EVALUATION OF TECHNIQUES TO COLLECT ILEAL DIGESTA IN PIGS

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EVALUATION OF TECHNIQUES TO COLLECT ILEAL DIGESTA IN PIGS

Proefschrift
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
op gezag de rector magnificus,
Dr. H.C. van der Plas,
in het openbaar te verdedigen
op vrijdag 17 Januari 1992
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

Köhler, T., 1992. **Evaluation of techniques to collect ileal digesta in pigs.** The results of some comparative experiments using different digesta collection techniques are presented. Physiological parameters such as growth performance, nitrogen and mineral balances and various blood variables were studied in pigs fitted with post-valve T-caecum (PVTC) cannulas and end-to-side ileo-rectal anastomosis (IRA). Digestibility measurements using different techniques (PVTC, IRA, simple T-cannula and re-entrant cannula) and different diets independently were carried out. Considerable effects of IRA on the physiological status of the animals were found. Digesta composition in IRA-pigs was in some aspects significant different from that in PVTC-pigs. This indicates an increased fermentative activity in IRA-pigs as well as some adaptative changes in mineral absorption as a result of the absence of hind-gut absorption. Differences in digestibility measurements between different cannulation techniques were small provided that the results were calculated on the basis of marker recoveries. The effect of a sugar/starch supplementation to the diet to compensate for the absence of the hind-gut functions in IRA-pigs was studied in PVTC-pigs. There was no effect on the apparent ileal digestibility of amino acids feeding a wheat based diet. The consequences of the different effects assosiated with the different techniques for digestibility measurements are discussed. PhD. Thesis, Department of Animal Nutrition, Wageningen Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands.

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Cover design : P.L. van der Togt
Illustrations : K. Boekhorst

STELLINGEN

I.

Physiological parameters such as digestibility should be measured by using techniques that have no or only minor effects on the physiological status of the animal.
(This thesis)

II.

The elimination of the digestive processes of the hind-gut of pigs fitted with an end-to-side ileo-rectal anastomosis has considerable effects on the physiological status of the animal.
(This thesis)

III.

Increased bacterial activity in the digesta of pigs fitted with an end-to-side ileo-rectal anastomosis has a clear effect on some digestibility parameters.
(This thesis)

IV.

In comparison with the re-entrant and the simple T-cannulation the post-valve T-caecum cannulation technique is a useful alternative.
(This thesis)

V.

The quantity of digesta recovered from pigs fitted with ileo-caecal re-entrant cannulae is hardly affected by dietary composition.
(This thesis)

VI.

Since the simple T-cannulation can be used only for spot-sampling the variations of the results obtained with this technique will certainly be higher than with other techniques.
(This thesis)

VII.

The ileo-rectal anastomosis (IRA) technique as we evaluated it should not be allowed in the future.

VIII.

Stamppotten zijn alleen goed tegen de honger.

IX.

Ernst zu nehmende Wissenschaftler nehmen sich in der Regel selbst nicht zu ernst.

X.

Bij fokken van dieren op hoge produktie dient men zich te realiseren dat niet alleen de voer opname maar ook de aard van het voer moet worden angepast.
(Tolkamp, B. & Ketelaars, J., 1991, Toward a new theory of feed intake regulation in ruminants. Thesis; University of Wageningen)

XI.

Onderzoekers die de IRA fistulatie technique zo hebben benoemd waren apolitiek

XII.

Die korrekte Anwendung präcaecaler Verdaulichkeitsparameter kann einen besseren Beitrag zur Verminderung der Umweltbelastungen durch tierische Exkremente leisten, als dies durch die Verwendung der fäkalen Verdaulichkeiten möglich ist.

Stellingen behorende bij het proefschrift "Evaluation of techniques to collect ileal digesta in pigs".

Torsten Köhler

Wageningen, 17 januari 1992

Aan Birgit, Vanessa en
mijn ouders.

Voorwoord

Dit proefschrift bestaat uit een vijftal wetenschappelijke artikelen, waarvoor het onderzoek is uitgevoerd bij de vakgroep Veevoeding van de Landbouwuniversiteit te Wageningen in samenwerkinig met het TNO Instituut voor Voeding en Fysiologie van Landbouwhuisdieren (ILOB) te Wageningen en het Institut für Tierernährung und Stoffwechselphysiologie van de Universiteit te Kiel (Duitsland).

Vanaf deze plaats wil ik een woord van dank richten aan iedereen die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift.

Mijn promotor prof. dr. ir. M.W.A. Verstegen dank ik voor de mogelijkheid om dit onderzoek hebben te kunnen verrichten en voor zijn bereidheid altijd over de resultaten en de manuscripten te willen discussiëren. Zonder zijn kritische commentaar zou dit proefschrift niet tot stand zijn gekomen.

De aanzet tot dit onderzoek is gegeven door mijn co-promotoren Dr. habl. R. Mosenthin en dr. ir. L.A. den Hartog zowel als dr. J. Huisman. Zonder hun inzet zou dit samenwerkingsproject tussen de vakgroep Veevoeding, het Institut für Tierernährung und Stoffwechselphysiologie en het ILOB-Instituut ondenkbaar zijn geweest.

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minder prettig zijn geweest.

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Dr. Laszlo Babinszky bedank ik voor het gezellig gezelschap op de buitenlanderkamer.

Verder dank ik allen die aan het proefschrift hebben meegewerkt, maar niet met name zijn genoemd.

Tenslotte wil ik het Deutsche Akademische Austauschdienst, het Wilhelm Schaumann Foundation for Agrian Sciences Promotion en het Wageningse Fond bedanken voor de financiële steun die zij hebben geleverd bij het tot stand komen of publicatie van dit proefschrift.

CONTENTS

General Introduction

General Introduction

For non ruminant animal production a well balanced diet is important to ensure economical production. Moreover, losses to the environment can only be minimized if the diet matches the requirement as good as possible. In the field of non ruminant nutrition this leads to an increased interest in providing animals with all nutrients and energy in adequate but not in excessive amounts. Only in that case an efficient growth is possible. However, nutrients present in feedstuffs as measured by chemical analyses are not completely available to the animal. Protein digestion and absorption may be incomplete since protein in feed is partly not accessible to proteolytic enzymes produced by the organism which may results from indigestible cell wall components and enzyme inhibition (e.g. the trypsin inhibitor in raw soyabeans) which decreases the efficiency of specific enzymes (Liener & Kakade, 1980). Moreover, some digestive enzymes like trypsin and chymotrypsin may also be adsorbed by dietary fibre (Schneemann, 1978). This may limit or prevent their proteolytic activity.

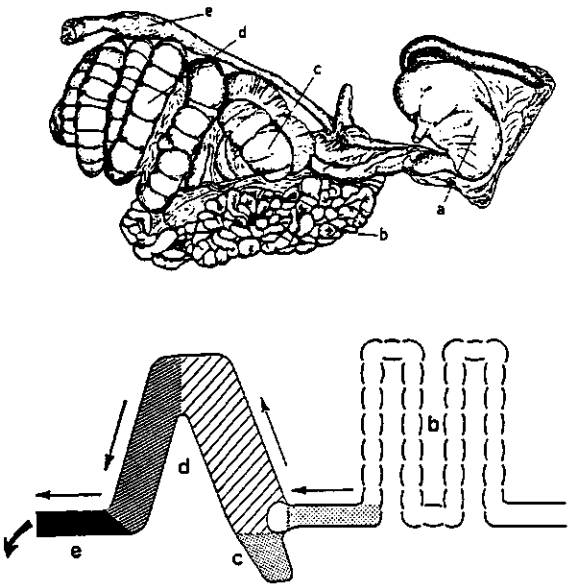
In order to supply the animals with an adequate and well balanced diet the amount of nutrients available to the animal must be known. In particular the relative quantities of individual amino acids available to the animal in each feedstuff has to be known. In order to adjust the dietary amino acid content amino acid digestibilities should be considered. It has to be pointed out, however, that amino acid digestibilities do not provide complete information about the differences in availability of amino acids. Digestibility of nutrients is defined as the difference between the nutrient intake with the feed and the excretion with the digesta or faeces divided by the intake with the feed. Availability is defined as the proportion of the nutrients in the diet that is absorbed in a form suitable for utilization in the intermediate metabolism. Consequently digestibility should be considered only as an indication of availability.

Digestibility measurements

A. The faecal analysis method

The classical method that has been used to determine amino acid digestibility is the faecal analysis method as described by Kuiken & Lyman (1948). This method for calculating the digestibility over the total digestive tract (Figure I) can be considered as the initial measurement of amino acid digestibility and it has been used extensively in studies with pigs (Dammers, 1964; Poppe et al., 1970; Eggum, 1973; Poppe, 1976). Studies by Zebrowska (1973, 1975) and Just et al. (1981) in which amino acids were infused into the caecum of pigs have shown that the nitrogen absorbed from the large intestine is nearly completely excreted in urine. This means that the hydrolysis of nitrogenous

Figure I. The intestinal tract of the pig:
a. stomach, b. small intestine, c. caecum, d. colon
e. rectum (according to Laplace et al., 1985)



compounds by microorganisms in the hind gut and their absorption as ammonia, amines and amides has almost no contribution to the protein status of the pig (Sauer, 1976; Hodgdon et al., 1977; Gargallo & Zimmermann, 1981; Just et al.,1981; Wünsche et al., 1982). In addition, faecal amino acid composition is affected by the synthesis of bacterial protein in the large intestine even when the pigs were fed diets that differ widely in amino acid composition. Bacterial protein represents 60-80% of the total nitrogen content of faeces (Mason et al., 1976; Mosenthin, 1987). As a consequence apparent faecal amino acid digestibilities are largely affected by the modifying activity of the microflora harboured in the large intestine. It has been accepted for a long time that bacterial activity is mainly restricted to the hind gut. Recent studies (Millard & Chesson, 1984; Graham et al., 1986; Buraczewska et al., 1988; Longland et al., 1988) have shown however, that there is also bacterial activity in the small intestine. This was concluded from the substantial degradation of non-starch polysaccharides prior to the terminal ileum. In addition Wünsche et al. (1991) reported DAPA-concentration in ileal digesta indicating that up to 25 % of the total N may be from bacterial protein. Therefore the ileal analysis method provides a more reliable estimation of digestibility, because it measures digestibility prior to microbial degradation and synthesis in the hind gut. The ileal, rather than faecal analysis method, should therefore be used to determine digestibility (Low, 1980; Tanksley & Knabe, 1984; Laplace et al., 1985; Sauer & Ozimek, 1986).

B. Digesta collection techniques

1. The slaughter technique

Digestibility measurement at the distal ileum requires collection of ileal digesta. The most simple method is to intercept digesta before it reaches the caecum. This can be done by slaughtering the animals (Cori, 1925, Payne et al., 1968; Kies et al., 1986; Moughan & Smith, 1987). However, using this technique, replicated observations in the same animal are not possible. This means, that individual animal variation can not be taken into account in the statistical analysis. Moreover, since digesta

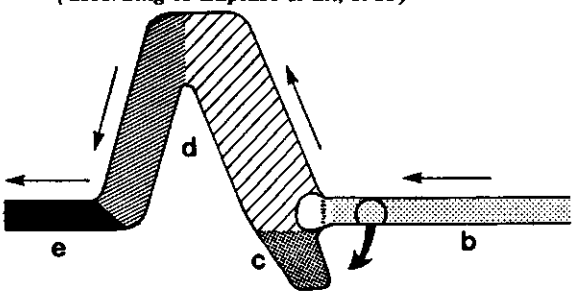
movement through the small intestine is intermittent, it may be necessary to pool digesta from several animals, because the amount of digesta per animal may be insufficient for analysis. As a consequence no information about the variation between animals are available. In addition, this approach is very expensive when applied to large species (Fuller, 1991). Furthermore, it can be expected that the shedding of mucosal cells at death into the intestinal lumen (Badawy et al.,1957; Fell, 1961) may have an influence on digestibility measurement of nitrogenous compounds. Therefore digesta samples have to be obtained by avoiding mucosal shedding. This can be done under anaesthesia or using for instance sodium pentobarbitone (Badawy, 1964).

2. Cannulation techniques

2.1 The simple T-cannulation

To avoid problems described for the slaughter technique various methods have been developed to collect digesta repeatedly from the same animal. The method that has been used most commonly until now is described by a surgical procedure which involves the implantation of a simple T-cannula, placed 5-15 cm anterior to the ileo-caecal valve (Figure II). With this technique digesta can be collected only by spot-sampling. This requires the use of an indigestible marker such as chromic oxide, titanium dioxide or barium sulphate. With regard to this technique, several concerns have been reported. There is some concern with regard to obtaining representative samples in T-cannulated pigs (Zebrowska, 1978; Sauer & Ozimek, 1986; Schröder et al., 1989; Leterme et al., 1990). A modification to improve digesta sampling using a suction tube has been described by Dierick et al. (1983). However, the use of this technique which requires additional devices (e.g. peristaltic pumps) complicates the collection of digesta.

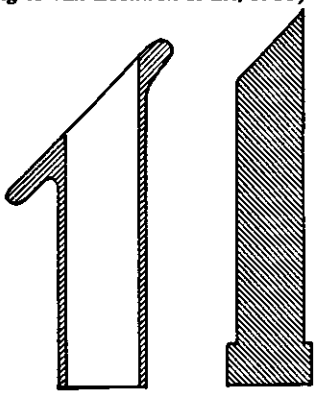
Figure II. The simple T-cannulation:
b. small intestine, c. caecum, d. colon, e. rectum
(according to Laplace et al., 1985)



The post-valve T-caecum cannulation technique

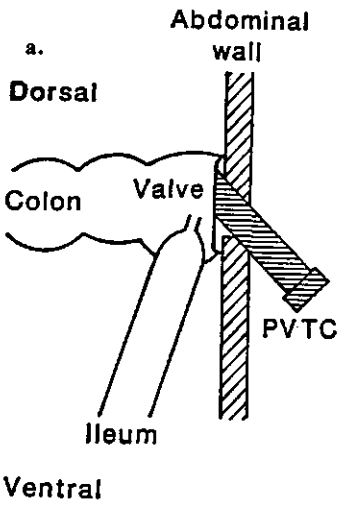
A modification of the simple T-cannulation has been developed by van Leeuwen et al. (1988). This technique which consists of only one large T-cannula uses the anatomy of the transition from the ileum into the caecum-colon (Figure III). After removal of the caecum the cannula is joined with the remnants of the caecum directly opposite to the ileo-caecal valve (Figures IVa). When the cannula is open the ileo-caecal valve, which is normally protruded into the caecum will now be protruded into

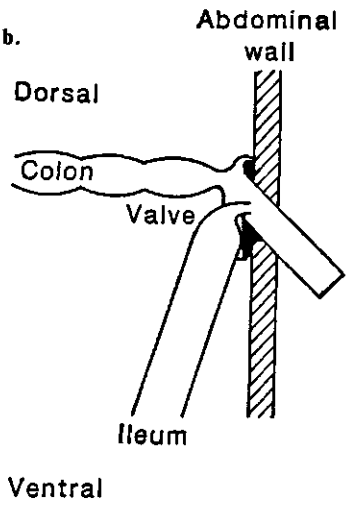
Figure III. The PVTC-cannula with plug.
(according to van Leeuwen et al., 1988)



the cannula (Figure IVb). The internal and external diameter of 25 and 30 mm, respectively guarantees a passage capacity that should be sufficient to collect digesta almost quantitatively. Preliminary results that have been presented by den Hartog et al. (1988) show that the apparent ileal digestibility of dry matter and nitrogen determined in pigs fitted with the PVTC-cannula are comparable with results determined in pigs fitted with simple or re-entrant cannulas and fed different diets.

Figure IV. ***Location of the post-valve T-caecum cannula***
a. The caecum is replaced by the cannula
b. The ileo-caecal valve is protruded into the cannula
(according to van Leeuwen et al., 1988)





The steered ileo-caecal valve cannulation

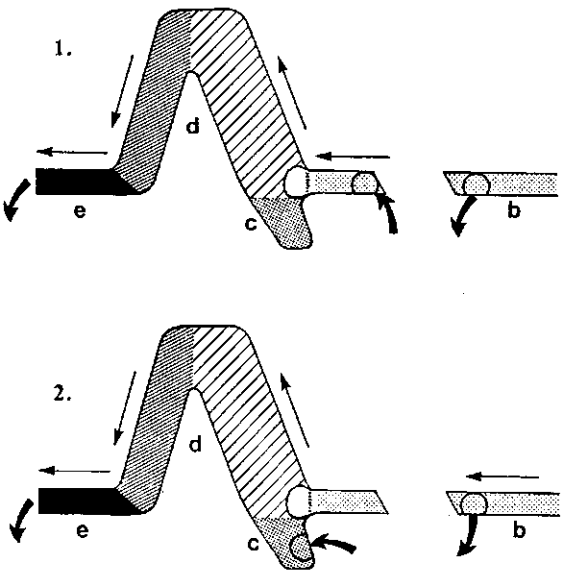
As a modification of the PVTC cannulation the so-called steered ileo-caecal valve (SICV) cannulation has been reported by Mroz et al. (1991). In contrast to the PVTC cannulation the caecectomy is omitted since the ileo-caecal valve can be steered into the T-shaped cannula, using two rings placed proximal to the ileo-caecal valve. One ring is fitted around the terminal ileum, close to the caecal wall, and another ring is introduced into the ileum proximal to the outer ring. Using a thread, that is connected with the inner ring, the ileo-caecal valve can be steered into the cannula. Post slaughter examination showed anatomical-pathological changes that were related to proliferation of fibrous tissues between the two rings and dilation of the distal ileum by muscular hypertrophy anterior to the ileo-caecal valve. Therefore Mroz et al. (1991) suggested a limited application of this technique of about 6-8 weeks.

2.2 The re-entrant cannulation

The ileo-ileal and ileo-caecal re-entrant cannulation

To collect digesta quantitatively earlier studies were carried out using pigs fitted with ileo-ileal or ileo-caecal re-entrant cannulas (Figure V) as described by Cunningham et al.(1962), Easter & Tanksley (1973) and Hazem & Drochner(1976). The method of bridging the ileo-caecal valve allows the collection of ileal digesta quantitatively via the proximal cannula. It also allows for the return of the remaining digesta into the animal via the distal cannula after sampling. On the other hand this technique requires complete transection of the small intestine, interrupting the transmission of the normal migrating myo-electric complex which is necessary for the normal digesta passage. Several researchers (Zebrowska, 1978; Sauer & Ozimek, 1986; Oslage et al., 1987; Schröder et al., 1989) have reported problems such as blockages proximal to the ileal cannula or between the proximal and distal cannula. In addition leakages around the cannulas have been reported by these authors. The extent to which blockage occurs depends on various

Figure V. The re-entrant cannulation:
1. ileo-ileal and 2. ileo-caecal; b. small intestine
c. caecum, d. colon, e. rectum
(according to Laplace et al., 1985)



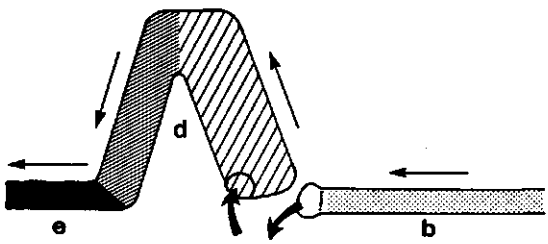
parameters: the fibre content of the diet, the fineness of grinding the diet, the viscosity of the digesta and the amount of digesta that has to pass via the cannula. To minimize problems caused by blockage van Leeuwen et al. (1987) showed that the infusion of a physiological saline solution into the proximal cannula reduced blockages. However, this modification is time consuming and makes handling more difficult.

Both re-entrant and T-cannulation cause a direct disturbance of the small intestine. This may have an influence on the processes of digestion and absorption. Studies in sheep (van Bruchem et al., 1983) and pigs (Buraczewska et al., 1979; Drochner et al.,1984; Huisman et al., 1985 and Metz et al., 1985) showed no effect of cannulation on the faecal digestibility. Results reported by Sauer et al. (1977) and Sauer and Aherne (1979), however, indicated that the insertion of ileo-caecal re-entrant cannulas results in higher faecal digestibility of amino acids than compared to intact animals. In agreement with these studies Jørgensen et al. (1985) also reported higher faecal digestibilities of dry matter, nitrogen and lysine in cannulated than in intact pigs.

The ileo-colic post-valve cannulation

To prevent problems associated with a direct manipulation of the small intestine a modification of the re-entrant cannulation described has been developed by Darcy et al. (1980 a,b) This technique preserves the functional role of the ileo-caecal valve since the proximal part of this re-entrant cannulation is formed into the remnants of the caecum (Figure VI). Using this technique disorders reported for the cannulation of the ileum (e.g. blockages proximal to the cannula, separation of digesta components) are avoided. Nevertheless using fibrous diets blockages of the proximal cannula still occur. In addition the surgical procedure is very complex and the handling is too time-consuming for routine measurements to be feasible (Darcy-Vrillon & Laplace, 1990).

Figure VI. The ileo-colic post-valve fistulation: b. small intestine, c. caecum, d. colon, e. rectum (according to Laplace et al., 1985)

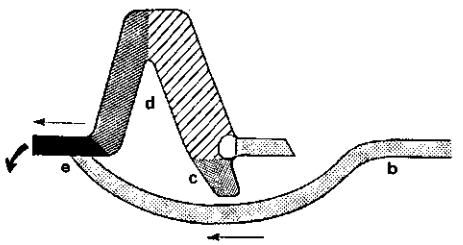


3. The ileo-rectal anastomosis technique

As an alternative to the different cannulation techniques the ileo-rectal anastomosis has been proposed initially by Fuller & Livingstone (1982). These workers fitted the ileum as a simple end-to-

Figure VII. The ileo-rectal anastomosis technique 1. simple end-to-side without ileo-caecal valve, 2. end-to-end without ileo-caecal valve, 3. end-to-side including ileo-caecal valve, 4. end-to-end including ileo-caecal valve; b. small intestine, c. caecum, d. colon, e. rectum (according to Laplace et al., 1985)

1.



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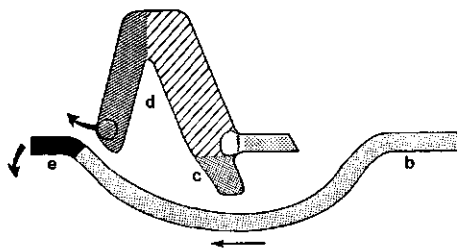

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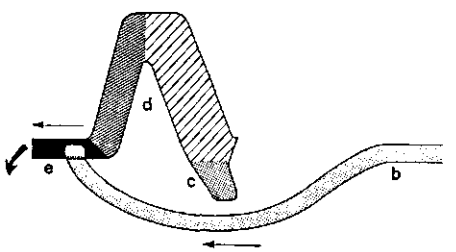

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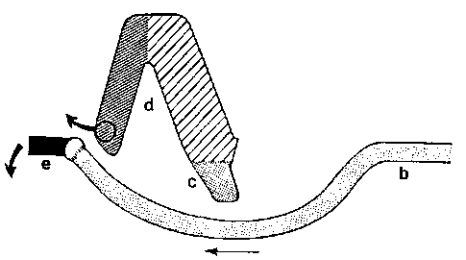

side anastomosis to the rectum, allowing residual digesta of the colon to be evacuated via the anus (Figure VII-1). Using this technique reflux of ileal digesta into the distal colon is still possible. Picard et al. (1984) modified this technique by separating the large intestine completely from the small intestine. This modification, that has been further adapted by Green et al. (1987) and Laplace et al. (1989) requires the insertion of a cannula for evacuation of residual digesta and gases out of the colon (Figure VII-2). Since the ileo-caecal valve has been suspected to restrict the passage of digesta and therefore a possible influence on digestibility (Laplace & Borgidia, 1976), Souffrant et al. (1985) recommended a modification maintaining the integrity of the ileum and the ileo-caecal valve by fitting the remaining part of the caecum immediately posterior to the ileo-caecal valve with the rectum. This technique carried out as an end-to-side ileo-rectal anastomosis maintains an intact junction between the colon and the rectum (Figure VII-3). A more recent modification of this post-valve ileo-rectal anastomosis has been reported by Green (1988). In this arrangement the ileum, including the ileo-caecal valve, is fitted to the rectum. To prevent a possible reflux of digesta into the colon, the large intestine is isolated and fitted with a cannula allowing the evacuation of residual digesta out of the colon (Figure VII-4).

Because of the functional destruction of the large intestine it is possible that alterations of the digestion process and in particular the absorption of nutrients and minerals occurs in

the small intestine and/or the rectum (Sauer & Ozimek, 1986). To compensate for the change in mineral absorption a mineral supplementation has been proposed by various authors (Picard, 1984; Hennig et al., 1986; Bengala-Freire et al., 1988; Darcy-Vrillon & Laplace, 1990; Leterme et al., 1990). Since the absorption of volatile fatty acids from the hind gut may account an energy source in pigs different authors have suggested a saccharose/starch supplementation of the diet of anastomized pigs (Hennig et al., 1986; Wünsche et al., 1987; Wünsche et al. 1988; Herrmann et al., 1988). Histological investigations at the terminal ileum did not show any changes of the ileal mucosa when conducted 8 weeks post-surgery (Souffrant et al., 1985). Fuller (1991) however, reported an increased number of goblet cells, hypertrophy of smooth muscle, elongation of the crypts and atrophy of the enterocytes at the villus type when measured 26 weeks post-surgery. In addition the concentration of volatile fatty acids in digesta recovered from anastomized pigs were higher than in digesta from pigs fitted with a simple T-cannula. On the basis of these results he concluded that there is an adaptation of the ileum in order to compensate for some of the functions of the bypassed large intestine.

