

Interaction between carbohydrates and fat in pigs

- Impact on energy evaluation of feeds -**

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BIJLAGE
Bijlage 1
Bijlage 2

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**Interaction between carbohydrates and fat in pigs:
impact on energy evaluation of feeds**

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Stellingen

1. De relatieve verhouding in netto energiegehalte tussen grondstoffen is afhankelijk van de huisvesting van varkens tijdens de bepaling.
Dit proefschrift
2. De betrouwbaarheid en nauwkeurigheid van een merkstof voor de bepaling van verteerbaarheid dient voor ieder laboratorium apart te worden vastgesteld.
Dit proefschrift
3. Bij de bepaling van het netto energiegehalte van fermenteerbare koolhydraten in varkens dient te worden gecorrigeerd voor de vluchtige vetzuren die in de mest worden uitgescheiden.
Dit proefschrift
4. Fermenteerbare koolhydraten hebben tegenstrijdige effecten op de hoeveelheid energie benodigd voor onderhoud in varkens:
 1. een lagere behoefte aan energie voor onderhoud door minder fysieke activiteit (*Schrama et al., 1996. J. Anim. Sci. 74: 2220-2225*);
 2. een hogere energiebehoefte voor onderhoud van het maagdarmweefsel (*dit proefschrift*).
5. Met het oog op welzijn en gezondheid van varkens, kwaliteit van het product, het milieu en een efficiënt gebruik van voer is kennis over de hoeveelheid opgenomen nutriënten en hun onderlinge verhouding belangrijker dan de totale hoeveelheid opgenomen netto energie. Het wordt dus tijd dat er een systeem ontwikkeld wordt gebaseerd op nutriëntenstromen.
6. Een regressievergelijking is slechts geldig binnen de range waarin deze is gemeten. Bij toepassing als energieformule suggereert men ten onrechte algemene geldigheid.
7. In studies naar het effect van 'ruwvoer' op het gedrag van dragende zeugen dient ook de energiebalans te worden gemeten.
8. Het onder de post 'materiële kosten' boekhoudkundig verwerken van de salarissen van medewerkers met een tijdelijk contract maakt het voor managers gemakkelijker te vergeten dat het om mensen gaat.
9. Het continu dragen van een identiteitspasje door medewerkers mag niet worden bevorderd door het een noodzakelijk instrument te laten zijn om bij de koffieautomaat te komen.
10. Door het gebruik van de motorfiets voor het woon-werkverkeer te bevorderen, kan de overheid het fileprobleem aanzienlijk verminderen.

Stellingen behorend bij het proefschrift

Interaction between carbohydrates and fat in pigs; Impact on energy evaluation of feeds

Gertrud C.M. Bakker, 4 december 1996

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General introduction and outline of the thesis

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Introduction

In the Netherlands, pigs receive in general (99%) compound feeds, containing all the nutrients they require (LEI, 1991). Cereals used to be the major ingredients. However, their proportion was reduced from 40% in 1970 to 15% in the eighties. They have been substituted by tapioca, and e.g. maize gluten feed, imported from the USA (LEI, 1991). In addition, increasing amounts of (mostly fibrous) by-products are used in pig feeds, originating from the food-processing industry, like milling and distillery by-products and also some forages.

The use of ingredients other than cereals or tapioca in compound feeds affected the chemical composition of the pig diet: from feeds with a large amount of starch towards feeds containing less starch but more fibrous polysaccharides, which are often called non-starch polysaccharides (NSP). Starch and NSP differ in many aspects: in chemical structure; in the type of nutrients they supply and their effect on other nutrients in the digestion process; efficiency of utilisation for energy gain; and other, non-nutritional, aspects.

In feed evaluation systems, each of the digestible nutrients is valorized after its supply of digestible (DE), metabolizable (ME) or net energy (NE) (as reviewed by Henry et al., 1988). In general, when the variation in ingredients used is relatively low, the less complex systems based on DE or ME will be sufficient. Otherwise, the NE-system is recommended. In the Netherlands, the NE-valorization of the carbohydrates as crude fibre and nitrogen-free extractives was criticized (Borggreve et al., 1976), and therefore evaluation of carbohydrates has been changed into separate evaluation of starch, sugar and fermentable carbohydrates (calculated as a residu by subtracting crude protein, crude lipid, starch and sugar from the organic matter content) (CVB, 1993).

To check the calculated NE from data in feedstuff tables with the actual NE, Van der Honing et al. (1982, 1985a and b) performed a series of experiments. They measured the NE-content of diets containing cereals, by-products and fat in pigs in respiratory chambers and concluded that efficiency of utilization of cereal-based diets was similar to NSP-rich diets. However, these NSP-rich diets also contained more fat than the cereal-based diet. In an experiment with almost similar diets as Van der Honing et al. (1982, 1985a and b) used, Jongbloed et al. (1986) demonstrated that pigs performed worse when offered diets with a similar calculated net energy supply but composed of by-products plus supplementary fat, compared to pigs given diets based on cereals or by-products without supplemented fat. It was suggested that either the energy contribution by fat was overestimated or fat and NSP interacted in energy supply.

Therefore, it is necessary to re-evaluate the present feed evaluation systems for their accuracy of predicting the feeding value of a pig feed, when large

amounts of NSP are present.

Outline of the thesis

This thesis aimed to investigate the interaction between amount of fat and source of carbohydrate, by quantifying the effects of adding incremental amounts of fat to starch or to different sources of fermentable carbohydrates.

First the literature was reviewed for the effects of fermentable carbohydrates on digestion and utilization of nutrients (Chapter 1). Then, a series of experiments are described (Chapters 2 to 7). Twelve diets were tested in a 4 x 3 factorial design with four amounts of animal fat and three sources of carbohydrate. The amounts of animal fat added to the diets were : 0 (o), 35 (l), 70 (m) and 105 (h) g per kg. The three sources of carbohydrate were maize starch (M), purified cellulose (C) and toasted soya bean hulls (S). The cellulose was used as a source of inert bulking material (Slavin and Marlett, 1980; Fleming and Lee, 1983; Ward and Reichert, 1986) and the soya bean hulls as a source of easily fermentable by-products (Stanogias and Pearce, 1985). Their maximum intake by pigs was ascertained in a preceding feeding trial. It was found that 320 g cellulose or 280 g soya bean hulls could be added per kg feed without problems. The amount of digestible nutrients in the same batch of soya bean hulls and cellulose and thus their calculated net energy (NE_d) content, were measured in separate digestibility trials.

Each kilogram of the control diet (Mo) contained 510 g basal diet, supplying all necessary amino acids, minerals and vitamins, and 490 g maize starch. In the other 11 experimental diets, an amount of the maize starch in the control diet (Mo) was exchanged with the amounts of fat, cellulose and soya bean hulls, to supply the equivalent net energy (NE). Figure 1.1 shows the amounts (g) of the different diets yielding the same total NE. As a result, the 12 diets had different estimated NE_d per kg.

The pigs received equal amounts of net energy during the whole growing-finishing period from 35 to 105 kg. Thus they received different amounts of feed and different amounts of the energy-supplying nutrients starch, NSP and fat, but similar amounts of the basal diet. With these diets, both ileal and total tract digestibility of nutrients were measured and the energy gain during the entire growing-finishing period. To enable a proper evaluation of the data obtained, it was necessary to check whether the techniques used, fitted with the standard techniques. In this respect, a new cannulation technique is described (Chapter 2), which leaves the hindgut intact, and thus enables measuring both ileal and total tract digestibility within the same pig. The total tract digestibility in ileum cannulated pigs and the use of a marker was compared with the data in non-cannulated pigs. In addition, two markers (chromium oxide and acid insoluble ash) were tested for their suitability as markers in pigs in pens and in metabolism

crates (Chapter 3).

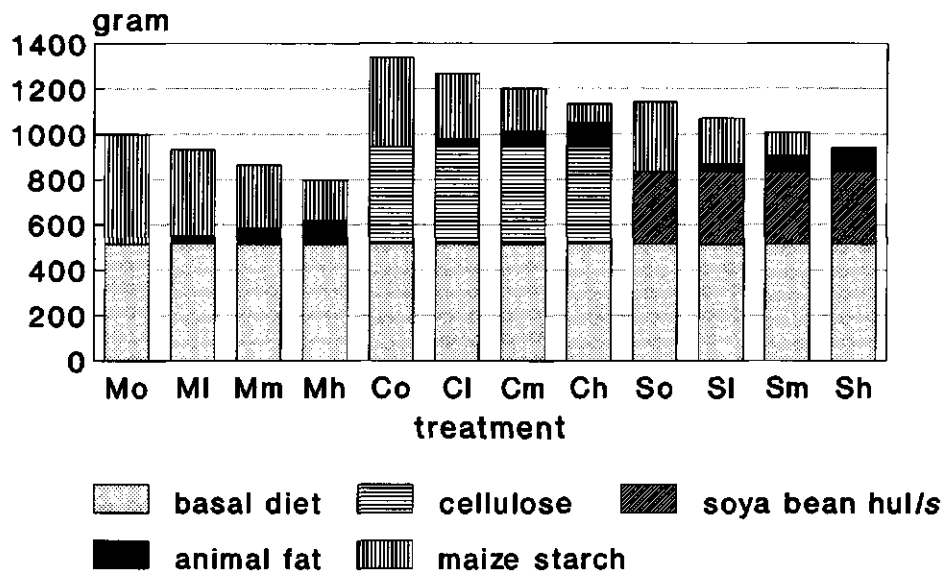


Figure 1.1
The amounts (g) of the 12 experimental diets yielding the same total assumed net energy

In group-housed pigs in pens, total tract digestibility of the 12 experimental diets was measured to check whether the calculated amounts of digestible nutrients (from the feedstuff table and preliminary experiments) corresponded with the actual supply of digestible nutrients (Chapter 4). Also, performance of the pigs was determined. In addition, with six of the twelve diets (only those diets with the zero (o) and the medium (m) fat addition levels) it was checked whether the assumption of additivity of digestible nutrients was valid at the terminal ileum and over the total tract (Chapter 5). To picture the fermentation process, with these same six diets the energy loss in methane, and the concentrations of volatile fatty acids at the terminal ileum and in faeces were determined (Chapter 6). With all the twelve experimental diets, the energy balance was measured by using the comparative slaughter technique (Chapter 7). The consequences for amounts of digestible nutrients and energy gain were discussed in the general discussion (Chapter 8).

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Chapter 1

Effect of different sources of non-starch polysaccharides on digestion of nutrients and efficiency of energy utilization in pigs

Effect of different sources of non-starch polysaccharides on digestion of nutrients and efficiency of energy utilization in pigs

Chemical composition of starch and non-starch polysaccharides

On average, pig diets contain 550-700 g carbohydrates per kg DM, of which 40-70 g per kg DM are low molecular weight sugars; 250-400 g starch per kg DM and 150-250 g non-starch polysaccharides (NSP) per kg DM (Bach Knudsen and Johansen, 1995). In the conventional Weende feed analysis, these carbohydrate components together with lignin represent the nitrogen-free extract and the crude fibre fractions (Figure 1.1). However, the analysed crude fibre fraction underestimates the NSP content with 70 to 370% (Schaller, 1978). Nowadays, in feed evaluation the carbohydrates are often identified as starch (& sugars) versus NSP.

Starch contains mainly two components: a minor component amylose, having an essentially linear structure, and a major component amylopectin, with a branched structure (Manners, 1979). Both components are built from glucose molecules. It is suggested that the ratio between these components determines the rate of degradation of the starch.

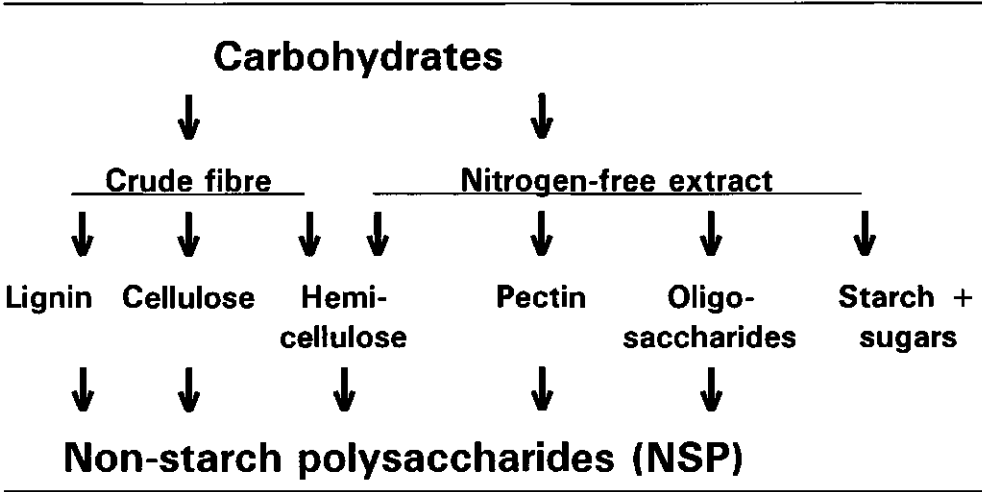


Figure 1.1
Classification of the carbohydrates

The NSP comprise a soluble fraction and a non-soluble fraction, which is analysed as neutral detergent fibre (NDF) at pH 7.0 (Van Soest, 1967; Figure 1). The soluble fraction contains pectin, some hemicellulose and oligosaccharides. The oligosaccharides contain one or more of the monomeric sugars (glucose, fructose, galactose). A well known oligosaccharide in barley and oats is β -glucan. It contains β -linked glucose molecules (1,3-D-glucose and 1,4-D-glucose). Pectin consists of a chain of α -1,4-linked D-galacturonic acids, partly esterified with methanol (Pilnik and Rombouts, 1979).

The non-soluble fraction comprises cellulose and most of the hemicellulose. The acid detergent fibre (ADF)-fraction in the NDF, corrected for lignin content (acid detergent lignin; ADL), corresponds with the cellulose content (Van Soest, 1967). Cellulose contains long chains of β -1,4-linked glucose molecules. The difference between NDF and ADF content, then, corresponds with the (insoluble) hemicellulose content. Although hemicellulose can be partly soluble, in general the insoluble fraction is considered to represent the whole hemicellulose fraction. Hemicelluloses rather are present as hetero polysaccharides consisting of at least two to four sugar residues (mainly pentoses like xylose and arabinose, but also linked with the hexoses galactose, mannose and glucose) in a branched structure (Dekker, 1979).

In human nutrition, a different classification is used. Dietary fibre is defined as the polysaccharides and lignin of plant material that resist hydrolysis by enzymes of the human digestive system (Trowell et al., 1976; Englyst et al., 1982). Van Soest (1985) concluded that the assumption in human nutrition that all fibres are the same is not correct. On the other hand, from a practical point of view, a single term for the fermentable carbohydrates is preferred over dealing with more terms. However, a single term is only allowed when the separate structures of NSP don't differ in the digestion and absorption process in the alimentary tract. In this respect, NDF is found to be not representative for the whole of NSP: it underestimates the amount of fermentable carbohydrates in most feedstuffs (Carré and Brillouet, 1986), and it gives inaccurate data on digestibility of NSP (Chabeauti et al., 1991; Vervaeke et al., 1991).

For a more detailed description of chemical structure and analytical procedures for determining fibre see Goering and van Soest (1970) and Theander and Åman (1979). Also, other techniques for determining cell wall structures are being developed, as reviewed by Casu (1985) and Boon (1989). However, their relevance for feed evaluation is not clear, yet.

In conclusion, on one hand the fermentable carbohydrates (NSP), calculated as organic matter minus starch, sugar, crude protein and crude fat (Åman and Hesselman, 1984; cited by Theander et al., 1989), might be included in feed evaluation as a single term. On the other hand, it might be crucial for an accurate feed evaluation to discriminate between, for example, soluble carbohydrates (NSP minus NDF), hemicellulose and cellulose. This will be discussed in the next sections.

Degradation of starch and NSP

Digestion of starch

Starch is mainly digested into oligosaccharides by pancreatic α -amylase in the intestinal lumen (Gray, 1992). Furthermore, enzymes in the intestinal surface membrane degrade these oligosaccharides into glucose (Manners, 1979). The extent of starch breakdown depends on a number of factors, such as: the chemical structure of the starch, its physical form, and the method of feed processing (Dreher et al., 1984; Williams and Chesson, 1989). In general, however, most of the starch in pig feeds has been disappeared at the terminal ileum (Keys and DeBarthe, 1974, Vervaeke et al., 1989; Table 1.1).

Fermentation of NSP

Monogastrics don't produce enzymes that degrade NSP. Therefore, this is performed by the microflora, mainly present in caecum and colon of monogastrics. The gastrointestinal microflora synthesize mainly volatile fatty acids (VFA) of which acetic acid, propionic acid and butyric acid are the most important ones (Bergman, 1990). They are absorbed through the gut wall and serve as a nutrient source for the pig. The variation in degradability of the NSP, however, is very large. Bach Knudsen et al. (1993) concluded that the order of breakdown of the various NSP constituents over the entire gastrointestinal tract was: β -glucans >> arabinoxylans > cellulose. However, for each of these constituents differences are found between sources. The degree of cell wall lignification is considered to play an important role for the breakdown of NSP (Bach Knudsen et al., 1993). In addition, particle size and retention time in the gut are also important aspects (Ehle et al., 1982; Drochner, 1984).

For a good fermentation of NSP in the large intestine an adequate supply of nitrogen is required to colonic bacteria (Bergner, 1982). In general, adequate levels of nitrogen are provided by residual feed protein escaping prececal digestion, endogenous nitrogen in mucus and epithelial cells and urea recycled into the gastrointestinal tract (Chesson, 1990).

Apparently, fermentation not only occurs in the hindgut; at the terminal ileum on average 26% (from -9 to 62%) of the NSP has disappeared (Table 1). For instance, on average 44 % of NSP in beet pulp is degraded at the terminal ileum, whereas it is less than 3% in wheat bran (Graham et al., 1986). It is concluded that this difference is related to the proportion of soluble NSP in the NSP. This corresponds with the ileal degradability of the insoluble cellulose being close to zero (Drochner, 1984; Partridge et al., 1986).

Volatile fatty acids (VFA)

The rate of fermentation is often related to the concentration of VFA in (parts of) the digestive tract and/or in faeces. The smallest concentration is found in the stomach and small intestine (up to 20 mmol/L); in the caecum and colon a

Table 1.1

Intake (g/d) and digestibility (% of intake) of starch and non-starch polysaccharides in the small intestine of pigs (Cited from Bach Knudsen and Johansen, 1995; for the references the reader is referred to their study)

Diet	Starch		NSP		Reference
	Intake	Digestibility	Intake	Digestibility	
Wheat-barley-oats	556	96.0	182	20	(1)
+ wheat bran	310	95.7	351	11	(1)
+ sugar beet fibre	193	95.3	633	37	(1)
Barley-soyabean meal	816	95.5	296	35	(2)
+ β -glucanase	816	96.7	296	41	(2)
Cereal-peas-soyabean meal	556	96.0	181	20	(3)
+ peas-early	348	92.9	338	28	(3)
+ peas-late	348	92.9	338	28	(3)
Barley-soyabean meal	492	83.7	181	28	(4)
Barley-soyabean meal extruded	457	96.9	184	25	(4)
Barley-soyabean meal	588	90.3	168	57	(5)
+ pelleting	588	94.9	168	55	(5)
+ enzymes	588	92.7	168	58	(5)
+ pelleting and enzymes	588	95.8	168	62	(5)
Barley-soyabean meal	601	94.8	182	50	(6)
+ baked HMLT	564	98.6	196	62	(6)
+ baked LMHT	612	96.9	184	60	(6)
Wheat flour	978	99.4	45	3	(7)
+ aleurone	1120	99.3	83	-4	(7)
+ pericarp/testa	999	99.5	86	-4	(7)
+ wheat bran	1003	98.7	86	10	(7)
Wheat flour	944	97.8	49	18	(7)
+ oat bran	1086	98.6	77	36	(7)
Rolled oats	891	96.8	119	36	(7)
+ oat bran	1000	96.6	162	34	(7)
Oat groats	885	97.0	123	21	(8)
Oat flour	878	98.6	81	25	(8)
Oat flour coarse	860	98.5	141	24	(8)
Oat bran	814	98.9	202	15	(8)
Wheat flour	911	100.0	44	30	(9)
+ β -glucan conc.	904	99.0	97	27	(9)
+ insoluble residues	1086	98.0	74	27	(9)
+ oat bran	1021	100.0	88	28	(9)
Barley-soyabean meal	642	92.5	206	21	(10)
+ peas-dark	600	91.7	206	29	(10)
+ peas-light	618	90.7	209	30	(10)
Barley-wheat starch	1573	100.0	118	9	(11)
+ pea fibre and pectin	1127	95.0	663	1	(11)
Peas-dried	402	92.9	169	-9	(12)
Peas-toasted	399	94.2	154	15	(12)
Peas-dried	457	88.9	225	40	(12)
Peas-toasted	443	85.7	197	24	(12)
Cereal-soyabean meal	248	98.8	120	8	(13)
Cereal-peas-soybean meal-rapeseed cake	216	95.5	125	1	(13)

HMLT, high moisture baked at low temperature; LMHT, lower moisture baked at high temperature.

Data from: (1) Graham et al. (1986b); (2) Graham et al. (1986a); (3) Graham and Åman (1986b); (4) Fadel et al. (1988); (5) Graham et al. (1989); (6) Fadel et al. (1989); (7) Bach Knudsen and Hansen (1991); (8) Bach Knudsen et al. (1993a); (9) Bach Knudsen et al. (1993b); (10) Abrahamsson et al. (1993); (11) Jørgensen et al. (1995); (12) Canibe and Bach Knudsen (1995a); (13) Gdala et al. (1995).

higher concentration of 170 to 230 mmol/L is found (Clemens et al., 1975). Within the hindgut, the concentration of VFA is highest in the caecum and gradually decreases along the colon (Bolduan et al., 1991; Vervaeke et al., 1989). The concentration increases with the amount of fermentable NSP in the diet (Kass et al., 1980; Gargallo and Zimmerman, 1981). It should be noted that measuring concentration of VFA in the gut can be used as a qualitative parameter for ranking fermentable NSP sources. However, it cannot be used as a quantitative parameter for determining the total amount of VFA produced, because both the production and the absorption of VFA depend on many factors. The concentration of VFA can be used, however, to determine quantitatively the difference between the amounts of VFA produced and absorbed. For this, the concentration needs to be multiplied by the digesta flow. To know the total amount of VFA produced, the amount of absorbed VFA should be known. The rate of absorption depends on the place in the gastrointestinal tract, and the type of VFA (Von Engelhardt et al., 1989). In addition, absorption of VFA is a passive process. It increases linearly with concentration (Von Engelhardt et al., 1989), and thus a higher amount of digesta might reduce the absorption rate because of its diluting effect. Measuring the flow of VFA in the portal vein underestimates the total amount of VFA absorbed, because some of the VFA are used as a energy source by the gut tissue (Latymer et al., 1991).

Not only the concentration, but also the relative ratios in which the separate VFA (mainly acetic acid, propionic acid and butyric acid) are found, are considered to change with the source of NSP (Ehle et al., 1982; Imoto and Namioka, 1983; Zebrowska, 1988; Schnabel et al., 1990). In general, highly fermentable carbohydrates tend to result in a high propionate to acetate ratio (Bergman, 1990). Conversely, cellulose-rich diets increase the proportion of acetate. In addition, β -glucans might enhance the proportion of butyric acid (Bach Knudsen et al., 1993).

For reviews on microbial biology of the large intestine of pigs the reader is referred to Cranwell, 1968; Ratcliffe, 1985; Varel, 1987; Allison, 1989; and Fonty and Gouet, 1989.

Effect of NSP on the digestion of other nutrients

General

For a maximum benefit of the nutrients supplied by starch and sugar, protein and fat, they need to be absorbed before the terminal ileum. However, there are indications that NSP have a negative effect on the precaecal digestion and absorption process of these nutrients. To illustrate the effects of NSP on both ileal and total tract digestible nutrients, Drochner (1984) measured these in diets with an identical basal diet but with different sources of added NSP (Table 1.2). From this study it can be concluded that addition of NSP reduced the apparent digestibility of protein and fat both at the terminal ileum and over the total tract.

Table 1.2

Effect of source of NSP on ileal and total tract apparent digestibility of nutrients (%) (data from Drochner, 1984; based on 20 kg minipigs)

	control diet A	A + 5% wood fibre	A + 5% purified cellulose	A + 5% pectin
intake (g/d)				
starch	183.1	190.5	190.1	189.7
crude protein	115.2	113.1	114.4	112.5
crude fat	25.6	24.6	24.4	24.6
soluble NSP ¹	91.5	74.2	73.4	105.8
hemicellulose	49.3	50.0	45.7	31.5
cellulose	10.7	24.1	31.6	10.6
lignin	5.1	10.8	5.9	5.3
ileal digestibility (%) ²				
starch	95.6	94.3 ^{NS}	94.8 ^{NS}	93.0 ^{NS}
crude protein	72.8	65.1 ^{***}	68.3 ^{**}	65.8 ^{***}
crude fat	73.2	47.3 ^{***}	67.8 [*]	68.2 [*]
soluble NSP ¹	63.1	49.7	54.8	21.2
hemicellulose	27.3	12.2 ^{***}	12.0 ^{***}	42.5 ^{***}
cellulose	-3.7	1.7 ^{NS}	5.7 ^{NS}	-9.4 ^{NS}
total tract digestibility (%) ²				
starch	100.0	100.0	100.0	100.0
crude protein	83.8	77.8 ^{***}	80.0 ^{***}	79.6 ^{***}
crude fat	63.8	30.8 ^{***}	51.4 ^{**}	67.0 ^{**}
soluble NSP ¹	88.7	79.8	85.3	81.9
hemicellulose	61.1	51.5 ^{NS}	58.4 ^{NS}	69.9 [*]
cellulose	22.2	8.3 ^{**}	33.6 [*]	39.3 ^{***}

¹ calculated as: nitrogen free extract + crude fibre - starch - NDF; Statistical analysis could not be performed, because individual data are not published.

² Statistical analysis was performed as pairwise differences of each treatment with the control diet. ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$; NS: $P > 0.05$.

These effects of NSP are related to reduced absorption, increased secretion or synthesis of fat, protein and minerals, and the retention time of the digesta in the gastrointestinal tract.

The absorption of glucose was reduced to 50% when guar gum was added to a glucose solution that was perfused through isolated loops of pig jejunum (Rainbird et al., 1984). Guar gum is a soluble NSP and increases the viscosity of the digesta. Therefore, the reduced absorption of glucose is thought to be associated with reduced diffusion from the intestinal lumen to the epithelial cells

or inhibition of the absorption process. Moreover, this effect might not only affect glucose absorption, but also reduce absorption of amino acids and fat. Indeed, it is shown that inclusion of a high amount of soluble NSP is accompanied with a reduced apparent fat and protein digestibility at the terminal ileum (Graham et al., 1986; Bach Knudsen et al., 1993).

Low (1989) concluded that increasing ileocaecal flow of water, electrolytes, secretory products from liver, pancreas and mucosa cells was positively correlated with increasing fibre levels in the diet. This negatively affects apparent digestibility of minerals, protein and fat.

It is found that NSP shorten the time the digesta retains in the gastrointestinal tract (Kass et al., 1980; Ehle et al., 1982). It is often suggested that this allows the digestive enzymes less time for degradation. However, passage time is only reduced in the hindgut; the precaecal retention time is either not affected or even prolonged (Drochner, 1984; Den Hartog et al., 1985; Latymer et al., 1990). Therefore, the effect on transit time is caused by the hindgut. The total transit time is shorter with increasing amounts of digesta that flows into the caecum (Kesting et al., 1991).

Starch and sugars

In general, no effects of NSP are found on starch digestibility at the terminal ileum (Tables 1.1 and 1.2). However, NSP are found to have a negative effect on the activities of the disaccharases sucrase, maltase and lactase in the rat (Khokhar, 1994). Apparently, in the relatively long small intestine this effect is corrected for, prior to the terminal ileum. Another option is that part of the starch is degraded by fermentation. However, until now it has not been possible to distinguish between fermentation of NSP and starch in the small intestine.

Moreover, there are indications that diets with lower concentrations of fermentable carbohydrates result in increased concentrations of lactic acid, produced by the increased population of Lactobacilli in stomach, first and second half of the small intestine (Koch et al., 1972). This seems to be supported by the (although not significant) larger amounts of lactic acid appearing in the portal vein with a diet containing 60% corn starch compared to diets in which part of the maize starch was exchanged for cellulose, alfalfa meal or lactose (Giusi-Perier et al., 1989). It remains uncertain, however, whether the higher net energy supply with the low-NSP diets in these experiments induced the lactic acid production.

Protein

Apparent protein digestibility is significantly reduced both at the terminal ileum and over the total tract when fermentable carbohydrates are present (Dierick et al., 1989; Shi and Noblet, 1993; Table 1.2). These effects might be caused by:

1. an increased endogenous secretion of nitrogen or an increased amount of

Table 1.3

Effect of source of NSP on ileal and total tract flow of N (g/d) (data from Drochner, 1984; based on 20 kg minipigs)

	control diet A	A + 5% wood fibre	A + 5% purified cellulose	A + 5% pectin
Intake	29.3	30.5	30.4	30.4
Excreted into caecum	8.0	10.7	9.6	10.2
NPN ¹	2.5	2.5	3.5	4.8
Real protein-N	5.5	8.2	6.1	5.4
N in fibre	0.73	0.68	1.69	2.59
N in bacteria	1.86	4.25	2.87	3.45
N in glycoproteins	2.18	2.84	2.4	ND ²
Excreted in faeces	4.7	6.8	6.0	6.2
NPN	0.4	2.2	0.4	0.3
Real protein-N	4.3	4.6	5.6	5.9
N in fibre	0.45	0.48	0.60	0.37
N in bacteria	2.42	2.96	3.71	3.98
N in glycoproteins	1.27	0.85	1.06	1.99

¹NPN: non-protein nitrogen, for example urea, ammonia, free amino acids, etc

²ND: not determined

sloughed cells from the gut wall (as glycoprotein; Table 1.3)

The amount of endogenous secreted nitrogen is linearly related to the amount of consumed NDF (Furuya and Kaji, 1992; Schulze, 1994) or DM intake (Butts et al., 1993). The amount of endogenous secreted protein reported in literature ranges between 16 to 39 g/kg feed (de Lange et al., 1989; de Lange et al., 1990; Boisen and Fernández, 1995).

2. a reduced digestion of dietary and/or endogenous crude protein

Roberfroid (1993) concluded that the effect of NSP on protein absorption depends on the type of fibre: soluble NSP (like pectins) are expected to have a larger negative effect than insoluble NSP (NDF). The net result of the processes 1. and 2. is usually expressed as a linear relationship with NDF intake (Schulze, 1994) or ADF intake (Shi and Noblet, 1993).

3. presence of active microflora

Differences in bacterial protein are not expected to be a crucial factor for the differences in ileal digestibility between the sources of carbohydrates (Sauer et al., 1991). However, this might depend on the type of NSP (Table 1.3). In the hindgut, apparent synthesis of protein is reported in the range of 49 to 62

g bacterial protein per kg fermented NSP (Gargallo and Zimmerman, 1981; Kirchgeßner et al., 1989; Mosenthin et al., 1992; Bakker et al., 1995). In addition, N from the blood (as urea) might be secreted into the gastrointestinal lumen (Mosenthin et al., 1992; Bakker et al., 1995). This will decrease the apparent total tract protein digestibility.

4. the presence of N in the NDF matrix, unavailable when NDF is not fermented (Schulze, 1994; N in fibre in Table 1.3)
5. the presence of trypsin inhibitors, for example in soya products, which have a negative effect on protein digestibility (Huisman, 1990). However, adequate toasting the ingredient eliminates this negative effect (Van der Poel, 1990).

Fat

Similarly as with protein, apparent fat digestibility is significantly reduced both at the terminal ileum and over the total tract when fermentable carbohydrates are present (Dierick et al., 1989; Shi and Noblet, 1993; Table 1.2). Furda (1990) suggested two possible mechanisms of NSP that especially affect apparent fat digestion. (1) A reduced dietary fat digestion, as a direct effect, by affecting the bile acids and other micellar components. (2) An increased secretion of fat in the faeces, as an indirect effect, by increasing the excretion of bile acids. Furda (1990) concluded that there are large differences between the fibres in the magnitude in which they affect fat absorption. Therefore, more information is needed on the specific source of fibre and its specific effects. Indeed, increasing amounts of oat hulls and wheat bran significantly reduced total tract fat digestibility, whereas increasing amounts of ground barley straw had no effect (Just et al., 1980). Moreover, Mason and Just (1976) found a reduced fat digestibility both over the total tract and at the terminal ileum, when they included raw potato starch in the diet. They speculated that this effect was caused by a reduced true digestibility of the dietary fat and/or by increased endogenous secretion by the pig and bacterial synthesis of fatty acids. In addition, supply of fibre is usually accompanied with an increased DM intake. A loss of 4.7 g fat, as endogenous fat, is expected per kg DM intake (Jørgensen et al., 1993). More quantitative results of fermentable NSP on apparent fat digestibility, however, are hardly found, probably because of the large number of mechanisms involved.

On the other hand, fat might affect the process of fermentation. If it escapes the precaecal digestion and enters the large intestine, it reduces the number of fermentation bacteria (Mallett and Rowland, 1983). This was confirmed by a reduced methane production in pigs which received fat in their diets (Christensen and Thorbeck, 1987).

Minerals

Lower absorption of minerals might occur in the presence of NSP (Partridge, 1978; Drochner, 1984; Ward and Reichert, 1986; Jongbloed, 1987). Whether

inclusion of NSP cause mineral deficiencies depends on the type and the amount of NSP and the dietary supply of the minerals. Very little is known on the quantitative aspects (Kornegay and Moore, 1986). Especially in geographical areas where pollution problems induce lower mineral concentrations in pig feeds, it should be known whether mineral requirements are still met when (large amounts of) fermentable NSP are supplied.

Efficiency of utilization of digestible energy from starch and non-starch polysaccharides

Theory

The proportion of metabolizable energy (ME) in digestible energy (DE) is smaller when the DE is supplied by fermentable carbohydrates compared with supplying starch, because during fermentation energy will be lost as methane (Figure 1.2). In addition, loss of energy occurs as fermentation heat.

33 hexose	+ 66 H ₂ O	→ 66 HAc	+ 66 CO ₂	+ 132 H ₂	
12 hexose	+ 24 H ₂	→ 24 HProp	+ 24 H ₂ O		
10 hexose		→ 10 HBut	+ 20 CO ₂	+ 20 H ₂	
128 H ₂	+ 32 CO ₂	→ 32 CH ₄	+ 64 H ₂ O		

55 hexose	→ 66 HAc	+ 24 HProp	+ 10 HBut	+ 32 CH ₄	+ 54 CO ₂	+ 22 H ₂ O
(MJ):154.5	57.9	36.8	21.8	28.1		

Figure 1.2

The supply of energy in volatile fatty acids, methane and heat by fermentation of carbohydrates, according to the stoichiometry of Hungate (1966)

According to stoichiometry (Hungate, 1966), the total amount of degraded NSP and the relative ratios of VFA can predict energy losses in methane and heat (Figure 1.2). For calculating the equations in Figure 1.2, it was assumed that the VFA acetic acid (HAc), propionic acid (HProp) and butyric acid (HBut) were synthesized in the molar proportions of 66:24:10 (Gädeken et al., 1989; Müller et al., 1989). From the equation, it can be concluded that theoretically 6.4% of the available energy (MJ) is lost as heat and 18% is lost as methane (CH₄). Overall, theoretically, a loss of energy of 20 to 25% of the DE can be expected when hexoses are fermented, compared to enzymatically digestion.

Furthermore, when ME from VFA is used for maintenance or growth,

efficiency of utilization of energy is on average 15% lower than ME from glucose (Vermorel, 1968; cited by R  rat, 1978). Calculated from the amount of ATP per mol fermentable carbohydrate, the total (net) energy supply from fermented carbohydrates is 63% compared to enzymatically digested starch (Figure 1.2).

In practice, however, energy lost as heat will be higher due to losses in other metabolic processes in bacteria. Conversely, energy lost as methane is found to be not as high as theoretically calculated (Zhu et al., 1993). Moreover, M  ller and Kirchge  sner (1983 and 1989) measured energy losses in methane ranging from 1 to 10% of the digestible energy (DE). It is emphasized, that this stoichiometry is based on hexose; when pentoses are included, which are present in hemicellulose, the energy loss might be larger. Therefore, it is concluded that Hungate's stoichiometry is not precise enough to substitute in vivo measurements in pigs (Longland et al., 1988; Zhu et al., 1993).

Energetic efficiency of utilization of energy as measured in vivo

In vivo, large variations in utilization of energy from degradable NSP (dNSP) and/or VFA are found (Table 1.4); for dNSP, the utilization ranged from 0 to 79%. However, these experiments showed large differences in diets and substrates, techniques used, feeding level, physiological status of the pigs, adaptation periods, etc.

The utilization of energy (k, in %) of pure volatile fatty acids (nr. 1, 2, 3, 4, 15; Table 1.4) does not include the loss of energy in fermentation heat, and might, therefore, overestimate the k of ME from NSP. Moreover, infusion of substrate in the hindgut (nr. 1, 10, 15; Table 1.4) does not include the negative effect of NSP on digestion and absorption of other nutrients before the terminal ileum, which might overestimate the net energy content of a diet. This is confirmed by giving the same substrates in the feed as infused in the hindgut (nr. 9 versus nr. 10; Table 1.4). In some studies, k is determined by relating the variation in proportion of energy that disappeared in the hindgut to energy gain (nr. 7, 8, 11; Table 1.4). However, this method ignores the NSP that is disappeared before the terminal ileum. In addition, the technique used to measure hindgut digestibility might affect the maintenance requirement of the pigs and thus affect the amount of retained energy (Herrmann et al., 1989; Longland et al., 1989). The factor k might overestimate the value for growing pigs when it is measured with NSP given to adult sows, fed close to maintenance (nr. 9, 10, 12, 13, 14, 15, 18; Table 1.4). These sows use the fermentation heat for maintaining their body temperature, while this heat is not of any use for thermoneutral housed pigs receiving a feeding level of more than 2 x maintenance. A sufficient length of the period for adaptation of at least four weeks (Walter et al., 1986) to the diet is important, especially with dNSP-rich diets. In addition, adult pigs are found to have a larger capacity for fermentation than younger pigs (Shi and Noblet, 1993). Therefore, the zero energy gain from

Table 1.4

Efficiency of utilization of energy from starch, degradable non-starch polysaccharides (dNSP) or the separate volatile fatty acids (VFA), acetic acid (HAc), propionic acid (HProp) or butyric acid (HBut) for growth and/or maintenance in pigs

no	starch	NSP	VFA			Method	Authors
			HAc	HProp	HBut		
1.			65	71	67	Infusion of VFA-containing solutions in the hindgut of growing pigs; feeding level 2.5 x M	Gädeken et al. (1985)
2.			60				Jentsch et al. (1968)
3.			56 - 59				Imoto and Namioka (1983)
4.			79	75			Roth et al. (1988)
5.	76						Schiemann et al. (1961)
6.						Calculated as $\Delta RE/\Delta ME$ in growing pigs, receiving diets containing: . raw versus steamed potatoes, 40% . raw versus steamed potatoes, 50% . raw versus steamed sugar beets, 40% Obtained from an equation relating energy gain to the proportion of hindgut fermentation Equation obtained with 24 feeds in 90-180 kg pigs Obtained from an equation relating NE/ME (%) to the proportion of hindgut fermentation in growing pigs. Equation obtained by exchange of casein + maize starch for soya bean meal, potato starch and cellulose Substrates given in feeds to adult sows . Cellulose	Herrmann et al. (1989)
7.		63 79 61 56					Jentsch et al. (1988)
8.		25					Just et al. (1983)
9.		41					Kirchgeßner and Müller (1991)

49	. Pectin			
64	. Xylose			
66	. Lactose			
65	. Starch			
10.	Substrates infused in hindgut of adult sows			Müller et al. (1989)
64	. Cellulose			
62	. Pectin			
70	. Xylose			
73	. Lactose			
69	. Starch			
59	Calculated from IRA versus intact growing pigs (ΔRE/ΔME) with diets containing beet pulp			Longland et al. (1989)
12.	Exchange of cereals (w/w) to increasing amounts of beet pulp, in feeds for growing pigs			Longland et al. (1991)
77	. sows			
79	Cellulose in 190 kg sows, fed at 1.2 to 1.4 * M.			Müller and Kirchgeßner, 1983a
68	Straw in 200 kg sows.			Müller and Kirchgeßner, 1983b
14.	Infusion of a mixture of HAC (75%) and HProp (25%) in hindgut of 180 kg sow			Müller and Kirchgeßner (1991)
70	25 feeds in 90-180 kg pigs; calculated as NE/DE			Schiemann et al. (1989)
16. 74	. soluble NSP	63		
	. insoluble NSP	59		
17. 82	61 feeds in 35-45 kg pigs; calculated as NE/DE	69		Noblet et al. (1994)
	10 d. adaptation period to the diet			
	. soluble NSP	77		
	. hemicellulose	66		
	. cellulose (ADF)	0		
18. 82	14 feeds in adult sows; calculated as NE/ME	56		Noblet et al. (1993)
	17 d. adaptation period to the diet			

cellulose (ADF; nr. 17; Table 1.4) might be due to both the relatively short adaptation period and the young age of the pigs.

In general, it can be concluded from Table 1.4 (nr. 16 and 17) that soluble dNSP (calculated as dNSP minus dNDF) tend to result in a higher k than the insoluble dNSP. Furthermore, measuring efficiency of utilization of energy for feed evaluation purposes should be performed with the 'target'-animals, with the appropriate feeding level, housing conditions, and a sufficient period of adaptation, preferably the entire period of production (meat, reproduction, lactation).

