Nitrogen metabolism in gastrointestinal tissue of the pig

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The protein content of the gastrointestinal tract is not more than 7 % of whole-body protein, while the amount of protein synthesized daily exceeds 20 % of whole-body protein synthesis. Considering this active protein metabolism in the gastrointestinal tract as well as the role of the gastrointestinal tract in digestion and absorption, we have investigated fluxes of N and amino acids (AA) in relation to diet composition. This work was carried out within the framework of looking at possibilities of further increasing the efficiency of N utilization in growing pigs, which has both economic and environmental implications.

The present article reviews N metabolism in the gastrointestinal tract of the pig and fluxes of N and AA after digestion and absorption by the gastrointestinal tract in relation to diet composition. AA and peptide transport by the intestine has been reviewed recently (Daniel *et al.* 1994; Munck & Munck, 1994; Mailliard *et al.* 1995) and is not considered here.

Measurements of nitrogen metabolism in the pig

In the pig, information relating to N metabolism in gastrointestinal tissues is mostly derived from *in vitro* measurements using isolated enterocytes and *in vivo* measurements of the net flux of nutrients across the gastrointestinal tract. For nutrient digestion and absorption, the mucosa of the small intestine, and the enterocytes in particular, are crucial. It is obvious that *in vitro* research has focused on enterocytes. However, it is relevant that these epithelial cells only form part of the small intestine, and that the gastrointestinal tract is more than only the small intestine.

Measurement of the net flux of nutrients across the gastrointestinal tract requires chronic indwelling catheters in the portal vein and in an artery (Rérat *et al.* 1980; Yen & Killefer, 1987; van Leeuwen *et al.* 1995). Portal–arterial concentration differences of a nutrient are measured and multiplied by the corresponding portal vein blood flow or plasma flow to give an estimate of the combined effects of absorption and metabolism by the gastrointestinal tissue. A positive net flux indicates absorption from the gastrointestinal lumen in excess of (an unknown amount of) metabolism in gastrointestinal tissues. A negative net flux indicates metabolism in gastrointestinal tissues in excess of absorption from the gastrointestinal lumen, or transport into the gastrointestinal lumen. Since the portal vein drains not only the stomach, the small intestine and the large intestine, but also the spleen and the pancreas, the net flux across the gastrointestinal tract comprises all the portal-drained viscera (PDV), rather than the gastrointestinal tract tissues only.

PROTEIN AND AMINO ACID METABOLISM

Gastrointestinal protein synthesis

As stated previously, protein metabolism in gastrointestinal tissues is very active. Gastrointestinal tissues exhibit one of the highest fractional protein synthesis rates, (the

proportion of the protein pool synthesized daily). In growing pigs the fractional rate of protein synthesis in the gastrointestinal tract varies from 18 to 42 %/d, and is much higher than the fractional synthesis rate of skeletal muscle (Table 1). Protein in the gastrointestinal tract accounts for only 6–7 % of whole-body protein. However, the amount of protein synthesized daily in the gastrointestinal tract is more than 20 % of whole-body protein synthesis, of which the small intestine accounts for the largest part (64 %; Table 1). Within the small intestine the rate of protein synthesis is higher in the mucosa than in the serosa (McNurlan *et al.* 1979). This higher protein synthesis rate is indicative of the high cell turnover in the mucosa and also of the intracellular synthesis of secretory proteins (McNurlan *et al.* 1979; Lobley, 1993). *In vitro*, approximately 10 % of absorbed AA are incorporated into mucosal protein (Bronk & Parsons, 1966). AA absorbed from the intestinal lumen are utilized more readily than plasma AA (Hirschfield & Kern, 1969) and provide AA for protein synthesis in the villus region, while AA from plasma supply the crypt region (Alpers, 1972).

The high protein synthesis rate of the gastrointestinal tissue can be explained partly by the synthesis of so-called endogenous proteins. Endogenous proteins are defined as proteins which are synthesized by the animal and are secreted into the lumen of the gastrointestinal tract. Endogenous proteins are not synthesized only by gastrointestinal tissues. In addition to gastric and intestinal juice and mucosa, proteins are secreted in saliva, bile and pancreatic juice. Intestinal juice accounts for approximately 70 % of endogenous N entering the lumen of the small intestine (Low & Zebrowska, 1989). The process of synthesis and secretion of endogenous proteins is largely influenced by feeding level (DM content) and diet composition. Anti-nutritional factors (protease inhibitors, lectins and tannins) and NSP can increase the synthesis and secretion of endogenous proteins in the digestive tract (Table 2). There is evidence that a significant amount of the endogenous proteins (70–79 %) is re-absorbed in the small intestine (Krawielitzki *et al.* 1990; Souffrant *et al.* 1993).

It can be concluded that both feeding level and diet composition influence protein synthesis in the gastrointestinal tract.

