. In "The Germ-free Animal in Biomedical Research". (Eds. M.E. Coates and B.E. Gustafsson.) Laboratry Animals Ltd:London. p.47-78.
MURALIDHARA, K.S. 1974. Diss.Abstr.Int. 34B:6125-B.
PARTRIDGE, I.G., KEAL, H.D. & MITCHELL, K.G. 1982. Anim.Prod. 35;209-214.
PASTEUR, L. 1885. C.r.hebd.Seanc.Acad.Sci. 100:68.
POND, W.G. & HOUPT, K.A. 1978. "Biology of the Pig". Comstock Publ.Assoc:New York.
RADISSON, J. 1982. Advances Vet.Med. 33;91-95.
RAIBAUD, P., CAULET, M., GALPIN, J.V. & MOCQUOT, G. 1961. J.appl.Vact, 24:285-306.
RAIBAUD, P., DUCLUZEAU, R., MOREAU, C., HUDAULT, S., NICOLAS, J.D. & DUBOS, F. 1982. Advances Vet.Med. 33:15-22.
RATCLIFFE, B. & FORDHAM, J.P. 1984. Lab.Anim. 18:337.
RATCLIFFE, B. & LOW, A.G. 1985. In "Proceedings of VIIIth Int.Symp.Gnotobiology". A.R. Liss, Inc:New York.

RERAT, A. 1978. J.Anim.Sci. 46:1808-1837.
ROBINSON, I.M., ALLISON, M.J. & BUCKLIN, J.A. 1981. Appl.Environ.Microbiol. 41:950-955.
RUSSELL, E.G. 1979. Appl.Environ.Microbiol. 37:187-193.
SALANITRO, J.P., BLAKE, I.G. & MUIRHEAD, P.A. 1977. Appl.Environ.Microbiol. 33:79-84.
SALTER, D.N. 1984. In "The Germ-free Animal in Biomedical Research". (Eds. M.E. Coates and B.E. Gustafsson) Laboratory Animals Ltd:London. p.235-263.
SAMBROOK, I.E. 1980. In "Current Concepts of Digestion and Absorption in Pigs". (Eds. A.G. Low and I.G. Partridge) NIRD:Reading. Tech.Bulletin 3. p.78-93.
SAVAGE. D.C. 1977. Ann.Rev.Microbiol. 31:107-133.
SAVAGE, D.C. 1982. Advances Vet.Med. 33:23-31.
SINKOVICS, G. 1974. Zbl.Vet.Med.B. 21:706-714.
SISSONS, M.J., CRANWELL, P.D. & BELL, A.W. 1983. Proc.Nutr.Soc. 42:67A.
SMITH, H.W. 1965. J.Path.Bact. 89:95-122.
SOJKA, W.J. 1965. "Escherichia coli in Animals". CAB Review #7.
SPECK, R.S., CALLOWAY, D.H. & HADLEY, K. 1970. Am.J.Clin.Nutr. 23:1488-1494.
SZABO, J. 1979. In "Clinical and Experimental Gnotobiotics". (Eds. T. Fliedner, H. Heit, D. Niethammer and H. Pflieger) Zbl.Bakt.Suppl.7. Gustav Fischer Verlag:Stuttgart. p.125-128.
TANNOCK, G.W. & SMITH, J.M.B. 1970. J.Comp.Path. 80:359-367.
UNDERDAHL, N.R., TORRES-MEDINA, A. & DOSTER, A.R. 1982. Am.J.Vet.Res. 43:2227-2232.
VAN DER WAAIJ, D., BERGHUIS-DE-VRIES, J.M. & LEKKERKERK, J.E.C. 1971. J.Hygiene. 69:405-411.
VAN ELDERE, J. & EYSSEN, H. 1984. In "The Germ-free Animal in Biomedical Research". (Eds. M.E. Coates and B.E. Gustafsson) Laboratory Animals Ltd:London. p.317-332.
VARGA, J. & MESZAROS, J. 1974. Magyar.Allatorv.Lapha. 29:301.
VARTIOVAARA, U. 1947. Proc.4th.Int.Congr.Microbiol.Copenhagen. p.528-529.
VARTIOVAARA, U. & ROINE, P. 1942. J.Sci.Agric.Soc.Finland. 14:127.
VARTIOVAARA, U., ROINE, P. & POIJARVI, I. 1944. J.Sci.Agric.Soc.Finland. 16:75.
VINCE, A., KILLINGLEY, M. & WRONG, O.M. 1978. Gastroenterology. 74:544-549.
VISEK, W.J. 1962. Am.J.Vet.Res. 23:569-574.
VISEK, W.J. 1978. J.Anim.Sci. 46:1447-1469.
VISEK, W.J. 1982. Advances Vet.Med. 33:64-76.
WALLACE, H.D. 1970. J.Anim.Sci. 31:1118-1126.
WALTON, J.R. 1982. Advances Vet.Med. 33:77-82.
WOSTMANN, B.S., BEAVER, M. & MADSEN, D. 1979. In "Clinical and Experimental Gnotobiotics". (Eds. T. Fliedner, H. Heit, D. Niethammer and H. Pfieger) Zbl.Bakt.Suppl.7. Gustav Fischer Verlag:Stuttgart. p.121-123.
YOSHIDA, T., PLEASANTS, J.R., REDDY, B.S. & WOSTMANN, B.S. 1968. Br.J.Nutr. 22:723-737.
ZEBROWSKA, T. 1982. In "Digestive Physiology in the Pig". (Eds. J.P. Laplace, T. Corring and A. Rerat) INRA:Paris. p.225-236.
ZEBROWSKA, T. 1973. Roczn.Nauk roln. 95B(3):85-90.

DEAMINATION OF AMINO ACIDS IN THE INTESTINAL DIGESTA OF PIGS

LUCYNA BURACZEWSKA, S. BURACZEWSKI
Institute of Animal Physiology and Nutrition, 05-110 Jabłonna, Poland

SUMMARY

The extent of bacterial deamination of amino acids was compared in the ileal and caecal digesta of pigs, incubated with or without casein hydrolysate as a source of free amino acids. The samples of digesta were incubated 2 and 4 h under anaerobic conditions. Negligible changes in amino acids and ammonia were observed when both kind of digesta were incubated without hydrolysate. All supplementing amino acids were at least partly deaminated during incubation with caecal but only aspartic and glutamic acids, serine, lysine, histidine and arginine with ileal digesta. After 4 h incubation the sum of amino acids in ileal digesta decreased by 8 and in caecal digesta by 24%.

INTRODUCTION

Although the bacterial population in the small intestine of pigs was found to be quite rich (Cranwell, 1968; Boucourt and Ly, 1975) its nutritional significance has not been sufficiently established. Keys and DeBarthe (1974a) mentioned that some of fibre material can be digested in the stomach and the small intestine in situations where there is slow flow through the upper digestive tract (DT). Drochner (1984) found that the ileal digesta of mini pigs contained large amount of bacterial protein.

The study was undertaken to obtain more informations on the extent of disappearance of amino acids in digesta from the ileum as compared to that from caecum. Digesta obtained from those regions of DT was incubated with or without addition of casein hydrolysate and changes in amino acids and ammonia concentrations were studied.

MATERIAL AND METHODS

Six castrated 50 kg pigs fitted with simple cannulas of the distal ileum were fed on diet with 15.4% crude protein containing 83.4% barley, 14.0% protein concentrate (soybean oilmeal, groundnut oilmeal, fish meal and yeast) and 2.6% mineral-vitamin mixture

The feed was offered in two equal meals at 7.00 and 19.00 h mixed 1:1 with water. After six days of preliminary period the ileal digesta was collected under CO_2 for four days, 12 h every day between meals. On the same day, after collection 600 g of each digesta, mixed or not with casein hydrolysate[1/] (1.2 g hydrolysate N per 1 kg digesta), was divided into 3 portions and incubated for 0, 2 or 4 h at 38°C under CO_2. After incubation a small part was left for pH measurement and the rest was freezed at -18°C. For nitrogen and amino acid analysis pooled samples from two days for each pig were prepared and freeze-dried. The digesta from the caecum was obtained at slaughter and after 2-fold dilution with 0.9% NaCl was treated in similar way as ileal digesta. Amino acids and ammonia were estimated after hydrolysis of freeze-dried digesta, using Beckman "Unichrom" amino acid analyzer.

RESULTS AND DISCUSSION

The pH of the ileal digesta before and after 2 and 4 h of incubation was 6.56, 5.25 and 5.09. The respective values 6.74, 5.75 and 5.41 for digesta supplemented with casein hydrolysate were somewhat higher but also decreasing with time of incubation. Similarly decreasing values of pH were observed after incubation of caecal digesta. All the values differed significantly and may be inpterpreted as a result of the fermentation process.

As it is shown in Table 1 and 2 the changes in amino acid and ammonia composition during incubation were negligible in the unsupplemented ileal and caecal digesta. The above results show that the processes of degradation and synthesis of amino acids did not influence markedly on their total content in digesta.

In both digesta supplemented with casein hydrolysate fermentation and deamination resulted in pronounced changes in amino acid content but the rate of deamination of amino acids in the ileal digesta was about one third of that in caecal. After 2 and 4 h of incubation the sum of amino acids (per 16 g N) decreased by 4.5 and 8% in the ileal and by 13 and 24% in the caecal digesta. Ammonia content after 4 h incubation increased 19 and 107%, respectively. Statistically significant decrease of the content of hydrolysate amino acids in the ileal digesta was found for aspartic and gluta-

1/ Acid hydrolysed casein containing all amino acids except Cys and Trp; Tyr content was reduced to 0,6 g/16 g N. (Bacto Casamino Acids, Difco Laboratories, USA).

mic acids, serine, lysine, histidine and arginine. In contrast, all the added amino acids were deaminated at least partly during incubation with the caecal digesta.

TABLE 1. Amino acid composition (g/16 g N) of ileal digesta incubated with and without casein hydrolysate at 38° C during different time

Digesta	without hydrolysate			with hydrolysate				
Incubation, h	0	2	4	0	2		4	
Asp	8.75	8.36	8.21	8.08	6.75^{x} [1]	(84) [2]	6.22^{xx}	(77)
Thr	4.58	4.42	4.39	4.35	4.27	(98)	4.25	(98)
Ser	4.55	4.29	4.19	4.40	4.10^{x}	(93)	3.86^{xx}	(88)
Glu	12.60	12.07	11.72	16.13	15.52	(96)	14.89	(92)
Pro	5.80	5.74	5.60	7.46	7.33	(98)	7.18	(96)
Gly	6.14	5.99	6.01	5.04	4.98^{x}	(99)	5.02	(100)
Ala	4.90	5.04	5.10	4.50	4.73^{x}	(105)	4.88^{xx}	(108)
Val	4.60	4.38	4.34	5.13	5.11	(100)	5.11	(100)
Ile	3.45	3.25	3.26	3.81	3.74	(98)	3.74	(98)
Leu	5.83	5.55	5.50	6.28	6.13	(98)	6.13	(98)
Tyr	2.59	2.43	2.42	1.95	1.92	(98)	1.87	(96)
Phe	3.67	3.43	3.43	3.46	3.43	(99)	3.36	(97)
Lys	5.06	4.80	4.85	5.62	5.39$_{x}$	(96)	4.52^{x}	(80)
His	1.99	1.86	1.80	2.07	1.94^{x}	(94)	1.69^{xx}	(82)
Arg	3.88	3.66	3.55	3.64	2.80^{x}	(77)	2.57^{xx}	(70)
Cys	1.99	1.89	1.85	1.42	1.41	(99)	1.38	(97)
Met	1.29	1.23	1.20	1.38	1.38	(100)	1.39	(100)
Trp	1.57	1.48	1.53	1.16	1.15	(99)	1.09	(94)
Sum	83.24	79.87	78.95	85.90	82.08$_{x}$	(96)	79.15$_{xx}$	(92)
N-NH_3	2.11	2.12	2.20	1.87	2.09^{x}	(112)	2.22^{xx}	(119)

[1] Differences significant at $P < 0.05$ (x) or $P < 0.01$ (xx) as compare to "0" time values

[2] Expressed in per cent of "0" time values

Alanine was the only amino acid the quantity of which increased during incubation of ileal digesta. This result indicates that not only degradation but also transformation and synthesis of amino acids takes place in the digesta.

Ammonia-N in both kind of digesta accounted for about 2 g per 16 g N what gives 12-13% in the sampled material before incubation. Those values even if overestimated (due to hydrolysis of digesta with 6 N HCl) correspond with low sum of amino acids per 16 g N. The values obtained were 83.2 for ileal and 76.7 for caecal digesta. In our recent estimation of ileal digestibility of amino acids of six different barleys the respective average value was 79.6. It

means that approximately 20% of total nitrogen in the ileal digesta was not the amino nitrogen.

TABLE 2. Amino acid composition (g/16 g N) of caecal digesta incubated with and without casein hydrolysate at 38° C during different time

Digesta	without hydrolysate			with hydrolysate					
Incubation, h	0	2	4	0	2			4	
Asp	8.34	8.10	8.10	8.35	6.32^{xx} [1]	(78) [2]		6.16^{xx}	(74)
Thr	3.98	3.97	3.91	3.96	3.53^{xx}	(89)		2.97^{xx}	(75)
Ser	3.43	3.38	3.36	3.81	2.62^{xx}	(69)		2.50^{xx}	(66)
Glu	10.58	10.32	10.16	15.06	13.76^{x}	(91)		11.04^{xx}	(73)
Pro	3.35	3.32	3.29$_{x}$ [2]	6.06	4.91^{xx}	(81)		3.49^{xx}	(58)
Gly	4.73	4.59	4.51^{x}	4.12	3.79^{x}	(92)		3.45^{xx}	(84)
Ala	5.35	5.25	5.16	4.95	4.82	(97)		4.24^{xx}	(86)
Val	4.78	4.77	4.73	5.60	4.82^{xx}	(86)		4.08^{xx}	(73)
Ile	3.88	3.83	3.78	3.89	3.31^{xx}	(85)		2.98^{xx}	(77)
Leu	5.82	5.71	5.64	5.44	4.70^{xx}	(86)		4.36^{xx}	(80)
Tyr	2.91	2.79	2.83$_{x}$	2.27	2.24	(99)		2.23	(98)
Phe	3.52	3.40	3.38^{x}	3.27	3.10	(95)		2.79^{xx}	(85)
Lys	5.50	5.66	5.59$_{x}$	6.69	6.09^{x}	(91)		5.23^{xx}	(78)
His	1.92	1.90	1.79^{x}	2.11	1.83^{x}	(87)		1.51^{xx}	(72)
Arg	4.02	3.83	3.83	3.68	3.07^{xx}	(83)		3.00^{xx}	(82)
Cys	1.47	1.41	1.43	1.15	1.14	(99)		1.13$_{x}$	(98)
Met	1.87	1.86	1.85	1.69	1.46^{xx}	(86)		1.44^{x}	(85)
Trp	1.26	1.33	1.33	1.02	1.03	(100)		1.02	(99)
Sum	76.71	75.51	74.67	83.12	72.55	(87)		63.62$_{xx}$	(76)
$N-NH_3$	1.98	2.08	2.17	1.55	2.60^{xx}	(168)		3.21^{xx}	(207)

[1] Differences significant at P 0.05 (x) or P 0.01 (xx) as compared to "0" time values

[2] Expressed in per cent of "0" time values

Above observations support an assumption that the transit time along the upper DT which according to Keys and DeBarthe (1974) lasts 7-14 h allows for some extent of degradation of amino acids before they are absorbed.

REFERENCES

BOUCOURT, R. & LY, J. 1975. Cuban J. Agric. Sci. 9: 163-170.

CRANWELL, P.D. 1968. Nutr. Abs. Rev. 38: 721-730.

DROCHNER, W., 1984. Advances in Animal Physiology and Animal Nutrition 14, Verlag Paul Parey. Hamburg u. Berlin.

KEYS, J.E. & DeBARTHE, J.V. 1974a. J. Anim. Sci. 39: 53-56.

KEYS, J.E. & DeBARTHE, J.V. 1974b. J. Anim. Sci. 39: 57-62.

FIBRE DIGESTION AND BACTERIOLOGY OF THE DIGESTIVE TRACT OF PIGS FED CEREAL AND VEGETABLE FIBRE

A. CHESSON, A.J. RICHARDSON, J.A. ROBERTSON
Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, U.K.

SUMMARY

Results of a bacteriological examination of digesta taken from selected sites along the digestive tract of pigs fed a bran- or swede-based diet are shown. Pectinolytic and cellulolytic anaerobes were detected in both the fore- and hindgut of swede-fed animals. Cellulolytic activity was absent in bran-fed animals and pectinolytic organisms were restricted to the hindgut. The major organic acids found were lactate in the foregut and VFA in the hindgut. The increased concentration of VFA in the hindgut corresponded to the observed disappearance of dietary fibre.

INTRODUCTION

Ethical and practical difficulties often point to the need for animal models to study some aspects of human nutrition. Results from this and other laboratories suggest that the gastro-intestinal tract of the pig is particularly suited to examining the digestion and physiological role of dietary fibre.

Dietary fibre, taken to include material of plant origin not degraded by host enzymes, is continually modified during passage through the digestive tract by both host endogenous secretions and the action of microorganisms. While it is generally acknowledged that fibre is extensively degraded in the hindgut, less well recognised are the substantial changes that also can occur to the chemical composition and hence properties of some fibres in the foregut. In animals fed a swede diet, approximately 70% of pectic substances and 15% of cellulose ingested was lost from digesta recovered at the terminal ileum while in bran-fed animals little change in fibre composition was detected (Millard and Chesson, 1984a,b; Robertson et al., 1985). Similar losses of pectic substances have been reported from digesta recovered from ileostomy patients (Holloway et al., 1983).

Estimates of the loss of fibre components in the foregut were made by the recovery of digesta from animals cannulated at the terminal ileum. Inevitably surgery, whether to humans or animals, disturbs digesta flow and allows airborne microorganisms access to this point in the gut. To confirm that the loss of fibre components detected in cannulated animals was not an artefact of surgery

and that fibre-degrading bacteria form part of the normal ileal flora, a series of sacrifice experiments was performed on unmodified animals preconditioned to swede- and bran-based diets.

MATERIALS AND METHODS

Four Large White x (Large White x Landrace) female pigs (85-90 kg), two maintained on a bran-based diet and two a swede-based diet were sacrificed by injection and over-sedation. The diets, their preparation and their fibre contents, have been described previously (Millard and Chesson, 1984a; Robertson et al., 1985). The entire gastro-intestinal tract was removed from each animal and the gut ligated and sectioned as rapidly as possible. Sections of the gut were opened under CO_2 and samples taken for bacteriological examination and for chemical analysis. Analytical methods were those of Millard and Chesson (1984 a,b).

Samples of known wet weight were homogenised for one minute under CO_2 and a dilution series prepared. Viable counts of anaerobic bacteria were made on roll-tubes inoculated in triplicate with suitable dilutions using the standard anaerobic Hungate technique. The basal medium contained (g l^{-1}) yeast extract, 2.5; bacto-casitone, 10; agar, 20; cysteine HCl, 1.0; $NaHCO_3$ 4; with 20% filtered rumen fluid and 30% mixed salts solution. Glucose (20g), maltose (20g) and cello-biose (20g) were added to the basal medium prepared for total counts and pectin, (10g), or powdered cellulose (0.5g), were added to media for pectinolytic and cellulolytic counts respectively. Resazurin was included as redox indicator. Roll-tubes were incubated at 38°C and total viable counts made after 2 days incubation. Pectinolytic organisms were detected and counted after 5 days by flooding tubes with 40% cetavalon. Zones of clearing produced by cellulolytic bacteria were counted after 21 days incubation. Total counts were made using an optical microscope and counting chamber.

RESULTS AND DISCUSSION

The overall digestibility of dietary fibre was 81% for swede and 40% for bran. Loss of specific fibre components at various sites in the gut was comparable to that found using cannulated animals.

Results obtained from the bacteriological examination of digesta from selected sites in the digestive tract are shown in Table 1. Although total

Table 1. Anaerobic bacteriology of selected sites in the digestive tract of pigs fed bran (B)- or swede (S)-based diets.

		Feed	Terminal ileum	Caecum	Rectum
g dry weight g^{-1} fresh	B	1.0	0.12	0.12	0.18
weight.	S	0.15	0.14	0.06	0.23
Log_{10} total bacteria	B	-	10.24	10.88	10.66
g^{-1} dry matter	S	-	9.88	11.28	11.04
Log_{10} total viable anaerobes	B	2.85	10.53	10.44	10.34
g^{-1} dry matter	S	5.45	8.18	11.19	10.04
Log_{10} total cellulolytic	B	0	0	0	0
anaerobes g^{-1} dry matter	S	0	4.26	6.83	8.74
Log_{10} total pectinolytic	B	0	0	8.42	8.91
anaerobes g^{-1} dry matter	S	0	4.26	>10.00	>9.40

Table 2. Organic acid concentration in samples taken from selected sites in the digestive tract of pigs fed bran (B)- or swede (S)-based diets. Values are given as mmol kg^{-1} dry matter.

Organic acid		Feed[a]	Stomach	Terminal ileum	Caecum[b]	Rectum
Acetate	B	131	18	85	876	111
	S	29	110	187	2607	235
Propionate	B	0	3	26	196	38
	S	0	19	4	261	51
Butyrate	B	0	4	13	108	14
	S	0	22	0	167	67
Lactate	B	187	113	757	0	0
	S	85	1058	191	303	30

[a] iso-butyrate present in feed and early foregut samples

[b] valerate and iso-valerate present in hindgut samples.

counts and numbers of viable anaerobes present in the lower third of the ileum were similar to those found in the hindgut the two populations showed distinct morphological differences. Large Gram +ve rods were predominant in all samples taken from the foregut, while populations in hindgut samples were of a more mixed appearance with large numbers of cocci and Gram -ve rods also present.

These differences in population were reflected in the relative amounts of organic acids detected (Table 2). Lactate, characteristic of digesta taken from the foregut, was absent from the hindgut of bran-fed animals and much reduced in those fed the swede diet. In contrast VFA production was low in samples taken from the foregut but increased dramatically in samples from the hindgut. Acetate was the major VFA present. The concentration of VFA present in digesta gave a good indication of the extent of fibre digestion and bacterial proliferation.

Pectinolytic anaerobes were found both in the hindgut of bran-fed and, in higher numbers, swede-fed animals. No pectinolytic organisms could be detected in samples taken from the lower ileum of bran-fed animals but they were consistently present in animals fed the swede-based diet. Anaerobic cellulolytic bacteria were apparently absent from the entire digestive tract of bran-fed animals but were found in the fore- and hindgut of those fed the swede diet.

It was earlier reported that substantial losses of pectic substances and limited degradation of cellulose took place in the foregut of pigs fed a swede-based diet (Millard and Chesson 1984a,b). No such modifications occurred in the foregut of bran-fed animals. Microbiological examination of samples taken from the digestive tract of intact animals fed the same diets produced results consistent with these initial observations and supports the view that these earlier findings were not an artefact caused by surgery. Thus both pectinolytic and cellulolytic activities were detected at the terminal ileum of swede-fed animals not in bran-fed animals. No cellulolytic anaerobes could be detected in animals maintained on bran despite the observed loss of some cellulose in the hindgut. The faster whole gut transit time found in bran-fed compared to swede-fed pigs may mediate against a stable cellulolytic population in these animals.

REFERENCES

HOLLOWAY, W.D., TASMAN-JONES, C. & MAHER, K. 1983. Am.J.Clin.Nutr. 37: 53-56.

MILLARD, P. & CHESSON, A. 1984a. Br.J.Nutr. 52: 583-594.

MILLARD, P. & CHESSON, A. 1984b. Eur.J.Biochem. 142: 367-369.

ROBERTSON, J.A., BAIN, SANDRA D. & CHESSON, A. 1985. Br.J.Nutr. (in press).

D(-) LACTIC ACID METABOLISM IN THE YOUNG PIG

P.D. CRANWELL, M.J. SISSONS, A.W. BELL
School of Agriculture, La Trobe University,
Bundoora, Victoria 3083, Australia.

SUMMARY

In two experiments lactic acid was given to young pigs, either by bolus intravenous injection or repeated intragastric injections, and the plasma concentrations of both isomers were measured over a period of time. The L(+) isomer of lactic acid was utilised rapidly and to a greater extent than the D(-) isomer but the tissues of the pig appear capable of utilising most of the D(-) isomer likely to be produced by fermentation in the gastrointestinal tract.

INTRODUCTION

The stomach contents of sucking pigs reared under farm type conditions can contain large amounts of L(+) and D(-) lactic acid (Cranwell *et al.* 1976). Both isomers are absorbed rapidly and at about the same rate in the pig, which can utilise small quantities of exogenously given D(-) lactic acid (Christie & Cranwell, 1976). The aim of this study was to measure the utilisation of large quantities of both isomers given intravenously or by repeated intragastric injection of amounts similar to those found in the stomach of the sucking pig.

MATERIALS AND METHODS

Pigs and their treatment

Experiment 1. Six Large White pigs, 8 - 10 weeks, 8 - 14 kg liveweight were each prepared with a catheter in the jugular vein. They were fed *ad lib.* on Pig Starter Ration (Barastoc, Victoria, Australia).

Experiment 2. Five Large White X Landrace pigs reared entirely by the sow were each prepared with a catheter in the jugular vein and a gastric fistula at 2 - 3 weeks of age.

Experimental and analytical procedures.

Experiment 1. On three separate occasions during a 3 week period each pig was fasted for 4 h and received a bolus intravenous injec-

tion of one of the following sterile solutions: physiological saline (NaCl, 9g/L), pure sodium L(+) lactate (pH 7.1), sodium D(-) lactate (pH 7.0) in racemic misture (0.54 L(+): 0.46 D(-)). The two lactate solutions were given at the rate of 5.77 mmol/$kg^{0.75}$ of L(+) or D(-) lactic acid. Blood samples (3.5 ml) were collected at intervals prior to and for up to 300 min following the bolus injection.

Experiment 2. On three separate occasions during a 4 week period each pig was fasted for 6 h and received twenty, hourly intragastric injections of one of the following solutions (15 ml/$kg^{0.75}$ per h): cows' milk, 250 mmol/L DL- lactic acid in cows' milk, 500 mmol/L DL-lactic acid in cows' milk. Blood samples (3.5 ml) were collected at intervals prior to and for up to 29 h following the commencement of intragastric injections. Urine was collected as voided for 7 - 27 h after the last injection was given.

Plasma, urine and infusion solutions were assayed for L(+) lactate by the method of Lundholm *et al.* (1963) and D(-) lactate by the method of Brandt *et al.* (1980).

RESULTS AND DISCUSSION

Experiment 1. Plasma L(+) and D(-) lactate concentrations were unchanged after saline injection. Immediately after injection of lactic acid solutions, plasma concentrations of the injected isomer reached a maximum of 8 - 39 mmol/L and then declined at an exponential rate from 5 - 10 min after injection. The plasma L(+) lactate concentration reached pre-injection values within 40 min after the bolus was given compared to 170 min for the D(-) isomer (Fig. 1). The exponential declines in lactate concentrations are described by the following equations: for L(+), (L) = 0.0431t + 1.748 (t, 5-35 min), r^2 0.897 ($P<0.01$) and for D(-) two exponential equations provided the best fit of the data, (D) = 0.0199t + 1.946 (t, 10-65 min), r^2 0.721 ($P<0.01$), and (D) = 0.0258t + 2.272 (t, 80-170 min), r^2 0.891 ($P<0.01$): where t is time after injections (min) and (L) and (D) are ln plasma concentrations (mmol/L) of L(+) and D(-) lactate respectively. Assuming first order kinetics, estimated half lives were for L(+) lactate, 16 min and for D(-) lactate, 35 min (t, 10-65 min) and 27 min (t, 80-170 min). The L(+) isomer was removed from plasma 1.7-2.0 times more rapidly than the D(-) isomer ($P<0.05$).

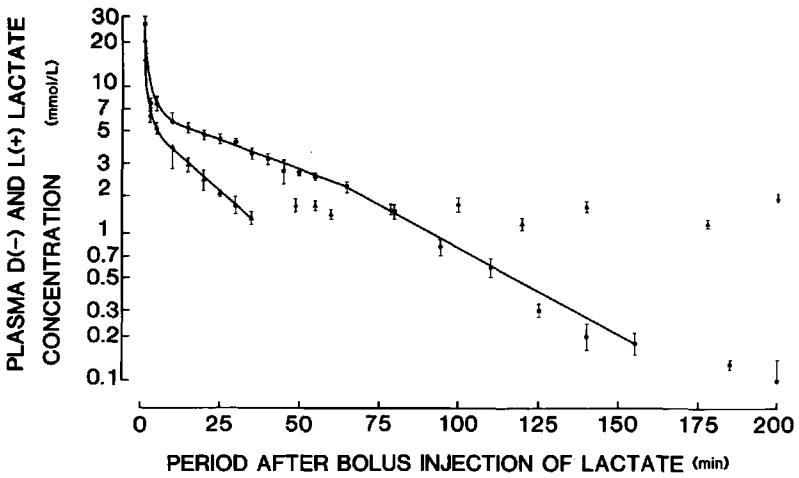


Fig. 1. Plasma L(+) (▲) and D(-) (●) lactate concentrations following a bolus injection of either pure L(+) or D(-) lactic acid (in a racemic mixture) (5.77mmol/kg$^{0.75}$). Points are mean values, with their standard errors represented by vertical bars.

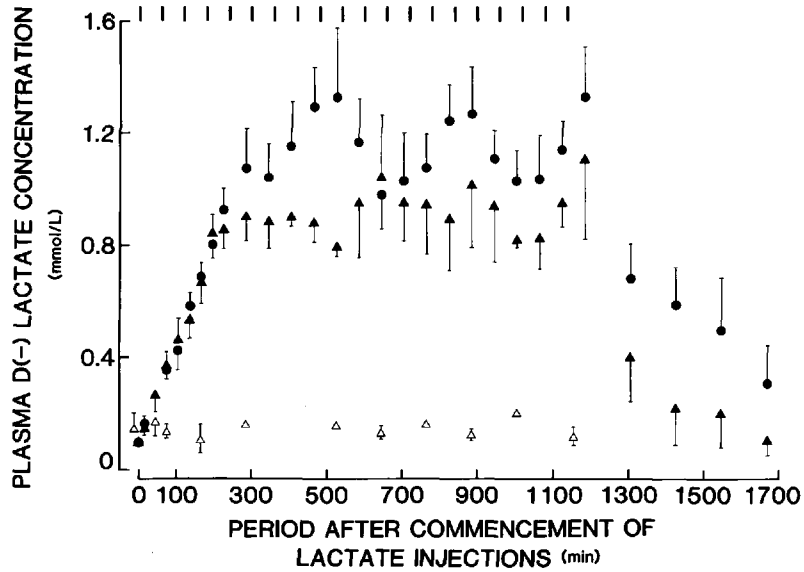


Fig. 2. Plasma D(-) lactate concentrations following hourly intragastric injections (I) of: cows'milk (△), 250 mM (▲), 500 mM (●) DL-lactic acid in cows' milk (15ml/kg$^{0.75}$ per h). Points are mean values with their standard errors represented by vertical bars.

Experiment 2. The mean plasma D(-) and L(+) lactate concentrations during control injections were 0.15 (SE 0.01) mmol/L and 1.46 (SE 0.06) mmol/L, respectively. During intragastric injections of 250 mmol/L racemic lactic acid, plasma D(-) lactate reached a plateau concentration (0.92, SE 0.02 mmol/L) 6 fold above pre-injection values 195 min after the first injection, and decreased to pre-injection values 280-405 min after the last injection (Fig. 2). The D(-) lactate concentration increased at a similar rate during injections of 500 mmol/L racemic lactic acid but, compared with the 250 mmol/L values, reached a significantly higher plateau concentration (1.13, SE 0.02 mmol/L; $P<0.001$) 7.5 fold above pre-injection values. The concentrations plateaued 225 min after the first injection and decreased steadily after the last injection but did not reach pre-injection values within 530 min. Neither of the two racemic lactic acid solutions significantly changed plasma L(+) lactate concentration relative to control values. However the mean urinary concentration of L(+) lactic acid was elevated 19 fold following injection of 250 mmol/L racemic lactic acid (3.69, SE 0.33 mmol/L) and 57 fold following injection of 500 mmol/L (10.79, SE 1.81 mmol/L). About 3 - 4 times more D(-) lactic acid was excreted in urine. The proportion of the total dose of each isomer given which was excreted in the urine was similar for both dose rates (Table 1).

Table 1. Urinary excretion of total, L(+) and D(-) lactate up to 28 h from the start of lactate injections (% injected).