Effect of digesta collection techniques on digestibility measurements

Each digesta collection method is associated with a specific interference with the intestine and several comparisons have been made to investigate their specific effects on digestibility. Moughan & Smith (1987) compared the apparent ileal digestibility of amino acids in ground barley using the simple T-cannulation and the slaughter technique and found no differences ($P > .05$). Zebrowska et al. (1978) found no differences in most instances when they compared amino acid digestibility in simple and re-entrant cannulated pigs fed four different diets. Taverner et al. (1983) reported no differences between the apparent ileal digestibility of dry matter, nitrogen and an average of amino acids in re-entrant and T-cannulated pigs that were fed three different diets. However, Schröder et al. (1989) found considerable differences between the apparent ileal digestibility of dry matter and nitrogen in various diets when these were determined in pigs fitted with T- or with re-entrant cannulas. Leterme et al. (1990) compared T-cannulation with the end-to-end ileo-rectal anastomosis technique and they reported systematically higher apparent digestibilities for organic matter, crude protein, ADF, NDF, starch, energy and different amino acids in T-cannulated pigs, but lower values for tryptophan and glycine in the T-cannulated animals. These differences which were only significant for ADF, NDF, methionine, proline and tryptophan were attributed to problems inherent in the use of cannulas. Picard et al. (1984) compared digestibility measurement in pigs fitted with the end-to-end ileo-rectal anastomosis and re-entrant cannulas. Apart from cystine there were no differences between the apparent digestibility of amino acids. Hennig et al. (1990) determined the digestibility of organic matter, crude protein, crude fat, crude fibre, NFE and some amino acids in

anastomized pigs, either end-to-side or end-to-end, and pigs fitted with ileo-caecal re-entrant cannulas. With the exception of isoleucine and lysine in a barley-lupin diet there were no differences between the treatments. Darcy-Vrillon & Laplace (1990) compared digestibilities determined in pigs provided with end-to-end ileo-rectal anastomosis and ileo-colic post-valve cannulas in a standard diet, a wheat bran diet and a sugar beet pulp diet. The apparent digestibility of dry matter in the wheat bran diet was significantly lower in anastomized pigs than in ileo-colic cannulated pigs. In addition, the apparent ileal digestibilities of dry matter, nitrogen and of the total of the amino acids in the sugar beet pulp diet were significantly lower ($P< 0.001$) in pigs with an ileo-rectal anastomosis. When pre-valve end-to-end ileo-rectal anastomosis were compared with post-valve end-to-end ileo-rectal anastomosis (Green, 1988) no differences were observed for the digestibility of nitrogen and amino acids.

It can be concluded, that the collection of digesta via a cannula anterior to the ileo-caecal valve may cause some changes in digestion and absorption as well as the possibility of obtaining unrepresentative samples. Therefore digesta collection posterior to the ileo-caecal valve is preferable. The ileo-rectal anastomosis that has been commonly used to collect digesta with minor manipulation of the small intestine eliminates hind gut digestion and absorption which may also have some influence on digestibility. The ileo-colic post-valve fistulation, an alternative to conserve both the small intestine including the ileo-caecal valve and the digestive processes of the large intestine tends to block easily, especially with fibrous diets. In addition, the surgery is complex and digesta collection is too difficult and time-consuming for this to be of routine use (Darcy-Vrillon and Laplace, 1990).

Although the manipulation of the gastro-intestinal tract has been proposed as a reliable method for measuring digestibility it should be realized that a physiological parameter such as digestibility will be effected to some degree by the technique applied. This means that the evaluation of a digesta collection technique should not be restricted to the comparability of the digestibility. Physiological aspects should also be considered and therefore the technique that causes the least alteration of the physiological status of the animal has to be preferred. The experiments described in this thesis have been performed to investigate the effect of the post-valve T-caecum cannulation in pigs with respect to the digestibility measurement, and also it's effect on the physiological status of the animals. Therefore physiological criteria such as growth performance, blood components, nitrogen-, sodium- and potassium-balances were determined in comparison with intact animals as well as with pigs fitted with an end-to-side ileo-rectal anastomosis (Chapter I and II). Since the PVTC cannulation technique has been specifically developed to determine the digestibilities of the nutrients at the terminal ileum, digestibility measurement will be compared with other techniques (Chapter III and IV). As mentioned above a saccharose/starch supplementation has been proposed to compensate for the missing energy

utilization from the large intestine in pigs fitted with an ileo-rectal anastomosis. On the other hand such a supplementation (100 g per kg dry matter intake) represents a different diet that may have an influence on digestibility measurement especially with regard to the amino acid digestibilities. Therefore Chapter V is focused on the effect of a saccharose/starch supplementation on amino acid digestibilities in a wheat based diet and using pigs fitted with PVTC cannulas.

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Chapter I

Effect of ileo-rectal anastomosis and post-valve T-caecum cannulation on growing pigs. 1. Growth performance, N-balance and intestinal adaptation[a]

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Effect of ileo-rectal anastomosis and post-valve T-caecum cannulation on growing pigs. 1. Growth performance, N-balance and intestinal adaptation

Abstract

The effects of post-valve T-caecum (PVTC) cannulation and end-to-side ileo-rectal anastomosis (IRA) on growth performance, nitrogen-retention and intestinal fermentation in growing pigs were compared with findings for a control group intact animals. There were no differences between PVTC-pigs and intact pigs in growth performance and N-balance. In IRA-animals reduced growth ($P< 0.01$), less efficient feed conversion ($P< 0.01$) and decreased N-retention ($P< 0.001$) were found. Indices of fermentation measured in ileal digesta of PVTC- and IRA-pigs were considerably different. In IRA- animals the concentration of volatile fatty acids (VFA) was about 112-162 mmol/l, higher ($P< 0.001$) than in digesta of PVTC-pigs (20-31 mmol/l). The molar proportions of acetate and propionate depended ($P< 0.01$ and $P< 0.001$ respectively) on the digesta collection technique. Concentrations and ratios of VFA measured in PVTC-pigs were similar to reported values (Argenzio & Southworth, 1974, Drochner, 1984, Mosenthin, 1987). Diaminopimelic acid (DAPA) concentration and N:DAPA ratios measured in digesta were significantly ($P< 0.05$ and $P< 0.001$ respectively) different between treatments. All digesta variables measured showed increased microbial activity in digesta of IRA-pigs, thus an influence on digestibility measurement can be assumed.

Introduction

Digestibility measured at the terminal ileum has been shown to be the best method for calculating the availability of amino acids in the pig (Zebrowska 1973). Therefore, three new methods for collection of intestinal digesta at the terminal ileum have been developed during the last decade. Ileo-colic post-valve fistulation (Darcy et al., 1980) has been described as very difficult and time consuming for routine measurement (Darcy et al., 1980). Ileo-rectal anastomosis (IRA) has been proposed as an alternative collection method (Picard et al., 1984; Darcy-Vrillon & Laplace, 1985; Souffrant et al., 1985; Hennig et al., 1986). The most important advantage of IRA is that digesta can be collected quantitatively via the anus. Using this technique problems such as blockages and leakages which have been described for re-entrant cannulation (Sauer, 1976; Just et al.,1980) do not occur. This finding could be of interest, especially using practical diets containing large quantities of fibrous byproducts. The most recent technique to be developed is the post-valve T-caecum (PVTC) cannula

described by van Leeuwen et al. (1988, 1991). This new technique uses the site of the ileo-caecal valve which normally protrudes into the caecum. The caecum is replaced by a large T-cannula with an internal and external diameter of 25 and 30 mm respectively. The relatively large size of the cannula permits the use of fibre-rich diets without the problem of blockages proximal to the cannula. In addition the samples from the PVTC cannula can be assumed to be representative because marker recovery is about 100% (van Leeuwen et al. 1991). In a previous experiment (Köhler et al., 1990) it was shown that there are no differences in digestibility measurement between the PVTC cannula and the simple T-cannula described by Just et al. (1985) and the ileo-caecal re-entrant cannula described by van Leeuwen et al.(1987). A new digesta collection method can also be evaluated from its influence on the physiological state of the animals. In the present experiment a long term study was made of some physiological effects of the new PVTC cannulation technique in comparison with the end-to-side IRA and with findings in intact pigs. The main criteria were growth, N-retention and intestinal adaptation.

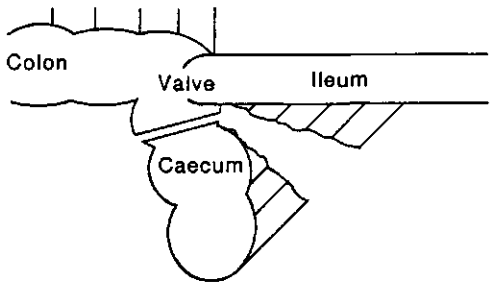
Materials and methods

Twenty-two crossbred barrows with an average live weight (LW) of approximately 30 kg were used in the study. Seven pigs were fitted with a PVTC cannula as described by van Leeuwen et al. (1988) and nine were prepared with an end-to-side IRA. The latter group was fasted preoperatively for 48 h to minimize contamination with digesta during surgery. The animals were given magnesium sulphate (75 g 2 d before surgery) as well as Prunacolon[1] (100 ml one day before surgery) to stimulate emptying of the colon. The animals were anaesthetized by inhalation anaesthesia as described by Kik et al.(1988). An incision (about 150 mm) was made in the lower abdominal wall ventral median following the linea alba, starting 100 mm behind the breastbone, opening the abdominal cavity. The caecal apex and the terminal portion of the ileum were located and exteriorized and the main part of the caecum was removed using a GIA 50 Premium[2] instrument (Fig.Ia). Subsequently the colon was cut using a TA Premium[2] instrument, so that a pouch of caecal tissue was built keeping the vascularity of the pouch intact (Fig.Ib). Afterwards the side of anastomosis on the pouch was identified. A purse string was placed there using a Purstring[2] instrument and the excess tissue was excised. An EEA[2] instrument, without anvil was introduced transanally into the rectum. An incision (about 2 mm) was made in the rectal tissue 100 mm proximal to the anus. The centre rod was advanced through this incision and the anvil was placed on its top. Next the Purstring[2] instrument

[1]Centrafarm, NL

[2]Auto Suture Instruments/United States Surgical Corporation-Norwalk. CT 06850

(a)



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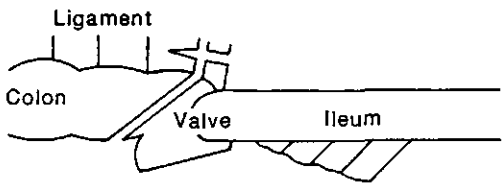

(c)

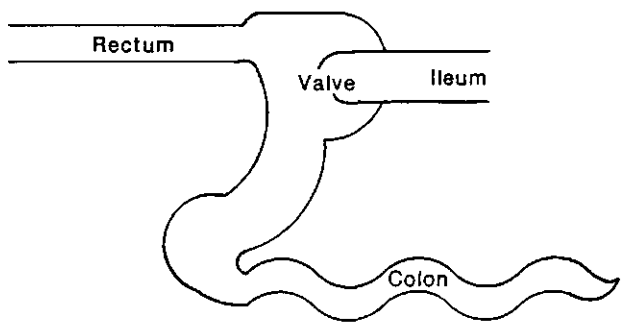

Fig.1. Stages in the surgical procedure used to establish an ileo-rectal anastomosis. a. Caecum is removed, b. the colon is cut, c. ileum, including ileo-caecal valve is connected to the rectum.

was removed and the purse string suture was tied and the EEA[2] instrument closed. Following up, the staples were fired and the IRA was created (Fig.Ic). The post-operative medication was 1 ml analgesic (Finadyne[3])/d for 3 d and 1 ml antibiotic (Tribrisse[4])/d for 5 d. After surgery, the pigs were allowed to recover for a period of 2 weeks. Six of the animals were not subjected to any surgical interference (intact group0. All the pigs were individually housed in adjustable cages in an environmentally controlled metabolism unit with continuous light and at an air temperature in the range of 19-21^0.

Diet.

The composition of the experimental diet is given in Table 1. Chromic oxide was included (2.5 g/kg) as a marker for the solid phase. The pigs were fed at about 2.4 times the maintenance requirement for energy (Agricultural Research Council, 1981) divided into two meals fed daily at 08.00 and 20.00 hours. Water was administered with the feed at a ratio of 2.5:1 (w/w). The anastomized pigs also received an electrolyte solution (200 ml/20 kg LW per d) twice daily as described by Hennig et al.(1986). In contrast to the PVTC-pigs IRA-pigs had continuous access to fresh water.

LW gain

The LW of the animals were recorded every 2 weeks.

Nitrogen-balances.

At 2 weeks before surgery as well as during the fifth and eleventh week after surgery N-balances were carried out over 5 d. The experimental groups were allocated to treatments after the first N-balance.

Digesta collection.

The ileal digesta were collected quantitatively for 5 d for 12 h/d from 09.00 to 21.00 hours, 3, 9 and 12 weeks after surgery. The digesta from the IRA-animals were collected in faeces boxes and immediately frozen at -20^0. The digesta from the pigs fitted with a PVTC cannula were collected continuously through polyethylene tubing into a collection container packed with crushed ice. All samples were stored by -20^0 until required for analysis.

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[3]Schering Corporation, USA

[4]Coopers Agrovet, NL

Table 1. Composition of the experimental diet (g/kg)

Ingrdients	
Maize	32.95
Barley	6.00
Wheat	10.50
Soyabean meal solv.extracted	22.50
Molasses	4.00
Potato pulp	9.20
Beet pulp	10.00
Animal fat	1.00
L-Lysine HCL	0.18
Methionine DL	0.12
Vitamins and minerals*	1.00
$Ca(H_2Po_4)*H_2O$	1.50
$CaCo_3$	0.50
NaCl	0.30
Cr_2O_3	0.25

* Contributed the following per kg of diet: retinol, 9000 IU; cholecalciferol, 1800 IU; α-tocopherol, 40 mg; menadione, 3 mg; riboflavin, 5 mg; cobalamine, 40 μg; nicotinic acid, 30 mg; D-pantothenic acid, 12 mg; choline chloride, 150 mg; ascorbine acid, 50 mg; KJ, 500 μg; $CoSO_4*7H_2O$ 2.5 mg; Na_2SeO_3, 0.2 mg; $FeSO_4*7H_2O$, 0.40 g; $CuSO_4*5H_2O$, 0.1 g; MnO_2, 0.07 g; $ZnSO_4*H_2O$, 0.2 g. This mixture also supplied 20 mg virginiamycin/kg to the diet. The mixture was made up to 1 kg with ground maize.

Chemical composition (g/kg dry matter)	
as analysed	
Crude protein (nitrogen x 6.25)	199.00
Gross energy	18.04 MJ kg^{-1} DM
Sodium	1.60
Potassium	13.00
Crude fibre+	65.00

+ Calculated value

Chemical and statistical analysis.

Digesta, faeces and urine samples per animal and per period were pooled before chemical analyses. Analyses for N and dry matter were carried out according to International Organization for Standardization (1979, 1983).

Volatile fatty acids (VFA) were determined as described by Kaufmann & Hagemeister (1969). Diaminopimelic acid (DAPA) analyses were carried out according to Ahrens et al.(1985). Differences among treatment means were tested according to the Tukey range test procedure of SAS (1990).

Results

LW Gain.

Fig. 2 shows the cumulative LW gain from 2 weeks after surgery until the 13th week after surgery. The average LW gain of the IRA-pigs was significantly lower ($P<0.01$) than for the intact pigs. The average daily gain of the animals with an IRA was only 221 g/d, whereas the average daily gain of the pigs with a PVTC cannula was 572 g/d and for the intact pigs it was 656 (SEM=21.7) g/d. The difference in daily gain between the PVTC-animals and the intact pigs was not significant ($P>0.05$).

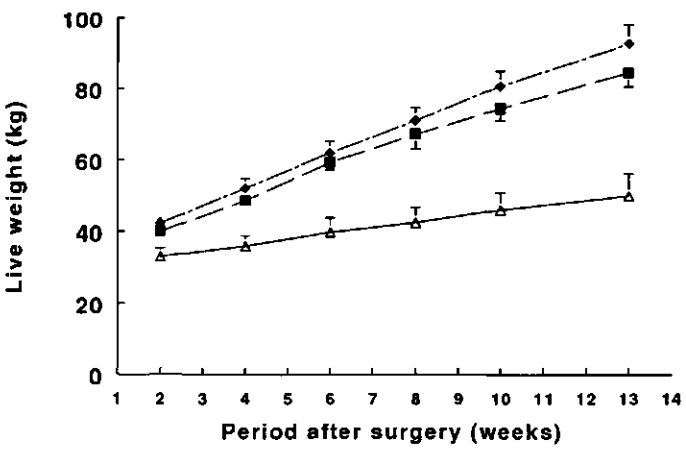

Fig. 2. Live weight gain of pigs fitted with ileo-rectal anastomosis (-Δ-), post-valve T-caecum cannula (-■-) and intact intestinal tract (-♦-). Values are means and standard deviations represented by vertical bars

The effect of the IRA on growth rate was significant (P< 0.01), as was the effect on feed conversion (P< 0.01). For the IRA-pigs it was 5.19 (2.3 times higher than for the intact pigs 2.29). The average feed conversion calculated for the animals fitted with a PVTC cannula was 2.56.

N-retention

Table 2 shows values for the three N balances. Before surgical intervention there were no significant differences between the groups. After surgery, however, N retention of IRA-pigs was considerablly lower than that of the other animals (P< 0.001). N retention of the intact and PVTC cannulated animals were about twice that of IRA- animals and decreased with time. After surgery N retention of the IRA-pigs remained almost constant throughout the experiment. Intact and PVTC-animals showed the normal increase in the accretion of N. Valued for faecal and urinary N excretion for IRA-pigs were constant and, apart from the renal N excretion in period three, significantly higher (P< 0.001) than those in the other two groups.

Table 2. Nitrogen balances (g N/d per kg metabolic live weight ($LW^{0.75}$)) in pigs fitted with ileo-rectal anastomosis (IRA), post-valve T-caecum (PVTC) cannula and intact pigs (INT).

	$LW^{0.75}$	*N-Intake*	*N-excretion*		*N-retention*
			faeces	*urine*	
2 weeks pre-surgery					
IRA	12.05	2.28	0.43	0.49	1.36
PVTC	11.79	2.28	0.43	0.53	1.32
INT	11.67	2.29	0.42	0.53	1.34
SEM	0.202	0.004	0.031	0.021	0.026
5 weeks post-surgery					
IRA	14.69	2.22	0.65	1.10	0.45
PVTC	18.62***	2.18***	0.34***	0.63***	1.21***
INT	19.38***	2.18***	0.28***	0.76***	1.14***
SEM	0.327	0.004	0.022	0.054	0.060
11 weeks post-surgery					
IRA	17.77	2.25	0.68	1.14	0.43
PVTC	25.36***	2.22***	0.37***	0.90*	0.95***
INT	26.94***	2.22***	0.33***	0.99	0.90***
SEM	0.439	0.003	0.034	0.059	0.076

† Standard error of the mean

Mean values were significantly different from those for IRA-animals: * P< 0.05, *** P< 0.001

VFA

The total amount of VFA are shown in Table 3. In all three periods the concentration of VFA measured in digesta from IRA- animals were significantly ($P< 0.001$) higher than those in digesta of PVTC- pigs. In PVTC-pigs the highest concentration of each VFA was observed 3 weeks after surgery. At 6 and 13 weeks after surgery the concentrations were lower and similar. In IRA-animals the absolute amount of acetate, propionate and bytyrate increased during the experiment. Thus, 13 weeks after surgery the concentration of acetate in the digesta of IRA-animals was about six times higher than that in digesta of PVTC-animals. For propionate and butyrate this factor was about 20 and for valerate 35.

Table 3. Contents of volatile fatty acids (VFA; mmol/l) and their molare proportion (mol/100 mol) in digesta of pigs fitted with ileo-rectal anastomosis (IRA) and post-valve T-caecum cannula (PVTC) 3, 9 and 12 weeks after surgery.

Period after surgery (weeks)	*total VFA*	*Molar proportions (mol/100mol) of* C_2	C_3	C_4	C_5	$C_2 : C_3$
3						
IRA	108.0	56.3	32.1	9.1	2.5	1.76
PVTC	31.2***	74.2***	18.9***	5.7**	1.3**	4.1***
SEM†	*	1.66	1.13	0.68	0.29	0.31
9						
IRA	116.4	58.2	31.8	8.2	1.8	1.84
PVTC	21.4***	79.5***	15.3***	4.6***	0.7***	5.80***
SEM	*	1.35	1.12	0.49	0.19	0.69
12						
IRA	162.7	58.1	31.3	8.5	2.1	1.86
PVTC	20.5***	83.3***	12.5***	3.5***	0.7**	7.13***
SEM	*	1.38	0.85	0.53	0.29	0.57

Mean values were significantly different from those for IRA-animals: ** $P< 0.01$, *** $P< 0.001$

† Standard error of the mean * could not be given as SE differed mainly between IRA-pigs (9.2, 11.5 and 5.8 respectively) and PVTC-pigs (3.7, 2.9 and 2.6 respectively) at 3, 9 and 12 weeks respectively.

Table 3 also presents the molar proportions of VFA in digesta. In both treatments acetate was present in the highest ($P< 0.05$) amount followed by propionate, butyrate and valerate. In PVTC-animals the amount of acetate was significantly ($P< 0.001$) higher (74-83 mol/100 mol) than that in IRA-animals (55-58 mol/100 mol) and increased with time. On the other hand in IRA-animals the amounts of propionate and butyrate (31 and 8-9 mol/100 mol) were significantly ($P< 0.001$ and $P< 0.01$ respectively) higher than those in PVTC-animals (12-19 and 3.0-5.2 mmol/100 mmol). These differences resulted in a different C_2:C_3 ratio. Thus, in PVTC-animals this ratio was 4.10 - 7.13 and increased during the experiment; in IRA-animals it was about 1.80.

DAPA

DAPA concentration in digesta recovered from PVTC- and IRA-animals are presented in Table 4. In all three digesta collection periods the concentration of DAPA in the digesta of the IRA-pigs was significantly higher ($P< 0.05$ and $P< 0.001$) than those in digesta of PVTC-pigs. In addition in IRA-animals the concentration of this amino acid increased with time. In digesta collected from PVTC-pigs the highest DAPA concentration was observed 3 weeks after surgery. Results measured in week 9 and week 12 post-surgery were smaller and similar. N:DAPA ratios are given in Table 4. In weeks 9 and 12 after surgery the differences between the treatments were significant ($P< 0.001$). In digesta of IRA-animals this ratio became smaller with time ($P< 0.01$), thus, the amount of microbial N increased. In PVTC-animals this ratio was similar ($P> 0.05$) throughout the experiment.

Digesta flow.

Fig.III shows the average digesta flow calculated over 5 d and expressed as percentage of the total dry matter outflow over 12 hours for the first digesta collection period. The between-hours digesta flow was less variable in PVTC-pigs compared with IRA-pigs and seemed to be reduced from the 10^{th} to 13^{th} hour after feeding. However, during the 8^{th} hour after feeding the digesta flow increased by 3 percentage units to 11% of the total outflow. For PVTC-pigs the within-hours mean standard deviation was 4.75 %. In IRA-pigs the hourly digesta flow was relatively irregular. Thus, the calculated hourly digesta flow varied between 3.7 and 12.6 % of the total outflow over 12 h. As with the PVTC-pigs the digesta flow decreased from the 8^{th} hour after feeding. In contrast to the results obtained with PVTC-pigs the mean variation for IRA-pigs was about two times higher.

Discussion

It is questionable whether IRA-animals and intact animals have similar metabolic rates. An increased metabolic rate as a result of discomfort might be expected. MacRae et al. (1982) found an

increased metabolic rate in some of the sheep provided with re-entrant cannulas when compared with intact animals. With the IRA-technique an increased metabolic rate would result in a lower rate of energy gain and an increased feed:gain ratio on a similar metabolizable energy (ME) intake. Hennig et al. (1986) reported a 55 % reduction in LW gain for IRA-pigs and a feed conversion which was 2.3 times higher than that for intact pigs. These workers suggested that energy utilization was reduced as a result of the absence of the large intestine. In the present investigation pigs were fed at a level of 2.4 times the maintenance requirement for energy (2.4 X 420 kJ/kg $LW^{0.75}$), this is about 90 % of the Agricultural Research Council (1981) standards. Herrmann et al. (1989) measured the energy digestibility and metabolizability in anastomized pigs in comparison with intact animals. They found that IRA-pigs have a 15 % lower metabolizability of energy compared with intact animals. Thus, when applied to our animals, this means that compared with the control group which had 2.4 X 420 = 1008 kJ ME kg $LW^{0.75}$ the IRA-animals had a feeding level of 15 % less or 857 kJ ME kg $LW^{0.75}$. Furthermore, Herrmann et al. (1989) found that maintenance requirement for energy was increased by 12 % in IRA-pigs. For IRA-pigs they calculated a maintenance requirement for energy of 504 kJ/kg $LW^{0.75}$. In our experiment the control pigs had 1008 - 420 = 588 kJ ME per kg $LW^{0.75}$ available for performance. In IRA- animals both the reduced metabolizability and the increased maintenance requirement for energy would resulted in energy available for growth of 857 - 504 = 353 kJ ME kg $LW^{0.75}$; this is much less than that for intact animals. Based on values for gain expressed per unit metabolic LW ($LW^{0.75}$) the IRA-animals had an average gain of 13.9 g / kg $LW^{0.75}$ and the intact pigs 29.5 g / kg $LW^{0.75}$. PVTC-pigs had an average LW gain of about 27.4 g / kg $LW^{0.75}$. Compared with the intact animals the IRA-animals gained 47 % and the PVTC-pigs 93 %. Based on energy supply and a similar composition of gain IRA-animals could gain about 60 % of that of the intact animals. The difference of 3.8 g / kg $LW^{0.75}$ between IRA- and intact pigs can be partly explained by the different diet in our study compared with that of Herrmann et al. (1989). In addition, the composition of gain may be different. In general our findings compared favourably with those of Herrmann et al.(1989). The somewhat reduced gain in PVTC-animals can be attributed to the digesta collection. Digesta to be collected does not contribute to large intestine digestion. With our technique of feeding, based on $LW^{0.75}$, a small reduction in rate of gain would result in less feed for the rest of the experiment.

