METHODOLOGICAL ASPECTS OF THE *IN VIVO* MEASUREMENT OF ILEAL AMINO ACID DIGESTIBILITY IN PIGS — A REVIEW —

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Summary

Much recent research on protein and amino acid (AA) digestive physiology of pigs has been concerned with measurement of the ileal apparent and true digestion and absorption. For measurement of the ileal apparent digestibility of AA, the steered ileo-caecal valve cannulation (SICV) and the ileo-rectal anastomosis (IRA) techniques appear to be the more reliable and simple methods, when compared with any methods requiring use of a marker for calculation of digestibility, or with the complex techniques of ileo-caecal re-entrant cannula (ICRC) and the postvalve ileo-colic re-entrant cannula (IPVC). On the other hand, the peptide alimentation ultrafiltration methods might be a better choice for measurement of the ileal endogenous nitrogen (N) and AA flow in a routine feedstuff analysis, although the classical method of ^{15}N-isotope dilution method is still a standard method for N and AA nutrition research in pigs.
(**Key Words**: Amino Acid, Digestibility, Pigs)

Introduction

Animals require balanced diets that provide an adequate level of all nutrients, including amino acids (AA). In order to provide an adequate, but not excessive level of AA, ileal digestible instead of total or faecal digestible AA supply should be considered (Sauer and Ozimek, 1986). In the last three decades, the AA digestive physiology of pigs has become the focus of interest of research in the developed and developing countries, and there have been major developments in our understanding of pig physiology. This is of benefit, not only to animal production, but also to basic science and as a model for research on problems of human nutrition.

Excellent reviews on the concept of digestible AA, measurement techniques and their use in practice have been previously presented by Low (1982), Sauer and Ozimek (1986) and Bock et al. (1989). However, since that time, there have been major developments in this area.

The purpose of this review is to provide a detailed outline of the novel techniques of determination of the ileal digestibility of AA (e.g. the SICV, peptide alimentation ultrafitration, ^{15}N-isotope dillution, IRA) and methods of measurement of the ileal endogenous N and AA flow for calculation of the true digestibility of AA.

Research on the Ileal Digestibility of AA Using the Cannulation and Anastomosis Methods

It is well established that the microflora in the large intestine ferment undigested dietary protein residues to yield products of no nutritional value to the pig; this has led to the view that the apparent digestion of AA is better estimated in the terminal ileum than in faeces (Zebrowska, 1973). Based on this consideration, much research on methods for collecting the ileal digesta has been done in recent years.

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Cannulation techniques

The classical methods of the ileal simple "T" cannula and the ileo-caecal re-entrant cannual (ICRC) have been introduced by Low (1980). Compared with the ileal simple "T" cannula, the ICRC method is more accurate in measuring nutrient digestibilities, since it involves the total collection of the ileal digesta and obviates the use of a marker such as chromic oxide. The use of a marker is likely to introduce errors with heterogeneous digesta. A major disadvantage of the ICRC method is that the small intestine is severed leading to possible blockage of the cannula with coarsely ground or fibrous materials, particulary with re-entrant cannulas inserted in the terminal ileum. The advantages of using the simple cannula technique are that simpler surgical procedures and post-operative animal care are required (Yin et al., 1991).

During the past years, a number of investigations have been carried out to compare the ileal digestibility of AA in pigs fitted with the ICRC versus simple cannulas. Studies by Zebrowska et al. (1977) showed no differences in most instances; however, there were significant differences ($p < 0.05$) in 12 out of 72 comparisons (for different diets, 18 AA). Taverner et al. (1983) found no differences ($p > 0.05$) in apparent ileal digestibility of AA of DM and N in pigs fitted with ICRC and simple "T" cannulas, when wheat, wheat-lupin or wheat-meat and bone meal diets were used in their experiment. Schroeder et al. (1989) reported that there were remarkable differences in ileal digestibilities of N and DM between the ICRC and "T" cannula methods, using diets based on barley, wheat, maize and a commercial diet. This was proved by a higher chromic oxide and a lower N concentration in the digesta of the spotsampling ("T" cannula method), which showed, unlike quantitative sampling, higher digestibility values. Koehler et al. (1990) found also somewhat lower Cr recovery with the simple "T" cannula pigs than with the ICRC cannula pigs, and the standard error of digestibility data was greater for the simple "T" cannula pigs. Yin et al. (1991) reported that, with the exception of one maize diet and maize plus peanut meal diet, the "T" cannula method gave higher ($p < 0.05$) digestibility values for DM, N and AA in a commercial diet and in diets containing oil-tea camellia cake, rapeseed meal, or a high lysine maize than those determined with the ICRC method. Conversely, the digestibility values of neutral detergent fibre, acid detergent fibre and acid detergent lignin measured with the "T" cannula method were considerably lower than those measured with the ICRC technique.