	Total Lactate		L(+)		D(-)	
Dose	Mean	SE	Mean	SE	Mean	SE
250 mmol/L	6.25	0.75	2.28	0.33	11.18	1.79
500 mmol/L	5.60	0.72	1.94	0.46	9.96	1.14

REFERENCES

BRANDT, R.B., SEIGEL, S.A., WATERS, M.G. & BLOCH, M.H. 1980. Anal. Biochem. 102: 39-46.

CHRISTIE, A.A. & CRANWELL, P.D. 1976. Proc. Nutr. Soc. 35: 28A.

CRANWELL, P.D., NOAKES, D.E. & HILL, K.J. 1976. Br.J.Nutr. 26: 71-86.

LUNDHOLM, L., MOHME-LUNDHOLME, E. & VAMOS, N. 1963, Acta Physiol. Scand. 58: 243-249

INFUSION OF NUTRIENTS INTO THE CECUM OF GROWING PIGS -A MODEL FOR THE FERMENTATIVE DIARRHEA ?

W.DROCHNER,E.SCHMIDT ,J.KAMPHUES
Institute for animal nutrition
Tierärztliche Hochschule Hannover
Bischofsholer Damm 15,3000 Hannover 1,Bundesrepublik Deutschland

SUMMARY

Infusion trials were carried out for the sake of examinating the effects of a nutrient load to the large intestine(24 fistulated animals,minipig,breed:Gottingen strain,6 weeks-8 months old,weighing 3-26 kg). The reaction of parameters in the feces were observed. The following results should be pointed out:

1) The tolerance for water ,infused into the cecum,is very high. The level of water-load,tolerated without any detrimental effects for the physiology of the intestine was 18,75 ml per kg bodyweight and hour. A level of 25 ml resulted in a sharp decrease of the dry matter content of the feces.

2) High sodium-chloride levels given up to an amount of 150mg/kg bw and hour were tolerated without negative effects for the dry matter content of the feces.

3) Infusion of different levels of starch induced an augmentation of the dry matter content of the feces and a slight increment of the concentration of volatile fatty acids .

4) After a period of starvation for 24 hours,ad lib.-feeding resulted in high feed intake(5-8% of b.w.)within 1 hour. Consecutively 8-9 hours thereafter,a typical fermentative diarrhea resulted,characterized by high levels of lactic acid in the feces(10-20 mmol/l) and a decline of fecal pH.

5) A parallel survey of parameters of the cecalchymus showed a dramatic increasing of the lactic acid concentration,especially of D-lactic acid,paralleled by a decline of the pH.

6) Extremely high levels of potatoe-starch(1250 mg/kg b.w.and hour) resulted in sporadic diarrhea of the fermetative type in the period 6-12 hours after beginning of the infusion.

7) Stomach-content of sacrificed animals,having severe diarrhea- caused by abundant high feed intake, was infused at different levels.In all cases, a pronounced reduction of the fecal dry matter content resulted,combined with an increasing level of lactic acid.

9) Infusions of lactic acid resulted in severe diarrhea ,when levels ,higher than 112 mg/kg bw. and hour were used. (Infusion time: 8 hours).

INTRODUCTION

The intracecal infusion of nutrients and metabolites of bacterial metabolism may be used as a model for physiological conditions,developing in cases,when animals show an unusual high feed intake. Ubandantly high feed intake causes a special form of fermentative diarrhea,which might be induced by fermentation of undigested nutrients,entering the large intestine.Increasing amounts of microbial end-products of metabolism might be responsible for the so-called "fermentative diarrhea"(for instance ammonia,lactic acid,volatile fatty acids,amines etc).

MATERIAL AND METHODS

The investigations were carried out with 24 fistulated (cecal fistulas) animals, belonging to the minipig-breed,strain Gottingen,6 weeks to 8 months old with a weight from 6 kg to 26 kg. The animals,housed in metabolism cages or in slatted floor-stables ,received their feed two times a day(8^{00} and 19^{00}) at a level of 1-2% of bodyweight.The water -feed - relation was 3 : 1 .The feed was rich in crude protein(21 %) ,main ingredients were wheat-meal,meat and bone-meal and soya.The infusions were performed according to the fig presentated.

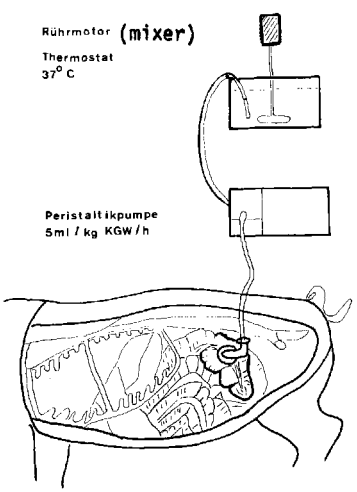


Fig.: Technique of infusions

The volume,infunded within a period of 8 hours after the morning-meal was 6,25 ml water per kg body weight and hour.Stomach-content was too viscous to be infused in the same manner. Therefore this material was infunded by means of a great syringe discontinously.

The animals were controlled for their physical health-status during the total time.No deviations were observed.

Feces and ileal chymus were collected hourly. The pH was measured directly by a pH-meter, ammonia was controlled by a semi-selective electrode, lactic acid by an enzymatic test (D- and L-form),volatile fatty acids by means of gaschromatography. The other parameters were determined by conventional methods(crude nutrients,dry matter - content according to the "Weende-method").

RESULTS AND DISCUSSION

The abilty of the large intestine to absorb water is very high. Concepts arguing with limited water absorbtion and high water flow from the small intestine to the large as a basis for diarrhea must fail,as a water load of 18,75 ml per kg bodyweight and hour is tolerated quite well.

In fermentative diarrhea,a lack of sodium-absorbtion-capacity of the colon has been discussed as well. The capadty of the large intestine is great.In the presentated trials,a load of 150 mg sodium chloride to the cecum(per kg body weight and hour) was tolerated without any negative effects for the dry matter content of the feces.

Excessively high feed intake as consequence of ad lib feeding following a period of starvation causes a decline of the pH in cecal cyhmus and feces(tab.1)

Tab.1: Parameters of cecal chymus ,samples collected after excessively high feed intake

time pp. h	n	pH	lactate total	lactate D-form mmol/l	ammonia mg/l
0-2	29	6,71(±0,5)	6,42(±3,1)	1,61(1,4)	145(55)
2-4	30	6,41(0,4)	7,02(3,2)	3,15(1,3)	78(52)
4-6	30	5,93(0,3)	16,70(5,5)	5,33(5,1)	67(19)
6-8	25	5,68(0,3)	18,94(6,2)	9,05(3,6)	65(7)
8-10	29	5,56(0,4)	25,18(6,4)	11,53(6,4)	54(19)

The decline of the pH in cecal chymus is correlated to low values in the feces. Thelactate-concentration in cecal chymus ,normally near to zero,augments rapidly. The ammonia-level remains low .D-lactate increases faster than total lactate. High doses of maize-starch,applicated intracecally induced a period of less defecations and a slight increment of the fecal dry matter content.

Excessively high amounts of potatoe-starch(1875 mg/kg bodyweight and hour) only sporadically induced wet feces. As will be shown in tab. 2,the infusion of stomach-content from animals ,sacrificed in the status of intensive diarrhea , caused an obvious decline of the dry matter content of the feces.In contrast to these observations,infusion of sterilized stomach content failed to reduce pH and dry matter content of the feces(sporadic cases of diarrhea).

A high capacity of lactobacilli to produce lactic acid in the stomach after intake of high levels of feed by the animal has been demonstrated recently by Kamphues(1984).

Tab. 2: Parameters of the feces ,samples collected after infusion of stomach-content into the cecum("spending" animals sacrificed in the status of severe diarrhea)(dose: 6,25 ml/kg body-weight and hour)

time pp. h	n	dry mat. %	pH	lactate total	lactate D-form mmol/l	ammonia mg/l
0-6	7	29,9(±3,4)	7,48(0,2)	3,34(1,41)	0,57(0,17)	118(34)
6-12	19	21,6(±6,6)	6,25(1,0)	17,61(18,64)	8,94(8,90)	115(37)
12-18	1	17,9(±)	5,86	24,51	11,88	103
18-24	2	30,4(±0,29)	6,67(0,3)	3,42(0,09)	2,20(0,0)	163(27)

Concentration of dry matter declined as a consequence of the infusion obviously. The standard-deviation however was high(values in brackets).The pH-values in the feces were affected in the same manner as those of the cecal chymus after excessive feed intake(see tab. 1).

The decline of the dry matter content is correlated to increasing levels of lactate in the feces.As had been observed for ileal chymus,the D-form of lactate increases faster than total lactate. Ammonia was not affected. Infusion of lactic acid resulted in severe diarrhea,when a level of 112 mg/kg body-weight and hour was exceeded. The effects for parameters of the feces were compareable to those presentated in tab.2.

Conclusions:

A sudden and excessive feed intake of young piglets results in a type of diarrhea resembling those effects,which can be induced by infusion of lactic acid or stomach content of animals,sacrificed in the status of severe diarrhea.It can be assumed,that stomach content,containing high levels of starch and lactobacilli, initiates a lactic-acid fermentation in the large intestine.The failure of normal starch to induce diarrhea-when infused into the cecum-might be correlated to the type of fermentation which takes place under these conditions.Normally ,this will be a fermentation resulting in production of high amounts of volatile fatty acids,which are absorbed with high capacity(Imoto,1978) from the large intestine.In consequence,the absorbtion of water and sodium under these conditions is very effective.

High lactic acid levels and low pH depress production of vfa.Additionally, osmotic values of lactic acid and direct detrimental effects for the mucosal membrane induce influx of fluid,resulting in diarrhea.

REFERENCES

KAMPHUES,J.(1984): Prakt.Tierarzt 65:1116-1122.
IMOTO,S.& NAMIOKA,S. 1978. J.Anim.Sci. 47:467-478
SCHMIDT,E.1984.Diss.med.vet. Hannover(West Germany)

THE INFLUENCE OF DIFFERENTLY TREATED BARLEY STRAW, NUTRIENT DENSITY AND VIRGINIAMYCIN ON THE FERMENTATION POTENTIAL OF HIND GUT DIGESTA

J.A. FERNÁNDEZ, H. JØRGENSEN, A. JUST and J.O. ANDERSEN
National Institute of Animal Science
Roligheds vej 25, 1958 Copenhagen V, Denmark

SUMMARY

Digesta samples from the hind gut of pigs slaughtered at 90 kg liveweight were incubated for up to 6 hours. Inclusion of straw in the diets tended to enhance the production of VFA. Increased nutrient concentration was positive related to fermentation, whereas inclusion of Virginiamycin reduced the production of the organic acids. Regardless of treatment the incubation time did not change the molar proportions of acetic, propionic, and butyric acid of total VFA.

These results are consistent with results achieved in combined N-balance-slaughter experiments and digestibility experiments with ileal cannulated pigs fed the same diets.

INTRODUCTION

It is a well established fact that active fermentation processes occur throughout the large intestinal tract (Argenzio, 1982). The concentration of volatile fatty acids (VFA) are widely taken as an indirect estimate of microbial activity. This is, however, a statical condition and not necessarily connected with the dynamic process as fermentation really is.

Differences in diet composition alter the concentration of VFA in ileal digesta and faeces (Just, 1983). The present study was undertaken in connection with a running investigation (Just et al., 1985) as an attempt to facilitate the understanding of fermentation processes as affected over time by different dietary treatment.

MATERIAL AND METHODS

Pigs from a running balance-slaughter experiment (Just et al., 1985) comprising twelve litters of 4 females each were used. The pigs were fed diets composed of barley, wheat, soybean meal, meat and bone meal, and 15% either untreated, NH_3- or NaOH-treated barley straw. In addition, the diets were formulated to contain either the standard amounts of digestive nutrients per Net Energy Unit or 20 percent in excess of the standard. Half of the pigs received 50 mg Virginiamycin per kg diet daily during the growth period 20-90 kg.

Immediately after slaughtering, the digesta was removed from a hind gut segment located 50 to about 100 cm distal to the apex caecalis. After mixing, the digesta was divided in several subsamples for incubation and dry matter determinations, respectively. About 20 g of digesta were diluted with a buffer solution, and were then incubated anaerobically at 37°C. Samples from half of the pigs were incubated for 0, 1, 2 and 3 hours, and the remaining samples were incubated for 0, 3 and 6 hours. Incubation was interrupted by addition of H_2SO_4-$MgSO_4$ solution.

The study also comprised bacteriological determinations of the digesta microflora as well as determination of DNA and ATP. Details about these results will be published elsewhere.

RESULTS AND DISCUSSION

Initial and final molar concentration as well as the molar production per hour of acetic, propionic and butyric acid as well as total VFA are shown in Table 1.

Dietary inclusion of barley straw tended to increase the initial concentration of propionic acid and total VFA, whereas the concentration of acetic acid was significantly increased. The highest and lowest concentrations were found for NaOH- and NH_3-treated straw, respectively. These results are in accordance with the higher proportions of nutrients disappearing from the hind gut in fibrous diets found by Just et al. (1983) but not completely with those found by Just et al. (1985).

Nutrient density had a positive and Virginiamycin a negative,

TABLE 1. The effect of differently treated barley straw, nutrient density and Virginiamycin on the concentration at 0 and 6 hours incubation of colon digesta and the production per hour of acetic, propionic and butyric acid as well as total VFA.

		15% barley straw			Nutrient density		Virginiamycin	
Diets	Basal diet	Untreated	NH_3-tr.	NaOH-tr.	100%	120%	-	+
Incubation 0 hour: mmol/kg colon digesta DM								
Acetic acid	334[a]	437[b]	412[b]	427[b]	369	390	385	374
Propionic acid	160	182	157	197	166	172	170	168
Butyric acid	94	80	77	101	84	96	93	87
Total VFA	621	719	664	747	646	685	676	654
Incubation 6 hours: mmol/kg colon digesta DM								
Acetic acid	665	863	809	815	691	804	776	716
Propionic acid	337	384	344	379	331	377	375	332
Butyric acid	182	158	166	210	163	197	195	165
Total VFA	1280	1457	1370	1466	1255	1460	1432	1281
Incubation 0-6 hours: production of organic acids mmol/hour/kg colon digesta DM								
Acetic acid	68	79	79	92	52[a]	69[b]	74	64
Propionic acid	34	38	36	39	27[a]	34[b]	41[a]	34[b]
Butyric acid	16	15	16	19	13	16	20[a]	15[b]
Total VFA	120	129	129	147	99[a]	129[b]	148[a]	120[b]

a, b, c: Different superscripts on the same line within treatments denote statistical difference: $P < 0.05$

though non-significant, relationship to the concentration of these organic acids. The influence of all treatments on molar concentrations after 6 hours incubation was very much the same as described above.

The molar proportion of acetic acid (57% of VFA), propionic acid (25% of VFA) and butyric acid (12% of VFA), regardless of treatment and incubation time, resemble that found in faeces samples by Just (1983). However, it is noteworthy that the straw containing diets tended to increase the proportion of acetic acid.

Production of VFA per hour was significantly increased by nutrient density and decreased by the addition of Virginiamycin The overall effect of incubation was an increasing production of the organic acids in question in a linear fashion as shown by the equations below (corrected for dietary treatments effect):

mmol VFA = 725 + 107 x incubation time, R^2 = 0.65

s_b 18 6

mmol acetic acid = 409 + 56 x incubation time, R^2 = 0.60

s_b 10 3

mmol propionic acid = 185 + 29 x incubation time, R^2 = 0.66

s_b 5 2

The equations show that the production of these fermentation products increase with time, under the circumstances described here, and even after 6 hours incubation. The pH of the samples, due to the buffer solution remained fairly constant at around 6.8 regardless of treatment and incubation time.

REFERENCES

ARGENZIO, R.A. 1982. In: Digestive Physiology in the Pig. (Eds. J.P. Laplace, T. Corring, A. Rerat). Les colloques de L'INRA. 12: 207-215.

JUST, A. 1983. 4th Int. Symp. Protein metabolism and nutrition. Clermont-Ferrand. Les colloques de L'INRA. 16. EAAP Publ. 31: 289-309.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A. 1983. Livest. Prod. Sci. 10: 171-186.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A. 1985. In: Digestive Physiology in the Pig. (Eds. A. Just, H. Jørgensen and J.A. Fernández). Report No. 580 Nat.Inst.Anim.Sci. Copenhagen, pp. 292-299

PERSISTENCE IN THE GUT OF SUCKING PIGLETS OF A LACTOBACILLUS STRAIN AND ITS INFLUENCE ON PERFORMANCE AND HEALTH

E. JONSSON
Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences
S-750 07 Uppsala, Sweden

SUMMARY

A Lactobacillus strain, isolated from the adhering lactobacilli in a pig stomach was fed to newborn piglets (day 0 and 1). It did not establish permanently in the gut of the piglets, although it showed adhesion ability to stomach epithelium (pars oesophagea) when tested in vitro. It did not adhere to the epithelium in vivo, but it survived the passage through the gut. It could be detected in high frequencies in the feces on the 2nd day and still after one week, but not after 18 days. Administrations at 3 and 6 weeks resulted in a low but stable level of the strain in the feces. The performance was not affected by the treatment.

INTRODUCTION

Antibiotics are today frequently used in animal feeds to control diseases and as general growth promoters. In many countries their use might be abolished in the future and in this context lactic acid bacteria are being discussed and tested as replacements. Although long in use, lactic acid bacteria as dietary adjuncts have not given consistent beneficial effects. Their possible mode of action and their function in the microflora of the anterior gut of the pig are not known. The stomach contents of pigs contain large numbers of lactobacilli. These bacteria originate from remains of earlier meals and from squamous cells with adhering lactobacilli shed from the pars oesophagea area of the stomach. They continuously inoculate the small intestine when passing out from the stomach. This experiment was made to study if lactobacilli from the indigenous microflora and with adhering ability could influence the establishment of lactobacilli in the gut of neonatal piglets and if this would promote health and performance of the animals.

MATERIAL AND METHODS

Twelve litters of mixed breeds (Swedish Landrace X Swedish Yorkshire) were used in a split litter trial. Washed overnight cultures of a heterofermentative Lactobacillus sp. strain, Po 22 were used. Treated piglets were given skim milk with bacteria (10^9 per day), and the control piglets skim milk without bacteria. Creep feed was supplied on the floor ad lib. The piglets were weaned at 6 weeks. Treatment took place on days 0-1, 19-22 and from 2 days before to 1 day after weaning. Samples were taken on days adjoining the treatment periods, and on day 7. Nine of the litters were used for studies of the fecal lactobacilli, performance and health, while piglets from the 3 remaining litters were slaughtered for studies of the lactobacilli in the gut. Performance and health were registered during 9 weeks. Enumeration of lactobacilli was done on Rogosa agar in anaerobic atmosphere. The administered strain was detected by means of agglutinating antibodies (cf Jonsson et al., 1985). Assessment of adhesion in the piglets was done by washing 1 cm^2 pieces of pars oesophagea area 3 times and homogenizing the tissue. If the count of lactobacilli in the macerate exceeded that of the third wash, adhesion was assumed (Fuller & Turvey, 1971).

RESULTS AND DISCUSSION

The administered Lactobacillus strain Po 22 was isolated from the microflora from the pig stomach. It could adhere to squamous epithelial cells when tested in vitro. When fed to newborn piglets, it was detected in the feces at a high frequency on day 2 and still on day 7. On day 19, however, it could not be detected any more. When fed again at 3 weeks of age the strain was again found in the feces and remained on a rather stable level until 6 weeks of age (Table 1). A certain cross-contamination within the litter occurred as the strain could also be detected in the feces of the control piglets. This could be caused by the change of teats between piglets during the first days (Hemsworth et al., 1976). The detection frequency was on a low level, though. The treatment did not influence the performance of the piglets (Table 2).

Table 1. Number of lactobacilli and detection frequency of Lactobacillus strain Po 22 in feces of piglets fed and not fed the strain

AGE	TREATED GROUP				CONTROL GROUP			
(days)	n	m	se	f	n	m	se	f
2	20	9.0§	0.20	55§	15	8.4§	0.34	20§
7	22	10.3	0.18	32	20	10.3	0.13	10
19	22	9.8	0.20	0	20	9.4	0.18	0
23	22	10.2	0.16	9	20	10.2	0.18	5
40	20	9.2	0.17	15	16	9.2	0.17	0
43	20	9.4	0.12	10	19	9.4	0.19	0

n = number of piglets
m = geometric mean of number of lactobacilli per gram feces
se = standard error
f = frequency (% of n) of detected Po 22 strain
§ = significant difference, p less than 0.05

Table 2. Weight of piglets fed and not fed Lactobacillus strain Po 22

AGE	TREATED GROUP		CONTROL GROUP	
(days)	n	m	n	m
0	46	1.4	42	1.4
7	42	2.5	39	2.6
21	40	5.0	39	5.1
42	40	8.2	38	8.7
63	39	10.5	37	11.3

n = number of piglets
m = least-square mean value of weight (kg)

The strain Po 22 was able to survive in the gut of piglets as well as in the gut of older pigs (Jonsson et al., 1985). On the 2nd day of life it constituted ca 20 % of the total number of lactobacilli in the feces, when calculated as a mean for the whole group. This proportion was great enough to raise the number of total lactobacilli significantly compared to the control piglets. The strain persisted until day 7 and constituted ca 10 % of the lactobacilli at this time. This was not enough to change the total count of lactobacilli significantly. By day 19 the strain had disappeared.

The persistence was not due to attachment of the strain to the non-secreting epithelium of the stomach. Although a high level of lactobacilli was found adhering to the pars oesophagea area already on day 2, Po 22 was not found in any of the treated piglets. Possibly the strain did not produce the extracellular substances needed for adhesion (Fuller, 1975) in vivo, or other factors such as pretreatment of the strain might have affected the adhesion. On the other hand, the strain might have grown fast enough in the digesta to overcome being washed out by peristalsis. A too low multiplication rate could then explain why the strain disappeared between day 7 and 19. The changes in digestive physiology, and competition from the indigenous microflora could also be involved.

It is not surprising that no effect on performance and health could be observed as the strain did not establish itself, thereby improving (if possible) the lactic acid bacterial flora of the young pig. The strain had also not been selected according to antibacterial criteria. Too little is yet known about the role of lactic acid bacteria in the gut microflora and its importance to the animal to get consistent effects by feeding lactic acid bacteria either to young or older animals.

REFERENCES

FULLER, R. 1975. J. gen. Microbiol. 87: 245-250.

FULLER, R. & TURVEY, A. 1971. J. appl. Bacteriol 34: 617-622.

HEMSWORTH, P.H., WINFIELD, C.G. & MULLANEY, P.D. 1976. Appl. anim. Ethol. 2: 225-233.

JONSSON, E., BJÖRCK, L. & CLAESSON, C.O. 1985. Livest. Prod. Sci. In press.

THE INFLUENCE OF VIRGINIAMYCIN ON THE ILEAL AND FAECAL DIGESTIBILITY OF NUTRIENTS IN DIFFERENTLY COMPOSED DIETS AND THE UTILIZATION OF DIGESTIBLE CRUDE PROTEIN AND ENERGY.

A. JUST, J. A. FERNÅNDEZ AND H. JØRGENSEN
National Institute of Animal Science
Rolighedsvej 25, 1958 Copenhagen V, Denmark

SUMMARY

On an average the addition of virginiamycin to differently composed diets improved the ileal digestibility of crude protein and energy by 1.0% and 6.1%, respectively, improved the utilization of digested crude protein and energy by 0.5% and 1.7%, respectively, and improved the overall value (digestibility x utilization of digested) of both crude protein and energy by 2.3%. The effect of virginiamycin on the concentration of VFA's and lactic acid in ileal digesta and faeces interacted with diet composition.

INTRODUCTION

Numerous feeding trials with growing pigs have shown that antibiotics or feed additives improve growth rate and feed conversion efficiency. The explanation for the beneficial effect of antibiotics is commonly believed to be an improved health status of the pigs. Howeve several investigations quoted by Just (1980, 1983) and investigations described by Just et al. (1980, 1981a,b) have shown that antibiotics improve the absorption of nutrients (and energy) in the small intestin and depress the fermentative processes as well as the digestibility of nutrients especially energy in the hind-gut.

Thus, addition of antibiotics to diets should be nutritionally beneficial, especially when their content of essential nutrients is below the requirements of the pigs. However, a negative effect particularly on energy digestibility could be expected in diets rich in fibrous material as the fermentative processes in many cases are depressed by the antibiotics added to the diets.

Therefore, the objective of the present investigation was to elucidate in more detail the effects of virginiamycin on the digestion of nutrients in the small intestine and the caecum-colon, respective-

ly, and the utilization of digested crude protein and -energy from differently composed diets.

MATERIALS AND METHODS

The diets used were composed of barley, wheat, soya bean meal, meat and bone meal and either 15% untreated, NH_3-treated or NaOH-treated barley straw. The chemical composition of the diets is given in Table 1.

Table 1. The chemical composition of the diet dry matter.

Diets	Basal diet	Diets including 15% straw: Untreated	NH_3-treated	NaOH-treated
Crude protein	20.2	19.2	19.6	19.0
Crude fat	3.2	3.1	3.1	3.1
Crude fibre	4.6	11.0	10.9	10.1
NFE-substances	65.7	60.5	60.2	60.7
Soluble carbohydrate	53.4	44.9	44.5	45.3

All diets were added 0.5% chromic oxide as marker. To half of the pigs the diets were added 50 ppm virginiamycin. The virginiamycin was weighed separately in small plastic bags, i.e. one bag per meal/pig.

Simple cannulae were inserted at the terminal ileum 3-5 cm anterior to the ileo-caecal valve on 16 female pigs (4 litters each of four pigs) at about 40 kg live weight. Three digestibility experiments were performed with each pig during the growth period from 50 to 80 kg live weight.

The pigs were fed three times daily exactly 8 hours apart and daily feed intake was about 90% of the Danish standard. Each digestibility experiment lasted 12 days, i.e. a five day adaptation period and a seven day collection period. In the first four days of the collection period faeces were collected quantitatively two times daily and then ileal digesta was collected from 7 to 9 a.m. and 11 a.m. to 1 p.m., from 9 to 11 a.m. and from 1 to 3 p.m. and from 8 to 10 a.m. and 12 a.m. to 2 p.m. on day 5, 6 and 7, respectively. Urine was collected by using bladder catheters.

The utilization of digested crude protein and energy was studied using the same diets in a combined N-balance-slaughter investigation comprising 60 pigs during the growth period from 20 to 90 kg. An iden-

Table 2. The influence of virginiamycin on the ileal (I) and faecal (F) digestibility of nutrients and the utilization of digested crude protein and energy.

Diets	Basal diet				Diets including 15% straw:												Significance of antibiotics	
					Untreated				NH_3-treated				NaOH-treated					
Virginiamycin	-		+		-		+		-		+		-		+			
Site	I	F	I	F	I	F	I	F	I	F	I	F	I	F	I	F	I	F
Crude protein	77	83	80	84	75	73	68	73	74	74	77	76	74	75	74	73	NS	NS
Lysine	86	83	88	84	84	76	78	76	84	75	87	78	84	77	84	72	NS	NS
Threonine	75	81	78	82	74	73	62	73	72	73	76	75	73	73	73	68	NS	NS
Crude fat	58	60	61	62	52	51	51	54	60	57	57	61	59	57	60	58	NS	NS
Crude fibre	-13	40	- 8	37	- 4	15	16	10	6	16	- 3	21	- 8	26	-10	22	NS	NS
NFE-substances	71	91	74	92	64	82	74	84	67	84	69	85	65	84	67	85	**	***
Soluble carbohydr.	90	100	91	100	91	99	95	99	94	100	94	99	91	99	91	99	NS	NS
Energy	67	84	70	85	57	69	66	70	60	70	61	73	58	73	59	72	*	*
Stearic acid	68	-202	71	-166	61	-54	35	-10	64	-45	70	-44	72	-70	74	-60	NS	NS
Linoleic acid	74	97	76	97	80	95	85	94	80	96	79	96	79	96	78	96	NS	NS
Concentration of VFA and lactic acid, mmol/kg dry matter intake																		
Acetic acid	59	59	48	47	35	119	73	114	34	117	33	102	31	103	31	100	NS	NS
Propionic acid	6	19	3	15	4	41	7	36	5	41	3	33	4	34	2	32	NS	**
VFA	67	93	52	73	39	183	87	172	41	189	37	159	36	162	34	154	NS	**
Lactic acid	109	68	36	42	112	118	137	121	121	386	137	343	147	255	157	226	NS	NS
Deposited percent of digested																		
Crude protein		40		41		44		41		44		41		43		47		NS
Energy		30		30		27		30		27		29		28		26		NS

tical factorial design was used for the investigations with cannulated pigs and for the combined N-balance-slaughter investigations. More details of these investigations will be published elsewhere.

RESULTS AND DISCUSSION

The main results are shown in Table 2. Except for energy the addition of virginiamycin had no statistical significant influence on the ileal and faecal digestibilities, but the data indicate an improved absorption of all nutrients in the small intestine. The ileal digestibility of crude protein was on an average improved by 1.0% and that of energy by 6.1%.

The effect of virginiamycin on the concentrations of VFA's and lactic acid interacted with diet composition. In the basal diet and the diet with untreated straw the concentrations of VFA's and lactic acid were increased in ileal digesta, whereas they were decreased in the diets with NH_3- and NaOH-treated straw. The faecal concentrations were decreased except for the diet with untreated straw.

In the balance-slaughter experiments addition of virginiamycin to the diets improved the utilization of digested crude protein and digested energy by 0.5% and 1.7%, respectively. The overall improvement i.e. digestibility x utilization of the digested amounted to 2.3% for crude protein and 2.3% for energy.

REFERENCES

JUST, A. 1980. In: Current Concepts of Digestion and Absorption in Pigs. Technical Bulletin 3 (Eds. A. G. Low and I. G. Partridge). National Institute for Research in Dairying, Shinfield, Reading, p. 66-75.

JUST, A. 1983. 4th Int. Symposium on Protein Metabolism and Nutrition, Clermont-Ferrand. Les Colloques de l'INRA, 16. EAAP Publ. 31:289-309.

JUST, A., ANDERSEN, J. O. & JØRGENSEN, H. 1980. Z. Tierphysiol. Tierernährg. u. Futtermittelkde. 44: 82-92.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J. A. 1981a. Brit. J. Nutr. 46: 209-219.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J. A. 1981b. Proc. VI. Symposium on Amino Acids. Warsaw, 5 pp.

COMPARISON OF THE AMINO ACID COMPOSITION OF PURE ENDOGENOUS AND MICROBIAL PROTEINS IN THE G.I. TRACT OF THE PIG

LAPLACE J.P., DARCY-VRILLON B., DUVAL Y.*, RAIBAUD P.*
Laboratoire de Physiologie de la Nutrition
* Laboratoire d'Ecologie Microbienne
I.N.R.A. - Centre de Recherches Zootechniques
F. 78350 Jouy-en-Josas - France

SUMMARY

The aminoacid (A.A.) composition of pure endogenous and microbial proteins in the gastrointestinal tract of the pig is described; meconium from small and large intestine and faeces from germ-free piglets as representative of endogenous fractions ; bacteria isolated from the faeces of pigs fed two different diets and pure culture of E. Coli as representative of microbial proteins. Estimates of the proportions of endogenous and microbial proteins in faeces of the Pig are derived from these data.

INTRODUCTION

Faeces or ileal digesta collected for protein digestibility measurements result from a mixture of several fractions of dietary, endogenous and bacterial origin. For that purpose, it is necessary to measure or estimate the respective amounts of these fractions in the samples analysed. Among available methods, it is possible to make estimations based on the comparison of the A.A. composition of the mixture collected and of accurate reference fractions. The present work aims at studying the A.A. composition of pure endogenous and microbial samples.

MATERIALS AND METHODS

Samples representative of endogenous and microbial proteins were analysed : 1) meconium of small and large intestine of piglets at birth; 2) faeces from germ-free piglets (1 to 5 days of age) reared in isolators ; 3) isolated faecal bacteria ; 4) pure culture of E. Coli. The faecal bacteria were isolated from faeces of 50 kg live weight pigs fed either a standard diet (barley : 60.0, maize : 15.0, soya-bean meal : 15.0, lucerne meal : 6.5 per cent) or a purified

diet (purified maize starch : 68.3, fish-meal : 21.6 per cent). The composition of both type of faeces was determined for comparison. Separation of bacteria was performed using several successive centrifugations. Concentration of bacteria was checked at each step by microscopic examination and bacterial count. The A.A. content of the samples was determined by ion-exchange chromatography after acid hydrolysis (24, 48 hrs, and oxidized preparation for sulfur A.A.). Diamino pimelic acid (DAP) was also analysed according to MZIK et al. (1978). The A.A. profiles of the various protein sources studied, were compared by calculation of the χ^2 distance (GUILLOTEAU et al., 1983 ; DARCY et al., 1983).