Digestibility measurement at the distal ileum have been shown to be more sensitive than faecal measurement, because microbical activity in the hind-gut of pigs influences the composition of faeces (Mason, 1984). On the other hand, with the IRA-procedure digesta flows through the rectum, which may permit some fermentation to take place. In PVTC-animals the cannula is placed post-valvularly, thus, it is possible that digesta from the colon may have an effect on digesta composition. As an indirect indication of microbical activity, VFA levels in digesta from IRA- and PVTC-animals were

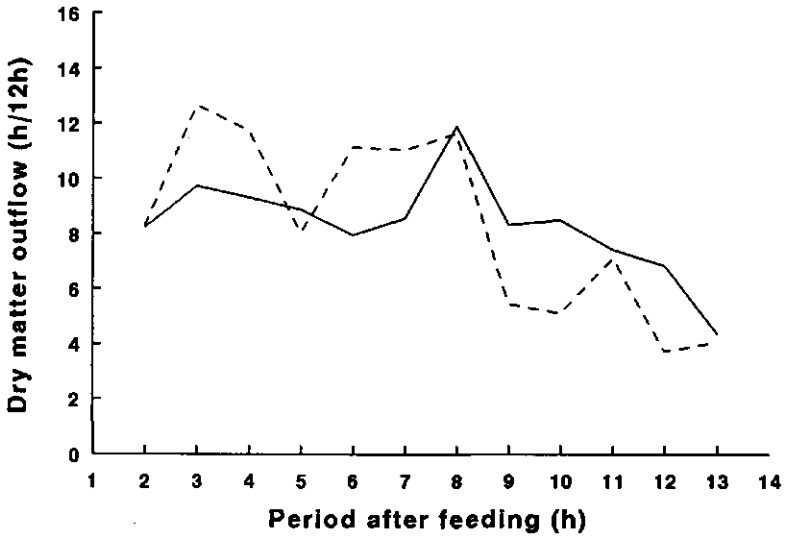

Fig. 3. Digesta flow (relative dry matter outflow per h over 12 h) in pigs fitted with ileo-rectal anastomosis (----) or post-valve T-caecum cannula (———). Sd IRA = 9.43, Sd PVTC = 4.75

measured (Table 3). In PVTC-animals the concentrations of VFA in ileal digesta were comparable with reported values (Clemens et al., 1975; Argenzio and Southworth, 1975; Drochner, 1984; Mosenthin, 1987). Values obtained with IRA-pigs were quite different from those for PVTC-pigs. In IRA-animals concentrations of VFA were about three to eight times higher and the molar proportions of acetate and propionate were quite different. While in PVTC-pigs the percentage of acetate was in a range of 74-83 %, the corresponding value was about 58 % in digesta recovered from IRA-animals . In the literature acetate:propionate ratios measured in different segments of the intestinal tract were considerably different (Clemens et al., 1975; Argenzio & Southworth, 1975; Drochner, 1984; Mosenthin, 1987). Differences between these sections of the intestinal tract were similar to those found between PVTC- and IRA-animals. Thus, molar proportions of VFA in ileal digesta of PVTC-pigs were similar to reported values when VFA concentration were measured in ileal digesta. Molar proportions of acetate of about 50-60 mol/100 mol as we found in digesta of IRA-pigs have also been reported for faeces (Argenzio & Southworth, 1974; Drochner, 1984; Sauer et al., 1991) and in digesta collected from the colon (Münchow et al., 1989). As an additional indication of microbical activity DAPA was determined (Table 4). DAPA concentrations in digesta of IRA-animals were 1.3 - 2.3 times higher than those in digesta collected from PVTC-pigs. Differences between

Table 4. Diaminopimelic acid (DAPA) concentration (mg/g DM) and the nitrogen (mg/100 mg DM) : DAPA (mg/100 mg DM) ratio in digesta of pigs fitted with ileo-rectal anastomosis (IRA) or post-valve T-caecum (PVTC) cannula 3, 9 and 12 weeks post-surgery.

Period after		*DAPA*			*N:DAPA*	
surgery (weeks)	*IRA*	*PVTC*	*SEM*†	*IRA*	*PVTC*	*SEM*
3	0.60	0.47*	0.04	51.8	59.8	3.97
9	0.68	0.34***	0.04	43.4	71.7***	3.91
12	0.80	0.34***	0.04	36.3	68.6***	3.36

† Standard error of the mean

Mean values were significantly different from those for IRA-animals * $P< 0.05$, *** $P< 0.001$

treatments with regard to both VFA and DAPA concentrations indicate increased microbial activity in the digesta recovered from IRA-pigs. This fact may be especially important for amino acid digestibility measurements, because it can be assumed the amino acid composition of microbial protein is different from that of digesta. The relative amount of microbial protein contributing to total protein is given as the N : DAPA ratio (Table 4). While in digesta from PVTC-animals this ratio slightly increased with time, in IRA-pigs the value decreased. Thus, the proportion of microbial protein relative to total protein in digesta from IRA-pigs increased with time. This increase in the population of micro-organisms corresponds with the increase in the concentration of VFA.

As mentioned previously, in the IRA-technique the ileo-caecal valve is used, as proposed by Souffrant (1985), to prevent a backflow of digesta from the rectum into the small intestine. On the other hand it can be accepted that there is no synchronization of the ileo-caecal valve and the anus, so that the digesta outflow via the anus is not similar to that in cannulated animals (Fig. III). This interruption of digesta flow in IRA-pigs resulted in an irregular digesta outflow and a significantly ($P< 0.05$) higher variation between the IRA-animals. An accumulation of ileal digesta in the rectum represents optimal conditions for fermentation. Although Green (1988) found no differences in the digestibility of amino acids between IRA-pigs in the presence or absence of the ileo-caecal valve, our findings for IRA-pigs with an intact sphincter suggest high microbial digestion which might influence digesta composition.

Differences between treatments in N losses from urine were not expected as urine was first collected in funnels, then in a container, in presence of sulphuric acid which maintained the pH of

the urine below 2. Overestimation of N retention has been reported previously (Oslage et al, 1987, Walz & Pallauf, 1989). In the present experiment, N retention was overestimated, the values being higher for PVTC-animals and intact animals than for IRA-animals. Thus, more N may have been lost into the air from faeces of PVTC- and intact animals than from faeces of IRA-animals.

It can be concluded that PVTC-cannulation has no significant influence on growth performance. Fermentation variables were similar in value to those given in the literature; thus, the influence of site (post-valvular) can be excluded. In IRA-pigs the effects of this technique the growth were considerable and in agreement with previously reported findings. Values for VFA and DAPA levels indicate a respectable level of microbial activity. The latter may have an influehce on digestibility measurements. Further studies will be necessary to investigate digestibility measurements in IRA-pigs, especially with regard to the digestibility of amino acids and carbohydrates, because the amino acid profile may be changed and a part of the carbohydrate may be broken down due to microbial fermentation.

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Chapter II

Effect of ileo-rectal anastomosis and post valve T-caecum cannulation on growing pigs. 2. Blood variables and mineral balances[a]

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Effect of ileo-rectal anastomosis and post-valve T-caecum cannulation on growing pigs. 2. Blood variables and mineral balances

Abstract

In a long term study nine ileo-rectally anastomosed (IRA) and seven post-valve T-caecum (PVTC) cannulated pigs were compared with six intact pigs with regard to different blood variables, sodium and potassium retention and weights of selected organs. After surgery apart from urea and K measured 13 weeks post-surgery there were no differences in the blood variables between the PVTC-pigs and intact pigs. In IRA-pigs concentrations of creatinine ($P< 0.01$), Na ($P< 0.001$), base excess ($P< 0.001$), pH ($P< 0.01$) and bicarbonate ($P< 0.001$) in blood were lower than those in INT-pigs. At 13 weeks after surgery the blood K concentration in IRA-pigs was higher ($P< 0.001$) than that in PVTC-pigs or intact pigs. At 6 weeks after surgery the blood urea concentration in IRA-pigs was higher ($P< 0.001$) than that in intact and PVTC-pigs. At 13 weeks after surgery the urea concentration in PVTC-pigs was higher ($P< 0.001$) than those in IRA-pigs or intact pigs. The Na and K balances in IRA-pigs were lower ($P< 0.01$ for Na at week 11 post-surgery and $P< 0.05$ and $P< 0.001$ for K at weeks 5 and 11 post-surgery respectively) than those in intact animals. Na retention was negative for IRA-animals 11 weeks after surgery. Na and K retentions were similar in PVTC-pigs and in intact pigs. The urinary:faecal excretion of Na differed slightly between PVTC-animals and intact animals. At 13 weeks after surgery there were no differences in organ weights between the PVTC-pigs and intact animals. In the IRA-pigs the weights of the liver ($P> 0.05$), the kidneys ($P> 0.05$) and the adrenal glands were higher ($P< 0.001$) than those in the intact animals.

Introduction

The post-valve T-caecum (PVTC) cannula, as described by van Leeuwen et al. (1988), has been shown to provide reliable measurements of digestibilities at the distal ileum of pigs (Köhler et al.,1990; Van Leeuwen et al., 1991). Ileo-rectal anastomosis (IRA) has been proposed as an alternative technique for cannulation (Picard et al., 1984; Laplace et al., 1985; Souffrant et al., 1985; Green, 1988). Both methods may affect the physiological state of the animals. Digestibility as a physiological variable has to be determined in animals in a normal physiological state. Findings for growth performance, digesta flow and intestinal fermentation have been presented previously (Köhler et al.,

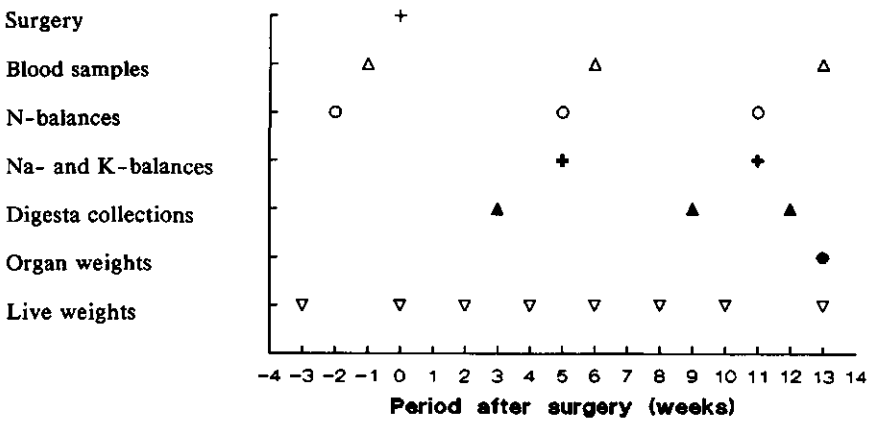

Fig. 1. Experimental design

1991). The objective of the present study was to compare the recently developed PVTC cannulation technique with the end-to-side IRA technique with regard to different blood variables, sodium and potassium balances and selected organ weights.

Materials and Methods

Twenty-two Crossbred castrates (Yorkshire x Dutch Landrace) with an average body weight of 30 kg were used. Nine pigs were provided with an end-to-side IRA and seven pigs with a PVTC cannula. Details of surgical techniques and the experimental procedure have been reported previously (Köhler et al., 1991). The experimental design is shown schematically in Fig. I. The animals were fed at 2.4 times maintenance requirement for energy (Agricultural Research Council, 1981). The composition of the experimental diet is shown in Table 1.

Blood-samples.

At 6 and 13 weeks after surgery blood samples were obtained 3 h post-prandially by puncture of the vena jugularis externa. The following analysis in blood were carried out: Na, K, chloride, calcium, magnesium, urea, creatinine, pH, active bicarbonate and the base excess. In addition Na, urea and creatinine levels were determined 1 week before surgery.

Analysis of blood samples.

Urea, creatinine, Na, K and Cl concentrations were measured in serum obtained after

Table 1.	Composition of the experimental diet (g/kg)
Ingredients	
Maize	32.95
Barley	6.00
Wheat	10.50
Soya-bean meal solv.extracted	22.50
Molasses	4.00
Potato pulp	9.20
Sugar-beet pulp	10.00
Animal fat	1.00
L-lysine HCL	0.18
Methionine DL	0.12
Vitamins and minerals*	1.00
$Ca(H_2Po_4)*H_2O$	1.50
$CaCo_3$	0.50
NaCl	0.30
Cr_2O_3	0.25

* Contributed the following vitamin and mineral sources per kg of diet: retinol, 2.7 mg; cholecalciferol, 45 μg, α-tocopherol, 40 mg; menadione, 3 mg; riboflavin, 5 mg; cobalamine, 40 μg; nicotinic acid, 30 mg; D-pantothenic acid, 12 mg; choline chloride, 150 mg; ascorbine acid, 50 mg; KJ, 500 μg; $CoSO_4*7H_2O$ 2.5 mg; Na_2SeO_3, 0.2 mg; $FeSO_4*7H_2O$, 0.40 g; $CuSO_4*5H_2O$, 0.1 g; MnO_2, 0.07 g; $ZnSO_4*H_2O$, 0.2 g. This mixture also supplied 20 mg virginiamycin/kg diet. The mixture was made up to 1 kg with ground maize

Chemical composition: Analysed (g/kg dry matter)	
Crude protein	199.0
Gross energy	18.04 MJ
Sodium	1.6
Potassium	13.0
Calculated	
Crude fibre	65.0
Chloride	1.9
Magnesium	1.6
Calcium	2.6

centrifugation of the blood samples at 3000 rev./min for 20 min. Urea and creatinine concentration were estimated using Biomerieux kits, Na and K were measured using a flame photometer (model 243), Cl using a Chlor-O-Counter Laméris. Mg was measured in heparinized plasma using an atomic absorption spectrophotometer (Model 305 B, Perkin Elmer). Plasma Ca concentration was measured

according to Willis (1960). pH, bicarbonate and base excess were measured in blood, air-free sampled in a syringe, kept at 0^0 in ice water until analysed using a blood gas pH meter (ABL2 acid base laboratory radiometer). These analysis were carried out within 90 min of collection of the blood.

Na and K balances

At 5 and 11 weeks after surgery the Na and K balances were determined over a period of 5 d.

Organ weights.

At 13 weeks after surgery the animals were slaughtered and the weights of the liver, kidneys and the adrenal glands as well as the girth of the rectum were registered.

Results

Blood variables

Values for blood variables are shown in Table 2. After surgery the blood creatinine contents of pigs fitted with an IRA were significantly lower than those in intact animals or in animals fitted with a PVTC cannula ($P< 0.01$). Pre-operative urea concentrations were not different between the treatment groups. At 6 weeks after surgery urea levels in the IRA-animals were increased and higher ($P< 0.001$) than in the other treatments. At 13 weeks after surgery blood urea levels for IRA-animals and intact pigs were similar; however, PVTC cannulated animals had higher ($P< 0.001$) levels compared with those for the other two treatments. At 6 and 13 weeks after surgery the blood Na levels of IRA-animals were lower than those of the intact animals ($P< 0.001$ and $P< 0.01$ respectively). At 13 weeks after surgery the blood K levels of the IRA-pigs were higher than those of the other animals ($P< 0.001$). In PVTC-pigs blood K levels were intermediate relative to the other treatments and significantly higher ($P< 0.001$) than levels for intact pigs. No significant differences between treatments were observed for the blood Cl, Ca and Mg contents. At 6 weeks after surgery the blood base excess of IRA-pigs was ($P< 0.001$) lower than that of intact pigs. The PVTC-animals had intermediate levels between IRA- and intact animals. At 13 weeks after surgery the blood base excess of IRA-pigs was at a very low level and significantly different ($P< 0.01$) from that of the other two treatments. At 13 weeks after surgery serum pH for IRA-animals differed ($P< 0.01$) from that for the other two treatments. At both 6 and 13 weeks post-operatively the amounts of active bicarbonates for IRA-pigs were lower ($P< 0.001$ and $P< 0.01$ respectively) than those for PVTC-pigs and intact pigs.

Na balance

The results of the Na balances are given in Table 3. For the intact and PVTC-cannulated pigs the Na retention (mg/d) decreased with time but remained slightly positive. At 5 weeks after surgery

Table 2. Biochemical characteristics in serum of pigs with ileo-rectal anastomosis (IRA), post-valve T-caecum cannulae (PVTC) and intact intestinal tract (INT)

	IRA	*PVTC*	*INT*	*SEM*[†]
creatinine µmol/l				
pre-surgical	112.22	124.71	111.33	4.20
6 weeks p.s.[‡]	118.78	145.71**	137.00	5.15
13 weeks p.s.	127.11	149.71**	148.33**	4.78
urea mmol/l				
pre-surgical	3.75	3.66	3.92	0.19
6 weeks p.s.	6.77	3.97***	4.53***	0.33
13 weeks p.s.	6.02	8.36***	6.37	0.32
sodium mmol/l				
pre-surgical	146.78	147.57	147.17	0.65
6 weeks p.s.	140.11	150.71***	148.17***	1.34
13 weeks p.s.	139.33	144.71**	146.33**	1.39
potassium mmol/l				
6 weeks p.s.	5.79	5.50	5.00	0.25
13 weeks p.s.	5.61	5.09***	4.75†††	0.09
chlorine mmol/l				
6 weeks p.s.	95.11	99.86	98.50	1.37
13 weeks p.s.	92.22	91.86	95.00	1.31
calcium mmol/l				
6 weeks p.s.	2.60	2.53	2.60	0.03
13 weeks p.s.	2.60	2.53	2.60	0.04
magnesium mmol/l				
6 weeks p.s.	0.84	0.87	0.87	0.02
13 weeks p.s.	0.83	0.90	0.85	0.02
base excess mmol/l				
6 weeks p.s.	1.19	3.14	5.18***	0.50
13 weeks p.s.	-0.96	5.24**	6.50**	1.37
pH				
6 weeks p.s.	7.32	7.31	7.37	0.41
13 weeks p.s.	7.26	7.37**	7.38**	0.03
active bicarbonate mmol/l				
6 weeks p.s.	28.63	32.37***	32.30***	0.61
13 weeks p.s.	27.89	32.36**	33.88**	1.04

[†] Standard error of the mean; [‡] post-surgery
Mean values were significantly different from those for IRA-pigs: ** $P< 0.01$, *** $P< 0.001$
Mean values for intact pigs were significantly different from those for PVTC-pigs and IRA-pigs: ††† $P< 0.001$

the Na retention (mg/d) of IRA-pigs was in the same range as values obtained for the other treatments. At 11 weeks after surgery the Na balance of the IRA-pigs was ($P< 0.01$) lower than those of the other treatments. At both 5 and 11 weeks the Na retention of PVTC- animals was similar to that of intact pigs. Table 3 also shows that in intact and PVTC cannulated animals the amount of Na excreted in the urine was higher than that excreted in the faeces. In IRA-animals the renal:intestinal Na ratio was reversed, which finally resulted in a negative ileal digestibility for Na in IRA-pigs.

K balance

The K balance (Table 4) shows differences similar to those for Na balance. The apparent faecal digestibility of K in the PVTC cannulated and intact pigs was about 86 %. The intestinal utilization of K in IRA-pigs was 34 %. As for Na, the renal:intestinal K loss in IRA-animals did not follow the pattern found for intact and PVTC-animals. The amount of K excreted in urine of intact and PVTC-pigs was higher than that excreted in the faeces ($P< 0.001$). In IRA-pigs more K was excreted in the digesta than in the urine. At 5 weeks after surgery K retention (mg/d) of IRA-pigs was lower ($P< 0.05$) than that of intact animals. At 11 weeks after surgery the K retention (mg/d) of PVTC- and intact animals was higher ($P< 0.01$) than that of IRA-pigs. K retention expressed as a percentage of intake shows no differences between the treatments.

Organ weights.

Table 5 shows the relative weights of the liver, the kidneys and the adrenal glands at 13 weeks after surgery. Weights are expressed in g/kg live weight$^{0.75}$. For the IRA-pigs the weights of the organs were higher than for the other animals. For the adrenal glands these differences were significant ($P< 0.001$). In addition to the organ weights the circumference of the rectum was measured. In IRA-animals the circumference of the rectum was larger ($P < 0.001$) than that in PVTC- or intact animals.

Discussion

Steger et al.(1976) reported that the blood creatinine content of growing pigs is positively correlated with the weight of the animals. In agreement with this statement Duggal and Eggum (1978) reported, that the daily creatine and creatinine excretion of growing pigs was positively related to body weight and to nitrogen retention. In addition, Murlin et al. (1953) concluded from their results that high urinary creatinine excretion indicated high N utilization. It may assumed from these three studies that high blood creatinine content may be associated with high urinary creatinine excretion; furthermore it may also be relate to high N utilization and high muscle mass. Only a limited amount of information is available in the literature on blood creatinine levels of pigs. These findings are

summarized in Table 6. The present results which were in the range 111-149 μmol/l (Table 2) are in agreement with corresponding values from the literature.

Table 3. Sodium balances in pigs fitted with ileo-rectal anastomosis (IRA), post-valve T-caecum (PVTC) cannulae or intact intestinal tract (INT).

	IRA	*PVTC*	*INT*	*SEM*[†]
Intake mg				
5 weeks p.s.[‡]	3015	2078***	2160***	36.74
11 weeks p.s.	3314	2759***	2924***	46.17
Excretion in faeces mg[§]				
5 weeks p.s.	2319	512***	376***	100.85
11 weeks p.s.	3672	1065***	960***	161.33
Excretion urine mg				
5 weeks p.s.	32	867***	1122***	66.79
11 weeks p.s.	48	1206***	1575†††	87.57
Retention mg				
5 weeks p.s.	663	699	662	117.06
11 weeks p.s.	-407	488**	390**	181.48
Retention in %				
5 weeks p.s.	21.9	33.8	30.7	4.43
11 weeks p.s.	-12.4	17.9**	13.3**	5.82
Total digestibility %				
5 weeks p.s.	23.0	75.5***	82.6***	3.61
11 weeks p.s.	-10.9	61.4***	67.2***	4.99

[†] Standard error of the mean; [‡] post-surgery.

[§] In PVTC-pigs and intact pigs: excretion in faeces; in IRA-pigs excretion in digesta.

Mean values were significantly different from those for IRA-pigs: ** $P < 0.01$, *** $P < 0.001$.

Mean values for intact-pigs were significantly different from those for PVTC-pigs and IRA-pigs: ††† $P < 0.01$.

A lower creatinine concentration ($P < 0.01$) and lower daily live weight gain ($P < 0.01$) was found for IRA-pigs than for PVTC- and intact pigs. The IRA-pigs also showed a lower N-retention ($P < 0.001$; Köhler et al. 1991). Thus, our findings for IRA-pigs were in accordance with values from

Table 4. Potassium balances in pigs fitted with ileo-rectal anastomosis (IRA), post-valve T-caecum (PVTC) cannula or intact intestinal tract (INT).

	IRA	*PVTC*	*INT*	*SEM*†
Intake mg				
5 weeks p.s.‡	13750	17066***	17776***	301.64
11 weeks p.s.	16911	23652***	25069***	395.59
Excretion faeces mg§				
5 weeks p.s.	9050	2316***	2280***	332.25
11 weeks p.s.	11290	3496***	3372***	412.05
Excretion urine mg				
5 weeks p.s.	2711	11752***	11922***	392.86
11 weeks p.s.	3400	15837***	16847***	610.38
Retention mg				
5 weeks p.s.	1989	2998	3574*	353.06
11 weeks p.s.	2221	4319***	4850**	515.74
Retention in %				
5 weeks p.s.	14.38	17.51	20.00	2.08
11 weeks p.s.	12.94	18.21	19.32	2.27
Total digestibility %				
5 weeks p.s.	34.02	86.47***	87.14***	2.51
11 weeks p.s.	32.99	85.17***	86.55***	2.45

† Standard error of the mean; ‡ post-surgery.

§ In PVTC-pigs and intact pigs: excretion in faeces; in IRA-pigs excretion in digesta.

Mean values were significantly different from those for IRA-pigs: * $P< 0.05$, ** $P< 0.01$, *** $P< 0.001$.

the literature for pigs with a reduced live weight, daily live weight gain and N utilization.

Berschauer (1977) found a clear negative correlation between the extent of protein utilization and the blood urea content. Berschauer (1977) measured blood urea in pigs fed on diets differing in energy:protein ratio and he concluded that an energy deficiency results in higher blood urea concentrations. Bolduan and Schneider (1986) reported that the normal blood urea content of pigs ranges from 4 to 7 mmol/l serum. About 90 % of urea is derived from deamination of amino acids

after absorption and 10 % is derived from ammonia originating from microbial activity and subsequent deamination in the intestine.

Table 5. Organ weights (per kg live weight$^{0.75}$) of pigs fitted with ileo-rectal anastomosis (IRA) or post-valve T-caecum cannulae (PVTC) and intact intestinal tract (INT) at week 13 post-surgery.

	IRA	*PVTC*	*INT*	*SEM*†
Liver in g	13.18	12.24	12.68	1.34
Kidneys in g	3.40	2.80	2.72	0.18
Adrenal glands in mg	93.00	60.00***	53.33***	0.01
Circumference of the rectum mm	149.40	99.30***	113.30***	0.68

† Standard error of the mean

Mean Values were significantly different from those for IRA-pigs: *** $P < 0.001$

Blood urea contents reported in the literature are given in different units of measurement. Table 7 gives a brief summary of these results as well as the corresponding values expressed in mmol/l. Our values are in the same range as those reported in the literature. There were significant differences ($P< 0.001$), however, between the treatments in our experiment (Table 2).

The blood urea content also depends on:

- the potential rate of protein gain (breed, sex, age),
- the protein quality (the composition of the amino acids),
- the level of protein intake
- the energy intake.