In order to overcome the disadvantages of the classical cannula methods, Darcy et al. (1980) developed the ileocolic post-valvular procedure (IPVC). This method preserves the functional role of the ileocaecal sphincter and allows for sampling of ileal digesta according to their normal arrival in the large intestine. With this method it is possible to measure the ileal nutrient digestibilities in fibre rich diets that have not been finely ground, which can not be done with the ICRC technique because blockage of the flow of digesta may occur in the ICRC cannulated pigs. However, no direct comparisons between the ICRC and IPVC methods have been found in the literature. Darcy (1989) reported that the IPVC method, like the ICRC cannula method, is too difficult and time-consuming for routine measurements. So, up to now, the methods most commonly used in practical feedstuff analysis are still the simple "T" cannula technique and sometimes the ICRC cannula technique.

Leeuwen et al. (1988, 1991) developed the post valve "T" caecum technique (PVTC). With this technique the anatomy of the transition from ileum into caecum-colon is used. Normally, the ileo-caecal valve protrudes into the caecum. In the PVTC method, the caecum is partially removed and replaced by the PVTC cannula, which is positioned opposite to the valve. When the cannula is opened, the valve protrudes into the cannula and thus digesta can be collected. Because the cannula is located in the colon, effects on motility in the ileum may be minimized. PVTC - caecum cannulated pigs can be used over a long period without signs of discomfort. Digesta collection with PVTC cannula pigs is nearly quantitative, but a marker is needed. This method is widely used in Holland and Belgium (Leterme et al., 1994; Schulze et al., 1994). In the literature, however, only one comparision experiment was reported (Koehler et al., 1990). They reported that the digestibilities of DM and cell wall constituents in a control diet containing of 23.6% corn, 19 % barley, 30% wheat and 18.4% soya bean meal, pectin-rich diet, crude fibre-rich diet and semisynthetic diet measured with the ICRC collection method were sometimes significantly higher ($p < 0.05$) than with the PVTC and the simple T cannula methods. But the N digestibility measured with the PVTC in the fibre-rich and semisynthetic diets was higher than that with the ICRC method. However, after correction to the 100% chromic oxide recovery for the simple "T" cannula and PVTC methods, the differences measured with the three different techniques were reduced to less than 5%. However, the results showed less variation wih the PVTC pigs compared to pigs fitted with simple T - cannulas.

The advantages of the PVTC method include: (1) no interference with the intestinal wall, ileal-caecal valve, or colon; (2) the ability to replace cannulas as the pig

develops; (3) the almost complete collection of digesta (Koehler et al., 1994).

A more recent and improved method for the quantitative collection of the ileal digesta developed from the PVTC cannula is the steered ileo-caecal valve cannula (SICV) technique (Mroz et al., 1994). The steering system consists of two stainless steel rings of which one (outer) is placed around the intestine, close to its junction with the caecum, and the second ring (inner), attached to a nylon cord is inserted into the lumen of the intestine. Since the outer ring is slightly smaller than the inner ring, by pulling or releasing the nylon cord bound to the inner ring, the ileo-caecal valve can be steered as required (Mroz, et al., 1994). The SICV technique appeared to be suitable for long term studies of the ileal digestibility of diets rich in cellulose or not. For example, although high amounts of soyabean hulls (317 g/kg) and pure cellulose (312 g/kg) were included in the experimental diets which were fed at libitum (Mroz et al., 1994). there were no blockages/obstructions in the flow of ileal digesta, which might occur in the ICRC method. Moreover, the SICV method might cause less discomfort to the animals and the surgery is much easier to perform compared to the ICRC method. Therefore on this basis, and in view of the accuracy and simplicity of handling, the SICV cannula may be a good alternative to the other cannulation methods, although further research is needed to comfirm these advantages of the SICV technique.