RESULTS AND DISCUSSION

The A.A. composition of the samples studied is given in table 1. The composition of meconium differs widely according to its origin : small vs. large intestine. The GLY, LEU and LYS contents are higher for the small intestine, whereas the THR, SER, PRO and CYS contents are higher for large intestine. There is no DAP in these samples. Faeces from germ - free piglets are rather similar to colic meconium as regards their A.A. composition. Their DAP content is very low. The A.A. profiles of isolated faecal bacteria do not differ much according to the diet. Moreover, this composition is not very far from that of pure E. Coli culture except for ARG content, which is very high in E. Coli. In addition, there is no major difference between the respective compositions of the total faeces and their paired bacterial isolates. In both cases, the total A.A. content is much higher in samples obtained in pigs fed the standard diet vs, the purified diet. Moreover the DAP content of faeces and bacteria obtained with the standard diet is twice as high as that of the homologous samples corresponding to the purified diet. The DAP content of E. Coli is 1.5 to 3 times that of isolated faecal bacteria. The calculations of χ^2 distance between the protein sources compared confirm all these observations. Using the same method, one can determine the combination of two protein sources which reproduces at best the registered composition of a sample studied. For both type of faeces, the best estimation corresponds to 90 per cent of the homologous isolated bacteria and 10 per cent of either meconium or germ-free faeces, with

Table 1. Aminoacid composition of some endogenous and microbial protein sources.

	Samples	Meconium		Germfree	Isolated faecal		E.Coli
		Small intestine	Large intestine	piglets faeces	Bacteria Standard	Bacteria Purified	pure culture
AMINO ACIDS - as per cent of their sum	ASX	9.08	7.38	7.80	11.18	14.18	11.14
	THR	7.14	14.50	11.48	5.47	5.06	5.11
	SER	5.99	9.16	8.20	4.81	5.51	3.99
	GLX	11.00	10.22	12.65	12.10	11.07	12.74
	PRO	5.72	10.26	9.23	4.28	3.46	3.59
	GLY	12.91	7.85	6.14	5.25	5.22	5.50
	ALA	5.59	5.70	5.07	6.98	6.83	7.50
	VAL	6.03	5.83	5.74	6.62	6.39	7.02
	ILE	3.41	2.48	3.40	6.00	5.80	5.51
	LEU	7.70	5.05	6.08	8.48	8.29	8.70
	TYR	3.21	2.11	2.81	4.64	4.02	3.80
	PHE	4.15	2.63	3.38	5.51	6.14	4.59
	LYS	6.13	3.98	5.21	7.45	6.55	7.30
	HIS	3.06	3.37	2.69	2.17	2.05	2.51
	ARG	5.33	3.99	4.93	5.24	4.87	7.27
	CYS	2.53	4.64	4.01	1.63	2.17	1.09
	MET	1.01	0.86	1.19	2.18	2.36	2.61
Sum 17 A.A*		16.30	21.64	24.97	35.57	25.70	59.80
Diamino pimelic* Acid		0.0000	0.0010	0.0075	0.1933	0.0938	0.2720

(*) as per cent of freeze-dried material.

a χ^2 value ranging from 6 to 14. The DAP to total nitrogen ratios allow to estimate the proportion of microbial proteins in the faecal protein, which would be 65 (standard) to 69 (purified) per cent.

Referring to bibliographical data, the meconium from small intestine and endogenous secretion from a perfused small intestine isolated loop (BURACZEWSKA, 1979) have closely similar A.A. composition, except for GLY content (absence of biliary glycoconjugates in an isolated loop). The colic meconium seems to predominate over that of small intestine in determining the composition of germ-free piglets faeces, which can be considered as totally endogenous. As regards faecal bacteria, our results can be compared to those supplied by MASON et al. (1976). Whatever the diet, the A.A. composition of isolated faecal bacteria remains nearly the same. It is also close to the profile of pure E. Coli, except for ARG content. Taking into account microscopic examinations and bacterial counts, these data suggest that the nature and amount of flora do not affect the A.A. composition of the total bacterial fraction. As compared to our estimations of the proportion of bacterial proteins in the total faecal nitrogen (90 per cent according to χ^2 calculations ; 65-69 per cent according to DAP), those given by MASON et al. (1976) range from 87-91 per cent (DAP) to 51-58 per cent (RNA). Thus bacterial proteins would dominate in the faecal nitrogen. However, the problem is to find an appropriate technique for an accurate measurement.

REFERENCES

BURACZEWSKA, L. 1979. Acta Physiol. Pol. 30 : 319-326.

DARCY, B., LAPLACE, J.P. & DUEE, P.H. 1983. Ann. Zootech. 32 : 315-340.

GUILLOTEAU, P., SAUVANT, D.& PATUREAU-MIRAND, P. 1983. Ann. Nutr. Met. 27 : 457-469.

MASON, V.C., JUST, A.& BECH-ANDERSEN S. 1976. Z. Tierphysiol. Tierernähr. Futtermittelkde 36 : 310-324.

MZIK, J., HOGAN, J.P., LINDSAY, J.R. & DAVIS, P. 1978. J. Chromatography. 152 : 269-271.

THE USE OF THE CANNULATED PIG TO STUDY THE EFFECT OF DIETARY FIBRE SUPPLEMENTS ON THE BACTERIAL FLORA OF THE PORCINE HINDGUT

Y.F. LIU[1], K. FADDEN[1], E. LATYMER[2], A.G. LOW[2] and M.J.HILL[1]
[1]PHLS, Centre for Applied Microbiology and Research, Bacterial Metabolism Research Laboratory, Porton Down, Salisbury, Wiltshire. SP4 0JG, U.K. [2]National Institute for Research in Dairying, Shinfield, Reading. RG2 9AT, U.K.

SUMMARY

The aim of the study was to determine the effect of dietary fibre on the bacterial flora of the terminal ileum, caecum, colon and faeces of the pig. The diets used were i) a standard control diet, ii) control diet supplemented with wheat bran, iii) pectin or iv) lactulose. Results indicate that these dietary fibre supplements had little effect on the bacterial flora of the porcine hindgut.

INTRODUCTION

As part of our studies on the causation of large bowel cancer, we are interested in the metabolism of dietary components, intestinal secretions and bile salts by the gut bacterial flora. It has been estimated that environmental factors are responsible for more than 80% of human cancers and that diet is responsible for almost 50% (1) In the case of gastrointestinal cancer it has been postulated that this relationship is mediated by bacteria. Many attempts have been made to relate the faecal bacterial flora to diet and these in general have been unsuccessful (2). However, studies in animals have shown a relation between caecal flora and diet (3) but all of these involved sacrifice of the animals and so only single time-points could be studied. Furthermore, it is suspected that the proximal gut is metabolically most active (4). As far as humans are concerned, there is no suitable way to study the proximal large bowel flora.

The pig is an excellent animal model because it is a natural omnivore with body weight and digestive physiology comparable to man. Young pigs fitted with cannulae in the terminal ileum, caecum and mid-colon were used in the study. This allowed continuous monitoring of the bacterial flora at these sites and of faeces

repeatedly during the course of dietary manipulation.

MATERIALS AND METHODS

Four Landrace x Large White boars were surgically cannulated with cannulae fitted in the terminal ileum, caecum and mid-colon. The diets used were: control diet (a standard pig diet) and control diet supplemented with either 10% wheat bran (WB), 5% pectin (P) or 5% lactulose (L). The experiment was designed as a Latin square with each dietary period lasting 2 weeks. Duplicate samples for bacteriological analyses were taken from each site (including faeces) on the last day of each dietary period. Samples were stored in cryoprotective glycerol broth at -40°C until analysis.

Quantitative bacteriological analyses were performed using the method of Hudson et al (5). This involved the inoculation of a range of selective and non-selective solid media with ten-fold dilutions of the sample. Colonies on various media were noted, counted, and representative numbers were subcultured and gram-stained. Aerobic and microaerophilic isolates were identified using conventional methods. Anaerobic isolates were identified to genus level by morphology, biochemical properties, fermentation end-products and growth on selective or differential media. Volatile fatty acid end-products were analysed by gas-liquid chromatography.

RESULTS AND DISCUSSION

The bacteriological findings for the pigs are shown in Table 1. For simplicity the table only shows the numerically most important organisms isolated. It has been observed that the terminal ileum of the pig is as heavily colonised as the large intestine; unlike the human ileum, which has between 10^6-10^7 colony forming units/g wet weight (cfu/g). This is in agreement with our findings. The bacterial flora at the cannulation sites from all 4 pigs when consuming the control diet was predominantly obligately anaerobic. The most commonly isolated organisms were Bacteroides spp. (principally Bacteroides fragilis) bifidobacteria, clostridia, veillonella, lactobacilli and anaerobic gram-positive non-sporing

Table 1. Bacterial counts (expressed as $\log_{10}$/g wet weight) of ileal, caecal and faecal samples on the various diets (control, WB, L, P).

Table 1a. Control diet

	Ileal	Caecal	Colon	Faeces
Total flora	8.02	7.95	8.30	8.42
Obligate anaerobes	7.23	7.81	8.16	8.30
Bacteroides	5.71	6.61	5.36	8.34
Anaerobic GPNSR	6.99	7.21	8.10	8.21
Clostridia	4.85	5.79	6.64	6.85
Total aerobes	7.75	7.54	6.83	7.29
Enterobacteria	6.82	6.69	6.39	6.91
Faecal Streptococci	7.43	6.69	6.22	6.29
Lactobacilli	6.70	7.81	8.10	7.91

Table 1b. Control Diet + Wheat Bran

	Ileal	Caecal	Colon	Faeces
Total flora	8.51	8.20	8.71	8.83
Obligate anaerobes	8.18	8.06	8.58	8.63
Bacteroides	6.62	5.92	6.59	6.77
Anaerobic GPNSR	8.10	7.89	8.56	8.09
Clostridia	5.73	5.59	6.54	6.54
Total aerobes	7.68	6.22	7.75	7.16
Enterobacteria	6.95	5.39	7.13	6.47
Faecal Streptococci	7.08	5.70	7.41	6.76
Lactobacilli	7.63	7.98	7.86	7.82

Table 1c. Control Diet + Lactulose

	Ileal	Caecal	Colon	Faeces
Total flora	8.17	8.49	8.75	8.63
Obligate anaerobes	7.92	8.37	8.50	8.56
Bacteroides	5.86	5.19	6.05	6.73
Anaerobic GPNSR	7.43	7.74	7.88	7.99
Clostridia	5.38	5.39	6.30	6.85
Total Aerobes	7.78	7.25	7.48	7.01
Enterobacteria	6.11	5.69	6.16	6.40
Faecal Streptococci	6.58	6.81	6.72	5.65
Lactobacilli	7.61	8.13	8.16	8.57

Table 1d. Control Diet and Pectin

	Ileal	Caecal	Colon	Faeces
Total flora	8.42	8.77	8.67	8.86
Obligate anaerobes	7.68	8.24	8.05	8.67
Bacteroides	6.29	5.77	4.94	5.18
Anaerobic GPNSR	7.37	7.15	7.40	8.28
Clostridia	5.67	5.38	6.71	6.72
Total Aerobes	8.63	8.58	7.96	8.16
Enterobacteria	8.49	8.58	7.62	7.41
Faecal Streptococci	8.03	7.77	7.97	7.67
Lactobacilli	7.33	7.50	7.99	8.24

rods (GPNSR). The most commonly isolated facultative organisms were the coliforms and faecal streptococci. Campylobacters and fusobacteria were present in small numbers, and oral streptococci were not isolated. Apart from the absence of oral streptococci, the bacterial flora isolated from the pig faeces was similar to that observed in human faeces. However, the concentrations of organisms is lower (by approximately 2log) than that found in human faeces. This may reflect the higher fibre content of the pig diets; which enable a faster intestinal transit and a dilution of colonic contents. Additionally, habitat simulating media (which may be expected to give higher recovery rates) were not used in this study.

Table 1 indicates that the dietary fibre supplements had minimal effect on the faecal flora, this finding is in agreement with the observations of Hill (2). The addition of WB, P or L had little effect on the gross individual components of the bacterial flora at any of the cannulation sites. However, there was an overall increase in the total obligate anaerobes.

Short dietary periods and a high residue control diet may in part explain the absence of notable changes in the gastrointestinal flora after dietary fibre administration. However, it is conceivable that diet may be important in altering the metabolic capacity of bacterial flora, rather than producing qualitative differences in the resident genera or species.

REFERENCES

WYNDER, E.L. and GORI, G.B. 1977. J. Natl. Cancer Inst. 58:825-832.

HILL, M.J. 1981. In Colon and Nutrition. Falk Symposium 32, 37-45. Eds. Kasper, H. and Goebell, H. MTP Press Ltd. The Hague.

MALLET, A.K., WISE, A. and ROWLAND, I.R. 1983. Arch. Toxicol. 52, 311.

BOWN, R.L., GIBSON, J.A., SLADEN, G.E., HICKS, B. and DAWSON, A.M. 1974. Gut, 15, 999.

HUDSON, M.J., HILL, M.J., ELLIOTT, P.R., BERGHOUSE, L.M., BURNHAM, W.R., and LENNARD-JONES, J.E. 1984. J. Med. Microbiol. 18, 335-345.

CLINICAL AND MICROBIOLOGICAL FIELD STUDIES ON DIGESTIVE TRACT DISORDERS OF PIGLETS AROUND THE TIME OF WEANING

M.J.A. NABUURS, F.G. van ZIJDERVELD, A. ROELOFS, J. HAAGSMA, P.W. de LEEUW
Central Veterinary Institute
P.O. Box 65, 8200 AB Lelystad, The Netherlands

SUMMARY

A clinical and microbiological study was performed in three swine herds involving six litters from one week before to three weeks post-weaning. The agents looked for were rotaviruses, *E. coli* and *Clostridium perfringens*.

Before weaning rotavirus excretion in the faeces was found in four litters, after weaning in all six litters. Many piglets had two episodes of virus shedding. Enterotoxigenic as well as non-enterotoxigenic *E. coli* strains were isolated in pure culture from all piglets but only after weaning. The different types appeared sequentially; the first strain was always a pathogenic one.

Clostridium perfringens type A was isolated from part of the piglets examined. The number of isolations before weaning was considerably higher than after weaning.

Although an over-all pattern relating the agents to diarrhoea could be shown, it proved difficult to find clear-cut relations in all individual piglets.

INTRODUCTION

Gastro-intestinal disturbances in piglets are of major importance in the period immediately after weaning. Several names are used that refer to the diarrhoea that occurs during this period, such as post-weaning diarrhoea, weanling diarrhoea, *E. coli* toxicosis etc. A lot of research has been done to elucidate the etiology of this syndrome. Specific *E. coli* strains are often isolated and they are generally considered to be responsible for the diarrhoea in the period immediately after weaning. Williams Smith and Hall found that some *E. coli* strains produced an enterotoxin and they suggested this to be the cause of the diarrhoea.

Food and feeding regimen have also been implicated in the etiology of diarrhoea in the weaning period.

In 1978 Tzipori *et al.* and Lecce *et al.* suggested that rotaviruses together with specific *E. coli* strains are of etiological importance.

Recently *Clostridium perfringens* type A (CPA) has been suggested to be involved in the etiology of piglet diarrhoea (Taylor and Olubonini, 1982; Nabuurs *et al.*, 1983).

Here we report preliminary results of an etiological investigation in three herds with a history of diarrhoea. Our attention was specifically focussed on rotavirus, *E. coli* and *CPA*.

MATERIALS AND METHODS

A. *Herds*

Three herds, A, B and C, were selected because of a history of severe diarrhoea in the post weaning period. In every herd two litters were chosen, each with a minimum of 10 piglets.

In herd A the litters under observation were housed together with 20 other litters in a farrowing house until weaning at an age of five weeks. After weaning piglets were moved to a weaning house whereby the two litters were mixed. They were penned on a slatted concrete floor.

Starting one week after birth piglets were given access to a commercially available milk replacer, after weaning a pre-starter diet was given.

In herd B the two litters were housed together with six other litters in one unit of the farrowing house until two weeks after weaning. In the weaning house the piglets were penned, without mixing the litters, on flat decks of a type that allowed an intensive contact between groups.

The piglets in herd B also had unlimited access to a milk replacer from one week after birth until one week before weaning when they received a pre-starter diet. Weaning was done at an age of five weeks.

In herd C the two litters were housed in a farrowing house, together with 16 other litters, until weaning. After weaning at an age of five weeks the piglets were moved to a weaning house where they were penned on flat decks. In this case there was only a direct contact between the two experimental litters. From the age of two weeks piglets had unlimited access to a commercially available pre-starter.

B. *Experimental design*

Collection of faeces was done daily starting one week before weaning until three weeks after weaning. Faeces were collected from the rectum with a small spoon. The spoon was placed in a tube and the samples were transported to the laboratory for bacteriological examination. They usually arrived within two hours after sampling. After bacteriological examination samples were frozen at -20°C.

The presence of diarrhoea was noted on the bases of clinical signs and visual inspection of the faeces. During the lactation period the excretion of yellow or brown pasty, thin or watery thin faeces was considered as diarrhoea, after weaning only the excretion of brown or yellow thin or watery thin faeces.

In all three herds four piglets per litter were sacrificed for histological examination of the intestinal tract. The first piglet in each litter was killed on the day of weaning, the others after 3, 7 and 10 days. The results of the histological examination will be dealt with in a separate paper.

C. *Bacteriological examination*

Examination for aerobic micro-organisms was done by inoculation of the faeces samples on sheep-blood agar plates. The plates were cultured for 20 hours at 37°C. Afterwards serological typing was done to establish the OK type of *E. coli* strains.

In each litter three animals were selected for examination for excretion of anaerobic micro-organisms. This was done, every second day, by inoculation of ten fold dilutions of faeces on

a sulfite containing selective medium. Sulfite reducing microbiological agents were subcultured for further identification. Obligate anaerobic growing isolates showing typical colonies on sheep blood agar and typical reactions on MacClung agar and in litmus milk were assumed to be *Clostridium perfringens*. Further typing of *Clostridium perfringens* was done by a mouse lethality assay followed by a neutralization test using specific antisera for the identification of the specific lethal toxins.

D. *Virological examination*

All faecal samples were tested for the presence of rotaviruses by an enzyme-linked immuno sorbent assay (ELISA) (Ellens and De Leeuw, 1977), using porcine serum instead of bovine serum. The ELISA results were occasionally verified by electron microscopy.

RESULTS

A. *Rotavirus excretion*

In figure 1 the percentage of piglets excreting rotavirus on the successive days is shown for all six litters. From herd A in one litter all 10 piglets excreted rotaviruses before weaning, in the other litter three out of ten piglets did so. After weaning five piglets in each litter were found to excrete rotavirus.

In the two litters of herd B only one pig excreted rotavirus before weaning, and all piglets except one excreted rotavirus after weaning.

Two piglets in one litter and three piglets in the other litter of herd C excreted rotavirus before weaning, six animals in one litter and seven in the other litter did so after weaning.

In table 1 a summary of these results is given and animals showing a rotavirus excretion episode before and one after weaning are presented.

Table 1. *Number of animals excreting rotavirus in particular periods*

Herd	Period		
	before weaning	after weaning	before and after weaning
A	8 (20)	5 (14)	5 (14)
B	0 (21)	15 (15)	1 (15)
C	2 (21)	13 (15)	2 (15)

In brackets total number of animals present in the period indicated.

B. *E. coli excretion*

In figure 2 the results of the *E. coli* examination are shown. In herd A two *E. coli* types were present one following up the other. The first one is a pathogenic type O139 K82, the second one has not yet serologically been identified because it is not a well-known pathogenic type.

FIGURE 1

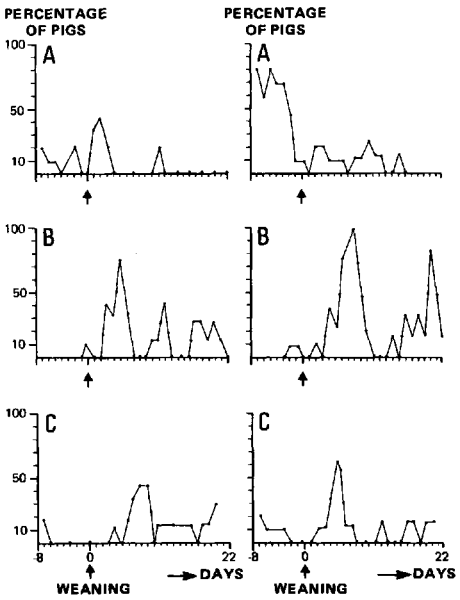


FIGURE 2

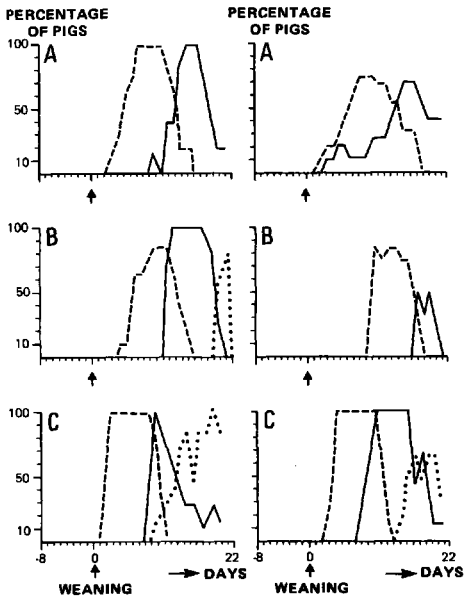


FIGURE 3

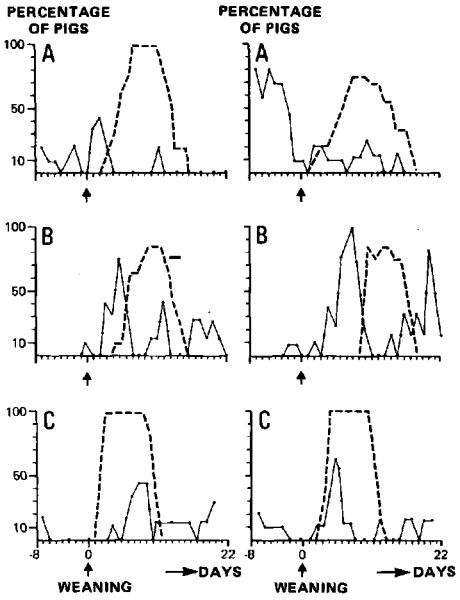


Figure 1 Rotavirus excretion in two litters of three herds

Percentage of faecal samples positive on a specific day are shown on the vertical axis, the days of investigation are on the horizontal axis.

Figure 2 *E. coli* isolation in two litters of three herds

Percentage of faecal samples found positive on a specific day for a recognized *E. coli* strain are shown on the vertical axis, days on the horizontal axis.

A ------ *E. coli* type O139 K82
——— *E. coli* not serologically identified
B ------ *E. coli* type O149 K91 K88
——— *E. coli* type O139 K82
. . . . . *E. coli* type O147 K89 K88
C ------ *E. coli* type O141 K85ae
——— *E. coli* auto agglutinable
. . . . . *E. coli* not serologically identified

Figure 3 Pattern of rotavirus excretion combined with the first isolated *E. coli* strain

——— rotavirus
------ *E. coli* strain

In herd B three different pathogenic types were isolated. The first one was identified as O149 K91 K88, the second one O139 K82 and the third one O147 K91 K88. This last type was isolated from only one litter.

In herd C also three different *E. coli* types were found. The first one was a pathogenic type O141 K85 ac, the second and the third one could not be identified yet because they were auto-agglutinable and of an uncommon serotype, respectively.

The combined pattern of rotavirus excretion and isolation of the first *E. coli* strain are shown per litter in figure 3. In all six litters rotavirus excretion occurred before pathogenic *E. coli* was found.

C. *CPA excretion*

Before weaning the percentage of CPA-positive samples was significantly higher in the three herds than after weaning (Table 2). All samples collected during the third week after weaning were negative for CPA.

Table 2. *Isolation of Clostridium perfringens type A from the faeces of piglets*

Herd	Number of days on which CPA was found/total number of days tested before weaning	after weaning
A	13/24 (54)*	3/66 (5)
B	11/24 (45)	18/72 (25)
C	5/24 (20)	1/60 (2)

* In brackets percentage of samples with CPA.

D. *Clinical findings and their relation with microbiological data*

During the period of investigation no disease symptoms were noted in the litters under investigation other than diarrhoea. However one piglet in one of the litters in herd A developed oedema disease (O.D.) two weeks after weaning (right litter in figure 1, 2 and 3). Clinical diagnosis was confirmed by post-mortem examination. In the litters that were not followed during the same period, about 3% of the piglets died of O.D. after weaning.

In herd B about 8% of the piglets that were not sampled, but also weaned during the investigation period, died with symptoms of post-weaning diarrhoea. Post-mortem examination confirmed that cardiovascular lesions and haemorrhagic lesions of the intestine were present.

In herd C there were no losses from several months before up to three months after the period of investigation.

It proved difficult to relate the, usually recurrent, diarrhoea episodes in individual pigs to the microbiological findings. This was mainly due to the fact that the onset of diarrhoea did not always coincide with the beginning of shedding of a particular agent. In addition, sometimes short diarrhoea episodes were observed without excretion of any of the agents we were looking for.

To present at least part of the data in a meaningful and yet clear manner, the number of days an agent was found in the faeces was related to the presence of diarrhoea on the day the samples were obtained (Table 3). In the case of rotavirus excretion, the diarrhoea percentage varied from 38–44, in the case of CPA from 25 to 53 per cent (Table 3). For the usually non enterotoxigenic *E. coli* strain O139 K82 the score for concomittant diarrhoea varied from 16 to 38 per cent. With the two usually enterotoxin positive *E. coli* strains O141 K85ac and O149 K91 K88 the highest values were found: 66 and 75 per cent respectively.

Table 3. *Total number of days a pathogenic micro-organism was detected in faecal samples and the percentage thereof with concomittant diarrhoea*

Herd	Agent									
	rotavirus		CPA		*E. coli* O149		*E. coli* O141		*E. coli* O139	
	N[1]	P[2]	N	P	N	P	N	P	N	P
A	74	– 44	16	– 25					100	– 16
B	76	– 37	28	– 53	68	– 75			60	– 38
C	47	– 38	6	– 33			116	– 66	3	– 33

1) N = total number

2) P = percentage

DISCUSSION

The feature of *E. coli* strains following up one another (Fig. 2) is striking. As far as we know similar observations have not been reported in the literature. After weaning the first *E. coli* strain detected in all litters was a pathogenic one. The next strain may be both, i.e. a pathogenic one (herd B) or a non pathogenic one (herds A and C). The reason for the presence of this pattern is unknown, but it may be of immunological origine. It may be that one type colonizes the gut until local immunity develops. Another strain may then colonize the gut, provided the circumstances are still favourable for colonization. The factors promoting colonization of the gut by most of these strains are unknown.

In almost all piglets of the six litters a pathogenic *E. coli* strain appeared in the faeces four to six days after weaning. For this phenomenon several explanations may be possible. Firstly alteration of the intestinal epithelium brush border by food components (Pusztai *et al.* 1979) or viral agents may play a role. Secondly disappearence of the lactogenic protection can be of importance as can be concluded from the work of Svendsen and Larsen (1977). Furthermore temporary shortage of enzymes, due to a not yet developed production or to epithelial damage, may result in ideal circumstances for *E. coli* to proliferate, because of an insufficient digestion and absorption of food

products.

As expected diarrhoea was most common on the days that enterotoxigenic *E. coli* was isolated from the faeces (Table 3).

In a considerable number of piglets we found two or more rotavirus excretion periods. This occurred in all six litters. Similar observations have been reported (De Leeuw *et al.* 1979; Lecce and King, 1980). Explanations put forward include immaturity of the immune system, an insufficient immune response and rotavirus strain differences.

In one litter of herd A a high rotavirus excretion peak was found before weaning, which may have resulted in a local intestinal immunity in most animals. It is likely piglets are then protected against a new infection with the same strain. In the other litter of herd A and both litters of herd C such an excretion peak was not found. However, the piglets that excreted rotavirus before weaning did so in the first days of the sampling period. Thus it may be that this represented the last days of an excretion period. In this respect it may be of significance that rather severe diarrhoea had occurred in all three litters before the investigation period started. In view of these findings we feel that future investigations along these lines should start earlier, preferably immediately after birth.

Results of these studies do not suggest that CPA is of major etiological significance in diarrhoea occurring after weaning (Table 2).

The most severe diarrhoea in the post-weaning period was observed in the litters of herds B and C. In these two herds rotavirus excretion was observed mainly after weaning and a usually enterotoxigenic *E. coli* strain was found after (B) or together with (C) the virus excretion. In herd A, little diarrhoea and little rotavirus excretion was observed after weaning. The *E. coli* strain isolated is usually non-enterotoxigenic. These findings lend some support to the suggestion put forward independently by Tzipori *et al.* (1980) and Lecce and King (1980), based on laboratory experiments, that rotaviruses together with specific *E. coli* strains are of etiological importance in the severe diarrhoea occurring after weaning. However, further studies are necessary to draw more firm conclusions.

REFERENCES

DE LEEUW, P.W., ELLENS, D.J. & HILBINK, F.W. 1979. Inserm 349-354.

ELLENS, D.J., DE LEEUW, P.W. 1977. J. Clin. Microbiol. **6** : 530-532.

LECCE, J.G. & KING, M.W. 1980. Proc. 3rd. Int. Symp. on Neonatal Diarrhoea, Oct. 6-8, Saskatchewan, 21-31.

LECCE, J.G., BALSBAUGH, R.K., CLARE, D.A. & KING, M.W. 1982. J. Clin. Microbiol. 715-723.

NABUURS, M.J.A., HAAGSMA, J., VAN DER MOLEN, E.J. & VAN DER HEIJDEN, Ph.J. 1983 Ann. Rech. Vét. **14** : 408-411.

PUSZTAI, A., CLARKE, E.M.W., KING, T.P. & STEWART, J.C. 1979. J. Food Sci. **30** : 843-848.

SVENDSEN, J. & LARSEN, J.L. 1977. Nord. Vet. Med. **29** : 533-538.

TAYLOR, D.J. & OLUBUNINI, P.A. 1982. Proc. IPVS July 26-31, Mexico, p. 66.

TZIPORI, S., CHANDLER, D;. MAKIN, T. & SMITH, H. 1980. Austr. Vet. J. **56** : 279-284.

WILLIAMS SMITH, H. & HALLS, S. 1967. J. Path. Bact. **93** : 531-543.

DOSE/RESPONSE EFFECT OF GROWTH PROMOTORS ON THE DIGESTIBILITY OF ENERGY IN GROWING PIGS

C. WENK
Institute of Animals Production, Nutrition Group,
ETH-Zentrum, CH-8092 Zürich

INTRODUCTION

The improvement of antimicrobial feed additives can partly be explained by a better health and stress resistance of the animals. But also a higher digestibility of nutrients can be observed with several substances (WENK, 1981).

The doses of the growth promotors, which are used in practice are commonly based on fattening experiments. Therefore ist is of interest to know whether the best growth performance can be observed at level with the highest digestibility of the nutrients.

In two separate experiments with growing pigs we studied the effect of two feed additives (Carbadoc [1)] and Bayo-n-ox [2)]) at 5 levels on the digestibility of the energy.

MATERIALS AND METHODS

An uniform basal ration, composed of barley, wheat, maize, heringmeal, soyabeanmeal, minerals, vitamins and celite 545 (carries of indicator) was used in both experiments. In table 1 mean composition of the rations and the additions of Carbadox and Bayo-n-ox are given.

As experimental animals two times 10 male castrates of the "Swiss Landrace" breed with a initial body weight (BW) of approximately 20 kg were used. The animals were fed restrictedly at a feeding level of about 110 g feed per kg BW and day in individual pens.

Both experiments were devided into 5 periods. In each period all feeds were offered to 2 pigs. The feeds were changed from period to period over the experiments corresponding to a latin square design, so that each animal got the feed of each treatment. Digestibility of energy was measured with the indicator method. In each period fresh faeces were collected over 4 days. HCl-unsoluble ash was used as indicator.

1) Mecaodx of PFIZER 2) BAYER

Table 1: Composition of the rations and experimental design of the experiments

Mean composition of the rations		Experiment 1 with Carbadox	Experiment 2 with Bayo-n-ox
Organic matter	g/kg feed	825	819
Crude protein	g/kg feed	183	196
Lipids	g/kg feed	50	49
Crude fiber	g/kg	58	61
Heat of combustion	MJ/kg feed	16.9	16.6

Experimental design		1	2	3	4	5
Experiment 1: Carbadox	ppm	0	25	50	75	100
Experiment 2: Bayo-n-ox	ppm	0	50	100	150	200

RESULTS AND DISCUSSION

In table 2 the digestibility of energy are presented for each treatment and period (mean of 2 values). Furthermore the mean values over the treatments and periods are given.