In the present experiment all these factors were standardized by assessing feed intake on a unit metabolic body weight basis. The blood urea content of intact pigs and in PVTC-cannulated pigs increased with time. This is possibly related to N-retention and growth intensity which decreased with time (Köhler et al., 1991). In IRA pigs the blood urea content during the 6th week after surgery was higher ($P< 0.001$) than that in intact or PVTC-cannulated pigs. These findings are in accordance with both the reduced N-retention and growth in the IRA-pigs. Rerat (1986) concluded that in intact pigs

the amount of urea which is taken up by the intestinal tract and excreted as microbial protein in the faeces was almost similar to the urinary excretion of urea. This recycled urea is excreted as microbial protein in the faeces. For pigs fitted with an IRA urea excretion as microbial protein is assumed

Table 6. Creatinine content in blood of pigs

mg/l	µmol/l	Reference	
0.90 - 1.30	80 - 100	Wrogemann and Holtz*	1972
2.25 - 2.37	199 - 209	Tumbleson et al.*	1972
1.59 - 1.80	141 - 159	Steger†	1976
1.15	102	Gregor*	1979
1.60 - 1.66	142 - 147	Witkowska†	1979
	111 - 150	Present study†	

* Mini pigs, † Growing pigs

Table 7. Urea content in blood of pigs

mg/l	mmol/l	Reference	
120 - 133	2.00 - 2.22	Tumbleson et al.*	1972
268 - 377	4.47 - 5.62	Steger et al.	1976
107 - 387 (Urea N)	3.83 - 13.83	Gregor*	1979
	4.50 - 6.20	Bolduan and Schneider	1986
300 - 409	5.00 - 6.82	Mosenthin et al.	1988
	4.20 - 6.35	Münchow et al.	1989
277.7	4.63	Wittenburg et al.	1989
	3.66 - 8.43	Present study	

* Mini pigs

impossible. Unless there is compensatory fermentation in the distal ileum or in the rectum, or both. Urea could also be excreted as urea with the ileal digesta, because Mosenthin et al. (1988) reported a higher urea concentration in ileal digesta after intravenous infusion of urea. Additional findings (Köhler, Verstegen, Mosenthin; unpublished results) showed that the N content in digesta from IRA-

gher than that in the ileal digesta of the PVTC-pigs. Thus, the composition of ileal digesta -animals is not similar to that of PVTC-animals. Also the amounts of volatile fatty acids iopimelic acid (DAPA) in the digesta of IRA-pigs were increased and were higher (P< 0.05 01 respectively) than those in PVTC-animals (Köhler et al., 1991). This shows that there d microbial activity in the ileum or rectum, or both, in IRA-pigs. As a consequence more e excreted because some urea may have been utilized by micro-organism. This could also e higher (P< 0.001) concentration of N in the digesta of the IRA-animals compared with -pigs. It may indicate a compensatory change of function in the ileum or rectum, or both, -animals. A compensatory change in digestive function in IRA-pigs was also suggested by tained for the dry matter contents of the digesta. The ileal dry matter content for IRA- /as higher (P< 0.001) than that of ileal digesta of PVTC-pigs (Köhler et al., 1992). In to these findings the larger (P< 0.001) circumference of the rectum (Table 5) and the digesta outflow (Köhler et al., 1991) indicate an increased transit time for digesta in the his may result in extra water absorption.

Sodium contents in urine (mg/l) 5 and 11 weeks post-surgery in pigs fitted with ileo-rectal anastomosis (IRA) or post-valve T-caecum cannulae (PVTC) and in intact pigs (INT)

	Treatment			
	IRA	*PVTC*	*INT*	*SEM*†
p.s.‡	15.00[a]	371.60[b]	416.00[b]	17.70
p.s.	12.38[a]	336.00[b]	406.83[c]	17.67

rd error of the means ‡ post-surgery

n values in the same row with unlike superscript letters were significantly different P< 0.001

cantara et al. (1980) reported that pigs fed on a low Na diet had a low blood Na concentration had an increased blood K concentration. There are reports that the ileal digestibility of Na ive and that Na is absorbed in the large intestine (Drochner, 1982; den Hartog et al., 1988; ge, 1978; Partridge et al.,1986). In IRA-pigs in the absence of absorption from the large

0.001 and P< 0.01) Na concentration in the blood of IRA-pigs at 6 and 13 weeks after surgery respectively (Table 2). In addition the blood K concentration of IRA-pigs was higher that those of the other treatments. Hyperkalaemia has been reported as a typical manifestation of Na deficiency (Black, 1960). Our findings in IRA-animals show the same tendences as those reported by Alcantara et al. (1980) who fed low-Na diets.

Münchow et al.(1989) reported serum Cl concentrations in the range 97.0 - 104.0 mmol/l for pigs fed on different diets. In accordance with these values our findings (91.9 - 99.9 mmol/l) were in the same range and they did not differ between treatments.

Hennig et al.(1986, 1988a) reported Ca concentrations in serum of intact and anastomized pigs in the range 2.59 - 2.94 mmol/l and Münchow et al.(1989) 2.52 - 2.73 mmol Ca /l serum. In the present experiment similar Ca concentrations were found. We found no differences between treatments.

For Mg there were no differences between the groups and the results were comparable with values reported in the literature. Nuoranne (1983) reported a normal post-absorptive Mg concentration of 0.80 - 0.96 mmol/l serum, this is in accordance with our values (0.83 - 0.90 mmol/l).

The IRA-technique had a considerable effect of on the base excess and the bicarbonate concentration; both variables were lower (P< 0.01 and P< 0.001 respectively) in pigs provided with an IRA. Patience et al.(1987) reported that reducing the dietary electrolyte balance decreased the serum pH and bicarbonate concentration. In accordance with these findings IRA-animals had a lower (P< 0.01) blood pH 13 weeks after surgery. Based on these findings a metabolic acidosis as the result of a reduced electrolyte balance can be expected for IRA-pigs. Findings for PVTC-cannulated pigs were similar to those for intact pigs.

In IRA-animals a significantly lower growth and N retention were observed (Köhler et al., 1991). Growth, N-retention and protein utilization depend on the level of energy intake. Na is an important element in the active absorption of different nutrients . Therefore, negative absorption of Na may be detrimental to the rate of gain. To compensate for the negative Na balance the IRA-pigs were supplied orally with extra electrolyte solution mixture, as described by Hennig et al.(1986). In addition, these authors proposed a sugar/starch supplement for IRA-animals to compensate for the absence of energy absorption from the large intestine. They reported similar N utilization and N balance in IRA-pigs and in intact pigs (Hennig, 1988). In the present investigation an electrolyte solution mixture, as described by Hennig et al.(1986), was added to the diet (200 ml/20 kg live weight

Table 9. Sodium contents in ileal digesta (g/100 g DM) 3, 9 and 12 weeks post-surgery in pigs fitted with ileo-rectal anastomosis (IRA) or post-valve T-caecum cannulae (PVTC)

Time	*Treatment*		
	IRA	*PVTC*	*SEM*†
3 weeks p.s.‡	1.02[a]	3.00[b]	0.07
9 weeks p.s.	0.96[a]	2.84[b]	0.06
12 weeks p.s.	0.95[a]	3.19[b]	0.04

† Standard error of the mean ‡ post-surgery

[ab] Mean values in the same row with inlike superscript letter were significantly different P< 0.001

balance in IRA-pigs and in intact pigs (Hennig, 1988). In the present investigation an electrolyte solution mixture, as described by Hennig et al.(1986), was added to the diet (200 ml/20 kg live weight per d), but the sugar/starch supplementwas not provided. Our findings showed that the addition of the electrolyte solution to the diet of the IRA-pigs was not sufficient to achieve the same level of N retention as intact or PVTC-cannulated animals. In IRA-pigs Na metabolism in the absence of the large intestine was different from that of the other groups (Table 3). Faecal Na digestibility of the intact and PVTC cannulated pigs ranged from 61.4 to 82.6 %. In IRA-pigs total Na digestibility was 23.0 and -10.9 %. Clarke et al. (1967) reported chronic Na depletion in humans fitted with an ileostomy. Their investigation by means of intravenous saline (9 g sodium chloride/l) infusion revealed an intestinal adaptation to conserve Na. Thus, after Na infusion Na concentration in ileal digesta increased and K concentration decreased. This post-infusion response suggests Na conservation in exchange for K. In our experiment Na loss in the urine was lowest in IRA-pigs. To compensate for the absence of sodium absorption in the large intestine of IRA-pigs the urinary Na loss was about thirty times lower than that in intact pigs (Table 8). In humans Clarke et al. (1967) concluded that intestinal adaptation is mediated by aldosterone. Increased aldosterone secretion may result in compensatory growth of the adrenal glands. In addition, kidney weights may have increased as the result of increased Na re-absorption. Table 5 shows that in IRA-animals weights of kidneys (P> 0.05) and adrenal glands were higher (P< 0.001) than those in PVTC- or intact animals. Values for PVTC-animals were similar to those for intact animals. The Na content of digesta from IRA-pigs

information indicates that animals fitted with an IRA may have altered Na metabolism. It seems that these animals have a hydro-electolyte disturbance due to the bypass of the colon.

For K a similar pattern was found (Table 4). The retention and the faecak:urinary K excretion of PVTC cannulated pigs was comparable to that of intact pigs. There was a considerable change in these variables for anastomized animals. Thus, IRA-pigs compensate for the absence of K absorption in the large intestine by reducing urinary excretion. The patterns of both Na and K balances demonstrate the important role of the large intestine in mineral absorption and were in agreement with the findings of Clarke et al.(1967).

In conclusion the findings of our experiment suggest that PVTC cannulation has no effect on the mineral balances, organ weights and blood variables measured. Differences between PVTC-pigs and intact pigs with regard to blood urea concentration suggested an effect of digesta collection on this variable. Further studies are necessary to investigate the effect of digesta collection on urea metabolism in PVTC-pigs. For IRA-pigs the findings suggest that Na supplementation as carried out in the present experiment was insufficient to compensate for the absence of mineral absorption from the large intestine.

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Chapter III

Digestibility measurements in pigs by using post-valve T-caecum cannulation or end-to-side ileo-rectal anastomosis

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Digestibility measurements in pigs by using post-valve T-caecum cannulation or end-to-side ileo-rectal anastomosis

Abstract

Digestibility measurement were carried out with pigs (30-90 kg LW) fitted with post-valve T-caecum (PVTC) cannulas or prepared with ileo-rectal anastomosis (IRA) at 3, 9 and 12 weeks p.s. The apparent ileal digestibilities of dry matter (DM), nitrogen (N), amino acids (AA), sodium (Na) and potassium (K) were determined. In addition, the concentration of DM, the recoveries of N, Na and K in the ileal digesta (per kg DM intake) and the AA composition in digesta were determined. The concentration of DM was higher ($P < .001$) in digesta from IRA-pigs than of PVTC-pigs. With the exception of arginine, lysine, valine and glutamic acid the amino acid composition was different between treatments ($P < .05$ - $P < .001$). With the exception of N, lysine, threonine, valine, aspartic acid, glutamic acid, glycine, proline, and serine the digestibilities were different. Digestibilities of DM and cystine were higher ($P < .01$ - $P < .001$) and digestibilities of arginine, histidine, isoleucine, leucine, methionine, phenylalanine, alanine and tyrosine lower ($P < .05$ - $P < .001$) in IRA- than in PVTC-pigs. Except for lysine and Na the digestibilities in PVTC-pigs were similar from week 3 until week 12 post-surgery. In IRA-pigs except for histidine, lysine, methionine, alanine and proline the digestibilities increased with time. While the digestibility of lysine decreased from wk 3 to 12 p.s., the methionine digestibility remained similar during the experimental period. The apparent Na digestibility was significantly higher ($P < .001$) in IRA-pigs (-8 to-29% vs -630 to-700%); the potassium digestibility was clearly ($P < .001$) lower in IRA- than in PVTC-pigs (26 - 37% vs 60 - 64%). In conclusion, the PVTC cannulation technique can be proposed as an alternative collection technique for ileal digesta in pigs. The IRA technique as applied in this study gives less consistent results. Results obtained with end-to-side IRA-pigs indicate intestinal adaptation as a result of the absence of digestion in the hind-gut.

Introduction

Digestibility measurements at the terminal ileum of pigs are thought to be more appropriate than faecal measurement (Zebrowska, 1973). Therefore different techniques have been developed during the last two decades to collect ileal digesta. The method most commonly used is via collection of digesta from a simple T-cannula, usually placed 5 to 10 cm anterior to the ileo-caecal valve. There are several concerns with this approach which include the question of obtaining representative samples and possible shortcomings of the digestibility markers. In addition, factors, such as the

internal diameter of the cannula, the fibre content of the diet, dry matter content and viscosity of the digesta should also be taken into account (Sauer and Ozimek, 1986). Because of these uncertainties studies with pigs fitted with re-entrant cannulas, either as ileo-ileal re-entrant or ileo-caecal re-entrant cannulas, are often preferred depending on the objectives and experimental conditions under which the studies are to be carried out. However, studies with pigs fitted with re-entrant cannulas are often hampered by problems that result from blockage of digesta; the extent to which this occurs increases with increasing particle size, crude fibre content, feed intake and factors that increase the viscosity of the digesta. A more recent technique for total collection of ileal digesta is the ileo-colic post-valve procedure which was developed by Darcy et al. (1980). This method maintains the integrity of the ileum and preserves the functional role of the ileo-caecal sphincter. The post-valve T-caecum (PVTC) cannulation technique, described by Van Leeuwen et al. (1988), and the various ileo-rectal anastomosis (IRA) techniques, (Fuller and Livingstone, 1982; Picard et al.,1984; Darcy-Vrillon and Laplace, 1985; Souffrant et al., 1985; Green, 1988), represent the most recent approaches for measurement of ileal digestibilities. These techniques were evaluated in a comparative study to determine their effects on growth performance, intestinal adaptation, blood parameters and mineral balance (Köhler et al.,1991 a and b). The objective of the present study was to determine the effect of the PVTC- and IRA-techniques on ileal digestibility at 3, 9 and 12 weeks (wk) post-surgery (p.s.).

Materials and Methods

Animals

16 barrows (Yorkshire X Dutch Landrace), average initial body weight of 30 kg, were used. Nine pigs were surgically modified according to the IRA-technique as described by Köhler et al. (1991a). Seven animals were fitted with a PVTC cannula according to procedures adapted from Van Leeuwen et al. (1991). The housing facilities, collection procedures and a detailed description of the experimental design were previously provided (Köhler et al. 1991a).

Diet

Following a recuperation period of two weeks the animals were fed twice daily at 08.00 and 20.00, equal amounts of the experimental diet. The total feed intake was 2.4 times the maintenance requirement of energy (2.4 x 420 kJ ME/$kg^{0.75}$; ARC, 1981). The formulation of the experimental diet is presented in tables 1. The chemical composition is given in table 2. Chromic oxide was included in the diet as digestibility marker. Water was administered with the feed at a ratio of 2.5:1. To prevent a mineral deficiency caused by the absence of hind-gut absorption, the IRA-pigs were supplied with an electrolyte solution (10ml kg LW^{-1}) as described by Hennig et al.(1986).

Chemical and statistical analysis

The digesta samples were pooled within animal and collection period prior to chemical analyses. Analyses for dry matter and nitrogen were carried out according to ISO 6496 (1983) and ISO 5983 (1979) methods. Amino acids, with the exception of methionine and cystine, were determined after acid hydrolysis (6N HCl for 22 h at 100^{0}C) according to Slump (1969). Methionine and cystine were determined as methionine sulfone and cysteic acid after oxidation with performic acid (Moore,1963). The oxidized samples were hydrolysed in the same manner as the samples that were not oxidized. The amino acid composition of the hydrolysate was determined using an automatic amino acid analyser (Biotronic LC 5001). Sodium and potassium were determined by atomic absorption according to NEN 3349 (1984). Chromic oxide was analysed as described by Bosch et al. (1988). Analyses for all parameters measured were carried out in duplicate. Differences among treatment parameters were tested according to the GLM procedure using SAS (1990).

TABLE 1. Composition of the experimental diet (%)

Ingredients	
Maize	32.95
Barley	6.00
Wheat	10.50
Soybean meal	22.50
Molasses	4.00
Potato pulp	9.20
Beet pulp	10.00
Animal fat	1.00
L-lysine HCL	0.18
DL-Methionine	0.12
Premix*	1.00
$Ca(H_2PO_4)*H_2O$	1.50
$CaCO_3$	0.50
NaCl	0.30
Cr_2O_3	0.25

* Contributed the following vitamin and mineral sources per kg of diet: retinol, 9000IU; cholecalciferol, 1800IU; α-tocopherol, 40mg; menadione, 3mg; ribovlavin, 5mg; cobalamine, 40μg; nicotinic acid, 30mg; D-pantothenic acid, 12mg; choline chloride, 150mg; ascorbine acid, 50mg; KJ, 500μg; $CoSO_4*7H_2O$, 2.5mg; Na_2SeO_3, 0.2mg; $FeSO_4*7H_2O$, 400mg; $CuSO_4*5H_2O$, 100mg; MnO_2, 70mg; $ZnSO_4*H_2O$, 200mg. This mixture also supplied 20mg virginiamycin to the diet.

TABLE 2. Chemical composition of the experimental diet (g/kg DM)

Crude protein	199.00
Gross energy	18.04 MJ kg^{-1} DM
Sodium	1.60
Potassium	13.00
Amino acids	
indispensable	
arginine	11.4
histidine	7.1
isoleucine	8.9
leucine	16.7
lysine	10.9
methionine	4.9
phenylalanine	9.9
threonine	7.9
valine	10.7
dispensable	
alanine	9.8
aspartic acid	20.1
cystine	4.3
glutamic acid	34.2
glycine	8.2
proline	14.4
serine	10.7
tyrosine	7.4

Results

The concentration of dry matter and the mean recovery of nitrogen, sodium and potassium (per kg dry matter intake) in digesta collected from IRA- and PVTC-cannulated pigs (3, 9 and 12 weeks p.s.) are presented in table 3. Since digesta collection was not quantitative (per period five days 12h d^{-1}) both the recoveries and the digestibilities of the nutrients were calculated on the basis of the Cr-recovery. This was on average 92.7% and 71.4% in IRA- and PVTC-pigs, respectively. Dry matter concentration in digesta collected from IRA-pigs was significantly ($P < .001$) higher than in PVTC-pigs from wk 3 to 12 p.s. Within both treatments this parameter was similar for the duration of the experiment. Three and nine wk p.s. the nitrogen recovery per kg dry matter intake tended to be higher ($P > .05$) in IRA- than in PVTC-pigs. However, 12 wk p.s. the nitrogen recovery was similar in both treatments. In all three collection periods, the values observed for sodium recovery were significantly ($P < .001$) lower and for potassium considerably ($P < .001$) higher in digesta of IRA- than of PVTC-pigs. A significant influence ($P < .05$ - $P < .001$) of collection period was observed in the

IRA-pigs for nitrogen, sodium and potassium, which means that the recoveries of these parameters decreased as the experiment continued. A period effect was observed for the sodium recovery in digesta collected from PVTC-pigs and which increased (P< .05) from wk 9 to 12.

Table 3. Dry matter concentration and the recovery (g/kg dry matter intake) of nitrogen, sodium and potassium in ileal digesta of pigs fitted with ileo-rectal anastomosis (IRA) or post-valve T-caecum (PVTC) cannulae at 3, 9 and 12 weeks post-surgery

	3 weeks p.s.[a]		*9 weeks p.s.*		*12 weeks p.s.*			*significance*[b]		
								treatment	period	
	IRA	*PVTC*	*IRA*	*PVTC*	*IRA*	*PVTC*	*SEM*[c]		*IRA*	*PVTC*
Dry matter	10.17	8.44	10.67	8.85	10.86	8.79	0.109	***		
Nitrogen	10.68	9.65	10.47	9.55	8.78	9.00	0.448		***	
Sodium	3.62	11.77	3.48	11.64	2.85	12.71	0.321	***	**	*
Potassium	10.16	5.08	10.03	5.47	8.62	4.89	0.519	***	*	

[a] post-surgery

[b] *= P< .05; **= P< .01; *** = P < .001

[c] standard error of the mean

The amino acid composition of ileal digesta collected from IRA- or PVTC-pigs is shown in table 4. With the exception of arginine, lysine, valine and glutamic acid the proportions of the amino acids were different (P< .05 - P< .001) between treatments. In IRA-pigs the results observed for threonine, aspartic acid, cystine, glycine, proline and serine were lower than in PVTC-pigs. Results calculated for the proportion of histidine, isoleucine, leucine, methionine, phenylalanine, alanine and tyrosine were higher in digesta of IRA- than in digesta of PVTC-pigs. The percentage of lysine increased in both treatments during the experiment (P< .001), whereas the percentage of threonine and serine decreased with time (P< .05 - P< .001). A significant influence of the collection period was found in IRA-pigs also for isoleucine, methionine, alanine and glycine (P< .01) and for leucine and tyrosine (P< .05) in PVTC-pigs.

Table 4. Amino acid composition in ileal digesta of pigs with ileo-rectal anastomosis (IRA) or post-valve T-caecum (PVTC) cannula at 3, 9 and 12 weeks post-surgery (% of total amino acids)

	3 weeks p.s.[a]		*9 weeks p.s.*		*12 weeks p.s.*			*significance*[b]		
								treatment	*period*	
	IRA	*PVTC*	*IRA*	*PVTC*	*IRA*	*PVTC*	*SEM*[c]		*IRA*	*PVTC*
Arginine	4.21	3.96	4.43	4.31	4.33	4.16	0.095			
Histidine	5.66	5.30	5.36	5.19	5.55	5.03	0.076	***		
Isoleucine	4.59	4.31	4.79	4.37	4.89	4.26	0.048	***	*	
Leucine	7.41	7.13	7.53	7.04	7.43	6.76	0.052	***		*
Lysine	4.38	4.38	4.48	4.76	5.68	5.36	0.124		***	***
Methionine	1.65	1.42	1.87	1.29	1.90	1.39	0.038	***	**	
Phenylalanine	4.55	4.41	4.69	4.36	4.63	4.44	0.033	***		
Threonine	5.56	5.97	5.50	5.92	5.22	5.66	0.045	***	***	**
Valine	6.29	6.33	6.45	6.35	6.28	6.17	0.042			
Alanine	6.77	6.32	6.84	6.22	7.33	6.38	0.080	***	**	*
Aspartic acid	11.35	11.67	11.76	11.83	11.11	11.80	0.115	*		
Cystine	3.30	3.90	3.10	3.56	3.17	3.68	0.058	***		
Glutamic acid	13.62	13.41	13.34	13.68	13.27	13.90	0.133			
Glycine	6.05	6.57	6.06	6.42	5.78	6.50	0.057	***	**	
Proline	5.27	5.20	4.62	6.02	4.52	5.28	0.191	*		
Serine	5.47	5.90	5.33	5.84	5.07	5.66	0.050	***	**	*
Tyrosine	3.87	3.82	3.89	3.59	3.86	3.57	0.033	***		*

[a] post-surgery

[b] * = P <.05; ** = P <.01; *** = P <.001

[c] standard error of the mean

The apparent ileal digestibilities of dry matter, nitrogen, amino acids, sodium and potassium are presented in table 5. Dry matter digestibility was higher (P< .001) in IRA- than in PVTC-pigs.

Table 5. Apparent digestibility of dry matter, nitrogen, sodium and potassium in pigs with ileo-rectal anastomosis (IRA) or post-valve T-caecum (PVTC) cannula, at 3, 9 and 12 weeks post-surgery.

	3 weeks p.s.[a]		9 weeks p.s.		12 weeks p.s.			significance[b]		
								treatment	period	
	IRA	PVTC	IRA	PVTC	IRA	PVTC	SEM[c]		IRA	PVTC
Dry matter	64.3	60.8	64.1	59.7	69.9	60.8	0.649	***	***	
Nitrogen	65.7	69.0	66.4	69.3	72.3	71.6	0.721		***	
Arginine	82.2	85.1	82.1	83.2	84.3	84.6	0.463	*	*	
Histidine	74.8	79.0	77.2	80.6	78.7	80.5	0.575	***		
Isoleucine	75.1	79.2	75.2	78.1	77.2	79.8	0.451	***	*	
Leucine	78.5	81.6	79.1	81.2	81.5	82.9	0.493	**	**	
Lysine	70.0	73.5	70.9	70.3	66.9	68.2	0.733		*	*
Methionine	83.5	87.4	82.3	88.1	83.8	87.9	0.425	***		
Phenylalanine	77.8	80.9	78.1	80.5	80.6	81.1	0.413	**	**	
Threonine	66.1	67.6	67.9	66.9	72.7	70.0	0.763		***	
Valine	71.6	74.5	72.1	73.6	75.7	75.7	0.560		**	
Alanine	66.6	72.1	67.5	73.1	68.9	72.5	0.637	***		
Aspartic acid	72.8	75.1	73.0	73.9	77.2	75.3	0.548		**	
Cystine	62.9	61.2	66.6	63.2	69.5	64.0	0.842	**	**	
Glutamic acid	80.9	83.2	82.0	82.2	83.9	82.9	0.401		*	
Glycine	64.3	65.6	65.8	65.2	70.7	66.6	0.745		**	
Proline	82.5	84.5	85.1	81.3	86.8	84.5	0.744			
Serine	75.3	76.3	76.9	75.7	80.3	77.7	0.583		**	
Tyrosine	74.7	77.7	75.6	78.3	78.3	79.6	0.501	**	**	
ΣAAS	75.5	78.2	76.6	77.5	79.0	78.6	0.459		**	
Sodium	-23.0	-640.4	-29.1	-632.2	-8.1	-699.6	8.559	***	*	*
Potassium	25.7	62.8	26.6	59.8	36.9	64.0	1.818	***	*	

[a] post-surgery; [b] *=P< .05; **=P< .01; ***=P< .001, [c] standard error of the mean

While in PVTC-pigs this parameter was similar from wk 3 to 12, in IRA-pigs it increased (P< .001) with time. Nitrogen digestibility measured in PVTC-pigs was similar to IRA-pigs. Nevertheless, the effect of period in IRA-pigs was significant (P< .001). This means that the Nitrogen digestibility in IRA- was lower than in PVTC-pigs 3 and 9 weeks p.s. and similar in week 12 p.s. With the exception of cystine amino acid digestibilities measured in IRA-pigs were lower than in PVTC-pigs 3 wk p.s. Amino acid digestibilities measured in IRA-pigs were slightly increased or in the same range at 9 weeks p.s. compared to 3 weeks p.s. The digestibility of methionine at wk 9 was lower than at 3 wk p.s. Except for lysine amino acid digestibilities measured in digesta of PVTC-pigs were similar at wk 3 and 9 p.s. With the exception of lysine the digestibilities of the indispensable amino acids increased in IRA-pigs from wk 9 to 12 p.s. Apparent ileal digestibility of lysine measured in IRA-pigs decreased from wk 9 to 12. Digestibilities of the indispensable amino acids measured in digesta of PVTC-pigs at wk 12 p.s. were higher than measured in digesta of IRA-pigs with the exception of threonine. The dispensable amino acid digestibilities were higher in IRA- than in PVTC-pigs with the exception of alanine and tyrosine. In IRA-pigs the effect of collection period on the digestibility of each amino acid was reflected by an increased (P<.01) digestibility for the total amount of amino acids from wk 3 to 12 p.s. Digestibility of the total amino acids in digesta of PVTC-pigs was similar in all three periods.