Adaptation

The period of adaptation to NSP-rich diets is of great importance for the interpretation of the results on supply of digestible energy and net energy from NSP to the pig. The period in which the gastrointestinal processes are completely adapted to the new diet is usually longer than with low fibrous diets (usually an adaptation period of seven to ten days). There are mainly two processes involved in this adaptation: the adaptation of the microflora in the gastrointestinal tract to the new substrate(s) (Edwards, 1993) and the adaptation of the gastrointestinal tract itself to the new absorbable products (Johnson, 1988).

In pigs receiving cellulose-rich diets, Giusi-Perier et al. (1989) observed significantly more VFA absorbed in the portal vein after 28 days compared to 21 days. Similarly, Gargallo and Zimmerman (1981) found that degradation of a high level of purified cellulose in a diet increased with time, with an optimum after 29 to 40d of adaptation. Adaptation seems to be related to a higher cellulase activity of the microflora (Varel, 1987). In addition, Anugwa et al. (1989) found more cellulolytic bacteria in the pig colon contents after feeding a diet containing 40% alfalfa for 34 d., but not after 17 d.

It has been reported several times that caecum and large intestine increase in length and/or weight (Kass et al., 1980; Stanogias and Pearce, 1985). Levrat et al. (1991) and Key and Mathers (1993) found in rats increasing weights of the empty caecum up to 21 days by feeding 30% soybean fibre (15% cellulose, 58% hemicellulose, 27% others) or up to 45% haricot beans (16.5% NSP, of which 92% non-cellulosic NSP), respectively. However, Anugwa et al. (1989) found no hypertrophic effect of alfalfa on the hindgut. The reason for a lack of hypertrophic effect of NSP in their study may be due to the larger contents of the mainly cellulose-containing alfalfa diets in pigs. These diets are more difficult to ferment than the easily degradable NSP in the rat studies. This hypothesis suggests that the amount of VFA plays an important role in the hypertrophy of the gastrointestinal tract, which is confirmed by infusion of separate VFA into the gastrointestinal tract (Sakata, 1987): VFA stimulated cell proliferation of

jejunum, caecum and colon. Effects of VFA were dose-dependent, and varied among acids (butyrate > propionate > acetate). It is suggested that hormonal mechanisms are behind this hypertrophy (Roberfroid, 1993). These heavier visceral organs may increase maintenance requirements for energy (Koong et al., 1983; Pekas, 1991) and thus reduce potential energy gain.

In conclusion, a too short adaptation period might underestimate the potential digestible energy supply by NSP and it might overestimate the potential energy gain of the pigs.

Non-nutritional aspects of non-starch polysaccharides

Apparently, including NSP-rich ingredients in pig diets has many disadvantages. It reduces apparent ileal and total tract digestibility of protein, fat, minerals and energy (Fernández and Jørgensen, 1986; Dierick et al., 1989). In addition, it will increase the amount of manure, due to its lower digestibility. Moreover, it will result in increased offals at slaughter, because the weight of offals at slaughter will be larger (both gut fill and empty gut weight; Kass et al., 1980; Stanogias and Pearce, 1985).

However, there are also quite a number of advantages for including NSP-rich ingredients in pig diets. A very important one is that these ingredients are usually relatively cheap. Moreover, when receiving these ingredients, the pig is not competing with humans for food ingredients. Because of the lower energy density, the pigs usually eat more bulk with these diets. It is suggested that this may improve the wellbeing of the pigs and it reduces the energy used for activity (Frazer, 1975; Broom and Potter, 1984; Matte et al., 1994; Schrama et al., 1996). Also, health of pigs may benefit from NSP in the diets. It has been found that a more coarsely-ground diet, as with NSP-rich ingredients, results in less gastric ulcers than with high energy density diets (Lee and Close, 1987). In addition, the lower pH caused by the fermentation products, can prevent pathogens from colonisation (Prohaszka, 1986; Wells et al., 1988). The fermentation processes both inside (in the gastrointestinal tract) and outside the pig (in the manure), might have beneficial effects on ammonia emission (Kreuzer and Machmüller, 1993; Scipioni et al., 1993; Canh et al., 1996; Bakker et al., 1996).

Consequences for feed evaluation systems

In general, feed evaluation systems are based on the amounts of digestible protein, fat and carbohydrates supplied by the separate ingredients. These amounts are generally obtained from feedstuff tables, that list all the ingredients with their average chemical composition and digestibility. Feed manufacturers

analyse usually each batch of ingredients more and more, prior to adding it to a compound feed. The digestibility of the nutrients is generally obtained from the feedstuff tables, and occasionally from a digestibility trial with the particular batch.

Thus, the methods by which chemical composition and digestibility are measured, determine the precision of diet formulation. The quality of analytical techniques can relatively easily be guaranteed by comparing the results on standards between the different laboratories. The data on digestibility in the feedstuff tables are mostly obtained with pigs housed under experimental conditions. For instance, the pigs are housed individually in metabolism crates. Housing pigs in pens compared to housing on metabolism crates was found to decrease retention time of digesta in the gut. This resulted in on average 1.8% lower digestibility coefficients for dry matter (Metz and Dekker, 1985). This effect was found to be enhanced by feeding a fibrous feed compared to a cereal feed.

The digestibilities of ingredients, which are listed in feedstuff tables, are usually obtained by the difference method: the ingredient is added to a basal diet of known digestibility (Šebek, 1989). The basal diet is usually made of relatively few ingredients, that are known not to affect digestibility of other nutrients. By using these data in diet formulation, it is assumed that they are representative for practical circumstances, where pigs are usually housed in groups and receive complex diets. However, when large amounts of NSP are included in the diets, the digestibility and energy supply from other ingredients might be reduced, as discussed in the previous sections of this chapter. Therefore, the present feed evaluation systems need to be re-evaluated for their accuracy of predicting the feeding value of a pig feed when large amounts of NSP are present.

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Chapter 2

Apparent digestibility of nutrients in diets with different energy density, as estimated by direct and marker methods in pigs with or without ileo-cecal cannulas

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Abstract

The objectives of this study were a) to compare the apparent total tract digestibility (TD) between non-cannulated (intact) and cannulated (steered ileo-cecal valve technique, SICV) pigs fed diets differing in energy density (Exp. 1), and b) to compare the direct vs marker (Cr_2O_3) methods for estimation of the TD and apparent ileal digestibility (ID) in SICV-cannulated pigs (Exp. 2). In Exp. 1, 24 intact and 18 SICV-cannulated castrates of approximately 40 kg initial BW were randomly assigned to six treatments in a $2 \times 3 \times 2$ factorial arrangement (two pig types, three carbohydrate sources, and two fat levels). In Exp. 2, the same SICV-cannulated pigs from Exp. 1 were given those treatments in a $2 \times 3 \times 2$ factorial arrangement (two methods of digestibility estimation, three carbohydrate sources, and two fat levels). In both experiments either maize starch, pure cellulose or soya bean hulls, without or with fat, were incorporated into a barley-soya bean meal-based diet to alter energy density. Daily rations were isoenergetic (based on NE_p), and water supply was 0.33 L/MJ of NE_p . In Exp. 1, the animal type effect on the TD of DM, OM, CP, and the pig type \times carbohydrate interactions for the TD of DM, OM and crude fiber (CF) were significant ($P \leq 0.05$), merely due to a larger difference found for the diet enriched with cellulose. In Exp. 2, the TD and ID evaluated with the marker method were significantly lower (except for the TD of CF) than with the direct method, mainly because Cr recovery was below 100%. Overall, the marker method seems to be superior because the TD means obtained from Cr ratios were closer to the TD obtained from intact pigs. In general, the SICV technique seems to be suitable for long term digestibility studies to measure the TD and ID in the same pig fed low- or high-fiber diets.

Key Words: Pigs, Cannulation, Digestibility, Energy

Introduction

There is contradictory evidence concerning total tract digestibility of proximate dietary nutrients in intact and cannulated pigs. In several studies, no impact of

simple T-cannulation of pigs on total tract digestion or on energy metabolism was found (Furuya et al., 1974; Close et al., 1984; Huisman et al., 1984; Moughan and Smith, 1987; Schröder et al., 1989). However, in other studies (Sauer et al., 1979; Jørgensen et al., 1985; Livingstone and McWilliam, 1985) there have been indications of adverse effects of cannulation on nutrient digestibility in the small intestine and/or in the whole gastrointestinal tract.

Comparisons of the direct vs marker methods (Cr_2O_3) in digestibility studies with cannulated pigs have not been extensively presented in the literature. Available techniques for quantitative collection of ileal digesta require removal of the cecum (post-valvular T-cecum cannulation of Van Leeuwen et al., 1988), or of the whole large intestine (ileo-rectal anastomosis) using various surgical procedures as described by Laplace et al. (1994), or affect gut motility by presence of two cannulas (re-entrant cannulation) as reported by Laplace (1980), and, therefore, normality of those pigs is questioned (Köhler et al., 1990; Fuller, 1991; Mroz et al., 1994). Moreover, larger amounts of fibrous products in diets for pigs with re-entrant or PVTC cannulas cause blockages and alterations of digesta passage rate (Huisman et al., 1984; Van der Meulen et al., 1993). Therefore, we developed a new T-technique named the steered ileo-cecal valve (SICV) cannulation that permits collection of ileal digesta quantitatively and that does not require any removal or transection of the gastrointestinal tract (Mroz et al., 1994). So far, application of this technique in pigs fed diets differing in energy density has not been tested. Therefore, the aim of the present work was a) to compare the apparent total tract digestibility of proximate nutrients between intact and SICV-cannulated pigs (Exp. 1) and b) to compare the direct and marker (Cr_2O_3) methods for estimation of the apparent total tract and ileal digestibilities of diets differing in energy density (Exp. 2).

Materials and methods

Animals and experimental design

Two digestibility experiments were carried out on crossbred castrates (Yorkshire x [Finnish Landrace x Dutch Landrace]) of approximately 40 kg initial BW and 105 kg final BW. In Exp. 1, the effect of cannulation on the apparent total tract digestibility (TD) of OM, ash, CP, ether extract (EE) and crude fiber (CF) was measured using 24 non-cannulated (intact) pigs and 18 pigs fitted with steered ileo-cecal valve (SICV) cannulas under inhalation anesthesia. The pigs were kept in the same building and randomly assigned to six treatments in a 2 x 3 x 2 factorial arrangement (two pig types, three carbohydrate sources, and two fat levels) to obtain four intact and three cannulated pigs per dietary treatment.

Experimental treatments were tested with the intact pigs in one series and the cannulated pigs in three series (six pigs per series). The intact pigs were housed in pens, except for the measurement period of 2 wk when they were transferred to

conventional stainless steel metabolism crates (1.2 m x .6 m), whereas the cannulated pigs were kept individually in pens of 1.35 m x 1.15 m throughout the entire study at an average ambient temperature of 18°C. In Exp. 2, the same SICV-cannulated pigs from Exp. 1 were used to measure the effect of direct vs marker (Cr_2O_3) methods for estimation of the TD and ileal digestibility (ID) of OM, ash, CP, EE, and CF in those treatments in a 2 x 3 x 2 factorial arrangement (two methods of digestibility estimation, three carbohydrate sources, and two fat levels). There were three pigs per dietary treatment.

Steered ileo-cecal valve cannulation technique and surgical procedure

The steered ileo-cecal valve cannulation (SICV) permits collection of ileal digesta quantitatively via a valve-steering system (Figure 2.1). Before surgery, each pig was held without feed for 36 h and had no access to water for 12 h. After premedication with Stresnil® (2 mg/kg s.c., Janssen Pharmaceutica BV, Tilburg, Holland) and Atropine (.05 mg/kg s.c., AUV, Cuijk, Holland), anesthesia was induced by intravenous injection of Nesdonal® (15 mg/kg i.v., Rhône Mérieux, Lyon, France) and maintained by inhalation of a mixture of halothane (1 to 4% as necessary) with O_2 -nitrous oxide via an endotracheal tube. Subsequently, the pig was placed on the left side and a laparotomy (a 70-mm incision) was made at the right hypochondrium.

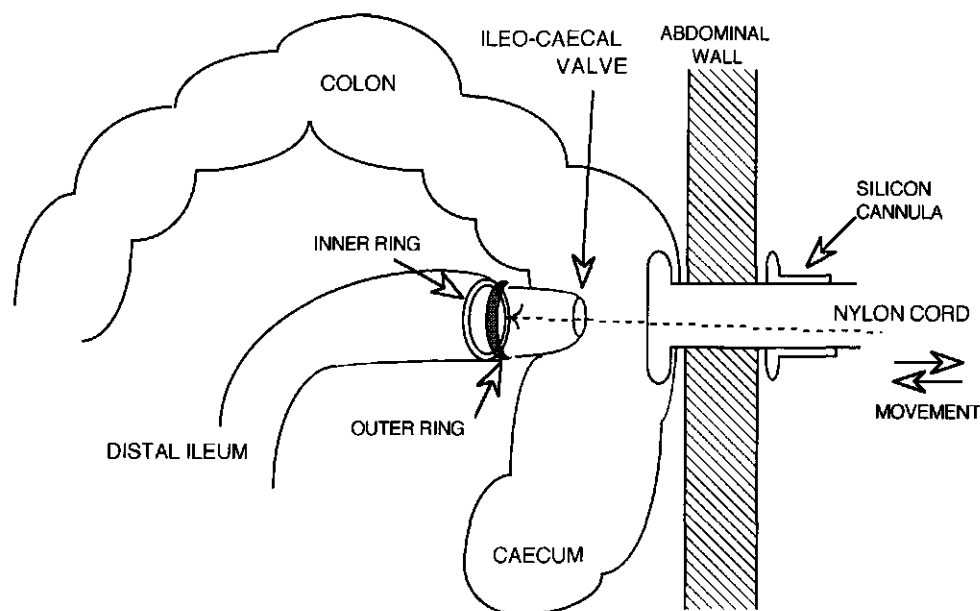


Figure 2.1.

A schematic view of the steered ileo-cecal valve (SICV) cannulation technique for quantitative collection of ileal digesta from pigs

The valve-steering system consists of two stainless steel rings of which one (outer, 2.0 mm thick and 34.0 mm o.d.) is placed around the intestine, close to its junction with the cecum, whereas the second ring (inner, 2.0 mm thick and 35.0 mm i.d.) is inserted into the lumen of the intestine. This inner ring is connected with a nylon cord, led outside of the animal through a silicone T-shaped cannula (barrel: 100 mm length, 26 mm i.d.; flange: 70 mm o.d.). Because the outer ring is slightly smaller than the inner ring, by pulling or releasing the nylon cord, the ileo-cecal valve can be "steered", as required.

First, the inner ring with the connected nylon cord was introduced into the lumen of the terminal ileum (approximately 15 cm from the ileo-cecal valve) via a small incision, which was afterward closed with Vicryl 3-0 (InstruVet BV, Amerongen, Holland) using the Lembert suture. Then, the outer ring was fixed close to the ileo-ceco-colonic junction for a permanent maintenance of the inner ring just anterior to the ileo-cecal valve. To fix the outer ring around the intestine, the ring was cut, and one of its ends was directed through the ileo-cecal mesentery, and a special safety-locker was made to close the ring firmly again. Successively, a 50 mm incision through the taenia coli of the cecum was made to introduce the silicone T-shaped cannula just across the ileo-cecal valve.

Next, the purse-string suture (Vicryl 2-0) was drawn tightly up around the cannula after guiding the nylon cord of the inner ring through the barrel. To secure the cannula, a second suture was made (peripheral to the previous one). Afterward, the cannula barrel was exteriorized by a stab wound made with a cork-borer (approximately 8 cm distal to the last rib). The cannula was held in place by a silicone, T-shaped, outer part (barrel: 30 mm i.d. and 40 mm length; flange: 26 mm o.d.). To prevent outflow of digesta, a polyvinyl chloride (PVC) cylindric stopper (80 mm length and 26 mm o.d.) was introduced into the lumen of the barrel. The abdominal cavity was closed by suturing, successively, the peritoneum, two muscle layers, subcutaneous tissues, and the skin. After surgery, the pigs were injected i.m. with Ampicillin (2 mL/50 kg BW, AUV, Cuijk, Holland) and Antipyranal® (3 mL/50 kg BW, Alfasan, Woerden, Holland) for 3 d to prevent postoperative infection and to minimize pain, respectively. The pigs were already adapted to the experimental diets before surgery (for a period of 4 wk). During the post-operative period of 10 d, the same diets were gradually increased approximately 100 g/d until the pre-surgical level of feed intake was achieved. Before and after the surgery the pigs were given ad libitum access to water.

Diets and feeding

In both experiments, six dietary treatments (Table 2.1) were arranged as a 3 x 2 factorial, that is, three carbohydrate sources (maize starch, purified cellulose [Arbocel B800, J. Rettenmaier, Holzmühle, Germany], and soya bean hulls), and two levels of animal fat added to a basal diet. The basal diet consisted of barley, soya bean meal, wheat middlings, and potato protein as major components (Table 2.2). Addition of maize starch in Treatments 1 and 2 was 35 and 25%,

Table 2.1

Ingredients, chemical composition (g/kg, as-fed basis), and energetic value of the dietary treatments

Carbohydrate:	Maize starch		Cellulose		Soya bean hulls	
Fat:	(-)	(+)	(-)	(+)	(-)	(+)
Treatment:	Mo	Mm	Co	Cm	So	Sm
Item	g/kg (as-fed basis)					
Basal diet	539	628	416	480	472	506
Maize starch	349	252	262	122	195	69
Toasted soya bean hulls	-	-	-	-	267	314
Cellulose (Arbocel [®])	-	-	261	310	-	-
Fat	-	59	-	48	-	61
Cane molasses	112	61	61	40	66	50
Analyzed composition						
DM	881	884	889	898	883	889
Ash	45	50	36	45	52	60
CP (N x 6.25)	155	180	119	133	166	183
Ether extract (EE)	26	87	20	69	27	92
Crude fiber (CF)	26	31	196	232	102	117
NDF	87	104	304	355	214	247
ADF	35	43	249	286	133	156
Starch	391	331	292	194	260	165
GE (MJ/kg)	16.1	17.6	16.1	17.3	16.2	17.7
NE _i (MJ/kg) ^a	9.7	10.7	7.7	8.2	8.5	9.6

^aNE_i = net energy

respectively.

Amounts of added purified cellulose in Treatments Co and Cm and of soya bean hulls in Treatments So and Sm were similar, i.e., 31 and 26%, respectively. No supplemental fat was given to Treatments Mo, Co and So, whereas Treatments Mm, Cm and Sm were supplemented with 5 to 6% of fat. This amount of fat was added in substitution of maize starch on the NE_i-equivalency (3 g of maize starch ≈ 1 g of fat). Chromic oxide (Cr₂O₃, 0.5 g/kg of basal diet) was used as an indigestible marker. Cane molasses was added to increase quality of pellets. Two isoenergetic daily meals were given in 12 h-intervals (0600 and 1800) in the amounts equivalent to 90% of the Dutch recommendations for net energy (NE_i) to pigs gaining 700 g/d (CVB, 1994). To have equal energy intake, daily rations differed among the treatments, but the amount of the basal diet in the daily rations was assumed to be the same, regardless of the treatment. Due to discrepancies between calculated and analyzed chemical compositions of the dietary treatments,

the applied amounts of the basal diet slightly deviated between the treatments (approximately $\pm 5\%$). This difference did not seem to influence our experimental goals and can be regarded as negligible. Water was added to each meal before feeding in equal proportion to NE_f intake (0.33 L/MJ). The pigs had no access to water between the meals.

Table 2.2
Ingredients of the basal diet

Ingredient	g/kg (as-fed basis)
Ground barley	290
Extracted soya bean meal (44% CP)	279
Potato protein	114
Wheat middlings	207
Soya oil	19
Alfalfa	41
Limestone	20
Calcium phosphate, dihydrate	16.9
Salt (NaCl)	4.2
Vitamin-trace mineral premix*	3.5
Choline chloride	.5
DL-Methionine	1.3
L-Lysine-HCl	1.5
Cr ₂ O ₃ -maize starch (1:3 wt/wt)	2.1

* Contained the following ingredients (per kilogram of the basal diet): 39 mg of vitamin AD₃ (19,500 IU and 3,900 IU, respectively); 39 mg of vitamin E; 10 mg of riboflavin; 49 mg of niacin; 19 mg of DL-Ca-pantothenate; 49 µg of vitamin B₁₂; 304 mg of antioxidant (4 to 5% of BHA, 4 to 5% of ethoxyquin, 4 to 5% of citric acid, 2 to 3% of orthophosphoric acid, 2 to 3% of E 471 fatty acid [esters of fatty acids and mono-/diglycerides, obtained after partial hydrolysis of fat from spices], and SiO₂ as carrier); 121 mg of MnO (93.6 mg of Mn); 376 mg of ZnSO₄·H₂O (136.9 mg of Zn); 5 mg of KI (3.8 mg of I); 1044 mg of FeSO₄·7H₂O (209.8 mg of Fe); 382 µg of Na₂SeO₃ (174.2 µg of Se).

Collection procedures

In both experiments, each dietary treatment was tested when the barrows weighed approximately 90 kg. Quantitative collection of feces was conducted during 10 d by gluing an adhesive tape with the Medical Adhesive B (Dow Corning®, 06904 Sophia Antipolis, France) around the anus of the pig. This adhesive tape was equipped with snap-fasteners for attaching polyethylene bags, and the bags with feces were replaced twice daily. Daily collections of feces were weighed and stored at -20°C. Afterward, two 24-h quantitative collections of ileal

digesta from the pigs were conducted at intervals of 5 d to avoid dehydration and/or electrolyte depletion. To enable digesta collection, the ileo-cecal valve was "steered" always successfully, into the lumen of the cannula (its proper position could be seen when digesta collection was accomplished), and the nylon cord was tied firmly around the outer part. Later, a special PVC joint was fixed temporarily to the cannula barrel. This joint permitted outflow of digesta even when the animals were resting on the right side, where the cannula was placed. Finally, a polyethylene tube of approximately 25 cm long was attached to the PVC joint. As soon as digesta appeared in this tube, it was emptied into a larger polyethylene bag kept permanently in Dry Ice. Hourly collections were weighed and stored at -20°C until chemical analysis. After digesta collection ended, the PVC joint was removed, the nylon cord released, and the cannula closed firmly with the cylindric stopper. The latter was secured with plastic zip-fasteners. Frozen cumulative samples of ileal digesta from both days were homogenized without thawing (by adding liquid N), freeze dried, and subsamples were taken for chemical analysis. Samples of feces were thawed, and after homogenization subsamples were air-dried.

Analytical procedures

All samples were analyzed in duplicate. Dry matter content in diets, feces, and ileal digesta was determined at 104°C, and nitrogen content (in fresh samples) was determined by the Kjeldahl method (AOAC, 1984). Crude protein was calculated as Kjeldahl N x 6.25. The samples were ashed at 550°C for calculation of percentage of ash. Crude fiber content was assayed by hydrolysis with 1.25% H₂SO₄ and 1.25% NaOH, whereas crude fat content was determined by boiling the sample in 3 N HCl for 3 h, and the filtered, washed, and dried residue was extracted using petroleum ether 40-60 (AOAC, 1984). Chromium content was assessed by atomic absorption spectrophotometry (Perkin-Elmer model 1100, at 357.9 nm) according to Williams et al. (1962). Dietary starch concentration was determined enzymatically (amylglucosidase/hexokinase method) with an autoanalyzer according to Bosma et al. (1987), and GE content in an adiabatic bomb calorimeter.

Statistical analysis

In both experiments each pig was the experimental unit. In Exp. 1, to evaluate the effect of cannulation on the total tract digestibility of dietary nutrients by the marker method the data were subjected to analysis of variance for a completely randomized design using the ANOVA procedure of Genstat 5 (Payne et al., 1989) according to the following 2 x 3 x 2 factorial model:

$$Y_{ijk} = \mu + T_i + C_j + F_k + (Tx C)_{ij} + (Tx F)_{ik} + (CxF)_{jk} + (Tx CxF)_{ijk} + \epsilon_{ijk}$$

where μ = overall mean, T = pig type (intact vs cannulated) effect, C = carbohydrate effect, F = dietary fat effect, and ϵ = error contribution with average 0 and variance σ^2 , and i = 1...2, j = 1...3, k = 1...2. Housing effect was not included in the model, because it is confounded with pig type. The SE was used

to test significance in comparisons of averages for intact vs cannulated pigs. No evidence ($P \leq 0.10$) of a TxCxM interaction was found.

In Exp. 2, to evaluate the effect of method (direct vs marker [Cr_2O_3]) on estimates of the apparent total tract and ileal digestibilities using SICV-cannulated pigs the following $2 \times 3 \times 2$ factorial model was fitted:

$Y_{ijkl} = \mu + S_i + C_j + F_k + M_l + (C \times F)_{jk} + (C \times M)_{il} + (F \times M)_{kl} + (C \times F \times M)_{jkl} + \epsilon_{ijkl}$
 where μ = overall mean, S = series effect, C = carbohydrate effect, F = fat effect, M = method effect, and ϵ = error contribution with average 0 and variance σ^2 , and $i = 1 \dots 3$, $j = 1 \dots 3$, $k = 1 \dots 2$ and $l = 1 \dots 2$. There was no evidence ($P \leq 0.10$) of a CxFxM interaction. The standard errors (SE) were calculated and significance of differences between means for direct vs marker methods was distinguished with the Student's t-test at $P \leq 0.05$, 0.01, and 0.001.

In the tabulated data are reported only the main effects of animal type, the method of digestibility estimation, and their interactions with carbohydrate sources, whereas the main effects of supplemental fat and interactions are discussed in the text of the results.

Results and discussion

Features and evaluation of the steered ileo-cecal valve cannulation technique

In this study, we tested a novel technique for quantitative ileal digesta collection by means of a newly developed steering system placed at the ileo-cecal junction. This technique has some advantages over other available quantitative techniques, in particular to the re-entrant (ileo-ileal or ileo-cecal) cannulation method. These advantages include: 1) only one T-shaped cannula is required, which disturbs to a lesser extent the normal intraluminal pressure that may result from two (re-entrant) cannulas; 2) there is no need for transection of the intestine with an interruption of the continuity of the muscle layers and the intraparietal plexuses, resulting in disruption of the myoelectric migrating complex (MMC) pattern distal to the cannula; 3) there is no need for permanent manipulations with the collection and re-introduction of digesta, which may affect the mechanisms controlling gastric emptying and intestinal peristalsis (Laplace, 1980); 4) the functional role of the ileo-cecal valve and of the cecum is maintained, and no detectable "invasion" of the small intestine by a ceco-colic type microflora takes place; 5) digesta samples for chemical analysis are fully representative because neither solid nor liquid fractions can "escape" into the large intestine during collection; and 6) there is no need to restrain the pigs even during sampling time (an important feature from the animal welfare point of view), and less labor input for the day-to-day maintenance and digesta collection is required.

In a very early phase of developing this technique, we indicated its application for a period of 6-8 weeks only (Mroz et al., 1991). Subsequently, however, our first prototype was substantially modified to eliminate this time limitation. In this

study, growing-finishing pigs equipped with the newly modified SICV cannulas were kept from 40 to 105 kg BW. During this period the pigs grew at a similar rate as the intact pigs on the respective treatments differing in energy density. Feeding in the post-operative (recovery) phase with voluminous and bulky diets containing soya bean hulls or cellulose was found to be most optimal when daily rations increased not more than 100 g feed/d.

Overall, the pigs did not show any signs of pain or discomfort when the ileo-cecal valve was steered into the lumen of the cannula for digesta collection, and they had no visible health problems throughout the study. Despite feeding relatively high amounts of fibrous feedstuffs (soya bean hulls and cellulose), obstructions or blockages in the flow of ileal digesta were not observed, while digesta leakage was low. To prevent inflammation at these leakage sites, the pigs were cleaned twice daily with a warm water-moistured sponge and soft-paper tissues. Afterward, the skin of this region was smeared with a special ointment containing furazolidon, zinc oxide and lidocaine (Alfasan, Woerden, Holland).

At postmortem examination, a good permanent serosal adhesion of the cecal-colic surface to the abdominal wall around the silicone cannula was present. The implanted steering system was found to be fully bio-inert; no immunologic reaction and no inflammatory cells could be detected. Around the outer ring, new collagen fibers were formed, without the presence of inflammatory cells. The inner ring was found to be bio-inert and mobile, as assumed. Neither visible alteration of the ileo-cecal valve histological structure, nor dilatation of the distal ileum, nor collateral passage (running side by side) of digesta was observed. All those characteristics fulfil the criteria that need to be met for reliable measurements of digesta kinetics from healthy pigs with a normally functioning alimentary tract.

Comparison of the apparent total tract digestibility between intact and steered ileo-cecal valve-cannulated pigs (Exp. 1)

Pigs fitted with T-shaped cannulas are often used to measure not only ileal, but also total tract (TD) digestibility, assuming their biocompatibility with intact pigs (Sauer et al., 1979; Den Hartog et al., 1988). This hypothesis was tested in our study with growing-finishing pigs of 90 kg BW fed diets differing in energy density and containing Cr as a marker. The overall mean apparent total tract digestibility (TD) estimated with the marker method for the intact pigs tended to be lower than for the SICV-cannulated pigs, regardless of the nutrient (Table 2.3). In the case of DM, OM and CP, the difference of three and two percentage units, respectively, was found to be meaningful ($P \leq 0.05$). The TD of OM and CF calculated from Cr ratios for the maize starch and soya bean hull treatments were similar for both types of pigs, but not for the cellulose treatments, where differences in the TD of OM and CF were equal to 8 and 22 percentage units in favor of the cannulated pigs ($P \leq 0.05$). In consequence, pig type x carbohydrate interaction was found to be significant for both nutrients.

Table 2.3

Apparent total tract digestibility of diets differing in carbohydrate source as estimated for intact vs SICV-cannulated pigs using the marker (Cr_2O_3) method (Exp. 1)

Carbohydrate: type of animal:	Maize starch		Cellulose		Soya bean hulls		Type ²	SED ¹ Carb ³	Type x Carb ⁴
	intact	SICV	intact	SICV	intact	SICV			
	percentage								
Digestibility:									
Dry matter	87	88	60	67	80	81	1.1*	1.3***	1.8*
Organic matter	90	90	61	69	82	83	1.1*	1.3***	1.8*
Ash	50	54	40	45	48	46	1.2	1.5***	2.1
Crude protein	88	90	75	76	77	80	0.8*	2.9***	1.4
Crude fat	75	79	70	71	69	69	1.4	1.7***	2.5
Crude fiber	36	34	9	31	65	64	3.6	4.4***	6.3*
Faecal Cr recovery	103	82	98	82	104	85	2.4***	2.9	4.2

¹ SED: standard error of differences of means

² Type: Intact (n=24) or SICV-cannulated (n=18) pigs of 90 kg BW.

³ Carb: Source of carbohydrate maize starch, cellulose or soya bean hulls

⁴ Type x Carb: interaction between type of animal and source of carbohydrate

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

In agreement with our findings, Jørgensen et al. (1985) reported that growing-finishing pigs fitted with T-shaped cannulas as compared to intact pigs, had a higher TD of CP and lysine, particularly when feeding diets with an increased CF content. A similar tendency was observed by Sauer et al. (1979).

However, with regard to the TD of ash, ether extract (EE) and CF in our studies, no effect ($P \leq 0.10$) of the pig type was found, although relatively larger differences were noted in the cellulose-containing treatments.

Addition of fat affected ($P \leq 0.001$) the apparent digestibility of DM, OM, ash, and EE, but its interactions (fat x carbohydrate, fat x pig type and fat x carbohydrate x pig type) were not statistically significant.

The differences in the TD of particular nutrients ascribed for the pig type effect in our study might have been confounded with effects of housing system, and perhaps with effects of surgery. The effect of the first factor (housing system) has been investigated by Metz and Dekker (1985) and Bakker and Jongbloed (1994), who concluded that the TD of DM evaluated from Cr ratios is by 1.8 percentage units lower in pigs housed in groups in pens than in pigs housed individually in metabolism crates. However, it remains uncertain to what extent this difference was affected by incidences of coprophagy (which affects Cr recovery) in group-housed pigs. So far, no comparative data on the TD in pigs (cannulated or not) kept

individually in pens vs metabolism crates were found. The second potentially confounding factor in our study (surgery) was eliminated in a similar methodological study by Close et al. (1984) by operating (laparotomizing) on the intact pigs, without cannulation. Besides, an effect of pig-related variation in passage rate, as well as in the day-to-day differences in gastrointestinal digestion and luminal influx/outflux of nutrients caused by varying activity, stress, and (or) deviations in myogenic-hormonal-neural regulation of digestive processes during the measurement periods cannot be excluded, despite assuming a "steady" flow of digesta.

In numerous nutritional studies, the TD is estimated from grab samples of feces and a Cr recovery check is not possible, and not necessary. We measured the recovery of Cr in feces, which ranged by the intact pigs from 94 to 106%, whereas by the cannulated pigs it was found to be approximately 20 percentage units less. This lower recovery of Cr in the cannulated pigs may be mainly attributed to its losses via digesta leakage, whereas a potential influence of several other factors (e.g., uneven marker dispersion in feed, differences in transit time of nutrients and the marker through the alimentary tract, representativity/homogeneity of samples, analytical precision in determination of nutrient and marker concentrations, and incidences of coprophagy) remains merely speculative.

Overall, a further comparative research under identical maintenance conditions of the intact and cannulated pigs, recording Cr losses due to digesta leakage seems desirable.

Comparison of the direct vs marker methods for estimation of the apparent total tract digestibility in steered ileo-cecal valve-cannulated pigs (Exp. 2)

By definition, using the direct or marker method should result in an identical estimation of the TD at a steady-state of the pig, independent of presence or absence of cannulas. In practice, however, some deviations may occur, mainly due to Cr recovery rates. In contrast to data on intact pigs, comparative data on the Cr recovery in feces of cannulated pigs are very limited in the available literature (presumably due to difficulties in quantification of digesta leakage). In this study, the recovery of Cr in feces of the SICV-cannulated pigs was found to be from 82 to 85% (Table 2.3). Köhler et al. (1990) reported that the Cr recovery in pigs fitted with either PVTC-, T-, or re-entrant cannulas, and fed a fiber-rich diet, was 71.6, 63.5 and 82.3%, respectively. In intact pigs, according to Ehle et al. (1982), it may range from 87 to 114%, whereas according to Petry and Enders (1974) it was from 78 to 95%. The latter authors reported also that with increasing amounts of DM excreted in feces, Cr recovery rate diminishes from 95 to 90%. However, both in a previous study by Bakker and Jongbloed (1994) and in this experiment, no interrelationship between excreted DM and Cr recovery was noticed.

We found that the overall mean TD calculated with the marker method was lower than with the direct method, regardless of the nutrient (Table 2.4). The difference was equal to three percentage units for OM ($P \leq 0.05$), nine percentage

Table 2.4

Apparent total tract digestibility of diets differing in carbohydrate source as estimated with the direct vs marker methods in cannulated pigs (Exp. 2)

Carbohydrate: Type of animal:	Maize starch		Cellulose		Soya bean hulls		SED ¹		Method Carb ⁴
	direct	marker	direct	marker	direct	marker	Method ²	Carb ³	
			percentage						
Dry matter	91	89	73	67	83	80	1.4*	1.7***	2.4
Organic matter	92	91	74	68	85	83	1.4*	1.8***	2.4
Ash	63	54	54	44	54	46	1.4***	1.7***	2.4
Crude protein	91	89	81	76	83	80	0.9**	1.1**	1.6
Crude fat	83	79	77	72	74	69	1.0***	1.3***	1.8
Crude fiber	46	35	41	30	68	63	4.2*	5.2***	7.3

¹ SED: standard error of differences of means

² Method: direct (calculated from quantitative collection of feces for 10 d) vs marker (calculated from Cr ratios in diets and feces) using 18 SICV-cannulated pigs of 90 kg BW

³ Carb: Source of carbohydrate maize starch, cellulose or soya bean hulls

⁴ Method x Carb: interaction between method and source of carbohydrate

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

units for ash ($P \leq 0.001$), three percentage units for CP ($P \leq 0.01$), five percentage units for EE ($P \leq 0.001$), and nine percentage units for CF. Our findings are in agreement with the results of Everts and Smits (1987), and Petry and Enders (1974), in which the TD calculated with the marker method seemed to be lower than with the direct method. Despite the differences in absolute values between both methods in our study, the effect of carbohydrate source on the TD of DM, OM, CP, and EE was distinguished statistically at the same probability (P) level (Table 2.4). However, the effects of carbohydrate sources on the TD of ash and CF evaluated via the direct method were statistically significant at $P \leq 0.05$, while via the marker method at $P \leq 0.01$. For the check of Cr recovery, the direct method is assumed to be absolutely faultless and serves as a reference method, whereas it is not always justified (Petry and Enders, 1974; Everts and Smits, 1987; Bakker and Jongbloed, 1994). To support this statement, the authors underlined that with the direct method there are inevitable losses of feed, digesta, and feces during measurement periods, and the results cannot be statistically controlled by the repeatability of the single measurements.

Neither main effects of supplemental fat nor interactions (fat x method, fat x carbohydrate, fat x carbohydrate x method) were found to be statistically significant.

In conclusion, it seems that despite the lower than 100% recovery rates of Cr as found in this study or reported by Köhler (1990), a preference should be given

to the marker method for evaluation of the TD of nutrients in future studies with SICV-cannulated pigs. In contrast to the direct method, the TD calculations from Cr ratios are independent of inevitable losses of feed, digesta and feces, and the results can be statistically controlled by the repeatability of the single measurements.

Comparison of the direct vs marker methods for estimation of the ileal digestibility in steered ileal-cecal valve cannulated pigs (Exp. 2)

Using the marker method in our study led usually to lower values of ID in comparison to the direct method, regardless of the treatment and nutrient (Table 2.5). These differences were six percentage units for OM ($P \leq 0.001$), 15 percentage units for ash ($P \leq 0.01$), four percentage units for CP ($P \leq 0.01$), four percentage units for EE ($P \leq 0.05$), and 20 percentage units for CF ($P \leq 0.001$). No statistically significant interactive effect of the method with carbohydrate source on the ID estimates for any nutrient was found.

Table 2.5

Apparent ileal digestibility of diets differing in carbohydrate source as estimated with the direct vs marker methods in cannulated pigs (Exp. 2)

Carbohydrate: Type of animal:	Maize starch		Cellulose		Soya bean hulls		Method ²	SED ¹	
	direct	marker	direct	marker	direct	marker		Carb ³	Method Carb ⁴
			percentage						
Dry matter	80	76	55	67	64	55	1.9***	2.4***	3.6
Organic matter	82	78	57	48	66	57	1.8***	2.3***	3.4
Ash	51	41	25	10	36	19	4.4**	5.5***	7.8
Crude protein	83	79	77	72	78	72	1.5**	1.9**	2.8
Crude fat	84	80	77	76	83	78	1.6*	2.0	2.9
Crude fiber	18	-1	18	-5	7	-12	5.4***	6.8***	10.1

¹ SED: standard error of differences of means

² Method: direct (calculated from quantitative collection of ileal digesta for 2 d) vs marker (calculated from Cr ratios in diets and ileal digesta) using 18 SICV-cannulated pigs of 90 kg BW

³ Carb: Source of carbohydrate maize starch, cellulose or soya bean hulls

⁴ Method x Carb: interaction between method and source of carbohydrate

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

As evaluated by both methods, supplemental fat exerted a positive effect on the ID of DM ($P \leq 0.01$), OM ($P \leq 0.01$) and EE ($P \leq 0.001$), but not on the ID of ash, CP and CF. No significant interactive effect of fat x carbohydrate, fat x method or fat x carbohydrate x method was found.

Methodological comparisons of the direct vs marker methods in estimation of the ID have not been extensively presented in the available literature. This is partly because the direct method is very laborious and can be employed only in animals with re-entrant cannulas, ileo-rectal anastomosis (IRA), and SICV-cannulated pigs. Theoretically, using the direct or marker method should result in an identical estimation of the ID at a steady-state of the pig, as already discussed in the case of the TD. However, due to an influence of some external and/or internal factors, this agreement between both methods (particularly at the ileal level, where collection periods are shorter than at the fecal level) is not always achieved. The discrepancies in the ID values calculated by both methods may arise due to a) sedimentation of Cr in the gastrointestinal tract (Latymer et al., 1990); b) between-animal variation in gastric emptying and flow patterns of ileal digesta (Laplace et al., 1983); c) a large circadian variation in the content of nutrients and Cr in ileal digesta (Graham and Åman, 1986); d) various sampling periods (Köhler et al., 1990); e) an extent of digesta leakage; and f) an analytical imperfection in determining feed and digesta composition. In contrast to the TD estimation procedure, collection of ileal digesta for the ID determination cannot last as long (7 to 10 d), because digesta must be re-introduced into the large intestine. In our study, estimations of the ID with the direct method was based on 48-h digesta collection (2 x 24, with 5 d intervals), and the collected material was not re-introduced. Average ileal recovery of Cr ranged from 71 to 85%. A similar range in the recovery rate of Cr in ileal digesta collected quantitatively for 3 d via re-entrant cannulas was reported by Köhler et al. (1990), who recovered 93.6 and 82.3% of Cr in ileal digesta of pigs fed control and fiber-rich diets, respectively. This ileal recovery of Cr of less than 100% may be ascribed for a day-to-day variation in the patterns and rates of gastric emptying, fluctuations in intestinal flow velocity due to differences in a cycle length of migrating myoelectric complexes through the small intestine, and duration of both quiescence and spiking activity phases (Rayner and Wenham, 1986). Also, Amidon et al. (1991) indicated that other physical factors such as exercise, temperature, particulate segregation within the pyloric antrum, and/or size, viscosity, osmolality and pH of particular gushes can specifically influence the rate of gastric emptying and flow velocity from one day to another. There is also some evidence that certain amounts of sedimented Cr (varying from one pig to another) always remain in the gastric (fundus) gland region of the stomach and in the crypts of the epithelium along the small intestine (C.R. Dove, University of Georgia, personal communication). It is well known that the crypts are not static but are dynamic and readily change dimension in response to any number of factors.

Overall, bearing in mind a large labour input, and a physiological limitation of time for a longer digesta collection to employ the direct method, the ID estimated from Cr ratios is advisable. Under conditions of this study the marker method seemed also to be more precise because the SD values were lower, regardless of the nutrient.