In practical pig rations Carbadox is used at a level of 50 ppm. Higher values above 100 ppm can have a detrimental effect on voluntary feed intake and therefore on the growth performance. Bayo-n-ox is commonly used at levels between 50 and 100 ppm. Negative effects of moderately higher levels are not known. The addition of the growth promotors caused a relative increase of the digestibility of energy of about 5 % with Carbadox and 1,5 % with Bayo-n-ox. Although the differences were generally small, in both experiments a positive weight or age effect of the digestibility of energy could be observed.

Above the lowest dose of the growth promotors no tendency of an increase or a decline was visible, although the highest dose was 4 times higher than the lowest.

The digestibility of the energy of the whole ration is a global value of the energy utilization in the digestive tract and

has signification in estimation of energy values of feeds. Nevertheless it cannot be excluded, that a possibly positive effect (e.g. higher nutrient absorption) at the high doses of the 2 antimicrobial substances above the practical application on the energy digestion was covered ba a negative influence (e.g. higher metabolic or microbial losses). Because the 2 growth promotors were tested in separate experiments a comparison of the substances is not valuable.

Table 2: Digestibility of energy

Treatment	1	2	3	4	5	mean
Experiment 1:						
Addition of Carbadox ppm	0	25	50	75	100	
period 1 (24 kg BW)	0.76	0.80	0.79	0.79	0.80	0.790
period 2 (35 kg BW)	0.75	0.81	0.80	0.81	0.80	0.794
period 3 (48 kg BW)	0.78	0.82	0.82	0.83	0.82	0.808
period 4 (61 kg BW)	0.76	0.80	0.80	0.81	0.81	0.796
period 5 (69 kg BW)	0.78	0.80	0.81	0.81	0.80	0.801
mean	0.766	0.806	0.798	0.811	0.805	0.797
(sd)	(0.013)	(0.009)	(0.011)	(0.017)	(0.009)	
relative to treatment 1	100	105	104	106	105	
Experiment 2:						
Addition of Bayo-n-ox ppm	0	40	100	150	200	
period 1 (26 kg BW)	0.77	0.79	0.78	0.78	0.80	0.785
period 2 (35 kg BW)	0.79	0.79	0.80	0.80	0.80	0.796
period 3 (44 kg BW)	0.78	0.82	0.78	0.81	0.80	0.798
period 4 (54 kg BW)	0.80	0.81	0.81	0.80	0.82	0.808
period 5 (64 kg BW)	0.82	0.82	0.81	0.81	0.81	0.814
mean	0.792	0.806	0.796	0.800	0.806	0.800
(sd)	(0.019)	(0.015)	(0.015)	(0.012)	(0.009)	
relative to treatment 1	100	102	101	101	102	

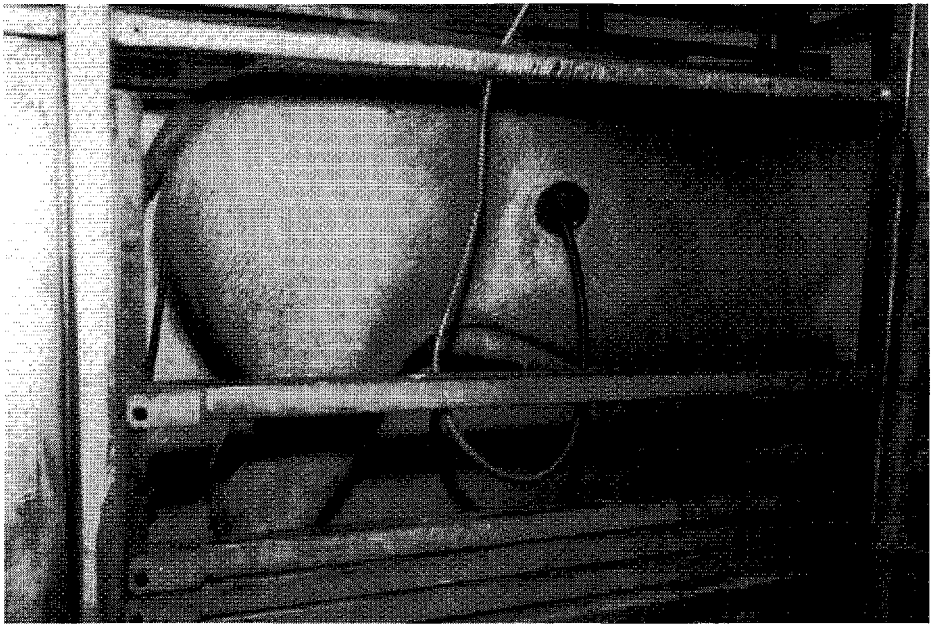
REFERENCES

WENK, C. 1981. Zur Verwertung der Energie beim wachsenden monogastrischen landwirtschaftlichen Nutztier. Habilitationsschrift ETH Zürich

SESSION 5

DIGESTION AND ABSORPTION OF NUTRIENTS: RESULTS AND THEIR PRACTICAL APPLICATIONS

Discussion leader: S. H. M. Metz



DIGESTION AND ABSORPTION OF NUTRIENTS: RESULTS AND THEIR PRACTICAL APPLICATIONS

WILLEM C. SAUER
Department of Animal Science
Faculty of Agricultural and Forestry
Edmonton, Alberta, Canada

DIFFICULTIES IN ASSESSING THE NUTRITIVE VALUE OF LUPIN-SEED MEALS AS SOURCES OF LYSINE AND ENERGY FOR GROWING PIGS

E.S. BATTERHAM
Department of Agriculture, Agricultural Research Centre
Wollongbar, NSW 2480, Australia

SUMMARY

The lysine in lupin-seed meal is highly digested, but has a low availability. Similarly, the digestible energy content is high, but estimates of net energy are considerably lower. The lower net energy value may have contributed to the low estimates of lysine availability in slope-ratio assays. Results with a growth experiment indicated that the differences in growth performance of pigs fed a lupin-seed meal compared to soyabean meal diet could not be overcome with a supplement of oil, designed to equalize estimated net energy contents. It is concluded that digestible energy is an adequate basis on which to formulate diets containing lupin-seed meal and that a deficiency of net energy is unlikely to have contributed to the low estimates of lysine availability.

INTRODUCTION

Techniques used to measure the nutritive value of lysine and energy in lupin-seed meals (L. albus and L. angustifolius) give conflicting results. The digestibility of lysine at the terminal ileum is high (0.82-0.93, Taverner, 1982; Taverner et al. 1983). However, the availability of lysine, as assessed with slope-ratio assays, is low (0.37-0.65, Batterham et al. 1984). The digestible energy content is high (ca. 15 MJ/kg, air-dry basis, Batterham, 1979). However, lupin-seed meal has a high crude fibre content (ca. 150 g/kg) and nearly half the energy is digested in the hind gut (Taverner and Curic, 1983; Taverner et al. 1983). Digestion involving hind gut fermentation is thought to be less efficient than digestion in the small intestine. Thus the net energy in lupin-seed meal may be considerably less than that indicated by the digestible energy value (Taverner and Curic, 1983).

The slope-ratio assays of Batterham et al. (1984) were formulated on a digestible energy basis, with dietary crude fibre

levels equalized with solka floc. If the net energy of the diets containing lupin-seed meal was over-estimated, this could have limited growth response and resulted in an under-estimation of lysine availability.

The objective of this study was to determine if a diet containing lupin-seed meal, when formulated on a digestible energy basis, was deficient in net energy.

MATERIALS AND METHODS

Wheat-based diets were formulated with lupin-seed meal or soyabean meal to be lysine deficient (8 g/kg) and to contain an estimated 14.5 MJ/kg digestible energy. The net energy in the lupin-seed meal and soyabean meal diets was estimated as 8.9 and 9.5 MJ/kg respectively, using the relationship between digestible and net energy reported by Taverner and Curic (1983). Soyabean oil was added to a lupin-seed meal diet to equalize the estimated net energy content with that of the soyabean meal diet.

The diets were offered to growing pigs on a restricted feeding scale between 20 to 45 kg live weight. At the completion, the pigs were slaughtered, and energy contents calculated from carcass nitrogen and fat contents.

RESULTS

Growth rates, particularly on a carcass basis, were lower for pigs fed the lupin-seed meal diets (Table 1). Energy retention was also lower ($P<0.01$). The addition of oil had no effect on growth rate ($P>0.05$), but improved food conversion ratio (carcass basis) and energy retention ($P<0.05$). However, energy retention was still lower than that of pigs fed the soyabean meal diet.

DISCUSSION

If energy was limiting in the lupin-seed meal diet, and lysine adequate, then a supplement of energy would increase amino acid utilization and would promote growth. However, if energy was adequate and lysine limiting, then a supplement of energy would not be used for protein synthesis, and growth would be either unaf-

Table 1. Growth rate, food conversion ratio (FCR) and energy retention of pigs fed diets containing lupin-seed meal, lupin-seed meal plus oil or soyabean meal

	1 Lupin	2 Lupin + Oil	3 Soya	SEM
Digestible energy (MJ/kg)	14.5	15.2	14.5	
Net energy (MJ/kg)	8.9	9.5	9.5	
Gain - g/day	607	609	636	10.1
Carcass gain - g/kg	440	434	508	6.1
FCR	2.2	2.1	2.1	0.03
FCR (carcass basis)	3.0	2.9	2.6	0.04
Energy retained: digestible energy intake	0.32	0.37	0.41	0.01

fected or depressed. The lack of a growth response to the oil supplement indicates that the lupin-seed meal diet was adequate in net energy and this was not the reason for the differences in growth between the lupin-seed and soyabean meal diets.

The lack of response to additional energy in the lupin-seed meal diet also indicates that, despite the high crude fibre content of lupin-seed meal, digestible energy appears an adequate basis on which to formulate diets involving its use.

The differences in the growth of pigs fed the diets is consistent with the differences in available lysine (5.0 and 6.3 g/kg for Diets 1 and 3 respectively). The reason for the low lysine availability in lupin-seed meal may be due to the presence of an unidentified anti-nutritional factor, or to the lysine being digested in a form that is inefficiently utilized.

REFERENCES

BATTERHAM, E.S. 1979. Aust. J. Agric. Res. 30: 369-375.

BATTERHAM, E.S., MURISON, R.D. & ANDERSEN, L.M. 1984. Br. J. Nutr. 51: 85-99.

TAVERNER, M.R. 1982. Proc. Aust. Soc. Anim. Prod. 14: 667.

TAVERNER, M.R. & CURIC, D.M. 1983. In "Feed Information and Animal Production". (Eds. G.E. Robards and R.G. Packham.) Commonwealth Agricultural Bureaux, Slough. p. 295-298.

TAVERNER, M.R., CURIC, D.M. & RAYNER, C.J. 1983. J. Sci. Food Agric. 34: 122-128.

PRAECAECAL DIGESTIBILITY OF AMINO ACIDS IN PIGS FED BARLEYS DIFFERING IN PROTEIN CONTENT

LUCYNA BURACZEWSKA*), E.SCHULZ AND HARRIET SCHRÖDER
Institute of Animal Nutrition, FAL
Bundesallee 50, D-3300 Braunschweig, F. R. Germany
*) Institute of Animal Physiology and Nutrition
05-110 Jablonna near Warsaw, Poland

SUMMARY

The apparent digestibility of nitrogen and amino acids was measured by the ileal and faecal methods in pigs fed nine barleys differing in nitrogen content (from 1.73 to 2.16 per cent/DM). A positive (r = 0.87) and significant relationship was found between the apparent nitrogen digestibility at the end of the ileum and the crude protein content of barley. Generally, the higher the protein of the grain, the higher the ileal digestibilities of the amino acids. For most amino acids the faecal values were greater than the ileal ones. This being specially so for threonine, tryptophan, histidine and cystine belonging to the nutritionally important amino acid group. When the relationship between the amino acid and nitrogen digestibilities at the level of ileum is considered, the values were found to be similar to that of nitrogen in the case of tryptophan, higher for isoleucine, valine (both 2-3 units), cystine, histidine, leucine, methionine (all 5-6 units) and phenylalanine (10 units) and lower only for lysine and threonine (7-8 dig.units).

INTRODUCTION

Barley provides a large proportion of dietary protein for pigs in many countries. It varies widely in total protein content and its digestibility. Eggum (1973) reviewed many studies and found that the variation in protein and amino acid digestibility of barley was related both to protein and to its tannin content. Since many years it has been generally recognized as important to estimate ileal rather than faecal digestibility of amino acid in feedstuffs as a measure of their availability to pigs. The results published up to now show large differences concerning the availability of nutritionally important amino acids of barley. The question arises whether and to what extent the ileal digestibility values are influenced by crude protein and fibre content of the grain.

The aim of this study was to determine the apparent digestibility of nitrogen and amino acids at the terminal ileum and over the entire digestive tract of growing pigs fed barley differing in nitrogen content.

MATERIALS AND METHODS

Male pigs in the range of live weight from 28 to 76 kg with simple cannula in the terminal ileum were fed with diets of nine different barleys (Table 1) as the only feed. The diets were supplemented with vitamins and minerals and chromium oxide was added. They were fed according to body weight for 12 days, each to 4, 5 or 6 pigs. The diets were offered in the wet form in two meals given every 12 h.

After 6 days of preliminary period, faeces were collected for 72 h from the seventh to the ninth and digesta for three 12 h periods from the tenth to the twelfth day. By using soft plastic tube the digesta was collected into a bottle immersed in an ice-water bath. Faeces and digesta were freeze-dried and pooled samples were also prepared for total collection period of digesta (36 h) from each pig on dry matter basis.

Chromium content was determined by a method based on that described by Petry a. Rapp (1970/71). Fiber fractions NDF and ADF were analyzed according to Fibertec System M. Samples for NDF determination were incubated with amylase after detergent treatment to remove the rest of starch from the crucible remaining.

RESULTS AND DISCUSSION

The contents of nitrogen, some amino acids and fibre fractions in different barleys are presented in Table 1. Within the barleys the amount of lysine decreased from 3.82 to 3.26 g/16 g N as the N content increased from 1.73 to 2.16 per cent/DM. Acid- and neutral-detergent fibre appeared not to be closely related indicating a variable content (from 8.0 to 10.6 %) of hemicellulose, calculated by difference between the NDF and ADF values. This fact is in agreement with observations made on wheats (Taverner et al., 1981).

Fig. 1 shows the results of apparent ileal digestibility of crude protein related to nitrogen content of eight barleys (excluding No. 9). The regression coefficient of the linear regression equation ($r = 0.87$) was positive and statistically significant. This is in agreement with Eggum (1973) who reported a positive relationship between the true digestibility and the content of protein

Table 1. Chemical characteritics of barleys (amino acids given in g/16 g N; others in per cent of DM)

No *)	1	2	3	4	5	6	7	8	9
Variety	Grit	Aramir	Gunhild **) Georgie	Diva	Aramir	Ingri **) Tapir Sonia	Aramir	Diva	Ingri **) Tapir
N	1.73	1.80	1.88	1.98	2.00	2.01	2.05	2.10	2.16
NDF	13.82	13.93	16.61	13.45	14.06	13.89	15.57	14.85	15.52
ADF	5.87	5.32	6.06	5.32	5.00	5.29	6.45	5.53	5.41
Crude fibre	4.85	4.74	5.19	4.36	4.79	4.59	5.20	5.24	4.91
Lys	3.82	3.79	3.66	3.63	3.67	3.47	3.50	3.53	3.26
Cys	2.29	2.11	2.09	2.12	2.23	2.10	2.13	2.08	2.15
Met	1.93	1.93	1.78	1.74	1.81	1.74	1.80	1.81	1.86
Trp	1.27	1.15	1.17	1.18	1.21	1.22	1.20	1.22	1.27
Thr	3.51	3.58	3.46	3.43	3.50	3.37	3.45	3.47	3.38
Ile	3.60	3.54	3.68	3.47	3.58	3.48	3.50	3.55	3.51

*) Nos 1,2,3,4,5,7 and 8 were summer barleys and no 6 and 9 winter barleys
**) Mixture of two or three varieties

in barley.

Generally, the higher the protein content in the grain the higher the apparent amino acid digestibility measured at the ileum. However, in our case the digestibilities for barley no 4 were somewhat higher than that of nos 5 and 7, and that of no 6 higher than that of nos 7 and 8, what was probably due to comparatively lower NDF content in barleys nos 4 and 6.

The average values for ileal and faecal apparent digestibilities are presented in Fig. 2. The graph shows that for nitrogen as well as for most of the amino acids the faecal values were greater than the ileal ones. In the case of nitrogen the faecal value exceeded in about 10 digestibility units what is in the range observed by other authors (from 8 to 15). The amino acids that disappeared to the largest extent in the large intestine among the dispensible amino acids were glycine, proline and serine. What the nutritionally more important amino acids is concerned, for cystine, valine, histidine, tryptophan and threonine faecal analysis originates overestimation ranging from 6 to 12 units and less

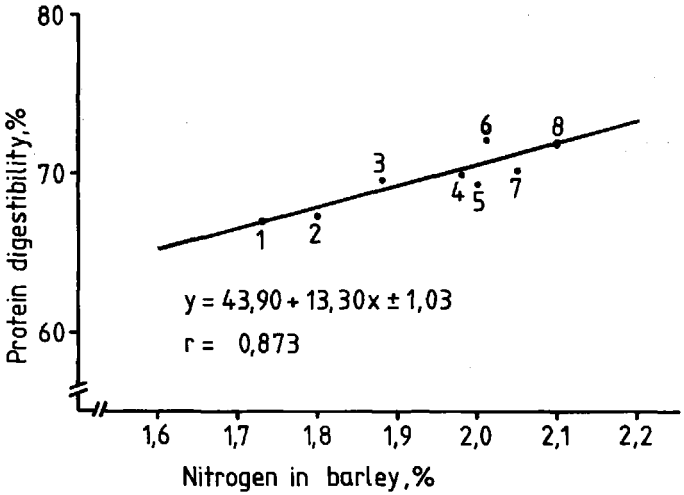


Fig. 1. Relationship between app. ileal digestibility of protein and nitrogen level in eight barleys (nos 1-8)

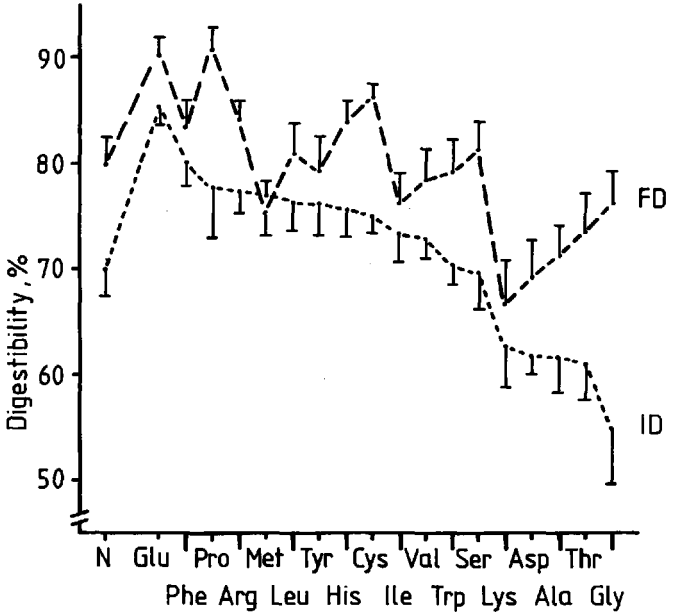


Fig. 2. App. ileal (ID) and faecal (FD) digestibility of amino acids in pigs fed eight barleys; (average values with standard deviation)

for other amino acids. For methionine the faecal as compared to ileal digestibility value indicates even a net increase which is consistent with the results reported by many authors (Cho & Bayley, 1972; Just et al., 1980; Taverner & Farrell, 1981; Zebrowska et al., 1981) and not only for grains but also for other feedstuffs. The differences of individual amino acids in the large intestine is thought to be primarily due to bacterial fermentation and synthesis in this part of the digestive tract. As the bacterial processes depend on the availability of nitrogen and energy, the differences between faecal and ileal values were somewhat smaller for the higher digestible barleys which contained more than 2 per cent N and less than 12 per cent NDF.

When comparison was made between the ileal amino acid and nitrogen digestibilities, which can be predicted from the equation given in Fig. 1, the value for tryptophan was similar to that of nitrogen, for lysine and threonine being 7-8 digestibility units lower, for isoleucine and valine 2-3 units higher, for cystine, histidine, leucine and methionine 5-6 units higher and in the case of phenylalanine even up to 10 digestibility units higher than the value for nitrogen.

REFERENCES

CHO,C.Y. & BAYLEY,H.S., 1972. Can.J.Physiol.Pharmac.50:513-518.

EGGUM,B.O. 1973. A study of certain factors influencing protein utilisation in rats and pigs. Rolighedsvey 26, 1958 København V.1973.

JUST,A., SAUER,W.C., BECH-ANDERSEN,S., JØRGENSEN,H.H. & EGGUM,B.O. 1980. Z.Tierphysiol.Tierernährg.Futtermittelkde. 43:83-91.

PETRY,H. & RAPP,W. 1970/71. Z.Tierphysiol.Tierernährg.Futtermittelkde. 27:181-189.

TAVERNER,M.R. & FARRELL,D.J. 1981. Brit.J.Nutr. 46:173-180.

TAVERNER,M.R., HUME,I.D. & FARRELL,D.J. 1981. Brit.J.Nutr. 46:159-171.

ŻEBROWSKA,T., BURACZEWSKA,L. & ŻEBROWSKA,H. 1981. VI. Intern.Symp. on Amino acids, Serock, Poland.

DIGESTION OF PROTEINS IN THE PIG : ILEAL AND FAECAL DIGESTIBILITIES AND ABSORPTION COEFFICIENTS OF AMINO ACIDS

DARCY-VRILLON B., SOUFFRANT* W.B., LAPLACE J.P., RERAT A.,
GEBHARDT* G., VAUGELADE P., JUNG J.
Laboratoire de Physiologie de la Nutrition
INRA - F 78350 Jouy-en-Josas (France)
*Wb Tierernährungsphysiologie-Futtermittelkunde
Karl-Marx Univ. - 7022 Leipzig (DDR)

SUMMARY

The present work was designed to compare three different methods to estimate the amino acid availability on the basis of their digestive utilisation : faecal and ileal digestibility and quantitative absorption. Tested for the same semi purified diet, these methods provided different results. That points out that each of them has a specific limitation. Ileal digestibility should be the best, but evaluation of endogenous nitrogen is required.

INTRODUCTION

For practical purposes, the estimation of amino acid availability is often based on their digestive utilisation which can be measured by different methods : amino acid disappearance from the lumen of the G.I. tract (apparent faecal or ileal digestibilities) or amino acid appearance in the intestinal efferent blood. These three different approaches were used concurrently for the same diet in the Pig. They were also combined with ^{15}N isotopic labelling to quantify endogenous supplies. This paper only aims at describing the apparent balances assessed by the three methods : faecal digestibility (F.D.), ileal digestibility (I.D.), and quantitative absorption coefficients (A.C.).

MATERIALS AND METHODS

Seven Large White pigs (52.6 $\pm$ 2.6 kg live weight) were fed a semi purified diet including 72.3 per cent purified wheat starch, and 16.1 per cent hydrochloric casein. Four of them were submitted to ileocolic postvalve fistulation (DARCY, LAPLACE, VILLIERS, 1980) to allow ileal digestibility measurements. The three others, used for faecal digestibility measurements, were also prepared to quantify

nutrient absorption, according to RERAT, VAUGELADE and VILLIERS (1980). Faecal digestibility values were established on 8 collection days in pigs fed twice a day. Ileal digestibility and absorption data were based on a 24h test period only for each pig fed once a day, due to the restraints resulting from isotopic labelling. Dry matter, total nitrogen and amino acid contents were determined in ileal digesta and faeces. Measurement of free amino acid concentrations in the blood of carotid artery and portal vein, along with that of portal blood flow, allowed to calculate the quantities of amino acids absorbed over 24 hrs.

RESULTS AND DISCUSSION

The present results (table 1) confirm some previous observations. Whatever the site of collection (ileum, faeces), nitrogen digestibility was lower than that of total amino acids. The previously observed hierarchy between the digestibilities of individual amino acids (DARCY, LAPLACE and DUEE, 1982) was also recorded at both levels for the casein diet used. However the range of the digestion coefficients for individual amino acids was smaller for faecal than for ileal digestibility. The average faecal digestibility of nitrogen and total amino acids was higher than the corresponding ileal values. The average deviation was small for the highly digestible diet used. Nevertheless it reached 10 points for some amino acids (CYS, GLY), or was suppressed or even inverted (MET) in some instances. Due to a large variability, mainly related to the experimental conditions (one test day only for ileal digestibility vs 8 days for faecal digestibility), most of these differences were not significant. As compared to these estimates of disappearance from the gut lumen, the absorption coefficients for nitrogen and amino acids were much higher, and often above 100 per cent. It means an additional recovery of endogenous matters within a 24 hrs period. Moreover there were large variations between the absorption coefficients of individual amino acids. That is probably related to the metabolism of some non essential amino acids within the intestinal cell wall. According to these results, the three methods provide rather different estimates. For dietary protein evaluation on the basis of their available amino acid content, each of them has got a specific defect :

Table 1. Mean faecal and ileal digestion coefficients, and mean absorption coefficients.

	THR	VAL	ILE	LEU	TYR	PHE	LYS	HIS	ARG	CYS
F.D.	96.3	97.1	96.4	97.7	98.9	97.2	97.8	97.9	96.9	85.1
I.D.	90.8	94.7	94.4	95.7	92.3	96.6	97.1	96.7	95.5	75.0
A.C.	113.6	109.5	95.2	82.2	95.9	104.3	129.4	112.8	141.3	76.3

	MET	ASX	SER	GLX	PRO	GLY	ALA	sum of 17 AA	Nitrogen
F.D.	96.6	95.9	96.3	98.3	97.4	92.6	94.1	97.3	95.2
I.D.	97.2	93.8	90.7	96.2	96.9	82.6	91.6	94.9	91.0
A.C.	113.1	109.4	126.6	3.7	88.5	250.1	477.0	107.3	125.9

1) faecal digestibility is mainly affected by the bacterial activity in the large intestine, resulting in amino acid disappearance without any benefit for the animal ; 2)ileal digestibility escapes this microbial influence but remains affected by the residual unabsorbed endogenous fraction ; 3) Absorption takes into account both the recycled endogenous nitrogen and the consequences of the gut wall metabolism.

Ileal digestibility is probably the best tool for practical purpose. The knowledge of endogenous nitrogen production and recycling as well as their variation factors remains a key step both for practical diet evaluation and for the understanding of digestive physiology. Our related ^{15}N results should allow such a progress.

REFERENCES

DARCY, B., LAPLACE, J.P. & DUEE, P.H. 1982. Ann. Zootech. 31 : 279-300.

DARCY, B., LAPLACE, J.P. & VILLIERS, P.A. 1980. Ann. Zootech. 29: 147-177.

RERAT, A., VAUGELADE, P. & VILLIERS, P.A. 1980. In "Current concepts of Digestion and Absorption in Pigs". (Eds. A.G. Low and I.G. Partridge.). Tech. Bull. 3, Nat. Inst. Res. Dairying, Reading : 177-214.

PROTEIN DIGESTION IN PIGS MEASURED IN VIVO AND IN VITRO

N. DIERICK, I. VERVAEKE, J. DECUYPERE and H. HENDERICKX
University of Ghent, Faculty of Agricultural Sciences
Laboratory of Nutrition and Hygiene,
Proefhoevestraat, 10, B-9230 Melle,
Belgium.

SUMMARY

Three two steps routine in vitro methods for predicting the protein digestibility of swine feeds and ingredients were proposed. The first method using acid pepsin and porcine jejunal fluid was a modification of the method of FURUYA et al. (1979). The second and third method were based on incubation with acid pepsin (4h)/pancreatin (4h) and acid pepsin (4h)/pronase (4h) respectively.

The first method requiring cannulated donor pigs could be replaced by the method using acid pepsin and pancreatin. A high correlation was found between the latter method (X) and in vivo fecal digestibility of protein (Y) for 30 swine feeds and ingredients : $Y = 7,256 + 0,854\ X$; $r = 0,87$; $rSD = 6,7$. A lesser correlation was found between this in vitro method (X) and in vivo ileal digestibility of protein (Y) from the same sources : $Y = 24,745 + 0,568\ X$; $r = 0,56$; $rSD = 6,7$. Although the proposed in vitro method is rapid and reproducible, the accuracy of the prediction equation can be improved if in the future more and own in vivo ileal N digestibility data corrected for endogenous N and in vivo fecal N digestibility data corrected for bacterial N are included.

INTRODUCTION

There is no doubt, digestibility experiments with cannulated (T-cannula, re-entrant) pigs are very work and time consuming. In order to enable ileal digestibility data to be widely used in diet formulation of swine, a future goal should be to predict protein digestibility in a feedstuff by a rapid and reliable in vitro procedure and next to predict amino acid digestibilities in a feedstuff from N digestibility. In monogastric animals such as the pig, the digestion of the protein is almost completed at the end of the small intestine. In order to simulate protein digestion in the small intestine, three in vitro methods were investigated :1) incubation of the test substrate

with acid pepsin followed by jejunal fluid obtained from cannulated pigs, based on the method of FURUYA et al. (1979) ; 2) incubation with acid pepsin followed by pancreatin ; 3) incubation with acid pepsin followed by pronase.
The obtained in vitro data are compared with own experimental in vivo data (ileal and fecal N digestibilities) or with literature data.

MATERIALS AND METHODS

1. Incubation procedure with pepsin/jejunal fluid.

This method was a modification of the method described by FURYA et al. (1979) and CLUNIES & LEESON (1984).
The first step of incubation was carried out by mixing an 150 mg protein containing sample, ground in a Cyclotec Sample Mill (Tecator, Sweden) with 20 mg of powdered pepsine (2000 FIP-U/g, Merck, Germany) in 20 ml HCl 0,075N + 1 drop Thimerosal (5% w/v in H_2O) and incubating in a shaking waterbath at 37°C for 4h in quadruplicate. Than, the mixture is brought to pH 7,5 with NaOH 0,2N. The second step of incubation is carried out after addition of 20 ml of prepared porcine jejunal fluid. Collection and preparation of jejunal fluid was as follows : 4 pigs (BL race, 30 kg) were cannulated with a simple T-cannula 0,5 m distal to the pancreasduct (DECUYPERE et al., 1977). The diet contained barley, corn and soya. Jejunal contents were collected during about 2 hours, every day, during several weeks. The collected fluids were centrifuged at 1250g. The supernatants were frozen and lyophilized. The lyophilized materials were thoroughly mixed, pooled and stored at -20°C. For Use, the material was reconstituted in a phosphate buffer (5g material/100ml P-buffer; 0,2M ; pH 7,5). The second step of incubation was carried out for 4h at 37°C The duration of the incubation during the first and second stage are comparable with the mean retention time of feed in the stomach and small intestine respectively. After acidifying the digest is treated with 10 ml phosphotungstic acid (0,02M) and centrifuged for 10 min. at 1250g. The undigested precipitate was analysed for N.

2. Incubation procedure with pepsin/pancreatin and pepsin/pronase.

The first step of the incubation with pepsin was as sub.1. In the second stage of the incubation, jejunal contents were replaced by 20 ml pancreatin solution (150mg pancreatin/100ml P-buffer; pancreatin

4 x NF Grade, VI, Sigma, St. Louis, USA) or by 20 ml pronase solution

Table 1. Comparison of digestibility of protein in vitro and in vivo (ileal and fecal) from swine feeds and ingredients.

	In vitro[1]	in vivo[2]	
		Ileal	Fecal
Feed ingredient			
Casein	99,8	87,1	93,3
Soya isolate	91,4	89,6	94,9
Soybeanmeal	93,7	78,6	88,8
Rapeseed meal	85,5	68,9	79,8
Cottonseed meal	88,1	73,1	77,0
Lineseed meal	80,8	-	78,0
Groundnutmeal	92,8	70,3	87,9
Fish meal	91,8	83,9	87,1
Meat meal	96,8	74,0	84,3
Pekilo SCP	88,5	-	85,1
Pruteen SCP	95,1	-	89,5
Peas	95,5	85,0	85,4
Wheat	89,7	82,8	86,6
Barley	87,0	73,8	80,2
Maize	81,7	73,6	83,2
Oats	88,4	62,0	74,0
Rye	87,8	68,0	79,0
Milo	78,3	75,0	81,8
Manioc	31,7	-	35,0
Maize glutenfeed	85,2	65,0	71,0
Wheat bran	74,5	60,0	70,0
Malt sprouts	79,4	-	65,0
Alfalfa meal	66,2	-	62,0
Dried sugarbeetpulp	55,9	-	39,0
Compound feeds			
A	86,2	70,4	82,1
B	84,9	74,6	81,6
C	76,3	64,0	75,5
D	89,1	73,6	79,5
E	67,4	69,6	82,1
F	77,5	76,4	84,1

1: samples containing 150mg protein, incubation method: acid pepsin/ pancreatin. 2: own and literature data (LENIS, 1983).