The sodium digestibility in IRA-pigs was slightly negativ. In PVTC-pigs, the ileal digestibility ranged from -632 to -700%. In IRA-pigs, the sodium digestibility increased towards the end of the experiment. In PVTC-pigs, the sodium digestibility decreased with time. Potassium digestibility in IRA-pigs was lower (P<.001) than in PVTC-pigs. While the ileal digestibility of potassium in PVTC-pigs was similar during the experimental period, it increased in IRA-pigs with time. The collection period affected sodium digestibility in PVTC-pigs. In IRA-pigs, sodium and potassium digestibilities changed with time.

Discussion

IRA has been recommended as a relatively new technique for measurement of ileal protein and amino acid digestibilities in pigs (Picard et al., 1984; Darcy-Vrillon & Laplace, 1985; Souffrant et al., 1985; Green, 1987). Nevertherless, a previous paper (Köhler et al., 1991a) reported an increased bacterial activity in digesta of IRA-pigs. The level of bacterial activity, i.e. the fermentation of fermentable substrates such as carbohydrates and the subsequent synthesis of bacterial cell mass, may affect the composition of digesta and thus digestibility. Darcy-Vrillon and Laplace (1990) compared the ileal digestibilities of dry matter, nitrogen and amino acids in pigs prepared with end-to-end ileo-rectal anastomosis or fitted with ileo-colic post-valve (ICPV) cannulas. Four weeks p.s. except for

proline, phenylalanine, cystine and methionine the digestibilities were significantly lower in IRA than in ICPV-pigs fed a beet pulp diet. Leterme et al. (1990) compared the apparent ileal digestibility of nitrogen and amino acids in end-to-end IRA-pigs and in pigs fitted with a simple T-cannula. They reported that except for tryptophan and glycine, the digestibilities were lower in IRA-pigs. Differences, however, were significant only for methionine, tryptophan and proline. As in the studies by Darcy-Vrillon and Laplace (1990), digestibility measurements by Leterme et al. (1990) were carried out about 4 weeks p.s. Digestibilities obtained in the present study indicate a significant change in amino acid digestibility throughout the 12 wk experimental period. Apart from cystine digestibility at 3 weeks p.s., values were lower in IRA- than in PVTC-pigs which is in agreement with reports in the literature (Darcy-Vrillon and Laplace; 1990, Leterme et al., 1990). Amino acid concentration (% in dry matter) in digesta of IRA-pigs increased from wk 3 to 12 p.s., which was associated with an increased dry matter digestibility. Except for methionine and lysine, these changes resulted in an increased amino acid digestibility in IRA-pigs from wk 3 to 12 p.s. In a previous study Köhler et al. (1991a) found a significantly higher concentration of diaminopimelic acid and volatile fatty acids in digesta of IRA- compared to PVTC-pigs. The increased dry matter digestibility in IRA-pigs may be due to an increase in processes of fermentation. Figure I presents differences for the recoveries of nitrogen and amino acids, per kg dry matter intake, in digesta of IRA- and PVTC-pigs. Recoveries measured in digesta of PVTC-pigs are indicated as 0 values. Bars above zero show higher recoveries while bars below zero show lower recoveries in digesta of IRA-pigs than in digesta of PVTC-pigs. With the exception of cystine, threonine, serine, proline and glycine the recoveries of all amino acids and nitrogen were higher in IRA- than in PVTC-pigs. This is not in accordance with a similar amino acid digestibility in IRA- and PVTC-pigs up to the terminal ileum. This may be explained by increased endogenous protein losses in IRA-pigs. A relatively large disappearance of cystine, threonine, serine, proline and glycine has been reported in the large intestine by several authors (e.g. Zebrowska and Buraczewski, 1977; Wünsche et al., 1979; Tanksley and Knabe, 1984; Sauer and Ozimek, 1986; Sauer et al., 1991). Sauer et al. (1991) postulated that this large disappearance of these amino acids in the hind-gut will result from a preferentially bacterial breakdown of non-digested endogenous protein. Taking into account the increased level of fermentation in digesta of IRA-pigs, this would explain the lower recoveries of these amino acids in digesta of these pigs. An increased bacterial activity in digesta of IRA-pigs may also explain the fact that in IRA-pigs methionine digestibility was similar from wk 3 to 12 p.s. since different authors (Sauer et al.,1982; Knabe et al.,1989) reported a net synthesis of methionine by microorganism in the hind-gut. Ammonia as the major endproduct of bacterial activity may be excreted with urine; an increased ($P< .001$ -$P< .05$) urinary nitrogen excretion was found in IRA-pigs (Köhler et al.,1991a).

The apparent ileal digestibility of lysine decreased with time ($P< .05$) in both treatments. These

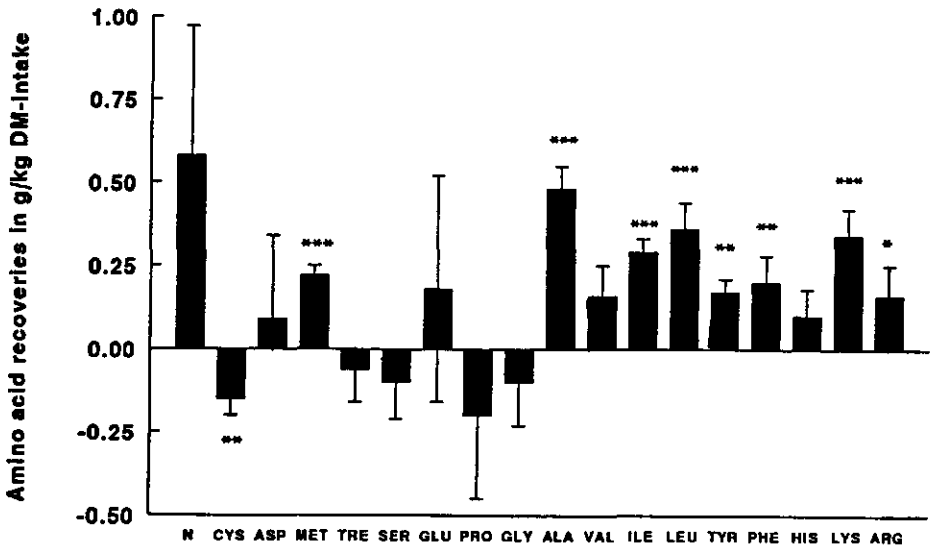

Fig. I. Differences in nitrogen and amino acid recoveries in digesta of PVTC- and IRA-pigs†

* = P< 0.05, ** = P< 0.01, *** = P< 0.001

† Recoveries measured in digesta of PVTC-pigs are indicated as 0 values. Bars above zero show higher recoveries while bars below zero show lower recoveries in digesta of IRA-pigs than in digesta of PVTC-pigs.

results are remarkable since lysine is often the 1st limiting amino acid in diets for pigs. A decreased apparent digestibility of lysine may be related to an increased endogenous lysine secretion. Nevertheless, in the literature no results were reported about the effect of age on the apparent ileal amino acid digestibility from 30-90 kg live weight measured in the same diet.

The recoveries of sodium and potassium in digesta of IRA- and PVTC-pigs (Table 3) demonstrate the importance of the large intestine in mineral absorption. For sodium a negative ileal digestibility was also reported by other investigators (Drochner, 1984; Den Hartog et al., 1988; Partridge, 1986). A sodium recovery of about 2.9 - 3.6 g per kg DM intake, which was found in digesta of IRA- compared to 11.6 - 12.7 g in PVTC-pigs can be explained by intestinal adaptation in IRA-pigs in order to conserve sodium. These findings are in agreement with the much lower (P<

absortion of sodium by the kidneys and an increased sodium absorption at the distal colon (rectum) can be attributed to increased aldosterone activity (Rechkemmer, 1990) this agrees with the higher (P< .001) weight of the adrenal glands which was found in IRA-pigs (Köhler et al. 1991b). The low sodium recovery in digesta of IRA-pigs resulted in a digestibility of -8% to -30% whereas in PVTC-pigs these values ranged between -650 and -700%, which is in agreement with results reported in literature previously referred. The potassium recovery in the digesta of IRA pigs was higher (P< .001) than in digesta of PVTC pigs in all three periods. These results are in agreement with findings reported by Heitzmann & Drochner (1990). These authors reported an increased potassium and decreased sodium concentration in ileal digesta when pigs were fed a diet with a low sodium content. The different recoveries of sodium and potassium in digesta of IRA- and PVTC-pigs resulted in significantly (P< .001) higher sodium and lower (P< .001) potassium digestibilities in IRA- than in PVTC-pigs. Sodium and potassium digestibilities measured in PVTC animals were similar to results reported in literature when pigs were fed different diets (Partridge et al.,1986; Den Hartog et al., 1988).

Conclusion

The results of the present study indicate an influence of the absence of hind-gut digestion on digestibility measurements of dry matter, different amino acids, sodium and potassium in IRA-pigs. Moreover, the intestinal adaptation in IRA-pigs resulted in a change in digestibilities during the experimental period. Results measured in PVTC-pigs were similar from wk 3 to 12 p.s. It can be concluded that PVTC-cannulation is a more suitable alternative for measuring digestibility at the terminal ileum in pigs than the end-to-side IRA as described in this study.

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Chapter IV

A comparison of different digesta collection methods to determine the apparent digestibilities of the nutrients at the terminal ileumin pigs

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A comparison of different digesta collection methods to determine the apparent digestibilities of the nutrients at the terminal ileum in pigs

Abstract

Three different cannulation techniques in pigs were tested with 15 crossbred castrates. Four pigs were fitted with a simple T-cannula, six pigs with a post-valve T-caecum cannula (PVTC cannula) and five pigs with ileocaecal re-entrant cannulas. Four diets were tested: a conventional diet, a pectin-rich diet, a crude fibre-rich diet and a semisynthetic diet. For quantitative collection of ileal digesta the PVTC cannulation technique was tested for homogeneity of sampling. Cr_2O_3 and TiO_2 were used as solid phase markers and Co-EDTA as a liquid phase marker. The digestibilities of dry matter, nitrogen, crude fibre, ADF and NDF were determined. Digestibilities measured with re-entrant cannula as a quantitative collection method were sometimes significantly higher ($P<0.05$) than with the other cannulas. After correction to 100% Cr recovery the differences were reduced to less than 5%. Nitrogen digestibility measured with the re-entrant cannula was lower than with the PVTC cannula in the fibre-rich and in the semisynthetic diet. The Cr recovery was mostly lower than 100%, except with PVTC cannulas and the semisynthetic diet. The recovery rates of Co were higher than of Cr. In the semisynthetic diet recoveries of Co and Cr were similar. The recovery rate of TiO_2, with the semisynthetic diet, was lower than the other two markers. The recovery rates of the markers depended on fibre contents of diets. With the pectin-rich and fibre-rich diets the Cr and Co recoveries were lower than with the control diet. In general, digestibility coefficients measured with the three different cannulation techniques were different. However, when corrections were made for marker recoveries there were only small differences.

(Key Words: Pigs, Comparison, Cannulation Techniques, Marker.)

Introduction

From a nutritional point of view terminal ileum digestibility is a better measure than faecal digestibility (Zebrowska ,1973). During the last two decades many studies regarding digesta collection have been performed to estimate the digestibility of the nutrients immediately before the ileo-caecal valve. Different methods for digesta collection are used. Most applied methods for quantitative collection are re-entrant cannulation or ileorectal anastomosis. Other methods involve the use of a

simple T-cannula for spot-sampling. These different cannulation techniques, however, each require a different collection method. Therefore results measured in these different ways may not be comparable. A few comparisons of the different methods have been performed to compare the results of these methods (Zebrowska et al.,1978; Taverner et al.,1983; Schröder et al.,1988). A new development is the post-valve T-caecum cannula (PVTC cannula) (v. Leeuwen et al.,1988; v.Leeuwen et al., unpublished). A comparison between the PVTC cannulation method, the re-entrant cannula and the simple T-cannula respectively, may show whether the results are comparable.

Therefore an experiment was designed to 1) compare various cannulation techniques using different collection techniques as they are described in literature as well as the digestibilities of different diets, some of which were extremely high in fibre, 2) to study the application of TiO_2 as a marker for the solid phase in comparison with Cr_2O_3 and with Co-EDTA as a marker for the liquid phase.

Materials and methods

Animals

Fifteen crossbred barrows of about 55 kg (Yorkshire X Norwegian X Dutch Landrace) were used. Five pigs were prepared with a re-entrant cannula as described by van Leeuwen et al. (1987). Four pigs were fitted with a simple T-cannula at the terminal ileum approximately 15 cm anterior to the ileo-caecal valve as described by Just et al. (1985). Six pigs were prepared with a post-valve T-caecum cannula (PVTC cannula) as described by van Leeuwen et al.(1988,1990 unpublished). The PVTC-technique uses the site of the ileo-caecal valve which is normally protruded into the caecum. The caecum is now replaced by a T-cannula. When the cannula is closed the digesta flows directly from the ileum into the colon. When the cannula is open the valve is protruded into the cannula due to overpressure in the abdomen. The digesta now flows directly into the cannula. All cannulas were made of medical tubing of silicon rubber[1]. The internal and external diameters of the T-cannula and re-entrant cannula were 19 and 24 mm, respectively. The PVTC cannulas were made with an internal diameter of 25 mm and an external diameter of 30 mm. The pigs were individually housed in adjustable cages in an environmentally controlled metabolism unit with continuous light and an air temperature in the range of 19 to 21°C. After surgery, the pigs were allowed to recover for three weeks.

[1] Tales, Ommen, The Netherlands

Table 1. Composition of experimental diets

Diet components, %	*Diet*			
	Control diet	*Pectin -rich diet*	*Fibre -rich diet*	*Semi synthetic diet*
Corn	23.6	25.9	23.8	
Barley	19.0	8.0	12.3	
Wheat	30.0	15.0		
Soybean meal	18.4	18.1	18.1	
Molasses	5.0	5.0	5.0	
Potato pulp		10.2		
Beet pulp		10.2		
Apple pectin		4.0		
Oats			15.0	
Oathusk meal			10.0	
Alfalfa			9.0	
Wheat straw meal			3.3	
Soya isolate				22.3
Wheat starch				28.2
Corn starch				28.2
Arbocel B 800[1]				6.0
Soyaoil				4.0
Anim. fat				4.0
L-lysine	0.13	0.19	0.06	
DL-methionine	0.07	0.04	0.07	
Vitamin-trace element mixture[2]	1.00	1.00	1.00	1.00
Mineral mixture[3]	0.37	0.37	0.37	0.37
$CaHPO_4*2H_2O$	1.50	1.44	1.30	
$Ca(H_2PO_4)*H_2O$				2.40
$CaCO_3$	0.97	0.50	0.79	1.00
$KHCO_3$				1.50
Cr_2O_3	0.05	0.05	0.05	0.50
TiO_2				0.50

[1] 100% cellulose

[2] Contributed the following vitamin sources per kg of diet: Retinol, 9000 IU; cholecalciferol,1800 IU; α-tocopherol,40 mg; menadione,3 mg; riboflavin,5 mg; cobalamine,40 μg; nicotinic acid,30 mg; D-pantothenic acid,12 mg; choline chloride,150 mg; ascorbine acid,50 mg; KJ,500 μg; $CoSO_4*7H_2O$ 2.5 mg and Na_2SeO_3. The remainder was up of ground corn

[3] Contributed the following mineral sources per kg of diet: NaCl,3 g; $FeSO_4*7H_2O$,0.40 g; $CuSO_4*5H_2O$,0.1 g; MnO_2,0.07 g; $ZnSO_4*H_2O$, 0.2 g. This mixture also supplied 20 ppm virginiamycin to the diet.

Diets

Four diets were tested. In period 1 a control diet was used whereas in periods 2, 3 and 4 a pectin-rich diet, a crude fibre-rich diet and a semisynthetic diet, respectively, were tested. The composition of the experimental diets and the chemical analysis of the diets are given in Table 1 and 2, respectively.

Table 2. Chemical analysis of the experimental diets (g kg^{-1} DM)

	Diet			
	Control diet	*Pectin -rich diet*	*Fibre -rich diet*	*Semi synthetic diet*
Crude protein	185	167	174	211
Crude fat	30	25	29	90
Crude fibre	35	60	107	48
Ash	64	62	71	65
ADF	50	85	131	58
NDF	136	167	251	63

Each animal received diets 1, 2 and 3. Animals with the re-entrant cannula and the PVTC cannula also received diet 4. Inclusion of Cr_2O_3 as a marker for the solid phase was different between the diets. In diet 4 the amount of Cr_2O_3 was 0.5% whereas the other diets contained only 0.05% Cr_2O_3. In addition, diet 4 contained 0.5% TiO_2 as an alternative marker for the solid phase. As marker for the liquid phase 0.2 g Co-EDTA per pig per day was administered with the feed. All animals were fed at 2.4 times maintenance requirement for energy (ARC,1981) divided into two meals fed daily at 08.00 and 20.00. Water was administered with the feed at a ratio of 2.5:1 (v/v).

Digesta-collection.

The ileal digesta of the pigs with re-entrant cannulas were collected quantitatively on days 1, 3 and 5 of each collection period 24 h day^{-1} as described by Van Leeuwen et al. (1987). For the pigs with T-cannulas, ileal digesta were collected for a total of 24 h per period, from 09.00 to 11.00, 13.00 to 15.00 and 17.00 to 19.00 on day 1 and from 11.00 to 13.00, 15.00 to 17.00 and 19.00 to 21.00 on day

2. This procedure was repeated on days 4 and 5, respectively of each collection period. The collection procedure in pigs with T-cannulas was similar to procedures used by Jørgensen et al. (1984). For the pigs with PVTC cannula the ileal digesta were collected quantitatively during 5 d for 12 h day^{-1} from 09.00 to 21.00.

Chemical and statistical analysis

Prior to chemical analyses digesta samples per animal and per diet were pooled, freeze dried and ground through a 1-mm mesh screen. Analyses of dry matter and nitrogen were done according to ISO 6496 (1983) and ISO 5983 (1979). Crude fibre was analysed as described by NEN 5417 (1985). ADF and NDF were analysed according to Goering and van Soest (1975), chromic oxide and Co as described by Bosch et al. (1988) and TiO_2 content was measured as described by Brandt and Allam (1987). Differences among treatment parameters were tested according to Tukey using SAS (1985).

Results

Digestibility coefficients

The digestibility coefficients of dry matter, crude fibre, ADF and NDF of the different diets and for each method are presented in table 3. With the control, pectin-rich and crude fibre-rich diets, the digestibilities for dry matter measured with re-entrant cannula were higher ($P< 0.05$) than when using the other two methods. Moreover in the crude-fibre-rich diet the digestibilities of crude fibre, ADF and NDF as well as of ADF in the semisynthetic diet determined with the re- entrant cannula were also significantly higher ($P< 0.05$) than measured with the PVTC cannula or the T-cannula (Table 3). There was wide variation in the fibre-rich diet especially measured with the T-cannula and the re-entrant cannula. With the high amount of fibre components, lower digestibility of the nutrients was found. Apart from nitrogen the differences between the digestibilities measured with the PVTC cannula and re-entrant techniques increased. A relatively high digestibility of the fibre components was found with all methods using diets 1, 2 and 3.

Recovery rates of the markers

The recovery of the markers were calculated and expressed per 24 h. Table 4 presents the recovery of the markers for the three experimental groups and for each diet.

Table 3. The apparent ileal digestibilities of dry matter, nitrogen, crude fibre. ADF and NDF for each diet and each collection technique (data for T-cannula and PVTC cannula are calculated to 100% Cr recovery)

	Collection technique					
	PVTC cannula		*T-cannula*		*Re-entrant*	
Control diet						
DM	73.41	±0.32[a]	73.00	±0.54[a]	78.26	±0.95[b]
Nitrogen	79.57	±0.43	78.79	±0.45	80.02	±1.02
Crude fibre	8.36	±2.35	10.11	±1.97	16.55	±3.10
ADF	18.18	±1.74	19.45	±1.29	24.35	±3.20
NDF	30.51	±1.37	31.77	±1.20	35.53	±2.96
Pectin-rich diet						
DM	57.99	±0.32[a]	56.55	±1.98[a]	66.72	±2.48[b]
Nitrogen	69.62	±1.19	70.52	±1.84	73.11	±3.15
Crude fibre	-1.06	±2.00	-1.60	±4.58	10.47	±8.71
ADF	6.88	±1.55	6.43	±3.78	16.10	±6.12
NDF	21.26	±1.19	19.70	±3.68	28.48	±4.86
Crude fibre-rich diet						
DM	57.43	±0.84[a]	54.40	±1.64[a]	66.65	±1.98[b]
Nitrogen	71.63	±1.07	66.09	±3.25	72.86	±2.31
Crude fibre	5.27	±1.20[a]	4.33	±4.92[a]	19.01	±4.43[b]
ADF	5.41	±1.03[a]	5.15	±4.79[a]	20.03	±4.02[b]
NDF	11.93	±1.20[a]	11.15	±4.43[a]	24.11	±3.98[b]
Semisynthetic diet						
DM	86.76	±0.31			87.55	±0.18
Nitrogen	92.35	±0.30			92.23	±0.17
Crude fibre	-1.88	±2.49			4.74	±1.82
ADF	2.03	±2.01[a]			10.04	±1.32[b]
NDF	1.25	±1.34			9.50	±0.72

ab Means (± SE) in the same row with different superscripts differ ($P < .05$)

Cr recovery

With the exception of animals fitted with PVTC cannula and fed the semisynthetic diet, the Cr recovery was lower than 100% for all treatments. A comparison of the recovery rates between the dietary treatments showed lower values with the pectin-rich and the fibre-rich diets than with the control diet. The differences between the cannulation methods were not significant ($P > 0.05$). The re-entrant method showed a lower recovery than the PVTC cannulation technique with the semisynthetic diet ($P < 0.01$). Table 4 shows that the recovery rate of Cr depends on the diet. Diets high in crude-fibre show a low recovery rate of Cr.

Table 4. Recovery of the markers

Diet	*Marker*	*Cannula type*		
		PVTC cannula	*T-cannula*	*Re-entrant*
Control diet	Cr	90.8 ±4.38	90.5 ± 7.32	93.6 ±4.37
	Co	103.5 ±2.74	103.4 ± 4.42	103.5 ±1.75
Pectin-rich diet	Cr	71.9 ±4.17	72.3 ± 9.97	89.2 ±8.85
	Co	84.5 ±4.24	86.4 ±13.95	102.2 ±7.73
Fibre-rich diet	Cr	71.6 ±5.96	63.5 ± 8.82	82.3 ±4.88
	Co	76.8 ±5.08	71.3 ±10.90	86.5 ±4.61
Semisynthetic diet	Cr	106.4 ±2.80 [a]		90.6 ±1.76 [b]
	Co	103.0 ±1.86 [a]		88.0 ±1.22 [b]
	TiO_2	91.2 ±2.11 [a]		80.2 ±1.97 [b]

Means (± SE) in the same row with different superscripts differ ($P < 0.01$)

Co recovery

There was a tendency for the Co recovery to be higher than the Cr recovery with the control, pectin-rich and fibre-rich diets. With PVTC cannulas and fed the pectin-rich diet this difference was significant ($P < 0.05$). Data from Co and Cr recoveries indicate a similar dependence on the amount of fibre. With the semisynthetic diet Co recovery was about the same level as Cr recovery. With the re-entrant cannula the Co and Cr recoveries were lower than with PVTC ($P < 0.01$).

TiO_2 recovery

The recovery rate of TiO_2, determined for the semisynthetic diet only, was less than 100%. This was lower than the recovery rates for the other two markers. The TiO_2 recovery with the re-entrant method, however, was lower compared with the PVTC cannula ($P < 0.01$).

The Influence of the Markers on the Digestibility measurement

The main assumption in the cannulation methods is that the re-entrant cannula is a quantitative

method. The use of a marker will be unnecessary. In the present study the digestibilities measured with the re-entrant cannula were also calculated to 100% Cr recovery. The corrected results for each diet and each method are given in Table 5. In this way the differences between the methods were reduced to less than 3%. The digestibilities for nitrogen and NDF calculated with the re-entrant and with the PVTC cannulation technique differed significantly ($P< 0.05$) in the fibre-rich diet. Differences in nitrogen digestibility ($P< 0.05$) were found in the semisynthetic diet between the re-entrant and the PVTC cannulas.

Cr and Co recoveries found with the PVTC in the pectin-rich diet were different ($P < 0.05$). Recoveries of TiO_2 on one hand and Cr and Co-EDTA on the other hand differed significantly ($P< .05$) between PVTC cannula and re-entrant cannula in the semisynthetic diet. The digestibility of dry matter (calculated to 100% recovery) showed a difference in relation to the markers used (Table 6). The results suggest that, when Co-EDTA was used, the digestibility would be higher than when calculated with Cr. With the semisynthetic diet the recoveries of Cr and Co did not differ. Moreover, even when the digestibility data were calculated to 100 % TiO_2 recovery, they were lower ($P < 0.05$) than with Cr or with Co-EDTA. With the T-cannula the largest variation in recovery data was found.