Implications

The newly modified steered ileo-cecal valve cannulation of pigs maintained the functional physiological role of the whole gastrointestinal tract, and the implanted steering system is bio-inert (no immunologic reaction or inflammatory cells). It seems to be a reliable technique for quantitative collection of ileal digesta to measure apparent ileal digestibility of low- and high-fiber diets fed to pigs from 40 to 105 kg of body weight. For practical evaluation of feeding values at the ileal and fecal levels using the same cannulated pig, a preference for the marker method is suggested.

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Chapter 3

The effect of housing system on apparent digestibility in pigs, using the classical and marker (chromic oxide, acid insoluble ash) techniques, in relation to dietary composition

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G.C.M. Bakker and A.W. Jongbloed

Abstract

The present study examined differences in faecal digestibilities of organic matter (dOM) and crude protein (dXP), between growing pigs housed either in pens as groups or individually in metabolism cages. In addition, a study was made of the influence of dietary composition on these observed differences. Four experimental diets were composed by iso-energetic exchange of maize starch in the control diet with purified cellulose (260 g kg⁻¹), toasted soya bean hulls (280 g kg⁻¹) or animal fat (67 g kg⁻¹), respectively. Both in pens and in metabolism cages, dOM and dXP were measured, using both chromic oxide (Cr₂O₃) and acid-insoluble ash (AIA) as markers. In metabolism cages, the results of the marker method were compared to those of the classical method, where dOM and dXP were measured by collecting faeces quantitatively. Recoveries of both markers were measured, after a period of 10 and 3 days.

With Cr₂O₃ as marker, pen-housing resulted in a dOM which was on average 2.5 (1.7 - 4.5) units lower and a dXP averaging 4.5 (3.0 - 5.8) units lower than measured in the metabolism cages (P<0.001). No significant interactions were demonstrated between housing system and dietary composition. In contrast, with AIA as marker significantly higher digestibility values were observed for pigs in pens, when fed the cellulose diet. With this diet, the dOM was on average 14.7 units higher and the dXP was on average 10.9 units higher for the penned pigs. For the pigs fed one of the other three diets, the dOM was on average 1.5 (0.1 - 2.7) units lower and the dXP was on average 3.7 (1.9 - 5.7) units lower. Thus, with AIA as a marker, the effect of housing system on digestibility interacted with the type of diet (P<0.05 on dOM and P<0.01 on dXP).

Comparison between the marker method with the classical method, showed that Cr₂O₃ provided similar results. In contrast, AIA displayed significantly higher dOM and dXP, except with the cellulose diet. This could be explained by differences in the recoveries of both markers. For Cr₂O₃ this was close to 100% and for AIA it varied from 97% on the cellulose diet to 183% on the control diet. The analytical procedure for AIA requires more research. Shortening the measuring period from 10 to 3 days did not prove to be significant, but increased the standard deviations. It was concluded that for practical

application, faecal digestibilities should be measured with penned pigs. According to the findings, under such conditions Cr_2O_3 is a good marker. AIA was found to be unsuitable.

Key words: faecal digestibility, pigs, housing (pen, cage), markers, chromic oxide, acid-insoluble ash, dietary composition

Introduction

Digestibility coefficients are the most important parameters in evaluation of the nutritive value of feeds for pigs. It is therefore essential that the method used to estimate the digestibility is both accurate and reliable. The most commonly used method for estimating digestibility, relies on quantitative collection of faeces for several consecutive days from animals kept in metabolism cages (classical method). However, this method is laborious and requires specialised equipment. This method of housing restricts the animals freedom of movement and is subjected to criticism on animal welfare grounds. Moreover, it is not known whether animals housed in cages perform similarly to those housed in pens which allow more freedom of movement. However, to collect fresh faeces quantitatively from a group of animals housed in pens is no easy task. Excretion of indigestible substances in faeces can be estimated, by using indigestible external (chromic oxide) or internal (acid-insoluble ash) markers. The quality of a marker is indicated by its recovery. This indicates the fraction of the given marker, which is recovered in the faeces. Kotb and Luckey (1972) concluded that chromic oxide (Cr_2O_3) is not toxic and is almost quantitatively recovered from the faeces of man and animals. Therefore, it can be used as an inert marker in digestibility studies. Acid-insoluble ash (AIA) is often used as an internal marker. Many nutritional studies have evaluated Cr_2O_3 (Moore 1957; Petry and Enders 1974; McCarthy et al 1974 and 1977; Sugimoto 1984; Moughan et al 1991) and AIA (McCarthy et al 1974 and 1977; Sugimoto 1984; Rundgren and Haglund 1988; Moughan et al 1991; Rowan et al 1991).

In these studies, the classical method has been compared with the marker method, using pigs in metabolism cages. The marker method, however, can also be applied for pigs in pens. McCarthy et al (1977) observed lower digestibility coefficients obtained with a marker for pigs in pens than those obtained with the classical method. It is not clear, to what extent this effect is due to the marker method (including the method of sampling) or to differences in housing system (metabolism cages or pens). The type of diet may have an effect on the marker method, by influencing the rate of passage. However, only a few digestibility studies have been performed with markers in high-fibre diets. Petry and Enders (1974) found that the recovery of Cr_2O_3 was lowered with

increasing crude fibre content, while Sugimoto (1984) found no effect. With AIA, Sugimoto (1984) showed a 10% lower recovery at high levels of crude fibre in comparison to lower crude fibre contents. In contrast, Rundgren and Haglund (1988) found no effect of crude fibre content on AIA recovery. Therefore stepwise comparisons are needed to elucidate these effects.

In the Netherlands many fibrous feedstuffs are used in pig feeds. Moore (1956) showed a similar excretion pattern for Cr_2O_3 , Cr_2O_3 -free ash and crude protein in dry matter. These excretion patterns were inversely related to the excretion pattern of crude fibre. Thus, it was indicated, that different organic components can have different passage rates, which may interfere with the method of measuring digestibility. In the present experiment high-fibre diets were included. For this study the authors chose apparent digestibility of crude protein next to organic matter as dependent variables.

The aim of the present study was to investigate apparent digestibility in relation to dietary composition, for pigs receiving the diets in metabolism cages or in pens, using classical and marker (Cr_2O_3 , AIA) methods.

Materials and method

Feeds and animals

Four diets were tested; a control diet (diet 1); two other diets comprising different sources of additional fibrous carbohydrates; cellulose (Arbocel B800, J. Rettenmaier, Holzmühle, Germany) 260 g kg^{-1} diet (diet 2) or soya bean hulls 280 g kg^{-1} diet (diet 3), and a fourth diet (diet 4) with added animal fat (67 g kg^{-1}). The control diet contained 540 g kg^{-1} of a basal feed, supplying the necessary protein/amino acids, minerals and vitamins, and 460 g kg^{-1} maize starch. In the other three experimental diets, maize starch was substituted iso-energetically on the basis of assumed net energy by cellulose, soya bean hulls or fat. The four feeds had different protein and net energy (NE_e) densities (see Table 3.1). Thus, the animals fed a low-density feed had to eat more kilograms of this feed to consume the same amount of net energy, than the animals fed a high-density feed. The feeds were manufactured and pelleted at the same time in an amount sufficient for all trials, and stored at -18°C .

Barrows, from a three-way cross Yorkshire δ x (Finnish Landrace δ x Dutch Landrace f) f , were distributed upon one of the four diets which were fed continuously from 30 to 105 kg live weight. The animals were fed twice daily. The daily allowance was fed in two similar portions. The feeding level was based on 90% of the energetic requirement to maintain a growth rate of 700 g day^{-1} (CVB, 1988). Water was supplied on the basis of a fixed water:net energy ratio of 0.33 litres (MJ NE_e) $^{-1}$, and was added to the feed before feeding.

Table 3.1

Analysed chemical composition (g kg⁻¹ DM), concentration of the markers (chromium (Cr; mg kg⁻¹ DM) and acid-insoluble ash (AIA; g kg⁻¹ DM)) and estimated net energy (NE, in MJ kg⁻¹ DM) of the feeds (n = 6; SD in parentheses)

Composition	Control	Dietary components		
		Cellulose	Soya bean hulls	Fat
Organic matter (OM)	949.0 (0.3)	959.9 (0.6)	940.0 (0.7)	943.2 (1.2)
Crude protein (XP)	177.6 (0.5)	136.7 (1.9)	191.0 (0.9)	203.7 (0.9)
Crude fat	29.3 (1.5)	21.2 (1.1)	30.8 (0.9)	95.9 (2.6)
Crude fibre	28.5 (2.0)	222.1 (10.0)	115.0 (0.9)	37.6 (4.9)
AIA	1.7 (0.4)	1.2 (0.5)	3.5 (0.1)	2.0 (0.2)
Cr	195.4 (6.8)	144.2 (5.0)	168.8 (6.9)	219.3 (9.8)
NE,	11.0	8.7	9.6	12.1

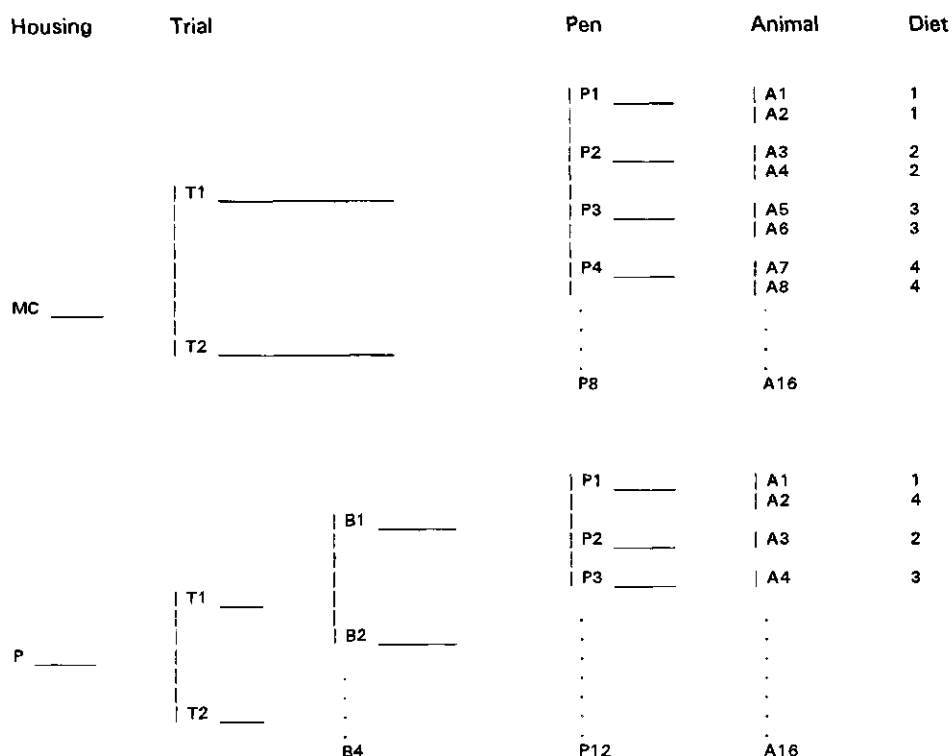
The markers

Cr₂O₃ was added at an amount of 0.5 g kg⁻¹ to the basal feed. This was to ensure a concentration of chromium of between 0.5 and 2.0 g kg⁻¹ in the dry faeces, in order to minimise the risk of errors related to a higher dilution factor with analysis (Lee et al 1986). However, due to the differing proportions of basal feed in the experimental diets, the concentration of Cr₂O₃ per kg feed differed between diets (Table 3.1). Before addition to the basal feed, Cr₂O₃ was mixed thoroughly with maize starch as carrier in the proportions (w/w) 1:3 and ground through a 0.5 mm sieve, to ensure a more even distribution in the feed. No extra AIA was added, since it was not found to be necessary (McCarthy et al 1974, 1977). Chromium is a part of the AIA-fraction, but it was assumed that this did not have any effect on the separate evaluation of the markers.

Experimental design and measurements

Comparative evaluation of apparent digestibility in the four feeds, was carried out using both the classical method (CM), collecting faeces quantitatively during a fixed period, and the marker method (MM), using either Cr₂O₃ or AIA as marker.

The classical method was carried out in two trials using two animals per feed per trial (Scheme 3.1). One trial started in spring and one in autumn. Within each trial the animals had the same father and had approximately the same age. The animals were kept in metabolism cages (CMmc) from 10 days before the start until the end of the collection period (another 10 days). Otherwise the animals were housed in pairs (per feed) in pens in the same department (Scheme 3.1).



Scheme 3.1

Experimental design per housing system, with MC = metabolism crate, P = pen, T = trial, B = block and A = animal

Faeces were collected during a period of 10 consecutive days at 90 kg live weight. Twice daily (after each feeding time) all faeces were collected and stored at -18 °C. The recovery of both markers, based on collection of 10 days, was calculated by dividing the total amount of marker in faeces by the total amount of marker given in the diet (Petry and Enders 1974).

After the collection period, the same pigs remained in the metabolism cages for three more days, in order to collect faeces for estimating digestibility using the marker method (MMmc). During these three days grab samples of fresh faeces were collected six times daily. The samples were collected twice during the hour preceding feeding and four times during 30 min, 2 h and 5 h after feeding. The faeces excreted outside these periods, the extra-faeces, were collected separately. The collected samples were stored in a refrigerator in a closed container for each pig. After each collection day, the samples were stored at -18 °C in the closed containers. Calculations were performed on the

grab sampled faeces (MMmc-g) and on the total faeces, being the sum of grab sampled faeces and the extra-faeces. The total faeces allowed a calculation of the recovery of both markers, based on collection for three days.

The grab sampling method was also carried out on 16 other animals housed in pens (MMP-g). Two trials were performed with two blocks in each trial (see Scheme 3.1). Both trials were performed at about the same time. Within each trial, the animals were selected with similar date of birth and genotype. Within each trial the animals between blocks differed only in live weight. Each block comprised four animals, fed the same four diets described previously. These animals were housed in groups of four animals and fed individually. Since this experiment was part of a larger experiment, these animals were not housed together in one pen. Within each block, the animals fed the control feed and the feed with added fat, were housed in one pen; the animals fed the cellulose feed and the soya bean hulls-feed were housed in a second and a third pen respectively (Scheme 3.1). Digestibility measurements were performed at 90 kg live weight. Faeces were collected and stored as for MMmc-g.

After finishing the collection period, the faeces were weighed, defrosted, mixed and a sample was taken for analyzing dry matter and crude protein content. The remainder was air-dried, ground through a 1 mm sieve and analysed for organic matter, chromium and AIA content.

All the digestibility coefficients obtained with the markers are the values as such and are not corrected for recovery.

Analytical procedures

Feed and faecal samples from each method were analysed in duplicate, so in total six analyses were done in the feed (two samples from each trial in metabolism cages; one sample from the trials in pens). The analyses were performed according to official Dutch procedures (NNI 1992), of which most procedures are comparable to those of the AOAC (1984). Nitrogen (N) content was determined by the Kjeldahl method. In faeces this analysis was done in fresh samples. All other analyses were done in air dried samples. Crude protein was calculated as Kjeldahl N x 6.25. Dry matter was determined by drying to constant weight at 103°C. Ash content was determined by ashing the sample for 3 h at 550°C. AIA content was determined by following the same procedure as for analyzing ash content. Subsequently the obtained ash was boiled in 3M HCl for 15 minutes. After filtering through an ash-free filter and washing with boiling water until free of acid, the sample was ashed again at 550°C for 30 minutes. Crude fibre content was determined by boiling the sample first in 0.13 M H₂SO₄ and then in 0.23M KOH. The residue was dried and ashed for 1 h at 550°C. Crude fat content was determined by boiling the sample in 3M HCl for 3 h. The filtered, washed and dried residue was extracted using petroleum ether bp 40-60°C. Because of the high fat content, the feed with added fat was pre-treated with petroleum ether. Chromium content was determined by an atomic

absorption spectrophotometer (Perkin-Elmer model 1100, at 357.9 nm), after Cr(III) was oxidised to Cr(VI) and the sample had been subsequently ashed at 550 °C.

Statistical analysis

The apparent digestibility coefficients of organic matter (dOM) and crude protein (dXP) were statistically analysed at two levels; the effect of the composition of the feed on the (difference between) methods and the effect of method within the feed. This was performed using the statistical software GENSTAT (Payne et al 1987).

Firstly, the effect of the dietary composition on dOM and dXP in relation to the method applied, was tested statistically in two models. For effect of housing system (method between animals) the following model was used:

$$Y_{ij} = \mu + H_i + D_j + (H \times D)_{ij} + e_{ij} \quad (1)$$

where μ is the mean; H is the housing system ($i = 1,2$); D is the diet ($j = 1,2,3,4$), $(H \times D)$ is the interaction; and e represents the residual error involved. For the recovery (R) and the methods within animals (metabolism cages), the difference between methods in dOM and dXP (δ dOM and δ dXP, respectively) and between recoveries obtained after collecting faeces for 10 or for 3 days (δ R), were tested using the model:

$$Y_{ij} = \mu + T_i + D_j + e_{ij} \quad (2)$$

where μ is the mean; T is the trial ($i = 1,2$), D is the diet ($j = 1,2,3,4$); and e is the error.

Secondly, within the feed treatments the different methods for obtaining dOM and dXP were compared using a Students t -test. Therefore, comparisons between animals (housing system) involved the comparison of the means of both methods. Differences between methods applied within animals (in metabolism cages) were tested against the hypothesis that there was no difference. Therefore, it was examined whether dOM and dXP were influenced by:

- (1) housing system (individually in metabolism cages or in groups in pens)
 - (2) method applied (classical method or marker method, including faeces collection method)
 - (3) marker recovery (Cr_2O_3 or AIA, including the required number of days to collect faeces),
- in relation to dietary composition.

Results and discussion

Using the grab sampling procedure, sometimes no faeces were collected from the pigs fed the low fibre feeds. In such cases, faeces were collected for one or two more days only from those animals in pens (MMp-g). For MMmc-g this resulted in one missing value for the control feed.

Effect of housing system

In general, lower values were observed for both dOM and dXP for pigs in pens (MMp-g), compared to pigs in metabolism cages (MMmc-g) (See Table 3.3). The lowering effect of housing in pens on dXP was larger than the effect on dOM. Much higher values of dOM and dXP were recorded with pigs housed in cages fed cellulose feed with AIA as a marker (significant effect of interaction between housing system and dietary composition using AIA ($P < 0.05$ for dOM and $P < 0.01$ for dXP; Table 3.2). With this cellulose diet, large differences were found in faecal AIA concentration between animals.

Table 3.2

Statistical analysis of effects of dietary composition (feed), housing system and the interaction on organic matter and crude protein digestibilities (dOM and dXP respectively) and the effect of dietary composition on marker recovery (recovery, R); the length of the faecal collecting period on recovery (length, δR) and the method of measurement (method; classical method versus marker method) on dOM and dXP (δdOM and δdXP , respectively) in relation to the marker, chromic oxide (Cr_2O_3) or acid-insoluble ash (AIA)¹

Statistical factor	Feed		Housing system		Feed.Housing	
	Cr_2O_3	AIA	Cr_2O_3	AIA	Cr_2O_3	AIA
<i>Between animals</i>						
dOM	***	***	***	ns	ns	*
dXP	***	***	***	ns	ns	**
<i>Within animals</i>						
Method:						
δdOM	ns	ns				
δdXP	ns	*				
Recovery (R)	ns	***				
Length (δR)	ns	ns				

¹ ns: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Table 3.3

Mean dOM and dXP (SD in parentheses) obtained by the marker method with pigs in metabolism cages (MMmc-g) or in pens (MMp-g), with chromic oxide (Cr_2O_3) and acid-insoluble ash (AIA) as markers¹

		dOM		dXP	
		MMmc-g	MMp-g	MMmc-g	MMp-g
Control feed	Cr_2O_3	90.7 ^a (0.03)	88.4 ^b (0.60)	87.9 ^a (0.74)	82.1 ^b (2.06)
	AIA	94.8 ^a (1.85)	92.1 ^a (0.79)	93.4 ^a (1.92)	87.7 ^b (1.94)
Cellulose	Cr_2O_3	67.1 ^a (2.31)	62.6 ^b (1.12)	75.2 ^a (3.79)	72.2 ^a (2.63)
	AIA	66.6 ^a (15.22)	81.3 ^a (1.69)	75.3 ^a (9.61)	86.2 ^a (0.99)
Soya bean hulls	Cr_2O_3	85.7 ^a (1.16)	83.5 ^b (0.88)	77.5 ^a (2.84)	72.9 ^b (2.22)
	AIA	90.8 ^a (0.37)	89.1 ^b (1.12)	85.6 ^a (1.37)	82.2 ^a (2.38)
Animal fat	Cr_2O_3	88.9 ^a (0.77)	87.2 ^a (1.50)	89.1 ^a (1.04)	84.4 ^b (3.03)
	AIA	91.7 ^a (1.66)	91.8 ^a (1.61)	91.9 ^a (1.93)	90.0 ^a (2.74)

¹ Means with different superscript in the same row differ significantly ($P < 0.05$) for the same digestibility coefficient

This variation was much larger between the animals in metabolism cages as between the animals in pens. This might explain the apparently contradictory effects with this feed, compared to the other feeds.

With all feeds, lower digestibilities were observed using Cr_2O_3 , for pigs in pens ($P < 0.001$ for both dOM and dXP; Table 3.2). However, with added fat only dXP was significantly lower in the pigs housed in pens (Table 3.3). Likewise, with the cellulose feed only dOM was significantly lower. No interactions between dietary composition and housing system were observed, using Cr_2O_3 (Table 3.2).

Animals housed in pens are more active than in metabolism cages. This difference may be enhanced by being housed in groups versus being housed individually. Metz and Dekker (1985) found that due to housing in pens, retention time of digesta in the gut decreased, which resulted in on average 1.8 % lower digestibility coefficients for dry matter. This effect was found to be enhanced by feeding a fibrous feed (46% hominy feed, 29% coconut expeller, 10% dried potato pulp) compared to a cereal feed (54% barley, 30% wheat). So, not only the housing system, but the fibrous feed itself also can have a depressing effect on retention time (Kass et al 1980) and on digestibility. On the other hand, Mateos and Sell (1981) showed that adding fat to a diet decreased

rate of food passage in laying hens. In the present trials using Cr_2O_3 , the reduction in dOM was largest on the cellulose diet and smallest on the fat diet. This indicates that the effects on passage rate of feed and housing system are not additive, although no significant interaction was observed between dietary composition and housing system (Table 3.2). Thus, no simple correction factor can be calculated to adjust for differences between digestibility coefficients obtained for pigs in metabolism cages or for pigs in pens.

It can be concluded that when evaluated with Cr_2O_3 as marker, the digestibility was lower in pigs housed in groups in pens compared to pigs housed individually in metabolism cages. A larger depression was observed in dXP, than in dOM. The results obtained with AIA varied considerably (Table 3.3).

Comparison between the classical method and the marker method

Comparing the marker method (MMmc-g) to the classical method (CMmc-t), significantly higher digestibility coefficients ($P < 0.05$) were obtained, ranging from 3.5 to 6.5 units for δdOM and from 4.8 to 9.5 units for δdXP , when AIA was used as a marker (Table 3.4).

Table 3.4

Mean dOM and dXP (SD in parentheses) obtained from pigs in metabolism cages, using the classical (CMmc-t) or the marker method (MMmc-g) with chromic oxide (Cr_2O_3) and acid-insoluble ash (AIA) as markers¹

		dOM		dXP	
		CMmc-t ²	MMmc-g	CMmc-t ²	MMmc-g
Control feed	Cr_2O_3	90.7*(0.43)	90.7*(0.03)	87.9*(0.73)	87.9*(0.74)
	AIA	90.7*(0.43)	94.8 ^b (1.85)	87.9*(0.73)	93.4 ^b (1.92)
Cellulose	Cr_2O_3	66.4*(2.27)	67.1* (2.31)	76.9*(2.39)	75.2*(3.79)
	AIA	66.4*(2.27)	66.6*(15.22)	76.9*(2.39)	75.3*(9.61)
Soya bean hulls	Cr_2O_3	84.3*(1.53)	85.7*(1.16)	76.1*(1.52)	77.5*(2.84)
	AIA	84.3*(1.53)	90.8 ^b (0.37)	76.1*(1.52)	85.6 ^b (1.37)
Animal fat	Cr_2O_3	88.2*(1.41)	88.9*(0.77)	87.1*(2.01)	89.1*(1.04)
	AIA	88.2*(1.41)	91.7 ^b (1.66)	87.1*(2.01)	91.9 ^b (1.93)

¹ Means with different superscript in the same row differ significantly ($P < 0.05$) for the same digestibility coefficient

² The value obtained with CM is independent on the type of marker.

For both parameters the largest differences were observed with feed containing soya bean hulls and the smallest with the feed containing extra fat. No significant differences were observed on the cellulose feed, but large standard deviations were measured, as mentioned in the previous section. Moreover, there was a significant effect ($P < 0.05$) of dietary composition on δdXP (Table 3.2). In contrast, with Cr_2O_3 no significant differences were observed between both methods for each feed.

Differences between the classical method and the marker method can be attributed to either the method itself (collecting faeces quantitatively for 10 days and collecting grab samples during 3 days, respectively) or the quality of the marker. Recoveries of both markers will be discussed in the next paragraph. Concerning the method, no literature was found indicating differences in digestibility coefficients due to the sampling method. McCarthy et al (1977), Furuya et al (1982) and Rundgren and Haglund (1988) showed no differences in results obtained with a marker when faeces were obtained either by grab sampling or by quantitative collection. However, Moughan et al (1991) suggested that grab samples of faeces should be collected for at least 5 consecutive days. Their conclusion might have been influenced by the fact that they collected only 20 g faeces per day. In the present trials, the minimum amount of fresh faeces excreted by the animals at any one time (of the six times on each of the three collection days) was 27 g. After the collection period was completed, the minimum weight of the accumulated sample was 241 g. So the grab sampling collection procedure for 3 days is considered to be representative for collecting faeces quantitatively with both markers in pigs.

It can be concluded that for pigs in metabolism cages, the Cr_2O_3 marker method resulted in similar digestibility coefficients as compared to the classical method, irrespective of dietary composition. The marker AIA gave either much higher values or large standard deviations in case of the cellulose diet (Table 3.3).

Recovery of the markers

The recoveries (calculated after collecting faeces for 10 days) of AIA, ranged from 97% on the cellulose feed up to 183% on the control feed and showed large standard deviations (Table 3.5). Compared to the control feed, increasing the crude fibre content reduced the recovery of AIA significantly (dietary composition ($P < 0.001$); Table 3.2). The recoveries of Cr_2O_3 however, varied between 99% for the feed with extra fat to 106% for the control feed as well as the feed with soya bean hulls (Table 3.4). No significant effect of dietary composition was shown (Table 3.2).

Similarly, Sugimoto (1984) showed a 10 % lower recovery of AIA and no effect on recovery of Cr_2O_3 , when increasing the crude fibre content by adding 20% beet pulp to a control diet. In contrast, Rundgren and Haglund (1988) found no significant difference in recovery for AIA when increasing the crude

Table 3.5

Mean recoveries (in %) of chromic oxide (Cr_2O_3) and acid-insoluble ash (AIA) (SD in parentheses) after collecting faeces for 10 or 3 days¹

		10 days		3 days	
Control feed	Cr_2O_3	106*	(7.7)	108*	(17.2)
	AIA	183*	(47.4)	151*	(82.0)
Cellulose	Cr_2O_3	102*	(4.6)	98*	(7.7)
	AIA	97*	(32.6)	105*	(25.2)
Soya bean hulls	Cr_2O_3	106*	(3.2)	109*	(13.6)
	AIA	164*	(5.0)	166*	(19.5)
Animal fat	Cr_2O_3	99*	(10.3)	103*	(13.1)
	AIA	140*	(29.5)	153*	(49.7)

¹ Means with different superscript in the same row differ significantly ($P < 0.05$)

fibre content from 35 to 75 g kg⁻¹ and Petry and Enders (1974) showed a 2-5% lower recovery of Cr_2O_3 , by increasing the crude fibre content to 200 g kg⁻¹ by adding polyethylene to a control diet.

Recoveries of both markers in this study are not in line with those reported in literature. Usually AIA recoveries were found to be closer to 100% and concluded to be a better marker, than Cr_2O_3 (McCarthy et al.; 1974; Sugimoto; 1984), except when high-fibre diets were used (Sugimoto, 1984).

There are three main aspects in which the described studies differ from the present ones. Firstly, most recoveries of markers were calculated using the classical method as well as the marker method, based on faeces quantitatively collected during 3-5 days. Jongbloed (1987) indicated that the variance in dry matter digestibility coefficients more than doubled with a collection period of 5 days compared to 10 days. Since the recovery is a ratio between the classical and marker method (Petry and Enders 1974, Wünsche et al 1984), both methods influence the recovery. When recovery was calculated after collecting faeces quantitatively for 3 days, the differences with the recovery obtained after 10 days did not differ significantly (Table 3.2). However, in all but one case, the standard deviations were much larger (Table 3.5). This could, for example, be explained by the daily variation in faeces excretion or to systematic errors in weighing feed or faeces. Therefore, in order to perform a correct test using markers, quantitative collection of faeces should last more than 3 days.

Secondly, the analytical methods applied to determine the markers were

different. In the literature, the common method of analysis for AIA, is the method described by McCarthy et al (1974). According to this method, the sample as such is added to 4M boiling HCl. According to Dutch standard procedures, ash obtained by proximate analysis, is added to 3M boiling HCl. Almost all recoveries of AIA obtained were higher than 100%, indicating more AIA was apparently recovered in faeces than was given by the feed. In the gastro-intestinal tract of pigs many salts are formed, which could disturb the analysis. Using a strong acid, these salts are meant to be dissolved and only AIA should remain. However, in the present method, these salts probably remain insoluble, while with the other method they might have been soluble. Den Hartog et al (1988) also found significantly higher recoveries (although not as high as in the present study) using AIA (analyzed with the same procedure as we used) as compared to either Cr_2O_3 or the classical method.

In the literature, the effects of analytical methods for Cr_2O_3 were also observed. For example, Saha and Gilbreath (1991) showed significant effects on recovery when varying the concentration of Ca, P or Mg. Since minerals were supplied with the basal feed, in accordance with animal requirements and not in excess, the detrimental effects that PO_4 can have on recovery of chromium may have been reduced.

Thirdly, Cr_2O_3 was added to the feed after mixing with a carrier, to improve a thorough distribution in the feed. Similarly, using Cr_2O_3 paper (as compared with powder) resulted in a better recovery of Cr_2O_3 (Furuya et al 1982). The effect of both pretreatments are probably the result of a more homogenous mixture of the marker in the feed (Corbett et al 1960).

Using described techniques and methods, it can be concluded that Cr_2O_3 has a good recovery, which is not affected by dietary composition. Under these conditions, AIA can not be considered as suitable.

Conclusions

For pigs housed as groups in pens, digestibility was found to be lower compared with pigs housed individually in metabolism cages. When using Cr_2O_3 as a marker, this effect averaged 2.5 (1.7 - 4.5) units for dOM and 4.5 (3.0 - 5.8) units for dXP. Particularly for high-fibre diets, the difference in apparent digestibility is highest. This can result in a reduction of up to 5% in energy value (NE_i) of the feed for pigs in pens. Therefore, it can be concluded that for practical application, in practice, digestibility coefficients should be estimated with pigs housed as groups in pens. Whether this is also true for pigs, which are individually housed in pens, is not known.

Estimating digestibility in pens can be done accurately by using chromic oxide, which is found to be a good marker with a recovery approximating 100% irrespective of the dietary composition. AIA is considered to be unsuitable,

because recovery of AIA is dependent upon dietary composition and much larger between-animal variations were observed, than with chromic oxide. More research is needed to evaluate the effect of type of analytical method on the results for AIA.

For estimating the recovery of a marker, a period of more than 3 days should be allowed for total collection of faeces.

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Chapter 4

Nutrient apparent digestibility and the performance of growing fattening pigs as affected by incremental additions of fat to starch or non-starch polysaccharides

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Nutrient apparent digestibility and the performance of growing fattening pigs as affected by incremental additions of fat to starch or non-starch polysaccharides

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Abstract

In a factorial design, animal fat was added incrementally (0, 35, 70 and 105 g/kg) to maize starch (M) or to two sources of fermentable carbohydrates (260 g purified cellulose (C) per kg or 270 g soya bean hulls (S) per kg). The 12 experimental diets were formulated by replacing maize starch in the control diet with fat, cellulose and soya bean hulls of equivalent estimated net energy. Ninety-six castrated males were given these diets according to net energy. Apparent digestibilities of crude protein, crude lipid, crude fibre and nitrogen-free extract were measured and were compared with those expected from the separate ingredients. Net energy conversion ratio (nECR) was also measured. Results showed that in the C and the S diets prediction of the apparent digestibility of nutrients was worse than in the M diets (significant effect of source of carbohydrate). Prediction of apparent digestibility of crude protein and crude lipid improved as the added fat increased (significant effect of amount of fat), except in the C and S diets where for digestible crude lipid the 70 g added fat per kg gave the worst prediction (significant effect of the interaction). The net energy calculated from the experimental data on apparent digestibility was proportionately between 0.83 and 0.98 of that calculated from the expected data. The pigs on the C and S treatments showed a significant lower nECR when calculated from the expected apparent digestibility coefficients, but not when calculated from those which were measured. At the highest fat addition, the nECR was poorest. It is concluded that the amounts of digestible components in compound feeds cannot always be obtained from those in the separate ingredients.

Keywords: carbohydrates, digestibility, fat, performance, pigs

Introduction

In many countries the energy of pig feeds is evaluated by considering the differential contribution of digestible nutrients to energy supply. This evaluation

is based on two assumptions: (1) that both the chemical composition and the digestibility coefficients of ingredients in a feed are known accurately and can be derived from feedstuff tables; and (2) that the amounts of digestible nutrients in the ingredients are additive and that there are no interactions between ingredients (Centraal Veevoederbureau (CVB), 1988).

For example, the Rostock method (Schiemann, Nehring, Hoffmann, Jentsch and Chudy, 1971) is based on net energy and resulted from research on diets consisting mainly of cereals. In those diets, as in conventional diets, the main source of carbohydrate was starch. However, increased proportions of by-products are now being used in compound feeds. These by-products may contain a large proportion of non-starch polysaccharides (NSP), so to obtain a sufficient concentration of energy in compound feeds they are often supplemented with fat. However, Jongbloed, van Diepen and Smits (1986) demonstrated that pigs performed differently when offered diets with a similar calculated net energy supply but composed of different ingredients. Pigs given the diets composed of by-products plus supplementary fat (50 g/kg) performed worse than pigs given diets based on cereals or by-products without supplemented fat. This disparity in performance was attributed either to an overestimation of fat or to an interaction between fat and NSP.

The study reported here aimed to resolve this uncertainty by quantifying the effects of adding incremental amounts of fat to starch or to two different sources of fermentable carbohydrates, and by investigating the interaction between amount of fat and source of carbohydrate. Therefore, a factorially designed experiment was initiated and data were collected on the apparent digestibility of those nutrients that contribute to energy supply and on performance.

Material and methods

Feeds and feeding

Twelve diets were tested in a 4*3 factorial design with four amounts of animal fat and three sources of carbohydrate. The amounts of animal fat added to the diets were : 0 (o), 35 (l), 70 (m) and 105 (h) g per kg. The fatty acid composition of the animal fat used was: 26 g/kg < C16:0; 223 g/kg C16:0; 49 g/kg C16:1/C18:0 iso; 138 g/kg C18:0; 393 g/kg C18:1; 128 g/kg C18:2 and 44 g/kg > C18:2. The three sources of carbohydrate were maize starch (M), purified cellulose (C) (Arbocel B800, J. Rettenmaier, Holzmühle, Germany) and toasted soya bean hulls (S). The cellulose was used as a source of poorly fermentable by-products and the soya bean hulls as a source of easily fermentable by-products. Their maximum intake by pigs was ascertained in a preceding feeding trial. It was found that 320 g cellulose or 280 g soya bean hulls could be added per kg feed without problems. The amount of digestible

nutrients in the same batch of soya bean hulls and cellulose and thus their calculated net energy (NE_e) content, were measured in separate digestibility trials, using the method described by Šebek (1989).

Each kilogram of the control diet (Mo) contained 510 g basal diet, supplying all necessary amino acids, minerals, vitamins and chromium III oxide (Table 4.1), and 490 g maize starch. The chromium III oxide was used as an indigestible marker, to enable the apparent digestibility of the diets to be determined. It was added to the diet according the method described by Bakker and Jongbloed (1994). To ensure that the composition of the basal diet was identical in all experimental treatments, one large batch sufficient for the whole experiment containing all the ingredients except the cane molasses, was mixed. The correct proportion of molasses was added to the final diets.

Table 4.1
Ingredients (g) per kg basal diet

Barley	274
Soya bean flour	263
Potato protein	107
Wheat middlings	195
Soya oil	18
Cane molasses	58
Alfalfa	39
Limestone	19
Ca(H ₂ PO ₄) ₂ · 1H ₂ O	15.6
NaCl	3.9
Trace mineral/vitamin pre-mix ¹	3.2
Choline chloride	0.5
S-DL methionine	1.2
L-lysine HCL	1.4
Cr ₂ O ₃ -starch mixture	2.0

¹ Trace mineral/vitamin pre-mix (mg/kg basal diet): 983 FeSO₄ · 7H₂O, 114 MnO, 354 ZnSO₄ · 1H₂O, 5 KI, 37 retinol/cholecalciferol (500/100 IU/mg), 11 riboflavin (0.80), 46 cyanocobalamin, 37 α-tocopherol, 46 niacin, 18 Ca pantothenate, 16 Na₂SeO₃, 286 antioxidant and 1247 carrier.

In the other 11 experimental diets, an amount of the maize starch in the control diet (Mo) was exchanged with the amounts of fat, cellulose and soya bean hulls, to supply the equivalent net energy (NE). Figure 4.1 shows the amounts (g) of the different diets yielding the same total NE.

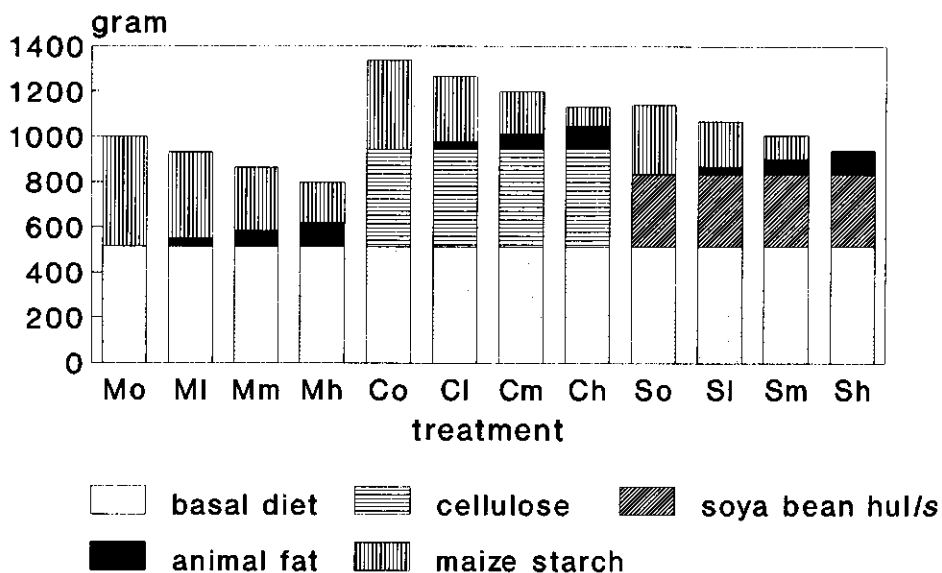


Figure 4.1

The amounts (g) of the 12 experimental diets yielding the same total assumed net energy

As a result, the 12 diets had different estimated NE_i per kg. Immediately after the diets had been mixed, samples were taken for chemical analysis. All the diets were made up simultaneously in an amount sufficient for all trials. They were pelleted without adding steam and stored at -18°C until use.

The daily feed allowance was offered in two equal portions; one at 7.00 h and one at 14.00 h. The amount supplied was based on the energy required to maintain a growth rate of 700 g/day (CVB, 1988). This was intended to supply 14.8 MJ NE_i per day at 40 kg live weight, 23.1 MJ at 70 kg live weight and 27.8 MJ at 100 kg live weight. Before it was offered, the feed was soaked in water, using a fixed ratio of water to assumed net energy supply of 0.38 l/MJ NE_i. The pigs had no access to other sources of water supply.

Animals and housing

Castrated male pigs were used, because they can eat more than boars and gilts and they enable urine and faeces to be collected separately. The 96 pigs used were from a three-way cross Yorkshire ♂ x (Finnish Landrace ♂ x Dutch Landrace ♀)♀. They were each assigned to one of the 12 diets and received that diet continuously from 30 to 105 kg live weight. They were housed in groups of

four in a pen on a partially slatted floor (2.4 m² solid floor and 1.8 m² slatted floor). At each feeding time the pigs were admitted to their individual feeding pens (0.5 m² each), were locked in and given their feed. Approximately 30 min later, when they had finished eating, the troughs were closed and the pigs were moved back to their pens. The feed and the water for the next meal were then put into the trough. This ensured that there was adequate soaking of the feed and that the pigs could start eating at the same time. Each pig had its own individual feeding pen during the experiment and was housed with the same penmates.

Experimental design

The treatments were assigned according to a split-plot design (Figure 4.2). Each block comprising the 12 treatments represented three pens and each pen represented one source of carbohydrate. Within each pen of four pigs, each pig was assigned one of the four amounts of fat. One replicate represented two blocks; two trials were performed with two replicates in each trial. One trial started in spring and one in autumn.