(150 mg pronase/100 ml P-buffer ; protease from Streptomyces griseus; type XIV, Sigma, St. Louis USA) respectively. Further treatments were as sub. 1.

RESULTS AND DISCUSSION

Comparing the three in vitro methods it was found that the jejunal fluid as proposed by FURUYA et al. (1979) can be replaced by an appropriate pancreatin solution without loss of accuracy. Using the latter method the in vitro protein digestibility from 30 swine feed ingredients and compound feeds was compared with in vivo ileal and fecal N digestibility coefficients obtained in our laboratory (DIERICK et al. 1984) and from the literature (LENIS, 1983). The results are presented in table 1. The relationship between the in vivo fecal (Y) and in vitro N digestibility (X) was as follows : $Y = 7,256 + 0,854\ X$ ($r = 0,87$, $R^2 = 0,76$; rSD = 6,7). The lower accuracy of the present method compared with that of FURUYA et al. (1979) is probably due to the lack of own in vivo data of our samples. This was especially true for predicting the ileal N digestibility (Y) from the in vitro incubation method (X). The regression equation for the latter was : $Y = 24,745 + 0,568\ X$; $r = 0,56$; $R^2 = 0,31$; rSD = 6,7. Because the in vitro method simulates the protein digestion in the small intestine, one should expect a better correlation with ileal digestibilities than with overall fecal digestibilities. However the in vitro method does not take into account the great amounts of endogenous nitrogen at ileal level. Therefore the accuracy of the prediction equation can be increased in the future if more and own in vivo data corrected for endogenous N at ileal level and for bacterial N at fecal level are included in the regression equation. This research is in progress now.

REFERENCES

CLUNIES, M. & LEESON, S. 1984. Poultry Science. 63 : 1033-1039.
DECUYPERE, J., VERVAEKE, I., HENDERICKX, H. & DIERICK, N. 1977. J. Anim. Sci. 46 : 463-468.
DIERICK, N., VERVAEKE, I., DECUYPERE, J. & HENDERICKX, H. 1984. In : "Results of pig research" Eds. IWONL-IRSIA, Brussels. p. 239-248.
FURUYA, S., SAKAMOTO, K. & TAKAHASHI, S. 1979. Br. J. Nutr. 41 : 511-520.
LENIS, N. 1983. In : "Les Colloques de l'INRA, n°16", France p.385-389.

CIRCADIAN VARIATION IN CHEMICAL COMPOSITION OF DUODENAL AND TERMINAL ILEAL DIGESTA

H. GRAHAM, K. HESSELMAN*, P. ÅMAN
Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences
S-750 07 Uppsala, Sweden

SUMMARY

A large circadian variation in the content of starch, crude protein, fat, ash and pH was observed in digesta collected from duodenum and ileum of pigs fitted with replaceable T-cannulas. The largest relative variation was, however, noted for chromic oxide which was included as a marker in the diet.

INTRODUCTION

During recent years the importance of kinetics in digestion has been widely recognized in animal nutrition. The site of digestion in the gastro-intestinal tract and how to affect this digestion is of great interest. The use of cannulated pig and improved chemical method has contributed to a better understanding of the processes of digestion.

However, any sophisticated method used is dependent on correct sampling techniques. In the present investigation the variation in basic chemical composition of duodenal and ileal contents, as influenced by time of collection in relation to feeding as well as by the age of the animal is studied. This knowledge is essential as a prelude to future work with replaceable T-cannulas in pigs, partly presented at this symposia.

MATERIALS AND METHODS

Three pigs, all fitted with replaceable T-cannula (Björnhag and Jonsson, 1984) close to the ileo-caecal junction were used. Two of the pigs also had a second T-cannula in the duodenum just distal to the pancreatic and bile ducts. The cannulas were made of stainless steel with inner diameters of 12 mm (duodenum) and 17 mm (ileum) respectively. The pigs were fed a ration equivalent to 2 % of body weight both at 08.00 and 16.00. The diet was cereal based with the following composition; 26.6 % barley, 26.6 % oats, 26.6 % wheat, 6.0 % peas, 6.0 % soybeans, 5.0 % fishmeal, 1.4 % dicalcium phos-

phate, 1.0 % vitamine premix 0.5 % calcium oxide and 0.3 % sodium chloride. Each pig was allowed an adaption period of seven days prior to collection. During day eight and ten, hourly samples of digesta was collected from the ileal and duodenal cannulas.

Feed was mixed with water (1:1, v/v) and chromic oxide (0.5 %) immediateley prior to feeding. The pigs weighed 25-30 kg and 65-70 kg at the time of the two occasions of sampling. Prior to freezing, the pH of the sample was measured. Each sample was freeze dried and ground in a Wiley mill to pass a 1 mm sieve. Ash, chromic oxide, crude protein (Nx6.25), crude fat after acid hydrolysis and starch were analyzed according to our standard procedures (Åman and Hesselman, 1984). Chromium was determined with atomic absorption. The relative composition of the digesta is presented as the mean for two pigs (duodenum) or three pigs (ileum).

RESULTS AND DISCUSSION

Circadian variation in pH and the content of starch, crude protein, fat, ash and chromic oxide in duodenal and ileal digesta is apparent in Figure 1 and 2. The pH in the duodenal samples varied considerably with anotable decrease 3-4 hours after feeding. The range of pH in the duodenal content was between 3.6 and 6.4 which is in full accordance with results from pigs fitted with duodenal re-entrant cannulas (Braude et al., 1976; Low et al., 1978). The effective buffering capacity of the small intestine reduce variation in terminal ileum. The pH varied at this position between 6.5 and 7.1 in accordance with Livingstone et al. (1976). A maximal starch content of about 40 % was found 3 to 5 hours after feeding. In the ileal samples the starch content was less than 10 %. The content of starch was higher, when the pigs weighed 70 kg, except around the afternoon feeding. A similar tendency was found in the duodenal samples. Mean starch content in the ileal samples was 7.4 % for the heavier pigs and 6.3 % for the small pigs. The absolute variation for crude protein and fat is not so pronounced although spot samples using chromic axide as a marker for digestibility studies seems to be an unprecise method.

The largest relative variation was found for the marker (Cr_2O_3). This means that spot samples from duodenum or ileum to determine apparent digestibilities, when chromic oxide is included in the diet as a marker, will give results with a low accuracy. Total collection

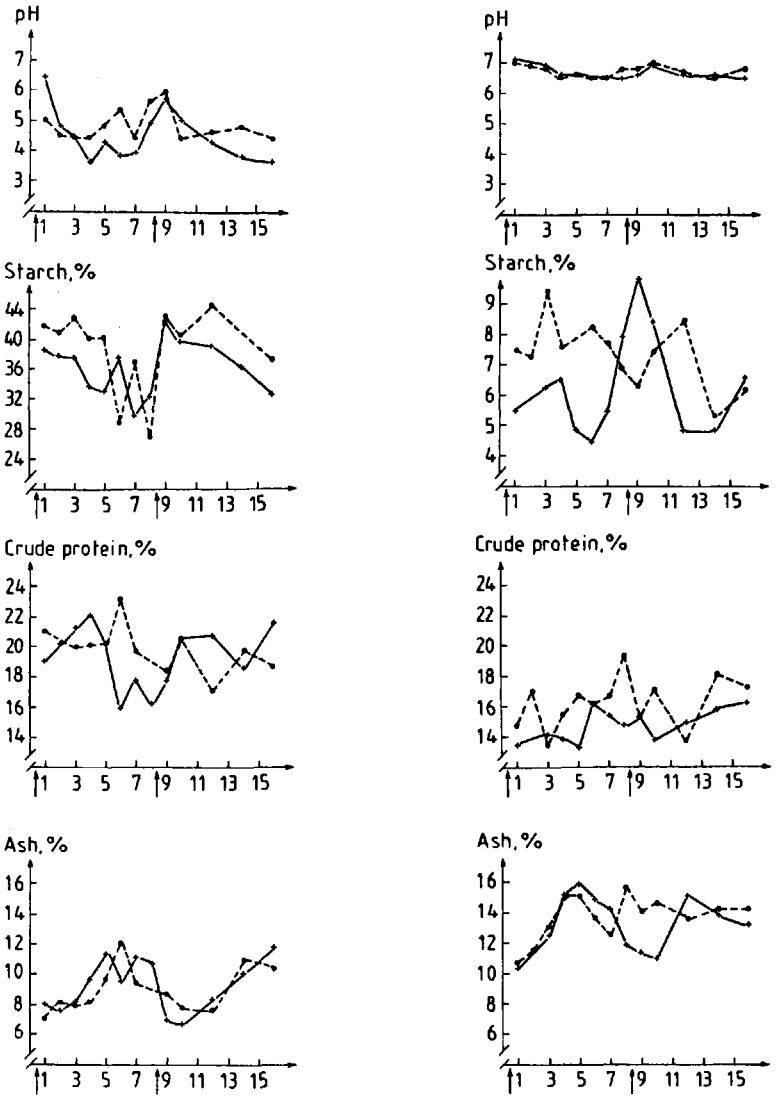


Figure 1. Circadian variation of pH, starch, crude protein and ash in duodenal (left column) and ileal (right column) digesta (% of DM) in relation to feeding (arrows). Weight interval 25-30 kg +—+; weight interval 65-70 kg ●---●.

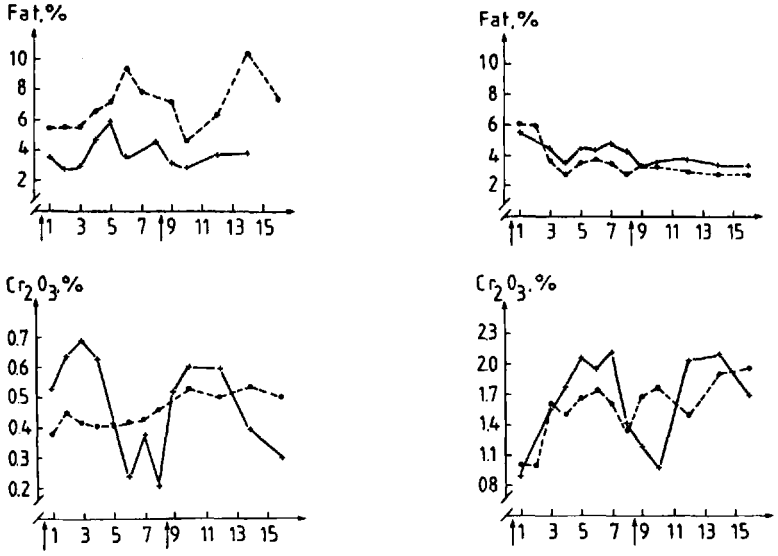


Figure 2. Circadian variation of fat and chromic oxide in duodenal (left column) and ileal (right column) digesta (% of DM) in relation to feeding (arrows). Weight interval 25-30 kg +—+; weight interval 65-70 kg ●---●.

using re-entrant cannulas is a complicated technique and does not allow fibrous feeds to the same extent as is possible with the cannulas used in this study. It is also possible to change to larger cannulas when the pig grow, enabling comparison to be made between different feeds at different ages.

REFERENCES

BJÖRNHAG, G. & JONSSON, E. 1984. Livest. Prod. Sci. 11: 179-184.

BRAUDE, R., FULFORD, R.J. & LOW, A.G. 1976. Br. J. Nutr. 36: 497-510.

LIVINGSTONE, R.M., BAIRD, B.A., ATKINSON, T. & CROFTS, R.M.J. 1980. J. agric. Sci. Camb. 94: 399-405.

LOW, A.G., PARTRIDGE, I.G. & SAMBROOK, I.E. 1978. Br. J. Nutr. 39: 515-526.

ÅMAN, P. & HESSELMAN, K. 1984. Swedish J. Agric. Res. 14: 135-139.

THE USE OF NYLON BAG AND IN VITRO TECHNIQUES FOR PREDICTING FEED DIGESTIBILITY FOR PIGS

H. GRAHAM*, K. HESSELMAN, P. ÅMAN, M. RUNDGREN, S. THOMKE
Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences
S-750 07 Uppsala, Sweden

SUMMARY

Nylon bag (in sacco) and in vitro (with ileal inocula) techniques for predicting digestibility were compared with the conventional in vivo total collection method for 9 pig feeds. In vivo apparent organic matter digestibility could be predicted ($P<0.001$) by both methods and also from the neutral detergent fibre content of the feed. In sacco and in vitro crude protein disappearences were not related to in vivo apparent crude protein digestibility, which they exceeded by up to 40 percentage units. However, in vivo apparent digestion of crude protein was closely correlated to in vivo apparent organic matter digestibility and neutral detergent fibre content ($P<0.01$). Energy digestibilities as determined by the three methods were positively correlated. The respective merits of the in sacco and in vitro methods are discussed.

INTRODUCTION

The determination of feed digestibility by conventional faecal collection methods is both costly and time consuming. However, Petry and Handlos (1978) demonstrated that feed digestibility for pigs could be predicted by passing a small sample, contained in a nylon bag, through the gastrointestinal tract. An in vitro method applicable to low fibre diets and employing duodenal digesta has also been suggested (Furuya et al., 1979), whereas Ehle et al. (1982) have investigated the degradation in vitro of plant cell wall components by microbial cultures from the large intestine and faeces of pigs. In this report the disappearance of organic matter, crude protein and energy from samples passed through the intestinal tract in nylon bags or incubated in vitro with ileal inocula is compared with in vivo apparent digestibility data for 9 pig feeds.

MATERIALS AND METHODS

In vivo data on the 9 feeds were obtained by total faecal

collection digestion trials with pigs weighing between 14 and 85 kg (Table 1). At least 6 pigs were used for each feed.

Two pigs, weighing about 150 kg and fitted with T-cannula in the duodenum and terminal ileum, were used in the in sacco and in vitro studies. The pigs were fed a standard barley-based fattening diet. Nylon bags (25x40 mm; pore size 20 µm) containing 1.0 g feed sample were introduced into the duodenum at feeding (8.00 and 16.00) and retrieved immediately on defecation, carefully washed and freeze-dried (Graham et al., 1985). For in vitro studies a 0.5 g sample of feed was incubated under anaerobic conditions for 60 h with ileal digesta (filtered through a double layer of cheese-cloth), diluted to 20 % in CO_2 purged physiological buffer (pH 6.9) as previously described for ruminant studies (Lindgren, 1979). After incubation the samples were isolated by filtration, washed with hot water and acetone, and air dried. Organic matter and crude protein disappearances reported (Table 2) are the means of at least 8 and 4 replicates respectively, whereas energy content of the degraded residues was determined on a pooled sample. Organic matter (OM), crude protein (CP; Nx6.25), energy and neutral detergent fibre (NDF; see Graham et al., 1985) were determined by standard methods. All feeds were ground to pass a 1 mm screen prior to in vitro and in sacco studies (Graham et al., 1985).

Table 1. Composition and major components of feeds (% dry matter), and weight of pigs for in vivo trials.

Feed	Major components	Pig weight (kg)	NDF (%)	Crude protein (%)	Gross energy kJ/kg
1[a]	barley (58 %), oats (21 %), soy bean oil meal (11 %)	45-85	14.5	19.5	18.6
2[a]	feed 1 + oat hulls (14 %)	45-85	19.8	17.9	18.3
3[a]	feed 1 + brewers grains (14 %)	33-48	19.0	21.4	18.7
4[a]	feed 1 + beet pulp (14 %)	33-48	16.1	18.0	17.9
5[a]	barley, (49 %), wheat (18 %), bran, peas + grass (10, 5, 5 %)	45-85	20.0	18.1	18.4
6	barley (Bomi)	14-24	15.6	11.3	18.7
7	barley (Risø 1508, high lysine)	14-24	19.3	13.2	19.3
8	corn cob meal	20	12.8	10.8	19.0
9[b]	whole crop peas	30-78	35.2	15.2	18.4

[a]including minerals and vitamins; [b]determined by difference

Table 2. In vivo apparent digestibility, and in sacco and in vitro disappearance of organic matter, crude protein and energy.

Feed	Organic matter			Crude protein			Enery		
	in vivo	in sacco	in vitro	in vivo	in sacco	in vitro	in vivo	in sacco	in vitro
1	85	90.3	82.0	82	96.8	94.8	84	89.8	88.7
2	79	88.4	75.2	80	96.1	94.7	77	82.7	80.1
3	82	88.0	78.9	80	96.0	92.8	80	87.1	84.3
4	86	91.0	83.8	78	95.1	93.3	84	90.9	87.9
5	77	83.7	75.2	77	96.3	93.5	75	81.8	80.6
6	86	90.5	83.0	78	94.8	92.6	83	87.9	86.6
7	84	88.0	81.6	73	93.2	90.7	81	84.9	83.4
8	86	92.8	84.1	74	92.9	88.0	82	90.6	85.7
9	59	72.0	67.2	52	92.7	92.3	N.D.	68.3	68.5
average									
S.E.M.	1.1	1.5	0.9	2.2	1.2	0.8	N.D.	N.D.	N.D.

N.D. - not determined

RESULTS AND DISCUSSION

The mean transit time of the bags was 41.6 h (range 26-62) and they were not retrieved in the order that they were introduced into the pigs. An in vitro incubation time of 60 h was chosen as degradation of most feeds would by then have reached an end-point. The use of mature pigs provides an established microflora and prolongs the usefulness of surgically altered animals.

In sacco OM disappearance was 4-13 percentage units greater than both in vivo digestibility and in vitro disappearance (Table 2). These higher figures probably result from particle loss from the bags and the absence of metabolic faecal material, whereas the in vitro system may be partially inhibited by the build-up of end-products inherent to this method. However, in vivo OM digestibility was closely related ($P<0.001$) to both in sacco and in vitro OM disappearance, and could also be predicted from the NDF content of the feed (Table 3).

Crude protein disappearances in sacco and in vitro were similar, and exceeded in vivo CP digestion, to which they were poorely correlated, by up to 40 % units (Petry & Handlos, 1978; Graham et al., 1985). Although some of this difference could be due to the presence of soluble but indigestible nitrogen compounds, the close relationship ($P<0.01$) and high coefficient of regression between in vivo apparent CP digestibility, and NDF and in vivo OM digestibility (Table 3) clearly demonstrates the influence of undegraded feed residues on faecal nitrogen content. The high in sacco and in vitro

Table 3. First order regression analysis between neutral detergent fibre (NDF), in vivo apparent digestibility, in sacco disappearance and in vitro disappearance of organic matter (OM), crude protein (CP) and energy (E).

Independent variable	Dependent variable	Intercept	Coefficient of regression	Coefficient of determination (R^2)
in vivo OM	NDF	105.1	-1.29	0.94***
	in sacco OM	-39.2	1.36	0.96***
	in vitro OM	-37.5	1.49	0.92***
in vivo CP	NDF	97.9	-1.20	0.75**
	in sacco CP	-323.1	4.19	0.54*
	in vitro CP	-45.4	1.30	0.09
	in vivo OM	2.8	0.90	0.74**
in vivo E	in sacco E	6.9	0.85	0.83**
	in vitro E	-2.8	0.99	0.91***
	in vivo OM	5.1	0.91	0.93***

*P 0.05, **P 0.01, ***P 0.001

CP disappearances demonstrate that indigestible, fibre-bound protein is not an important component of cereal-based pig feeds.

The positive correlation (R^2>0.83) between in vivo, in sacco and in vitro energy digestibilities (Table 3) would be expected as the OM disappearances for all 3 methods are closely related.

Both methods investigated could be used for the routine evaluation of large numbers of pig feeds; however neither method would seem to be superior to the more simple NDF determination. Of the two methods, the in vitro system is more practical to operate and allows control of the degradation time. Thus results obtained by the technique were more reproducible (see S.E.M., Table 2). Analysis of undegraded in sacco and in vitro residues could provide valuable information on the influence of treatments and chemical composition on the rate and extent of feed digestion.

REFERENCES

EHLE, F.R., JERACI, J. L., ROBERTSON, J.B. & VAN SOEST, P.J. 1982. J. Anim. Sci. 55: 1071-1081.

FURUYA, S., SAKAMOTO, K. & TAKAHASHI, S. 1979. Br. J. Nutr. 41: 511-520.

GRAHAM, H., ÅMAN, P., NEWMAN, R.K. & NEWMAN, C.W. (1985) to be published.

LINDGREN, E. 1979. Report 45. Department of Animal Nutrition, Swedish University of Agricultural Sciences.

PETRY, H. & HANDLOS, B.M. 1978. Arch. Tierernährung. 28: 531-543.

THE EFFECT OF INSERTION OF RE-ENTRANT CANNULAE ON DIGESTIVE PROCESSES

J. HUISMAN[1], E.J. VAN WEERDEN[1], G. HOF[2], K.K. VAN HELLEMOND[1] AND P. VAN LEEUWEN[1]
1) ILOB/IGMB, Institute for Animal Nutrition Research - TNO
P.O. Box 15, 6700 AA Wageningen, The Netherlands
2) Department of Animal Nutrition; Agricultural University
Haagsteeg 4, 6708 PM Wageningen, The Netherlands

SUMMARY

The effect of insertion of re-entrant cannulae on digestive processes was studied in experiments with fattening pigs and milk-fed calves.
The faecal digestibility of organic matter, crude protein and amino acids, crude fibre and NFE was not effected by insertion of re-entrant ileo-caecal cannulae in pigs. Rate of passage of chromium in faeces in pigs fitted with a re-entrant ileo-caecal cannula was identical to that in pigs fitted with a simple T-cannula in the distal ileum. The insertion of re-entrant cannulae in the duodenum did not effect the rate of passage of chromium through the distal ileum, the ileal dry matter digestibility and bloodsugar contents in veal calves.

INTRODUCTION

Studies are carried out at ILOB/IGMB on the rate of passage of digesta in the duodenum and ileum in pigs, veal calves and sheep. In addition, the ileal and faecal digestibility coefficients are determined. The method of total collection with re-entrant cannulae is used in the digestibility studies. The alternative method with single T-cannulae has the advantage to involve less labour but there are drawbacks. Major concerns are the questions on how representative are the samples collected via the single cannula and the shortcomings of the digestibility markers that have to be used.

A major point in studies with cannulated animals is the question whether cannulation effects the digestive processes. Various experiments with pigs and veal calves were carried out to answer this question.

MATERIALS AND METHODS

Pig experiment 1

Three pigs of approximately 65 kg live weight were fed a diet containing 14.2% crude protein and 6.2% crude fibre. The faecal digestibility of organic matter, crude protein, crude fibre, NFE and amino acids was determined before and after insertion of re-entrant ileo-caecal cannulae.

Pig experiment 2

The experiment was subdivided in two subexperiments.

a. Six pigs (initial weight appr. 40 kg) were fitted with a re-entrant ileo-caecal cannula. Two diets were fed according to a change over design. The first diet contained 5.2% crude fibre (c.f.) and 17.7% crude protein (c.p.). The second diet contained 9.2% c.f. and 16.4% c.p.
Cr-EDTA was added to the feed. The rate of passage of Cr from Cr-EDTA in the distal ileum was measured each hour for each pig during 3 days, 24 hours a day. The rate of passage was found to be at maximum at appr. four hours after oral administration in both diets.

b. Seven pigs (initial weight appr. 30 kg) were fitted with a simple T-cannula at the distal ileum. Three of these pigs were fed the 5.2% c.f. diet. The other four pigs were fed the 9.2% c.f. diet. Cr-EDTA was added to the feed. Rate of passage in faeces was determined. Faeces were collected every 3 hours during two periods of 48 hours for each pig and analysed for Cr content. Following these determinations Cr-EDTA was infused into the distal ileum via the simple T-cannula. The Cr-EDTA solution was infused all at once at four hours after the morning feeding. The infusion time was based on the results of rate of passage determined at the distal ileum in the pigs of subexperiment a. fitted with re-entrant ileo-caecal cannulae. In that experiment Cr-passage rate at the distal ileum was found to be at maximum at four hours after oral administration. This maximum peak in Cr-passage rate was in the simple cannulated pigs simulated by infusion of the Cr-EDTA. Faeces were collected according to the same procedure as described.

Veal calf experiment 1

Eight veal calves were fitted with a re-entrant cannula in the distal ileum. Four of these calves were also provided with a re-entrant cannula in the duodenum. The calves were milk-fed. Cr-EDTA was added to the milk. Ileal digesta were collected hourly during 4 days (24 hours/day). The age of the calves was appr. 8 weeks at the start of the experiment. In addition the ileal dry matter digestibility was determined.

Veal calf experiment 2

Eight veal calves were involved in this experiment. Four of these calves were provided with a re-entrant cannula in the duodenum, the other four calves were not cannulated. Bloodsamples were taken from the jugular vein and analysed for bloodsugar contents. Bloodsamples were taken for each calf during four days at the following times: 0.5 hour before and at 0.5, 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 6.0 and 7.0 hours after morning feeding (at 0800 a.m.). The age of the calves was appr. 7 weeks at the start of the experiment.

RESULTS AND DISCUSSION

There were no significant differences ($P < 0.05$) with respect to faecal digestibility of organic matter, crude protein, crude fibre, NFE and amino acids determined before and after insertion of re-entrant ileo-caecal cannulae in pigs. Drochner (1984) found also no significant effect of re-entrant cannulation in the distal ileum on the faecal digestibility of crude protein, crude fat, NFE and sodium in mini-pigs. These results are in concordance with Buraczewska et al. (1979). They found no effects on N-retention in pigs fitted with a re-entrant cannula in the duodenum and the distal ileum. This observation indicates that the process of protein and amino acid absorption was not effected by double re-entrant cannulation.

There were no significant differences in passage rate of Cr in faeces when in pigs fitted with a simple cannula Cr was administered with the feed or was infused in the distal ileum at 4 hours after feeding (see description of experiment 2b.). These results indicate that the rate of passage of digesta through the whole digestive tract was the same in pigs fitted with a re-entrant ileo-caecal cannula or fitted with a single T-cannula. There are many reports in literature which indicate that simple cannulation does not effect digestive processes. Assuming that simple T-cannulation did not effect ileal passage rate of digesta it can be stated that re-entrant cannulation did not effect ileal passage rate of digesta either.

There were no significant differences in Cr-passage rate at the distal ileum between the calves fitted with a re-entrant cannula in the duodenum and those who were not provided with a re-entrant cannula in the duodenum. Ileal digestibility of dry matter was also not different between both groups of calves.

In studies with milk-fed veal calves it was observed that bloodglucose contents respond sharply and quickly after ingestion of the milk. If the insertion of re-entrant cannulae in the duodenum should effect the abomasal emptying and/or duodenal flow then, as a result, bloodsugar contents should also be effected. In veal calf experiment 2 it was found that bloodsugar contents were not effected by the insertion of re-entrant cannulation in the duodenum.

In summary the insertion of re-entrant cannulae in the duodenum or at the distal ileum has no effect on the digestive processes.

REFERENCES

BURACZEWSKA, L., ZEBROWSKA, T., WÜNSCHE, J., HENNIG, U., KRAWIELITZKI, K., KREIENBRING, F., MEINL, M., BORGMANN, E. & BOCH, H.D. 1979. Arch.Tierernährung, Bd 29, 437.

DROCHNER, W. 1984. Supplement to Journal of Animal Physiology and Animal Nutrition, vol. 14.

EVALUATION OF DIETARY PROTEIN THROUGH ILEAL AND FAECAL DIGESTIBILITY (PIGS) AND NITROGEN RETENTION (PIGS AND CHICKS)

S. JAGGER, D.J.A. COLE, J. WISEMAN
Department of Agriculture and Horticulture
University of Nottingham School of Agriculture
Sutton Bonington, Loughborough, Leics. LE12 5RD, U.K.

SUMMARY

The protein quality of diets containing (i) untreated fishmeal, (ii) fishmeal heated to 130°C and (iii) fishmeal heated to 160°C was evaluated by estimating digestibility of nitrogen and nitrogen retention in the pig and chick. The digestibility and retention of nitrogen in the pig were determined by conducting a balance study with 4 gilts cannulated at the terminal ileum. Nitrogen retention data obtained from the chick were analysed by the slope-ratio assay technique. Mean values obtained from the pig trial for the 3 fishmeals were:- faecal digestibility of nitrogen (%) - (i) 83.42, (ii) 82.00, (iii) 71.10; ileal digestibility of nitrogen (%) - (i) 72.00, (ii) 71.30, (iii) 63.30; nitrogen retention (%) - (i) 50.70, (ii) 53.70, (iii) 39.60. The results from the chick slope-ratio assay were as follows:- (ii)/(i) = 0.90, (iii)/(i) = 0.77. The chick and pig data indicated that fishmeal heated to 160°C had been significantly damaged but only the chick detected a change in fishmeal heated to 130°C.

INTRODUCTION

In order to examine methods of evaluating protein, diets must be formulated to provide a range of protein quality. However, to reduce unnecessary variation arising when a number of high-protein ingredients are used one source of protein, fishmeal, was modified by heat treatment.

MATERIALS AND METHODS

Fishmeal, taken from a single batch, was subjected to a temperature of 130°C for a period of 3 hours or 160°C for a period of 0.5 hours. These treatments did not alter the chemically analysed composition of the fishmeal but they reduced the 2.4.6 trinitrobenzenesul-

phonic acid 'reactive' lysine value. The treated and untreated fishmeal samples were incorporated into diets for pigs and chicks.

a. Pig trial: Four diets were formulated:

a) Basal (Maize, barley, wheat starch and tallow)
b) Basal + 10% untreated fishmeal
c) Basal + 10% fishmeal heated to 130°C
d) Basal + 10% fishmeal heated to 160°C

Each diet was fed to 4 Landrace x (Landrace x Large White) gilts, initially weighing 40-45 kg liveweight, in an experiment designed as a 4 x 4 Latin square. All animals had previously been surgically fitted with simple, T-piece cannulae at the terminal ileum to allow collection of digesta. Each diet was offered with water, in the ratio of 1:2 (w/v), every 12 hours at a level of 2.5 x maintenance. Eight days were allowed for the pigs to acclimatize to each diet containing the inert marker titanium dioxide which was included at a level of 0.5% of the diet. Faeces and urine were collected continuously for 5 days during each period. Ileal digesta were collected on the 1st, 3rd and 5th day of the faecal collection period at 2, 6 and 10 hours after feeding. The nitrogen content of faeces, urine and ileal digesta was determined to allow the digestibility and retention of nitrogen to be calculated for each diet.

b. Chick trial: A slope ratio assay was conducted with 12 treatments:

	Level of inclusion of fishmeal(%)*			
(a) Basal + untreated fishmeal	3	6	9	12
(b) Basal + fishmeal heated to 130°C	3	6	9	12
(c) Basal + fishmeal heated to 160°C	3	6	9	12

(* fishmeal was included at the expense of wheat starch)

After an acclimatization period of 7 days each diet was offered ad libitum to 6 cages of chicks (each cage contained 3 chicks) for 14 days. During the last 4 days of the trial the amount of food eaten and the weight of excreta produced was recorded. Excreta were analysed for nitrogen and nitrogen retention was calculated. The results were analysed by the slope-ratio technique of Finney (1964).

RESULTS AND DISCUSSION

In the pig the digestibility of nitrogen (Table 1) obtained for fishmeal heated to 160°C was significantly lower than the untreated fishmeal and the fishmeal heated to 130°C. No significant difference was detected between the digestibility of nitrogen in the untreated fishmeal and the fishmeal treated to 130°C. This pattern was observed with both ileal and faecal digestibilities. However, the digestibility of nitrogen determined from ileal digesta was significantly lower than that determined from faeces, but this difference was not affected by treatment.

TABLE 1. Digestibility of nitrogen (%) in fishmeal

	Untreated	Treated at 130°C	SED	Mean of untreated and 130°C treated	Treated at 160°C	SED
Faecal digestibility	92.21	88.51	2.2	90.36	70.09	1.9
Ileal digestibility	83.40	79.70	6.2	81.55	65.90	5.4

The values obtained for nitrogen retention in the pig were 50.70% 53.70% and 39.60% for the diets containing untreated fishmeal, fishmeal heated to 130°C and fishmeal heated to 160°C, respectively. Clearly the diet containing fishmeal heated to 160°C was inferior to those containing untreated fishmeal and fishmeal heated to 130°C. No difference was found between the last two diets which agrees with the results obtained for the digestibility of nitrogen.

Results of the chick slope-ratio assay(Figure 1) indicated that there was a significant difference between treatment. The ratio of fishmeal heated to 130°C to untreated fishmeal was 0.90 ± 0.03 and that of fishmeal heated to 160°C to untreated fishmeal was 0.77 ± 0.04.

The chick assay therefore is a more sensitive test for the quality of protein than the pig balance study. However, ileal and faecal digestibility values in the pig indicated that fishmeal heated to 130°C had a lower nutritive value than the untreated fishmeal but these values did not differ significantly. The nitrogen digestibility values determined at the ileum of the pig were consistently below those determined from faeces. Therefore, faecal digestibility would overestimate the digestibility of nitrogen assuming that protein entering the large intestine has no nutritional value.