Ileo-rectal anastomosis method

The ileo-rectal anastomosis (IRA) method, which consists of linking by surgery the extremity of the small intestine to the rectum end to side or end to end, facilitates total collection of digesta at the distal ileum, without disturbance to the animal. There are less disadvantages than the cannulation methods. (Souffrant et al., 1985; Hennig et al., 1986; Darcy et al., 1990 and Yin et al., 1993).

However, there is also some controversy about the IRA method. First the colon of the IRA pigs has no absorption digestive function in context to the absorption of water, mineral elements and energy in normal pigs.

TABLE 1. APPARENT ILEAL DIGESTIBILITIES (%) OF NUTREINTS FOR DIETS IN PIGS WITH THE ILEORECTAL ANASTOMOSIS (IRA) AND ILEO-CAECAL RE-ENTRANT CANNULA (ICRC: HENNIG ET AL., 1991 AND YIN ET AL., 1993)

Diets	Organic matter		Crude protein		Energy		Dry matter	
	IRA	ICRC	IRA	ICRC	IRA	ICRC	IRA	ICRC
1. Barley 1 + Lupin 1	76	70	77	75				
2. Barley 1 + Lupin 2	73	70	75	77				
3. Lupin 1	80	82	84	86				
4. Lupin 2	70	64*	83	80*				
5. Field bean 1	83	75*	78	73				
6. Field 2	84	77	80	76				
7. Rapeseed meal	72	73	73	69				
8. Mixture A	82	81	81	81				
9. Mixture B	82	81	80	78				
10. N-free mixture	77	75						
11. **Soyabean meal	81	75	81	70*	77	73	79	72*
12. **Rapeseed meal	80	73*	72	72	74	72	79	79*
13. **Commercial diet	82	72	82	73*	76	77	79	72
14. **Fish meal	92	91	89	85	91	91	88	89
15. **Raw soyabean	83	83	60	59	80	80	81	82
16. **Single cell protein	79	79	67	66	73	77	74	74

* Values of IRA and ICRC were significantly different ($p < 0.05$).

** Diets from Yin et al. (1993).

This bypass, instead, may affect the physiological state of the animal and may cause some compensatory adaptation of the small intestine (Koehler et al., 1990). Second, in either the end to end or the end to side IRA method there might be a role of digesta reflux, a possibility which needs to be more thoroughly examined (Fuller et al., 1994). Finally, the IRA technique should be questioned as a routine means of collecting ileal digesta from the ethical standpoint (Koehler et al., 1991). However Souffrant et al. (1985) and Hennig et al. (1986) reported, from X-ray examination of pigs prepared with the end to side anastomosis, that there was little reflux of digesta into the descending colon and no physiological histological changes found in the IRA pigs, compared with the intact pigs. Yin et al. (1993) reported that there were no differences in growth and development or weight and bulk of the digestive tract organs between IRA pigs and intact pigs. Despite the loss of function of the colon, it would not affect nutrient digestion anterior to the ileum, especially for measurement of ileal digestibility of AA which are almost absorbed at the end of ileum (Zebrowska, 1973). In order to correct for loss of the colon function, extra water, sodium chloride (5 g kg^{-1} diet), sodium bicarbonate (5 g kg^{-1} diet), mineral elements and a vitamin mix (1 g kg^{-1} diet) were provided in diets during the experiments (Souffrant et al., 1985; Henning et al., 1986; Yin et al., 1993).

TABLE 2. APPARENT ILEAL DIGESTIBILITIES OF AMINO ACIDS WITH SIGNIFICANT ($p < 0.05$) DIFFERENCES IN 16 KINDS OF FEEDSTUFFS (HENNIG ET AL., 1992; YIN ET AL., 1993)

I		II	
IRA<ICRC	Diets	IRA>ICRC	Diets
Met	1, 2, 3, 5, 6, 10	Phe	1, 4, 8, 10
Lys	1, 2	Lys	4, 8
Ile	2, 8	Tm	4, 8, 10
Leu	2	Thr	10
Val	2	Arg	10
Ala	2	His	10
		Glu	4, 13
		Gly	4, 9
Asp	2	Asp	10
Pro	8	Pro	5
		Val	13
		Ala	13