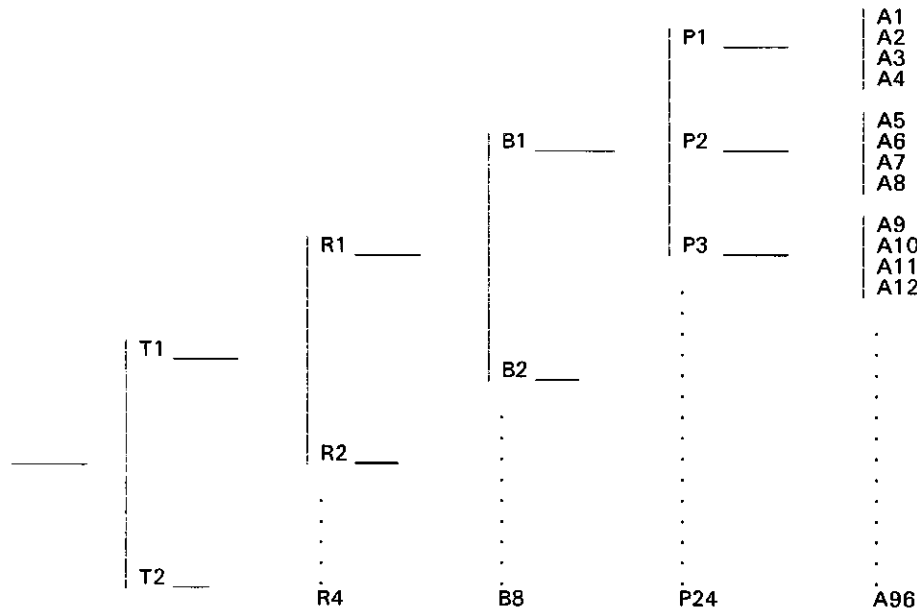


Figure 4.2
 Experimental design, with T = trial, R = replicate, B = block, P = pen and A = animal; within one block the three sources of carbohydrates were allotted to the three pens; within each pen the four amounts of fat were allotted to the four animals (split plot design)

For each replicate, 28 pigs were selected from a batch of eight or nine litters. The pigs were approximately the same age and had as few sires as possible. When between 15 and 20 kg live weight, the selected 28 pigs were split into two groups of 14 pigs, one lighter than the other. Within each group, two pigs were reference animals and the other 12 were allotted to the 12 treatments of one block. The reference pigs were zero controls for a slaughter experiment, which was performed with the same pigs. The results of this slaughter experiment and the methods applied are described elsewhere (Bakker et al., 1996). After the pigs were assigned, they were housed in their pens. They remained at least 2 weeks on the same starter feed as before assignment. This enabled them to become accustomed to the pen, their penmates and the feeding system. At about 30 kg, the pigs were weighed (initial live weight) and were gradually introduced to the experimental diets by being given increasing amounts of them and decreasing amounts of the starter feed over a period of 4 days.

Measurements

The animals were weighed weekly, and their feed was allocated on the basis of this weight and the growth expected in the following week. In each trial, composite samples of each diet were taken while the feed portions were being prepared, for chemical analysis. Feed not eaten were collected daily from the troughs, stored at 4 °C and for each animal the totals of 1 week were weighed and analysed for dry matter (DM) content. Apparent digestibility was measured at two live weights, 60 and 90 kg, using chromium III oxide as a marker. The faeces were collected and stored as described by Bakker and Jongbloed (1994). At the end of each period of 3 days, the faeces were defrosted, mixed and a sample was taken for analysing the contents of air-DM, DM and nitrogen (N). Air-dried faeces of two pigs with the same treatment within one replicate were pooled by combining similar amounts of air-DM. This pooled sample was ground through a 1-mm sieve and analysed for ash, crude fat (XL), crude fibre (XF) and chromium content. The mean contents in air-DM of DM and N were calculated from the contents in the samples of each of the two pigs involved. Four measurements of apparent digestibility per treatment were obtained at 60 kg, and four at 90 kg live weight. For each treatment eight observations on performance were obtained. The apparent digestibility coefficients were used together with data from chemical analysis, to calculate the NE_i from the equation:

$$NE_i \text{ (kJ/kg)} = 10.84 \cdot DXP + 36.11 \cdot DXL + 6.28 \cdot DXF + 12.68 \cdot DXX \quad (1)$$

(DXP, DXL, DXF and DXX in g per kg; CVB, 1988)

The following three NE_i values, which varied in the combination of the source of variables, were compared; (1) the assumed NE_i , for which the chemical composition of the separate ingredients and their digestibility coefficients were obtained from the Dutch feedstuff table (CVB, 1988); (2) the estimated NE_i , for which the chemical composition of the final experimental diets was obtained from analysis from the present experiment and the digestibility coefficients of the separate ingredients were obtained from the feedstuff table; (3) the calculated NE_i , for which the chemical composition and the apparent digestibility coefficients of the final experimental diets were obtained from the present experiment.

When the fastest grower had reached a live weight of about 105 kg, the total amount of assumed net energy consumed during the trial by this pig became the standard for the other pigs. Once an animal had consumed this standard amount of assumed net energy, its final live weight was measured 3 to 4 hours after the morning feeding. It was expected that the different sources of carbohydrate would result in large differences in gut fill (Kass, Van Soest, Pond, Lewis and McDowell, 1980; Jongbloed and Hoekstra, 1985), making the rates of gain less comparable between treatments. Therefore, in order to exclude the effect of gut fill, the empty body weight was measured. This was done by euthanizing the animals with barbiturate immediately after the final weighing, removing the gastrointestinal tract and the bladder, and then weighing these before and after emptying.

At the beginning of the experiment the 16 reference pigs (two pigs per block) were also euthanized and their intestinal content was weighed. The ratio between empty body weight and live weight of these young pigs was 0.91 (s.e. 0.02). The empty body weight gain (EBG) for the experimental pigs was calculated by subtracting the calculated initial empty body weight ($0.91 \times \text{initial live weight}$) from the measured final empty body weight. The EBG per day (EBGd) was calculated. Two types of net energy conversion ratio (NECR) were calculated by dividing the total intake of estimated net energy or calculated net energy, respectively, by EBG.

Chemical analysis

This experiment was part of a larger project. When this experiment was completed, three feed samples for each treatment were chemically analysed. These are listed in Table 4.2. However, in all, between three and 18 feed samples were analysed per treatment in the entire project. Feed and faecal samples from each treatment were analysed in duplicate, following official Dutch protocols (Nederlands Normalisatie Instituut, 1992) (NEN), most of which are comparable with those of the Association of Official Analytical Chemists (AOAC, 1984). All samples were air-dried before analysis, except in the case of faecal samples, in which N content was analysed (NEN 3145) when fresh. The air-dried samples were analysed for DM (NEN 3332), crude fibre (NEN 5415),

crude lipid (EG L15/29, method B (pre-treatment with hydrochloric acid)), chromium (as described by Bakker and Jongbloed, 1994), starch (NEN 3574) and sugar (NEN 3571). The starch and sugar content were subtracted from the nitrogen-free extraction fraction (XX) to give the non-starch fraction (nsXX).

Statistical analysis

One pig was the experimental unit for assessing performance (mean EBGd and nECR; see Figure 4.2). An analysis of variance was performed, using GENSTAT (Payne, Lane, Ainsley, Bicknell, Digby, Harding, Leech, Simpson, Todd, Verrier and White, 1987) and the following model:

$$Y_{hiklm} = \mu + T_h + R_{(th)i} + B_{(th)ij} + Carb_k + e1_{hijk} + F_l + (Carb * F)_{kl} + e2_{hiklm} \quad (2)$$

where μ = overall mean; T_h = effect of trial; $R_{(th)i}$ = effect of replicate within trial; $B_{(th)ij}$ = effect of block within replicate within trial; $Carb_k$ = effect of source of carbohydrate; $e1_{hijk}$ = error contributed by the main plot (T, R, B and Carb); F_l = effect of amount of added fat; $(Carb * F)_{kl}$ = interaction between source of carbohydrate and amount of added fat; $e2_{hiklm}$ = error contributed by the subplot (F) and the interaction; $h = 1, 2$; $i = 1, 2$; $j = 1, 2$; $k = 1 \dots 3$ and $l = 1 \dots 4$.

The experimental unit for assessing the apparent digestibility coefficients was two pigs subjected to the same treatment within one replicate. Therefore, the following model was used:

$$Y_{hiklm} = \mu + T_h + R_{(th)i} + Carb_k + e1_{hik} + F_l + (Carb * F)_{kl} + e2_{hiklm} \quad (3)$$

(variables as described for model 1). Firstly, it was tested whether *Carb*, *F* or *Carb * F* had a significant effect on the difference between apparent digestibility coefficients measured at 60 kg and at 90 kg, to check if it was permissible to use the mean of both figures.

Results

General

The animals in replicate 1 systematically left some of their feed. Therefore, the amount of feed was proportionately reduced to 0.90 of the initial scheme and the water supply was reduced from 0.38 to 0.33 l/MJ NE_p. This meant that the average feeding level was lowered from 3.2 to 2.8 times maintenance. The measurements at 60 kg live weight in the first replicate were obtained before the scheme was changed, but no differences in apparent digestibilities were expected between these feeding levels (Smits, Jongbloed and Šebek, 1994).

Four animals had to be removed from the trials. In the first trial one animal

was euthanized prematurely (treatment Mo), because severe leg problems suddenly appeared after the apparent digestibility measurements had been obtained. In the second trial two animals had to be removed (treatments Sl and Sh) because of an infection, and one animal was excluded (treatment Ch), because it appeared to be uncastrated.

Feed composition

Table 4.2 gives the analysed chemical composition of the 12 diets. In the course of the trials, chemical analysis of the diets revealed some discrepancy between the actual and intended composition. The composition of the diets (Table 4.3) was recalculated from the results of the chemical analysis (the average for the whole project) and the separate ingredients, using linear programming. The results showed that the C diets contained less cellulose than intended (from 26 g/kg less for Cl to 82 g/kg less for Ch). The fat content of the diets was also slightly less than intended, particularly in the m (medium fat) diets: 18 g/kg less for Mm, 8 g/kg less for Cm and 5 g/kg less for Sm.

Table 4.2

Analysed chemical composition of the experimental diets (g/kg dry matter (no. = 6) unless otherwise stated)

Diet ¹	Dry matter (g/kg)	Organic matter	Crude protein	Crude lipid	Crude fibre	nsXX ²	Starch	Sugar	NDF ³
Mo	892	950	174	30	29	265	453	87	91
Ml	887	948	186	66	31	255	414	81	102
Mm	894	943	203	101	35	192	392	68	119
Mh	906	940	209	169	35	212	286	75	129
Co	895	960	134	23	221	236	346	53	336
Cl	905	959	134	51	258	268	251	58	372
Cm	912	950	150	74	251	254	223	44	380
Ch	916	952	162	125	238	254	158	56	375
So	894	942	186	33	113	295	306	66	242
Sl	897	938	194	67	126	301	252	61	269
Sm	901	934	202	103	136	309	187	58	275
Sh	916	933	214	152	148	287	120	53	308

¹Diet: maize starch (M), cellulose (C) or soya bean hulls (S) were combined with four amounts of added fat (0 (o), 35 (l), 70 (m) and 105 (h) g/kg, respectively)

²nsXX = non-starch XX (=nitrogen free extract) minus (starch & sugar (no. = 2))

³NDF = neutral detergent fibre (no. = 2)

Table 4.3

Composition of the feeds; ingredients (g/kg feed), net energy value (NE_i) and estimated digestibility coefficients as recalculated by linear programming

Diet ¹	Basal diet	Maize starch	Cellulose	Soya bean hulls	Fat	Cane molasses	NE _i (MJ/kg feed)	dXP ²	dXL ²	dXF ²	dXX ²
Mo	538	349	-	-	-	112	9.6	0.84	0.76	0.30	0.93
MI	584	298	-	-	31	86	10.2	0.85	0.83	0.30	0.92
Mm	628	252	-	-	59	61	10.6	0.85	0.85	0.30	0.92
Mh	649	152	-	-	122	77	11.9	0.85	0.87	0.30	0.90
Co	417	262	261	-	-	61	7.6	0.84	0.74	0.42	0.84
CI	422	173	304	-	26	76	7.9	0.84	0.83	0.42	0.80
Cm	480	122	310	-	48	40	8.1	0.85	0.85	0.42	0.77
Ch	513	64	288	-	92	43	9.3	0.85	0.87	0.42	0.76
So	472	195	-	267	-	66	8.5	0.76	0.65	0.71	0.89
SI	485	139	-	290	33	54	9.1	0.76	0.78	0.72	0.88
Sm	506	69	-	314	61	50	9.5	0.76	0.82	0.73	0.86
Sh	523	-	-	343	108	26	10.5	0.76	0.84	0.73	0.83

¹For explanation of the abbreviations used, see Table 4.2

²dXP = digestible crude protein; dXL = digestible crude lipid; dXF = digestible crude fibre; dXX = digestible nitrogen-free extract

Hence the experimental diets contained slightly more basal diet than intended: on average, 41 g/kg (range 25 to 52 g/kg) for the M diets, 50 g/kg (range 26 to 71 g/kg) for the C diets and 17 g/kg (range -8 to +33 g/kg) for the S diets. From the preceding digestibility trials the apparent digestibility coefficients for cellulose and soya bean hulls were used and for the other nutrients from the Dutch feedstuff table (CVB, 1988) to calculate a corrected NE_i value (Table 4.3). This estimated NE_i was used in all subsequent calculations.

Apparent digestibility

The results on performance and apparent digestibility per parameter are discussed in the following order: effect of source of carbohydrate, effect of amount of fat and the effect of the interaction.

Statistical analysis of the differences between apparent digestibility coefficients at 60 kg and 90 kg live weight showed that the amount of fat, source of carbohydrate and the interaction significantly affected them all, except for digestible crude fibre (dXF). Therefore, in the Tables 4.4, 4.5 and 4.6 the results are given separately for both live weights. At 60 kg the apparent digestibility coefficients were lower than at 90 kg. The statistical effects of source of carbohydrate (Table 4.4), amount of fat (Table 4.5) or the interaction

Table 4.4

The effect of source of carbohydrate on the apparent digestibility of nutrients at 60 and at 90 kg live weight

Source of carbohydrate	Apparent digestibility coefficient ¹			
	dXP	dXL	dXF	dXX
At 60 kg live weight				
Mean				
Maize starch	0.81	0.74	0.01	0.92
Cellulose	0.72	0.71	0.07	0.78
Soya bean hulls	0.70	0.70	0.44	0.85
S.e.d.	0.004	0.014	0.086	0.005
F-probability	***	*	**	***
At 90 kg live weight				
Mean				
Maize starch	0.83	0.78	0.14	0.92
Cellulose	0.74	0.74	0.08	0.78
Soya bean hulls	0.74	0.71	0.60	0.87
S.e.d.	0.009	0.006	0.061	0.007
F-probability	***	***	***	***

¹ For explanation of the abbreviations see Table 4.3

Table 4.5

The effect of amount of fat on the apparent digestibility of nutrients at 60 and at 90 kg live weight

Amount of added fat	Apparent digestibility coefficient ¹		
	dXP	dXL	dXX
At 60 kg live weight			
Mean			
0 g per kg	0.73	0.59	0.88
30 g per kg	0.73	0.72	0.86
70 g per kg	0.74	0.75	0.83
105 g per kg	0.77	0.81	0.82
S.e.d.	0.006	0.011	0.006
F-probability	***	***	***
At 90 kg live weight			
Mean			
0 g per kg	0.76	0.63	0.89
30 g per kg	0.77	0.76	0.88
70 g per kg	0.77	0.76	0.84
105 g per kg	0.79	0.82	0.83
S.e.d.	0.007	0.010	0.005
F-probability	**	***	***

¹ For explanation of the abbreviations used, see Table 4.3

(Table 4.6) were also less significant at 60 kg than at 90 kg.

On average, the digestible crude protein (dXP) of the fibrous C and S diets was 0.09 units lower than that of the starch-rich M diets (effect of source of carbohydrate; $P < 0.001$; Table 4.4). The dXP increased at the largest amount of added fat (effect of amount of fat; $P < 0.001$ at 60 kg and $P < 0.01$ at 90 kg; Table 4.5). No significant effect of the interaction on dXP was found.

On average, at 60 kg, both fibrous diets showed a digestible crude lipid (dXL) 0.03 units lower than the M diets (effect of source of carbohydrate; $P < 0.05$; Table 4.4). At 90 kg this difference between the M diets and the S diets had increased to up to 0.07 units, resulting in an even more significant effect of source of carbohydrate ($P < 0.001$; Table 4.4). dXL was lowest at the zero fat addition (o) and increased concomitantly with the amount of added fat (effect of amount of fat; $P < 0.001$; Table 4.5). This increase from the zero added fat (o) to h fat addition was on average 0.08 units more in the M and the S diets than in the C diets (Table 4.6). It was not equally distributed among each amount of added fat, and it differed for the three sources of carbohydrate; hence interaction had a significant effect ($P < 0.05$ at 60 kg and $P < 0.001$ at 90 kg).

Table 4.6

Significant effects of the interaction between source of carbohydrate, amount of fat and the interaction on the apparent digestibility of nutrients at 60 and at 90 kg live weight

Diet ¹	Apparent digestibility coefficient ¹			
	at 60 kg live weight		at 90 kg live weight	
	dXL	dXX	dXL	dXX
Mean				
Mo	0.59	0.93	0.65	0.93
MI	0.75	0.93	0.78	0.93
Mm	0.79	0.91	0.81	0.92
Mh	0.84	0.90	0.86	0.91
Co	0.62	0.82	0.67	0.83
CI	0.70	0.79	0.76	0.81
Cm	0.74	0.75	0.74	0.73
Ch	0.79	0.74	0.80	0.75
So	0.55	0.88	0.58	0.90
SI	0.71	0.87	0.73	0.89
Sm	0.72	0.84	0.74	0.87
Sh	0.80	0.81	0.80	0.83
s.e.d.				
Carb	0.014	0.005	0.006	0.007
F	0.011	0.006	0.010	0.005
Carb*F	0.022	0.010	0.016	0.010
F probability				
Carb	*	***	***	***
F	***	***	***	***
Carb*F	*	**	***	***

¹ For explanations of the abbreviations used, see Tables 4.2 and 4.3; also Carb = source of carbohydrate; F = amount of added fat; Carb*F = the interaction between source of carbohydrate and amount of added fat.

The dXF was higher in the S diets than in the M diets and the C diets (effect of source of carbohydrate; $P < 0.01$ at 60 kg and $P < 0.001$ at 90 kg; Table 4.4). No effects of amount of fat or the interaction were found.

On average, compared to the M diets the mean digestible nitrogen free extract (dXX) was 0.06 units lower for the S diets and 0.14 units lower for the C diets (effect of source of carbohydrate; for both live weights $P < 0.001$; Table

4.4). As added fat increased, dXX fell with 0.06 units from the zero (o) to the h fat addition (effect of amount of fat; for both live weights $P < 0.001$; Table 4.5). At the largest amount of added fat the dXX was 0.07 units lower for the S diets and 0.08 units lower for the C diets (Table 4.6). On the other hand, only a small reduction of 0.03 units was found for the M diets (effect of interaction; $P < 0.01$ at 60 kg and $P < 0.001$ at 90 kg; Table 4.6).

Table 4.7

The effect of source of carbohydrate on empty body weight gain per day and estimated net energy conversion ratio

Source of carbohydrate	EBGd ¹ (g/day)	Estimated nECR ² (MJ NE _e /kg)
Mean		
Maize starch	744	26.4
Cellulose	702	29.7
Soya bean hulls	752	27.6
S.e.d.	14.1	0.42
F-probability	**	***

¹ EBGd = empty body weight gain per day

² nECR = net energy conversion ratio

Table 4.8

The effect of amount of fat on empty body weight gain per day and both the estimated and calculated net energy conversion ratio

Amount of added fat	EBGd ¹ (g/day)	Estimated nECR ¹ (MJ NE _e per kg)	Calculated nECR (MJ NE _e per kg)
Mean			
0 g/kg	761	27.2	25.8
30 g/kg	750	27.2	25.9
70 g/kg	725	27.6	25.5
105 g/kg	696	29.4	27.6
S.e.d.	15.6	0.53	0.49
F-probability	***	***	***

¹ for explanation of the abbreviations used, see Table 4.7

Performance

Data on EBGd and net energy conversion ratio (nECR) are given in the Tables 4.7 and 4.8.

The S diets resulted in the largest EBGd and the C diets in the smallest (effect of source of carbohydrate; $P < 0.01$; Table 4.7). The EBGd fell as amount of fat rose: this inverse relationship was especially marked for the Ch and Sh diets (effect of amount of fat; $P < 0.001$; Table 4.8).

The estimated nECR was poorest for the C treatments and best for the M treatments (effect of source of carbohydrate; $P \leq 0.001$; Table 4.7). In contrast, the calculated nECR (mean = 26.2 MJ NE_e/kg; s.e.d. = 0.41) was not significantly affected by source of carbohydrate. The poorest values for nECR occurred systematically in the h treatments (effect of amount of fat; $P < 0.001$; Table 4.8). No significant effect of interaction was observed.

Discussion

The study reported here aimed to quantify the effects on apparent digestibility and on performance of growing pigs, when they received similar amounts of estimated NE but from different ingredients. Therefore, diets were formulated with incremental amounts of fat added to starch or to two different sources of fermentable carbohydrates. In addition, it was aimed to investigate the interaction between amount of fat and source of carbohydrate. This issue was studied in two phases. First, the effect was studied of using the analysed chemical composition of the experimental feeds for obtaining the estimated NE_e, rather than using the data from the separate ingredients listed in the Dutch feedstuff table (CVB, 1988) as for the assumed NE_e. Secondly, the effect was studied of using the measured apparent digestibility coefficients of the experimental feeds to obtain the calculated NE_e, rather than using the data from the separate ingredients from the feedstuff table or from preliminary studies (Table 4.3) as for the estimated NE_e. Finally, the performance of pigs was studied in relation to energy conversion using the measured rather than the estimated amounts of digestible nutrients.

Feed composition

Although when preparing the experimental diets the exact amounts of the various components were added to the basal diet, the composition of the diets deviated from what was intended (as in Figure 4.1). However, when composition was recalculated, using data from the chemical analysis of the individual ingredients and of the final diet (Table 4.3), it was found that the integrity of the experimental design was retained even though the concentrations of ingredients were different. Moreover, the diets had been supplemented with sufficient amounts of basal diet and therefore all the pigs

received at least the amount of essential nutrients intended. Thus the differences were only in the energy supply. The cellulose diets contained 1.01 to 1.10 of the NE assumed initially, because the lower cellulose content was compensated by a higher content of basal diet, which had a higher NE content than cellulose. Because the pigs were given feed according to assumed NE, those fed the C diets received somewhat more NE than intended. On average, the estimated NE_i values of the M and the S diets were as intended, though in the Mm treatment the energy supply was proportionately 0.96 of the target, because the fat content was less than in the design.

From the above we conclude that it is difficult to predict the chemical composition of the final diet accurately. However, in the present experiment the diet compositions achieved are sufficiently valid for the results to be evaluated meaningfully.

Digestible nutrients

The apparent digestibility coefficients of feedstuffs are assumed to be known and to be constant throughout the growing period. However, Roth and Kirchgeßner (1984) demonstrated that apparent digestibility coefficients increase concomitantly with live weight; this experiment corroborates this. Moreover, significant effects of the dietary factors were found on the difference between the measurements at both live weights. However, further discussion of the effect of live weight is beyond the scope of this paper.

The changes in apparent digestibility coefficients throughout the growing period cannot be derived from our experimental design. Therefore, a mean apparent digestibility coefficient was calculated as the best available alternative. This coefficient, the chemical composition of the diets (Table 4.2), the DM intake and equation (1) were used to calculate the total amount of calculated NE from each digestible nutrient (DXP, DXL, DXF, DXX). Figure 4.3 shows the contribution of the individual digestible nutrients to the total estimated and calculated NE_i supply of the diets.

Additional calculations were performed for dXX. In the XX fraction, the amounts of digested starch and sugar differed in all the experimental diets, because fat, cellulose and soya bean hulls were exchanged for maize starch. Therefore the effects of the amount of fat and the source of carbohydrate on DXX are not mutually independent. Therefore, the DXX fraction was divided into a starch & sugar fraction (tStarch, with an assumed digestibility coefficient of 1.00) and a DnsXX fraction (Digestible non-starch XX). The relative differences (dif) between the expected (Table 4.3) and the measured apparent digestibility coefficients which were significantly affected by the dietary factors are presented in Table 4.9.

$$(\text{dif} = \frac{\text{expected} - \text{measured}}{\text{expected}})$$

Table 4.9

Effects of source of carbohydrate, amount of added fat or the interaction on the relative difference in nutrient digestibility

expected - measured

$$(\text{dif} = \frac{\text{expected} - \text{measured}}{\text{expected}}).$$

Diet ¹	difXP ¹	difXL ¹	difXF ¹	difnsXX ¹
Mean				
Mo	0.04	0.18	1.22	-0.03
MI	0.04	0.09	0.75	-0.04
Mm	0.03	0.06	0.56	-0.01
Mh	0.01	0.03	0.49	-0.03
Co	0.14	0.12	0.79	0.07
CI	0.14	0.12	0.69	0.00
Cm	0.15	0.12	0.90	0.13
Ch	0.13	0.08	0.90	0.06
So	0.07	0.13	0.25	0.00
SI	0.06	0.08	0.25	-0.01
Sm	0.06	0.10	0.23	0.00
Sh	0.02	0.05	0.35	0.03
s.e.d.				
Carb	0.007	0.012	0.23	0.02
F	0.007	0.011	0.09	0.02
Carb*F	0.012	0.020	0.26	0.03
F-probability				
Carb	***	ns	ns	**
F	***	***	ns	*
Carb*F	ns	**	**	ns

¹ For explanations of the abbreviations used, see Tables 4.2, 4.3 and 4.6

In this way, the effects of different ratios of basal diet to experimental ingredients were excluded.

From Figure 4.3 and Table 4.9 it can be concluded that the measured apparent digestibility in all diets was less than expected. Moreover, from the results with the C and the S treatments it can be concluded that the measured apparent digestibility coefficients were lower than those obtained in the preceding digestibility trials with the same batches of cellulose and soya bean hulls, which were obtained from pigs housed in metabolism crates. The difference in apparent digestibility might therefore be attributable to the housing

system. Bakker and Jongbloed (1994) suggested that the larger freedom to move in pens than in metabolism crates might have resulted in an increased passage rate of the digesta in the gastrointestinal tract and hence in the observed decreased apparent digestibility. In their study the effect of methodological differences were eliminated and could not have caused the differences in digestibility between both housing systems. This difference ranged from 0.02 to 0.05 units for organic matter and from 0.03 to 0.06 for XP.

It can be concluded that for practical purposes (e.g. for preparing a feedstuff table) apparent digestibility should be measured in pigs kept in groups in pens, until a factor is known to convert digestibility in one housing system to another.

Effect of source of carbohydrate on apparent digestibility

Fermentable carbohydrates and especially the C treatments significantly increased difXP (significant effect of source of carbohydrate; $P < 0.001$; Table 4.9). It is known that fermentation of carbohydrates increases excretion of microbial nitrogen, which results in reduced apparent faecal XP digestibility (Sauer, Mosenthin, Ahrens and den Hartog, 1991; Furuya and Kaji, 1992).

In contrast to the findings of Just, Andersen and Jørgensen (1980), it was not possible to demonstrate that fermentable carbohydrates significantly increased difXL (Table 4.9). Although lower digestibility coefficients were measured with the C treatments than with the M treatments (Table 4.4) while similar values were expected (Table 4.3), the relatively low value in case of the Mo treatment probably reduced the differences between means (Table 4.6).

Similarly as with difXP, difnsXX appeared to be the largest in case of the C treatments (significant effect of source of carbohydrate; $P < 0.01$; Table 4.9). In the M diets the difnsXX had a negative sign, indicating that a larger amount of nsXX had been digested than was expected.

Effect of amount of fat on apparent digestibility

It is not clear what caused the decrease in difXP at the largest addition of fat (effect of amount of fat, $P < 0.001$), or in other words, why dXP was increased at this fat level (Table 4.5).

With regard to difXL, the large value of 0.14 for the control treatments (o) (Table 4.9) indicates that the fat in the basal diet was less digestible than had been expected from the feedstuff table (CVB, 1988; Table 4.3). The reason for this might be the technique used for analysing crude lipid content. Unlike the studies summarized in the feedstuff table, in this study crude lipid in feed and faeces samples was measured after pre-treating with HCl. As Just et al. (1980) indicated, some of the lipid in faeces is in the form of mineral soaps. Thus, simple extraction with petroleum ether will not remove these soaps, and will result in overestimated digestibility coefficients for crude lipid. This relative contribution of the overestimation of the amount of DXL in the basal diet (as

shown in the o treatments) declined as the amount of fat increased; hence difXL was lower when more fat was added (Table 4.9; effect of amount of fat $P < 0.001$). It can be concluded that the analytical techniques should be similar if data from an experiment or from a feedstuff table are to be used as the basis for another experiment or for interpreting another experiment.

The dXL as such increased as amount of added fat increased (Table 4.5); this is in line with the results obtained by Just (1982). The explanation is partly that added fat is more digestible than the fat in the basal diet, and partly that endogenous fat has less impact in high-fat diets than in low-fat diets (Just et al., 1980; Just, 1982).

In contrast to difnsXX (effect of amount of fat, $P < 0.01$; Table 4.9), no significant effect of amount of fat was observed on difXF. This indicates that the effect of fat might be influenced by the physical structure of the fermentable carbohydrates. At the two highest fat addition levels m and h, nsXX appeared to be less digestible than at the two lower addition levels (o and l) compared with what was expected (Table 4.9).

Effect of the interaction between source of carbohydrate and amount of fat on apparent digestibility

In the two fibrous diets (C and S) the medium amount of fat (m) resulted in a higher difXL than in the case of the other amounts of fat or the M diets (effect of interaction, $P < 0.001$; Table 4.9). This indicates the dXL was depressed even more with these carbohydrates at this particular amount of added fat. None of the other studies in the literature demonstrate this effect. In this study it is uncertain to what extent the effect is caused by reduced digestibility of the fatty acids in the added fat, or by enhanced excretion of endogenous or microbial fat into the gut.

At increasing amounts of fat, difXF diminished within the M diets, but increased slightly within the C and the S diets (interaction effect; $P < 0.01$; Table 4.9). From the literature on ruminants it is well known that a higher fat content in the diet can inhibit fermentation by gut microflora (Brooks, Garner, Gehrke, Muhrer and Pfander, 1954) or reduce the microflora population (Mallett and Rowland, 1983). However, in contrast to difXF, no significant interaction effect was observed on difnsXX. This effect of fraction of carbohydrates corroborates with the studies on rats of Key and Mathers (1993).

Performance

In this experiment pigs received different amounts of estimated NE (Figure 4.3; first bar of each treatment). Therefore, their performance was influenced not only by the different nutrients, but also by the total supply of NE. Only the Mm, the CI and the Cm treatments, however, showed a significantly reduced supply of calculated NE (Figure 4.3; second bar of each treatment).

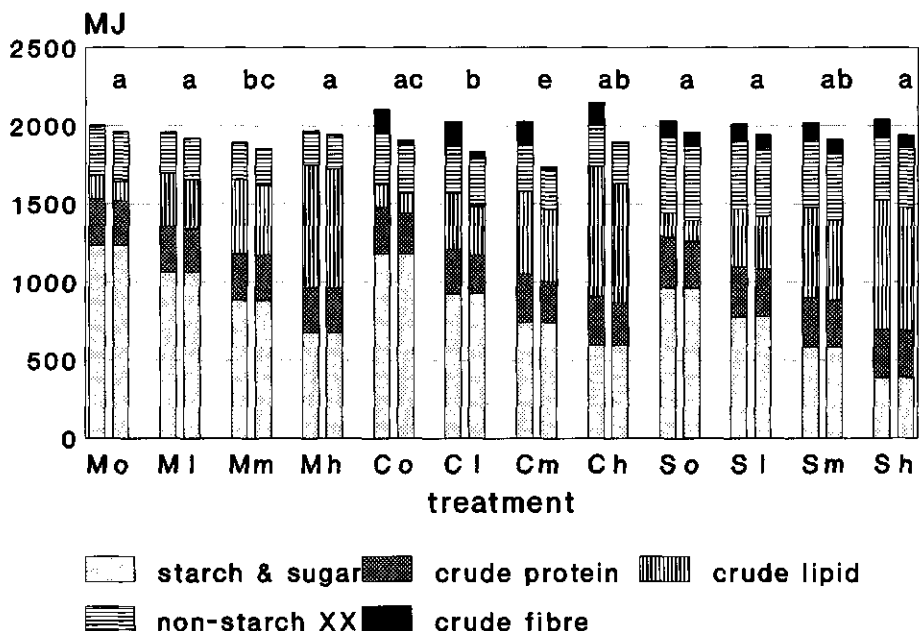


Figure 4.3

Contribution to total NE, consumed per treatment of expected (first bar) and measured (second bar) digestible nutrients. Second bars with a different superscript differ significantly ($P < 0.05$)

Using the apparent digestibility coefficients as measured in calculating NE instead of the expected digestibility, resulted in an absence of an effect of source carbohydrates on the net energy conversion ratio (nECR; Table 4.7). It was anticipated that fermentation of part of the XX (the nsXX fraction) would result in lower weight gain with fibrous carbohydrates (Dierick, Vervaeke, Demeyer, Decuyper, 1989). However, body gain and energy gain do not always respond identically to the treatments. Because the EBG was used to calculate nECR, it is possible that the amount of energy retained still differed between the treatments because of differences in body composition (fat and protein). In an experiment yielding data on energy retention with similar feeds a lower energy retention was found with the fibrous carbohydrates (Bakker et al., 1996). Moreover, the equation to calculate NE might not be correct. Further discussion on energy retention and on the equation to calculate net energy is beyond the scope of this paper.

Both the estimated and the calculated nECR were worst in animals receiving the most fat (Table 4.8). Because nECR is the ratio of NE to EBG, this effect on nECR was either due to differences in calculated net energy intake or in EBG, or

a combination of both. The NE intake for these treatments was calculated as being similar to the control treatment within the same source of carbohydrate (Figure 4.3). As concluded from Table 4.8, EBGd was poorest at the largest addition of fat. The results indicate that this large supply of dietary fat exceeded the animal's capacity to deposit fat. Therefore, some of the dietary fat might also have been used for energy for maintenance. This is a less efficient process than using energy from fat for fat deposition (Chudy and Schiemann, 1969). Further research on energy utilization and on prediction of net energy might shed more light on the individual effects of fat and sources of carbohydrates.

Conclusions

In this study, fermentable carbohydrates reduced the apparent digestibility of XP (on average by 0.04 units in the case of soya bean hulls and by 0.12 units in the case of cellulose) and the apparent digestibility of XL (on average by 0.07 units in the case of soya bean hulls and by 0.10 units in the case of cellulose). It was not possible to explain unexpected reduction in dXL at the medium amount of added fat with the fibrous carbohydrates. It is therefore concluded that it is difficult to predict accurately the chemical composition of the final feed, even if weighed amounts of ingredients are mixed into the feeds. Furthermore, apparent digestibility coefficients cannot always be obtained from preceding trials or feedstuff tables, especially if the housing system and analytical techniques used are not exactly identical. Moreover, the amounts of digestible nutrients of the separate ingredients in the diets are not additive; this is especially true for fermentable carbohydrates. Therefore, for practical purposes, the amounts of digestible nutrients from ingredients cannot always be obtained from digestibility trials with one ingredient and from feedstuff tables; the effects of nutrients on endogenous secretion and on microbial processes in the gut are among the factors that should be taken into account.

These effects on digestibility result in a lower NE supply than was expected. The NE calculated with the data on digestibility in this study (the calculated NE_c) was proportionately between 0.83 and 0.98 of those calculated with data from the preceding digestibility trial and from the feedstuff table (the estimated NE_e). Relating NE intake to performance, in contrast to estimated nECR no differences were found in calculated nECR between sources of carbohydrates. To explain this effect, data on energy retention and an evaluation of the equation to calculate NE are needed.

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Chapter 5

Amounts of apparent ileal and total tract digested nutrients from various combinations of fat and non-starch polysaccharides in growing fattening pigs

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Abstract

This study aimed to quantify the effect of fat and different sources of carbohydrates fed alone and in combinations on the ileal and total tract nutrient supply. In a 2 x 3 factorial arrangement, animal fat was added (0 and 70 g/kg) to maize starch and to each of two sources of fermentable carbohydrates: 260 g/kg purified cellulose or 270 g/kg soya bean hulls. In the six experimental diets maize starch was replaced with fat, cellulose and soya bean hulls of equivalent net energy. Apparent ileal and total tract digestibilities (ID and TD, respectively) of nutrients were measured in 18 ileum cannulated castrates weighing on average 90 kg. Results showed a large variation in TD of fermentable carbohydrates (FERM) among pigs within treatment, that may cause large differences in performance. Combinations of animal fat with soya bean hulls or cellulose supplied 2 to 5 percentage units less energy at the terminal ileum than calculated from the separate ingredients, mainly due to a 4 to 7 percentage units lower ID of fat. Also, starch in the combination of fat with soya bean hulls was slightly less digestible. In the hindgut, the added fat tended to reduce TD of FERM in the soya bean hulls with 5 percentage units. Therefore, the assumption of additivity of (both ileal and total tract) digestible nutrients is incorrect in mixed diets with a high fat and fibre content. It is concluded that in feed formulation, measuring ID and TD of this combination will be necessary for an accurate determination of the energy value of the feed.

Key words: pigs, carbohydrates, fat, digestibility, interaction

Introduction

In general, the potential energy supply of a pig diet is calculated as the sum of the amounts of (total tract) digestible nutrients supplied by the separate feedstuffs used as ingredients in that diet. Hence, it is assumed that the different digestion processes of these ingredients do not interact with each other. This assumption is correct for low fat diets with a common (40 g per kg)

dietary fibre level (Hansen et al., 1991). However, when large amounts of fermentable carbohydrates and fat are added, this assumption is not correct (Bakker et al., 1995); added fat interacted with type of carbohydrate (starch versus fermentable carbohydrates), which effect resulted in lower apparent digestibilities of fat and crude fibre. In addition, the performance of the pigs fed fat and/or fermentable carbohydrates was lower than expected from the calculated net energy supply.

In our previous study, it was not investigated whether the nutrients which contribute to energy supply disappeared from the digestive tract prior to or beyond the terminal ileum. In order to have maximum benefit of their potential nutrient supply, most nutrients need to be digested and absorbed before reaching the terminal ileum. These nutrients are: amino acids from protein, fatty acids from lipids, and glucose from starch and sugars. If they disappear in the large intestine, the value of the nutrients will be lower, resulting in a reduced feeding value of the feed. In addition, when these undigested nutrients enter the large intestine, they may interfere with the fermentation process (Mallett and Rowland, 1983). Thus, it is important to know whether the interactive effect between fat and fermentable carbohydrates takes place prior to the terminal ileum or in the hindgut of the pig.

The present study aimed to determine the nutrient supply of animal fat and two different sources of fermentable carbohydrates individually and in combination at the terminal ileum and over the total digestive tract.

Material and methods

Diets

A basal diet was formulated to supply all the required amino acids, minerals and vitamins (Table 5.1). To ensure that the composition of the basal diet was identical in all experimental diets, one large batch sufficient for the whole experiment containing all the ingredients except the cane molasses, was mixed. The correct proportion of molasses was added to the final diets. Each kilogram of the control diet (maize starch without added fat; Mo) contained 540 g basal diet (molasses included) and 460 g maize starch. In the experimental diets, a certain amount of the maize starch in the control diet was replaced with fat, cellulose and soya bean hulls of equivalent net energy (Figure 5.1).

The six diets were tested in a 2 x 3 factorial arrangement with two inclusion levels of animal fat and three sources of carbohydrates. Of the factor animal fat either none (o) or 70 (m) g per kg was incorporated in the diet. The three sources of carbohydrates were: raw maize starch (M), purified cellulose (C; 260 g per kg diet; Arbocel B800, J. Rettenmaier, Holzmühle, Germany), and toasted soya bean hulls (S; 270 g per kg diet). Maize starch was assumed to be digested completely by the gastrointestinal enzymes. Cellulose was included as

an example of a poorly fermentable carbohydrate, and soya bean hulls were used as an easily fermentable carbohydrate.

Table 5.1
Composition of the basal diet (g/kg)

Barley	290
Soya bean flour	279
Potato protein	114
Wheat middlings	207
Soya oil	19
Alfalfa	41
Limestone	20
Ca(H ₂ PO ₄) ₂ ·1H ₂ O	16.9
NaCl	4.2
Trace mineral/vitamin pre-mix ¹	3.5
Choline chloride	0.5
S-DL methionine	1.3
L-lysine HCL	1.5
Cr ₂ O ₃ -starch mixture	2.1

¹ Contained the following ingredients (mg/kg basal diet, unless otherwise stated): 39 vit. AD₃ (19,500 IU and 3,900 IU, respectively); 39 vit. E; 10 riboflavin; 49 niacin; 19 DL-Ca-pantothenate; 49 µg/kg of vit. B₁₂; 304 antioxidant (4 to 5% of BHA, 4 to 5% of ethoxyquin, 4 to 5% of citric acid, 2 to 3% of orthophosphoric acid, 2 to 3% of E 471 fatty acid, and SiO₂ as carrier); 121 MnO (93.6 Mn); 376 ZnSO₄·H₂O (136.9 Zn); 5 KI (3.8 I); 1044 FeSO₄·7H₂O (209.8 Fe); 382 µg/kg of Na₂SeO₃ (174.2 µg/kg of Se)

Table 5.2
Composition of the diets; ingredients (g/kg as fed)

Diet	Basal diet	Maize starch	Cellulose	Soya bean hulls	Fat ¹	Cane molasses
Mo	538	349	-	-	-	112
Mm	628	252	-	-	59	61
Co	417	262	261	-	-	61
Cm	480	122	310	-	48	40
So	472	195	-	267	-	66
Sm	506	69	-	314	61	50

¹The fatty acid pattern in the animal fat used was: 26 g/kg < C16:0; 223 g/kg C16:0; 49 g/kg C16:1/C18:0 iso; 138 g/kg C18:0; 393 g/kg C18:1; 128 g/kg C18:2 and 44 g/kg > C18:2.