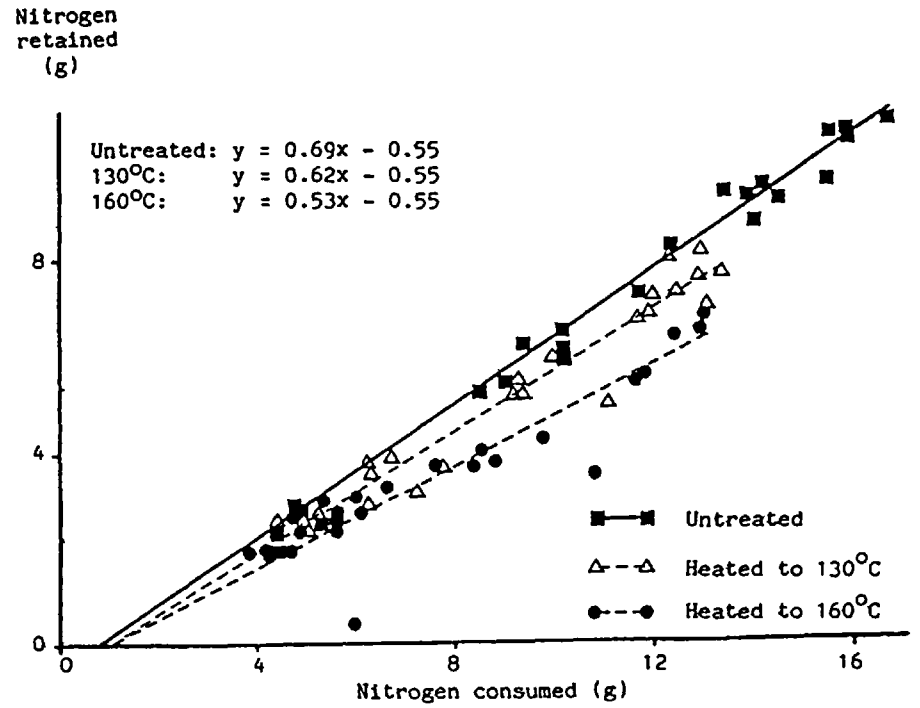


Figure 1. Chick slope-ratio assay of nitrogen retention against nitrogen consumed

REFERENCES

FINNEY, D.J. 1964. Statistical methods in biological assay. 2nd edition. London.

COMPARATIVE DIGESTIBILITY EXPERIMENTS WITH NORMAL AND CANNULATED PIGS

H. JØRGENSEN, J.A. FERNÁNDEZ, A. JUST
National Institute of Animal Science
Rolighedsvej 25, DK-1958 Copenhagen V, Denmark

SUMMARY

Comparison of 156 normal and 46 cannulated pigs comprising 38 different diets varying in feedstuff and chemical composition showed that the cannulated pigs had higher overall digestibility. Although the differences were small i.e. 0.5, 1.8 and 1.3 percent units for dry matter, crude protein and lysine, respectively, the differences were statistically significant except for dry matter. The highest differences were found in diets with high crude fibre content and diets including potato starch.

INTRODUCTION

Cannulation of the intestine has enabled experimenters to measure various functions of the gut and much valuable information on fundamental biochemical, physiological and nutritional processes have been obtained. Reentrant cannulation that involve a transection of the intestine cause more disturbance of the gut motility (Laplace, 1980) than insertion of a simple T-shaped cannulae (Wenham & Wyburn, 1980). However, all kind of cannulations cause some disturbance as at the place of cannulation the intestine is brought up to the body wall, which most likely decreases the rate of passage of digesta.

In the present paper a comparison is done between normal and cannulated pigs' ability to digest various diets and thereby evaluate whether the results obtained with cannulated pigs can be applied universally and valid conclusions can be drawn from experiments with cannulated pigs.

MATERIAL AND METHODS

In 3 of the experiments with cannulated pigs carried out at this institute the same diets have for various reasons been fed both to

normal and cannulated pigs thus making comparison possible.

A total of 38 diets varying in feedstuff and chemical composition have been used, involving 156 normal pigs and 46 cannulated pigs. More details about these experiments are given by Just et al. (1983, 1985). The cannulated pigs were fitted with simple T-cannulas 3-5 cm cranial (visual) to the ileal-caecal junction at about 40 kg. The digestibility experiments both with normal and cannulated pigs were by and large performed simultaneously from about 45 kg up to about 80 kg liveweight.

The digestibility data was calculated from quantitative collection of faeces. The collection period for the normal pigs lasted 7 days. For the cannulated pigs the collection period varied from 4 to 6 days prior to the collection of ileal digesta. In experiment 2 faeces from cannulated pigs were only collected for 2 days. Therefore digestibility was calculated on basis of chromic oxide determinations and adjusted to quantitative collection.

In some cases weight differences between normal and cannulated pigs occurred (5-10 kg) and although the influence on the digestibility was small the data were adjusted to identical weight.

RESULTS AND DISCUSSION

Daily gain was measured only in periods of 14 days. In general the growth rate of normal and cannulated pigs has been very much the same, in average 500-700 g daily, depending on type of diet, thus indicating that cannulated pigs physiologically behaved as normal pigs.

In Table 1 the results are given from experiments 1 and 2 in which it was possible to compare the digestibility of dry matter, crude protein and lysine. Table 2 shows the results from experiment 3 where the digestibility of dry matter and crude protein are compared. Independent of type of diet the relationship between the digestibility of normal and cannulated pigs were generally alike. The cannulated pigs digested in average dry matter 0.5, crude protein 1.8 and lysine 1.3 percent units higher than normal pigs. These differences were significant for crude protein and lysine but not for dry matter.

Some of the differences could be derived from a slightly lower feed intake of the cannulated pigs, but as shown by Sauer et al. (1982) there is hardly any effect on digestibility within this range

Table 1. Comparison of dry matter, crude protein and lysine digestibility measured on normal (N) and cannulated (C) pigs

	Dry matter			Crude protein			Lysine		
	N	C	N-C	N	C	N-C	N	C	N-C
Type of diet exp. 1:									
Basal	85	85	0	83	84	-1	83	84	-1
Basal + 20% nutrients	84	85	-1	84	86	-2	84	86	-2
15% untreated straw	70	73	-3	73	74	-1	75	78	-3
15% untreated straw + 20% nutrients	70	72	-2	74	78	-4	75	79	-4
15% NH_3-treated straw	71	74	-3	73	76	-3	74	77	-3
15% NH_3-treated straw +20% nutrients	72	72	0	75	76	-1	78	78	0
15% NaOH-treated straw	76	76	0	74	76	-2	76	78	-2
15% NaOH-treated straw +20% nutrients	74	76	-2	74	77	-3	74	76	-2
Type of diet exp. 2:									
2% cellulose, 0% potato starch	91	91	0	93	95	-2	96	97	-1
5% " , 5% "	88	88	0	90	92	-2	94	94	0
8% " , 8% "	86	87	-1	88	88	0	92	91	1
10% " ,10% "	84	84	0	84	85	-1	89	89	0
13% " ,13% "	81	80	1	82	83	-1	87	86	1
16% " ,16% "	78	79	-1	77	79	-2	82	82	0

of feed intake. The explanation for the slightly higher overall digestibility of cannulated pigs is most likely due to a slower rate of passage. As discussed by Laplace (1980) there seems not to be any disturbance of the gut motility anterior to the cannulae but more disturbance is induced with transection of the intestine in re-entrant cannulation. The simple T-cannulae used in this study causes less disturbance than re-entrant cannulae (Wenham & Wyburn, 1980). Insertion of a cannulae in the intestinal lumen together with that the normally free moving intestine is anchored to the body wall must inevitably interfere with intestinal contractions and obstructs the rate of passage to some extent. The largest differences in digestibility are found in diets high in crude fibre or diets including large amounts of potato starch.

Table 2. Comparison of dry matter and crude protein digestibility measured on normal (N) and cannulated (C) pigs

	Dry matter			Crude protein		
	N	C	N-C	N	C	N-C
Type of diet exp. 3:						
Barley	80	81	-1	75	77	-2
Barley hull meal	57	54	3	62	64	-2
Oats	68	69	-1	77	78	-1
Wheat	86	86	0	83	87	-4
Wheat bran	67	66	1	65	70	-5
Rye	84	84	0	74	76	-2
Skimmilk, dried	95	94	1	94	93	1
Meat and bone meal	82	82	0	78	79	-1
Rapeseed meal	79	78	1	75	75	0
Sunflower meal	76	74	2	80	79	1
Soybean meal	91	89	2	85	83	2
Grass meal	69	71	-2	47	45	2
4% crude fibre, 0% potato starch	82	80	2	80	79	1
" , 20% "	83	84	-1	72	78	-6
8% " , 0% "	74	77	-3	74	77	-3
" , 20% "	76	78	-2	66	73	-7
12% " , 0% "	68	67	1	71	72	-1
" , 20% "	70	71	-1	64	67	-3
8% crude protein, 5 g lysine	85	84	1	80	81	-1
" , 7 g "	84	84	0	80	80	0
11% " , 5 g "	81	82	-1	80	82	-2
" , 7 g "	81	83	-2	80	83	-3
14% " , 5 g "	76	77	-1	79	82	-3
" , 7 g "	76	78	-1	80	82	-2

REFERENCES

JUST, A., FERNÁNDEZ, J.A. & JØRGENSEN, H. 1983. Livest. Prod. Sci. 10:171-186.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A. 1985. Livest. Prod. Sci. 12: xx-xx.

LAPLACE, J.P. 1980. In: Current Concepts of Digestion and Absorption in Pigs. (Eds. A.G. Low and I.G. Partridge). Tech. Bull. 3, Nat. Inst. Res. Dairying, Reading, p. 24-47.

SAUER, W.C., JUST, A. & JØRGENSEN, H. 1982. Z. Tierphysiol., Tierernährg. u. Futtermittelkde. 48: 177-182.

WENHAM, G. & WYBURN, R.S. 1980. J. Agric. Sci., Camb. 95: 539-546.

RELATION BETWEEN ILEAL AND FAECAL DIGESTIBLE NUTRIENTS IN 96 DIETS FOR PIGS

H. JØRGENSEN, J.A. FERNÁNDEZ, A. JUST
National Institute of Animal Science
Rolighedsvej 25, DK-1958 Copenhagen V, Denmark

SUMMARY

Ileal and overall digestibility of nutrients have been measured in 96 diets and feedstuffs. In general most of the crude fat was digested in the small intestine, whereas the crude fibre disappeared in the hind gut. A net synthesis of lysine and methionine took place in caecum-colon and a substantial amount of cystine and threonine disappeared. In average of all the diets overall digestibility of crude protein was very similar to especially ileal digestibility of lysine and methionine. There were found reasonably good prediction equations of ileal digestibility of lysine, methionine, cystine and threonine on basis of faecal digestibility and the equations except for cystine were improved by adding dietary crude fibre.

INTRODUCTION

Many studies have been carried out with cannulated pigs to obtain more detailed information on the digestive processes. These experiments have shown that the microflora of the hind gut has a modifying effect on nutrient digestibility, which can vary with the supply and composition of substrate (Just et al., 1981).

It has been shown that the digestibility of amino acids estimated at the terminal ileum has a slightly higher correlation to N-balance than values estimated over the whole tract (Just et al., 1985).

The objective of the present investigation was to study in more details the relation between ileal and faecal digestibility values.

MATERIAL AND METHODS

The data used orignate from experiments carried out at the department for research in pigs. In total 96 different diets and feedstuffs have been investigated. In some cases, for instance where the purpose has been to investigate the value of a protein source, semi-

synthetic diets have been used.

The pigs were usually surgically fitted with cannulas at about 40 kg live weight, and used for experiments from 45 kg up to about 90 kg. Fifteen pigs used in the first conducted experiments were fitted with re-entrant cannulas. The following 88 pigs used in the experiments were fitted with simple T-cannulas. All cannulas were placed at the terminal ileum about 4-5 cm anterior to the ileo-caecal junction.

Three to eight repeated collections were performed on each diet, i.e. in total 420 complete collections.

RESULTS AND DISCUSSION

The average digestibility pattern of all 96 diets is given in Table 1. Nearly all the digestion of crude fat take place in the small intestine, whereas crude fibre is digested in the hind gut largely by microbial fermentation.

On an average 20% of dietary carbohydrates and 8% of dietary soluble carbohydrate (starch + sugar) disappeared in the hind gut. The relative high amount of carbohydrate passing the small intestine is probably due to a number of diets containing potato starch.

As the largest part of energy originates from carbohydrates, the pattern of energy digestion follows that of carbohydrate i.e. 19% of dietary energy disappeared in caecum-colon. Just et al. (1983) found the value of energy disappearing from the caecum-colon to be only half of that of energy absorbed from the small intestine.

In the caecum-colon a net synthesis of lysine and methionine took place, whereas substantial amounts of cystine and threonine disappeared in that region. In average the overall digestibility of crude protein is very similar to the ileal digestibility of the important amino acids lysine and methionine in balanced diets (Sauer et al., 1980). In individual feedstuffs, for instance meat and bone meal, great differences between the ileal and overall digestibility of lysine may occur (Jørgensen et al., 1984).

Obtaining ileal digestibility values is quite laborious, i.e. surgery, collections, special care of pigs, etc. It would therefore be of great interest if the ileal digestibility of protein and amino acids could be predicted from the overall digestibility. To study

Table 1. Nutrient digestibility pattern measured at the terminal ileum, hind gut, and over the whole tract

	Digestion in:					
	Small intestine		Hind gut		Whole tract	
(n=96)	DC	% of digested	DC	% of digested	DC	% of digested
Crude fat	60	95	3	5	63	100
Crude fibre	-3	0	33	100	33	100
NFE-substances	71	80	18	20	89	100
Sol.carbohydrate	92	92	8	8	100	100
Energy	66	81	15	19	81	100
Crude protein	74	91	7	9	81	100
Lysine	84	104	-3	-4	81	100
Methionine	85	106	-5	-6	80	100
Cystine	70	83	14	17	84	100
Threonine	72	90	8	10	80	100

DC = digestibility coefficient

this possibility the correlation coefficients of overall to ileal digestibility of crude protein and the four most important amino acids lysine, methionine, cystine and threonine are shown in Table 2. Relatively high correlations were found, especially of crude protein to lysine (r = 0.73). The highest correlations were otherwise found between the amino acids themselves. Inclusion of dietary crude fibre in the prediction equation for the ileal digestibility of lysine improved the predicting value from 69% to 73% of the variation.

Ileal DC of lysine =

$$33.2 + 0.6 * \text{overall DC (Lysine)} + 0.04 * \text{crude fibre (g/kg DM)}$$

$$s_b \quad 0.04 \qquad\qquad 0.01 \qquad\qquad R^2 = 0.73$$

Table 2. Correlation of overall to ileal digestibility in 96 diets

	Overall digestibility				
	Crude protein	Lysine	Methionine	Cystine	Threonine
Ileal digestibility					
Crude protein	0.83				
Lysine	0.73	0.83			
Methionine	0.70	0.77	0.76		
Cystine	0.46	0.38	0.32	0.82	
Threonine	0.69	0.76	0.60	0.49	0.73

The same improvement in predicting value by addition of crude fibre as a predictor was found for methionine and threonine but no improvement was found for cystine.

In Table 3 is shown the relationship between dietary carbohydrate components and the amounts disappearing in the hind gut.

Except for cystine the amounts of crude protein and amino acids disappearing in the caecum-colon were negatively correlated to the carbohydrate fractions i.e. increasing levels of dietary fibre decrease the difference between the overall and the ileal digestibility. The explanation is that dietary fibre acts as an predictor of the nutrient supply to the caecum-colon, which in turn influences the microbial activity and thereby the ratio between degradation and synthesis of amino acids.

Table 3. Correlation of dietary fractions to the amounts of crude protein/amino acids (overall digestibility - ileal digestibility) disappearing from caecum-colon.

DC_overall - DC_ileal	Crude fibre	NDF[1)]	Soluble carbohydrate
Crude protein	-0.45	-0.25	-0.03
Lysine	-0.50	-0.44	-0.27
Methionine	-0.53	-0.49	-0.31
Cystine	-0.03	-0.04	-0.03
Threonine	-0.50	-0.32	-0.09

1) NDF = NFE-substances + crude fibre - soluble carbohydrate

REFERENCES

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A., 1981. Br.J.Nutr. 46:209-219.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A., 1983. Livest.Prod.Sci. 10:171-186.

JUST, A., JØRGENSEN, H. & FERNÁNDEZ, J.A., 1985. Livest.Prod.Sci. 12: xx - xx.

JØRGENSEN, H., SAUER, W.C. & THACKER, P.A., 1984. J.Anim.Sci. 58:926-934.

SAUER, W.C., JUST, A., JØRGENSEN, H., FEKADU, M. & EGGUM, B.O., 1980. Acta Agric.Scand. 30: 449-459.

THE EFFECT OF DIETARY FAT AND MINERAL LEVEL ON THE SITE OF ABSORPTION OF SOME NUTRIENTS AT DIFFERENT LEVELS OF CRUDE FIBRE AND CRUDE PROTEIN

H. JØRGENSEN, J.A. FERNÁNDEZ, A. JUST
National Institute of Animal Science
Roligheds vej 25, 1958 Copenhagen V, Denmark

SUMMARY

Crude fat added to diets for pigs increased both the ileal and faecal digestibility of crude protein and the amino acids lysine, methionine and threonine, whereas increasing dietary levels of minerals decreased the digestibility. The increased dietary mineral level decreased the digestibility of crude fat and stearic and linoleic acid indicating a binding of fat by minerals. High levels of dietary crude fibre increased the net synthesis of lysine and methionine in the hind gut. A higher dietary crude protein concentration caused increased digestibility both at the ileal and faecal level.

INTRODUCTION

For optimal growth and performance, pigs require balanced diets which provide adequate levels of all nutrients. A number of dietary factors influence the digestibility in the different regions of the digestive tract and thereby the amount of nutrients available for the metabolic processes in the pig. It has been shown that dietary factors as crude protein, crude fat and especially crude fibre have considerable impact on the amount of nutrients digested in the small intestine and hind gut, respectively (Just et al., 1980; Sauer et al., 1980; Just, 1983).

The aim of the present study was to elucidate the influence on the site of absorption of dietary factors as: crude fat, crude protein, crude fibre and level of minerals.

MATERIAL AND METHODS

Four litters of 7 female pigs were fitted with simple T-cannulae 5 cm cranial to the ileo-caecal junction. The surgery was carried out when the pigs weighed about 40 kg and the digestibility experi-

Table 1. Description of experimental diets

	3% crude fat			15% crude fat		
	Mineral level			Mineral level		
6.5% crude fibre 19.5% crude protein	50%	100%	150%	50%	100%	150%
10% crude fibre 19.5% crude protein	50%	100%	150%	50%	100%	150%
6.5% crude fibre 25% crude protein	50%	100%	150%	50%	100%	150%

ments were performed in the weight range 50-80 kg. The experimental diets are described in Table 1. They included three types of rations varying in crude fibre and digestible crude protein content. Each type of diet included two levels of fat (3 and 15%) and each level of fat was supplemented with three levels of minerals (50, 100 and 150% of the Danish Standards) in total 18 feed mixtures. Chromic oxide (0,5%) was added as a marker for digestibility determination. The pigs were housed in stainless steel metabolism cages and fitted with bladder catheters that allowed separate collection of urine and faeces.

The experimental pigs were fed equal amounts of feed every 8 hours and distilled water was given in a ratio 2.5:1. Between each balance period the pigs were kept in a normal pen during an 11 day period and fed a normal grower ration. With each pig three complete balances were carried out. A 5 day adjustment period was followed by 4 days collection of urine and 2 days collection of faeces. Ileum samples were collected the last 3 days of the collection period 2 x 2 hours every day covering the whole period between meals.

RESULTS AND DISCUSSION

As shown in Table 2 the level of crude fat increased both the ileal and faecal digestibility of crude protein and the four amino acids lysine, methionine, cystine and threonine by 1-2 percent units, which is in accordance with Sauer et al. (1980). Increasing level of minerals (Danish Standards of Ca, P, Mg, Na, K, Fe, Mn, Zn, Cu and

Table 2. The influence of dietary crude fat and level of minerals on the ileal (I) and faecal (F) digestibility nutrients

	Level of crude fat						Level of minerals							
	3%		15%		Signi-ficance		50%		100%		150%		Signi-ficance	
	I	F	I	F	I	F	I	F	I	F	I	F	I	F
Crude protein	79	84	80	86	**	**	81	86	79	85	74	83	**	**
Lysine	89	87	91	89	**	**	91	89	90	89	90	87	NS	**
Methionine	90	87	92	89	***	***	92	89	91	89	90	86	***	**
Cystine	69	83	70	84	NS	NS	70	85	70	84	69	82	NS	**
Threonine	78	84	79	86	NS	**	80	86	78	85	77	83	*	***
Crude fat	57	60	82	82	***	**	71	75	69	71	68	69	NS	**
Stearic acid	79	-4	73	52	*	***	80	32	74	23	74	19	*	NS
Linoleic acid	62	82	83	84	***	NS	74	86	74	83	71	81	NS	*

Significance within ileal (I) or faecal (F) digestibility.
NS: non significant, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

Se) decreased the ileal and faecal digestibility of crude protein and the four amino acids. In general the effect was more pronounced at the terminal ileum. Furthermore, there was found decreasing apparent digestibility of crude fat and fatty acids (stearic and linoleic), which indicates a binding of fat by the minerals.

In Table 3 the main results obtained with the three types of rations are shown. An increased concentration of dietary crude fibre decreased as expected the overall digestibility of crude protein and the four amino acids. Furthermore, the difference between the ileal and overall digestibility was diminished and for lysine and methionine a net synthesis in the hind gut took place which is in accordance with earlier experiments (Sauer et al., 1980; Just, 1983). In contrast to the earlier experiments no difference or an increased ileal digestibility occurred at the highest crude fibre level. This is partly due to the type of feedstuffs in the diets, and in addition the high fibre diet contained more casein which is a highly digestible protein source. Comparison of the diets containing 19.5 and 25% dietary crude protein, respectively, showed that the 25% crude

protein diet had the highest ileal and faecal digestibility. This is to be expected as the excretion of endogenous nitrogen into the digestive tract more or less is proportional with dietary dry matter intake and therefore accounts for a greater part of the faecal nitrogen at lower than at higher dietary crude protein concentrations.

Table 3. The influence of dietary crude fibre and crude protein on the ileal (I) and faecal (F) digestibility of nutrients

	6.5% crude fibre 19.5% crude protein		10% crude fibre 19.5% crude protein		6.5% crude fibre 25% crude protein	
	I	F	I	F	I	F
Crude protein	79^{a}	85^{b}	79^{a}	82^{b}	81^{b}	87^{c}
Lysine	89^{a}	88^{a}	90^{b}	87^{a}	91^{b}	91^{b}
Methionine	89^{a}	87^{a}	92^{b}	86^{a}	92^{b}	90^{b}
Cystine	71	85^{a}	68	81^{b}	70	86^{a}
Threonine	77^{a}	84^{a}	78^{a}	83^{a}	80^{b}	87^{b}
Crude fat	66	71^{a}	74	75^{b}	68	69^{ac}
Stearic acid	75	4^{a}	74	43^{b}	79	26^{c}
Linoleic acid	68^{a}	86^{a}	79^{b}	83^{ab}	70^{ac}	81^{b}

a, b, c: Different superscripts on the same line within ileal (I) or faecal (F) digestibility denotes statistical difference: $P < 0.05$.

REFERENCES

JUST, A. 1983. 4th Int.Symp. Protein Metabolism and Nutrition. Clermont-Ferrand. Les Colloques de l'INRA, 16. EAAP Publ. 31:289-309.

JUST, A., ANDERSEN, J.O. & JØRGENSEN, H. 1980. Tierphysiol., Tierernährg. u. Futtermittelkde. 44: 82-92.

SAUER, W.C., JUST, A., JØRGENSEN, H.H., FEKADU, M. & EGGUM, B.O. 1980. Acta Agric. Scand. 30: 449-459.

THE INFLUENCE OF DIETARY SUPPLY OF MINERALS ON APPARENT ABSORPTION AND RETENTION OF MINERALS IN GROWING PIGS

H. JØRGENSEN, A. JUST & J.A. FERNÁNDEZ
National Institute of Animal Science
Rolighedsvej 25, DK-1958 Copenhagen V, Denmark

SUMMARY

Retention of calcium and phosphorus increased with increasing dietary supply but to a less extent at the high dietary level, whereas retention of sodium showed a linear increase. Magnesium was found to be absorbed mainly in the hind gut. Furthermore a nearly constant amount of magnesium was excreted into the urine independent of intake. The amount of sodium that passed the terminal ileum was 3-7 times that of intake. Most of the sodium was reabsorbed in the hind gut. In contrast to sodium potassium was absorbed in the small intestine and secreted into the large intestine. The increased amount of potassium absorbed was excreted into the urine.

INTRODUCTION

In order to supply the pigs with adequate amounts of essential minerals it is necessary to obtain knowledge of the availability of minerals from different feedstuffs and mineral supplements. Mineral digestibility measurements are complicated by the fact that several minerals are secreted into the gastrointestinal tract. Many other factors influence digestibility: nutritional status of the pig, dietary factors, supply of minerals etc. (Just, 1972; Patridge, 1978; Jørgensen et al., 1979; Sauer et al., 1982; Jørgensen & Fernández, 1984).

In the present study some results are reported concerning the influence of dietary supply of minerals on retention and excretion of minerals in growing pigs.

MATERIAL AND METHODS

Four litters of 7 female pigs were fitted with simple T-cannulas about 5 cm cranial to the ileo-caecal junction. The surgery was

carried out when the pigs weighed about 40 kg and the digestibility experiments were performed in the weight range 50-80 kg. The experimental diets varied in chemical composition (crude fat, crude protein, crude fibre) and within each type of diet 3 levels of minerals (50, 100 and 150% of the Danish Standards) were applied, i.e. a total of 18 feed mixtures. Chromic oxide (0,5%) was added as marker. The pigs were housed in stainless steel cages and fitted with bladder catheters. The experimental pigs were fed equal amounts of feed every 8 hours and 2.5 litre of destilled water was given per kg diet. Between each balance period the pigs were kept in a normal pen during an 11 day period and fed a normal grower ration. With each pig 3 complete balances were carried out. A 5 day adjustment period was followed by 4 days collection of urine and 2 days collection of faeces. Ileum samples were collected the following 3 days of the collection period 2 x 2 hours every day covering the whole period between meals.

RESULTS AND DISCUSSION

The retained amount of crude protein and minerals are presented in Table 1. The lowest protein retention was found with a 50% dietary mineral level thus indicating that this mineral level was too low to ensure maximum growth.

Table 1. Protein and mineral retention at different levels of mineral intake

	Level of minerals							
	50%		100%		150%		Significance	
	Balance	%	Balance	%	Balance	%	Balance	%
Crude protein, g/day	108	38[1)2)]	136	48	137	49	**	*
Ca, g/day	3.5	46	6.4	42	7.8	34	***	**
P, g/day	2.8	43	4.6	35	5.6	29	***	***
Mg, g/day	0.25	20	0.41	23	0.34	14	**	**
Na, g/day	0.8	57	1.1	34	1.5	28	***	***
K, g/day	2.0	27	1.7	17	1.7	14	NS	***

1) Retained protein in percent of digested crude protein
2) Retained minerals in percent of intake

Generally the relative amount of minerals retained decreased with increasing dietary levels when expressed in percent of intake.

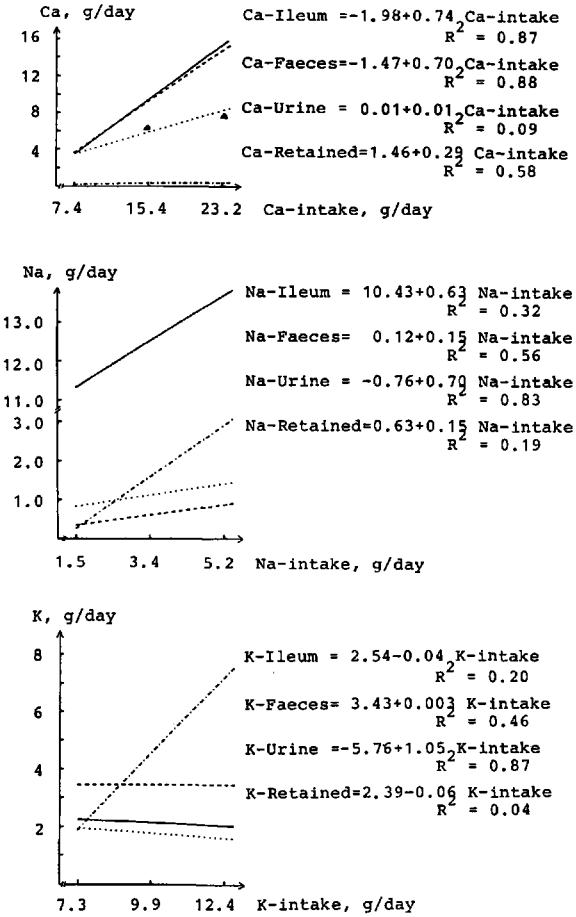


Figure 1. Passage of minerals in ileal digesta, excretion into faeces and urine and retention in relation to dietary supply of minerals.

——— Ileum – – – – – Faeces
—·—·— Urine ····· Retained

The daily retention of calcium and phosphorus increased with increasing dietary amount but to a less extent at the high dietary level. Daily retention of sodium showed a lineary increase with increasing dietary level of sodium.

Daily amounts of minerals in ileal digesta, faeces, urine and retained amounts of minerals in relation to dietary supply are shown in Figure 1. The relationship is described by regression of daily amount excreted or retained on daily intake when accounting for all known variations. The regression coefficients (b_1) express a ratio (output/intake) or what happens with each increment in daily supply of minerals.

Hardly any calcium is excreted into the urine (Figure 1) but 70% of each increment in the supply of calcium is excreted in faeces and 29% retained. The excretion in faeces and the retention of calcium increased and decreased, respectively, relative to dietary intake although the changes were more marked at the high intake level. Magnesium was found mainly to be absorbed in the hind gut, which is in agreement with Partridge (1978). Nearly a constant amount was excreted into the urine and the retention was highest on the normal level of minerals in the diet.

The amount of sodium passing the terminal ileum was 3 to 7 times higher than the sodium intake. The overall digestibility of sodium was almost the same independent of the concentration in the diet. The net absorption of sodium in the large intestine was about 8-9 g per day.

Potassium showed a complete different pattern to that of sodium. In the small intestine a net absorption took place but in the large intestine a secretion occurred. The increased dietary supply of potassium was completely excreted into the urine.

REFERENCES

JUST, A. 1972. Acta Agric. Scand. 22: 223-237.

JØRGENSEN, H.H. & FERNÁNDEZ, J.A. 1984. 35th Ann.Meet. EAAP. The Hauge. 4 pp.

JØRGENSEN, H., JUST, A. & FERNÁNDEZ, J.A. 1979. 30th Ann.Meet. EAAP. Harrogate. 4 pp.

PARTRIDGE, I.G. 1978. Br.J.Nutr. 39:527-537.

SAUER, W.C., JUST, A. & JØRGENSEN, H. 1982. Z.Tierphysiol., Tierernährg u. Futtermittelkde. 48:177-182.

USE OF THE FREE-MOVING DOUBLE NYLON BAG IN STUDIES OF DIGESTION IN THE LARGE INTESTINE OF PIGS

R.M. LIVINGSTONE
Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

SUMMARY

Double nylon bags inserted into the terminal ileum and recovered in faeces were superior to single bags for the study of fermentative digestion in the large intestine. The bags moved through the gut with the main flow of digesta. Digestibility of nutrients at the terminal ileum can be estimated without involving markers by using bags passing through the large bowel, together with a knowledge of the daily nutrient intake and output of the pig.

INTRODUCTION

Artificial fibre bags (dacron, nylon) are routinely used in the assessment of the nutritive value of feedstuffs for ruminants. The history of the development of the technique and its application to the study of rumen processes have been described by Ørskov et al. (1980). Rapp (1972) placed nylon bags in the gut of pigs by stomach tube and recovered them in faeces 48 h later. Digestibilities of grain mixtures found by the indicator method or by the use of bags were similar. However, the pyloric sphincter greatly restricts the size of bag which can be used. Sauer et al. (1983) reported results for protein digestibility arising from the use of a "mobile" bag (25 x 40 mm) which was inserted through a duodenal cannula after incubation of the feed samples in 0.01 N HCl and pepsin. The bags were recovered in faeces. The method has proved useful, but only for evaluating small samples of individual feedingstuffs encapsulated in the bag. Nylon bags suspended in the caecum of pigs have been used at the Rowett Institute by Robertson et al. (1985). This method will provide a good index of the rate and extent of fermentation of a sample of cell wall material for example, but such information obtained from one site in the gut may not be applicable to the entire digestive process which happens to dietary residues passing from the ileo-caecal junction to the terminal rectum.