I. Digestibility values in IRA pigs lower than in the ICRC pigs (Hennig et al., 1992; Yin et al., 1993).

II. Digestibility values in IRA pigs higher than in the ICRC pigs (Henning et al., 1992; Yin et al., 1993).

Different results have been reported from different laboratories. Fuller et al. (1994) found that the AA digestibility in diets containing barley, dried milk or a mixture of the two determined with the IRA pigs of 6 months after the surgery were lower than those determined with the IPVC pigs, but there was no significant difference in digestibility of AA between the IRA pigs of 3 weeks after surgery and the IPVC pigs. Darcy et al. (1990) found, with a standard cereal-based diet and two semi-synthetic diets enriched with either wheat bran or beet pulp fed to the IRA and the IPVC pigs, that there were no significant differences ($p > 0.05$) in the total N and AA digestibilities between the IRA and IPVC pigs fed the standard or wheat bran diet. But on the beet pulp diet, the IPVC method resulted in a significantly higher digestibility. They suggested that this was probably due to the by-pass of the ileo-caecal-colic sphincter in the IRA pigs. However, further investigation is needed to identify the reason for the differences.

Leterme et al. (1990) and Pascal et al. (1990) found that the digestibility data determined with the IRA technique, including AA and cell wall constituents, were more reliable than those determined with the cannulation techniques. More recently, Hennig et al. (1991) and Yin et al. (1993) reported that there were only minor differences in the determination of the ileal nutrients digestibilities between the IRA and IRRC methods (table 1 and table 2), according to their extensive experiments on a wide variety of feedstuffs.

Techniques for the Measurement of the True Ileal Digestibility of AA

^{15}N-Isotope dilution method

Quantification of the endogenous AA flow at the terminal ileum is not only important for the determination of AA requirement, but also for calculation of the true ileal digestibility of AA, using the following formula.

Ileal true digestibility of AA =

$$\frac{\text{dietary AA} - (\text{ileal AA-endogenous AA})}{\text{dietary AA}} \times \frac{100}{1}$$

Using conventional methods, the endogenous AA recovery at the end of ileum cannot be quantified when a protein-containing diet is fed. So, up to now, most of the ileal or faecal digestibilities of AA have been determined with the classical N-free method. This approach, however, is open to criticism (Low, 1980) due to the unphysiological nature of the protein-free state.

Souffrant et al. (1986) reported that the nondigested

dietary protein could be differentiated from the endogenous protein derived from the animal digestive tract by using the ^{15}N-isotope dilution technique. This technique is based on labelling the precursor pool of endogenous N by infusion of a ^{15}N-leucine solution into the peripheral blood. After a period of 8 days, a plateau was reached and the enrichment of endogenous N was assumed to be equal to that in the trichloracetic acid (TCA) soluble fraction of the blood. From the ratio between enrichment in the TCA-soluble fraction in blood and enrichment in the digesta, the endogenous part of N in the digesta can be derived.

In recent years, de Lange et al. (1989, 1990, 1992) further developed this method. They found that: 1. The AA composition of endogenous protein determined in pigs fed a protein-free diet and parentally administered with AA should provide a better estimation for the calculation of true AA digestibility, when based on the determination of the true N digestibility by the ^{15}N-isotope dilution technique; 2. Small but significant differences ($p < 0.05$) in AA composition of the endogenous protein were observed, when different fibre levels and sources of protein-free diets were fed, although the amounts of endogenous protein recovered in the digesta collected from the distal ileum were similar (25.5 to 27.7 g kg^{-1} dry matter intake for 4 different diets); 3. The recoveries of endogenous protein in ileal digesta and the true ileal protein and AA digestibilities were higher than those determined by feeding the protein-free diets.

Oral administration of the stable isotope technique is commonly used in human nutrition studies. In pig nutrition research, van Leeuwen (1994) used this method for the study of AA metabolism. They observed that the enrichments in the muscle tissue did not reveal significant differences between the ^{15}N oral intake and infusion techniques, but there was a clear difference in the enrichment of ileal digesta. In the animals dosed orally with ^{15}N, the ^{15}N enrichment was two to three times higher than in those dosed parentally. But there were no differences of the ileal true digestibility values of AA calculated based on enrichment of the TCA soluble fraction of the blood plasma for the group with the ^{15}N infusion and on a steady state model with N in digesta as end product for the group with the orally administered ^{15}N. Both methods resulted in similar estimates for true digestibility of N.

But, Leterme et al. (1994) reported that the ^{15}N-isotope infusion method seemed to overestimate the endogenous losses at the ileum of pigs whereas the ^{15}N-labelled diets seemed to underestimate them. Leterme et al. (1994) suggested that the rate of recycling in all of the endogenous secretions of the ^{15}N-infusion would be refined.