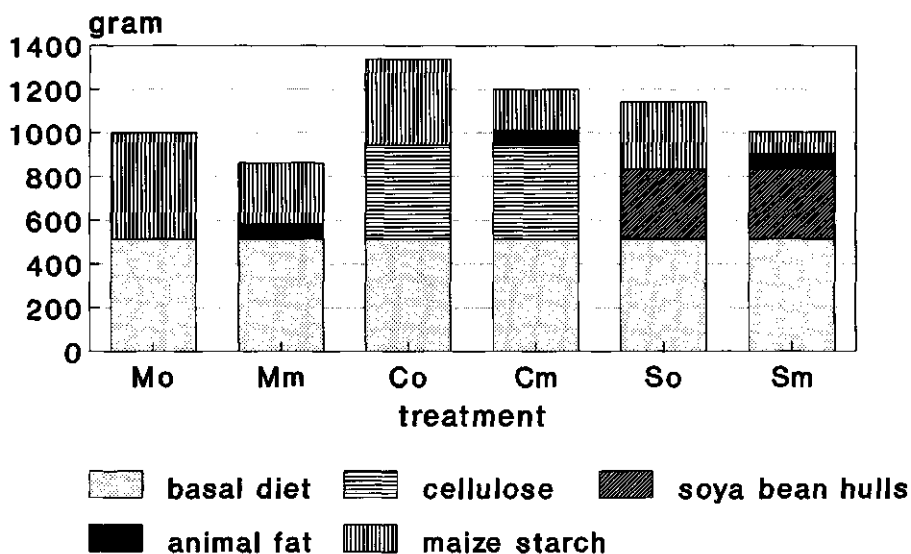


Figure 5.1

The amounts (g) of the six experimental diets yielding the same total net energy as 1000 g of control diet Mo

In the design, the factor fat was confounded with starch content; when the fat content was higher the starch content was lower. However, regardless the fat inclusion level, the amounts of cellulose or soya bean hulls were similar. Therefore, the factors fat and fibre are independent.

Details of preparation of these diets were reported by Bakker et al. (1995). As a result of the experimental design (Figure 5.1), the six diets differed in net energy (NE_p) densities and nutrient concentrations per kg (Table 5.2). Therefore, to consume the equivalent amount of NE_p , the pigs on a low density feed had to consume more feed than pigs on a high density feed. All feeds were made simultaneously in amounts sufficient for all experiments. They were cold-pelleted without adding steam and stored at -18°C until required. On average one week prior to each trial, feed was taken out of the freezer and stored at $+4^\circ\text{C}$.

Feeding and animals

The castrated males selected for the experiments were from a three-way cross Yorkshire δ x (Finnish Landrace δ x Dutch Landrace δ) δ . At selection, a uniform group for each trial was used with as few sires as possible. The pigs were assigned to one of the six diets and received that diet continuously from

30 to 105 kg live weight. At about 30 kg live weight the pigs were gradually introduced to the experimental diets by increasing the amount of experimental diet and reducing the starter feed over a period of four days. The pigs were weighed every Tuesday at 7.30 h. Their ration for the following week was based on this weight and the growth expectancy in the week to follow. The pigs were fed individually twice daily, receiving 50% of their daily feed allowance at each meal time. The feeding level aimed to supply 10.4 MJ NE_r per day at 30 kg live weight, 18.6 MJ NE_r per day at 60 kg and 24.1 MJ NE_r at 90 kg live weight (CVB, 1988). The pigs received 0.33 l water per MJ NE_r, that was added to the feed at least six hours before feeding. Composite samples were taken of each diet during preparation of the mealtime portions. These samples were then presented to the laboratory for chemical analysis. Occasional leftovers were collected daily from the troughs, stored at 4°C and were weighed and analysed for DM content as weekly amounts for each pig.

Measuring total tract and ileal apparent digestibility of nutrients

Unless otherwise stated **apparent** digestibility of the nutrients was measured. Eighteen ileum cannulated castrated males were used, which were housed individually in pens (1.6 m²). The floors in these pens were made of a metal grid covered by smooth synthetic material. Each pen had one wall made of perspex glass; the other three were made of wood. The pens were linked together in a row, so that the two neighbouring pigs could see each other through the perspex wall. The feeding trough was made of stainless steel and a cover allowed the feed and water to be put into the trough before the feeding time without the pig being able to eat it.

After having received the experimental diets from on average 30 kg onwards, at about 45 kg live weight, the 18 pigs were surgically fitted with a steered ileo-caecal valve (SICV) cannula as described by Mroz et al. (1996). It was demonstrated that the use of this technique results in correct values for total tract digestibility, because the hindgut remains intact. In each trial, two reserve pigs were also prepared, in accordance with our standard procedures. After surgery, incrementally amounts of experimental diet were supplied, until the pigs received the intended amounts.

Digestibility coefficients were determined for dry matter (DM), crude protein (XP), crude fat (XL), starch, fermentable carbohydrates (FERM; calculated as DOM - DXP - DXL - starch - sugar) and gross energy (GE). Total tract and ileal digestibilities (TD and ID, respectively) were measured at 90 kg live weight, using chromic oxide as a marker. In order to determine TD, all the excreted faeces were collected in a plastic bag attached to the pig's rear (Van Kleef et al., 1994), during a 10-day period, making it impossible for urine to contaminate the faeces. Faeces were collected at each feeding time by replacing the plastic bag containing faeces with an empty one. The bag and its contents were weighed and stored at -18°C.

To obtain ID, ileal digesta was collected on the first and sixth day after the faeces collection had been completed. The digesta was collected in a plastic bag attached to the cannula, continuously for 24 hours on each of these days (Mroz et al., 1996). The hourly amounts collected from each pig were weighed and stored at -18°C until required for chemical analysis.

Analytical procedures

When all the digesta and/or the faeces had been collected, the daily amounts from each pig were mixed together and samples were taken for analysis. Faeces were defrosted, but the digesta remained frozen. While mixing the digesta, liquid nitrogen was added to avoid thawing. This procedure guarantees a homogeneous sample and minimizes the risk of volatilization of ammonia from the digesta.

The samples from each diet were analysed in duplicate, following official Dutch protocols (NNI, 1992), most of which are similar to those of the Association of Official Analytical Chemists (AOAC, 1984). Samples of fresh material were obtained for nitrogen analysis (NEN 3145). The remaining faeces were air-dried and the digesta was freeze-dried. These samples were then ground through a 1 mm sieve and analysed for DM (NEN 3332), ash (NEN 3329), XL (EG L15/29, method B pre-treatment with hydrochloric acid) and chromium (as described by Bakker and Jongbloed, 1994). Analysis for GE content was performed with an adiabatic bomb calorimeter. All digesta samples were also analysed for starch content (NEN 3574; using the enzymes amyloglucosidase/hexokinase).

Table 5.3

Analysed chemical composition of the experimental diets (g/kg dry matter, unless otherwise stated)

Diets ¹ :	Mo	Mm	Co	Cm	So	Sm
Dry matter (DM, g/kg)	881	884	889	898	883	889
Ash	51	57	40	50	59	67
Crude protein (XP)	176	204	134	148	188	206
Crude fat (XL)	30	98	22	77	31	104
Starch	444	374	328	216	294	186
Sugar	87	68	53	44	66	57
FERM ²	213	201	424	465	362	381
Gross Energy (GE; MJ/kg DM)	18.3	19.9	18.1	19.3	18.3	19.9

¹ Diet: maize starch (M), cellulose (C) or soya bean hulls (S) were combined with two amounts of added fat (0 (o) and 70 (m) g/kg, respectively)

² FERM: fermentable carbohydrates calculated as: $1000 - \text{ash} - \text{XP} - \text{XL} - \text{starch} - \text{sugar}$

Faeces samples from at least one pig per treatment were analysed for starch content. If significant amounts of starch were found, the faeces samples of the other pigs were also analysed for starch. The FERM contents were calculated by subtraction of the amounts of XP, XL, starch and sugar from the OM content.

This experiment was the last one in the project. In all, between 3 and 18 feed samples were analysed per diet during the entire project (Bakker et al., 1993). In the present experiment on the cannulated pigs, feed samples were analysed in duplicate for DM, NDF, ADF and chromium content. The results from these analyses indicated that the diets used in the present experiment were within the range of those of the entire project. Therefore, the chemical composition of the diets as presented in Table 5.3 are the mean values of the entire project.

Calculating total tract and ileal digested amounts of ingredients

The experimental design allowed us to calculate nutrient and energy supply at the terminal ileum and the total tract of each of the ingredients by the difference method. The composition of the diets was taken as presented in Table 5.2. The nutrient supply by the basal diet as ingredient was calculated as the difference between the nutrients supply with the control diet Mo on one hand and those with the ingredients maize starch and cane molasses on the other hand. Certain assumptions were made for cane molasses and maize starch. The data for TD of nutrients for cane molasses and maize starch were obtained from CVB (1988). Furthermore, the ID of nutrients for cane molasses were taken as obtained by Figueroa et al. (1988). The digestibilities of starch in the basal diet and in the maize starch was assumed to be similar. Then, the amount of digested nutrients by animal fat was calculated as the difference between the results with diet Mm on one hand and the calculated values of the basal diet and the assumptions for digestibility of nutrients in cane molasses and maize starch on the other hand. Similarly, the amounts of digested nutrients by cellulose was calculated with the results of diet Co and those of soya bean hulls with the results of diet So.

Experimental design and statistical analysis

Three trials were performed to assess TD and ID of nutrients. In each trial the six treatments were assigned to six pigs. Thus, each pig was an experimental unit and each treatment was tested with three pigs. Differences between measured and calculated digestibility coefficients of the combination diets Cm and Sm were identified by confidence intervals (t-tests), as described by Hansen et al. (1991). The test statistic t was calculated as the difference between the expected and the measured digestibility, divided by $[sd/\sqrt{3}]$. The critical values (t_2) at various nominal significant levels (α) are: 2.92 at $\alpha=0.10$; 4.30 at $\alpha=0.05$ and 9.92 at $\alpha=0.01$.

Results

General

During the experiment, two pigs had to be removed from the first trial (one on diet Cm and one on diet So). One of these two pigs continued to leave some of its feed and the other one lost its cannula. In the second and the third trial no problems occurred. Therefore, at the start of the third trial two pigs were added to the six that had already been planned. They received the two missing diets in order to maintain three statistical units per diet as planned. Thus, the number of replicates per diet remained balanced. Because the factor trial had hardly any significant effects, the data were used as was intended.

Ileal and total tract digestibility of nutrients in the experimental diets

As a result of the experimental design, the experimental diets showed a large range in both ID and TD of DM and energy (Table 5.4). The DM and energy digestibilities of the C diets were the lowest; those of the M diets were the highest. The ID of the S diets were close to those of the C diets, but the TD were closer to those of the M diets. This indicates that, compared to the M diets, a larger proportion of the S diets was fermented in the hindgut. In all diets, starch was nearly completely digested at the terminal ileum. The TD of starch was assumed to be 100% in all treatments; this was checked and confirmed in the first trial. The ID and TD of FERM were the lowest for the C diets; those for the M and the S diets were nearly equal. Furthermore, the XP-digestibilities were highest for the M diets. For XL, the TD was lower than the ID, in contrast with all the other nutrients.

Table 5.4

Average ileal and total tract nutrient digestibility of the six experimental diets measured in pigs

Diets:	Ileal digestibility (%)						Total tract digestibility (%)					
	Mo	Mm	Co	Cm	So	Sm	Mo	Mm	Co	Cm	So	Sm
DM	76	75	52	42	58	52	89	87	68	66	83	78
GE ¹	77	77	55	47	60	58	89	88	69	68	83	79
XP ¹	78	80	74	72	72	72	89	90	78	74	79	80
XL ¹	74	86	74	78	74	81	72	85	68	75	60	79
Starch	99	99	99	99	99	97	100	100	100	100	100	100
FERM ¹	26	22	06	-2	24	9	72	66	39	46	77	68

¹ See Table 5.3 for explanation of the abbreviations

Digestibility of nutrients of the ingredients

Of the basal diet, 80% of the TD of energy (TDE) disappeared before reaching the caecum (Table 5.5). Of the remaining 20% in the hindgut most of the energy was supplied by FERM. Cellulose had a negative effect on the DE supply at the terminal ileum (IDE), mainly due to a negative effect on digestible XP and FERM. For soya bean hulls, only 32% of the TDE had disappeared before entering the caecum; of the FERM only 8% was digested at the terminal ileum, while the TD was 80%. In contrast to the other ingredients, the TDE supply of animal fat was lower than its IDE.

Table 5.5

The chemical composition¹ of each ingredient (in g/kg DM, energy in MJ/kg DM) and its ileal and total tract supply of digested nutrients (g/kg DM consumed) calculated with the difference method

ingredient	XP	XL	starch	sugar	FERM	GE
Basal diet						
chemical composition	310	53	223	56	270	19.4
ileal supply	251	39	219	50	54	13.0
total tract supply	282	40	223	56	203	16.3
Cane molasses						
chemical composition	55	-	-	633	186	15.2
ileal supply ²	0	-	-	570	37	10.6
total tract supply ³	23	-	-	633	104	12.8
Maize starch						
chemical composition	4	-	959	-	35	17.5
ileal supply	0	-	949	-	20	17.0
total tract supply ³	0	-	959	-	25	17.3
Cellulose						
chemical composition	1	2	-	-	994	17.2
ileal supply	-24	0	-	-	-48	-1.5
total tract supply	-52	-6	-	-	199	2.2
Soya bean hulls						
chemical composition	135	19	43	21	721	17.6
ileal supply	57	14	42	0	58	3.9
total tract supply	49	-3	43	21	577	12.3
Animal fat						
chemical composition	-	999	-	-	1	37.5
ileal supply	44	909	-	-	32	36.0
total tract supply	93	899	-	-	-206	33.8

¹ See Table 5.3 for explanation of the abbreviations

² Digestibility coefficients derived from Figueroa et al. (1988)

³ Derived from CVB (1988)

Although the fat did not contain any XP, addition of animal fat improved XP digestion both at the terminal ileum and in the hindgut. In the hindgut, the added fat reduced FERM and XL digestibility.

In order to check if the ingredients interact with each other in nutrient supply, we compared the measured digestibilities from each of the combination diets - cellulose + fat (diet Cm) and soya bean hulls + fat (diet Sm) - with the digestibilities calculated from the individual ingredients. This calculated digestibility was obtained by adding together the digestible nutrients supplied by the ingredients in each diet (Tables 5.2 and 5.5), divided by the chemical diet composition (Table 5.3). The calculated and measured ID and TD are presented in Tables 5.6 and 5.7, respectively.

Table 5.6

Nutrient ileal digestibility (%) of the two combination diets (fat combined with cellulose or soya bean hulls) as measured with the whole diet (mean and sd) and as calculated from the separate ingredients

nutrient diet with:	Measured digestibility (M)	SD ¹ (df 2)	Calculated digestibility (C)	difference: M minus C	significance of difference ²
XP					
Cellulose + fat	71.5	2.4	74.4	-2.9	NS
Soya bean hulls + fat	71.5	1.1	71.9	-0.4	NS
XL					
Cellulose + fat	77.6	2.9	85.0	-7.4	*
Soya bean hulls + fat	81.3	1.4	85.5	-4.2	*
Starch					
Cellulose + fat	98.7	0.4	98.5	+0.2	NS
Soya bean hulls + fat	97.1	0.3	98.2	-1.1	*
FERM					
Cellulose + fat	-2.3	6.6	3.4	-5.7	NS
Soya bean hulls + fat	9.2	2.8	13.4	-4.2	NS
Energy					
Cellulose + fat	46.9	2.8	51.5	-4.6	NS
Soya bean hulls + fat	57.8	1.2	60.1	-2.3	t

¹ SD: standard deviation of the measured values

² statistical significance: NS: $P > 0.10$; t: $P \leq 0.10$; *: $P \leq 0.05$; **: $P \leq 0.01$

The calculated ID were consistently higher than the measured ones, except for starch in the Cm diet (Table 5.6). In diet Cm, the measured ID differed more from its calculated values than in diet Sm, except for starch. Also, the standard

deviations were larger with the Cm diet than with the Sm diet. For both diets, the measured ID of XP was not significantly different from the calculated one. For the ID of XL, however, this difference of 4.2 (diet Sm) to 7.4 (diet Cm) units was significant for both diets. Also, the measured ID of starch in diet Sm was significantly 1.1 unit lower than was calculated from the separate ingredients. The measured ID of FERM were not significantly different from the calculated ones. As a result from the reduced ID of starch and XL, the measured IDE in diet Sm tended ($P \leq 0.10$) to be 2.3 units lower than calculated from the ingredients. With the Cm diet the difference between the measured IDE and the calculated one was 4.6 units and thus larger than with the Sm diet. But this difference was not statistically significant due to a larger standard deviation (Table 5.6).

Table 5.7

Nutrient total tract digestibility (%) of the two combination diets (fat combined with cellulose or soya bean hulls) as measured with the whole diet (mean and sd) and as calculated from the separate ingredients

nutrient diet	Measured digestibility (M)	SD ¹ (df 2)	Calculated digestibility (C)	difference M minus C	significance of difference ²
XP					
Cellulose + fat	74.2	1.3	78.9	-4.7	*
Soya bean hulls + fat	80.4	2.0	78.9	+1.5	NS
XL					
Cellulose + fat	75.1	3.2	82.2	-7.1	t
Soya bean hulls + fat	78.6	1.8	79.9	-1.3	NS
FERM					
Cellulose + fat	46.4	20.7	33.6	+12.8	NS
Soya bean hulls + fat	68.4	3.1	73.7	-5.3	t
Energy					
Cellulose + fat	67.8	8.8	65.9	+1.9	NS
Soya bean hulls + fat	79.2	1.5	81.7	-2.5	t

¹ SD: standard deviation of the measured values

² statistical significance: NS: $P > 0.10$; t: $P \leq 0.10$; *: $P \leq 0.05$; **: $P \leq 0.01$

Not only the measured ID, but also the measured TD differed from the calculated values of the diets Cm and Sm (Table 5.7). The differences between measured and calculated values were larger with TD than with ID, except for the energy digestibility of the Cm diet. Also, the standard deviations of TD were larger than those of ID, except for the XP digestibility of the Cm diet. For TD, the differences were not consistently negative. However, those differences that

were significant or tended to be significant were lower as measured than when calculated. This indicates that the negative effects were more pronounced than the positive ones.

The TD of XP in the Cm diet was significantly 4.7 units lower than when calculated from the separate ingredients. With the Sm diet, the measured TD of XP was not significantly different from the calculated one. With both diets a 1.3 (diet Sm) to 7.1 (diet Cm) units lower TD of XL was measured than was calculated from the separate ingredients. However, only with the Cm diet this difference tended to be significant. The mean TD of FERM in the Cm diet was 12.8 units higher than the calculated one. However, as the standard deviation was very large, this difference was not significant. In contrast, in the Sm diet the measured TD of FERM tended to be lower (5.3 units) than the calculated one. These effects, although less pronounced, were also shown in the TDE of both diets.

Discussion

The purpose of this study was to check whether adding fat to different sources of fermentable carbohydrates would supply the same amount of nutrients as when these ingredients were fed separately. Hansen et al. (1991) showed that for low fat diets with a regular dietary fibre concentration (40 g/kg) the assumption of additivity of TD of nutrients is correct. This might not be true, however, for diets with large concentrations of fat and FERM, because FERM and fat affect each other's TD (Just et al., 1980; Mallett and Rowland, 1983). Also, maize oil and type of FERM interact with regard to TD (Key and Mathers, 1993). This interaction was confirmed in a previous experiment with FERM and various levels of animal fat (Bakker et al., 1995). It is unknown, however, whether the assumption of additivity of nutrients is correct at the terminal ileum. Starch, sugar, protein and fat should have been absorbed before reaching the terminal ileum to take optimal advantage of their nutritive potential. Therefore, in the present experiment, both ID and TD were measured.

In the present experiment, addition of fat was confounded with withdrawal of starch. However, this is common in commercial diets: diets with a low starch content usually have a higher fat content to maintain a certain energy content. The comparison between the three sources of carbohydrates maize starch, cellulose and soya bean hulls is not confounded with any other effect, except for a difference in dry matter intake. Therefore, the design of the present experiment is correct.

Effect of source of carbohydrate on pre-caecal digestion

The present experimental design was based on the assumption that the maize starch is digested almost completely by the gastrointestinal enzymes in

the small intestine (Graham et al., 1986; Bach Knudsen et al., 1993); cellulose was used as an example of a poorly fermentable carbohydrate (Fahey et al., 1980) and the soya bean hulls were used as an easily fermentable carbohydrate (Stanogias and Pearce, 1985). These assumptions proved to be correct, as shown by the present results on digestibility of the mixed diets (Table 5.4) and of the individual carbohydrate sources as ingredients (Table 5.5).

The largest differences in chemical composition between the diets containing three sources of carbohydrates were observed in the starch and FERM contents (Table 5.3). The dietary starch nearly completely disappeared before the terminal ileum (Table 5.4). In general, it is assumed that the ileally digested starch is absorbed as glucose and not as volatile fatty acids (VFA). Although some of the organic matter was fermented, as can be concluded from the ID of FERM (Table 5.4), it was not possible to distinguish between digestion of starch into glucose by the gastrointestinal enzymes or fermentation of part of the starch by microbes into volatile fatty acids. This is an important issue, because the part that is fermented supplies less energy to the pig than when it is digested into glucose.

In addition, the starch digestion might be overestimated due to incomplete analysis. The FERM fraction comprised all the nutrients that are not starch, sugar, XL or XP. Thus, the intermediates of starch degradation (oligosaccharides) were identified as FERM, because the analysis for starch ignores such intermediates. Hence the amount of fermented FERM might be underestimated.

Although cellulose (FERM content 99%; Table 5.5) was nearly indigestible prior to the caecum (Table 5.4), it had a negative effect on the ID of other nutrients (Dierick et al., 1989; Shi and Noblet, 1993). This is most clearly illustrated by the negative contribution of cellulose to IDE (Table 5.5). For each kilogram of cellulose, apparently 24 g more XP was present in the digesta, accounting for one third of its negative contribution to IDE (Table 5.5). This extra XP might be the result of an increased endogenous flow of XP, sloughed cells from the gut wall, and/or a reduced absorption of dietary and/or endogenous XP (Shi and Noblet, 1993; Schulze, 1994). This endogenous XP is linearly related to DM intake (Butts et al., 1993): it is thus allowed for the present study to relate the extra XP flow (compared with the control Mo) at the terminal ileum with the extra daily DM intake of 808 g cellulose. The loss of 24 g XP/kg cellulose in the present study was within the range reported in literature: 16 - 39 g/kg (de Lange et al., 1989; de Lange et al., 1990; Boisen and Fernández, 1995). For the diet Co, this endogenous XP induced by cellulose reduced the ID of XP from 78 % to 73%. Therefore, this effect is too large to ignore.

A loss of 4.7 g fat, as endogenous fat, was expected per kg DM intake (Jørgensen et al., 1993). However, the present results showed no clear negative effect of the additional DM intake by cellulose on ID of fat. Apparently, the

amount of endogenous fat is not always linearly related to the amount of DM consumed. To our knowledge, there is no study reported in literature about the validity of the linear relationship between DM intake and ileal endogenous fat secretion. Because the basal diet and soya bean hulls contain some XP and XL, the endogenous losses will be accounted for in their digestibility coefficients. This way, the ingredients are individually 'punished' for their effects on endogenous losses.

In conclusion, the difference method determines accurately the supply of nutrients of the separate ingredients at the terminal ileum, provided that these ingredients contain all the nutrients used in feed evaluation. Otherwise, as with cellulose in the present study, an additional (positive or negative) factor is needed to account for positive or negative effects on the digestibility of nutrients of the other ingredients. For a correct evaluation of starch, the analysis should be evaluated. Moreover, it should be studied whether and how much of the starch is fermented in the small intestine.

Effect of source of carbohydrate on degradation of nutrients in the hindgut

In general, when the content of starch in the diet is decreased, the contribution of the hindgut to digestibility is increased and visa versa (Shi and Noblet, 1993). Therefore, in the present study, the exchange of maize starch with fibrous ingredients and/or animal fat resulted in increased contributions of the hindgut to the total tract digestibilities of the mixed feeds (Table 5.4; the difference between TD and ID). There was net disappearance of all the nutrients from the hindgut, except for XL. These results for XL may suggest that XL was synthesized in the hindgut. This corroborates the results of Mason and Just (1976). The amount of synthesized XL in the hindgut was not constant for each unit of fermented carbohydrate; thus no accurate relationship could be calculated. To estimate the supply of digestible fat to the pig, the ID of XL is preferred over its TD.

Similarly, a net synthesis of XP was observed in the hindgut per kg fermented FERM of cellulose and soya bean hulls, respectively (Table 5.5). During the fermentation process the bacteria incorporate N in their cell. This N originates from undigested protein in the NDF-matrix (Schulze, 1994) or from the blood (Mosenthin et al., 1992; Bakker et al., 1996). When more N is incorporated in bacteria in the hindgut, less N will be excreted in urine (Bakker et al., 1996). Apparently, the XP that is absorbed from the hindgut is almost totally excreted with the urine (Zebrowska, 1975; Just et al., 1981) and is therefore not of any value for the pig. Therefore, for feed(stuff) evaluation the supply of digestible XP should be determined at the terminal ileum.

In conclusion, in formulating diets after their energy supply, prediction of intake of digestible XL and XP should be based on their ID, especially when large amounts of fermentable carbohydrates are present.

Effect of adding fat on digestion and fermentation

Fat had a positive effect on the ID of XP and FERM (Table 5.5). This effect might be attributed to a slower transit time of digesta with fat (Mateos et al., 1982), allowing the intestinal enzymes more time for digestion. Imbeah and Sauer (1991) also found that fat had a positive effect on ID of XP. However, they could not confirm that this effect was related to transit time. In the present study, the relatively small amount of added fat (67 g/kg DM in diet Mm; Table 5.2) improved the ID of XP with 1.7 unit. The positive effect of fat on ID of FERM is in contrast to what was expected (Mallett and Rowland, 1983). In diet Mm, the added fat improved the ID of FERM with 2 units.

In contrast to the positive effects of the added animal fat on the ID of FERM and XP, in the hindgut the added fat reduced DE absorption by 2.2 MJ/kg, due to reduced FERM fermentation (Table 5.5). This might be caused by a reduced population of gastrointestinal microflora (Mallett and Rowland, 1983). In the present study, the added animal fat reduced TD of FERM in diet Mm with 8 units (from 74 to 66 %). This effect on fermentation might also explain the positive effect of the fat on XP digestibility in the hindgut (Table 5.5): when less carbohydrates are fermented in the hindgut less N will be incorporated in the bacteria (Bakker et al., 1996).

In conclusion, when adding animal fat to a diet, a positive effect on ileal XP digestibility should be taken into account. In addition, a negative effect on hindgut fermentation can be expected.

Effect of the combination of fermentable carbohydrate with fat on digestion and fermentation

The experimental design allowed to measure the nutrient and energy supplies of each combination at the terminal ileum and for the total tract; cellulose with animal fat (Cm) and soya bean hulls with animal fat (Sm) (Tables 5.6 and 5.7).

Calculated from the individual ingredients, the ID were expected to be higher than what was actually measured from the mixed diets (Table 5.6). Especially the ID of XL was reduced, with 4 to 7 units in the diets Sm and Cm, respectively. In addition, the ID of starch of diet Sm was one unit lower than expected (Table 5.6). As a result, the digestible energy supply prior to the caecum was reduced with 2 to 4 units by combining fat in the diets with soya bean hulls or cellulose, respectively. It could not be distinguished whether all of the digestible starch was digested into glucose or that a part was fermented into VFA. If part of the starch was fermented into VFA, the utilization of DE would be worse than when it was digested into glucose (Dierick et al., 1989).

In contrast, Shi and Noblet (1994) reported the reverse effect: they found that the combination of rapeseed oil and a 'fibre'-mixture (wheat bran, soya bean hulls, sugar beet pulp and wheat straw) resulted in a higher measured ileal DE supply than the calculated one. There is no clear explanation for the difference in results between their study and the present one; it may be the type

of fat being either vegetable oil or animal fat.

With regard to the TD (Table 5.7), the largest effects of the combinations were found on the TD of FERM: the combination of fat with cellulose resulted in an improved TD, while the combination with soya bean hulls gave a reduced TD. However, the reduced TD of Sm was for 80 % due to the reduced ID (calculated from the difference in ID (Table 5.6) as a percentage of the difference in TD (Table 5.7)). These results with the Sm diet partly corroborates the results of Shi and Noblet (1994). They also found that in the combination diet, with a 'fibre'-mixture and rapeseed oil, less DE disappeared from the hindgut than was calculated from the individual ingredients. However, as mentioned in the previous section, they found the opposite effect at the terminal ileum.

The difference in digestibility of XL in diet Sm at the terminal ileum became smaller in the hindgut; hence the measured TD of XL was not significantly different from the calculated one (Table 5.7). The reduced TDE of 2.5 units was for 92% due to the effects up to the terminal ileum. For the Cm diet, the TD of XP and XL were worse than expected. This was probably due to the higher TD of FERM than calculated from the separate ingredients. A higher fermentation rate results in a higher rate of microbial XP and XL synthesis. However, the standard deviation for TD of FERM was large. This may result in large differences in performance between individuals.

In conclusion, the assumption of additivity for feeding value when two or more ingredients are mixed together in one feed (Hansen et al., 1991) is incorrect for diets with a high fibre and high fat content. These effects can be identified by measuring both the ID and TD of the specific combination.

Conclusions

It is concluded that in formulating diets, the energy supply by nutrients that need to be digested before the terminal ileum (XP, XL and starch) might be overestimated when derived from TD of the separate ingredients. Therefore, prediction of intake of digestible XL and XP should be based on their ID, especially when large amounts of fermentable carbohydrates are present. For a correct evaluation of starch, the analysis should be evaluated, and it should be studied how much of the starch is fermented in the small intestine. With the difference method, the supply of nutrients of the separate ingredients at the terminal ileum can be obtained accurately, provided that these ingredients contain all the nutrients used in feed evaluation. Otherwise, the 5 units lower ID of XP by inclusion of 275 g cellulose or the nearly 2 units higher ID of XP by inclusion of 67 g animal fat per kg diet will be ignored.

The large variation in (total tract) fermentation among pigs within treatment may cause large differences in performance.

When adding animal fat to a low fibre diet a negative effect on hindgut

fermentation should be taken into account. When adding fat to a high fibre diet, the total tract fermentation tended to be even more reduced (5 units) than expected from the animal fat separately. The ID of fat was reduced with 4 to 7 units.

The assumption of additivity of ileal and total tract supply of nutrients for feeding value when two or more ingredients are mixed together in one feed (Hansen et al., 1991) is incorrect for diets with a high fibre and high fat content. These effects can be identified by measuring both the ID and TD of nutrients in the specific combination.

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Chapter 6

Amounts of methane and volatile fatty acids from various combinations of fat and non-starch polysaccharides in growing fattening pigs

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Abstract

This study aimed to quantify the effect of different sources of carbohydrates fed alone or in combination with fat on the amount of methane and on the concentration of volatile fatty acids (VFA) in the digesta and in faeces of pigs. In a 2 x 3 factorial arrangement two levels of animal fat (0 and 70 g/kg) were combined with three sources of carbohydrates: 350 g/kg maize starch (M), 260 g/kg purified cellulose (C) or 270 g/kg soya bean hulls (S). In the six experimental diets maize starch was replaced with fat, cellulose and soya bean hulls of equivalent net energy. Methane production was measured in 12 pairs of castrates. Concentrations of VFA in faeces and in digesta were measured in 18 ileum cannulated castrates. In both experiments, pigs received the experimental diets from 30 kg live weight onwards and measurements were performed when the pigs weighed on average 90 kg. Results showed the amounts of methane were highly correlated with the different amounts of total tract digestible energy of fermentable carbohydrates (DE-FERM) in the diets. Regression analysis showed that loss of energy as methane was 9.5% with each additional MJ of DE-FERM. Fat addition seemed to increase methane production. The mechanism behind it remains unsolved. VFA concentration was similar for most diets. It was higher, however, in faeces of both C diets. No clear relationship could be found between the concentration and the ratios of VFA in digesta and faeces on one hand and DFERM on the other hand. The potential energy supply by VFA from DE-FERM may vary from 81 to 90% of DE-FERM, when the energy lost in VFA in faeces and by methane are subtracted. Lactic acid concentration was enhanced in the digesta of the pigs receiving the M diets. The possible absorption of lactic acid instead of glucose did not considerably alter the availability of nutrients.

Key words: pigs, carbohydrates, fat, fermentation

Introduction

Digestible carbohydrates supply most of the dietary energy to the growing pig. They comprise starch and non-starch digestible carbohydrates (FERM).

FERM is defined as the organic matter minus the protein, fat, starch and sugar. Starch is assumed to be digested by enzymes in the animal's small intestine, and FERM can partly be fermented by microbes (DFERM), mainly in the animal's hindgut. Compared to starch, fat supplies more net energy and DFERM less. Therefore, to maintain a fixed energy density, DFERM-rich diets are often supplemented with fat. It is thus assumed that these ingredients do not interact with each other during digestion and absorption. This assumption is correct for low fat diets with a common dietary fibre level (40 g per kg) (Hansen et al., 1991). However, when large amounts of fermentable carbohydrates and fat are added, this assumption is not correct (Bakker et al., 1996). It was found that the ileal energy supply was between 1 to 4 percentage units lower than expected from adding up the values from the separate ingredients. Moreover, dietary fat appeared to reduce hindgut fermentation (Mallett and Rowland, 1983; Christensen and Thorbeck, 1987; Bakker et al., 1996). These effects may only partly explain the reduced performance of the pigs fed large amounts of fat and/or fermentable carbohydrates (Bakker et al., 1995).

It is often suggested that part of the starch is precaecally fermented into volatile fatty acids (VFA), instead of being hydrolysed and absorbed as glucose. When degraded into VFA, starch will supply less energy to the pig than when it is digested into glucose (Dierick et al., 1989). Although the starch digestibility at the terminal ileum of the diets was close to 100% (Bakker et al., 1996), it is still uncertain whether all starch was digested completely into glucose or that part was fermented into VFA.

The present study aimed to determine the potential energy supply by FERM and the effect of adding fat. Therefore, methane production and concentrations of fermentation products in faeces were measured. In addition, in the ileal digesta concentrations of fermentation products were measured to distinguish between enzymatic digestion of starch or partly degradation by fermentation.

Material and methods

Diets

A basal diet was formulated to supply all the required amino acids, minerals and vitamins (Table 6.1). To ensure that the composition of the basal diet was identical in all experimental diets, one large batch sufficient for the whole experiment containing all the ingredients except the cane molasses, was prepared and mixed. The correct proportion of molasses was added to the final diets. Six diets were tested in a 2 x 3 factorial design with two inclusion levels of animal fat and three sources of carbohydrates. Of the factor animal fat either none (o) or 70 (m) g per kg was added to the diet. The three sources of carbohydrates used as the second factor were: maize starch (M), purified cellulose (C; 260 g per kg diet; Arbocel B800, J. Rettenmaier, Holzmühle,

Germany), and toasted soya bean hulls (S; 270 g per kg diet). Maize starch was assumed to be digested completely by the gastro-intestinal enzymes.

Table 6.1
Ingredients (g) per kg basal diet

Barley	290
Soya bean flour	279
Potato protein	114
Wheat middlings	207
Soya bean oil	19
Alfalfa	41
Limestone	20
Ca(H ₂ PO ₄) ₂ ·1H ₂ O	16.9
NaCl	4.2
Trace mineral/vitamin pre-mix ¹	3.5
Choline chloride	0.5
S-DL methionine	1.3
L-lysine HCL	1.5
Cr ₂ O ₃ -starch mixture	2.1

¹ Contained the following ingredients (mg/kg basal diet, unless otherwise stated): 39 vit. AD₃ (19,500 IU and 3,900 IU, respectively); 39 vit. E; 10 riboflavin; 49 niacin; 19 DL-Ca-pantothenate; 49 µg/kg of vit. B₁₂; 304 antioxidant (4 to 5% of BHA, 4 to 5% of ethoxyquin, 4 to 5% of citric acid, 2 to 3% of orthophosphoric acid, 2 to 3% of E 471 fatty acid, and SiO₂ as carrier); 121 MnO (93.6 Mn); 376 ZnSO₄·H₂O (136.9 Zn); 5 KI (3.8 I); 1044 FeSO₄·7H₂O (209.8 Fe); 382 µg/kg of Na₂SeO₃ (174.2 µg/kg of Se)

Table 6.2
Composition of the diets; ingredients (g/kg diet as fed)

Diet	Basal diet	Maize starch	Cellulose	Soya bean hulls	Fat ¹	Cane molasses
Mo	538	349	-	-	-	112
Mm	628	252	-	-	59	61
Co	417	262	261	-	-	61
Cm	480	122	310	-	48	40
So	472	195	-	267	-	66
Sm	506	69	-	314	61	50

¹The fatty acid pattern in the animal fat used was: 26 g/kg < C16:0; 223 g/kg C16:0; 49 g/kg C16:1/C18:0 iso; 138 g/kg C18:0; 393 g/kg C18:1; 128 g/kg C18:2 and 44 g/kg > C18:2.

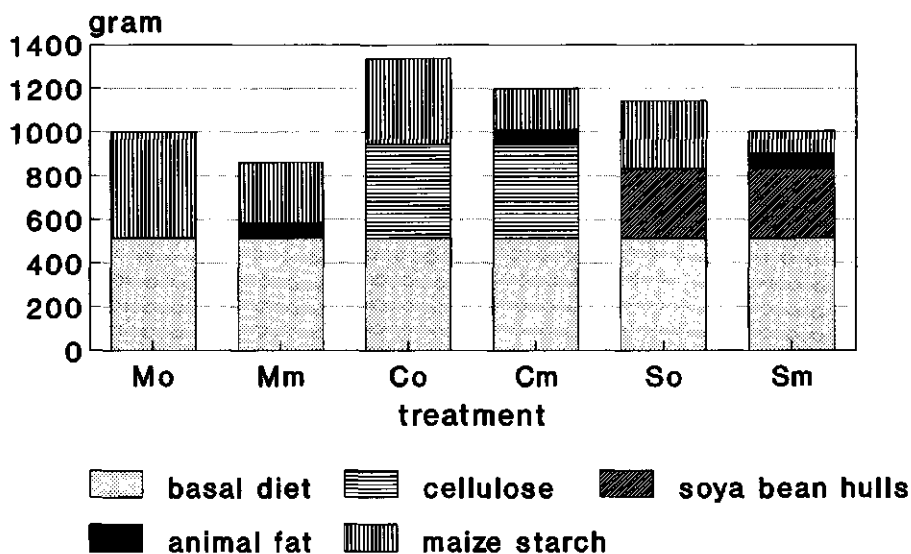


Figure 6.1

The amounts (g) of the six experimental diets yielding the same total net energy as 1000 g of control diet Mo

Cellulose was included as an example of a poorly fermentable carbohydrate and soya bean hulls were used as an easily fermentable carbohydrate source.

Each kilogram of the control diet (with maize starch, without added fat; Mo) contained 540 g of basal diet and 460 g of maize starch. In each of the five other experimental diets, a certain amount of the maize starch in the control diet was replaced with fat, cellulose and soya bean hulls of equivalent net energy (Figure 6.1). As a result, the six diets differed in net energy (NE_i) densities and nutrient concentrations per kg (Table 6.2). Thus, to consume the equivalent amount of NE_i the pigs on a low density feed needed to eat more than pigs on a high density feed. All the diets were made simultaneously in amounts sufficient for all experiments. They were cold-pelleted without adding steam and stored at -18°C until required. On average one week prior to each trial, feed was taken out of the freezer and stored at $+4^\circ\text{C}$.

In composing the diets, no corrections were made for the protein and fat present in the soya bean hulls. Correction for these "contaminations" would have a large impact on the diet composition. This would make comparison between the treatments even more complicated.

Feeding and animals

The castrated males selected for the experiments were from a three-way cross Yorkshire ♂ x (Finnish Landrace ♂ x Dutch Landrace ♀)♀. We aimed at selecting an uniform group for each trial with as few sires as possible. The pigs were assigned to one of the six diets and received that diet continuously from 30 to 105 kg live weight. At about 30 kg live weight the pigs were gradually introduced to the diets by increasing the amount of experimental diet and reducing the starter feed over a period of four days. The pigs were weighed every week. Their ration for the following week was based on this weight and on the growth expectancy in the week to follow. The pigs were fed individually twice daily, receiving 50% of their daily feed allowance at each meal time. The feeding level was aimed to supply 10.4 MJ NE_i per day at 30 kg live weight, 18.6 MJ NE_i per day at 60 kg and 24.1 MJ at 90 kg live weight (CVB, 1988). The pigs received 0.33 l water per MJ NE_i. Water was added to the feed at least six hours before feeding. Composite samples were taken of each diet during weighing of the meal portions, and presented to the laboratory for chemical analysis. Occasional leftovers were collected daily from the troughs, stored at 4°C, weighed and analysed for DM content.

Experiment 1: Methane production

In total, 24 castrated males (four per treatment) were used in two trials to measure methane production. Before the measurements, each pair of two castrated males receiving the same diet were housed together in a pen. At approximately 85 kg live weight, each pig was transferred to a metabolism crate. The two crates with pen mates were together placed in an open circuit respiration chamber (volume 12 m³), where the pigs were allowed one week to adapt to the new housing conditions. Then, all faeces excreted during the subsequent balance period of 10 days were collected and stored at -18 °C, at which time the pigs had a mean live weight of 90 kg. Samples of feed and faeces were analysed for dry matter (DM), ash, crude protein (XP), crude lipid (XL) and gross energy (GE). The amounts of digestible fermentable carbohydrates (DFERM) were calculated as DDM - DASH - DXP - DXL - starch - sugar). In this experiment, the digestible energy supply (DE) was calculated to investigate its relationship with the energy losses by methane production. In addition, methane was related to DE supply from the nutrients starch and sugar (calculated as: (starch + 0.94 x sugar [CRC, 1989]) x 17.6 MJ/kg), fat (DXL x 39.6 MJ/kg) and fermentable carbohydrates (DFERM x 17.6 MJ/kg). Methane was measured using the infra-red technique (Hartman & Braun, Uras G3) twice during the balance period of 10 days, each time continuously for 48 hours.