The present paper reports preliminary work with a large type of double nylon bag which was inserted through a simple ileal cannula and recovered in faeces. The objective was two-fold. Firstly, to develop a method to study fermentative digestion in the large intestine which would increase the rate of screening of diets and dietary constituents. Secondly, to enable the measurement of digestibility at the terminal ileum to be done using a simple cannula without use of markers.

MATERIALS AND METHODS

The bags. Aperture size is important and differences in degradation between pore sizes can be explained by differences in particulate matter losses. The upper limit to pore size is set by the risk of losing undegraded food particles. Materials with 20μ and 35μ were found to give smaller dry matter losses than 53 μ (Uden et al. 1974). A suitable minimum appears to be 10μ, which enables ready access for all bacteria. In the double bags the outer had a pore size of 53 μ and the inner 10μ. The dimensions of the bags were for the outer 160 x 60 mm, and for the inner 150 x 45 mm. The large bags enabled greater sample accuracy to be obtained and these bags were readily recovered from faeces. The outer bag kept the inner one clean and prevented blockage of the 10μ apertures during passage through the large intestine. On recovery from faeces the inner bag was removed, weighed and freeze dried.

Transit time. To study whether the presence of relatively large bags impeded the normal flow of food residues through the large intestine, transit time was measured when bags were present in the gut or not. A standard diet based on barley was used and polystyrene particles were used as a marker. The polystyrene was placed into the terminal ileum and particles recovered from faeces were plotted as a percentage of the total recovered during the collection period of 72 h. The time taken for 50% recovery of particles was the transit half-time.

Digestibility. The effects of diet type, double or single bags, time of placement in the cannula and washing of the bag plus sample were investigated in a separate experiment. The design is shown in Table 1.

Table 1. Experimental design

	1		2		3	
Period 1 control	a.m. p.m.	Single not washed	a.m. p.m.	Double not washed	a.m. p.m.	Single washed
Period 2 methyl cellulose	a.m. p.m.	Double not washed	a.m. p.m.	Single washed	a.m. p.m.	Double not washed
Period 3 methyl cellulose	a.m. p.m.	Single washed	a.m. p.m.	Double washed	a.m. p.m.	Single not washed
Period 4 control	a.m. p.m.	Double washed	a.m. p.m.	Single not washed	a.m. p.m.	Double washed

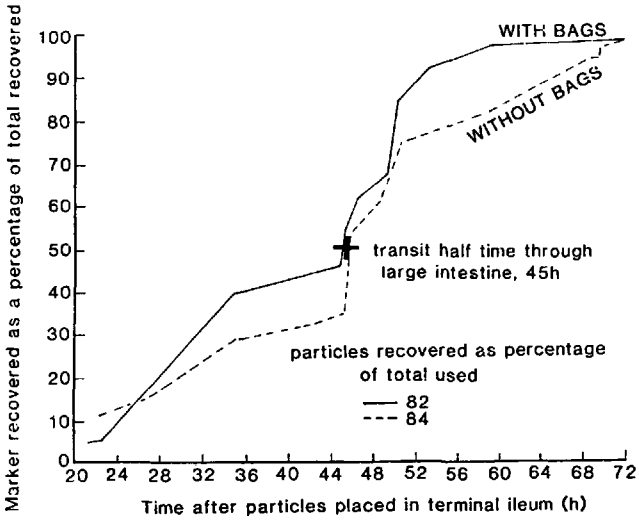


Figure 1. Transit time for food residues through the large intestine, with nylon bags present or not, in the gut.

Table 2. Apparent disappearance of organic matter and nitrogen from nylon bags passing through the large intestine. The bags contained ileal digesta.

Comparison	Organic matter		s.e.	sig.	Nitrogen		s.e.	sig.
Control v methyl cellulose	0.43	0.46	0.022	NS	0.52	0.57	0.032	NS
a.m. v p.m.	0.43	0.46	0.021	NS	0.52	0.55	0.033	NS
Washed v not washed	0.46	0.44	0.022	NS	0.58	0.55	0.031	NS
Double v single bag	0.47	0.42	0.020	*	0.59	0.49	0.025	***

Diets. Two diets were used. The first (control) contained (g/kg) ground barley 705, soyabean meal 160, maize starch 100, mineral and vitamin supplement 35. In the second diet methyl cellulose replaced 0.6 of the maize starch. The pigs were fed once daily at 0800 h. Each period as shown in Table 1 lasted 7 days and ileal digesta were collected from the pigs on day 3 of each period and were kept chilled until the following morning. Bags containing about 25 g of fresh digesta were placed in the terminal ileum of each pig on day 4 of each period, either 2 or 8h after the daily meal (a.m. or p.m. respectively in the table). The washing procedure before insertion in the gut, was agitation of the bag plus sample in water for 30 min, and was done to ensure that any losses from the bag in the gut would be due to the action of microorganisms and not simply to diffusion.

RESULTS AND DISCUSSION

The results for the transit time study are shown in Figure 1. A half time of about 45 h for food residues was obtained whether the bags were present in the gut or not, indicating that the bags did not interfere with the normal passage of residues through the large intestine. However, there was wide variation in the time which individual bags took to pass through the lower bowel, the range being 22 to 63 h.

The disappearance of organic matter and N from the bags is shown in Table 2. Of the comparisons made, only that of the double versus single bag revealed a significant difference with a higher digestibility for organic matter and for N resulting from use of the double bag.

The 50μ apertures of the outer bag remained free from obstruction but if used alone could have enabled particulate matter to be lost from the contents. The inner bag remained clean, permitted access by bacteria and prevented loss of particles. The higher digestibility found with the double compared with the single bag could be attributed to blockage of more than 50% of the 10μ apertures of the single bag by digestive secretions and food residues. The effect of time for passage of the bags through the large intestine on the digestibility of the contents requires

investigation. Ørskov et al. (1980) indicated that in the rumen, concentrates require 12 to 36 h and good quality roughages 24 to 60 h to reach their potential degradation. Therefore the contents of bags which pass more rapidly through the gut may not have been completely digested. Degradation curves are therefore required for different substrates.

An estimate of apparent digestibility at the terminal ileum can be obtained using pigs with simple T-cannulae without the use of a marker substance. This could be of great advantage with bulky and fibrous material such as grass, root or forage brassicas where even dispersion of a marker like chromic oxide in the food is difficult. Three factors have to be known. 1) The nutrient intake per unit of time: 2) The amount of nutrient voided in the faeces per unit of time: 3) The coefficient of disappearance of the nutrient from the large intestine. The latter can be obtained using free-moving nylon bags. Each bag would contain a representative sample of ileal digesta. Then $L = (T-F)/T$; $T = -F/(L-1)$; $D = (I-T)/I$; where L is (3) above, T is the amount of nutrient passing the terminal ileum per unit of time, F is (2) above, I is (1) above and D is apparent digestibility at the terminal ileum. The disadvantage is the capacity of the nylon bag. It was found with the T-cannula used, having an internal diameter of 16 mm, that 25 g of fresh digesta was about the maximum amount which could be inserted into the cannula in a double bag. This amount is equivalent to approximately 3 g DM. If half this DM disappears from the large intestine, the remaining sample for analysis is only 1.5 g DM. The advantage is that any number of bags can be used in succession until sufficient sample is obtained, and each bag provides an estimate of digestibility in the large intestine, thus consolidating the estimate of digestibility anterior to the terminal ileum.

REFERENCES

ØRSKOV, E.R., HOVELL, F.D.DeB. & MOULD, F. 1980. Trop. Anim. Prod. 5: 195-213.

RAPP, W. 1972. Thesis Ludwig-Maximilians-Universitat, Munchen, 82 pp.

ROBERTSON, J.A., BAIN, S.D. & CHESSON, A. 1985. Br.J.Nutr. (in press).

SAUER, W.C., JORGENSEN, H., JAIKARAN, S. & OZIMEK, L. 1983. Agric. & Forestry Bulletin, Special Issue, 115-117, En. 62nd Annual Feeders Day Report, Univ. Alberta.

UDEN, P., PARRA, R. & VAN SOEST, P.J. 1974. J. Dairy Sci. 57: 622, Abstr.

EFFECTS OF HOUSING ON GASTROINTESTINAL TRANSIT TIME AND DIGESTIBILITY OF FEEDS IN GROWING PIGS

S.H.M. METZ, R.A. DEKKER
Institute for Livestock Feeding and Nutrition Research,
P.O.Box 160, 8200 AD Lelystad, The Netherlands

SUMMARY

Transit time (TT) of digesta in growing pigs at a high level of feed intake fluctuated between 24 and 48 hours. For a fibrous diet this TT increased with liveweight of the pigs. TT depended upon housing system. For dry matter (DM), organic matter (OM) and N, digestibility was positively correlated with TT, being reflected in significant housing and liveweight effects upon digestibility.

INTRODUCTION

In the Netherlands increased utilization of fibrous byproducts in pig feeding has led to studies concerning the role of fibre in digestion. Both physiological aspects and the effects on digestibility and utilisation of digestible energy have been studied quite extensively. One important aspect is the gastrointestinal transit time (TT) of the digesta, which should be long enough to allow adequate fermentation of the fibrous components in the large intestine. No data are available yet concerning TT of digesta in growing pigs consuming rations composed of commercially available fibrous byproducts at a high level of feed intake. This paper deals with the TT of the digesta and its relation with digestion in fast-growing pigs, fed a cereal or a fibrous byproduct diet, under two housing systems.

MATERIALS AND METHODS

TT was measured in an experiment with 4 young boars, growing from 30 to 150 kg liveweight. The experiment included 4 periods, of 5 weeks each. Within a period the pigs were alternately grouphoused in a pen and individually housed in a metabolism crate; the pigs were fed individually. Simultaneously 2 pigs were fed a cereal diet and the 2 others were fed a byproduct diet. The cereal diet consisted of barley (54.5%), wheat (30%) and dried potato protein (9%); the byproduct diet was a mixture of hominy feed (46%), coconut expeller (29%), dried potato pulp (10%) and dried potato protein (9%).

Both diets contained some min. + vit., cane molasses and soyabean oil. The cereal diet contained per kg feed 551 g starch + sugar, 41 g crude fiber and 184 g NDF; the byproduct diet contained per kg 281 g starch + sugar, 92 g crude fiber and 344 g NDF. The cereal diet was fed to the pigs at a daily intake of about 100 g/kg$^{\frac{3}{4}}$ in the weight range till 100 kg and declining thereafter to about 80g/kg$^{\frac{3}{4}}$ at 150 kg liveweight. Daily feed supply was 5% more for the byproduct diet because of its lower net energy content). The diets were fed in two equal meals per day at 6.30 a.m. and 3.30 p.m., as a slurry (feed to water ratio of 1:2.5). Change-over of feed occurred during the first few days of each period.

The experimental scheme is shown in Table 1. Within periods for

Table 1. Experimental scheme

	period 1		period 2		period 3		period 4	
	pen	cage	cage	pen	pen	cage	cage	pen
pig 1	B*	B	A*	A	A	A	B	B
pig 2	B	B	B	B	A	A	A	A
pig 3	A	A	A	A	B	B	B	B
pig 4	A	A	B	B	B	B	A	A

* A = cereal diet; B = byproduct diet

each pig in each housing system the following observations were made
- TT of digesta, as weighted mean retention time of the marker that was excreted with the faeces (Warner, 1981), using polyethyleneglycol (PEG) and cubical polythene pellets (PE; size 1-2 mm) as markers for liquids and solids respectively
- digestion of the diets, by total collection of faeces during 96 hours + correction for a 100% excretion of marker, using Cr_2O_3
- activity of the pigs (lying, sitting, standing) during either 96 hours (in a pen) or 24 hours (in metabolism crates).

Observations were analysed statistically by regression analysis.

RESULTS AND DISCUSSION

TT of digesta fluctuated between about 24 and 48 hours (Table 2). For the cereal diet TT_{PE} was always longer than TT_{PEG}, on average 28%. TT_{PEG} and TT_{PE} differed not for the byproduct diet (average difference of 2%, with a SD of 6%). TT of digesta was significantly longer, based upon PEG on average 20%, during housing on

cages than during grouphousing in a pen. Diet effects on TT of a solid marker relative to TT of a liquid marker were also observed in a second experiment using the same diets, the same liquid marker and Cr-mordanted NDR as solid marker. The diet effects can be explained by a much higher water holding capacity of the digesta when pigs consume fibrous diets (Metz, unpublished results), leading to a closer joining of liquid and solid particle flow as compared with situations that pigs consume diets poor in fibre.

Table 2. Liveweight, TT_{PEG} and TT_{PE}*

liveweight(kg)		period	housing	TT_{PEG}		TT_{PE}	
cereal	byprod.			cereal	byprod	cereal	byprod.
36	35	1	pen	26.7	24.0	33.8	25.0
47	46	1	cage	30.9	25.9	44.9	25.0
69	64	2	cage	34.0	30.8	42.3	30.6
82	77	2	pen	28.3	26.7	38.3	26.2
97	105	3	pen	28.0	31.6	38.7	31.2
108	121	3	cage	29.1	41.2	38.7	38.9
136	143	4	cage	39.3	46.5	47.0	44.4
145	155	4	pen	26.1	36.1	32.0	31.4

* Each value represents the mean of 2 observations on 2 pigs.

TT increased significantly with liveweight (age) when the pigs were fed the byproduct diet, but not when the pigs received the cereal diet. Analysis of the data revealed that this increase with age was primarily an age effect and not an effect of feeding level (decreasing after 100 kg liveweight). The regression of TT_{PEG} on liveweight is shown in Table 3.

In order to investigate whether differences in TT had consequences for digestion, for both diets the relationship between digestion

Table 3. Effect of liveweight upon TT of digesta.

diet	housing	TT_{PEG} =	a	+	b x LW	SD_b	n	r
cereal	pen		27.6	-	0.00	0.03	8	-0.06
	cage		26.7	+	0.08	0.08	6	0.17
byprod.	pen		19.9	+	0.10	0.01	7	0.98
	cage		16.9	+	0.20	0.02	7	0.98

Table 4. Relationship between TT and digestion of OM and N.

diet	DC_{OM} =	a	+	b x TT_{PEG}	SD_b	n	r
cereal	=	82.2	+	0.09	0.04	14	0.53
byproduct	=	67.5	+	0.33	0.06	14	0.84
	DC_N						
cereal	=	73.8	+	0.26	0.08	14	0.70
byproduct	=	51.0	+	0.65	0.10	14	0.87

and TT was analysed. Digestion appeared to increase significantly with TT, irrespective whether increasing TT was due to difference in housing system or due to increasing liveweight of the pigs. The dependency of digestion upon TT could adequately be described by linear regression, the regression coefficients being higher for the fibrous diet as compared with the cereal diet (Table 4). Both for OM and N the relationship (r) was stronger for the byproduct than for the cereal diet, as might be expected.

Both the variation in TT, either due to growth of the pigs (for the byproduct diet) or due to housing system (for both diets), and the positive relationship between TT and digestion indicates that digestibility depends upon liveweight and housing system. Regression analysis of digestibility coefficients (using the factors animal, diet, housing system, liveweight and interactions) indicated that between animal variation and diet-housing interactions were not significant. There existed significant effects of diet, housing, liveweight and diet-liveweight interaction, which together explained 96% of the total variance in DC_{DM}. The liveweight effect was only significant ($P<0.05$) for the byproduct diet. Per diet the dependence of DM-digestibility upon housing and liveweight for the two diets was as follows :

cereal diet	DC_{DM}	=	79.3	+	1.81 x H*	+	0.011 x LW
byprod. diet	DC_{DM}	=	68.0	+	1.81 x H*	+	0.056 x LW

* In a pen: H = 1; in a cage: H = 2, indicating that the mean difference between cage and pen in DC_{DM} was on average 1.8 %.

REFERENCE

Warner, A.C.I. (1981). Nutr. Abstr. & Rev., Series B, 151: 789-820.

NYLON BAG AND IN VITRO TECHNIQUES TO PREDICT IN VIVO DIGESTIBILITY OF ORGANIC MATTER IN FEEDSTUFFS FOR PIGS

S.H.M. METZ, J.M. VAN DER MEER
Institute for Livestock Feeding and Nutrition Research,
P.O.Box 160, 8200 AD Lelystad, The Netherlands.

SUMMARY

Nylon bag and in vitro enzymatic methods are presented to imitate the in vivo organic matter digestibility (d_o) for pig feeds. Both methods show very promising results. Separation of the mixed feeds and feedstuffs in groups of similar products improves the accuracy of the regression equations for predicting d_o from the alternative methods.

INTRODUCTION

Up till now, feed evaluation for pigs is mainly based upon measurement of digestibility in vivo, using fattening pigs. Digestion trials, however, are expensive and laborious, take a long time and require large quantities of feed. Additionally, for many feedstuffs digestion trials can only be performed after mixing the feedstuff with a basal ration with known digestibility, resulting in a less accurate digestibility measurement for the feedstuff under investigation.

There is a need for alternative techniques to overcome some disadvantages of digestion trials. Two techniques have shown promising results in recent years: a nylon bag technique (Sauer et al. 1983) and an in vitro enzymatic assay (Decuypere et al., 1984). This paper deals with modifications of both techniques and their reliability for estimation of d_o.

MATERIALS AND METHODS

Feeds: For both techniques the studies were performed with a large number of feedstuffs and mixed feeds. All feeds had known d_o, measured in digestion trials with 4 fattening pigs.

Final methods are described for the techniques separately. Nylon bag technique: Animals: Two castrated male pigs, with a T-shape duodenal cannula at about 25 cm from the pylorus, growing from 50 till 100 kg liveweight. The animals were fed a

diet consisting of barley, solv.extr. soybean meal, min.+vit. (86:12:2, on a weight basis) at an intake level of 2.4 times maintenance. The diet was fed in two equal meals daily (at 8.00 a.m. and 4.00 p.m.) as a slurry (diet to water ratio 1:2.5).

Nylon bags: were prepared from monofil nylon (size 25 x 40 mm).

Experimental design: Using the same animals, two experiments were performed. The first experiment concerned methodological studies, the second experiment evaluation of the reliability of the method. The standard procedure in the 2nd experiment was: 10 bags (pore size 41 µm; 1 g feed/bag) per feed were incubated in a pepsin/HCl solution (1:10.000; 2.5g/l,pH 2.0, 5h) and stored at -18°C thereafter. Before putting the bags into the duodenum they were thawed. Each pig got 10 bags per day during 5 days a week (Monday till Friday), 2 at a time at 8, 10, 12, 14 and 16 o'clock. Bags were collected from the faeces after recording time of defaecation, roughly cleaned, freeze-dried and cleaned by brushing. Then bags + contents were analysed for dry matter (DM) and ash.

In vitro technique: About 0.5 g feed was incubated in scintered glass filtration tubes with 40 ml pepsin/HCl solution (1:10.000 2.5 g/l; pH 1.0, 40°C, 1h). The liquid was filled up with water to 50 ml, heated (80°C, ½h) and filtrated. Thereafter 40 ml of a potassium phosphate buffer containing per liter 2.5 g hog-pancreatine, 25 mg pancreatic lipase, 50 mg bilesalts, 2.5 ml Termamyl 60 L (NOVO; amyloglucosidase), was added to the residue and the mixture was incubated again (40°C,1h). After subsequent filtration the residue was incubated (40°C, 2h) in 40 ml acetate buffer (pH 5.0) containing 25 g Aspergillus Niger cellulase per liter and filtrated again. Then the residue was successively washed with water, ethanol and aceton, dried, weighed, ashed and weighed again.

Calculations: From the initial amounts and the losses during the experimental procedures the digestibility of organic matter in nylon bags (d_ONB) or in vitro ($d_O$vitro) were calculated. Then linear regression of d_O on d_ONB or $d_O$vitro was made, in order to estimate the adequacy of both techniques.

RESULTS AND DISCUSSION

The methodological studies resulted in the final procedures

described in materials and methods. The results obtained with these procedures showed (Table 1) that usually d_ONB was somewhat lower than d_O and $d_O$vitro was somewhat higher than d_O. In the nylon bag technique the mean transit time (TT) of the bags in pigs was 35.3 hours (between feed CV in TT was 11%) which was comparable to TT of digesta in growing pigs (Metz and Dekker, 1985). Variation in d_O was largely reflected by variation in d_ONB and in $d_O$vitro. Regression analysis indicated that reliability of both techniques for estimating d_O was promising but not yet accurate enough when applied to the whole material (see RSD in Table 2). Division of the feeds into product groups showed for both methods a good accuracy of d_O-estimation for the mixed feeds, although feeds varied considerably in both

Table 1. Organic matter digestibilities of the feeds

	single feeds			mixed feeds		
	d_O	d_ONB*	$d_O$vitro*	d_O	d_ONB*	$d_O$vitro*
peas	96.0	-11	- 7	89.1	- 1	+ 3
tapioca	92.4	- 5	+ 2	88.7	- 3	+ 1
tapiocameal	88.8	- 4	+ 4	86.7		+ 1
maize	88.2	- 3	+ 3	85.1	- 3	+ 4
soybean, solv.extr.	87.9	- 6	- 1	84.5	- 6	+ 2
groundnut expeller	87.8	- 4	+ 6	84.2	- 5	+ 2
citruspulp	84.8	-12	+ 4	83.7	- 2	+ 4
lupin	84.7	-15	- 6	82.6		+ 3
barley	83.4	- 1	+ 5	82.4	- 5	+ 2
coconut expeller	82.6	- 9	+ 1	81.8	+ 1	+ 6
coconut, solv.extr.	75.2	+ 3	- 1	81.7	- 4	+ 5
hominy feed	74.3	- 2	+ 2	81.4	- 0	+ 3
rice bran	72.0	- 8	+ 3	81.3		+ 3
linseed expeller	71.8	- 8	+ 3	78.2		+ 4
rapeseed	68.5		+ 2	77.9	+ 3	+ 9
rapeseed	68.1	- 3	+10	77.2	- 0	+ 3
wheat middlings	66.2	- 4	+ 9	77.1		+ 6
linseed, solv.extr.	63.4	- 1	+17	75.5	- 4	+ 2
cornsilage	58.0		+ 2	75.3	- 3	+ 0
sunflower, solv.extr.	49.9	- 0	+ 8	74.3	+ 1	+ 9
				72.2	- 4	+ 7

* deviation from d_O

feedstuff and chemical composition. For the subgroups of single feeds the estimation of d_o using the nylon bag technique was not better than for the whole set. Some improvement was obtained, however, by correction of d_oNB to a standard TT of 36 hours for the nylon bags in the pigs (to RSD=3.4 for oilseeds + residues and RSD=4.3 for other feedstuffs). This seems logical as there was variation in TT between the feeds, and within feeds TT was positively correlated with d_oNB. In the in vitro method do oilseeds + residues still need more attention, because of their filtration problems and in order to overcome too high values for some rapeseed and lineseed products. The RSD (1.3) for other single feedstuffs (mainly rich in starch) seemed excellent; the number of feedstuffs is still small, however.

To improve the accuracy of the regression equations, inclusion of proximate analyses (e.g. crude fat, crude fibre) in regression equations may be useful and will be examined in the near future.

Table 2. Regression of d_o on d_oNB and d_o vitro.

	n	d_o =	a	+	b x d_oNB	r	RSD
all feeds	34	=	9.2		0.93	0.90	4.0
mixed feeds	16	=	13.5		0.86	0.88	2.4
oilseeds*	8	=	-0.9		1.06	0.96	4.0
others	10	=	20.1		0.82	0.86	4.9
		=	a	+	b x $d_o$vitro		
all feeds	34	=	-1.8		0.99	0.91	3.8
mixed feeds	16	=	-1.		0.98	0.85	2.7
oilseeds*	8	=	-21.0		1.24	0.95	3.9
others**	8	=	2.6		0.93	0.99	1.3

*oilseeds + residues; ** Lupine and peas excluded;

REFERENCES

Decuypere J.A., et al., 1984. Proc. 8th Intern. Pig Veterinary Congress, Ghent, p. 330.

Sauer, W.C., Jorgensen, H. and Berzins, R. 1983. Can. J. anim. Sci. 63, p 233-237.

Metz, S.H.M. and Dekker, R.A. 1985. Proc. 3rd Int. Seminar on "Digestive Physiology in the pig". In press.

EVALUATION OF DISTILLERS BY-PRODUCTS FROM BARLEY AS PROTEIN SOURCE FOR PIGS

M. NÄSI
Department of Animal Husbandry, University of Helsinki
SF-00710 Helsinki, Finland

SUMMARY

The nutrient digestibility and protein utilization of barley derived distillery feeds were studied with growing pigs receiving one of five diets in which the protein sources were BDDGS, BDDG, BDS, WDDGS or SBM. The diets were formulated to contain 13.0 % DCP, 0.80 % lysine and 0.56 % S-amino acids. The distillery by-products contained crude protein 24.8 - 41.5 %, crude fat 6.3 - 8.4 % and crude fibre 7.8 - 10.2 % on a dry basis. Their lysine content was 0.63 - 1.36 and S-amino acids 0.58 - 1.36 %. The digestibilities of organic matter and crude protein were 63 - 83 and 63 - 76 %. The N retentions on the BDDGS, BDDG, BDS, WDDGS and SBM diets were, respectively, 21.7, 23.0, 17.7, 24.2 and 24.6 g/d and the biological values 55, 56, 55, 59 and 66. The daily gains varied from 700 to 762 g. The data indicated that distillery by-products could replace soybean meal quite satisfactorily as a protein source in amino acid fortified diets.

INTRODUCTION

The desire to increase the domestic protein supply for animal feeding in Finland has prompted the search for alternative protein sources. The protein content is high in distillery by-products left after the alcohol process. A new ethanol plant due to start operatin soon will use barley as raw material and employ an integrated ethanol-starch process. The increased supply of distillery feed fractions expected in the next few years stimulated the present study to evaluate these products, especially barley residues, for which little information is available regarding utilisation in pig diets.

MATERIALS AND METHODS

Dehulled barley derived distillers feedstuffs were obtained from the Koskenkorva factory of OY Alko AB. Large White castrated pigs weighing 33 to 82 kg were used to compare five barley based diets containing one of the following protein sources (%): barley distillers dried grains with solubles (BDDGS) 40.0 plus lysine 0.20; barley distillers dried grains (BDDG) 28.3 plus lysine 0.11; condensed distillers solubles (BDS) 61.0 plus lysine 0.21 plus methionine 0.05; wheat distillers dried grains with solubles (WDDGS) 27.7 plus lysine 0.39 and soybean meal (SBM) 14.4. The diets were formulated to contain 13.0 % DCP, 0.80 % lysine and 0.56 % S-amino acids. Five digestibility and balance trial were conducted using 6-day preliminary periods and 6-day collection periods in a 5 x 5 Latin square design. The pigs were kept in metal metabolism units equipped with collection trays for separate total collection of faeces and urine. The details of the procedures are the same as described by NÄSI (1984).

RESULTS AND DISCUSSION

During the ethanol process the starch is fermented and the other grain components concentrated. The dehydrated distillery products had a crude protein content of 34.4 to 41.5 %, but the protein of the condensed solubles was lower, 24.8 % of DM (Table 1). The fibre contents of the BDDGS and BDDG were only two-thirds of that of products of whole barley (NÄSI 1984). The lysine content of the products and its availability (58 - 83 %) were higher than in previous experiments. The pepsin-HCl solubility of the protein varied from 74 to 84 % of the samples indicating no serious denaturation in drying.

The organic matter and NFE digestibilities of BDS exceeded those of the other products ($P < 0.01$), 83 vs. 63 - 65 %, but the crude protein was less digestible ($P < 0.01$), 63 vs. 69 - 76 % (Table 2). The digestibilities and calculated feed values were considerably higher than those of whole barley distillers feeds (NÄSI 1984). The FU values of 0.75 - 0.79 per kg DM are sufficiently high for

inclusion in pig feed formulas at the level of 10 - 20 %. However, an evaluation also has to be made with production trials. The BDDG and WDDGS diets fortified with amino acids gave N retentions similar to that of the SBM diet ($P > 0.05$), but the BDDGS and BDS diets gave poorer results ($P < 0.01$). The biological values of the diets including distillery products were significantly lower than those of the SBM-barley diet ($P < 0.01$). The daily gains of 700 - 742 g for the pigs on barley-distillery product diets were satisfactory compared with the value of 762 g/d for the pigs fed SBM-barley.

The present experiment performed to assess the nutritive value of barley distillers by-products indicated that products obtained from dehulled barley are similar in value to products derived from wheat. According to the N-balance results distillers by-products could replace SBM quite satisfactorily, but further production experiments are needed. The integrated starch-ethanol process yields many feed fractions which can be processed and combined in different ways to give suitable feeds for domestic animals, including monogastrics.

Table 1. Chemical composition and feed values of distillery by-products from barley compared with those of wheat

In DM	BDDGS	BDDG	BDS	WDDGS
Crude protein	34.4	40.0	24.8	41.5
Crude fat	8.4	7.5	6.5	6.3
Crude fibre	7.8	10.2	1.4	9.9
ADF	22.0	22.0	-	18.2
NDF	38.0	47.6	-	40.9
NFE	43.9	39.1	53.5	38.5
Lysine	0.95	1.36	0.69	0.63
S-amino acids	0.88	1.36	0.58	1.03
Threonine	1.33	1.56	0.87	1.28
FU/kg DM	0.79	0.76	0.88	0.75
DCP in DM, %	23.6	30.3	15.5	30.2
ME MJ/kg DM	12.5	12.2	13.5	11.9

Table 2. Digestibility coefficients of distillery by-products and protein utilisation of the diets

	BDDGS	BDDG	BDS	WDDGS	SBM
Organic matter	65.7^{f}	63.0^{g}	82.8^{e}	62.9^{fg}	
Crude protein	68.7^{fg}	75.9^{f}	62.6^{gh}	72.8^{fg}	
NFE	66.3^{f}	54.3^{g}	92.6^{e}	57.1^{g}	
N intake, g	62.9^{ae}	61.5^{abe}	58.3^{be}	62.5^{abe}	51.4^{cf}
N in faeces, g	17.3^{g}	13.9^{gh}	20.0^{ef}	15.4^{fg}	8.9^{h}
N in urine, g	23.9^{a}	24.6^{a}	20.6^{a}	22.9^{a}	17.9^{a}
N retained, g	21.7^{fg}	23.0^{efg}	17.7^{h}	24.2^{ef}	24.6^{e}
Urinary urea-N, g	21.9^{e}	20.0^{fg}	12.9^{g}	20.2^{ef}	14.5^{fg}
Biological value	55.1^{g}	55.7^{fg}	54.7^{g}	58.6^{fg}	65.5^{e}
Daily gain, g	742^{a}	732^{a}	700^{a}	700^{a}	762^{a}

Means with different letters were significantly different:
a - d ($P < 0.05$), e - h ($P < 0.01$).

REFERENCES

NÄSI, M. 1984. J. Agric. Sci. Finl. 56: 221 - 226.

DIGESTIBILITY OF DIETS CONTAINING RAPESEED MEAL, SOYABEAN MEAL AND FISHMEAL IN PIGS WITH ILEAL CANNULAS

I.G. PARTRIDGE, A.G. LOW and J.J. MATTE
National Institute for Research in Dairying, Shinfield, Reading, U.K.

SUMMARY

The apparent ileal and faecal digestibility of dry matter (DM), total nitrogen (N) and 17 amino acids (AA) were measured with barley diets containing 'double zero' rapeseed meal (R), soyabean meal (S) or fishmeal (F), or combinations of soya + rapeseed (SR), soya + fishmeal (SF), rapeseed + fishmeal (RF) or soya + rapeseed + fishmeal (SRF). Seven pigs fitted with a 'T' cannula in the terminal ileum were used in a 7 x 7 Latin square design, using Cr_2O_3 as a marker. A semi-purified diet (RX) containing rapeseed meal as the only protein source was also evaluated. In all cases where treatment differences were significant, values were lower for diet R than for diet F ($P<.05$); in general, diet S was intermediate. When rapeseed was used in combination with fishmeal (RF) or with both fish and soyabean meal (SRF) the digestibility of N and all AA were not significantly reduced ($P>.05$), compared with fishmeal alone. The values obtained with diet RX were generally higher than the corresponding values for diet R.

INTRODUCTION

In recent years, the development of new varieties of rape has led to an increased use of rapeseed meal in pig diets. However, it is probably still inappropriate to use rapeseed meal as the only protein supplement; despite a low glucosinolate content, voluntary food intake may be reduced by about 10% when a diet containing rapeseed meal is compared to a diet containing soyabean meal (Lee, 1981; Lee & Hill, 1983). A lower level of rapeseed inclusion tends to reduce the detrimental effect on food intake (Singam & Lawrence, 1979; Fenwick, 1982). There is a need, therefore, to evaluate rapeseed meal in conjunction with complementary protein supplements for growing pigs.