Discussion

The method of re-entrant cannulation has been considered to be a reliable quantitative method, because the digesta must always follow the artificial connection between the cannulas. This means that 100% marker recovery is assumed. Schröder et al.(1988) reported 97% Cr recovery at the terminal ileum when they used 2 g Cr_2O_3 kg^{-1} diet. Drochner et al.(1984) found 99.7% recovery of Cr when the amount of the marker was 3 g kg^{-1} diet. On this basis digestibilities found with this method have not been corrected to 100% marker recovery. In contrast to this, the present results with the re-entrant cannula and Cr_2O_3 added to the diet showed recoveries that were always considerably below 100 %. It is remarkable that low recoveries were found for all four diets, ranging from the semisynthetic diet to the fibre-rich diet. The difference may be partly due to the relatively small proportion of Cr_2O_3 as well as to difficulties in obtaining representative samples with regard to this marker. During the adaptation period as well as during the collection period of the pectin-rich diet and the crude fibre-rich diet a few animals did not eat the whole ration. The low feed consumption may be caused by a temporary blockage of the fistulas, because we observed more leakage in pigs fed these diets. The differences between the marker recoveries for the solid and the liquid phases showed that there was

Table 5. The apparent ileal digestibilities of dry matter, nitrogen, crude fibre, ADF and NDF for each diet and for each type of cannula (calculated to 100% CR-recovery)

	Collection technique		
	PVTC cannula	*T-cannula*	*Re-entrant*
Control diet			
DM	73.4 ± .32	73.0 ± .54	73.3 ± .57
Nitrogen	79.6 ± .43	78.8 ± .46	78.6 ± .82
Crude fibre	8.4 ±2.34	10.1 ±1.97	10.6 ±1.96
ADF	18.2 ±1.74	19.5 ±1.29	19.1 ±1.09
NDF	30.5 ±1.37	31.8 ±1.20	31.1 ±1.34
Pectin-rich diet			
DM	58.0 ± .55	56.6 ±1.99	57.5 ±1.72
Nitrogen	69.6 ±1.19	70.5 ±1.56	69.9 ±1.84
Crude fibre	-1.0 ±2.00	-1.6 ±4.58	-0.6 ±4.33
ADF	6.9 ±1.55	6.4 ±3.78	5.2 ±4.87
NDF	21.3 ±1.19	19.7 ±3.68	19.2 ±3.71
Crude fibre-rich diet			
DM	57.1 ± .98	54.4 ±1.64	55.0 ± .03
Nitrogen	71.6 ±1.07[a]	66.1 ±3.25	67.1 ±1.15[b]
Crude fibre	5.3 ±1.20	4.3 ±4.92	1.4 ±2.42
ADF	5.4 ±1.03	5.2 ±4.79	2.7 ±2.20
NDF	11.9 ±1.20[a]	11.2 ±4.43	7.7 ±1.17[b]
Semisynthetic diet			
DM	86.8 ± .37		86.2 ± .31
Nitrogen	92.4 ± .30[a]		91.4 ± .28[b]
Crude fibre	-4.5 ±1.71		-5.2 ±1.76
ADF	2.0 ±2.01		0.6 ±2.04
NDF	1.3 ±1.34		0.0 ±1.38

ab Means (± SE) in the same row with different superscripts differ ($P < 0.05$)

a separation between the solid and liquid phases. This indicated that the ratios of these two phases were altered. This may also explain the relatively high digestibility coefficients sometimes found for NDF and ADF.

For Cr and Co the effect of the diets was considerable. With the pectin-rich and fibre-rich diets the Cr and Co recoveries were markedly lower than with the control diet. The Cr recovery was lower than the recovery rate of Co. This was not found with the semisynthetic diet. This may indicate that fibre components have an influence to the marker recoveries. For the diet rich in pectin the difference between the marker recoveries and the PVTC cannulation technique was significant ($P < 0.05$). This suggested that the PVTC cannulation technique cannot always be used as a quantitative

method to collect digesta at the terminal ileum and that it is necessary to use a marker. Moreover, the digestibility of crude fibre in the pectin-rich and the semisynthetic diets as measured with the PVTC cannula were nearly zero. However, in thecontrol diet the digestibility of crude fibre was about 8%. This variation between diets is less than that observed when the simple T-cannula is used. This suggested that the rate of marker recovery obtained with the PVTC cannula varies less than with the T-cannula. This may be explained by the fact that with the PVTC cannula the digesta will be pressed into the cannula with the normal ileal overpressure. It is possible that a blockage may occurs when the animal is lying on the cannula, which causes a counter-pressure in the cannula.

With the T-cannula we found somewhat lower Cr recovery than with the re-entrant cannula. The data also show that the variation in the results measured with the T-cannula was larger than when using the other methods. Likewise Co recovery was always higher than Cr recovery. This observation and the high variation within treatments point to a reduced homogeneity of the samples with the T-cannula. Schröder et al. (1988) suggested that this is caused by a change in pressure at the base of the cannula when it is opened, which results in separation of the coarse and fine particles. This separation may occur especially with diets rich in fibre components and would partly explain the large digestibility coefficients of NDF and ADF. These authors compared the simple T-cannula with the re-entrant cannula and they reported higher digestibility coefficients for dry matter, nitrogen and amino acids with the simple T-cannula. This may due to a separation in solid and liquid phase which in turn may reduce or increase marker concentration in samples. Similar to the results of Schröder et al. (1988) Just et al. (1985) and Oslage et al. (1987) found negative digestibility coefficients for fibre components. In contrast to this, our results sometimes showed high digestibilities for NDF and ADF. This indicates that the fibre components and Cr_2O_3 in the digesta may be separated.

From the present investigation the following conclusions can be drawn. With re-entrant cannulas a higher digestibility was found than with PVTC cannulas and T-cannulas. However, in all three methods marker recoveries were lower than 100%. When the digestibilities were corrected to 100% recovery there were only small differences between the methods. A striking point was that with re-entrant cannulas the marker recovery was below 100%, especially when fibre-rich diets were used. The results for the digestibilities determined with the T-cannula are comparable to results obtained with the PVTC cannula and to corrected results determined with re-entrant cannula. On the other hand the T-cannula measurements showed a greater within-treatment variation. This means the possibility of miscalculating the digestibility will be increased with the T-cannula when only a few animals are used.

Table 6. The apparent ileal digestibility of dry matter calculated to 100 % Cr, CO-EDTA and TiO_2 recovery

Diet and type of cannula	*Marker*		
	Cr	*Co*	*TiO_2*
Control diet			
PVTC cannula	73.41 ±0.32 [a]	76.73 ±0.64 [b]	
Re-entrant	73.33 ±0.57	75.86 ±1.23	
T-cannula	73.00 ±0.54 [a]	76.51 ±0.62 [b]	
Pectin-rich diet			
PVTC cannula	57.99 ±0.54[a]	64.35 ±0.33[b]	
Re-entrant	57.50 ±1.72[a]	63.12 ±0.64[b]	
T-cannula	56.55 ±1.98	63.27 ±2.70	
Crude fibre-rich diet			
PVTC cannula	57.06 ±0.98[a]	60.25 ±0.78[b]	
Re-entrant	55.04 ±0.03[a]	57.27 ±0.68[b]	
T-cannula	54.40 ±1.64	58.96 ±4.23	
Semisynthetic diet			
PVTC cannula	86.76 ±0.31[a]	86.35 ±0.24[a]	84.89 ±0.13[b]
Re-entrant	86.23 ±0.37[a]	85.88 ±0.19[a]	84.46 ±0.17[b]

[ab] Means (± SE) in the same row with different superscripts differ ($P < 0.05$)

Our results also suggested that with a re-entrant cannula it is necessary to employ a marker (e.g., Cr_2O_3) to get a representative sample. This was especially important for diets with extremely high amount of fibre.

Although there were some clear differences in the digestibility coefficients measured with the PVTC cannula and with the re-entrant cannula, it can be concluded that the results measured with the PVTC technique were comparable with data measured with the re-entrant or the simple T-cannula. We observed that the PVTC cannulation may cause less discomfort to the animals. Moreover the surgery is much easier to perform compared with the re-entrant technique. In the cannula techniques, the results show less variation with PVTC cannula compared to the simple T-cannula. Therefore on this basis, and in view of the simplicity of handling, the PVTC cannula is a good alternative to the other methods. Further studies have to be carried out to compare this new technique under the same collection conditions to get answers about the results for marker recoveries as well as about the influence of the cannulas on the digesta passage rate.

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Chapter V

A note on the effect of a saccharose/corn starch supplementation on the apparent ileal amino acid digestibility in pigs

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A note on the effect of a saccharose/corn starch supplementation on the apparent ileal amino acid digestibility in pigs

Abstract

The ileo-rectal anastomosis technique in pigs causes a decreased availability of energy and also an increased maintenance requirement for energy. To compensate for that, a supplementation of the experimental diet with a saccharose/starch mixture has been proposed by different authors. Supplementation with a saccharose/starch mixture may affect the ileal digestibility of various nutrients. To study this 10 barrows (initial body weight 98 kg) fitted with post-valve T-caecum cannulas (PVTC) were used to investigate the influence of a saccharose/corn starch supplementation on the apparent ileal digestibility of dry matter, nitrogen and amino acids in a wheat based diet (83.4 % wheat, 4.5 % soybean meal, 4.5% soya flour, 4.5 % herring meal). Five animals were fed the experimental diet and 5 animals received in addition a saccharose/corn starch supplementation. The supplementation was added at a level of 100g per kg dry matter intake with the experimental diet. The apparent ileal digestibility of dry matter, nitrogen and amino acids was not influenced by the saccharose/starch supplementation (P> .05). However, digestibilities of nitrogen and proline tended to be lower in pigs that received the supplementation.

Zusammenfassung

Ileorektostomie bei Schweinen verringert die Umsetzbarkeit der Energie und erhöht den Energieerhaltungsbedarf. Zum Ausgleich wird oftmals eine Supplementierung der Ration durch eine zusätzliche Gabe an Saccharose und Stärke empfohlen. Es ist nicht auszuschließen, daß eine derartige Ergänzung einen Einfluß auf die Verdaulichkeitsbestimmung haben könnte. In einem Versuch mit 10 Börgen (Anfangsgewicht 98 kg LM), die mit Post-Valve T-Caecum (PVTC) Kanülen versehen waren, wurde der Einfluß einer solchen Supplementierung auf die scheinbare praecaecale Verdaulichkeit der Trockenmasse, des Stickstoffs und der Aminosäuren untersucht. Als Versuchsfutter kam eine Weizenration (83.4 % Weizen,4.5 % Sojaschrot, 4.5 %Sojamehl, 4.5 % Heringsmehl) zum Einsatz. 5 Tiere erhielten zusätzlich pro kg aufgenommene Futtertrockenmasse 100 g einer Mischung aus Saccharose und Maisstärke. Die scheinbare praecaecale Verdaulichkeit der Trockensubstanz, des Stickstoffs und der Aminosäuren wurde nicht beeinflußt (P> 0.05). Bei den Schweinen mit Saccharose/Maisstärke-Supplementierung waren die Verdaulichkeitswerte für Stickstoff und Proline etwas geringer als in der Kontrolgruppe.

Introduction

The measurement of amino acid digestibility at the terminal ileum has been accepted to be more related to the absorption of amino acids than the corresponding faecal measurement (Zebrowska, 1973). Ileal digestibility measurements require the collection of ileal digesta and thus a technique without or only minor effects on the physiological status of the animals. The ileo-rectal anastomosis (IRA) technique has been proposed as an alternative to the re-entrant- or simple T-cannulation of the ileum (Fuller & Livingstone, 1982; Picard et al., 1984; Souffrant et al., 1985; Laplace et al., 1985; Hennig et al., 1986; Green et al., 1987). This technique, which consists of a bypass of the colon, allows to collect ileal digesta directly via the anus. However, omitting the hindgut digestion resulted in an absence of the specific digestion and absorption of nutrients from this intestinal segment. This is especially related to the absorption of some minerals and also of volatile fatty acids. The latter are known to be an energy source in pigs. As a consequence Herrmann et al. (1989) reported that the energy metabolizability in 12 different diets composed of various feedstuffs (barley, fish meal, gras meal, lupin, raw and steamed potatoes, raw and steamed sugar beets, sucrose and apple pectin) was about 15% lower in anastomized pigs than in intact pigs. In addition to this observation, they found that the maintenance requirement for energy is about 12% higher in IRA than in intact pigs. These workers suggested that this may be due to some changes in the thermoregulation in IRA-pigs. It may also be suggested that this extra requirement is partly associated with stress. Such a change in energy requirements is in agreement with a reduction in growth performance of IRA-pigs as described also by Hennig et al. (1986), Bengala Freire et al. (1988) and Köhler et al. (1991).

TABLE 1. *COMPOSITION OF THE EXPERIMENTAL DIET (%)*

Wheat	83.4
Soybean meal	4.5
Soya flour	4.5
Herring meal	4.5
Premix*	1.00
$CaCO_3$	1.2
NaCl	0.25
$KHCO_3$	0.20
$NaHCO_3$	0.20
Cr_2O_3	0.25
Supplement	
Saccharose	50.00
Corn starch	50.00

* Contributed the following vitamin and mineral sources per kg of diet: retinol, 9000 IU; cholecalciferol, 1800 IU; α-tocopherol, 40 mg; menadione, 3 mg; riboflavin, 5 mg; cobalamine, 40 μg; nicotinic acid, 30 mg; D-pantothenic acid, 12 mg; choline chloride, 150 mg; ascorbine acid, 50 mg; KJ, 500 μg; $CoSO_4 * 7H_2O$, 2.5 mg; Na_2SeO_3, 0.2 mg; $FeSO_4 * 7 H_2O$, 400mg; $CuSO_4 * 5 H_2O$, 100 mg; MnO_2, 70 mg; $ZnSO_4 * H_2O$, 200 mg. This mixture also supplied 20 mg virginiamycin to the diet.

To compensate for the extra energy requirement in IRA-pigs different authors supply the diet of these animals with a saccharose/starch mixture (ratio of 1:1). It was proposed to add 10% of this mixture on the basis of the daily dry matter intake given with the experimental diet (Hennig et al., 1986; Wünsche et al., 1987; Wünsche et al., 1988, Herrmann et al., 1988). The diet supplemented in this way represents a change in diet and therefore a change in the secretion of digestive enzymes may be possible. Such a change may influence the apparent ileal digestibility of amino acids. Therefore an experiment was carried out to investigate the effect of a saccharose/corn-starch supplementation (100 g per kg dry matter intake) on the apparent ileal digestibility of dry matter, nitrogen and amino acids in a wheat based diet.

TABLE 2. Nitrogen and amino acid concentration in the experimental diet (% i. DM)

Nitrogen	3.32
Amino acids	
Indispensable	
Arginine	1.09
Histidine	0.70
Isoleucine	0.82
Leucine	1.40
Lysine	0.89
Methionine	0.41
Phenylalanine	0.94
Threonine	0.68
Valine	1.08
Dispensable	
Alanine	1.03
Aspartic acid	1.48
Cystine	0.41
Glutamic acid	4.57
Glycine	0.87
Proline	1.37
Serine	0.97
Tyrosine	0.61

Materials and Methods

10 male castrates (Yorkshire X Dutch Landrace) fitted with a post-valve T-caecum cannula as described by Van Leeuwen et al. (1991) were used. The average initial body weight was 98 kg. The animals were housed individually in stainless steel metabolic crates in a temperature-controlled barn (19° C).

The formulation of the experimental diet has been given in Table 1. The chemical composition is presented in Table 2. Chromic oxide was included in the diet as digestibility marker. The animals of the control group I (n=5) were fed at 2.4 times the energy required for maintenance (2.4 x 420 kJ ME/$kg^{0.75}$; ARC, 1981) while the animals of group II also received a saccharose/corn-starch mixture. The amount of this supplementation was 10 % of the daily dry matter intake, given with the experimental diet. Pigs were fed equal amounts of the feed twice daily at 8.00 and 20.00 h. Water was administered with the feed at a ratio of 2.5:1. After an adaptation period of 10 days to the experimental diet ileal digesta were collected quantitatively over 24 hours at days 11, 13, 15 and 17. Collection started one hour after the morning meal.

TABLE 3. Apparent ileal digestibility of dry matter, nitrogen and amino acids in pigs fed a wheat based diet with or without saccharose/corn starch supplementation (calculated to 100% Cr-recovery)

Treatments	*Control*	*Supplementation*	*SEM**
Dry matter	75.0	74.8	1.04
Nitrogen	77.5	75.5	1.74
Amino acids			
Indispensable			
Arginine	85.5	86.3	0.94
Histidine	79.4	79.9	0.98
Isoleucine	81.0	81.1	1.65
Leucine	82.5	82.5	1.54
Lysine	73.3	73.0	2.98
Methionine	83.6	82.3	1.23
Phenylalanine	83.7	83.9	1.26
Threonine	68.6	69.1	2.65
Valine	78.3	78.6	1.77
Dispensable			
Alanine	78.2	78.5	2.06
Aspartic acid	69.8	70.4	2.21
Cystine	70.2	71.1	1.49
Glutamic acid	89.7	89.1	1.01
Glycine	71.4	69.9	2.62
Proline	85.4	83.1	1.29
Serine	77.8	77.3	1.98
Tyrosine	82.9	83.5	1.55

* Standard error of the mean

The digesta were pooled within animals and sampled prior to chemical analyses. Dry matter and nitrogen were analyzed immediately according to ISO 6469 (1983) and ISO 5983 (1979). Chromic oxide and amino acids were analyzed in freeze-dried material. Chromic oxide was determined as described by Bosch et al. (1988). Apart from methionine and cystine, the amino acids were determined after hydrolysis (6N HCl for 22 h at 100^0 C) as described by Slump (1969). Methionine and cystine were determined as methionine sulphone and cysteic acid after oxidation with performic acid (Moore, 1963). The oxidized samples were hydrolysed in the same manner as the samples that were not oxidized. The amino acid composition of the hydrolysate was determined using an automatic amino acid analyzer (Biotronic LC 5001). Analyses for all parameters were carried out in duplicate. Differences among treatment parameters were tested according to the GLM procedure using SAS (1990).

Results and Discussion

The results of the apparent ileal digestibility of dry matter, nitrogen and amino acids of the presented experiment are given in table 3. All digestibilities were calculated to 100% Cr-recovery. Between treatments no significant ($P > 0.05$) differences were observed. With the exception of dry matter, threonine, alanine, aspartic acid, cystine, glycine, proline and serine digestibilities observed were in agreement with the results, that have been reported by Herrmann et al. (1988). These workers determined the apparent ileal digestibility in pigs fitted with an end-to-side ileo-rectal anastomosis fed a diet that was nearly similar with the diet fed in the present experiment (87.9% wheat, 6% soya bean meal and 4% fish meal).

Our results showed some differences between treatments for the digestibilities of nitrogen and proline (digestibility in diets with supplement were 2 and 2.3 percentage units lower). Buraczewska (1981) showed that the absorption of most amino acids was depressed when solutions containing casein hydrolysates and different sugars were perfused in isolated segments of the small intestine. In addition it is known that the different kinds of dietary starch have a different effect on the apparent ileal digestibility of amino acids (Darcy et al., 1981, Darcy-Vrillon & Laplace, 1984). Rerat (1991) reported that non fibre carbohydrates may have an effect on the amount of endogenous nitrogen secretion. As a result there may be a variation in the true and apparent digestibility of crude protein and amino acids. In addition this author reported some indications that the transport of amino acids by the enterocytes may be inhibited by non fibre carbohydrates. Results of the present experiment sowed that the effect of the supplementation on the secretion of additional digestive enzymes apparently was only minor. It did not affect the apparent ileal digestibility of dry matter, nitrogen and amino acids. The average daily gain of the pigs that received the supplementation was 577g/d and about 14.5% higher than for the control group (504g/d). With consideration to the presented results a

supplementation of a saccharose/corn-starch mixture to the experimental diet may be a method suitable to compensate for the decreased growth in anastomized pigs.

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General discussion

General discussion

Introduction

Present understanding of digestive and metabolic processes has been mainly derived from data obtained from animal experiments. For most criteria concerning digestive processes the human is ranging closer to the dog or the pig than to other species (Giesecke, 1990). Therefore a large number of studies on digestive processes have been carried out in pigs because the results obtained can be used to improve both pig and human nutrition. In pigs similar to the humans as a monogastric species the gastro-intestinal (GIT) tract consists of two parts. In the proximal part, that is composed of the mouth, the oesophagus, the stomach and the small intestine, the enzymatical digestion and most of the absorption of the dietary nutrients takes place. In the small intestine the digestive processes require the secretion of digestive enzymes that are produced by the salivary glands, the gastric mucosa, the bile, the pancreas and the mucosa of the small intestine. The distal part of the GIT, which is characterized by fermentative digestion and absorption of some minerals, especially sodium and magnesium, volatile fatty acids and water, consists of the caecum, the colon and the rectum. In human and animal nutrition more knowledge of the intestinal processes at different sites of the GIT is necessary with respect to the following points:

- → in human nutrition, a better understanding of dietary aspects is required with regard to metabolic diseases (disease of the liver, the kidneys etc.); or diseases of the GIT itself including the removal of intestinal segments (e.i. cancer of the bowel, ileostomy).
- → improvement of animal nutrition to reduce environmental pollution caused by animal excretions (manure) and to ensure animal production as economical as possible.
- → an increased interest into pharmacokinetics

Such studies require, at least to a certain extent, the surgical manipulation of animals.

Lawful foundations of animal experiments

In the Netherlands and in Germany the performance of animal experiments is directed by the 'Wet op de dierproeven' (1977) and the 'Bundestierschutzgesetz' (1986), respectively. In both countries the statutory regulations are similar. Animal experiments require official permission. According to '§ 7' of the 'Bundestiersch utzgesetz' and 'Artikel 1' of the 'Wet op de dierproeven' animal experiments are permitted:

I. to prevent, to recognize and to treat diseases in human and in animals,

II. to recognize physiological functions in humans and in animals,

III. for toxicological and pharmacological experiments

IV. for basic research in general.

Additionally, the number of animals must be restricted to a minimum (§ 9 Abs.2.2, Bundestierschutzgesetz) and it is not permitted to inflict pain, suffering or damage to the animal in

order to save time, money or work (§ 9 Abs. 2.3, Bundestierschutzgesetz). With consideration to the experimental aims and to Artikel 4 of the resolution to the 'Wet op de dierproeven' (1985) the physiological and ethological requirements of the animals should be taken into account. This means that the study of the physiological processes of digestion should be determined in animals that are in a normal physiological status and are subjected to a minimal level of stress as a result of environmental or experimental conditions. As mentioned in this thesis, the PVTC-cannulation has been developed to avoid the effect of cannulation on intestinal processes up to the terminal ileum by collecting digesta post-valvular and by preventing the dissection of the intestine. The PVTC-cannulation is a relatively new technique and it was necessary to evaluate this method with respect to its influence on several physiological and digestive parameters. Therefore, the PVTC-cannulation has been used in each experiment as a reference technique for comparisons with other different techniques.

The physiological status in pigs provided with different techniques to collect ileal digesta.

Since the collection of digesta requires the manipulation of the GIT there is a fundamental interest not to change the physiological status of the animals, so that the results measured can be transferred to intact animals. In Chapter I and II of this thesis some physiological parameters in PVTC cannulated pigs and in IRA-pigs were compared with corresponding values obtained in intact animals. Livingstone and McWilliam (1985) reported a growth depression in T-cannulated pigs of about 7 % compared to intact pigs when gain was expressed in g per day. Jørgensen et al. (1985a) reported a growth rate in T-cannulated pigs that was similar to that in intact pigs. In PVTC-cannulated and in anastomized animals a depression in growth performance indicated a different effect of these techniques on the physiological status of the animals. A reduction of the average daily gain per $kg^{0.75}$ of about 53 % in IRA pigs compared to intact animals indicated a considerably greater influence than the reduction of about 7 % that was reported in PVTC pigs. The reduced growth in PVTC cannulated pigs, that was calculated over the whole experimental period, can be explained as a result of loss of digesta during the three digesta collection periods, because the digesta collected did not contribute to the hind gut digestion. Based on the assumption that the faecal digestibility of energy is about 10 percentage units higher than the ileal digestibility, the PVTC-pigs would have had 13 % less energy absorption during the collection periods. Since digesta was collected totally over 8 % of the experimental period the losses of energy due to the digesta collection would only be 1 % of the total energy intake. This does not explain the reported growth reduction of 7%. However, using our feeding strategy (dietary energy intake was calculated on the basis of $kg^{0.75}$), a small reduction in rate

of gain will result in less feed for the rest of the experiment. This means that our data observed in PVTC pigs are in agreement with data reported by Livingstone & McWilliam (1985). The reduced growth found in IRA pigs indicated that this technique may cause a strong disturbance of the energy metabolism. This has been demonstrated previously by Herrmann et al. (1989). Moreover, additional physiological investigations on the blood composition, the nitrogen, sodium and potassium balances and the weight of some selected organs as described in Chapter I and II, clearly indicate a number of significant differences between animals fitted with an end-to-side IRA and intact animals. In accordance with the differences in growth performance, the results of the nitrogen balances in IRA pigs were quite different compared to PVTC and INT animals. Although the diet of the IRA pigs was supplemented with an extra electrolyte solution, similar to that described by Hennig et al. (1986), the absence of hind gut digestion resulted in reduced sodium and potassium balances. The reduced retention of sodium and potassium in IRA pigs corresponded with some changes in the blood levels. Patience et al. (1987) reported that a reduction in dietary electrolyte balance decreased the pH and bicarbonate concentration in the serum of pigs. In accordance with these authors the blood values for the base excess, the pH and the active bicarbonate in IRA-pigs were lower than in PVTC- or INT-pigs. As a consequence of the disturbed hydro-electrolyte status of the IRA-pigs and the increased reabsorption of sodium and potassium by the kidneys, the weights of the adrenal glands ($P< 0.05$) and the kidneys ($P> 0.05$) were higher than in PVTC- or INT-pigs. Differences in the weight of the liver ($P> 0.05$) may be a result of the change in nitrogen metabolism. Differences that were found for the girth of the rectum may be attributed to an increased fermentation and/or to changes in the flow rate of digesta.

To prevent negative effects of the ileo-rectal anastomosis technique on growth performance and mineral balances, supplementation of the experimental diet with extra minerals and easily soluble carbohydrates such as saccharose and starch has been proposed by different authors (Hennig et al. 1986, Wünsche et al. 1987, Herrmann et al., 1988, Wünsche et al., 1988). However, each supplementation has to be considered as a modification of the diet. As mentioned in Chapter V the inclusion of different sugars in the diet may have an influence on amino acid digestibility (Buraczewska, 1981). According to Darcy et al. (1981) and Darcy-Vrillon & Laplace (1984) an effect of a starch supplementation can be expected depending on the type of starch used. It has been reviewed recently by Rerat (1991) that non fibre carbohydrates may have an effect on the amount of endogenous nitrogen secretion. As a result there may be a difference between the true and apparent ileal digestibilities of crude protein and amino acids. According to Rerat (1991), there may also be an inhibition of the transport of amino acids by the enterocytes. In pigs supplementation and deficiency of minerals have a negative effect on the apparent ileal digestibility of crude protein, amino acids and crude fat (Jørgensen et al. 1985b). This is thought to be associated with some changes

of the osmolarity and thus in the homeostase across the gut wall. However, an additional supply of electrolytes is necessary to prevent a deficiency of certain minerals, which has been reported in IRA pigs, and in humans that were provided with an ileostomy (Clarke et al., 1967, Hennig, 1988). Hennig (1988) reported significantly higher apparent ileal digestibilities of isoleucine, leucine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, alanine, aspartic acid, glycine, proline and serine in IRA-pigs (30-40 kg LW) when they received an extra electrolyte supplementation containing 1.94 g sodium per day. With respect to the results of Jørgensen et al. (1985b) this improvement of the apparent ileal digestibility can be considered as a result of a mineral deficiency in IRA-pigs which receive no mineral supplementation. Results presented in Chapter II showed that a sodium supplementation as provided in our experiment (0.66 g sodium * d^{-1} * 20 kg LW^{-1}) was not sufficient to maintain a positive sodium balance. To prevent a negative effect of a sodium deficiency on the sodium balance and on digestibility of some particular nutrients, different authors have proposed a higher sodium supplementation (Laplace et al., 1989, Darcy-Vrillon & Laplace, 1990, Leterme et al., 1990). However, until now no reports on the effect of such supplementations on sodium balances are available.