Experiment 2: Concentration of fermentation products in faeces and ileal digesta in relation to the amounts of digested nutrients

Eighteen ileum-cannulated castrated males (three per treatment) were used to

measure apparent total tract and ileal digested amounts of nutrients, and the concentration of fermentation products in faeces and in digesta. The pigs were housed individually in pens (1.6 m²; described by Bakker et al., 1996). After having received the experimental diets from on average 30 kg onwards, at about 45 kg live weight, the 18 pigs were surgically fitted with a steered ileal-caecal valve (SICV) cannula as described by Mroz et al. (1996). Use of this technique results in reliable values of total tract digestibility, because the hindgut remains intact. In each trial two reserve pigs were also prepared, in accordance with our standard procedures. After surgery, incrementally amounts of experimental diets were supplied, until the pigs received the intended amounts for the experiments.

Faeces and digesta were collected at 90 kg live weight. All the excreted faeces were collected in a plastic bag attached to the pig's rear (Van Kleef et al., 1994), during a 10 day period. This method of collection makes it possible to collect the faeces without contamination with urine. Faeces were collected at each feeding time by replacing the plastic bag containing faeces with an empty one. The bag and its contents were weighed and stored at -18°C. Digesta was collected on the first and sixth day after faeces collection had been completed. It was collected in a plastic bag attached to the cannula, continuously for 24 hours on each of these days (Mroz et al., 1996). The hourly amounts produced by each pig were weighed and stored at -18 °C until required for chemical analysis.

With these samples total tract and ileal digestibility (TD and ID, respectively) of DM, ASH, XP, XL, neutral detergent fibre (NDF), acid detergent fibre (ADF), starch and gross energy (GE) were determined using chromic oxide as a marker. The daily amounts of total tract and ileal digested nutrients were obtained by multiplying the daily amount of each nutrient consumed with its TD and ID, respectively. The concentrations of volatile fatty acids (VFA), lactic acid and alcohol were measured in the faeces as well as in the digesta.

Analytical procedures

When all the digesta and/or the faeces had been collected, the daily amounts from each pig were mixed together and samples were taken for analysis. Faeces were defrosted, but the digesta remained frozen. While mixing the digesta, liquid nitrogen was added to avoid thawing. This procedure minimizes the risk of evaporation of ammonia from the digesta.

The samples from each diet were analysed in duplicate, following official Dutch protocols (NNI, 1992), most of which are identical to those of the Association of Official Analytical Chemists (AOAC, 1984).

Samples of fresh material were obtained for nitrogen analysis (NEN 3145), VFA and lactic acid (Robinson et al., 1986). The remaining faeces were air-dried and the digesta was freeze-dried. These samples were then ground through a 1 mm sieve and analysed for DM (NEN 3332), ash (NEN 3329), XF (NEN 5415), XL (EG L15/29, method B pre-treatment with hydrochloric acid) and chromium

(as described by Bakker and Jongbloed, 1994). Analysis for NDF and ADF content was performed using the method described by McQueen and Nicholson (1979) and GE content was measured with an adiabatic bomb calorimeter. The FERM contents were calculated by subtracting the amounts of ash, XP, XL, starch and sugar from the DM content. The hemicellulose fraction was calculated as the difference between NDF and ADF. All digesta samples were analysed enzymatically for starch content (amyloglucosidase/hexokinase method; NEN 3574). Faeces samples from at least one pig per treatment were analysed for starch content. If significant amounts of starch were found, the samples of the other pigs in the same treatment were also analysed for starch.

This experiment was the last one in the project. In all, between 3 and 18 feed samples were analysed per diet during the entire project (Bakker et al., 1993). In the present experiment on the cannulated pigs, feed samples were analysed in duplicate for DM, NDF, ADF, and chromium content. The results from these analyses indicated that the diets used in the present experiment were within the range (max. 3%) of the mean in the entire project. Therefore, the chemical composition of the diets as presented in Table 6.3, were the mean values of the entire project.

Table 6.3

Analysed chemical composition of the experimental diets (g/kg DM, unless otherwise stated)

Diets ¹ :	Mo	Mm	Co	Cm	So	Sm
Dry matter (DM, g/kg)	881	884	889	898	883	889
Ash	51	57	40	50	59	67
Crude protein	176	204	134	148	188	206
Crude lipid	30	98	22	77	31	104
Crude fibre	29	35	220	258	115	132
Starch	444	374	328	216	294	186
Sugar	87	68	53	44	66	57
NDF	99	118	342	395	242	278
ADF	40	49	280	319	151	176
Hemicellulose	59	69	62	76	91	102
FERM ²	213	201	424	465	362	381
Gross Energy (MJ/kg DM)	18.3	19.9	18.1	19.3	18.3	19.9

¹ Diet: maize starch (M), cellulose (C) or soya bean hulls (S) were combined with two amounts of added fat (0 (o) and 70 (m) g/kg, respectively)

² FERM: fermentable carbohydrates calculated as: DM - Ash - XP - XL - Starch - Sugar

Experimental design and statistical analysis

In Experiment 1, data on methane production and total tract digestible energy in intact pigs were obtained from a statistically balanced experiment. Two trials were performed, each one comprising two pigs per treatment. Each pair of pigs formed the experimental unit for measuring methane production (two observations per diet). For the amounts of digested energy, each pig was the experimental unit (four observations per diet). Statistical analysis was performed by analysis of variance using GENSTAT (Payne et al., 1987) according to the following model:

$$Y_{hijk} = \mu + T_h + Carb_i + F_j + (Carb \times F)_{ij} + e_{hijk}$$

where μ = overall mean;

T_h ($h = 1, 2$) = effect of trial;

$Carb_i$ ($i = 1 \dots 3$) = effect of source of carbohydrate;

F_j ($j = 1, 2$) = effect of fat;

$(Carb \times F)_{ij}$ = interaction between carbohydrates and fat;

e_{hijk} = error.

In Experiment 2, data on total tract and ileal digested nutrients and on concentration of fermentation products (methane excluded) in faeces and chyme were obtained. Three trials were performed, each one comprising one pig per treatment. Thus, each pig was an experimental unit. For statistical analysis in this experiment, the same statistical analysis model as mentioned above was used, with the slight alteration that h in the factor T was now 1, 2 or 3.

Results

General

In Experiment 1, measurements of methane production in the intact pigs went according to plan. In Experiment 2, with the cannulated pigs, however, two pigs had to be removed from the first trial (one on diet Cm and one on diet So); one of these two pigs continued to leave some of its feed and the other one lost its cannula. In the second and the third trials no problems occurred. In the third trial two pigs were added to the six that already had been planned. They received the two missing diets in order to maintain three statistical units per diet as planned. Thus, the number of replicates per diet remained balanced.

In both Experiments 1 and 2, TD of energy and DM were measured. Results showed that the TD of DM and OM were on average 3 percentage units higher in the cannulated pigs, compared to intact pigs. The comparison between these two types of pigs has been described in detail by Mroz et al. (1996).

On average, the pigs consumed 2271 g DM/day of diet Mo (sd 33.2); 1990 g DM/day of diet Mm (sd 40.0); 3079 g DM/day of diet Co (sd 63.0); 2746 g DM/day of diet Cm (sd 69.8); 2626 g DM/day of diet So (sd 23.1); and 2368 g DM/day of diet Sm (sd 41.0).

Experiment 1: methane production

The differences between diets in DE, DE-XL, and DE-(starch&sugar) (Table 6.4) were due to the experimental design: maize starch from the control diet (Mo) was exchanged with similar amounts of net energy from fat and/or cellulose or soya bean hulls.

Table 6.4

Amounts of digestible energy (MJ/d) supplied by the whole diet (DE) and separately by fat (DE-XL), starch and sugar (DE-[starch and sugar]) and fermentable carbohydrates (DE-FERM), the energy loss by methane (MJ/d), and the ratios (%) of methane to DE and DE-FERM with the six experimental diets (Experiment 1)

	Mo	Mm	Co	Cm	So	Sm	r.s.d. ¹	Significance ¹
DE	37.25 ^c	33.80 ^{ab}	36.60 ^{bc}	31.75 ^a	39.00 ^c	36.35 ^{bc}	1.32	Carb [*] , Fat ^{**}
DE-XL	1.70 ^a	5.57 ^b	1.59 ^a	5.94 ^b	1.75 ^a	7.07 ^c		Carb ^{**} , Fat ^{***}
DE-(starch&sugar)	21.04 ^f	15.10 ^c	20.23 ^e	12.35 ^d	16.26 ^d	9.79 ^a		Carb ^{***} , Fat ^{***} , Carb x Fat ^{**}
DE-FERM	6.23 ^{ab}	4.73 ^a	7.52 ^b	5.98 ^{ab}	12.50 ^c	10.72 ^c	0.82	Carb ^{***} , Fat [*]
Methane	0.25 ^a	0.25 ^a	0.40 ^a	0.30 ^a	0.85 ^b	0.85 ^b	0.15	Carb ^{**}
Methane/DE	0.7 ^a	0.7 ^a	1.1 ^a	0.9 ^a	2.2 ^b	2.3 ^b	0.4	Carb ^{**}
Methane/DE-FERM	4.0	5.3	5.4	4.6	6.8	7.9	1.8	

¹From the analysis of variance with the effects of source of carbohydrate (Carb), addition of fat (Fat) and the interaction between source of carbohydrate and fat addition (Carb x Fat). r.s.d.: residual standard deviation. Statistical significance: *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$

^{a,b,c,d,e,f} Means with different superscript in the same row differ significantly ($P < 0.05$)

The pigs with the S diets, received more DE from fermentable carbohydrates (DE-FERM) than those with the C or the M diets. In addition, they produced significantly more methane than those receiving the M or the C diets (Table 6.4). Neither fat nor the interaction between fat and source of carbohydrate had a significant effect on methane production. The effect of source of carbohydrate was significant when methane production was expressed per kJ DE, but the effect, however, was not significant, when expressed per kJ DE from FERM (DE_{ferm}).

Regression analysis showed a significant linear relation between methane energy (Y-axis) and the amount of DE from fermentable carbohydrates (Figure 6.2 and equation 1).

$$\text{Methane energy (kJ/d)} = -269.3^* (95.4) + 94.7^{***} (1.13) \times \text{DE-FERM (MJ/d)} \\ \text{adj. } R^2 = 86.3\%, \text{ rsd} = 110 \quad (1)$$

Figure 6.2 shows that diets with added fat (m) apparently gave a higher methane production than those without added fat (o). Regression analysis with DE from fat as an additional variable in the model gave the following equation:

$$\text{Methane (kJ/d)} = -399.1^{**} (98.8) + 97.9^{***} (0.96) \times \text{DE-FERM (MJ/d)} + 26.6^* (1.18) \times \text{DE-XL (MJ/d)} \quad (2)$$

adj. $R^2 = 90.2\%$, $\text{rsd} = 93$

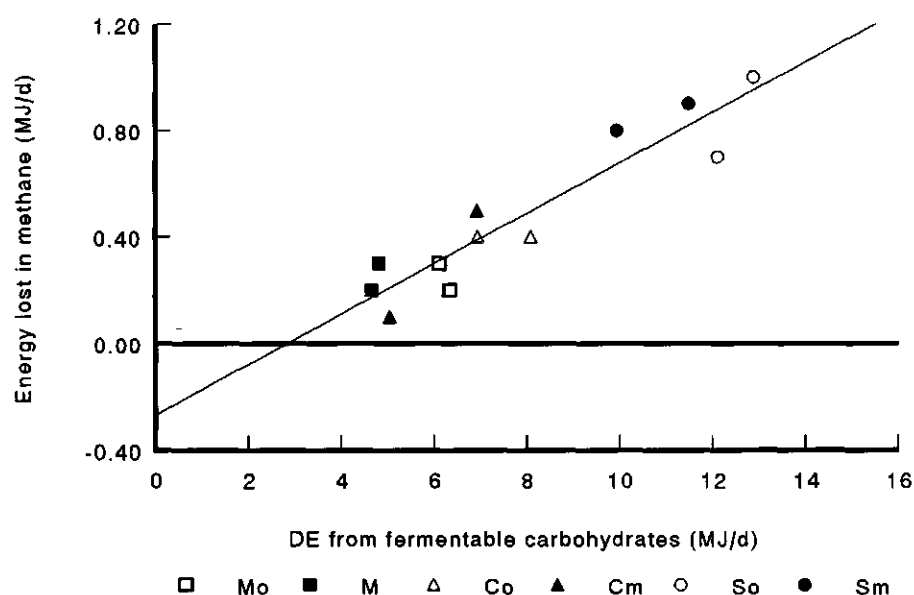


Figure 6.2

The relationship between methane production (Y-axis) and DE from fermentable carbohydrates (DE-FERM; X-axis), according to the equation:

$$\text{Methane energy (MJ/d)} = -0.269^* + 0.0947^{***} \times \text{DE-FERM (MJ/d)}$$

adj. $R^2 = 86.3\%$, $\text{rsd} = 0.110$

Equation (2) gave a better fit of the data than equation (1): the adj. R^2 was higher and the residual standard error of the observations was reduced from 110 with equation (1) to 93 with equation (2). The coefficient for DE-fat reached significance at $P = 0.05$.

Experiment 2: Concentration of fermentation products in faeces in relation to the amounts of digested nutrients

The TD of starch was found to be 100%. Similarly as in Experiment 1, there were large differences between the treatments in amounts of digested fat and starch (Table 6.5), as a result of the experimental design.

Table 6.5

Amounts of total tract digested nutrients (g/d) in the six experimental diets, the concentrations of alcohols, lactic acid and volatile fatty acids in fresh faeces (mmol/kg), and the ratio of acetic, propionic and butyric acid in the volatile fatty acids (%) (Experiment 2)

	Mo	Mm	Co	Cm	So	Sm	r.s.d. ¹	Significance ¹
Total tract digested nutrients (g/d)								
DM	2031 ^{bc}	1747 ^a	2118 ^c	1808 ^{ab}	2168 ^c	1859 ^{ab}	131.8	Fat***
Protein	354 ^b	365 ^b	322 ^a	305 ^a	395 ^c	391 ^c	12.7	Carb***
Fat	49 ^a	166 ^b	46 ^a	160 ^b	50 ^a	193 ^c	4.6	Carb***, Fat***, Carb x Fat***
Starch	1008 ^a	744 ^c	1010 ^a	593 ^b	772 ^d	440 ^a	11.7	Carb***, Fat*** Carb x Fat***
Sugar	198 ^a	135 ^b	163 ^c	121 ^a	173 ^d	135 ^b	2.3	Carb***, Fat*** Carb x Fat***
FERM	361 ^{ab}	278 ^a	521 ^{bc}	571 ^{bc}	704 ^c	632 ^c	121.5	Carb**
NDF	135 ^a	145 ^a	379 ^{ab}	504 ^b	443 ^b	453 ^b	139.7	Carb**
ADF	47 ^a	52 ^{ab}	237 ^{abc}	345 ^c	260 ^{bc}	264 ^c	112.3	Carb**
Hemicellulose ²	88 ^a	93 ^{ab}	142 ^{bc}	159 ^c	182 ^c	189 ^c	28.6	Carb***
Concentrations of fermentation products in faeces (mmol/kg)								
Lactic acid	4.6	6.0	7.7	5.7	9.2	5.9	3.60	
Alcohols	0.2	0.3	-0.3	54.5	3.4	27.7	37.09	
Volatile fatty acids	49.8 ^a	56.7 ^a	92.1 ^{ab}	138.9 ^b	137.3 ^b	42.1 ^a	29.76	Carb*, Carb x Fat**
% acetic acid	71.9	66.2	68.2	67.0	62.8	53.8	13.24	
% propionic acid	19.7	21.7	21.0	22.7	23.9	29.1	9.04	
% butyric acid	4.4 ^a	6.4 ^a	8.1 ^{ab}	8.4 ^{ab}	8.6 ^{ab}	11.6 ^b	2.86	Carb*
% isobutyric acid	2.0	3.0	1.1	0.7	2.0	2.3	1.40	
% valeric acid	2.1	2.7	1.8	1.2	2.7	3.2	1.73	

¹a,b,c see Table 6.4

² Hemicellulose was calculated as NDF minus ADF

The lowest amounts of DFERM were found with the M diets; the highest amounts with the C and S diets. The C diets tended to supply more digestible ADF, the S diets more hemicellulose. Within each fat inclusion level, the amounts of digestible fat were not different between the sources of carbohydrate, except for diet Sm.

In the faeces, the concentrations of lactic acid were not different between the treatments (Table 6.5) and with most treatments, the concentrations of alcohols in the faeces were not significantly different from 0. Only with Cm and Sm alcohols were detected in faeces, but this effect of treatment was not

statistically significant due to the very large variation between pigs.

In faeces, the concentrations of volatile fatty acids (VFA) were significantly different between the treatments (Table 6.5). On average, in the faeces of the pigs fed the C diets the highest concentration of VFA were measured and in the faeces of those fed the M diets the lowest concentration. The larger concentration of VFA with the C and S diets was accompanied by an increased ratio of butyric acid. Addition of fat had no effect of the VFA-concentration in faeces with the M-diets, whereas it increased the concentration with the C-diets and reduced it with the S-diets.

Experiment 2: Concentration of fermentation products in ileal digesta in relation to the amounts of digested nutrients

The dietary treatments varied in supply of both fat and starch at the terminal ileum (Table 6.6), as a result from the experimental design. At this site, starch was almost completely digested, irrespective of the diet supplied.

The amounts of ileally digested crude protein were slightly higher with the S diets. This extra protein originates from the soya bean hulls, which was not corrected for in the experimental design.

The amounts of DFERM at the terminal ileum were similar for all diets: only with the Cm diet no FERM was disappeared before the terminal ileum. Also, NDF disappearance was lowest with both C diets; especially the ADF digestion was very low. The amounts of FERM, NDF and ADF consumed, however, were by far the largest with the C diets. Conversely, the amounts of ileally digested hemicellulose (calculated as NDF minus ADF) were less different between the treatments. Thus, the low amounts of ileally digested FERM, NDF and ADF with the C diets compared to the M diets and the S diets is mainly associated with ADF.

When fat was added, the amounts of ileally digested FERM tended to be reduced. However, with these diets also total consumption of FERM was slightly less.

Of the three types of endproducts from fermentation, lactic acid, alcohols and VFA, at the terminal ileum only the concentration of lactic acid was significantly affected (Table 6.6): with the M diets the highest concentrations were found compared to the C and the S diets. The concentration of lactic acid in the digesta was on average four times higher than found in faeces (Table 6.5).

In the digesta, concentration of alcohols were on average double the concentration in the faeces. The concentrations compared to lactic acid and VFA, however, were still relatively low. Compared to the concentrations in faeces, the VFA concentrations in digesta were at similar level in both M-diets, but they were more than two or three times lower with the S-diets and the C-diets. The concentration of VFA in digesta was not significantly different between treatments. Also, the relative ratios of the separate VFA were not

significantly different between treatments. The concentrations of isobutyric acid and valeric acid were negligible.

Table 6.6
Amounts of ileally digested nutrients (g/d) in the six experimental diets and the concentrations of alcohols, lactic acid and volatile fatty acids in fresh digesta (mmol/kg), and the ratio of acetic, propionic and butyric acid in the volatile fatty acids (%) (Experiment 2)

	Mo	Mm	Co	Cm	So	Sm	r.s.d. ¹	Significance ¹
Ileally digested nutrients (g/d)								
DM	1728 ^d	1493 ^b	1613 ^{cd}	1148 ^a	1520 ^{bc}	1223 ^a	63.8	Carb ^{***} , Fat ^{***} , CarbxFat [†]
Protein	311 ^{ab}	324 ^b	303 ^{ab}	292 ^a	356 ^c	348 ^c	12.6	Carb ^{***}
Fat	50 ^a	167 ^b	50 ^a	165 ^b	61 ^a	200 ^c	6.5	Carb ^{***} , Fat ^{***} , CarbxFat [†]
Starch	996 ^a	735 ^c	1000 ^a	585 ^b	764 ^d	428 ^a	12.4	Carb ^{***} , Fat ^{***} , CarbxFat ^{***}
Sugar	198 ^a	135 ^b	163 ^c	121 ^a	173 ^d	135 ^b	2.3	Carb ^{***} , Fat ^{***} , CarbxFat ^{***}
FERM	127 ^b	90 ^b	82 ^b	-28 ^a	135 ^b	87 ^b	47.0	Carb [†] , Fat [†]
NDF	50 ^{bc}	64 ^{bc}	37 ^b	-26 ^a	88 ^{bc}	94 ^c	30.2	Carb ^{**}
ADF	-2 ^b	10 ^b	-37 ^b	-96 ^a	5 ^b	-11 ^b	25.7	Carb ^{**}
Hemicellulose ²	53 ^a	55 ^a	74 ^b	70 ^{ab}	83 ^b	105 ^c	9.4	Carb ^{***}
Concentrations of fermentation products in digesta (mmol/kg)								
Lactic acid	57.5 ^b	32.5 ^{ab}	8.7 ^a	18.7 ^a	31.5 ^{ab}	20.1 ^a	14.52	Carb [†]
Alcohols	5.8	3.4	20.5	4.8	5.4	9.7	11.04	
Volatile fatty acids	42.9	64.6	49.7	52.0	54.8	51.2	19.34	
% acetic acid	76.3	74.3	76.4	74.7	76.4	73.5	4.99	
% propionic acid	16.9	21.5	17.9	19.8	20.1	21.6	4.42	
% butyric acid	5.7	4.1	5.8	5.5	3.6	4.8	2.41	
% isobutyric acid	0	0	0	0	0	0	0	
% valeric acid	1.0	0.0	0.0	0.0	0.0	0.0	0.72	

¹,^a,^b,^c,^d,^e see Table 6.4, ² see Table 6.5

Discussion

The purpose of this study was to determine the potential energy supply of DFERM and its relation with the amounts of the nutrients fat and starch & sugar. The motive for this experiment originates from a previous experiment with the identical diets (Bakker et al., 1996), in which it was concluded that the

combination of fermentable carbohydrates (cellulose and soya bean hulls) with fat supplied less digestible energy at the terminal ileum and over the total tract than expected from the separate ingredients. This explained partly the reduced performance of the pigs receiving these diets (Bakker et al., 1995). An additional explanation for this effect might be found in the endproducts of fermentation. In addition, in the previous study (Bakker et al., 1996), starch might have been fermented precaecally. However, this could not be confirmed, because at the terminal nearly all starch was apparently digested (Bakker et al., 1996). By measuring fermentation products in the digesta and methane production we aim to answer this question.

Methane

The present results on methane production shows a clear relation between the energy from fermented carbohydrates (DE-FERM) and methane production (Table 6.4 and equation 1). Apparently, on average 9.5% of each extra MJ DE-FERM was lost as methane. This corroborates the results with ruminants, where losses of energy as methane were measured up to 10% of the DE (Beever et al., 1991). However, Kirchgeßner et al. (1991) concluded that the loss of methane in pigs was only up to 4% of each extra DE-FERM (equation 3, adapted from Kirchgeßner et al., 1991)

$$\text{Methane production (kJ/d)} = 161.2 + 41.1 \times \text{DE-FERM (MJ/d)} \quad (3)$$

$$R^2 = 59\%$$

Both equations (1) and (3) predict similar methane production at DE-FERM = 8 MJ/d. Equation (3) is based on lower amounts (both the upper and the lower limits of the range) of DE-FERM (all less than 8 MJ/d) than equation (1). Apparently, at lower DE-supply (equation 3), the equation is less steeper than when DE-supply is higher (equation 1). These results indicate, that the relation between DE-FERM and methane production might not be linear, especially in the lower range. Similarly, also in other studies where the pigs received less DE-FERM than in the present study (Zhu et al., 1993; Shi and Noblet, 1994) a lower proportional loss of energy in methane was found: 5% as recalculated from their measured values.

In addition, a lower methane production might have been caused by a shorter adaptation period of seven (Zhu et al., 1993), eight (Shi and Noblet, 1994) to (at least) ten days (Kirchgeßner et al., 1991). In the present experiment the pigs received the diets at least ten weeks before methane production was measured.

In the present experiment, adding fat to the diet increased methane production (equation 2). This was the opposite to what was expected (Christensen and Thorbek, 1987). There is no explanation why fat addition increased methane production. Addition of fat was confounded with starch removal (Table 6.2), but it is not likely that a higher proportion of the starch was

fermented when the total supply was lower. There is only an explanation why the fat did not reduce methane production in the present study. The main difference between the present study and that of Christensen and Thorbeck (1987) concerned the type of fat added: we used animal fat while they used vegetable oil. The animal fat contained a large quantity of saturated fatty acids, while the vegetable oil contained mainly unsaturated fatty acids. Synthesis of methane is a pathway for binding excessive H_2 (Hungate, 1966, pp 266-270). Jensen and Jørgensen (1994) hypothesized that the soya oil in the study of Christensen and Thorbeck (1987) formed a sink for H_2 in the gastrointestinal tract resulting in less methane synthesis. This explains also why the animal fat in the present study did not reduce methane production.

In conclusion, the present results show that the amounts of methane was highly correlated with the different amounts of total tract DE-FERM in the diets. Regression analysis showed that loss of energy as methane was 9.5% with each additional MJ of DE-FERM. It is suggested that the relation between methane and DE-FERM might not be linear, but also adaptation time to the diet might have a large impact. Fat addition seemed to increase methane production.

Volatile fatty acids

In the digesta, the dietary factors had no significant effects on the VFA ratios (acetic acid:propionic acid:butyric acid) which averaged 75:20:5 (Table 6.6). In contrast, Drochner (1984) found a significant increase in acetic acid percentage in digesta from 75 to 78% by adding different sources of fibre to a control diet. When large amounts of starch would have been degraded by fermentation, it would have affected the ratios of VFA.

The absence of any effect on both VFA concentration and ratios in digesta corresponded with ileal FERM digestion (in Table 6.6): at the terminal ileum, the diets did not significantly differ in amounts of FERM that had disappeared. The flow of digesta into the hindgut, however, was very different between the sources of carbohydrates. As a result, the daily flow of VFA into the hindgut was highest with the C diets and lowest with the M diets. However, it is expected that this will have no effect on the energy supply to the pig, because these VFA can be absorbed in the hindgut.

In faeces, the acetic acid:propionic acid:butyric acid ratios of on average 71:22:7 were comparable to what was reported by most others; except Müller et al. (1989) reported 66:24:10 and Zhu et al. (1993) reported 59:24:17. The ratios of acids formed, generally depend on the type of the available nutrients. The shift in the ratios between different sources of carbohydrate in the present study, however, was not substantial. Similarly, Vervaeke et al. (1989) measured slightly different ratios for each of their eight different diets (range: 164-270 g NDF per kg and 66 - 113 g ADF per kg), averaging 65:21:14. In addition, the ratios reported by Zhu et al. (1993) of 59:24:17 in their basal diet were not changed when they added sugar beet pulp. In the present study, the

fermentable carbohydrates significantly increased the proportion of butyric acid in the VFA in faeces from 5 to 9%. Bach Knudsen et al. (1993) found an enhanced ratio of butyric acid when oat bran was given instead of oat flour or wheat products. In general, it appears to be difficult to relate the VFA ratios to the type of available nutrients.

When the VFA concentration in faeces is multiplied by the total excretion of faeces, the total amount of excreted VFA is obtained. This measured amount of VFA in faeces is the difference between the amounts of VFA produced and absorbed. According to Kenelly et al. (1981), the amount of VFA in digesta and faeces represent only a small fraction of the total amount of VFA that was formed, because VFA is absorbed very rapidly. Similarly as at the terminal ileum, at the rectum the differences in amounts of faeces leaving the hindgut was very large between the diets. This indicates that there were large differences in amounts of VFA excreted in the faeces. The energy of VFA in faeces is not available to the pig anymore. Therefore, it is calculated how much of the DE-FERM was available as an energy source to the pig (Table 6.7). It was assumed that the amount of energy supplied by VFA was equal to the amount of DE-FERM minus the energy lost as methane and (Table 6.4) the energy lost as heat (6% according to the stoichiometry of Hungate, 1966). Then the amount of energy in VFA lost in faeces was subtracted.

Table 6.7

Calculated amounts of energy absorbed as volatile fatty acids (VFA) in the gastrointestinal tract of pigs (all data in MJ/d)

	Mo	Mm	Co	Cm	So	Sm
DE-FERM (Table 6.4)	6.23	4.73	7.52	5.98	12.50	10.72
Lost as methane (Table 6.4)	0.25	0.25	0.40	0.30	0.85	0.85
Lost as heat (6%; Hungate, 1966)	0.37	0.28	0.45	0.36	0.75	0.64
VFA ¹						
acetic acid	3.41	2.29	3.60	2.76	5.37	3.67
propionic acid	1.68	1.36	2.00	1.69	3.69	3.58
butyric acid	0.52	0.55	1.07	0.86	1.84	1.98
VFA lost in faeces ²	0.03	0.04	0.13	0.44	0.24	0.08
Energy from VFA	5.58	4.16	6.54	4.87	10.66	9.15
in % of DEferm	89.6	88.0	87.0	81.4	85.3	85.4

¹ Energy content of acetic acid 0.873 MJ/mole; propionic acid 1.575 MJ/mole and butyric acid 2.179 MJ/mole; relative ratios obtained from Table 6.5

² Calculated as amount of fresh faeces (kg/d) x concentration of VFA-energy in faeces (MJ/mmol x mmol/kg)

It was concluded that the amount of energy absorbed as VFA in the pig was different between the sources of carbohydrates. For the M diets, it was on average 89%, while for the C and the S diets it was on average 84 and 85%, respectively.

In conclusion, no clear relationship could be found between the concentrations and the ratios of volatile fatty acids in chyme and faeces and the amount of fermented carbohydrates. When calculating the potential (net) energy supply from VFA, the amounts lost in faeces and methane should be subtracted.

Lactic acid

In the digesta of the pigs receiving the M diets a higher concentration of lactic acid was measured. With these diets the amounts of ileally digested starch and sugar were the highest (Table 6.6). This might indicate that (parts of) this starch and sugar was not entirely digested into glucose but were partly used for synthesis of lactic acid. This could result in a reduced energy gain, because the pig retains more fat from glucose than from lactic acid (Vermorel, 1968; cited by Rérat, 1978). The performance of the pigs with the M diets was, however, higher compared to those with the other diets (Bakker et al., 1995). Therefore, the possible absorption of lactic acid instead of glucose did not considerably alter the availability of nutrients. It was quantitatively not very important. However, this does not include qualitative effects on gut health.

Conclusions

In conclusion, the amounts of methane could be estimated from the different amounts of total tract DE-FERM in the diets. Loss of energy through methane could increase up to 10% of the DE-FERM. However, it is suggested that the relation between methane and DE-FERM might not be linear. The effects on methane production of amount of DE-FERM, added fat, type of fat and adaptation time should be investigated more in detail, to enable an accurate prediction of methane production.

No clear relationship could be found between the concentrations and the ratios of VFA in chyme and faeces on one hand and DFERM on the other hand. When calculating the potential energy supply by VFA, the amounts lost in faeces and methane should be subtracted. Of the DE-FERM, between 81 to 90% is absorbed as VFA.

The possible absorption of lactic acid instead of glucose did not considerably alter the availability of nutrients. It was quantitatively not very important. However, this does not include qualitative effects on gut health.

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Chapter 7

Energy gain in pigs receiving diets containing large amounts of fat and fermentable carbohydrates

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Abstract

This study aimed to determine the utilization of digestible nutrients for energy retention, as affected by the amount of fat and fermentable carbohydrates. A factorial design was made with 12 treatments. These were composed by iso-energetical exchange of maize starch from the control diet with purified cellulose (260 g/kg), with toasted soya bean hulls (270 g/kg) and with four amounts of animal fat (0, 30, 60 and 105 g/kg). Ninety-six individually fed castrates received these experimental diets from 30 to about 105 kg live weight (on average 100 d) until they had consumed a fixed amount of estimated net energy. The amounts of retained energy, fat and protein in the pigs were determined using the comparative slaughter technique. In addition, weights of full and empty parts of the gastrointestinal tract were measured. Results showed that both amounts of retained energy and fat were lowest in pigs receiving the cellulose containing diets. Incrementally additions of fat lowered energy and fat gain, especially at the highest fat addition level. The pigs on these treatments retained less fat than the amount of digestible fat they consumed. Retained protein was not significantly different between diets. The weight of the empty hindgut was increased with 23 g per kg of fermentable carbohydrates consumed during on average 100 d. The K_{pf} varied from 53% for a cellulose diet to 66 % for two maize starch diets. From regression analysis, it was concluded that the relatively low efficiency of utilization of 0.43 for FERM was due to its enhancing effects on maintenance requirement. For starch this coefficient was 0.71.

Keywords: pigs, energy retention, carbohydrates, animal fat, gut weight

Introduction

The major nutrients that supply energy to the animal are glucose, lipids, volatile fatty acids and amino acids. Equations that predict potential energy gain in pigs are, therefore, mainly based on the amounts of each of these apparently digested nutrients in diets. The contents of these digestible nutrients in feed ingredients are obtained from feedstuff tables. Moreover, the contents of

digestible nutrients from the separate ingredients in a feed are assumed to be additive. Bakker et al. (1995) showed, however, that this assumption does not hold for feeds with large proportions of fermentable carbohydrates and fat.

The efficiency of utilization of digestible energy (DE) for retained energy (RE) depends on the type of nutrients (Dierick et al., 1989) and also on the composition of energy gain; the protein to fat ratio (Kielanowski, 1965; Webster, 1985). In prediction equations, coefficients for utilization for each of the digestible nutrients and the maintenance requirement per kg metabolic weight are considered constant, irrespective of the ratios of the digestible nutrients included in the diet. Moreover, these values are included in formulas predicting the dietary (net) energy content (ARC, 1981; CVB, 1988; Noblet et al., 1989 and 1994). This assumption is used for common compound feeds, which have a limited variation in digestible nutrients (Noblet et al., 1994). However, for diets which differ largely in chemical composition of the diet or for individual feedstuffs it is doubtful if this assumption is correct (Van der Honing et al., 1984).

From results of a previous study (Bakker et al., 1995), it was concluded that digestibility of feeds with large amounts of fermentable carbohydrates and fat should be measured values. Calculated values from the separate ingredients gave considerable different results. In the present study with the same diets, it is aimed to check whether the utilization of the measured amounts of these digestible nutrients also depend on the ratios of the nutrients included in the diet or if the efficiencies given in literature can be used.

Material and methods

Experimental diets

Twelve diets were tested in a 4*3 factorial design with four amounts of added animal fat¹ (coded: o, l, m and h) and three sources of carbohydrates (coded: M, C and S). The three sources of carbohydrates were maize starch (M), purified cellulose (C; Arbocel B800, J. Rettenmaier, Holzmühle, Germany) and toasted soya bean hulls (S). It was assumed that maize starch was digested completely by the gastrointestinal enzymes. Cellulose was used as a source of poorly fermentable carbohydrates (Fahey et al., 1980). Soya bean hulls were used as a source of easily fermentable carbohydrates (Stanogias and Pearce, 1985). The maximum amounts of cellulose and soya bean hulls to be included in the experimental diets had been determined in previous trials (Bakker et al., 1995). In addition, the net energy contents (NE_e) of cellulose and soya bean

¹ The fatty acid composition of the fat used was: 2.6% < C16:0; 22.3% C16:0; 4.9% C16:1/C18:0 iso; 13.8% C18:0; 39.3% C18:1; 12.8% C18:2; and 4.4% > C18:2.

hulls were calculated by using their measured amounts of digestible nutrients and the NE-equation (Nehring et al., 1969; CVB, 1988; equation 1).

$$NE_i = 10.8 * DXP + 36.1 * DXL + 6.3 * DXF + 12.7 * DXX \quad (1)$$

Each kilogram of the control diet (Mo) contained 540 g of a basal diet that supplied all necessary amino acids, minerals, vitamins and essential fatty acids. The experiment was aimed to give similar protein deposition in the different treatments. Therefore, the amounts of amino acids in the basal diet were based on this goal. The basal diet contained 29 g fat per kg. The remaining 460 g of the control diet was maize starch. In the other 11 diets, 440 g cellulose (C) or 310 g soya bean hulls (S), and 0 (o), 35 (l), 70 (m) or 110 (h) g fat were added to the control diet, at the expense of the equivalent amount of net energy from maize starch (see Figure 7.1).

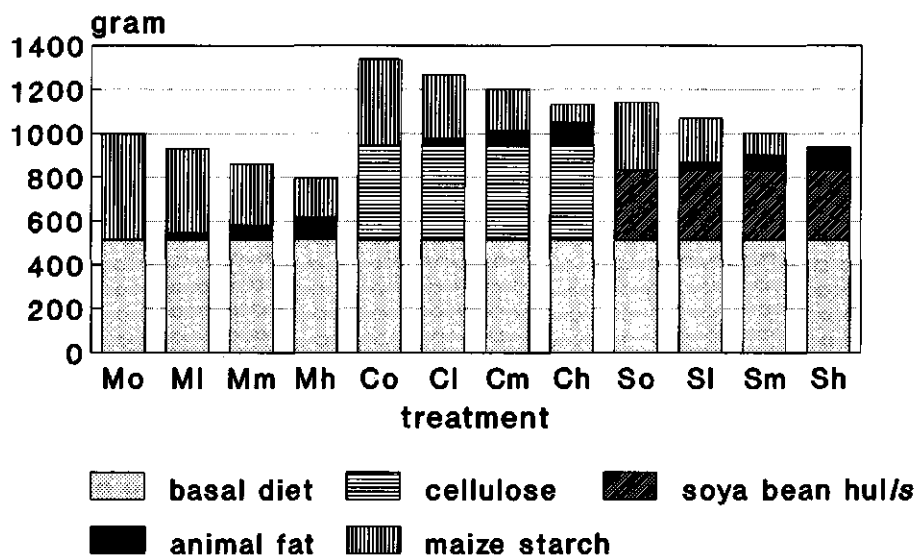


Figure 7.1

Amounts (g) of the 12 experimental diets yielding the same total assumed net energy. This net energy (NE_i) is based on the equation:

$NE_i = 10.8 * DXP + 36.1 * DXL + 6.3 * DXF + 12.7 * DXX$, with the amounts of digestible nutrients in the ingredients obtained from CVB (1988).

As a consequence of this design, the 12 diets had different net energy densities per kg and it thus resulted in an inverse relationship between the dietary concentrations of starch on one hand and those of lipids (XL), crude fibre (XF) and NDF on the other hand. The starch content in the 12 diets ranged

from 453 to 120 g/kg DM; the XL content ranged from 23 to 169 g/kg DM; the XF content ranged from 29 to 258 g/kg DM and the NDF content ranged from 91 to 380 g/kg DM. The analysed chemical composition and the measured digestibility coefficients of the diets were described elsewhere (Bakker et al., 1995). The measured amounts of digestible nutrients consumed (g/pig/day) are presented in Table 7.1. The amounts of feed consumed during the experiment were determined for each pig separately; the digestibility coefficients were the mean values per diet.

The starch and sugar were almost completely disappeared at the terminal ileum (Bakker et al, 1996a). It is assumed that no parts of starch or sugar are precaecally fermented (Bakker et al., 1996b).

Table 7.1

Determined mean daily intakes of digested protein (DXP), fat (DXL), FERM (DFERM), starch & sugar and total DE for each experimental diet during the experimental period of the pigs (30 - 105 kg live weight)

	DXP	DXL	DFERM ¹	starch & sugar ²	DE
Diet ³	g/day				MJ/day
Mo	251	34	232	965	27.8
MI	256	85	211	834	27.6
Mm	259	125	152	706	25.4
Mh	254	207	177	515	26.2
Co	235	36	243	953	27.9
CI	221	85	314	698	26.3
Cm	239	121	218	585	24.4
Ch	245	202	231	429	25.2
So	281	40	486	784	30.2
SI	279	96	479	616	29.4
Sm	272	141	479	455	28.2
Sh	276	210	415	292	27.1

¹ DFERM: Calculated as the daily amount of digested organic matter - DXP - DXL - (starch & sugar)

² Starch & sugar: Sugar was converted to equivalent amounts of net energy of starch by the factor 0.94 (CRC Handbook of Chemistry and Physics, 1989)

³ Diet: maize starch (M), cellulose (C) or soya bean hulls (S) were combined with four amounts of added fat (0 (o), 30 (l), 60 (m) and 105 (h) g/kg, respectively)

Feeding, pigs and housing

To enable faeces to be collected separately from urine male castrates were used. Moreover, castrates will eat more than boars. We used 112 pigs in the experiment, of which we used 16 to determine the initial body composition in this comparative slaughter experiment. The 96 other castrates were assigned to one of the 12 feeds and received their experimental diet continuously from 30 to about 105 kg live weight. They were housed in groups of four in a pen and were fed individually as described by Bakker et al. (1995). The feed allowance was given in two equal meals per day. The feeding level meant to supply 13.3 MJ NE_i per day at 40 kg, 20.8 MJ NE_i at 70 kg and 25.0 MJ NE_i per day at 100 kg live weight. These amounts were about 90% of the net energy considered for a mean growth rate of 700 g per day (CVB, 1988).

Prior to the start of the experiment, the NE_i contents of the diets were estimated from the NE_i equation (Nehring et al., 1969; CVB, 1988) and the concentration of digestible nutrients in the ingredients. The amounts of digestible nutrients in cellulose and soya bean hulls were determined in previous digestibility trials; those in the other ingredients were obtained from the feedstuff table (CVB, 1988).

The pigs received 0.33 l water per MJ NE_i, which was used to soak the feed. The pigs were weekly weighed, and their ration was based on this weight and the expected growth in the following week. Leftovers were collected daily from the troughs, stored at 4°C and for each pig the totals of one week were weighed and analysed for dry matter content.