EXPERIMENTAL PROCEDURE

Seven LW x (LW x LR) pigs weighing about 30 kg were surgically fitted with an ileal 'T' cannula, 30 cm anterior to the ileo-caecal junction. They were assigned to a 7 x 7 Latin square design and received each of seven barley diets containing rapeseed meal (R), soyabean meal (S), fishmeal (F) or combinations of these, SR, SF, RF or SRF (Table 1).

Table 1. Composition of the diets (g/kg)

	S	R	F	SR	SF	RF	SRF	RX
Barley	702.3	562.2	857.5	632.3	780.0	709.9	707.4	-
Maize starch	-	-	-	-	-	-	-	549.3
Soyabean meal	250.0	-	-	125.0	125.0	-	83.3	-
Fishmeal	-	-	125.0	-	62.5	62.5	41.7	-
Rapeseed meal	-	375.0	-	187.5	-	187.5	125.0	375.0
Soyabean oil	10.0	31.0	-	20.5	5.0	15.5	13.7	30.0
Salt	2.5	2.7	-	2.6	1.2	1.3	1.7	4.5
Limestone	3.7	6.0	-	4.8	1.8	3.0	3.2	6.0
Dical. phosphate	14.0	5.6	-	9.8	7.0	2.8	6.5	18.2
Min/Vit. suppl.	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.0
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Composition based on ingredient analysis								
DE (MJ/kg)	13.1	13.1	13.1	13.1	13.1	13.1	13.1	14.9
Lysine	8.8	8.8	8.8	8.8	8.8	8.8	8.8	6.8
Threonine	6.3	7.3	6.0	6.8	6.2	6.6	6.5	5.3
Met. + Cyst.	5.3	7.3	5.6	6.3	5.4	6.4	6.0	5.3
Crude protein	180	192	176	186	178	184	183	130
Crude fibre	51	71	44	61	48	58	55	43

All diets contained (g/kg): Ca 9.8; P 6.6; NaCl 5.2.

The diets were fed wet (1.0 kg:2.5 l. water) twice daily at 08.30 and 20.30. Feed was restricted to about 4% of liveweight per day. Each period lasted 7 days. Faeces and ileal digesta were collected from 08.30 to 20.30 on day 5 and 7 of each period. The diets and all freeze-dried ileal digesta samples were analysed for dry matter (DM), total nitrogen (N), amino acids (AA) and Cr_2O_3 and ileal and faecal apparent digestibility values were calculated.

The data were subjected to analysis of variance; differences among treatment means were tested by the Student-Newman-Keuls multiple range test (Snedecor & Cochran, 1971).

At the end of the seven-week period, the pigs received for one week the semi-purified diet RX described in Table 1. All the procedures for feeding, ileal digesta collection faeces collection and laboratory analysis were identical to those described above.

RESULTS AND DISCUSSION

The ileal and faecal apparent digestibility values for DM, N and AA in the barley diets are presented in Table 2. Where significant differences were observed between diets, values for R were always lower (P<.05) than those for F. With few exceptions the values for diet S were intermediate. When protein supplements were combined, SR showed apparent digestibility values in the range of those observed with S and R; RF was similar (P>.05) to diet F except for a small difference in the apparent faecal digestibility of DM; SF and SRF showed no significant differences (P>.05) from diet F.

Table 2. Ileal and faecal apparent digestibility of DM, N and AA in barley diets. Values are means for 7 pigs in a Latin Square design. (Means in the same row with a common superscript letter do not differ significantly (P>.05))

	Diet							
	S	R	F	SR	SF	RF	SRF	SED
Ileal DM	0.55b	0.50a	0.64c	0.56c	0.60bc	0.59bc	0.57bc	0.025*
Faecal DM	0.80c	0.75a	0.81c	0.78b	0.80c	0.79bc	0.80c	0.007*
Ileal N	0.70	0.66	0.71	0.69	0.71	0.70	0.69	0.020
Faecal N	0.78b	0.72a	0.78b	0.75ab	0.79b	0.76b	0.78b	0.015*
Ileal AA								
Asp	0.72	0.65	0.70	0.70	0.71	0.69	0.70	0.020
Thr	0.65	0.62	0.70	0.66	0.68	0.68	0.67	0.024
Ser	0.71	0.67	0.73	0.71	0.73	0.71	0.71	0.020
Glu	0.80	0.78	0.82	0.80	0.82	0.82	0.80	0.013
Pro	0.80b	0.72a	0.81b	0.77b	0.80b	0.78b	0.78b	0.017*
Gly	0.53	0.58	0.62	0.59	0.58	0.61	0.58	0.026
Ala	0.66a	0.69a	0.75b	0.69a	0.71ab	0.74b	0.71ab	0.021*
Cys	0.61	0.60	0.63	0.64	0.64	0.65	0.62	0.024
Val	0.73	0.70	0.77	0.72	0.74	0.74	0.74	0.019
Met	0.81	0.84	0.84	0.84	0.83	0.85	0.83	0.013
Ileu	0.76b	0.71a	0.79b	0.74ab	0.76b	0.76b	0.76b	0.016*
Leu	0.78	0.77	0.82	0.78	0.79	0.80	0.80	0.016
Tyr	0.77ab	0.72a	0.80b	0.76ab	0.78ab	0.77ab	0.76ab	0.018*
Phe	0.77	0.74	0.80	0.76	0.78	0.78	0.77	0.017
His	0.75b	0.66a	0.72b	0.70b	0.73b	0.72b	0.72b	0.017*
Lys	0.75bc	0.68a	0.80c	0.72b	0.77bc	0.76bc	0.75bc	0.018*
Arg	0.84	0.80	0.83	0.83	0.83	0.83	0.83	0.011

The corresponding values for diet RX are presented in Table 3. In most cases, the values were higher than those observed with diet R. This could be due to a lower digestibility of AA in barley and/or reduced overall digestibility due to the higher fibre content of the barley-based diet.

Table 3. Ileal and faecal apparent digestibility of DM, N and AA in the semi-purified diet (RX). Values are means for 7 pigs ± standard deviations.

Ileal DM: 0.72±0.018 Faecal DM: 0.86±0.002

Ileal N: 0.67±0.035 Faecal N: 0.78±0.031

Ileal AA

Asp	0.67±0.036	Pro	0.69±0.028	Val	0.71±0.038	Tyr	0.69±0.035
Thr	0.64±0.041	Gly	0.65±0.050	Met	0.87±0.018	Phe	0.73±0.045
Ser	0.68±0.033	Ala	0.74±0.041	Ileu	0.73±0.036	His	0.68±0.038
Glu	0.82±0.022	Cys	0.70±0.022	Leu	0.79±0.042	Lys	0.72±0.031
						Arg	0.84±0.024

Similarly, the apparent digestibility values for diets S, R and F tended to be lower to those observed with semi-purified diets containing soya (Tanksley & Knabe, 1984), soya or canola meal (Sauer et al., 1982) or fishmeal (Darcy et al., 1982). The faecal apparent digestibility values of DM and N in diets S and R are in the same range as those reported by Singam & Lawrence (1979) with similar diets based on barley.

In conclusion, the use of European 'double zero' rapeseed meal as the only protein supplement in practical diets would probably cause a significant reduction in apparent AA digestibility compared with fishmeal and, to a lesser extent, soyabean meal. However, if similar proportions of the required protein supplement were provided by rapeseed and fishmeal or by rapeseed, fishmeal and soyabean meal, the digestibility of N and AA would probably not be significantly lower than with fishmeal alone.

REFERENCES

DARCY, B., LAPLACE, J.P. & DUEE, P.H. 1982. Annls Zootech. 31: 279-300.

FENWICK, G.R. 1982. Proc. Nutr. Soc. 41: 277-288.

LEE, P.A. 1981. PhD Thesis: Univ. London.

LEE, P.A. & HILL, R. 1983. Br. J. Nutr. 50: 661-671.

SINGAM, A.D.R. & LAWRENCE, T.L.J. 1979. J. Sci. Fd Agric. 30: 21-26.

SNEDECOR, G.W. & COCHRAN, W.G. 1971. In: Statistical Methods. 6th Ed. Iowa State Univ. Press, Ames, U.S.A.

SAUER, W.C., CICHON, R. & MISIR, R. 1982. J. Anim. Sci. 54: 292-301.

TANKSLEY, T.D. & KNABE, D.A. 1984. In: Recent Advances in Animal Nutrition. W. Haresign & D.J.A. Cole, Ed. Butterworths:London.

EVALUATION OF THE MOBILE NYLON BAG TECHNIQUE FOR MEASURING THE DIGESTIBILITY OF PIG FEEDS

M.R. TAVERNER, R.G. CAMPBELL
Animal Research Institute
Werribee, 3030, Australia

SUMMARY

The digestibility by pigs of dietary dry matter was measured in 39 diets using the conventional technique of total faecal collection. Comparison with estimates made using a mobile nylon bag technique (MNBT) revealed that the MNBT underestimated ($P<0.001$) digestibility. The average (±SD) values were 76.9 (±6.92) vs 81.8 (±5.97)% for MNBT and total collection respectively. However, the values were linearly related ($r^2 = 0.89$) in the following manner: Digestibility (%) = 21.62 + 0.78 MNBT (%).

Two basal diets were fed to pigs during test measurements using MNBT. The diets differed predominantly in their fibre contents. The digestibility values of test feeds obtained by the MNBT were affected ($P<0.001$) by the basal diet which the pigs received. For seven diets with an average (±SD) digestibility of 83.8 (± 4.12)%, the average MNBT values were 75.0 (± 5.67) and 77.6 (± 5.36)% when the pigs were fed during the assay with diets containing 5.9 and 4.6% crude fibre, respectively.

INTRODUCTION

Conventional digestibility techniques involving the total collection of faeces from individually housed pigs are impractical for rapid and routine analyses of pig feeds. A more practical method may be provided however, by the mobile nylon bag technique (MNBT) described by Sauer *et al.* (1983a). The latter authors enclosed feed samples inside fine-mesh nylon bags and, following *in vitro* peptic pre-digestion, inserted the bags into the small intestine of a pig prepared with a duodenal cannula. The bag and residual or undigested feed was recovered from the pig faeces within 48 hours. The MNBT showed promise in determining the apparent digestibilities of dietary protein (Sauer *et al.* 1983a), dry matter (DM) and energy (Sauer *et al.* 1983b).

This paper reports experiments designed to investigate both the accuracy of digestibility estimates obtained using the MNBT over a wide range of test diets and the influence on these values of the type of diet given to pigs receiving the nylon bags.

MATERIALS AND METHODS

The conventional or total faecal collection method of measuring digestibility involved entire male pigs between 40 and 70 kg liveweight housed in individual metabolism crates. Each test diet was offered for a period of 14 days and during the last 5 days the faecal output was collected daily from each pig and stored at 5°C. The total output from each pig was then weighed, mixed and sampled for DM analysis.

The procedures used for the MNBT were similar to those described by Sauer et al. (1983a). Rigid cannulae (23 mm i.d. x 60 mm length) were inserted into the duodenum of entire male pigs at approximately 40 kg liveweight. Following a period of at least two weeks recovery to normal appetite, the pigs were given from 1.5 to 2.0 kg/d (adjusted according to liveweight) of one of the two basal diets. The major ingredients of basal diet A were triticale and lupins and in basal diet B they were wheat and soyabean meal. The crude and acid detergent fibre contents of the diets were 5.9 and 4.6% and 6.6 and 4.0% respectively. The DM digestibilities of the diets were 81.0 and 86.7% for diets A and B respectively.

Small bags, 25 x 45 mm, of monofilament nylon with a 50 um mesh were made using a heat sealing technique. Test feeds were milled pass a 1 mm screen. Weighed samples of approximately 1 g of test feed were sealed into the bags which were identified by an indelible marker. Each bag was placed in 50 ml of a solution containing 0.01N HCl and pepsin (1 g/l) and agitated at 37°C for 2.5 h prior to its insertion.

The pigs were given their total daily feed allowance over a period of approximately 2 hr during which time the bags were inserted. A bag was inserted as feeding commenced and another bag inserted as the preceding bag moved along the intestines. Approximately 4 bags were inserted into each pig on each day. The bags were recovered in the faeces usually within 48 h

and were then frozen. The feed sample inside the bag was recovered after freeze-drying and was weighed and sampled for DM analysis.

The effects of the basal diet on nylon bag measurements were assessed in the first experiment. The DM digestibilities of seven diets were determined and compared with MNBT values for the same diets obtained with pigs fed on each of the two basal diets.

The accuracy of MNBT to predict digestibility was examined for 39 test diets which provided a wide range in protein, fat and fibre contents. For MNBT estimates a total of eight pigs were used. Pigs were fed basal diet B and over 500 bags of the test diets were inserted. The same test diets were fed to noncannulated pigs to provide a total of more than 200 diestibility measurements by total faecal collection.

RESULTS AND DISCUSSION

The average DM digestibilities of the seven diets in the first experiment were 83.8, 75.0 and 77.6% when determined using total faecal collection and the MNBT with basal diets A and B respectively. There were significant ($P < 0.001$) differences between all three values. Thus the MNBT underestimated DM digestibility. Furthermore, the values obtained by the MNBT were significantly affected by the basal diet given to the pigs receiving the bags. However, the average values determined by MNBT (NB%) were significantly ($P < 0.05$) related to the DM digestibility values determined by total faecal collection. The regression equations are:

Assay with diet A

$$\text{Digestibility (\%)} = 26.65 + 0.76\ (\pm\ 0.170)\ \text{NB\%} \qquad r = 0.89 \quad rsd = 2.05 \qquad (1)$$

Assay with diet B

$$\text{Digestibility (\%)} = 23.19 + 0.78\ (\pm\ 0.198)\ \text{NB\%} \qquad r = 0.87 \quad rsd = 2.26 \qquad (2)$$

The average MNBT values for all 39 diets were again less ($P < 0.001$) than DM digestibility values (81.8 and 76.9% for total faecal collection and MNBT, respectively). The variation associated with the MNBT was higher (CV 9.0%) than that of the digestibility measurement (CV 7.3%). These results

are in contrast to those of Sauer et al. (1983b) who found no difference between these techniques for the DM digestibilities of three barley samples (83.2 and 83.1% for MNBT and total collection, respectively).

The digestibility values of the 39 diets ranged from 62.8 to 91.5% and again there was a significant linear regression of the average MNBT values and the average digestibility values for all diets

Digestibility (%) = 21.62 + 0.78 (± 0.084) NB% (3)

r = 0.84 rsd = 3.31

These results show that the MNBT underestimates DM digestibility. In part the failure of the MNBT to achieve the correct digestibility of the test feeds appears to be due to factors associated with the dietary material accompanying the bags through the intestines. In the present experiments the test sample was less digested when it accompanied a diet that was higher in fibre and thus itself less digestible. However, this did not appear to influence the relative digestibilities of diets as shown by the similarities between the slopes of equations 1 and 2. Thus, despite the MNBT underestimating DM digestibility, the use of prediction equations such as equation 3 provides another means of predicting the digestibility and ultimately the digestible nutrient contents of pig diets. Neveretheless the conditions of the assay, and particularly the basal diet given to the test animals, must be carefully standardized. Further work is required to compare the MNBT to the more conventional techniques of predicting the available nutrient levels of pig feeds.

ACKNOWLEDGEMENTS

The financial assistance of the Australian Pig Industry Research Committee and the skilled technical assistance of Mrs. E. Speirs are both gratefully acknowledged.

REFERENCES

Sauer, W.C., Jorgensen, H. and Berzins, R. (1983)
Can. J. Anim. Sci. 63: 233-237.

Sauer, W.C., Ozimek, L. and den Hartog, L.A. (1983)
J. Anim. Sci. 59 Supplmt 1 : 271-272.

IN VITRO APPROXIMATION OF THE ENERGY DIGESTIBILITY OF PIG DIETS.

I. VERVAEKE, N. DIERICK, J. DECUYPERE and H. HENDERICKX.
University of Ghent, Faculty of Agricultural Sciences,
Laboratory of Nutrition and Hygiene,
Proefhoevestraat, 10, B-9230 Melle,
Belgium

SUMMARY

Comparing in vivo and in vitro energy digestibility data at the ileal and fecal level of two diets with different fiber content we can summarize our calculations as follows :

1. differences in digestibility between diets are more pronounced at the ileal than at the fecal level, and using in vitro techniques.
2. ileal apparent digestibility coefficients from in vivo experiments are underestimated true values.
3. conclusion 2 includes that the endogenous fraction at the ileal level is quantitatively very important.
4. the energy supply from the hindgut processes can be approximated from in vitro VFA production rates.

INTRODUCTION

Last years we note an increased tendency in research to evaluate pig diets by ileal apparent digestibility coefficients (AD) together with fecal ones. Reasons for these are the nutritional less efficient processes occurring in the hindgut compared to those in the small intestine.

In our laboratory we tried to elaborate in vitro techniques to imitate small and large intestinal digestion. The in vitro technique for ileal digestibility is based on the method developed by FURUYA (1979), using intestinal enzymes isolated from jejunal fluid. Quantification of the large intestinal digestion in vitro on the other hand imitating in vivo conditions is more difficult for reason of its microbial characteristics. Measuring production rates of VFA in terms of µmol/h/g O.M. however seems to be a relevant technique of approximation. Present experiments were set up to evaluate the in vitro results so obtained by comparison with in vivo results. The digestibility of the gross energy in the small and large intestine was choosen as a base of discussion.

MATERIALS AND METHODS

Two diets were prepared of which the AD in the small and large intestine was expected to be quite different. Diet I was a conventional one, containing barley (48.5 %), corn (20 %), soy bean meal (25 %) ; diet II contained manioc(43.5 %), dried sugar beet pulp (20 %), SCP (17.5 %) and meat and bone meal (10 %). Following experiments were set up using both diets.

Exp. 1. In vivo determination of ileal and fecal AD of the major nutrients and some fiber fractions, according to the experimental set up of DIERICK et al. (1983).

Exp. 2. In vitro determination of the small intestinal digestion using the FURUYA technique (1979).

Exp. 3. Production rates of VFA from cecal contents were determined as the parameter characterizing the fermentation in the whole large intestine. For that purpose a technique permitting rapid sampling of cecal contents was developed (VERVAEKE et al. 1985).

RESULTS AND DISCUSSION

Our results are briefly summarized in table 1.

Table 1. Comparison of the energy digestion (kJ/d) from in vivo and in vitro experiments.

	energy intake/day	energy digested ileal	energy digested in the hindgut	total
I. Calculations from in vivo experiments - kJ (%)				
diet I	38568	23865 (61.9)	8082 (20.9)	31947(82.8)
diet II	37944	19923 (52.5)	9915 (26.1)	29838(78.6)
II. Calculations from in vitro experiments - kJ (%)				
diet I	38551	29091 (75.5)	2797 (7.1)	31888(82.6)
diet II	35886	20082 (56.0)	5031 (14.0)	25113(70.0)

From these in vivo and in vitro evaluations of the two diets with different fiber composition we suggest following interpretations can be given

- studying the energy supply from protein, fat and carbohydrates

using in vivo and in vitro digestibilities, greater differences between the two diets are found at the ileal level than at the fecal level.

- the total energy digested (%) at the fecal level from the in vivo approximation (small intestinal + hindgut digestion) agrees very well with the apparent digestibility of OM. The supply of energy from the feed at the ileal level should actually be higher by reason of an important endogenous secretion (± 4000 kJ/day, according to the data of JUSTE (1982)).The energy supply from the hindgut fermentation of 8082 and 9915 kJ for both diets is actually an overestimation as the energy disappeared as CH_4 and CO_2 is incorporated in these figures.
- concerning the in vitro results a difference of 19.5 % in the supply of gross energy at the ileal level and of 12.6 % at the fecal level was calculated. The ileal values are higher than the in vivo values fact which most likely is caused by the abscence of the endogenous fraction. The hindgut data in vitro probably approximate better the net energy supply (from VFA) to the pig as CH_4 and CO_2 productions are not involved.
- our results suggest that the approximation of ileal digestibility data by in vitro techniques can facilitate the evaluation of pig diets permitting firstly rapid experimentation. Secondly more reproducible and more pronounced differences of nutritional characteristics are obtained.

REFERENCES

FURUYA, S., SAKAMOTO, K. and TAKAHASHI, S. 1979. B.J.N. 41,511.

DIERICK, N., DECUYPERE, J., LANNOYE, J., VERVAEKE, I. en HENDERICKX H. 1983. Landbouwtijdschr., 36, 6, 1683.

VERVAEKE, I., DIERICK, N., DECUYPERE, J., COSIJN, G. 1985. Archiv. Tierernähr. 35, 175.

JUSTE, C. 1982. Les colloques de l'INRA, 12, Paris, p. 155.

The financial support of the IWONL Brussels is gratefully acknowledged.

THE SENSITIVITY OF THE ILEAL DIGESTIBILITY METHOD AS COMPARED TO THE FAECAL DIGESTIBILITY METHOD

E.J. VAN WEERDEN, J. HUISMAN, P. VAN LEEUWEN, P. SLUMP
ILOB/IGMB, Institute for Animal Nutrition Research - TNO
P.O. Box 15, 6700 AA Wageningen, The Netherlands

SUMMARY

In three batches of soyabean meal (normally toasted, slightly undertoasted and slightly overtoasted, respectively) ileal and faecal digestibility of the most important parameters, including the amino acids, were determined. In the faecal digestibilities no adverse effects of the incorrect toasting procedures were observed. However, the effect of under- and overtoasting is clearly evident in the ileal digestibilities of organic matter, crude protein, lysine, S-amino acids, threonine and tryptophan. Ileal digestibility of the carbohydrates was in the incorrectly toasted batches reduced with 35-50%.

In three commercial batches of maize faecal digestibility was not different, but in one of the batches ileal digestibilities of protein and amino acids were clearly lower than in the other batches, especially for tryptophan.

The results indicate that ileal digestibility is a more sensitive criterion than faecal digestibility for detecting negative effects on protein quality of feedstuffs.

INTRODUCTION

It is generally accepted now that, in principle, the ileal digestibility of protein/amino acids is a better measure of the nutritional value of a protein source for pigs than the faecal digestibility. The amino acids released in the large intestine are not absorbed as such, but are converted to ammonia and/or amines and excreted via the urine (Zebrowska, 1973; Just et al., 1981). Whether the consequence of this finding should be such that in practical diet formulation ileal instead of faecal digestibility coefficients of amino acids should be used, is still a matter of debate. The main question to be answered in this respect is: are the differences between ileal and faecal digestibility coefficients so important that the valuation of the nutritional characteristics of the different feedstuffs changes and diet composition is effected? In order to answer this question,

in a long-term project sponsored by the combined mixed feed industry in The Netherlands, comparisons are made between ileal and faecal digestibilities of the individual amino acids in feedstuffs used in practical pig diet formulation. Different batches of soyabean meal and maize were examined in the present study.

MATERIALS AND METHODS

The experiments were carried out with barrows (Dutch Landrace x Yorkshire) of 40-60 kg live weight. Half of the barrows were provided with ileo-caecal re-entrant cannulas, the other half (littermates of the cannulated animals) remained intact. After a recovery period of about 3 weeks for the cannulated pigs, the animals were adapted to the experimental diets. After an adaptation period of 10 days ileal digesta were collected quantitatively in the cannulated animals during 3 x 24 h. In the normal pigs, faeces were collected during 5 x 24 h.

The maize was fed as a complete feed (95.4% maize + 4.6% minerals + vitamins); soyabean meal was given as part of a diet that contained 75.4% maize, 20% soyabean meal and 4.6% minerals + vitamins.

Three batches of soyabean meal were tested: a normally toasted product, a slightly undertoasted and a slightly overtoasted product (analysed criteria in Table 1).

Table 1. Analysed criteria in 3 batches of soyabean meal

toasted:	normal	under	over
Crude protein (%)	44.1	50.6	51.5
Crude fibre (%)	6.8	3.6	3.7
Protein Digestibility Index	26	27	9
Urease activity (mg N/g/min at 30°C)	0.09	0.11	0.01
Trypsin inhibitor (mg/g product)	< 5.0	4.9	2.0

The normally toasted product was a commercial 44% cp soyabean meal; both the over- and undertoasted products were from the same batch of soya flour.

The three batches of maize that were tested were bought via normal commercial channels. Batch 1 contained 9.0% cp and 2.3% crude fibre, batch 2, 9.4% cp and 2.8% crude fibre and batch 3, 9.3% cp and 2.4% crude fibre.

RESULTS AND DISCUSSION

The ileal and faecal digestibility coefficients in the three batches of soyabean meal of the most important parameters are shown in Table 2.

Table 2. Faecal and ileal digestibility of three soyabean meals

	faecal			ileal		
toasted:	normal	under	over	normal	under	over
Organic matter	88	95	100	62	54	57
Carbohydrates	93	100	100	43	22	28
Crude protein	90	93	95	83	78	77
Lysine	91	94	96	87	84	83
Methionine + Cystine	88	93	95	81	78	74
Threonine	88	92	94	83	78	76
Tryptophan	92	92	93	87	80	79

The faecal digestibility coefficients were slightly higher in both the under- and overtoasted product than in the normal soyabean meal. However, the effect of over- and undertoasting is evident in the ileal digestibilities, in which the coefficients for organic matter, crude protein as well as those for the most important amino acids are lower than in normal soyabean meal. The most striking adverse effect of the incorrect toasting procedure is observed in the ileal digestibility of carbohydrate.

The results presented confirm the findings of Vandergrift et al. (1983) and Rudolph et al. (1983) that the effects of inadequate toasting of soya can be detected more precisely by the determination of ileal rather than feacal digestibility. In the present study with only slightly under- and overtoasted soyabean meal the adverse effects were only visible when the ileal digestibility but not when the faecal digestibility coefficients were determined.

The results of the digestibility determinations in the three commercial batches of maize are shown in Table 3. With respect to the faecal digestibility coefficients, there were hardly any differences between the batches. The differences in ileal digestibilities between batches 1 and 2 were also small, but batch 3 showed a consistent lower ileal digestibility of protein and amino acids than both

other batches; especially for tryptophan, the difference is very marked. Because the origin of the batches of maize could not be traced back, the cause of the different results with batch 3 remains unknown, but these findings indicate again that ileal digestibility is a more sensitive criterion than faecal digestibility for detecting negative effects on protein quality of feedstuffs.

Table 3. Faecal and ileal digestibility of maize

	faecal			ileal		
	batch 1	batch 2	batch 3	batch 1	batch 2	batch 3
Organic matter	90	90	90	80	81	79
Carbohydrates	94	93	94	85	86	85
Crude protein	80	82	80	70	70	60
Lysine	63	65	66	57	59	45
Methionine + Cystine	83	84	83	76	79	62
Threonine	76	76	74	61	65	51
Tryptophan	70	74	72	48	54	24

REFERENCES

JUST, A., JØRGENSEN, H. & FERNANDEZ, J.A.,1981. Br.J.Nutr. 46: 209-219.

RUDOLPH, B.C., BOGGS, L.S., KNABE, D.A., TANKSLEY, Jr. T.D. & ANDERSON, S.A. 1983. J.Anim.Sci. 57(2): 373-386.

VANDERGRIFT, W.L., KNABE, D.A., TANKSLEY, Jr. T.D. & ANDERSON, S.A., 1983. J.Anim.Sci. 57(5): 1215-1224.

ZEBROWSKA, T., 1973. Roczn.Nauk.Rol. B95, 85

GENETIC VARIATION OF THE DIGESTIBILITY OF ENERGY IN GROWING PIGS

C. WENK, P. MOREL
Institute for Animal Production, Nutrition Group,
ETH-Zentrum, CH-8092 Zürich

INTRODUCTION

A constant digestion capacity of our pigs is usually accepted in our energy evaluation systems.

Out of a selection experiment over 7 generations for growth performance and backfat thickness in pigs the datas for this study were collected.

MATERIALS AND METHODS

Over the period of 7 generations pigs of the "Landrace" breed were selected for growth performance as well as for thin and thick backfat thickness (WAEFLER, 1982). Furthermore, the slow growing animals with the high backfat thickness (-line) consist of mainly halothane negative animals and the fast growing animals with small backfat thickness (+line) of about 60 % halothane positive animals.

In the experiment (7th and 8th generation) 128 growing pigs were housed in single pens. Growth performance was studied over the period in which the pigs, after 25 kg BW, ingested 210 kg feed. The animals were fed ad libitum or restricted with about 110 g per kg metabolic body weight ($BW^{3/4}$). The feed consisted of barley, maize, soybeanmeal and heringmeal, supplemented with minerals, vitamins and celite 545. It contained 820 g organic matter, 214 crude protein and 40 g crude fiber per kg dry matter.

In the 8th generation the following body weight gains after 25 kg BW and the ingestion of 210 kg feed per animals were achieved.

Table 1: Daily body weight gain of the pigs in the 8th generation

feeding level		ad libitum	restricted
+ line	♀	774	710
	♂	862	718
- line	♀	734	610
	♂	841	582

The digestibility of the energy was measured with the indicator method (HCl-unsoluble ash). For this purpose fresh faeces were collected over two following days at the body weights of about 30 to 70 kg. The following experimental design was obtained.

Table 2: Experimental design

Selection lines:	positive (+)		negative (-)
Sexes:	females	and	castrated males
Feeding level:	ad libitum		restricted
Body weight:	30 kg		70 kg

RESULTS AND DISCUSSION

In the first experiment (7th generation) all 128 individual animals were taken into account at 30 kg BW. In the second period at 70 kg BW only 3 mean samples could be analysed per treatment. The measurements of digestibility in the second experiment (8th generation) were done with 8 individuals per treatments at both weights. The experiments will be continued with the 9th generation of the two selection lines. In table 3 results of the 7th and 8th generation are presented as digestibilities of energy.

Table 3: Digestibility of energy as a function of selection line, sex, feeding level and body weight

Body weight (kg)	30		70	
Sex	♀	♂	♀	♂
7th generation				
+line				
restricted	0.802	0.809	0.788	0.793
ad libitum	0.792	0.805	0.799	0.797
-line				
restricted	0.795	0.800	0.793	0.806
ad libitum	0.782	0.779	0.789	0.786
8th generation				
+line				
restricted	0.791	0.795	0.817	0.810
ad libitum	0.799	0.795	0.807	0.808
-line				
restricted	0.784	0.789	0.812	0.810
ad libitum	0.787	0.788	0.801	0.801

The variation of the measured digestibility of energy was small in all experiments. Nevertheless significant differences between the lines, feeding level and body weight could be observed, as can be seen in table 4.

The +line showed a slightly but significantly higher digestibility of energy than the -line. Similar observations were found by WENK (1982) in other experiments with the same selection line. SUNDSTØL et al. (1979) found in their breeding experiments, that the fatter animals had a higher nutrient digestibility than the leaner pigs.

There was no significant difference in the digestibiliy of energy between castrates and females. But in the 7th generation and also as a tendency in the 8th generation the restricted fed animals digested the feed energy better than the ad libitum fed, although the difference in the mean feeding level was not big (MOREL and GERWIG, 1984).

While in the 7th generation no clear body weight effect could be observed, there was a highly significant increase of the digestibility of energy with a higher body weight in the 8th generation, an observation, which could be confirmed in many other experiments (WENK, 1981, FERNANDEZ et al. 1979, and others).

Table 4: Statistical interpretation of digestibility of energy

	selection lines	sex	feeding level	body weight
7th generation				
at 30 kg BW	***	ns	***	-
8th generation				
at 30 kg BW	**	ns	ns	-
at 70 kg BW	ns	ns	ns	-
all values	*	ns	($p < 0.07$)	***

*** significant at $p < 0.001$ ns not significant

** significant at $p < 0.01$ - not calculated

* significant at $p < 0.05$

REFERENCES

FERNANDEZ, J.A., JUST, H. & JORGENSEN, H. 1979. Proc. 30th Annual Meeting of the EAAP, Harrogate.

MOREL, P. & Gerwig, C. 1984. Vorläufige Beobachtungen eines laufenden Versuches am Versuchsgut der ETH, Chamau, über Appetit und Fleischansatzvermögen beim Schwein. Schweiz. landwirtschaftl. Monatshefte, 62: 343-348.

SUNDSTØL, F., STANDAL, H. & VANEN, O. 1979. Acta agric. Scand. 29: 337-345.

WAEFLER, P. 1982. Dissertation ETH Zürich Nr. 6979.

WENK, C. 1981. Zur Verwertung der Energie beim wachsenden monogastrischen landwirtschaftlichen Nutztier. Habilitationsschrift ETH Zürich

WENK, C. 1982. Genetisch bedingte Variation der Verdauung beim wachsenden Schwein. Seminar für Haustiergenetik Zürich, 11./12. März 1982

INDEX OF AUTHORS