With respect to the physiological status of the pigs it can be concluded that the end-to-side ileo-rectal anastomosis used in the present experiments has a considerable influence on various metabolic parameters. The supplementation of the diet with an electrolyte solution as proposed by Hennig et al. (1986) is not sufficient to maintain the sodium and potassium balances on the level required for optimum performance and maintenance of good health. Although no significant differences were found in the apparent ileal digestibility of nitrogen and amino acids (Chapter V) the supplementation of the diet of IRA pigs with non fibre carbohydrates should be considered carefully with respect to the measurement of intestinal parameters. The most significant advantage of the ileo-rectal anastomosis is saving of time and labour during the collection period. However, because of considerable alterations of the the physiological status of the animals and because of ethical considerations, (it is extremely difficult to avoid the pigs suffering discomfort from the frequent outpouring of digesta from the anus), the IRA-technique should be questioned to be used in digestibility studies.

Data from PVTC pigs suggested only some slight effects on the physiological status of the animal. There was only one remarkable change concerning the urea level in blood, i.e. the urea level in the blood of PVTC pigs at week 13 post-surgery was significantly higher ($P< 0.001$) than in intact animals (8.36 versus 6.37 mmol/l). According to Gargallo and Zimmermann (1981) cecectomy does not affect the plasma urea level. It can be speculated if the increase in blood urea concentration of PVTC-pigs is a result of the digesta collection which has been completed two days before blood samples were taken. With respect to the disruption of the digesta flow and the disturbed bacterial

activity in the hind gut throughout this period an increased ammonia absorption from the large intestine is possible. In addition, digesta collection as carried out during these investigations may also be responsible for some slight effects on sodium balances that were recorded in PVTC pigs. This means that the absence of the hindgut absorption of sodium during the collection periods might be responsible for the lower renal excretion of sodium. A modification of the collection procedure, including the provision of recovery days between consecutive collection times may be helpful to stabilize the sodium status of the animals. An additional supply of NaCl after each collection should be considered in order to compensate for the absence of sodium absorption from the hindgut during the collection. Most of the data presented in Chapter I and II suggest that PVTC-cannulation maintains a more stable physiological status when compared to the IRA-technique. However, further investigations are necessary to obtain more information about the effect of the digesta collection per sé on the sodium status and urea metabolism.

Digestibility measurement in pigs fitted with different techniques.

The enzymatical digestion, and the majority of absorption of dietary nutrients occur in the proximal part of the GIT up to the terminal ileum. The bacterial degradation of the remaining nutrients, including the synthesis of bacterial cell mass will follow in the hind gut. Therefore faeces composition provides no direct measure about the absorption of the major nutrients in the small intestine. To exclude the effect of the bacterial activity in the hind gut, the ileal rather than the faecal analysis method should be used to measure digestibilities. Since each collection technique involves a specific manipulation of the GIT, digestibility measurement may differ depending on the technique used. For this reason two experiments were carried out to compare the effect of the end-to-side IRA, the PVTC-, the re-entrant- and the simple T-cannulation on the digestibility of different nutrients (Chapters III and IV). Data in Chapter III show that the digestibilities of dry matter, arginine, histidine, isoleucine, leucine, methionine, phenylalanine, alanine, cysteine, tyrosine, sodium and potassium measured in pigs that were fitted with PVTC cannulas or end-to-side IRA were significantly different at week 3, 9 and 12 post-surgery, respectively. In addition the digestibility coefficients were similar in PVTC-pigs from week 3 to week 12 post-surgery, except for lysine and sodium, which were slightly decreased. In IRA-pigs however, digestibilities of dry matter, most of the amino acids (except histidine, lysine, methionine, alanine and proline), sodium and potassium increased with time. The decreased digestibility of lysine that was found in both treatments is in accordance with the results reported by Pietruschka (1987) in pigs fitted with an end-to-side IRA and fed a wheat-based diet. In contrary to the results given in Chapter III he also found slightly decreased digestibilities of aspartic acid, threonine, serine, glutamic acid, alanine and nitrogen. Differences between IRA and PVTC can be explained in general as a consequence of some adaptative changes of

the intestinal processes in IRA-pigs. Notably nitrogen and amino acid digestibilities were associated with the increased bacterial activity that was found in the digesta of IRA-pigs (Chapter I). This progressive modification of the function of the terminal ileum has also been reported by Fuller (1991) when he compared IRA-pigs with T-cannulated pigs. The results reported by this author showed histological changes as well as an increased bacterial activity in the ileum of IRA-pigs at week 26 post-surgery. In our experiment the dynamics of this adaptation was also reflected in a change of digesta composition and thus in digestibilities. The decreased nitrogen recovery in digesta of IRA-pigs, that was particullary observed between week 9 and week 12 post-surgery resulted in an increased apparent digestibility of nitrogen and amino acids. The nitrogen retention and/or nitrogen excretion with urine at week 11 post-surgery did not increase when compared with data that were measured at week 5 post-surgery. However, the present data give evidence that nitrogen was absorbed increasingly. With regard to the microbial activity this might occur as ammonia, amines and amides. Differences between IRA and PVTC-pigs were also found for the digestibilities of sodium and potassium. These values must be considered in close relation to their urinary excretion, which was hardly reduced in IRA-pigs. Partridge (1978) reported a high secretion of sodium anterior to the mid-jejunum and dependant on the type of diet, a sodium absorption up to the terminal ileum that was considerably efficient. Apart from one diet that consisted of maize starch, sucrose and casein, differences in secretion and absorption resulted in a net secretion of sodium anterior to the distal part of the small intestine. Net secretion and thus a negative apparent digestibility of sodium has also been reported by Drochner (1984), den Hartog et al. (1988) and Partridge (1986). However, sodium absorption in the large intestine is related to a positive sodium balance at the end of the GIT. Since in IRA-pigs there is no absorption of sodium from the large intestine, parameters measured in IRA-pigs indicated a strong metabolic adaptation to conserve sodium partly in exchange for potassium as described in Chapter II and III. While results found in PVTC-pigs gave no indication concerning a possible effect of cannulation on digestibility, parameters measured in IRA-pigs indicated that digesta composition is affected by this technique. As a consequence data reported in the literature obtained from pigs fitted with and end-to-side IRA should be interpreted carefully. This concerns in particular the apparent digestibilities of lysine and methionine for which a net synthesis in the hind gut has been reported by different authores (Sauer et al., 1982; Bergner, 1984; Knabe et al., 1989), but also the digestibilitites of threonine, glycine and proline which are known to be degradaded by micro-organisms to a greater extent than other amino acids.

In addition to the studies described in Chapter III the main interest of Chapter IV was focused on the specific problems that occur when different cannulation techniques are used for measuring ileal digestibilities. Therefore digestibility measurements in PVTC cannulated pigs were compared with those in pigs fitted with ileo-caecal re-entrant or simple T-cannulas. Since problems described

for cannulation techniques are associated notably with the fibre content of the diet, digestibilities of four diets that differed in their fibre contents as well as in the source of fibre were determined. Another objective that was taken into account was the use of different digesta collection procedures as described in the literature. With respect to the problems concerning cannulation techniques (blockages, leakages, separation) different markers (Cr_2O_3, Co-EDTA and TiO_2) were added to the diet. The most important criteria of any digesta collection is to obtain representative samples. Different authors reported a circadian variation of digesta composition (Livingstone et al., 1980; Graham and Åman, 1986). Therefore an adequate collection of digesta is needed to obtain a representative samples.

It has been agreed for a long time that digestibility measurements using non quantitative methods require the addition of an inert, non absorbable substance for time-correcting the faecal excretion of a material under study (Edin et al., 1944; Kreula, 1947; Schürch et al., 1950; Carroll et al., 1952). For the digestibility measurement at the distal ileum or any other site of the GIT the use of a marker is also needed, in particular when simple T-cannulas and also PVTC cannulas are being used. In re-entrant cannulated pigs the use of a marker is also necessary in the case of spot-sampling or investigations on the passage rate of digesta (Horszczaruk, 1971 a,b; Holmes, 1974; Zebrowska et al., 1978; Sauer et al., 1981; Taverner et al., 1983). Such a marker is chromium (Cr) which can be used as chromium oxide (Cr_2O_3) or as chromium-neutral detergenz fibre (Cr-NDF) to mark the solid phase, or as chromiumr-ethylenediamine tetraacetic acid (Cr-EDTA) to mark the liquid phase. In each case chromium can also be used as ^{51}Cr to simplify the analysis (Bergner and Klenke, 1985; Krawielitski et al.,1987). Other markers for the solid phase are TiO_2, Fe_2O_3 or ^{103}Ru-phenanthrolin. In addition, cobalt-ethylenediamine tetraacetic (Co-EDTA) and polyethylenglycol (PEG) can be used as alternative markers for the liquid phase. To ensure the accuracy of calculating the digesta passage different requirements for a marker have to be met as formulated by Kotb and Luckey (1972). A positive correlation between chromium and dry matter concentration in both duodenal and ileal digesta has been reported by different authors (Moore, 1957; Partridge, 1985; Graham and Åman, 1986;). This means that Cr_2O_3 will generally follow the flow of dietary matter through the GIT. Faichney (1980) introduced the double-marker method to take into account the separation of dietary components within the GIT. He suggested the use of two markers, one for the solid phase and one for the liquid phase. Using this technique Latymer et al. (1985) reported that the liquid phase of the digesta tended to move faster than the solid phase through the small intestine, except when pectin (50g/kg diet) or guar gum (60g/kg diet) were added to the diet. However, both phases arrived more or less at the same in faeces. Using the double-marker method differences in the passage-rates for both phases through the small intestine creates differences in the digestibility coefficients when these are calculated on basis of the recovery-rates of the two different markers (Jongbloed et al., 1991;

Chapter IV of this thesis). Jongbloed et al. (1991) reported ileal digestibility coefficients of dry matter, total P and phytic acid for a maize/soybean meal diet and these were markedly lower ($P < 0.05$) when calculated to 100% cobalt-recovery and compared with results that were calculated on 100% chromium-recovery. On the other hand digestibilities were very similar when calculated for both markers in a tapioca/maize gluten diet. In contrast to this our results presented in Chapter IV of this thesis showed dry matter digestibilities that were mostly higher when calculated for cobalt-recovery and compared to the results based on chromium-recovery. Since only a few comparisons of the different markers are available, no clear explanation can be given if these differences are affected by the type of marker, the collection procedure or even by the diet per sé.

In our study described in Chapter IV, different cannulation techniques were used in combination with different collection procedures. This means that digesta in PVTC-pigs were collected on 5 consecutive days, 12 h per day. In the re-entrant pigs digesta were collected on 3 alternate days 24 h per day, as described by van Leeuwen et al. (1987). Digesta collection in T-cannulated pigs was carried out on 4 days, for three periods of 2 h per day as described by Jørgensen et al. (1984) As proposed in the literature, digestibilities in T-cannulated pigs were calculated for 100 % marker-recovery whereas digesta collection in re-entrant pigs was expected to be quantitative. In PVTC-pigs digestibility markers were also used to study the homogeneity of sampling. In this way differences between re-entrant cannulation, and PVTC- and T-cannulation became obvious. As described in Chapter IV we used four different diets (a control diet, a pectin-rich diet, a fibre-rich diet and a semisynthetic diet). In re-entrant cannulated pigs the recovery rates of chromium were about 7-18 percentage units below 100 % for all four diets. In addition, apart from the semi-synthetic diet, the recovery-rate of cobalt was higher than that of chromium. This can be explained by blockages of fibrous components anterior to the proximal cannula. The liquid phase including Co-EDTA passed the blockages along the gut wall, which may also explain why more liquid leakages were observed with diets containing a higher fibre content. This difference in marker-recoveries was also observed for the control and the semi-synthetic diet, but to a smaller extent. Digestibilities in the re-entrant pigs were higher than in PVTC or T-cannulated pigs that were calculated to 100 % chromium-recovery. When digestibilities in re-entrant cannulated pigs were also corrected to 100% chromium-recovery, all digestibilities apart from nitrogen and NDF in the fibre-rich diet were similar.

In PVTC- and T-cannulated pigs digestibilities were similar when calculated to 100% chromium-recovery. Nevertheless, marker recoveries indicated differences between the recoveries of the solid and the liquid phase. These differences between marker-recoveries within treatments was greater for the T-cannula than for the PVTC-cannula. As mentioned in Chapter IV, in T-cannulated pigs digesta was collected totally for a 24 h per period, whereas in PVTC and re-entrant pigs digesta

were collected for 60 and 72 h, respectively. This means that differences between marker-recoveries may be a result of both the different digesta collection procedures and/or a separation of digesta components in T-cannulated pigs. Feeding the fibre-rich diets the rate of marker-recovery decreased more in T-cannulated pigs than in PVTC- or re-entrant pigs. This may be due to temporary blockages with fibrous material or the more viscous nature of the digesta after feeding the pectin-rich diet. Moreover, with an increase of fibrous components in the diet as well as an increased amount of feed with the live-weight the amount of digesta that had to pass through the cannula was increased. This means that during the collection of digesta the size of the cannulas (the inner diameter in PVTC and T-cannulas was 25 and 19 mm respectively) may have restricted the passage capacity through the cannula because digesta movement is performed in portions caused by the myo-electric complex. For the semisynthetic diet, the increased digestibility of dry matter and thus decreased amount of digesta resulted in recovery rates of both chromium and cobalt that were much higher than for the other three diets. Differences in marker-recoveries between PVTC- and re-entrant cannulated pigs and for the semisynthetic diet may be due to the different sizes of the cannulas. It is also be possible that in re-entrant pigs the greater disturbance of the myo-electric complex and the loss of the intestinal overpressure after opening of the cannula resulted in a small blockage anterior to the ileal cannula. On the basis of the results reported in Chapter IV it can be concluded that digestibility measurements in PVTC cannulated pigs show similar results when compared with re-entrant - or T-cannulated pigs provided that the results were corrected to 100% marker-recovery.

The accuracy of measurement however is reflected by the variation of the results measured. Data from Chapter IV showed that within treatments greatest variation for marker recovery-rates and digestibility coefficients were observed in T-cannulated pigs. With respect to the marker recovery-rates in T-cannulated pigs, the greatest variation (given as standard deviation) was found with the pectin-rich and the fibre-rich diets. Differences of variation between treatments can be explained partly as a result of different numbers of observations within treatments. Nevertheless, they indicate that calculation of digestibility in PVTC- and re-entrant cannulated pigs were more accurate than in T-cannulated pigs. In re-entrant pigs the greatest variation in marker-recoveries was found with the pectin-rich diet. Since the viscosity of the digesta was increased after feeding the pectin-rich diet, it might be possible that the infusion of extra saline into the proximal cannula was not sufficient to improve the digesta flow equally in all five pigs. However, using different digesta collection procedures the variation within treatments indicated that digesta collection in PVTC and re-entrant pigs is more homogeneous than in T-cannulated pigs. Using the double-marker method more information was available to explain differences of digestibilities between different techniques. In addition, differences between marker-recoveries gave indications as to the collection procedure that is necessary to obtain more homogeneous samples. Further investigations are still necessary to

standardize the collection procedure as well as the use of markers to improve the comparability of results measured at different institutes.

Table 1. Variation of the apparent ileal digestibility of nitrogen and indispensable amino acids in wheat

References	1	2	3	4	5	6	7
Nitrogen	85.6	82.9	86.0	84.8	76.2	75.0	79.7
Amino acids							
Argenine	90.0	85.8	87.3	90.4	86.3	86.8	79.7
Histidine	92.0	89.1	86.3	88.0	81.9	82.4	80.2
Isoleucine	85.0	85.3	87.4	88.0	76.9	76.1	81.2
Leucine	85.0	86.9	88.8	85.5	80.7	80.4	83.0
Lysine	78.0	75.7	76.7	84.2	53.2	50.6	63.4
Methionine	83.0	86.6	82.1	89.9	78.2	82.6	83.6
Phenylalanine	92.0	88.8	87.8	92.4	81.1	82.3	84.2
Threonine	78.0	76.5	76.5	77.5	69.8	65.7	65.0
Valine	85.0	82.8	80.1	86.0	78.5	74.8	78.1

1 Ivan & Farrell, 1976 re-entrant
2 Sauer et al., 1977 re-entrant
3 Sauer et al., 1981 re-entrant
4 Lin et al., 1987 T-cannula
5 Pietruschka, 1987 end-to-side IRA
6 Wünsche et al., 1987 end-to-side IRA
7 Green et al., 1987 end-to-end IRA

Digesta composition, composition and amount of endogenous amino acids, digesta passage, the activity of digestive enzymes and thus digestibility measurement may all be affected by the diet and/or separate dietary components as it has been reviewed by Low (1982), Austic (1983), Tanksley and Knabe (1984), and Sauer and Ozimek (1986). Additionally, results measured may be affected by the use of different procedures between laboratories. The variation between samples of different batches of feedstuffs on the apparent ileal digestibilities of nitrogen, and of the indispensable amino acids for wheat and barley as reported by different authors are presented in Tables 1 and 2. It is important to consider the variation within each of these cereal grains. For example, in wheat the apparent digestibilities of lysine and methionine ranged from 50.6 to 84.2% and from 78.2 to 89.9%, respectively and in barley from 54.2 to 79.4% and 72.2 to 86.9%. The use of these data for formulating

diets should be considered critically considering the wide variation between the measurements which can be attributed more to differences between the batches and/or laboratories than to differences between the techniques used.

Table 2. Variation of the apparent ileal digestibility of nitrogen and indispensable amino acids in barley

References	1	2	3	4	5	6	7	8
Nitrogen	74.9		75.4	67.6	80.0	75.0	71.3	70.2
Amino acids								
Argenine	81.5	83.0	79.7	82.7	80.0	85.7	77.3	78.9
Histidine	80.4	83.0	76.4	72.6	77.0	83.1	76.3	77.0
Isoleucine	79.1	78.0	76.9	78.4	78.0	83.2	75.4	71.8
Leucine	81.5	82.0	79.5	76.3	79.5	82.7	78.1	77.7
Lysine	73.3	79.0	69.6	68.7	75.0	79.4	67.1	54.2
Methionine	80.4		76.8	75.1	86.9	83.0	78.3	77.2
Phenylalanine	82.2	83.0	81.4	82.1	82.0	88.7	78.1	80.6
Threonine	71.2	70.0	66.8	59.5	74.0	73.5	63.6	66.5
Valine	78.0	78.0	69.6	75.0	77.0	82.1	75.5	74.3

1 Sauer et al., 1977 re-entrant
2 Just et al., 1980 re-entrant
3 Sauer et al., 1981 re-entrant
4 Poppe et al., 1983 re-entrant
5 Moughan and Smith, 1987 T-cannula
6 Lin et al., 1987 T-cannula
7 Green et al., 1987 end-to-end IRA
8 Wünsche et al., 1987 end-to-side IRA

Conclusions

Investigations of digestive processes require the collection of digesta at different sites of the GIT. This can be carried out using various different collection techniques. Different collection techniques have been developed to collect digesta at the distal ileum. Using the end-to-side IRA which bypasses the large intestine results in a strong effect on the growth performance, the nitrogen balance, the sodium and potassium metabolism, different blood parameters and the digesta composition. Although an increased intestinal fermentation may be restricted to the end-to-side anastomosis, problems concerning the sodium and potassium metabolism as well as the discomfort that is associated with the outpouring of digesta from the anus can be accepted to be similar using the

end-to-end method. Therefore, this technique has to be considered as unsuitable for the measurement of digestibilities. For the collection of ileal digesta the simple T-cannula, the PVTC-cannula and the ileo-caecal re-entrant cannula showed similar results after correction to 100% marker-recovery. Nevertheless, with regard to the accuracy, i.e. the homogeneity of the sample, the re-entrant- and the PVTC-cannulation should be preferred. In PVTC-cannulated animals the small intestine remains intact and the function of the ileo-caecal valve can be maintained. As a consequence only minor changes were observed for the growth performance, the sodium balance and the urea-level in blood. This may be due to the feeding strategy and the digesta collection procedure used in this experiment. Using the re-entrant cannulation, however, the dissection of the intestine and the bridging of the ileo-caecal valve may have an influence on the digesta flow and thus on intestinal parameters. In re-entrant pigs the occurrence of blockages in front of the proximal cannula suggest the use of the PVTC-cannulation, especially in the case of fibre-rich diets. Moreover, compared to the re-entrant and the simple T-cannulas, the PVTC-technique requires a more simple surgery without any disturbance of the small intestine. Results measured in PVTC-pigs indicated an accuracy of measurement that is sufficient to enable the number of animals to be restricted to a minimum. The results of this thesis show that the re-entrant cannulation-technique can not be considered furthermore as a reliable quantitative collection technique.

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General abstract

General abstract

Investigations in the field of digestive processes are important to improve human and animal nutrition. During the last decades an increased number of studies on these subjects related on digestion were carried out in pigs. Since digestive processes which take place in the proximal part of the gastro-intestinal tract are quite different from those in the distal part, digesta collection at the terminal ileum becomes more important. However, all techniques that have been developed at different institutes are associated with some specific handicaps. To improve digesta collection at the terminal ileum, the post-valve T-caecum cannulation has been developed at the ILOB Institute in Wageningen. Investigations were necessary to evaluate this new technique in comparison with different other techniques. Physiological and digestive parameters in pigs were studied by using different digesta collection techniques and presented in this thesis.

In the first chapter investigations were focused on the growth performance and nitrogen balances in pigs fitted with a PVTC-cannula or end-to-side ileo-rectal anastomosis (IRA). Data observed were compared with corresponding values determined in intact pigs (INT). It was found that PVTC-cannulation resulted in a decreased daily gain of about 7 % when compared with INT. This was explained with the digesta collection periods that were carried out during the experimental period, because digesta collected did not contribute to the energy status of the animal. In addition, using our feeding strategy (dietary energy intake was calculated on the basis of metabolic live weight), a small reduction in rate of gain will result in less feed for the rest of the experiment. A reduction of the daily gain of about 53 % that was observed in IRA-pigs indicated a strong influence of this technique on the energy metabolism which was in agreement with the literature. Data observed for the nitrogen-balances were close to that for the growth performance. This means that N-retention in PVTC-pigs was similar to that in INT-pigs. Data found for IRA-pigs were distinctly different, which means that N-excretion in the digesta and in the urine of these pigs was significantly higher than in PVTC- or INT-pigs. As a consequence N-retention was considerably lower in IRA-pigs.

In addition to these studies the fermentative processes in the ileal digesta collected with both techniques were under study. Volatile fatty acids and diaminopimelic acid were determined as indicative parameters of the bacterial activity. Results found in PVTC-pigs were similar to that reported in the literature when determined in T- or re-entrant cannulated pigs. Data measured in digesta from IRA-pigs were considerably higher and more similar to faecal data. This was explained with an adaptative change of digestive processes in the terminal ileum and/or rectum of IRA-animals.

Since PVTC-cannulation requires the removal of the caecum and the IRA-technique excludes the hind gut digestion up to the rectum, this may have an influence especially on the absorption of some minerals. Changes in digestive processes may have an influence on blood-parameters. Therefore, investigations of blood-parameters such as creatinine, urea, sodium, potassium, chloride, calcium, magnesium, base excess, pH and active bicarbonate were carried out in PVTC- and IRA-pigs and were compared with corresponding values obtained in INT-pigs (Chapter II). In addition the sodium- and potassium-balances were determined in these animals and compared with a reference group of INT-animals. Blood-parameters found in PVTC-pigs were similar to that in INT-pigs, except for urea and potassium at week 13 post-surgery. With regard to these differences it was concluded that they may have been affected by the digesta collection that was completed only two days before blood-samples were obtained. In IRA-pigs, however, changes in the blood levels of creatinine, urea, sodium, potassium, base-excess, pH and active bicarbonate were found when compared to INT-pigs. These differences may be explained fundamentally with the absence of the hind gut digestion. This means that the increased urea level in blood of IRA-pigs at week 6 post-surgery can be accepted as a a result of the absence of the microbial utilization of urea in the large intestine. After adaptation to an increased fermentation in the terminal ileum and/or rectum, ammonia may be absorbed decreasingly resulting in a blood level that was similar to that in INT-pigs at week 13 post-surgery. In IRA-pigs differences found for the base-excess, the pH and the level of active bicarbonate can be accepted as a result of the changed sodium- and potassium-balances. Whereas results obtained in PVTC-pigs were mostly similar to that in INT-pigs (apart from the renal sodium excretion, that was lower in PVTC- than in INT-pigs at week 11 post-surgery), balances for both minerals were changed in IRA-pigs. This means, that the intestinal sodium excretion was much higher in IRA-pigs than with the faeces in PVTC- or INT-pig. In addition the renal excretion of sodium was considerable lower in IRA-pigs when compared with the other treatments. The sodium retention in IRA-pigs, however, was negative at week 11 post-surgery. As a result of the changed sodium metabolism the potassium-balances in IRA-pigs were different when compared with PVTC- or INT-pigs. Although the renal excretion was reduced in IRA-pigs the retention (in % of the intake) tended to be lower than in the other treatments. Differences in the renal excretion for these minerals might be associated with an increased aldosterone activity in IRA-pigs, because the weights of the adrenal glands were significantly higher than those from PVTC- or INT-pigs.