For each week, the pig's mean metabolic weight was calculated as the average of the two weighings, expressed as $W^{0.75}$. The mean metabolic weight during the whole experiment was calculated by averaging the weekly mean metabolic weights.

Experimental design

The treatments were assigned according to a split plot design (Figure 7.2). Each block comprising the 12 treatments represented three pens and each pen represented one source of carbohydrate. Within each pen of four pigs, each pig was assigned to one of the four amounts of fat. Two trials were performed (one in spring and one in autumn), of which each one comprised two replicates. Each replicate comprised two blocks with pigs of the same age. The pigs in the first block were selected for their higher live weight than those in the second block (see also Bakker et al., 1995). As a result, at selection the average live weight of the pigs in the first block was on average 4 kg more than of their litter mates in the second block.

When the 112 selected pigs weighed between 15 and 20 kg, they were allotted to the 12 treatments and to the zero controls (16 animals). After the pigs had been selected and housed in their pens, they were allowed at least two weeks to adapt to the pens, the penmates and the feeding system.

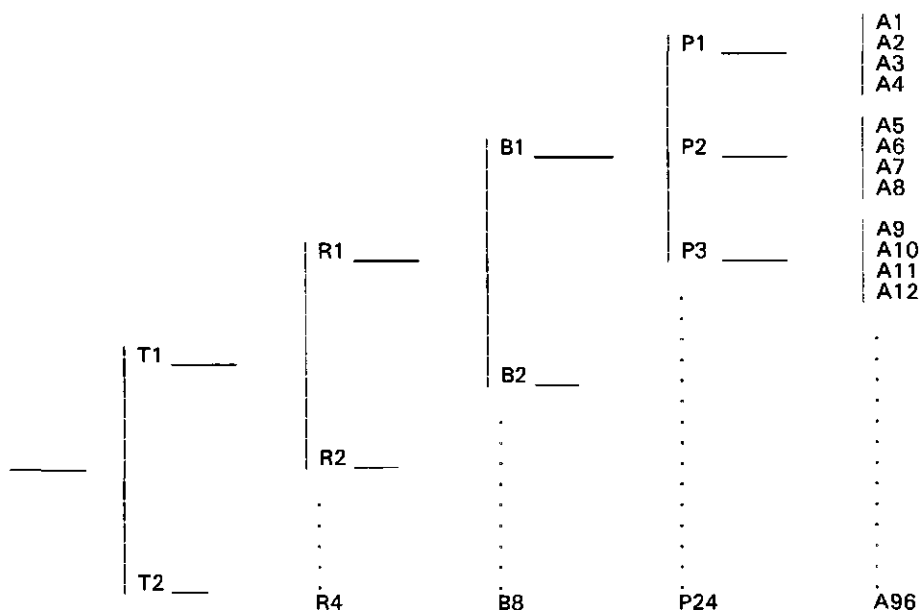


Figure 7.2

Experimental design, with T = trial, R = replicate, B = block, P = pen and A = animal; within one block the three sources of carbohydrates were allotted to the three pens; within each pen the four amounts of fat were allotted to the four animals (split plot design)

When the pigs weighed on average 30 kg (= initial live weight), over a period of four days they were gradually introduced to the experimental diets by being given increased amounts of the experimental diets and less of the starter feed, which was their diet before. The amount of net energy from this starter feed during these four days was included in the total consumed NE_f during the experiment. NE_f content of the starter feed was obtained from the feed manufacturer.

All pigs were fed the same total amount of net energy during the experiment. We had defined this fixed amount as the total amount of net energy consumed during the first replicate by the fastest grower, when it had reached a live weight of approximately 105 kg. Once a pig had consumed this standard amount of NE_f , the pig was euthanised and the amount of retained energy, fat and protein in the empty body was determined by using the comparative slaughter technique.

Comparative slaughter technique

One or two days before slaughter, the pig was shorn (except the head). The obtained hairs were weighed air-dry for each pig. We assumed that the air dry hair contained 810 g protein per kg (Oslage, 1965). Between three to four hours after the last morning feeding, the final live weight of the pig was measured. Immediately thereafter, the pig was euthanised by injecting barbiturate into the vena jugularis. By exsanguination and collecting the blood, the loss of blood during taking out the gastrointestinal tract was minimized. The blood of each pig was collected in a container. The gastrointestinal tract was removed and divided into four segments: stomach, small intestine, caecum and large intestine. These segments were weighed, emptied, rinsed carefully with water and reweighed after the excess of water was wiped off carefully. The bladder was also taken out, weighed, emptied and reweighed. The empty body and the closed container for each pig with the blood and the empty entrails were frozen separately at -20°C and stored until the whole trial had been completed. The final empty body weight was calculated as the final live weight plus the amount of the shorn hair minus the contents of the intestine and the bladder.

The 16 zero controls (two pigs per block) were euthanized at the beginning of the experiment. Similarly as with the experimental pigs, their intestinal content was weighed and removed, and the empty body was stored at -20°C until required for chemical analyses. The ratio between their empty body weight and their live weight was 0.91 ± 0.02 . We defined the empty body weight gain (EBG) for the pigs fed the experimental diets to be the difference between the initial empty body weight ($0.91 \times \text{initial live weight}$) and the final empty body weight. In the comparative slaughter technique, it is assumed that the chemical composition of the reference animals is identical to those of the experimental pigs at the start of the experiment.

For chemical analysis of the body, in each trial samples were obtained from the controls and the experimental pigs at the same time. The whole body, except the jaws with the teeth and molars, was treated and sampled as described by Everts and Dekker (1994). The samples were analysed for dry matter, ash, nitrogen, fat and gross energy. The part of the head with the teeth and molars (referred to as 'jaws') was cut out as small as possible, weighed and stored at -20°C . We assumed that its chemical composition was not affected by the treatments. In addition, in each trial, 20 jaws of the largest weight range as available were autoclaved during 5 hours. Then the teeth and molars were taken out and weighed. We assumed them to consist of 100% ash. The remaining part of the jaw was ground and mixed by an ultra-turrax; samples were taken and analysed similar to the carcass samples. The average composition was multiplied with the jaws weight of each pig in the same trial, and added to its chemical composition.

Analytical procedures

All analyses on feed and faeces samples were performed in duplicate and on carcass samples in triplicate. Analysis were following official Dutch procedures (Nederlands Normalisatie Instituut, 1992), most of which are comparable with those of the Association of Official Analytical Chemists (AOAC, 1984) and are described more in detail by Bakker et al. (1995). The carcass samples of the experimental pigs and the reference pigs of each trial were prepared and analysed within one week. In the carcass samples all analyses were done in fresh material. The differences in the weights of the jaws was too small to have an effect on chemical composition. Therefore, in each trial we used the relevant mean composition. The mean chemical composition of the jaws was: In the first trial: 410 g DM/kg (sd. 10.7); 149 g ash/kg (sd. 10.4); 28 g N/kg (sd. 0.6); 89 g XL/kg (sd 9.0); 7571 kJ GE/kg (sd 371.2). In the second trial: 393 g DM/kg (sd. 8.4); 134 g ash/kg (sd. 12.2); 27 g N/kg (sd. 0.5); 89 g XL/kg (sd 11.6); 7337 kJ GE/kg (sd 460.1).

Calculating the efficiency of utilization of digestible nutrients for growth

The efficiency of utilization of digestible nutrients for growth was calculated from two different methods:

- (1) In the present experiment, RE was measured at different DE intakes for the twelve diets (Table 7.1). Therefore, the DE intake should be corrected for DE required for maintenance, to provide an equal basis for comparing the efficiency of energy gain between the treatments. Therefore, the amount of DE available for growth (DE_g) was calculated from the total amount of DE minus the amount of DE required for maintenance (DE_m = 440 kJ/W^{0.75}; calculated from 420 kJ/W^{0.75} ME_m (Verstegen et al., 1973) divided by 0.955 (ratio ME/DE; ARC, 1981)).
- (2) The ratio RE/DE_g does not provide information about the efficiency of utilization of the individual digested nutrients. Therefore, the contribution of the separate digested nutrients to NE_i (= RE + 293 kJ x W^{0.75}) was calculated by regression analysis. Because of our experimental design with limited variation in intake of DXP (Table 7.1) and ME, we were not able to estimate independently the efficiency coefficient for DXP and the amount of energy required for maintenance, respectively. Therefore, we adopted the values for DXP of 10.8 kJ NE and for NE_m of 293 kJ NE per W^{0.75} from equation 1.

Subsequently, we regressed the remaining net energy (e.g. RE + NE_m - 10.8 x DXP) on DXL, starch & sugar and DFERM.

Statistical analysis

For statistical analysis, analysis of variance was performed (see Figure 7.2), using the statistical software GENSTAT (Payne et al., 1987) and the following model:

$$Y_{hijkl} = \mu + T_h + R_{(hi)i} + B_{(hij)j} + Carb_k + e1_{hijk} + F_l + (Carb * F)_{kl} + e2_{hijkl} \quad (2)$$

where μ = overall mean;

T_h ($h = 1, 2$) = effect of trial;

$R_{(hi)i}$ ($i = 1, 2$) = effect of replicate within trial;

$B_{(hij)j}$ ($j = 1, 2$) = effect of block within replicate within trial;

$Carb_k$ ($k = 1 \dots 3$) = effect of source of carbohydrate;

$e1_{hijk}$ = error contribution of the main plot;

F_l ($l = 1 \dots 4$) = effect of amount of added fat;

$(Carb * F)_{kl}$ = interaction between source of carbohydrate and amount of added fat;

$e2_{hijkl}$ = error contribution of the subplot and the interaction.

Results

Four pigs had to be excluded from the experiment. In the first trial, one pig had to be euthanized prematurely (treatment Mo), because severe leg problems suddenly appeared. In the second trial two pigs had to be removed (treatments SI and Sh), because of an infection and one pig was excluded (treatment Ch), because it was not castrated properly.

It should be emphasized that the data on the weight of the empty gastrointestinal tract and its content were obtained at the end of the experiment, while the amounts of retained energy, fat and protein were obtained as the difference between the contents at the start of the experiment (on average 30 kg) and the end (on average 105 kg).

Body composition

Trial, replicate within trial, and block within replicate within trial did not affect any of the results on body composition; therefore, they are not presented separately. None of the variables was significantly affected by the interaction between amount of fat and source of carbohydrate (Table 7.2).

The pigs being fed the M diets retained the highest amount of energy per day (10.8 MJ/d). The difference with pigs fed the S diets (10.4 MJ/d) was not significant, while the pigs being fed the C diets (8.9 MJ/d) retained on average 20% less energy than those being fed the M diets ($P \leq 0.001$; Table 7.2). Incrementally fat additions to the diets lowered energy retention from on average 10.8 MJ per day with no extra fat addition (o) to an average of 9.5 MJ per day with 105 g fat/kg (h diets; $P \leq 0.001$; Table 7.2). The retained energy comprised both protein and fat. That protein and fat are the main energy depots, is confirmed by comparing the calculated energy gain ($39.6 \text{ (MJ/g)} \times \text{retained fat (g/d)} + 23.8 \text{ (MJ/g)} \times \text{retained protein (g/d)}$) with the measured energy gain (Table 7.2).

The mean daily protein deposition per pig was not significantly affected by the experimental dietary factors and averaged 121 g per day. Therefore, the effects on energy retention were due to effects on fat retention. The M treatments resulted in the highest fat retention (202 g/day) and the C diets in the lowest one (156 g/day) ($P \leq 0.001$; Table 7.2).

The fat retention of the pigs fed the S diets (191 g/day) was in between those two treatments, but closer to the M diets than to the C diets.

Table 7.2

Effects of source of carbohydrate and/or amount of included fat in pig diets on mean retained energy, mean retained protein, mean retained fat and mean metabolic weight during the growing finishing period (30-105 kg)

	retained energy MJ/pig/day	retained protein g/pig/day	retained fat g/pig/day	metabolic weight kg W ^{0.75}
Mean				
Mo	11.6 ^f	121	223 ^h	23.3 ^{abc}
MI	11.2 ^{ef}	119	208 ^{gh}	23.8 ^{bc}
Mm	10.4 ^{cde}	118	195 ^{efg}	23.0 ^{ab}
Mh	9.9 ^{cd}	122	176 ^{cde}	23.0 ^{ab}
Co	9.6 ^{bc}	125	170 ^{bcd}	23.5 ^{abc}
CI	8.4 ^a	122	143 ^a	23.1 ^{ab}
Cm	8.7 ^a	123	148 ^{ab}	23.5 ^{abc}
Ch	8.8 ^{ab}	113	159 ^{abc}	22.7 ^a
So	11.1 ^{ef}	125	206 ^{gh}	24.3 ^c
SI	10.7 ^{def}	131	192 ^{defg}	24.3 ^c
Sm	10.1 ^{cd}	124	185 ^{def}	24.2 ^c
Sh	9.7 ^{bc}	114	178 ^{cde}	23.1 ^{ab}
S.e.d.				
Carb*Fat	0.42	6.5	10.9	0.46
Carb	0.28	3.4	4.2	0.21
Fat	0.25	3.7	6.7	0.27
F-probability				
Carb*Fat	NS	NS	NS	NS
Carb	***	NS	***	**
Fat	***	NS	***	*

^{a,b,c,d,e,f,g} Different superscripts within the same row indicate significant differences ($P \leq 0.05$)

Statistical significance: NS: $P > 0.05$; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$

Incrementally amounts of fat in the diets lowered the fat retention in the pigs from on average 201 g per day at the zero fat addition (o) to 172 g per day at the h fat addition ($P \leq 0.001$; Table 7.2).

The mean metabolic weight was similar for the M and the C treatments and averaged 23.3 kg throughout the whole experiment. It was 0.7 kg higher in case of the S treatments ($P \leq 0.01$; Table 7.2). Fat addition lowered mean metabolic weight ($P \leq 0.05$; Table 7.2): at the highest fat addition level the metabolic weight was 22.9 kg, while at the other three lower addition levels it was on average 23.7 kg.

Utilization of digestible nutrients for growth

The efficiency of utilization of DEg for RE was significantly affected by the source of carbohydrate, the amount of added fat and the interaction (Table 7.3). The DEg of the M diets was utilized with an efficiency of 65%, while for the C as well as the S diets it was 57% (Table 7.3; $P \leq 0.001$). At the two highest fat addition levels RE/DEg ratio was higher (62 % at m and 60% at h) than at the two lower levels (59% at the control and 58% at l) (Table 7.3; $P \leq 0.05$). Within the M and the S diets the differences in efficiencies between the fat addition levels were not statistically significant. Within the C diets, however, the lower fat addition levels o and l gave lower efficiencies than the higher fat addition levels m and h (Table 7.3, effect of interaction, $P \leq 0.05$).

Table 7.3

Effect of source of carbohydrate, the amount of fat and the interaction on the ratio between retained energy (RE) and digestible energy for growth (DEg) in growing pigs (30 - 105 kg)

amount of added fat ^{2,3}	RE/DEg (%)		
	source of carbohydrate ^{1,3}		
	maize starch (M)	cellulose (C)	soya bean hulls (S)
0 g per kg (o)	66.1 ^{ef}	55.1 ^{ab}	56.7 ^{ab}
30 g per kg (l)	65.0 ^{ef}	52.8 ^a	57.0 ^b
70 g per kg (m)	66.3 ^f	62.0 ^{cde}	57.8 ^b
105 g per kg (h)	62.4 ^{def}	58.9 ^{bcd}	57.4 ^{bc}

¹ significant effect of source of carbohydrate, $P \leq 0.001$, s.e.d. = 0.78

² significant effect of amount of fat, $P \leq 0.05$, s.e.d. = 1.18

³ significant effect of the interaction between source of carbohydrate and amount of fat, $P \leq 0.05$, s.e.d. = 2.05

^{a,b,c,d,e,f} Different superscripts indicate significant differences ($P \leq 0.05$)

The relatively high value for Cm might be due to an overestimated digestible nutrient supply (Bakker et al., 1995). Reexamination of the data, however, gave no indication of errors in calculations or errors in analysis.

When efficiency of utilization of digestible nutrients for RE was calculated by regression analysis, the h fat addition was not included in the regression, because in these treatments more digestible fat was consumed than was deposited, which will result in lower efficiencies of utilization of fat (Chudy and Schiemann, 1969). The regression equation found was:

$$\begin{aligned} \text{NE (kJ)} - 10.8 \text{ DXP} &= 33.9 \text{ (se: 2.3) DXL} + 12.4 \text{ (se: 0.31) [starch \& sugar]} \\ &+ 7.8 \text{ (se: 0.9) DFERM} \quad (2) \\ (\text{adj. } R^2 &= 54\%; \text{rsd} = 908 \text{ kJ}; \text{CV} = 6.4\%; P \leq 0.001 \text{ for all coefficients;} \\ n &= 70) \end{aligned}$$

Thus, the DE from fat (39.6 kJ/g) was used with an efficiency of 86 %, the DE from starch (17.6 kJ/g) with 71 % and the DE from FERM (17.6 kJ/g) with 45 %. It needs to be pointed out that the adj. R^2 (54%) was not very high.

Gastrointestinal tract

Only source of carbohydrate affected the weights of the gut contents and the empty gut (Table 7.4). Furthermore, the effect of block within replicate within trial on empty gut weight was significant: at slaughter the pigs in the first block had a 8% heavier empty total gastrointestinal tract and 14% heavier empty large intestine than those in the second block. Apparently, the heavier live weight at selection was correlated with a heavier gastrointestinal tract at slaughter. When related to either final live weight or empty body weight, however, the significant effect of block on parts of the empty gut disappeared.

The increased gut content of 3 to 4 kg with the fibrous diets C and S compared to the M diets ($P \leq 0.001$) was mainly caused by the 2.3 kg higher content in the caecum and the large intestine (Table 7.4). In addition, the stomach of the pigs receiving the C diets contained 0.6 to 0.8 kg more digesta.

The empty gastrointestinal tract of the pigs receiving the soya bean hulls weighed 14% more than of those being fed the starch-rich M diets ($P \leq 0.001$; Table 7.4). With the cellulose containing diets, however, the weight of the empty gut increased only 4% compared to the M treatments. The heavier empty gut weight of the S treatments was mainly due to the large intestine ($P \leq 0.001$; Table 7.4). The weights of the empty stomach and small intestine were not affected by the experimental dietary factors. The stomach weighed on average 0.7 kg (s.e.d. Carb = 0.02) and the small intestine 2.6 kg (s.e.d. Carb = 0.08).

Table 7.4

Effect of source of carbohydrate in the diet on the weight of content and the empty tract of segments of the total gastrointestinal tract in growing pigs

source of carbohydrate	gut content (kg/pig)					empty gut (kg/pig)		
	stomach	small intestine	caecum	large intestine	total tract	caecum	large intestine	total tract
Mean								
Maize starch	2.6 ^a	1.0 ^a	0.4 ^a	2.0 ^a	6.0 ^a	0.19 ^a	1.6 ^a	5.0 ^a
Cellulose	3.4 ^b	1.8 ^b	0.6 ^b	4.1 ^b	10.0 ^b	0.20 ^a	1.7 ^a	5.2 ^a
Soya bean hulls	2.8 ^a	1.5 ^b	0.6 ^b	4.0 ^b	8.9 ^b	0.24 ^b	2.2 ^b	5.7 ^b
S.e.d.	0.17	0.15	0.07	0.20	0.34	0.016	0.05	0.13
F-probability	***	***	**	***	***	*	***	***

^{a,b,c} Different superscripts within the same row indicate significant differences ($P \leq 0.05$)

Statistical significance: NS: $P > 0.05$; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$

Discussion

Feed evaluation systems aim to predict potential retention of protein or energy as accurately as possible. Recently, Noblet et al. (1994) presented prediction equations for energy retention from compound feeds. In their approach the utilization of the digestible nutrients in the ingredients of a compound feed was independent of inclusion level of the ingredient. However, in high fat high fibre diets the yield of retained energy from digestible nutrients was less than expected (Van der Honing et al., 1984). The authors indicated that there might be an interaction between fat and fermentable carbohydrates on energy supply to the pig. Therefore, in this study we aimed to quantify the difference in utilization of DE for RE when DE was supplied by starch rich diets or by diets with large amounts of fermentable carbohydrates and/or animal fat. Experimental diets were composed in a factorial design, that were expected to supply a similar amount of net energy, but from different ratios of glucose, fat and volatile fatty acids. These ingredients have different energy densities, therefore, the total amounts of feed which the pigs had to eat were different between the treatments (Figure 7.1). This experimental design allowed a factorial analysis according to model 1. Only fat addition was confounded with starch withdrawal.

In the present study, we investigated the energy gain from the amount of total tract digestible nutrients as measured in the experimental diets (Table 7.1). In a previous study (Bakker et al., 1995), we found an interaction effect between fibrous carbohydrates and fat on digestibility. It was concluded that

both chemical composition and ileal and total tract digestibility of a compound feed should be measured, when the diet contains large amounts of fat and/or fibre (Bakker et al., 1996a).

The results of the digestibility measurements with the experimental feeds in these pigs showed that both the chemical composition and the digestibility of the feeds deviated somewhat from what was intended (Bakker et al., 1995). Thus, the NE_i calculated with these measured digested nutrients was lower than the initial one, and this difference was not similar for all the treatments. In addition, digestibility should be measured in the housing system to be applied. We showed that when the housing system is very different from those used to obtain data in the feedstuff table, different digestibility coefficients should be applied (Bakker and Jongbloed, 1994). The data obtained in the present experiment would be, therefore, an accurate basis to base the prediction of energy gain on.

Effect of digestible nutrients on empty gut weight

The fermentable carbohydrates, especially the S diets, increased the empty gut weight (Table 7.4), which corroborates the results of others (Kass et al., 1980; Anugwa et al., 1989). An increased empty gut weight or length might be related to the absorption of large amounts of nutrients. Studies on differences in empty gut weight or length between breeds (Petersson et al., 1979; McKay et al., 1984) also suggested this relation. Anugwa et al. (1989), however, found no effect on the empty large intestine. They concluded that feeding alfalfa during 34 d was too short for the microbial fermentation to reach its full capacity and cause hypertrophy of the large intestine. In our experiment, the growing fattening period lasted on average 100 d, which is the average length of the growing fattening period for pigs in the Netherlands. After that period, the pigs receiving the soya bean hulls diets had a heavier empty hindgut than those receiving the other diets. These S diets supplied far more fermentable nutrients (DFERM) than the other diets (Table 7.1), while the C diets resulted in the highest bulk intake (Figure 7.1). Therefore, the rate of fermentation seems to be more responsible for the empty gut gain than physical expansion. Regression analysis resulted in the following linear relationship between empty hindgut weight at slaughter (kg; caecum + large intestine) and the intake of fermentable carbohydrates (DFERM, kg) during the growing fattening period (35-110 kg live weight):

$$\text{empty hindgut (kg)} = 1.32 \text{ (se: 0.088)} + 0.023 \text{ (se: 0.003)} * \text{DFERM (kg)} \quad (4)$$

(adj. R² = 44%; rsd = 0.308 kg; CV = 15.3%; P ≤ 0.001 for both coefficients; n = 92)

Our data show that the hindgut was increased with 23 g per kg of DFERM consumed during the whole growing-finishing period of on average 100 d. The

total DFERM intake was between 15 and 49 kg for a 100d period (based on Table 7.2). The differences in amounts of DFERM between the treatments (Table 7.1) relate very well to the differences in empty gut weight (Table 7.4). Hence, the DFERM of cellulose gave relatively the similar gut growth as the DFERM of soya bean hulls. The DFERM induced growth of the hindgut is not a linear process during the whole growth period, but will probably take a relatively short period. Similarly, in a relatively short period of 14 to 32 days (Anugwa et al., 1989) the weight of the gut will be reduced to control values when no longer large amounts of fermentable carbohydrates are eaten. This effect has also been found with other metabolically active organs, like the liver (Bikker, 1994; Van den Hemel-Grooten, 1996).

It was concluded for both protein (Petersson et al., 1979; Anugwa et al., 1989) and fat (present study) that they had no effect on the hypertrophy of (parts of) the gastrointestinal tract. The absence of effect on empty gut weight of the interaction of fat with source of carbohydrate suggests that there was no effect of interaction on the absorption of fermentation products.

In the present experiment, large differences in gut fill were found between the sources of carbohydrates (Table 7.4). On the day of slaughter, the pigs received their usual ration. The ratio of the amount of gut fill to the amount of water and feed of the last feeding was at slaughter (between three to four hours after the last meal) on average 101% for the M diets; 156% for the C diets and 145% for the S diets. It is not known how this gut fill affects maintenance requirements, as discussed by Van Es (1982), but in the method of feeding pigs according to their (metabolic) live weight this gut fill is included.

It is concluded that the weight of the empty hindgut was increased with 23 g per kg of DFERM consumed during the whole growing-finishing period of on average 100 d. The other nutrients had no effect on empty gut weight. In addition, large differences in gut fill were found.

Utilization of digestible nutrients for energy retention: existing methods

In the present study, the retained protein (in g/pig/day; Table 7.2) was not affected by any dietary treatment. For the pig producer, however, it is important to know the amount of protein in lean tissue, whereas the amount of protein in offal, like gut tissue, are of no economical value. If it is assumed that the DM in gut tissue only contained protein and that the DM-content of gut tissue was 180 g/kg (McKay et al., 1984), 126 g protein would have been retained in the 700 g extra hindgut tissue with the S diets (Table 7.4) compared to the M diets. This was retained in a period of 100 d. The average protein gain in lean tissue would therefore be 1 g/d less in the S diets compared to the M diets (Table 7.2). This is a relatively small amount compared to the daily protein gain of 121 g. Therefore, the increased empty gut tissue did not contribute significantly to protein gain. Therefore, the differences between the dietary treatments in energy gain were mainly due to differences in fat retention (Table 7.2).

Energy retention in pigs was estimated in two ways:

(1) from the energy intake above maintenance (DEg). This is the classical method of calculating a coefficient Kpf or Kg (Kielanowski, 1965). Therefore, the ratio RE/DEg (Table 7.3) and the coefficients Kpf or Kg are identical.

We assumed that the experimental design would give similar amounts of RE from different ratios in the amounts of DE from glucose, fat or volatile fatty acids. Because these nutrients differ in efficiency of utilization (Black, 1995), different amounts of DE had to be given. In addition, due to interactions in digestibility, the supply of digestible nutrients was lower than expected and different among treatments (Bakker et al., 1995). This would, therefore, result in different ratios RE/DEg for our diets. This is confirmed by the present data, that show that the dietary treatments significantly interacted with each other on the ratio RE/DEg (Table 7.3); the ratio varied from 53 to 66%. However, with this method the effect of each nutrient separately cannot be quantified.

In addition, the efficiency of utilization of each nutrient depends on the process its energy is used for: maintenance, protein retention or fat retention (Black, 1995). Recently, some evidence has been presented that Kpf might be independent of the ratio Δ PD/LD (marginal ratio of protein deposition to lipid deposition) (Susenbeth et al., 1991; Bikker et al., 1994). But this might only be true for low fat low fibre diets, because from our data we can conclude that the PD/LD ratio varied from 54% with Mo to 85% for CI (Table 7.2), and Kpf varied also (Table 7.3).

In conclusion, for diets with large amounts of fermentable carbohydrates and/or fat, predicting energy gain from DE supply is not accurate.

(2) from the individual digestible nutrients. Also with this method a prediction equation with a relatively low level of accuracy was obtained (equation 3, adj R^2 = 54%). In compound feeds with much less variation in composition compared to ours, Noblet et al. (1994) obtained a much better fit in their prediction equations. Their experiments were performed with young pigs on metabolism crates, that were allowed a 2 week period to adapt to the new diet before the energy balance was measured. Therefore, possible effects of different maintenance requirements due to differences in gut weight or housing conditions could not be expressed.

It is concluded that with diets with large amounts of fermentable carbohydrates and fat, the prediction of energy gain in pigs could not be predicted accurately from digestible energy above maintenance nor from net energy.

Utilization of digestible nutrients for energy retention: difference between supplied and required ME

To evaluate the effects of the different nutrients on energy gain, the efficiency of utilization of the specific nutrient for either maintenance or retention of protein or fat should be taken into account. Both methods described

in the previous section do not discriminate between these energy consuming processes. Therefore, a third method is used that relates the nutrients supplied with the energy retained. First the ME supply by the digestible nutrients was calculated (Figure 7.2), and as a second step the energy used by the pig was calculated.

ME supplied by the nutrients:				
DXP ¹	* 23.8 ²	- E-urine ³	=	ME from digestible protein
DXL ¹	* 39.6 ²		=	ME from digestible fat
Dstarch ¹	* 17.6 ²		=	ME from starch
DFERM ¹	* 17.6 ²	- E-methane ⁴	=	ME from fermentable carbohydrates
				+ total ME-supply

¹ Table 7.1, g/d

² heat of combustion, kJ/g; ARC, 1981

³ Energy loss in urine (kJ/d), calculated as: 334 + N-urine * 30.6 (Just, 1982)

N-urine (g/d) was calculated as DXP - (retained protein/6.25)

⁴ Energy loss in methane (kJ), calculated as: -269.3 + (94.7xDFERM(MJ)x17.6) (Bakker et al. 1996)

Figure 7.2

The method for calculating ME supply from the consumed digestible nutrients

The ME supply can be calculated relatively accurately (Figure 7.2). However, it is not exactly known which nutrient is used for each of the ME-requiring processes in the pig. It is assumed that in the present experiment the efficiency of protein retention was constant, due to the small variation in amount of digested protein consumed (Table 7.1) and the similar amounts of protein retained (Table 7.2). In addition, it is assumed that this efficiency was not affected by the other nutrients present (Van den Hemel, 1996). Thus, the ME from digestible protein was completely used for protein retention in the pig. The other ME required for protein retention (y kJ) would have been supplied by y kJ ME from starch.

The ME from digestible fat is preferentially retained as fat in the body, and is retained with a high efficiency (Chudy and Schieman, 1969; Black, 1995). Therefore, 0.9 x ME from digestible fat was retained as fat. For the other part of the retained fat, ΔREfat, it is assumed that ME was supplied by starch and/or volatile fatty acids from fermentable carbohydrates. In case of the treatments with the highest fat inclusion levels (h diets) intake of digestible fat was higher than the amount of retained fat (Tables 7.1 and 7.2). The excess of ME from digestible fat was assumed to be used for maintenance with an efficiency of 0.66 (Chudy and Schieman, 1969; Black, 1995). For the remaining part of the

maintenance requirement, ΔNEm , ME was supplied by starch and/or fermentable carbohydrates.

Therefore, $(1-y) \times$ ME from starch and ME from fermentable carbohydrates were used for ΔRE_{fat} and ΔNEm . Regression analysis showed the following relation:

$$\Delta RE_{fat} + \Delta NEm \text{ (MJ/d)} = 0.70 * (1-y) * \text{ME from starch (MJ/d)} \\ + 0.43 * \text{ME from fermentable carbohydrates (MJ/d)}$$

adj. $R^2 = 90.0 \%$; $rsd = 896 \text{ MJ}$; $CV = 8.9\%$; $P \leq 0.001$ for both coefficients; $n = 92$)

This shows that by these assumptions, the ME from starch was used with an efficiency of 0.70 for fat retention and maintenance. This value fits well within the range reported in literature for glucose of 0.74 for fat retention and 0.68 for maintenance (Black, 1995). The efficiency of utilization of 0.43 from the ME from fermentable carbohydrates, however, is lower than the range reported in literature: 0.50 for maintenance and 0.62 for fat retention (Black, 1995).

An explanation might be found in the amount of energy assumed for maintenance. It is generally assumed that the maintenance requirements (NEm) are constant for all treatments. However, the maintenance requirement could have been affected by gut fill, empty gut weight and/or behaviour (Van Es, 1982). All these aspects are affected by fermentable carbohydrates. As mentioned before, it is not known whether gut fill affects maintenance requirement. However, it is included in the metabolic weight that is calculated from live weight of the pig. Tissue of organs like the intestinal tract are metabolically very active (Koong et al., 1983; Pekas, 1991), and probably increased maintenance requirements more than the increased metabolic weight accounted for. Furthermore, it is suggested that the behaviour of the pigs is affected by the type of nutrient, especially by fermentable carbohydrates (Frazer, 1975; Broom and Potter, 1984; Matte et al., 1994; Schrama et al., 1996). However, if this effect would have the most important effect, it would have lowered the maintenance requirement.

It is concluded that for a more accurate prediction of energy gain in pigs with diets containing large amounts of fermentable carbohydrates and fat, it should be distinguished which nutrient(s) were used for maintenance, protein retention and/or fat retention. The efficiency of utilization of ME from fermentable carbohydrates of 43% was relatively low. It is suggested that large amounts of fermentable carbohydrates enhance the energy requirements for maintenance.

Conclusions

With diets containing large amounts of fermentable carbohydrates and fat, the prediction of energy gain in pigs could not be predicted accurately from digestible energy above maintenance nor from net energy. It is concluded that for a more accurate prediction of energy gain in pigs with diets containing large amounts of fermentable carbohydrates and fat, it should be distinguished which nutrient(s) were used for maintenance, protein retention and/or fat retention. The efficiency of utilization of ME from fermentable carbohydrates of 43% was relatively low. It is suggested that large amounts of fermentable carbohydrates enhance the energy requirements for maintenance, due to increased empty gut weight and gut fill. The empty hindgut was increased with 23 g per kg of Dferm consumed during the whole growing finishing period of on average 100 d. The other nutrients had no effect on empty gut weight.

Therefore, prediction equations should only be applied for those feed(stuff)s on which they are calculated. When a different range of feedstuffs is used, new calculations should be performed and a new equation or system should be developed.

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Chapter 8

General discussion

General discussion

Introduction

An accurate prediction of energy gain in pigs from digestible nutrients is necessary for two main reasons:

- (1). If the daily energy gain is too high compared to protein gain the pig may deposit too much fat. Because the average consumer prefers lean meat, the pig producer will obtain a lower price per kg of meat.
- (2). If the daily energy gain is too low, the growth potential of the pig is not fully utilized. Therefore, the pig producer can market less pigs per year for slaughter and thus has a lower income.

In general, the energy of pig feeds is evaluated by considering the differential contribution of digestible nutrients to energy supply. This evaluation is based on three assumptions: (1) that both the chemical composition and the digestibility coefficients of ingredients in a feed are known and can be derived from feedstuff tables; (2) that the amounts of digestible nutrients in the different ingredients are additive and that there are no interactions between ingredients (CVB, 1988); and (3) that after digestion the contribution of each nutrient to energy supply is independent of the amounts of other nutrients. Therefore, it is important to check whether the assumption of additivity of digestible nutrients in ingredients within a diet is correct, especially when high fibrous ingredients are used. In addition, it is necessary to check whether the predicted energy gain based on the available digestible nutrients corresponds with the net energy gain as actually achieved.

Another aspect that should be considered is whether the techniques used to determine digestible nutrient supply by the separate ingredients, that are listed in feedstuff tables, are applicable to farm conditions, without restrictions. Most of the tabulated values are obtained under well-controlled laboratory conditions.

For diets with a common fat and dietary fibre content, Hansen et al. (1991) concluded that the assumption of additivity of ingredients was correct. Because of their relatively low price and some non-nutritional effects (e.g., beneficial for health and wellbeing of the pigs; Chapter 1) increasing amounts of NSP-rich diets are supplied to pigs. NSP-rich ingredients have a lower energy density compared to cereals, and therefore, pigs have to consume more DM to obtain the same amount of net energy (NE). Hence, diets containing large amounts of NSP-rich ingredients are often supplemented with fat, to compensate for the low (net) energy content.

From a series of experiments by Van der Honing et al. (1982, 1985) and Jongbloed et al. (1986) it was concluded that the pigs performed less on diets containing NSP-rich by-products supplemented with fat compared to those on diets containing cereals or by-products without added fat. It was suggested that

added fat and source of carbohydrate (cereals or by-products) interacted with each other.

From the literature (Chapter 1), it was concluded that fermentable carbohydrates may reduce the apparent digestibility of fat and protein. In addition, it was concluded that the utilization of fermented carbohydrates is lower, compared to enzymatically digested starch. However, the data showed a large range among the studies, due to differences in techniques used, feeding level, adaptation time, etc. Moreover, only occasionally both the effects on digestion and utilization were measured with the same diets.

Therefore, an experiment was performed with 12 diets, in a 4 x 3 factorial arrangement with four amounts of animal fat and three sources of carbohydrate. The amounts of animal fat added to the diets were : 0 (o), 35 (l), 70 (m) and 105 (h) g per kg. The three sources of carbohydrate were maize starch (M), purified cellulose (C) and toasted soya bean hulls (S). The cellulose was used as a source of poorly fermentable carbohydrate and the soya bean hulls as a source of easily fermentable carbohydrate. The amount of digestible nutrients in the same batch of soya bean hulls and cellulose, and thus their calculated net energy (NE_c) content, were measured in separate digestibility trials. The NE_c of the other ingredients were obtained from the feedstuff table (CVB, 1988). Each kilogram of the control diet (Mo) contained 510 g basal diet, supplying all necessary amino acids, minerals and vitamins, and 490 g maize starch. In the other 11 experimental diets, an amount of the maize starch in the control diet (Mo) was exchanged with the amounts of fat, cellulose and soya bean hulls of equivalent net energy (NE). Thus the NE-concentration was different between diets (Table 8.1), but the NE-supply, on live weight basis, was similar.

Growing fattening pigs received these diets continuously from 30 to 105 kg, receiving similar amounts of net energy. In these pigs, total tract nutrient digestibilities (Chapter 4) and energy gain (Chapter 7) were measured. With these data, net energy concentrations of the diets were calculated and compared with the ones as estimated from the feedstuff table (Table 8.1).

As a result, in Table 8.1, for each diet four NE values are presented. From NE1 to NE4 the number of measured data is increased:

NE1 is the **assumed** NE_c, for which the chemical compositions and the digestibility coefficients of the diets were based on the contribution of each ingredient. The data on the separate ingredients were obtained from the Dutch feedstuff table (CVB, 1988);

NE2 is the **estimated** NE_c, for which the chemical compositions of the experimental diets were obtained from analyses of the present experiments and the digestibility coefficients of the separate ingredients were obtained from the feedstuff table;

NE3 is the **calculated** NE_c, for which the chemical compositions and the apparent digestibility coefficients of the final experimental diets were obtained from the present experiments;

NE4 is the measured NE, for which the energy gain in the pigs were obtained from the present experiments.

When comparing these different NE it can be concluded that the NE concentration is reduced when increased numbers of measured data are applied (from NE2 to NE4; Table 8.1). Hence, both digestibility and energy gain are overestimated when data from a feedstuff table are applied.

Table 8.1

Comparison between the different levels of measured data of the diets for predicting net energy content¹ (MJ/kg DM) of the diet

	NE1 assumed	NE2 estimated	NE3 calculated	NE4 measured				
chemical composition ² :CVB ³		measured	measured	measured				
digestibility: CVB	CVB	CVB	measured	measured	NE2/ NE1 ⁴	NE3/ NE1 ⁵	NE4/ NE1 ⁶	
energy gain: CVB	CVB	CVB	CVB	measured				
Mo	10.8	10.9	10.7	10.3	101	(-2)	99	(-5) 94
MI	11.6	11.5	11.3	10.7	99	(-2)	97	(-5) 92
Mm	12.5	12.0	11.8	11.1	96	(-2)	94	(-5) 89
Mh	13.3	13.2	13.2	11.5	100	(-1)	99	(-13) 86
Co	8.1	8.6	7.8	6.9	106	(-10)	96	(-12) 84
Cl	8.4	8.7	7.8	6.6	104	(-11)	93	(-15) 77
Cm	8.9	9.0	7.6	7.0	101	(-16)	85	(-7) 78
Ch	9.3	10.2	9.0	7.6	110	(-13)	97	(-17) 80
So	9.5	9.6	9.2	8.5	102	(-5)	97	(-6) 91
Sl	9.9	10.2	9.8	8.9	103	(-4)	99	(-9) 90
Sm	10.6	10.7	10.1	9.1	101	(-6)	95	(-8) 87
Sh	11.1	11.5	10.9	9.5	104	(-6)	98	(-14) 84

Statistical significance⁷:

effect of source of carbohydrate:	***	***	***
effect of fat inclusion	***	***	***
interaction between source of carbohydrate and fat inclusion:	***	***	*

¹ NE_e-content calculated (for NE1, NE2 and NE3) as: 10.8xDXP + 36.1xDXL + 6.3xDXF + 12.7xDXX (Nehring et al., 1969)

² comprises both the effect of chemical analysis and the amount of added ingredients

³ CVB: Feedstuff table (1988)

⁴ NE2/NE1 (%): effect of measuring chemical composition

⁵ NE3/NE1 (%): + effect of measuring digestibility

⁶ NE4/NE1 (%): + accuracy of the prediction equation for NE_e

⁷ Statistical significance: NS: P>0.05; *: P≤0.05; **: P≤0.01; ***: P≤0.001

The ratio to NE1 of each additional data set applied (NE2, NE3 and NE4, respectively), indicates the relative (over)estimation of the data from the feedstuff table. The assumed net energy concentration (NE1) overestimated the measured NE (NE4) with 6 to 23% (NE4/NE1; Table 8.1). Of this ratio -4 to +10 percentage units is due to errors in chemical and ingredient composition (NE2/NE1 minus 100, Table 8.1). This difference was largest with the C diets. In addition, 1 to 16 percentage units of the reduced NE4/NE1 is caused by reduced digestibility (NE3/NE1 versus NE2/NE1; Table 8.1). This was on average 2 units for the M diets, 12 units for the C diets and 5 units for the S diets (diets Mh, Ch and Sh excluded). Another 5 to 17 percentage units of the lower ratio NE4/NE1 is caused by a lower energy gain than expected (NE4/NE1 compared to NE3/NE1; Table 8.1). This was on average 5 percentage units for the M diets, 12 percentage units for the C diets and 8 percentage units for the S diets (diets Mh, Ch and Sh excluded). In addition, the highest fat addition (h) resulted in a 7 percentage units lower energy gain compared to the mean of the other three fat addition levels.