In order to refine the ^{15}N-isotope dilution technique, Lien et al. (1994) studied the contribution of endogenous protein to total protein recovered at the distal ileum of pigs fed protein containing diets. They found that enrichments in some endogenous AA were overestimated in blood samples taken only at feeding. They confirmed again that the ^{15}N-isotope dilution method could provide a means of accurately assessing the recovery of endogenous AA in ileal digesta of pigs fed protein-containing diets. Moreover, the use of the ^{15}N- leucine dilution technique allowed for a sensitive means of determining the effect of diet manipulation on the composition of the endogenous protein.

Although the ^{15}N-isotope technique can be used to quantify the endogenous protein flow at the distal ileum of pigs, there are problems with this technique: (a) the surgery is complex; (b) the collection procedure is labour-intensive and complex; (c) a high level of hygiene is required especially at the time of ^{15}N-leucine infusion; (d) the ^{15}N-leucine is very expensive. However, the ^{15}N labelled feedstuff and the orally alimentated ^{15}N technique are much more expensive than that of the the ^{15}N-leucine dilution methods.

Peptide alimentation and ultrafitration method

The peptide alimentation ultrafiltration method developed inecent years might be used in routine feedstuff analysis. In this techniqe, the animal is fed a semi-synthetic diet containing enzymatically hydrolyzed casein as its sole nitrogen source; ileal digesta are collected and the N fraction is separated physically using a large-volume disposable Centriprep- 10 Ultrafiltration device. The high molecular weight (MW>10,000 Da) fraction resulting from the ultrafiltration provides a measure of endogenous AA flow. If some of the dietary AA and small peptides are not absorbed, they will be removed in the low molecular weight fraction (Moughan et al., 1990).

Butts et al. (1991, 1993) found that the enzymatically hydrolysed casein diet had a significantly higher level of endogenous AA flow than the protein-free diet fed to rats and pigs. They suggested that the enzymatically hydrolysed casein alimentation ultrafiltration method could be used in practical feedstuff analysis.

Efficiency of the peptide alimentation ultrafiltration method was compared with the traditional N-isotope dilution method by Schulz et al. (1994). They found that the endogenous ileal N flows were 3.12 and 3.64 g/kg dry matter intake ($p > 0.05$) respectively for the ^{15}N-isotope dilution and the peptide alimentation ultrafitration method. The proportion of endogenous N in the total N flow

passing the terminal ileum was 71 and 84% ($p > 0.05$) for the N-isotope dilution method and the peptide alimentation ultrafiltration method, respectively. They concluded that both methods were valuable approaches for measuring endogenous ileal N losses.

However, Leterme et al. (1994) reported that the endogenous N secretion was increased by the supplementation of the hydrolysed casein. They explained that it seemed that the hydrolyzed casein was not completely digested and the use of ultrafitration for the removal of the molecules with a molecular weight lower than 10 kDa was not appropriate. More than 20% of the endogenous N which reach the end of the ileum have a molecular weight lower than 10 kDa. The use of filters with a lower cut-off or a source of completely digestible peptides would be better suited to the objective of measuring the endogenous flow of AA and peptides in normal protein nutrition (Leterme et al., 1994).

Although it is not clear whether the peptide alimentation ultrafiltration method can be used for accurately measuring the ileal endogenous AA or not, it appears to have value for measuring endogenous ileal AA losses.

Overall Assessment

Collection of digesta from a simple "T"-cannula and PVTC is associated with doubt about obtaining representative samples of digesta and recovery of the marker, especially with a high-fibre diets. The quantitative techniques, however, such as the ICRC and IPVC cannulations may lead to disturbances of gastro- intestinal motility, blockages of digesta flow are time-consuming for routine measurements and the surgery is difficult.

The SICV technique may overcome some of these disadvantages. However, in comparison with the IRA technique, in which no cannula is needed, the SICV technique is more complex. With the IRA technique, specially for pigs a long time after surgery, (i.e. 6 months), the physiological changes must be taken into consideration.

Methodological aspects relating to the determination of the endogenous N and AA flow at the distal ileum were also discussed. There is a need for further studies on this topic, especially for the peptide alimentation ultrafiltration technique which might be used in the routine true AA digestibility measurement, if some questions are clarified.

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