In IRA-pigs the intestinal adaptation as well as differences in mineral balances resulted in different digestibilities of dry matter, some amino acids, sodium and potassium (Chapter III). In PVTC-pigs digestibilities were similar from week 3 to week 12 post-surgery, except for lysine and sodium that were slightly decreased. In IRA-pigs, however, apart from histidine, lysine, methionine, alanine and proline digestibilities increased with time. Similar than in PVTC-pigs the digestibility of

lysine decreased with time. In IRA-pigs changes in digestibility with time were related to an increase in intestinal fermentation processes. The strong differences between the collection techniques demonstrate the important role of the large intestine for the absorption of these minerals.

When digestibility measurements in PVTC-pigs were compared with that in T- or re-entrant cannulated pigs fed different diets, differences were found between the re-entrant cannulation on one hand and the PVTC- and T-cannulation on the other hand (Chapter IV). Comparison of digestibilities based on additional calculation to 100 % chromium-recovery in re-entrant pigs, showed that differences were much smaller, but still significant for nitrogen and NDF in a fibre-rich diet and for nitrogen in a semisynthetic diet. Marker-recoveries gave evidence that the digesta collection obtained by re-entrant canulation was not quantitatively. The use of the dual-marker-method indicated that the collection of the liquid phase was more quantitatively than the collection of the solid phase. This was in agreement with problems such as blockages and leakages, that were observed for all cannulation techniques especially after feeding pectin-rich and fibre-rich diets.

Investigations in IRA-pigs showed a daily gain that was much lower than in PVTC- or INT-pigs. Since in the literature this has been shown to be a result of the decreased energy metabolizability combined with an increased maintenance requirement for energy, the experimental diets for IRA-pigs are often supplied with extra easily soluble carbohydrates such as saccharose and starch. To prevent a possible influence of extra carbohydrates on digestibility measurement, IRA-pigs used in our experiments did not receive such a supplementation. However, when extra carbohydrates were supplied to a wheat based diet and fed to PVTC-pigs the apparent ileal digestibilities of dry matter, nitrogen and amino acids were not influenced (Chapter V). Therefore it was concluded that the supplementation of the experimental diet with a saccharose/starch mixture may be suitable to improve the energy-status of the animals.

In conclusion to the results presented in this thesis, the PVTC-cannulation technique can be accepted as a reliable alternative to different other digesta collection techniques. Moreover, this new technique maintains the physiological status of the animal and the surgical procedure as well as the handling of this technique are relatively simple. The end-to-side ileo-rectal anastomosis has to be considered as a technique which severely affects the physiological status of the animals as well as the intestinal processes to an unacceptable extent. Therefore this technique should not be used furthermore. The re-entrant cannulation that has been supposed as a quantitative collection method should be used critically, especially in the case of fibre-rich diets. The results presented suggest the necessity of the use of a digestibility marker with this technique. Parameters observed with the T-cannulation showed the greatest variation between animals indicating that digesta samples were less

homogenous than collected with the other techniques. Taking the disadvantages that have been described for the re-entrant and T-cannulation into account, the PVTC-cannulation should be preferred when digesta have to be collected at the terminal ileum.

Samenvatting

Samenvatting

Onderzoekingen op het gebied van de verteringsprocessen zijn belangrijk om de humane en de diervoeding te verbeteren. Gedurende de laatste decennia is veel onderzoek met varkens op dit terrein uitgevoerd. Omdat de verteringsprocessen die in het proximale deel van het maagdarmkanaal plaats vinden duidelijke verschillen vertonen met die in het distale deel, is het interessant om chymus te verzamelen uit het terminale ileum. Op verschillende instituten is een aantal technieken ontwikkeld om ileum chymus te verzamelen. Helaas heeft elke techniek een aantal specifieke nadelen. Om het verzamelen van chymus uit het terminale ileum te verbeteren is op het ILOB in Wageningen de "Post Valve T-Caecum" (PVTC) canule ontwikkeld. Onderzoek was nodig om de waarde van deze techniek te bepalen in vergelijking tot verschillende andere bestandetechnieken. Dit proefschrift beschrijft de fysiologische en verterings parameters, gemeten in varkens met behulp van verschillende technieken om chymus te verzamelen.

Het eerste hoofdstuk beschrijft de resultaten van proeven waarin de groei en de stikstof balans van varkens voorzien van een PVTC-canule of van een termino-laterale ileo-rectaal anastomose (IRA) werden gemeten. De gevonden waarden werden vergeleken met soortgelijke waarden bepaald in intakte (INT) dieren. PVTC-canulatie resulteerde in een verlaagde dagelijkse groei van ongeveer 7% in vergelijking tot INT-dieren. Dit werd verklaard doordat de chymus, verzameld gedurende de experimentele periode, niet kon bijdragen aan de energievoorziening van het dier. Door de gehanteerde voerstrategie (energieopname werd gebaseerd op $kg^{0.75}$), resulteert een iets lagere groei in minder voer voor het resterende deel van de experimentele periode. Een verlaging van de dagelijkse groei met ongeveer 53%, zoals die gevonden werd in IRA-varkens, wees op een sterke invloed van deze techniek op het energiemetabolisme. Deze resultaten kwamen overeen met de literatuur. De N-balans gaf dezelfde resultaten te zien als de groei. Dit betekent dat de N-balans van PVTC-varkens vergelijkbaar was met die voor INT-varkens. Voor de IRA-varkens was zowel de stikstof uitscheiding met de chymus als met de urine significant hoger dan voor PVTC- of INT-varkens. Dit resulteerde in een aanzienlijk lagere N-balans voor de IRA-varkens.

Aanvullend op deze proeven werden de fermentatie processen in ileum chymus, verzameld *m.b.v. beide methoden onderzocht. Vluchtige vetzuren en diaminopimelinezuur werden bepaald* als maat voor de bacteriële activiteit. De waarden gevonden in PVTC-varkens waren vergelijkbaar met in de literatuur vermelde waarden bepaald in T- of re-entrant gecanuleerde varkens. Cijfers gemeten in chymus van IRA-varkens waren aanzienlijk hoger en kwamen meer overeen met waarden zoals

deze in mest gevonden worden. Dit werd verklaard door een adaptieve verandering van verteringsprocessen in het terminale ileum en/of in het rectum van IRA-varkens.

Omdat bij de PVTC-canulatie het caecum wordt verwijderd en bij de IRA-techniek de vertering in caecum en colon wordt uitgesloten, kan dit een invloed hebben op de balans van met name enige mineralen. Veranderingen in verteringsprocessen kunnen een invloed hebben op bloedparameters. Daarom werd een aantal bloedparameters, zoals creatinine, ureum, natrium, kalium, chloor, calcium, magnesium, zuur-base overschot, pH en aktieve bicarbonaat, gemeten in PVTC- en IRA-varkens en vergeleken met dezelfde parameters gemeten in INT-varkens (Hoofdstuk II). Daarnaast werd in deze dieren de natrium en kalium balans gemeten en vergeleken met een referentie groep van INT-dieren. Bloedparameters gevonden in PVTC-varkens waren gelijk aan die in INT-varkens, met uitzondering van ureum en kalium in week 13 post-operatief. Met betrekking tot ureum en kalium werd geconcludeerd dat deze waarden beïnvloed konden zijn door het verzamelen van chymus dat plaatsvond tot slechts 2 dagen voor het nemen van de bloedmonsters. In vergelijking tot INT-varkens echter, werden in IRA-varkens veranderingen in bloedspiegels van creatinine, ureum, natrium, kalium, zuur-base overschot, pH en aktieve bicarbonaat gevonden. Deze verschillen kunnen verklaard worden door de afwezigheid van de vertering in het caecum en colon. Dit betekent dat de verhoogde ureum concentratie in het bloed van IRA-varkens in week 6 post-operatief een gevolg kan zijn van de afwezigheid van microbiële benutting van ureum in de dikke darm. Na adaptatie aan een verhoogde fermentatie in het terminale ileum en/of het rectum, zou de ammoniak absorptie kunnen afnemen, hetgeen resulteert in een bloedspiegel die gelijk is aan die in INT-varkens in week 13 post-operatief. De verschillen gevonden in IRA-varkens voor het zuur-base overschot, de pH en het gehalte aan aktieve bicarbonaat, kunnen veroorzaakt zijn door een verandering in de natrium- en kaliumbalans. Terwijl de resultaten verkregen met PVTC-varkens in hoge mate vergelijkbaar waren met INT-varkens (behalve voor de natrium uitscheiding door de nieren, welke lager was in PVTC-dieren dan in INT-dieren in week 11 post-operatief), weken de balansen van beide mineralen af in IRA-dieren van die van INT-dieren. Dit betekent dat de natrium uitscheiding in de darm veel hoger was in IRA-varkens dan de uitscheiding met de mest bij PVTC- of INT-varkens. Daarnaast was de uitscheiding van natrium via de nieren aanzienlijk lager voor IRA-varkens dan voor beide andere technieken. De natrium retentie in IRA-varkens was echter negatief in week 11 post-operatief. Vanwege de uitwisseling tegen natrium, verschilde ook de kalium balans in IRA-varkens van die in PVTC- of INT-varkens. Hoewel de uitscheidingen door de nieren verlaagd waren in IRA-varkens, was er een tendens tot een lagere retentie (in % van de opname) dan in de andere behandelingen, hetgeen de samenwerking tussen deze mineralen aangeeft. Verschillen

in de uitscheiding via de nieren van deze mineralen kunnen geassocieerd zijn met een verhoogde aldosteron aktiviteit in IRA-varkens. De gewichten van de bijnieren waren significant hoger voor IRA-varkens dan voor PVTC- of INT-varkens.

In IRA-varkens resulteerde de adaptatie van de dunne darm, evenals de verschillen in mineralen balansen, in verschillende verteerbaarheden voor droge stof, enkele aminozuren, natrium en kalium (Hoofdstuk III). In PVTC-varkens waren verteerbaarheden constant gedurende week 3 tot week 12 post-operatief, behalve voor lysine en natrium, welke licht daalden. Voor IRA-varkens echter, namen de verteerbaarheden toe met de tijd, behalve voor histidine, lysine, methionine, alanine en proline. Evenals in PVTC-varkens daalde de verteerbaarheid van lysine in de tijd. Voor IRA-varkens moeten de veranderingen in verteerbaarheid gezien worden in relatie tot de toename van fermentatie in de dunne darm. De belangrijke rol van de dikke darm voor de absorptie van natrium en kalium blijkt uit de grote verschillen in verteerbaarheden van deze mineralen tussen de gebruikte technieken.

Bij een vergelijking van verteerbaarheidsmetingen in PVTC-varkens met die in T- of re-entrant gecanuleerde varkens, werden verschillen gevonden tussen de re-entrant canulatie enerzijds en PVTC- en T-canulatie anderzijds (Hoofdstuk IV). Wanneer ook in re-entrant varkens de verteerbaarheden werden berekend op basis van 100% chroom recovery, waren de verschillen veel kleiner. De verschillen waren echter nog steeds significant voor N en NDF in het vezelrijk voer en voor N in het semi-synthetische voer. Resultaten van de recovery van merkstoffen toonden aan dat de re-entrant canulatie niet kwantitatief was. Het gebruik van twee merkstoffen gaf aan dat het verzamelen van de vloeibare fase meer kwantitatief was dan het verzamelen van de vaste fase. Dit kwam overeen met de problemen zoals verstoppingen en lekkages, die waargenomen werden voor alle technieken, vooral na het voeren van pectine rijke en vezelrijke rantsoenen.

IRA-varkens toonden een dagelijkse groei die veel lager was dan voor PVTC- of INT-varkens. Omdat in de literatuur aangegeven wordt dat dit een resultaat is van een verlaagde energie metaboliseerbaarheid in combinatie met een verhoogde onderhoudsbehoefte voor energie, worden proefrantsoenen voor IRA-varkens vaak verstrekt met extra gemakkelijk verteerbare koolhydraten zoals saccharose en zetmeel. Om een mogelijke invloed van extra koolhydraten op de verteerbaarheid van het voer te voorkomen, kregen de IRA-varkens die gebruikt werden in onze experimenten deze toevoeging niet. Wanneer echter extra koolhydraten werden toegevoegd aan een op tarwe gebaseerd voer dat aan PVTC-varkens werd verstrekt, werden de schijnbare ileale verteerbaarheid van droge

stof, stikstof en aminozuren niet beïnvloed (Hoofdstuk V). Daaruit werd geconcludeerd dat een toevoeging van een saccharose/zetmeel mengsel aan het proefvoeder bruikbaar kon zijn om de energie-status van de dieren te verbeteren.

Uit de resultaten beschreven in dit proefschrift kan geconcludeerd worden dat de PVTC-canulatie techniek gebruikt kan worden als een betrouwbaar alternatief voor andere verzamelmethoden van dunne darm chymus. Bovendien blijft bij deze nieuwe techniek de fysiologische toestand van de dieren nagenoeg normaal en is de chirurgische ingreep evenals het hanteren van de techniek eenvoudig. De termino-laterale ileo-rectaal anastomose moet gezien worden als een techniek waarbij de fysiologische toestand van de dieren evenals de processen in de darm in een niet te accepteren mate worden aangetast. Daarom zou deze techniek bij verteringsonderzoek niet gebruikt moeten worden. De re-entrant canulatie, die gezien werd als een kwantitative verzamelingsmethode, moet kritisch gebruikt worden, vooral wanneer vezelrijke voeders onderzocht worden. De hier gepresenteerde resultaten bevelen het gebruik van een indicator ook voor deze methode aan. De parameters verkregen m.b.v. de T-canule vertoonden de grootste spreiding tussen dieren. Dit duidt op minder homogene monsters dan verkregen met één van de andere technieken. Rekening houdend met de nadelen die beschreven werden voor de re-entrant en voor de T-canulatie techniek, zou de voorkeur gegeven moeten worden aan de PVTC-canulatie wanneer chymus verzameld moet worden uit het terminale ileum.

Zusammenfassung

Zusammenfassung

Im Hinblick auf eine verbesserte Ernährung von Mensch und Tier gewinnen verdauungsphysiologische Untersuchungen immer mehr an Bedeutung. Während der letzten Jahrzehnte wurden derartige Studien in zunehmendem Maße an Schweinen durchgeführt. Da sich die Verdauungsprozesse im proximalen Teil des Verdauungstraktes grundlegend von denen im distalen Teil unterscheiden, stand die Sammlung von Chymus am terminalen Ileum im Mittelpunkt des Interesses. Da alle Techniken zur Chymussammlung, die an verschiedenen Instituten entwickelt wurden, mit unterschiedlichen Nachteilen behaftet sind, wurde als weitere Alternative die sogenannte Post-Valve T-Caecum (PVTC) Kanulierung am ILOB Institut in Wageningen entwickelt. Eine Beurteilung dieser neuen Technik erfolgte durch vergleichende Untersuchungen mit anderen Techniken und zwar durch einen Vergleich unterschiedlicher verdauungsphysiologischer und stoffwechselphysiologischer Parameter. Die Resultate dieser Studien sind Grundlage der vorliegenden Dissertation.

Im ersten Kapitel wurde der Einfluß der PVTC-Kanulierung und der termino-lateralen Ileo-Rektal Anastomosis (IRA) auf die Wachstumsleistung und die Stickstoffbilanz von wachsenden Schweinen untersucht. Die hierbei erhobenen Daten wurden mit denen intakter Tiere (INT) verglichen. Im Vergleich zu den intakten Tieren wiesen die PVTC Tiere eine um 7% geringere tägliche Zunahme auf. Da der während der Sammelperioden entnommene Chymus keinen weiteren Beitrag zur Energieversorgung des Tieres leistet, wurde diese geringere Zuwachsleistung auf die während des Versuchs durchgeführten Chymussammelperioden zurückgeführt. Im Zusammenwirken mit der in diesem Experiment durchgeführten Fütterungsstrategie (die tägliche Energiezufuhr richtete sich nach dem metabolischem Körpergewicht), resultierte bereits eine geringe anfängliche Wachstumsdepression wieserum in einer geringeren Futtermengenzulage während der weiteren Versuchszeit. Die bei den IRA-Schweinen verzeichnete Wachstumsdepression von 53% weist darauf hin, daß diese Technik den Energiestoffwechsel stark beeinflußt. Diese Ergebnisse stimmen mit denen in der Literatur gefundenen Daten überein. Die Resutate der Stickstoffbilanzen entsprachen denen der Wachstumsleistung, das heißt, der N-Ansatz bei den PVTC-Schweinen war vergleichbar mit dem der INT-Schweinen, während bei den IRA-Schweine aufgrund der signifikant höheren N-Ausscheidungen sowohl im Chymus als auch im Harn der tägliche N-Ansatz entsprechend niedriger war.

Zusätzliche Untersuchungen betrafen die Fermentationsprozesse im Dünndarmchymus. Als indirekte Parameter zur Messung der bakteriellen Aktivität dienten die Gehalte an flüchtigen Fettsäuren und Diaminopimelinsäure. Die Ergebnisse im Chymus der PVTC-Schweine waren

vergleichbar mit den in der Literatur angegebenen Werten. Demgegnüber waren die im Chymus der IRA-Tiere gemessenen Konzentrationen erheblich höher und entsprachen annähernd Vergleichswerten, wie sie in der Literatur für die Faeces angegeben werden. Dies wurde auf eine adaptative Veränderung der Verdauungsprozesse in Ileum und/oder Rektum der IRA-Tiere zurückgeführt.

Während die PVTC-Kanulierung die Entfernung des Caecums erfordert wird bei der IRA-Technik die gesamte Dickdarmverdauung bis hin zum Rektum eleminiert. Die Auswirkungen auf die Natrium- und Kaliumbilanz wurde untersucht und verschiedene Blutkennwerte wurden erfaßt, um Hinweise auf eventuelle Stoffwechselstörungen zu erhalten. In Kapitel 2 wurden folgende Blutkennwerte bei Schweinen mit PVTC-Kanülen, IRA und INT-Tieren miteinander verglichen: Kreatinin, Harnstoff, Natrium, Kalium, Chlorid, Calzium, Magnesium, Basen-Überschuß, pH und aktives Bicarbonat. Zusätzlich wurden Natrium- und Kalium-Bilanzen durchgeführt.

Die Blutkennwerte der PVTC-Tiere waren vergleichbar mit denen der INT-Tiere. Jedoch waren 13 Wochen nach dem operativem Eingriff die Harnstoff- und Kaliumkonzentration im Serum der PVTC-Tiere zum Teil deutlich erhöht. Diese Unterschiede lassen sich möglicherweise durch die nur zwei Tage vor der Blutentnahme abgeschlossene Chymussammlung erklären. Bei den IRA-Tieren wichen folgende Blutkennwerte von denen der INT-Tieren ab: Kreatinin, Harnstoff, Natrium, Kalium, Basen-Überschuß, pH und aktives Bicarbonat. Diese Unterschiede können in erste Linie durch das Fehlen der Dickdarmverdauung bei den IRA-Tieren erklärt werden. Der 6 Wochen nach den Operationen gemessene deutlich höhere Harnstoffgehalt im Serum der IRA-Tiere läßt sich durch die fehlende mikrobielle Nutzung des Ammoniaks im nicht vorhandenen Dickdarm erklären. 13 Wochen nach der Operation wiederum waren die Harnstoffkonzentrationen im Serum der IRA-Tieren mit denen der INT-Tiere jedoch vergleichbar. Dieser Befund läßt sich mit der zunehmenden mikrobiellen Aktivität im Chymus der IRA-Tiere und der damit verbundenen verringerten Ammonium-Absorption erklären. Die im Serum der IRA-Tiere gemessenen abweichenden Werte für den Basenüberschuß, pH und aktives Bicarbonat müssen als Resultat der veränderten Natrium- und Kalium-bilanzen angesehen werden.

Während die Resultate der Natrium-bilanzen bei den PVTC-Tiere weitgehend identisch waren mit denen der INT-Tiere (mit Ausnahme der etwas verringerten renalen Natriumexkretion in der 11. Woche nach den Operationen) wichen die Resultate der IRA-Tiere von denen der INT-Tiere ab. Die IRA-Tieren zeigten eine weit höhere Natriumexkretion mit dem Chymus als dies bei den PVTC- und INT-Tiere mit dem Kot zu verzeichnen war. Zusätzlich war die renale Natriumexkretion bei den

IRA-Tieren erheblich vermindert. Diese Veränderungen führten 11 Wochen nach der Operation zu einer negativen Natrium-bilanz. Im Zusammenhang mit dem Natriumstoffwechsel veränderte sich bei den IRA-Tieren auch die Kalium-bilanz. Obwohl für die IRA-Tiere eine Verringerung der Kaliumexkretion mit dem Urin zu verzeichnen war, lagen die Werte der Kaliumretention (in % der Aufnahme) etwas unter denen der anderen Versuchsgruppen. Die unterschiedliche renale Mineralstoffexkretion der IRA-Tiere läßt sich möglicherweise mit einer veränderten Aldosteronaktivität erklären, da die Nebennierengewichte der IRA-Tiere deutlich höher waren als die der PVTC- oder INT-Tiere.

Bei den IRA-Tieren führten die adaptativen Veränderungen im Darmtrakt als auch die veränderten Mineralstoff-bilanzen zu abweichenden Verdaulichkeiten für die Trockenmasse, einige Aminosäuren, Natrium und Kalium (Kapitel 3). Bei den PVTC-Tieren wurden von der 3. bis zur 12 Woche nach der Operation gleiche Verdaulichkeiten gemessen. Dies galt jedoch nicht für Lysin und Natrium, deren Verdauungskoeffizienten im Zeitverlauf stetig abnahmen. Bei den IRA-Tieren wiederum stiegen die Verdauungskoeffizienten, mit Ausnahme für Histidin, Lysin, Methionin, Alanin und Prolin, im Zeitverlauf an. Wie auch bei den PVTC-Tieren nahm die Lysinverdaulichkeit bei den IRA-Tieren im Zeitverlauf ab. Die Veränderungen der Verdauungskoeffizienten bei den IRA-Tieren steht im direkten Zusammenhang mit der zunehmenden Fermentationsintensität im Darmtrakt. Die extrem unterschiedlichen Mineralstoffverdaulichkeiten, die für die IRA Technik im Vergleich zur PVTC Technik ermittelt wurden, unterstreichen die Bedeutung des Dickdarms im Hinblick auf die Absorption einzelner Mineralstoffe.

Im Kapitel 4 sind die Ergebnisse vergleichender Verdaulichkeitsbestimmungen in Abhängigkeit von der Rationszusammensetzung und der verwendeten Fisteltechnik beschrieben (PVTC-, einfache T- sowie ileo-caecale Brückenkanüle). Hierbei zeigten sich signifikante Unterschiede in der praecaecalen Verdaulichkeit einzelner Nährstoffe, wenn die ileo-caecalen Umleitungstechnik als quantitative Sammeltechnik verwendet wurde. Wurden die Verdaulichkeiten jedoch in gleicher Weise wie bei der PVTC- und T-Kanülen-Technik auf der Basis der Chrom-Wiederfindungsrate berechnet, so verringerten sich die Differenzen mit Ausnahme für Stickstoff und NDF in der rohfaser-reichen Ration sowie für Stickstoff in der semisynthetischen Ration. Die Wiederfindungsraten der Verdaulichkeitsmarker zeigten, daß die Chymussammlung mit der Umleitungstechnik nicht quantitativ war.

Bei den IRA-Tiere zeigten sich wesentlich geringere tägliche Zunahmen im Vergleich zu den PVTC- oder den INT-Tiere. In der Literatur wird dies mit einer verminderten Umsetzbarkeit der

Energie sowie mit einem erhöhten Energieerhaltungbedarf erklärt. Aus diesem Grunde wird eine Ergänzung der Versuchsration mit leicht löslichen Kohlenhydraten, wie z.B. Saccharose und Stärke empfohlen. Um einen möglichen Einfluß einer solchen Ergänzung auf die Verdaulichkeitsmessung auszuschließen wurde in den vorliegenden Versuchen mit IRA-Tieren auf eine solche Supplementierung verzichtet. Aus den Resultaten in Kapitel 5 geht jedoch hervor, daß die Supplementierung einer Weizenration mit zusätzlichen Kohlenhydraten keinen Einfluß auf die scheinbare precaecale Verdaulichkeit der Trockenmasse, des Stickstoffs sowie der Aminosäuren hat. Daraus läßt sich ableiten, daß eine Ergänzung der Versuchsration mit einer Saccharose/Stärke Mischung eine geeignete Maßnahme zur Verbesserung des Energiestatus der Tiere darstellt.

Auf Grund der in dieser Dissertation dargelegten Resultate kann die PVTC-Kanulierung als geeignete Alternative zur Sammlung von Chymus am terminalen Ileum angesehen werden. Darüber hinaus wird der physiologische Zustand des Tieres kaum verändert und die Operationmethodik sowie die Handhabung der Tiere sind vergleichsweise einfach. Die termino-laterale Ileo-Rektal Anastomosis hat einen erheblichen Einfluß auf den physiologischen Zustand des Tieres. Aus diesem Grund ist eine weitere Verwendung dieser Methode mit Einschränkungen zu versehen. Die Umleitungstechnik kann ihrem Anspruch, eine ausschließlich quantitative Methode zur Sammlung von Dünndarmchymus zu sein, nicht uneingeschränkt gerecht werden. Der Einsatz eines Verdaulichkeitsmarkers empfiehlt sich insbesondere beim Einsatz rohfaserreicher Rationen. Die mit der einfachen T-Kanülentechnik gemessenen Verdauungswerte zeigten die größte Variation zwischen den Tieren, was auf eine vergleichsweise geringere Homogenität der Proben hinweist. Unter Berücksichtigung der genannten Nachteile der konventionellen T- Kanülentechnik und der Umleitungstechnik empfiehlt sich die Verwendung der PVTC-Kanulierung zur Sammlung von precaecalem Dünndarmchymus als sinnvolle Alternative.

Curriculum vitae

Torsten Uwe Köhler werd op 17 mei 1960 geboren te Glückstadt. Hij behaalde in Juni 1980 de "Allgemeine Hochschulreife" aan het Fachgymnasium für Sozialwirtschaft te Kiel. In Oktober 1981 begon hij met zijn studie Agrarwissenschaften aan de Universiteit te Kiel met Veevoeding, Veehouderij, Veefokkerij and Huisdiergenetica als hoofdvakken en met Zoötechnische Economie, Landbouwtechniek en Graslandkunde als bijvakken. Na het afstuderen in Februari 1988 begon hij in April 1988 bij de Vakgroep Veevoeding als wetenschappelijk medewerker en hij werkte aan het onderzoek dat resulteerde in dit proefschrift.