In this chapter 8, we will discuss the aspects of chemical composition on NE only briefly. The aspects of digestible nutrients on NE, however, will be discussed more in detail. In this respect, the effect of housing system, the source of carbohydrate and its effects on the apparent fat, protein and starch digestibility, and the effect of live weight will be evaluated. In addition, the aspects of energy utilization and the prediction of energy gain will be discussed. Finally, the overall conclusions are formulated.

Estimation of the content of digestible nutrients in a feed (NE2/NE1 and NE3/NE1; Table 8.1)

For a correct estimation of the digestible nutrients in a feed, both the chemical composition and the nutrient digestibilities should be known. Otherwise, the net energy supply can be very different from the intended one. In the present experiments, the calculated energy content (NE3) was between 85 and 99% of the intended net energy supply (NE3/NE1 in Table 8.1).

It is becoming common practice in the Netherlands that feed manufacturers have the ingredients chemically analysed, prior to producing the feeds. The chemical composition of the mixed feed, then, is calculated from the ones of the separate ingredients and their concentration in the feed. In the experimental diets described in this thesis, the actual amounts of ingredients in the diets were slightly different from those intended (Chapter 4). Especially the fine-powdered cellulose was difficult to mix into the diets. It is not known, to what extent this phenomenon may affect practical feeds, but it may be less than in our semi-synthetic diets.

In the present experiments, the NE2 in Table 8.1 is based on the recalculated

contents of ingredients, using linear programming and the analysed chemical composition of the separate ingredients and the feeds, as described in Chapter 4.

The digestibility coefficients of nutrients in the ingredients are usually derived from a feedstuff table. These values were higher than the ones we measured in the mixed feeds (Chapter 4). When applying these measured digestibility coefficients in the NE-equation, instead of those from the feedstuff data, the largest effects on reduced energy supply seems to be associated with the sources of carbohydrates in the diets (NE3/NE1 versus NE2/NE1). Compared to NE2, NE3 reduced the ratio to NE1 with on average 2 units for the M-diets, with 12 units for the C-diets and with 5 units for the S-diets (Table 8.1). In Table 8.2 the effects of source of carbohydrates on the relative difference between measured and estimated digestibility are summarized.

Table 8.2
Effects of source of carbohydrates on the relative difference (dif) in nutrient digestibility (%; calculated as : $\frac{[\text{estimated} - \text{measured}]}{\text{estimated}} \times 100\%$; adapted from Table 4.9)

source of carbohydrate in the diet	difXP ¹	difXL ¹	difXF ¹	difnsXX ¹
maize starch	3	9	76	-3
cellulose	14	11	82	6
soya bean hulls	5	9	27	0

¹ XP: crude protein; XL: crude lipid; XF: crude fibre; and nsXX: non-starch nitrogen free extract

The largest differences between measured and estimated digestibilities were observed for XF digestibility (Table 8.2). However, because of its relatively low contribution to net energy supply, this might have only a small effect on NE. In addition, fat and protein were digested less than expected (Table 8.2).

One of the factors that may have affected these digestibilities is the type of housing system used (Chapter 2). In addition, the source of carbohydrate may affect the apparent fat, protein and starch digestibility (Chapters 4 and 5). The overall effects of housing system and source of carbohydrate on nutrient supply are evaluated in the next sections. In addition, the effect of live weight on digestibility is discussed, which will affect energy supply throughout the entire growing-finishing period.

Housing system

The data on supply of digestible nutrients from these diets (NE3) were obtained with group-housed pigs in pens, by using chromic oxide as a marker. In contrast, the digestibility data in feedstuff tables (NE2) are usually obtained by collecting faeces quantitatively from individually housed pigs under well controlled laboratory conditions (Šebek, 1989). Therefore, there are two main differences:

- (1) the methodology of using a marker and grab-sampling the faeces vs quantitative collection of faeces and
- (2) the housing conditions.

From our studies, it was concluded that the chromic oxide acted as a good marker (Chapters 2 and 3). Hence, the differences in digestibility between both housing systems were not caused by the marker. Therefore, it was concluded that housing pigs in groups in pens showed a reduced apparent digestibility of the measured nutrients, when compared to pigs housed on metabolism crates (Chapter 3). It should be pointed out that in this conclusion it is assumed, that there is no interaction between housing system and chromic oxide. It has been shown that, in contrast to metabolism crates, pigs in pens have the possibility to eat faeces (coprophagy). On one hand, this could improve digestibility because the nutrients pass the digestive tract for the second time (Kemmer et al., 1996). When the faeces are not digestible, the marker to nutrient ratio is not altered, and has therefore, no effect on the digestibility as such. On the other hand, increased amounts of bulk, like indigestible faeces, might reduce digestibility of other nutrients in the digestive tract. However, because of the relatively high feeding level in the present experiments, there may be only a small chance that coprophagy had occurred.

Between both housing systems the difference in the digestibility of the diets was larger with the high fibrous diets (Chapter 3), although a feed*housing system interaction could not statistically be confirmed. It was suggested that this housing effect might be due to a shorter passage time in the gastrointestinal tract (Metz and Dekker, 1985). The OM digestibility was reduced with 2.3 percentage units for the Mo diet, with 4.5 percentage units for the Co diet and with 2.2 percentage units for the So diet. When it is assumed that all the nutrients in the OM were affected similarly (although the effects on crude protein were more pronounced than on OM), it can be concluded that the lower NE3 with the M diets was due to the housing system. With the cellulose diets and the soya bean hulls diets a difference in NE of 7.5 (12 (Table 8.1) minus 4.5) and 2.8 (5 minus 2.2) percentage units, respectively, still could not be explained. Most likely, these effects are caused by the source of carbohydrate included.

Source of carbohydrate and apparent digestibility of nutrients

Non-starch polysaccharides reduce the apparent digestibility of protein and fat, both at the terminal ileum and over the total tract (as reviewed in Chapter 1). Prior to the terminal ileum, increased endogenous secretion of protein and fat and reduced absorption of dietary protein and fat may occur, whereas from the hindgut mainly increased amounts of bacterial protein and lipids are excreted in faeces. In the separate ingredients, these apparently undigested amounts of protein and fat are incorporated in the apparent digestibility coefficients as listed in feedstuff tables. However, with the cellulose and the soya bean hulls diets the apparent digestibility of protein and fat differed relatively more from the tabulated values than with the maize starch diet (Table 8.2). This might be due to larger amounts of endogenous losses and/or microbial synthesis or due to reduced digestibility of other ingredients such as the added fat. This will be outlined more in detail in the next paragraphs.

Apparent fat digestibility

The amount of endogenous fat excreted in faeces ranges between 4.4 to 33 g/kg DM intake (Freeman et al., 1968; Adams and Jensen, 1984; Jørgensen et al., 1993), and is found to depend on DM intake and on source and amount of dietary fat. In addition, in the hindgut the activity of microbes may affect apparent total tract digestibility of fat (Mason and Just, 1976; Just et al., 1980). Hence, in the present study, the large differences in DM intake (on average 1619 g/d with the M diets, 2233 g/d with the C diets and 1936 g/d with the S diets) and in amounts of carbohydrates fermented (on average 193 g/d with the M diets, 252 g/d with the C diets and 465 g/d with the S diets; Chapter 7) may have affected apparent fat digestibility. Because increasing amounts of fat were added, regression analysis with each source of carbohydrate would give the sum of endogenous + part of the microbial fat (related to the basal diet) on one hand and the digestibility of the added fat on the other hand (Jørgensen et al., 1993).

By regression analysis, the mean amount of fat consumed (X) was related to the mean amount of digested fat (Y).

M diets: $Y = -17.0 (1.50) + 0.91 (0.009) * X$; $n = 16$; $rsd = 2.7$; $adj R^2 = 99.8\%$
C diets: $Y = -11.4 (2.28) + 0.83 (0.013) * X$; $n = 16$; $rsd = 4.1$; $adj R^2 = 99.6\%$
S diets: $Y = -20.9 (2.75) + 0.87 (0.015) * X$; $n = 16$; $rsd = 4.6$; $adj R^2 = 99.6\%$
all coefficients were significant ($P \leq 0.001$)

The constants in these equations show that the amount of excreted endogenous fat (at fat intake = 0) was 17 g/d with the M-diets; 11 g/d with the C diets and 21 g/d with the S diets. However, due to the relatively large standard error the excreted amount of endogenous fat was only significantly different between the C diets and the S diets. This conclusion is based on the least significance

difference (lsd) of 8.25 g/d as calculated from the standard errors of the three constants. When related to the above mentioned daily DM intake, endogenous fat excretion was 10.5 g for the M diets, 4.9 g for the C diets and 10.8 g for the S diets. These data are in the lower part of the range of 4.4 to 33 g/kg DM intake as presented in literature (Freeman et al., 1968; Adams and Jensen, 1984; Jørgensen et al., 1993).

The coefficients in the equations show the apparent digestibility of the added fat. This was 0.91 for the M diets, whereas it was 0.83 for the C diets and 0.87 for the S diets. The difference in fat digestibility between the M and the C diets was significant (lsd=0.046). The reduced digestibility of the added fat with both the C and the S diets can be attributed both to reduced apparent ileal and total tract digestibility (Chapter 5). The reduced fat digestibility by the purified cellulose is in contrast with the results of Borel et al. (1995) in rats. However, they used larger amounts of fat (250 g/kg) and lower amounts of cellulose (100 g/kg). In addition, in their study the cellulose was added as a single dose, without any adaptation period.

It is concluded that both the endogenous secreted fat and the total tract digestibility of added fat is not constant, but depends on the source of carbohydrate used.

Apparent protein digestibility

With the C and the S diets, total tract protein digestibility was less than expected from the Feedstuff Table (Chapter 4). It was on average 3 % lower with the M diets, 14 % lower with the C diets and 5 % lower with the S diets (Table 8.2). Reduced protein digestibility may be related to a number of factors, like increased endogenous protein secretion, reduced absorption of protein, and synthesis of microbial protein (Chapter 1). Because the largest amounts of microbial protein are synthesized in the hindgut, it is preferred to evaluate protein digestibility and endogenous losses of protein at the terminal ileum. Endogenous losses of protein, as measured at the terminal ileum, depend mainly on the amount of consumed NDF (Schulze, 1994) or DM (Butts et al., 1993).

In the hindgut of pigs, the apparent digestion of protein may occur, but because this is almost completely excreted in the urine, the nutritional value of this protein is very low (Zebrowska, 1975; Just et al., 1981). Increasing rates of fermentation in the hindgut reduce the apparent digestion of protein, due to incorporation of the protein in the microbial protein (Gargallo and Zimmerman, 1981; Kirchgeßner et al., 1989). Hence, the rate of synthesis of microbial protein is related to the amount of fermentable carbohydrates. When the fermentation rate is very high, even net synthesis of protein in the hindgut might be found (Mosenthin et al., 1992; Bakker et al., 1996).

In the present experiments, the consumed amounts of protein were almost similar for the treatments. However, the digestibilities were very different, and thus the amounts of undigested protein were very different. As indicated for the

fat digestibility in the previous section, there were large differences between treatments in intake of DM and fermentable carbohydrates. Therefore, to explain the reduced total tract protein digestibility with the C and S diets, the undigested amounts of protein were regressed with the amounts of fermentable carbohydrates consumed (FERM; calculated as OM - Ash - XP - XL - Starch - Sugar) and the amount of fermented FERM (DFERM). The consumed FERM is expected to affect ileal endogenous losses and the DFERM is expected to affect synthesis of microbial protein in the hindgut). The regression equation obtained was:

$$\begin{aligned} \text{Undigested protein (g/d)} &= 22.8 (4.39) \\ &+ 36.5 (0.49) * \text{consumed FERM (kg/d)} \\ &+ 116.6 (1.12) * \text{fermented FERM (kg/d)} \\ n &= 48; \text{rsd} = 9.36; \text{adj } R^2 = 82.4\%; \text{all coefficients were significant (} P \leq 0.001 \text{)} \end{aligned}$$

According to this regression equation, endogenous losses of protein amounted 36.5 g/kg FERM consumed. This amount is within the range as published in literature of 16 to 39 g/kg feed (De Lange et al., 1989; De Lange et al., 1990; Boisen and Fernández, 1995).

The synthesis of bacterial protein amounted 116.6 g/kg FERM. This value is relatively high compared to values in literature of 49 to 62 g/kg FERM in pigs (Gargallo and Zimmerman, 1981; Kirchgeßner et al., 1989; Mosenthin et al., 1992; Bakker et al., 1996). Conversely, in ruminants an even higher synthesis is assumed of 150 g microbial protein per kg fermented organic matter (Tamminga et al., 1994). The values in pigs are often related to fermentation in the hindgut, thus ignoring fermentation prior to the terminal ileum. It is not known, to what extent pigs digest and absorb microbial protein in the small intestine.

It is concluded that both the intake of FERM as well as the rate of fermentation affect apparent protein digestion negatively, with 36.5 g/kg and 116.6 g/kg, respectively.

Apparent starch digestibility

In general, no effect of fermentable carbohydrates are found on ileal starch digestibility (Chapter 1). However, it has been suggested that viscous carbohydrates shifts the digestion of starch to more distal in the small intestine (Roberfroid, 1993). This might result in a more balanced supply of glucose to the intermediary metabolism over the day (Van der Meulen, 1996). To be able to confirm this hypothesis, measurements of metabolites in the portal blood should be performed. This was not performed in the present studies. However, purified cellulose and soya bean hulls (a combination of cellulose and hemicellulose) do not cause a high viscosity in the intestines (Roberfroid, 1993), and therefore no effect on starch digestion is expected.

It is sometimes suggested, that some of the starch is fermented in the small

intestine, especially when large amounts of FERM are present. In the present studies, both total tract DFERM and methane were measured (Chapter 5). It was found that methane production was closely related to DFERM, but no effect of amount of starch was found. When large quantities of starch would have been fermented it was expected to be noticed as an increased methane production. Therefore, there are no indications that in the present experiment part of the starch was fermented.

Live weight

When a pig becomes older and heavier, digestibility will improve (Roth and Kirchgeßner, 1984; Everts et al., 1986; Fernández and Jørgensen, 1986; Shi and Noblet, 1994; Chapter 4). Therefore, the measured net energy concentration of a feed will be lower when measured in a young pig than when measured in an older pig. This difference is relatively large when comparing measurements at 60 kg live weight with those at 90 to 100 kg live weight (Shi and Noblet, 1994; Chapter 4). In the present experiments it averaged 2.25% of the NE (calculated from Chapter 4).

In addition, type of diet may interact with live weight. Of the three sources of carbohydrates in our experiments, the soya bean hulls tended ($P=0.08$) to show the largest effect of live weight (60 kg vs 90 kg) of 3.7% in NE concentration. This was due to a 5% difference in digestibility of organic matter (Table 8.3). Therefore, in obtaining data on digestibility of ingredients for listing in a feedstuff table, often Latin square designs are used with pigs from 40 to 110 kg live weight. In this way, each diet is tested in pigs of different live weight. These measurements are usually performed in individually housed pigs in metabolism crates. It is assumed, then, that the difference in digestibility between 60 kg live weight and at 90 kg live weight is similar in metabolism crates and in pens. Unpublished data from the experiment described in Chapter 3, however, show that the difference in XP digestibility between 60 and 90 kg was larger in pen-housed pigs than in pigs housed in metabolism crates (Table 8.3); for OM this effect was not significant.

Furthermore, it is assumed that ileal digestibility is not affected by live weight (Furuya and Takahashi, 1980). Our (unpublished) data are in agreement with this statement.

In conclusion, for a correct estimation of the apparent total tract XP digestibility of a diet not only the type of feed, but also the housing system (Chapter 3) and age (Table 8.3) should be taken into account.

Table 8.3

Effect of live weight (60 kg versus 90 kg), housing system (metabolic crate versus pen) and the interaction on digestibility of organic matter (dOM) and crude protein (dXP)

	dOM				dXP		
	60 kg	90 kg	Δ		60 kg	90 kg	Δ
<u>metabolic crate</u> , total collection							
Mo	90.0	90.7	0.7 ^{ab}	t	86.2	87.9	2.0 ^{ab} **
Co	66.0	66.4	0.7 ^{ab}	NS	74.0	76.9	3.8 ^{bcd} t
So	82.7	84.3	1.9 ^{bc}	NS	73.3	76.1	3.8 ^{bcd} *
Mm	88.6	88.2	-0.5 ^{ab}	NS	88.1	87.1	-1.2 ^a NS
<u>pen</u> , marker method							
Mo	86.7	88.4	1.9 ^{bc}	*	76.8	82.1	6.6 ^{cd} ***
Co	63.7	62.6	-1.7 ^a	NS	70.5	72.2	2.4 ^{abc} NS
So	79.5	83.5	4.9 ^c	*	68.0	72.9	7.0 ^d t
Mm	85.3	87.2	2.2 ^{bc}	NS	81.1	84.4	4.0 ^{bcd} NS
Statistical significance:							
	Feed: *	s.e.d. 1.22		Feed: t	s.e.d. 1.53		
	Housing: NS	s.e.d. 0.86		Housing: *	s.e.d. 1.08		
	Feed*Housing: NS	s.e.d. 1.72		Feed*Housing: NS	s.e.d. 2.16		

Statistical significance: NS: P>0.10; t: P≤0.10; *: P≤0.05; **: P≤0.01

Effects on energy gain (NE4/NE1, Table 8.1)

In conventional pig diets, the main source of carbohydrate is starch, which originates mainly from cereals. However, increased proportions of by-products are now being used in compound feeds or in combination with a basal diet. These by-products may contain a large proportion of non-starch polysaccharides (NSP), that have a lower energy density than cereals. To obtain a sufficient concentration of energy, these compound feeds are often supplemented with fat. When supplying similar amounts of net energy with these diets to growing-finishing pigs, a similar performance and energy gain of the pigs can be expected. However, after correcting for chemical composition and lower digestibilities (NE3/NE1, Table 8.1), the measured NE (NE4) was still lower than NE3 and this difference was not similar for all the diets. The measured NE (NE4) gave 5 percentage units lower value for the M diets, than when calculated from the digestible nutrients (NE3). For the C diets this was even a 12 units lower

value, and a 8 units lower value for the S diets. In addition, at the highest fat addition level (h, 105 g/kg), measured NE was 7 percentage units lower, compared to the other three fat addition levels.

Prediction of energy gain

All NE4 contents were lower than NE3 contents (Table 8.1). When predicting NE3 the following equation was used:

$$NE_1 = 10.8 \times DXP + 36.1 \times DXL + 6.3 \times DXF + 12.7 \times DXX \text{ (Nehring et al., 1969)}$$

This equation was obtained with mainly fat gaining pigs (150-180 kg live weight), whereas in the present experiment young fast growing pigs were used. The data of Whittemore et al. (1988) revealed that the ratio of protein energy retention to total energy retention will be higher in growing-finishing pigs than in heavier pigs (> 110 kg live weight). In addition, Laswai et al. (1991) and Rao and McCracken (1991) found that the amount of energy required for maintenance significantly correlated with the rate of protein retention. Therefore, growing-finishing pigs require more energy for maintenance than adult pigs. In addition, Noblet et al. (1991) concluded that the most accurate equation for maintenance requirement is obtained when it is related to weight of lean tissue and weight of viscera in the body.

The differences in NE4/NE1 vs NE3/NE1 was not constant between the diets. This can be explained by the different types of nutrients supplied. From the present experiment (Chapter 7) the following equation was obtained:

$$NE = 10.8 \times DXP + 33.9 \times DXL + 12.4 \times [\text{starch} + \text{sugar}] + 7.8 \times \text{DFERM}$$

The starch in DXX received a slightly lower coefficient of utilization (from 12.7 to 12.4) than in the equation of Nehring et al. (1969). The coefficient for the fermentable carbohydrates in DXX (non-starch XX; DnsXX), however, was reduced by 40%: from 12.7 to 7.8. Conversely, the coefficient for the crude fibre (XF) was increased from 6.3 to 7.8. As a result, compared to NE4 the overestimation of NE3 was larger for the fibrous diets, due to lower coefficients of utilization for the fibrous components.

In Chapter 7, it was concluded that the efficiency of energy utilization was 0.71 for the starch and 0.43 for the DFERM. This value for DFERM is relatively low compared to literature values, being on average 0.50 for maintenance and 0.62 for fat retention (Black, 1995). It was concluded, however, that only between 0.81 and 0.90 of the digestible energy of FERM was available to the pig. This was due to energy losses in methane and in volatile fatty acids excreted in the faeces. When these losses are taken into account in calculating efficiency of utilization, then it increased to 0.50 for FERM. The efficiency for starch remained unaffected. This correction resulted in a smaller difference between the cellulose diets and the other diets. The r.s.d. however, remained unaffected.

Table 8.4

The differences between equations predicting mean retained energy per pig according to the format: $RE = \text{Constant} + \beta_1 * \text{digestible crude protein} + \beta_2 * \text{digestible crude fat} + \beta_3 * (\text{starch \& sugar}) + \beta_4 * \text{digestible crude fibre} + \beta_5 * \text{digestible non-starch XX} - (NE_m)$; digestible crude nutrients in gram per day

retained energy kJ/day	constant	kJ / gram				maintenance kJ / day	source
		β_1	β_2	β_3	β_4	β_5	
$RE_{Nehring} =$	0	10.8	36.1	12.7	6.3	12.7	Nehring, Schieman and Hoffmann, 1969
$RE_{Just}^1 =$	-2192	16.6	29.7	14.0	11.9	14.0	Just, Jørgensen and Fernandez, 1983
$RE_{Hoffmann} =$	0	11.0	34.0	12.9	10.4	10.4	Hoffmann et al., 1990
$RE_{CVB} =$	0	10.8	36.1	13.5	9.5	9.5	CVB, 1993
$RE_{Noblet} =$	0	11.3	35.0	14.4	0	12.1	Noblet et al., 1994

¹ RE_{Just}^1 calculated from $NE = (0.81 * ME) - 2200$, and $ME = 10 + 20.5 * DXP + 36.7 * DXL + 14.7 * DXF + 17.3 * DXX$

² aW (average body weight) calculated as described by Just, 1982. We calculated carcass weight to be the final live weight minus the weight of the gastrointestinal tract, fill included (Table 7.4), minus 13.6 kg other slaughter offal (Berende, pers. comm, 1994).

³ The factor 515 was calculated as the average value (in ME) from Table 8.1 in Hoffmann et al., 1990, and multiplied by 0.67 to convert the requirement for maintenance from ME to NE.

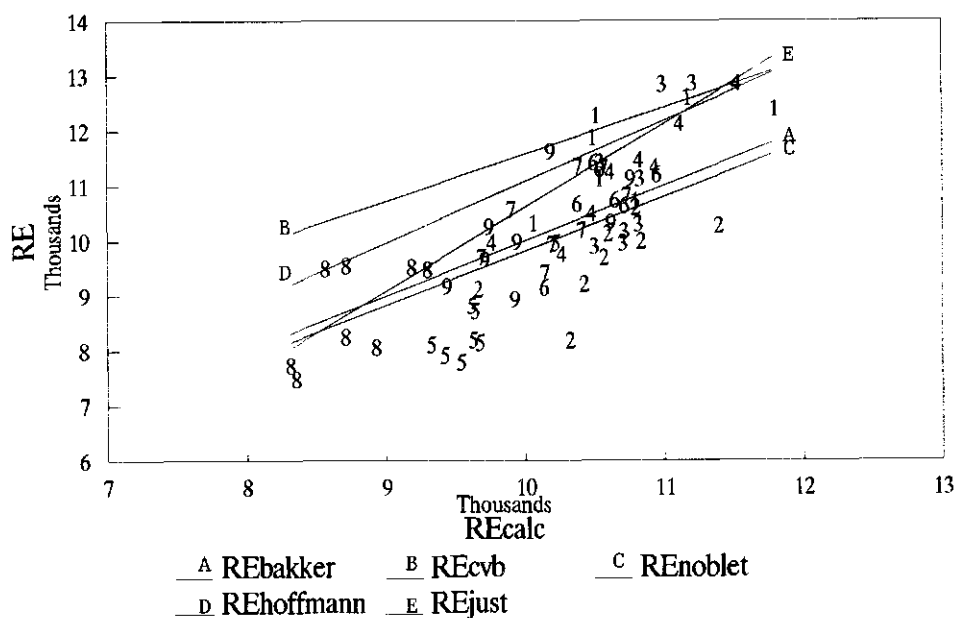


Figure 8.1

The ratio RE/RE_{calc} , when RE_{calc} is calculated with the equations as given in Table 8.4; 1 = Mo; 2 = Co; 3 = So; 4 = MI; 5 = CI; 6 = SI; 7 = Mm; 8 = Cm; 9 = Sm

Table 8.5

The relations between energy gain predicted from several European Net Energy equations (Table 8.4) with the measured energy gain

equation	adj. R ² %	r.s.d. kJ/day	C.V. %	Min ¹ kJ/day	Max ¹ kJ/day
RE = -3994** (1392) + 1.209***.RE _{Nehring} (0.118)	60	849	8.5	9565	13429
RE = 3020** (1049) + 0.658***.RE _{Just} (0.096)	40	1040	10.4	7892	13394
RE = + 0.903***.RE _{Hoffmann} (0.010)	52	925	9.2	8905	12908
RE = -3642* (1440) + 1.179***.RE _{cvb} (0.123)	57	879	8.8	9496	13434
RE = + 1.019***.RE _{Noblet} (0.104)	55	898	9.0	7829	12674

¹ Minimum and maximum values for the independent variable

In Europe, various equations for estimating net energy were developed with different dietary compositions, live weight of the pigs, techniques (comparative slaughter or respiration chamber) and assumed amount of maintenance requirements (Table 8.4). With each equation, the measured retained energy was compared with the ones predicted from the amounts of digestible nutrients from the present experiments (REcalc) and the values for maintenance (Table 8.4) (Table 8.5 and Figure 8.1).

With all equations the coefficient of variation (CV) varied only slightly: from 8.5 to 10.4%. It is concluded that with diets with large contents of fermentable carbohydrates and fat, none of the presently used net energy equations in Europe can predict energy gain accurately (Table 8.5 and Figure 8.1; Chapter 7). The equations of Nehring et al. (1969), Hoffmann et al. (1990) and CVB(1993) are all based on pigs gaining mainly fat. These equations predict higher energy gain (Figure 8.1) than data from Just et al. (1983), Noblet et al. (1994) and the present study; they obtained data with (younger) fast growing pigs.

For practical application, Just et al. (1983) adapted the calculation of metabolic weight into average metabolic weight by taking into account slaughter offal. In this way, high fibrous diets are calculated to have a higher requirement of energy for maintenance in order to punish those diets for decreasing the dressing percentage of the pig. Apparently, this equation fitted with our data (Figure 8.1). However, from Table 8.5, it can be concluded that this method has the largest *rsd* and a low *adj. R²*.

Conclusions

The maximum error in net energy content (all data from feedstuff tables vs all data measured) ranged between 6 to 23%. Of this ratio -4 to +10 percentage units was due to errors in chemical and ingredient composition. In addition, 2 to 16 units were due to effects on digestibility. This comprised housing system (2 units), apparent fat and protein digestibility.

Both the amount of endogenous secreted fat and the total tract digestibility of added fat were not constant, but depended on the source of carbohydrate used. In addition, both a larger intake of FERM as well as a higher rate of fermentation affected apparent protein digestion negatively, with 36.5 g/kg and 116.6 g/kg, respectively. For a correct estimation of the apparent total tract XP digestibility of a diet not only the type of feed (FERM content), but also the housing system and age should be taken into account.

The equation, used for calculating NE from the measured digestible nutrients, accounted for 5 to 12 units lower measured NE, than calculated. It is concluded that with diets containing large amounts of fermentable carbohydrates and fat, none of the presently used net energy equations in Europe can predict energy gain accurately (Table 8.5 and Figure 8.1; Chapter 7).

In conclusion, especially with high fibrous diets large effects can be expected on digestion and utilization. Therefore, the best method to obtain an accurate energy content is by measuring it under the conditions the feeds will be given to the pigs in practice.

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Summary

Summary

In marketing pigs, nearly 50% of the costs are those of the feed. Therefore, it is necessary to know the nutritional value as accurately as possible.

In the Netherlands, pigs receive in general (99%) compound feeds, containing all the nutrients they require. Cereals used to be the major ingredients. However, their proportion was reduced from 40% in 1970 to 15% in the eighties. The use of ingredients other than cereals or tapioca in compound feeds affected the chemical composition of the pig diet: from feeds with a large amount of starch towards feeds containing less starch but more fibrous polysaccharides, that are often called non-starch polysaccharides (NSP). Starch and NSP differ in many aspects: in chemical structure; in the type of nutrients they supply and their effect on other nutrients in the digestion process; efficiency of utilization for energy gain; and other, non-nutritional, aspects.

In order to have maximum benefit of their potential nutrient supply, most nutrients need to be digested and absorbed before reaching the terminal ileum. These nutrients are: amino acids from protein, fatty acids from lipids, and glucose from starch and sugars. If they disappear from the large intestine, the energy value of the nutrients will be lower, resulting in a reduced feeding value of the total feed. On the other hand, NSP are fermented mainly in the hindgut, supplying energy to the pig in the form of volatile fatty acids.

In general, the energy of pig feeds is evaluated by considering the differential contribution of digestible nutrients to energy supply. Energy evaluation is based on three assumptions: (1) that both the chemical composition and the digestibility coefficients of ingredients in a feed are known and can be derived from feedstuff tables; (2) that the amounts of digestible nutrients in the different ingredients are additive and that there are no interactions between ingredients; and (3) that after digestion the contribution of each nutrient to energy supply is independent of the amounts of other nutrients. Because NSP have a relatively low energy density, they are often supplemented with fat to maintain a certain energy density in the diet. Hence it is assumed that the feeding values are additive. It was found, however, that the combination of NSP rich by-products and fat resulted in less energy gain in pigs than an iso-energetic combination of cereals and fat or by-products separately. It was concluded that the NSP and fat interacted on energy supply to the pigs.

It is important to know whether the interactive effect between fat and fermentable carbohydrates takes place prior to the terminal ileum or in the hindgut of the pig. To be able to measure this, a new technique of ileo-cecal cannulation was developed: the steered ileo-cecal valve (SICV). In contrast to other techniques, in this technique the gut remains intact. After testing it with high fibrous diets, it was concluded that both ileal and total tract digestibility can be measured in the same pig. For this, the use of a marker is recommended.

To investigate the interaction between NSP and fat, an experiment was performed with 12 diets, in a 4 x 3 factorial arrangement with four amounts of

animal fat and three sources of carbohydrate. The amounts of animal fat added to the diets were : 0 (o), 35 (l), 70 (m) and 105 (h) g per kg. The three sources of carbohydrate were maize starch (M), purified cellulose (C) and toasted soya bean hulls (S). The cellulose was used as a source of poorly fermentable carbohydrate and the soya bean hulls as a source of easily fermentable carbohydrate. These diets were given to pen-housed pigs from 30 to 105 kg live weight, which period is the growing-finishing period in practice. In these pigs both digestibility of the diets and the energy gain were measured. In a separate set of pigs, the digestibility of nutrients at the terminal ileum was measured.

All the measured digestibility coefficients were lower than expected from the feedstuff table. This effect was partly attributed to differences in techniques for estimating digestibility between the present experiment (practical conditions) and the experiments supplying data for the feedstuff table. Most of the tabulated values are obtained under well-controlled laboratory conditions. It was found that housing pigs in groups in pens, as in common practice, reduced the digestibility of organic matter with 1.5 %-units, compared to pigs housed in metabolism crates. For protein this difference was larger: on average 3.7 %-units. Feeding high fibrous diets tended to increase these differences.

In addition, the assumption of additivity of digestible nutrients in ingredients within a diet was not correct, especially when high fibrous ingredients were used. When cellulose or soya bean hulls were included in the diet, digestibility of protein and fat was worse. It was concluded that intake of dry matter or fibrous material increased endogenous secreted protein with 36.5 g/d per kg NSP consumed, which reduced apparent digestibility. In addition, microbial protein synthesis of 116.6 g/d per kg fermented NSP also reduced apparent protein digestibility. Moreover, the added fat was less digestible at the terminal ileum when combined with cellulose or soya bean hulls in a diet, than when they were all fed separately. The total tract digestibility of the added fat was 91 % with the low fibre diet, but 83% when combined with cellulose and 87% when combined with soya bean hulls.

The energy gain predicted from the measured digestible nutrients was compared with the net energy gain as actually achieved. It was concluded that the utilization of energy from fermentable carbohydrates was relatively low: 0.43. This was partly ascribed to energy losses in methane and energy losses in volatile fatty acids in faeces. Of the digestible energy from fermentable carbohydrates 0.81 to 0.90 was available for energy gain in the form of volatile fatty acids. In addition, increased weight of the empty gastrointestinal tract was found, which may have required a large part of the available energy for maintenance, leaving less energy for growth.

It is concluded, that digestibility of nutrients should be measured both at the terminal ileum as over the total tract, when large amounts of fermentable carbohydrates are included in the diet. They should be measured under practical conditions. The variation in energy gain between pigs, however, remains relatively large.

Samenvatting

Samenvatting

In de vleesvarkenshouderij wordt bijna 50% van de kosten besteed aan voer. Het is daarom van groot belang de nutritionele waarde van een voer zo goed mogelijk te kennen.

In Nederland krijgen de varkens over het algemeen (99%) mengvoeders verstrekt, die alle voedingsstoffen bevatten die ze nodig hebben. Granen waren vroeger de belangrijkste grondstof in het voer. Het aandeel granen in het voer is echter verlaagd van 40% in 1970 tot 15% in de tachtiger jaren. Het gebruik van andere grondstoffen dan granen of tapioca in het voer heeft consequenties gehad voor de chemische samenstelling van het varkensvoer: relatief zetmeelrijke voeders maakten plaats voor voeders met minder zetmeel maar met meer niet-zetmeel koolhydraten (NSP). Zetmeel en NSP verschillen van elkaar in menig opzicht: in de chemische structuur; in de soort voedingsstof dat ze aan het dier leveren en hun effect op andere voedingsstoffen in het verteringsproces; de efficiëntie van de benutting van energie; en andere, niet-nutritionele aspecten.

Om de nutriënten maximaal te benutten, dienen eiwit, vet en zetmeel voor het einde van het ileum te zijn verteerd. Als deze in de dikke darm verdwijnen, zal de energiewaarde van deze nutriënten verminderen, waardoor de energiewaarde van het gehele voer lager wordt. De NSP worden daarentegen voor het belangrijkste deel in de dikke darm door microben verteerd tot vluchtige vetzuren, die door de darmwand van het varken worden geabsorbeerd.

Over het algemeen wordt de energie in een varkensvoer gewaardeerd naar de verschillende bijdragen van de nutriënten. Energiewaardering is gebaseerd op drie aannames: (1) dat de chemische samenstelling van een voer bekend is; (2) dat de hoeveelheden verteerbare nutriënten in de ingrediënten verkregen kunnen worden uit een voederwaardetabel, en dat er geen interacties zijn tussen ingrediënten; en (3) dat na vertering de bijdrage van elke nutriënt aan de energiebalans onafhankelijk is van de hoeveelheden van andere nutriënten. Vanwege het relatief lage energiegehalte van NSP wordt er vaak vet aan NSP-rijke voeders toegevoegd. Zo kan een bepaald energiegehalte van het rantsoen worden gehandhaaft, mits de energiewaarden van beide grondstoffen additief zijn. Een experiment heeft echter aangetoond dat een combinatie van NSP en vet in een rantsoen minder goede resultaten gaf dan eenzelfde hoeveelheid energie geleverd door een combinatie van granen met vet of NSP alleen. De conclusie werd getrokken, dat er sprake kon zijn van interactie tussen NSP en vet.

Het is van groot belang te weten of er interactie tussen vet en koolhydraten plaatsvindt, en of dit voor of na het einde van het dunne darm plaatsvindt. Om dit te kunnen meten is een nieuwe techniek ontwikkeld: de steered ileo-cecal valve (SICV)-techniek. In tegenstelling tot andere technieken laat deze techniek het maagdarmkanaal intact. Nadat het was getest met NSP-rijke voeders werd geconcludeerd dat zowel de ileale als de faecale verteerbaarheid gemeten kan worden in hetzelfde dier. Hiervoor wordt wel het gebruik van een merkstof aanbevolen.

Om de interactie nader te onderzoeken, zijn proeven gedaan met 12 voeders, in een 4 x 3 factorieel schema, met vier hoeveelheden vet en drie soorten koolhydraten. De hoeveelheden toegevoegd vet waren 0, 35, 70 en 105 g per kg voer. De drie soorten koolhydraat waren maiszetmeel, pure cellulose and getoaste sojahullen. De cellulose werd gebruikt als een relatief inert materiaal, terwijl de sojahullen een goed fermenteerbare koolhydraat is. Deze voeders werden gegeven aan varkens die waren gehuisvest onder omstandigheden die de praktijk zoveel mogelijk benaderden. Met deze varkens werd de verteerbaarheid en de energie-inhoud van de voeders bepaald.

Alle gemeten verteerbaarheden bleken lager dan verwacht op basis van de Veevoedertabel. Dit effect kon voor een deel worden toegeschreven aan het verschil in de technieken om de verteerbaarheid te bepalen tussen onze proeven en die waarmee de waarden in de veevoedertabel worden bepaald. Veel van deze 'Tabelwaarden' zijn namelijk gemeten met individueel gehuisveste dieren op stofwisselingskooien. Het bleek echter dat groepen varkens in grondhokken het voer slechter verteerden dan varkens op stofwisselingskooien. Dit verschil was 1,5 %-eenheden in de organische stof en 3,7%-eenheden in ruw eiwit. De NSP-rijke voeders leken dit verschil nog te vergroten.

Uit de resultaten van de proeven werd geconcludeerd dat de aanname van additiviteit van verteerbare nutriënten in ingrediënten niet juist is, vooral wanneer NSP-rijke grondstoffen worden gebruikt. De verteerbaarheid van vet en eiwit werd slechter wanneer cellulose of sojahullen aan het voer was toegevoegd. Elke kg extra opgenomen droge stof of NSP verhoogde de endogene secretie van eiwit met 36,5 gram/dag, waardoor de schijnbare verteerbaarheid werd verlaagd. De verteerbaarheid werd nog verder verlaagd met 116.6 gram/dag per kg gefermenteerde NSP, als gevolg van synthese van microbiel eiwit. Aan het einde van de dunne darm was het toegevoegde vet (verteerbaarheid in het controle voer 91%) slechter verteerbaar wanneer het werd toegevoegd aan een voer met cellulose (verteerbaarheid 83%) of sojahullen (verteerbaarheid 87%).

De verwachte energieaanzet berekend uit de gemeten verteerbare nutriënten werd vergeleken met de werkelijke energieaanzet. Het bleek dat de benutting van energie uit fermenteerbare koolhydraten lager was dan verwacht: 0.43. Dit kon deels worden toegeschreven aan energieverliezen in methaan en in vluchtige vetzuren die in de mest waren uitgescheiden. Door deze verliezen was 0.81 tot 0.90 van de energie uit fermenteerbare koolhydraten beschikbaar voor het dier. Bovendien bleek het lege maagdarmkanaal zwaarder als gevolg van de voeders met veel fermenteerbare koolhydraten. Dit weefsel heeft waarschijnlijk meer energie voor onderhoud gevraagd, waardoor er minder energie beschikbaar was voor groei van vlees.

De conclusie luidt dat zowel ileale als faecale verteerbaarheid van nutriënten gemeten moet worden, wanneer veel NSP-rijke grondstoffen in een varkensvoer zijn verwerkt. De proefomstandigheden dienen dan de praktijk zoveel mogelijk te benaderen. Daarnaast blijft de variatie in energieaanzet tussen varkens relatief groot.

Curriculum Vitae

Geertruida Cornelia Maria Bakker is geboren op 10 april 1963 te De Weere (gemeente Hoogwoud, N.H.). Na het behalen van het atheneum-B diploma aan de Scholengemeenschap Werenfridus te Hoorn in 1981, begon zij aan haar studie Zoötechniek aan de toenmalige Landbouw Hogeschool te Wageningen. Zij studeerde in januari 1989 af met de hoofdvakken Veehouderij, Algemene Agrarische Economie, en Veevoeding. Na een aantal maanden onbezoldigd te hebben gewerkt bij de vakgroep Veevoeding van de Landbouw Universiteit, werd zij per 1 oktober 1989 aangesteld als wetenschappelijk onderzoeker in tijdelijke dienst bij het toenmalig DLO-Instituut voor Veevoedingsonderzoek (IVVO-DLO) te Lelystad. Tot eind 1993 is het onderzoek uitgevoerd dat beschreven is in dit proefschrift. Per 1 september 1994 is zij wetenschappelijk onderzoeker in vaste dienst bij de afdeling Voedingsfysiologie Varkens en Pluimvee van het DLO-Instituut voor Dierhouderij en Diergezondheid (ID-DLO), waarvan het voormalige IVVO-DLO deel uitmaakt. Het werkterrein omvat voornamelijk de koolhydraten-, vet- en energiehuishouding.

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Dr Z. Mroz heeft de operaties verricht en is de eerste auteur van Hoofdstuk 2. Zdislaw, ik hoop dat we nog veel zullen samenwerken!

Hans van Diepen heeft me geleerd hoe een proefvoeder dient te worden gemaakt en wat er mogelijk is op het gebied van de software (Optisam, Lotus, Slide Write, etc.). Hans, dank je wel voor je collegialiteit.

Vincent Hindle heeft twee manuscripten op het (foutief) gebruik van de Engelse taal gecorrigeerd, waardoor ik beter Engels ben gaan schrijven. Vincent, many thanks.

Verder wil ik alle andere collega's, die ik niet met name heb genoemd maar die

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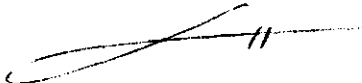
De medewerkers van de vakgroep Veevoeding wil ik danken voor hun open houding en hun medewerking.

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Het boekje gaat nu dicht.

Gertrud.

A handwritten signature, likely of Gertrud, consisting of a long, sweeping horizontal stroke with a small vertical tick at the end.