Glutamine metabolism

Glutamine is the most important respiratory fuel for intestinal cells (for review, see Duée *et al.* 1995). Measurements of arterial–venous differences across autoperfused jejunal

Table 1. *Protein content, fractional protein synthesis rate and amount of protein synthesized in various organs of a 44 kg pig* (Data taken from Simon, 1989)

	Protein content (g)	Fractional protein synthesis rate (%/d)	Protein synthesized (g/d)
Liver	211	19·7	41·6
Pancreas	21	81·7	17·2
Stomach	49	18·1	8·9
Small intestine	135	37·5	50·6
Caecum	8	42·1	3·4
Colon	54	30·8	16·6
Kidney	27	12·8	3·5
Skeletal muscle	2828	3·9	110·3
Heart	23	5·3	1·2
Skin	399	6·2	24·7

Table 2. *Effect of diet composition on ileal flow of endogenous crude protein (nitrogen × 6·25) in piglets (10–25 kg) as determined by the ^{15}N dilution technique*

Diet and protein source	Endogenous protein (g/kg DM intake)	Reference
N-free diet	7·5	Butts *et al.* (1993)
Soyabean concentrate:		Jansman *et al.* (1994)
Alone	15·0	
+ Soyabean lectins (mg/g): 0·16	19·5	
0·96	22·0	
Soyabean concentrate:		Schulze *et al.* (1995)
Alone	17·1	
+ Purified NDF (g): 155	20·1	
200	24·1	
+ Wheat bran (g): 300	23·3	
+ Sunflower hulls (g): 198	21·1	

NDF, neutral-detergent fibre.

segments of rats have shown that glutamine accounts for 77 % of total CO_2 production in the fed state and 35 % in the post-absorptive state (Windmueller & Spaeth, 1978,1980). Glutamine is taken up both from the intestinal lumen and from the arterial blood, especially from plasma because glutamine concentrations of erythrocytes circulating through the splanchnic bed are unchanged (Windmueller & Spaeth, 1975). Not only rat enterocytes but also rat colonocytes use glutamine as a fuel, although the latter prefer butyrate (Watford *et al.* 1979; Ardawi & Newsholme, 1985). The role of glutamine in the gastrointestinal tract is not limited to being a source of energy; in terms of N metabolism, glutamine provides amide-N for nucleotide synthesis and generates various nitrogenous substrates, e.g. alanine, proline and citrulline (Windmueller & Spaeth, 1975,1978).

In the growing pig the PDV flux of glutamine (and usually glutamic acid) is negative after feeding a wide range of diets: (semi)synthetic diets (Vaugelade *et al.* 1994; Prior & Gross, 1995), different amounts and sources of protein (Simoes Nunes *et al.* 1991; Rérat *et al.* 1992), different sources of starch (van der Meulen *et al.* 1997), or different amounts of fibre (Lenis *et al.* 1996). This indicates that in the pig there is a high utilization by the PDV of glutamine and glutamic acid originating from the intestinal lumen and the circulation. *In vitro*, enterocytes isolated from pigs of various ages (newborn up to 20 weeks old) oxidize glutamine (Posho *et al.* 1994; Vaugelade *et al.* 1994; Wu *et al.* 1994*b*,1995). In enterocytes isolated from 0- to 2-d-old pigs incubated with both glutamine and glucose, glutamine generates 1·4- to 3·8-fold higher amounts of ATP than glucose (Posho *et al.* 1994; Wu *et al.* 1995), indicating that glutamine is a preferred fuel for enterocytes of neonatal pigs compared with glucose (Wu *et al.* 1995). In enterocytes isolated from 14- and 21-d-old pigs, glutamine contributes less ATP than glucose in the presence of both glutamine and glucose, indicating that in older pigs intracellular fuels (potentially lipids) are used by the enterocytes, or that there is a diminished preference by enterocytes for glutamine as a fuel compared with glucose (Wu *et al.* 1995). Data from enterocytes isolated from growing pigs also indicate that glutamine may be less essential for the pig intestine, since after a 3 d fasting period glucose metabolism accounts for up to 47 % of the ATP, while the contribution of glutamine is only 36 % (Vaugelade *et al.* 1994). Glutamine is not only oxidized, but in terms of N metabolism it is mainly metabolized to NH_3, glutamic acid,

alanine, aspartic acid, citrulline, ornithine and proline (Posho *et al.* 1994; Vaugelade *et al.* 1994; Wu *et al.* 1994*b*,1995).

Colonocytes isolated from growing pigs also metabolize glutamine, yielding NH_3, glutamic acid, aspartic acid and alanine. However, oxidation of glutamine is limited since butyrate is the obligatory energy substrate for colonocytes (Darcy-Vrillon *et al.* 1993).

In vivo, synthesis of ornithine and citrulline by the enterocytes is shown by a positive PDV flux of ornithine and citrulline, both in the fed (Simoes Nunes *et al.* 1991; Rérat *et al.* 1992; Lenis *et al.* 1996; van der Meulen *et al.* 1997) and post-absorptive state (Wu *et al.* 1994*a*). In the post-absorptive state there is also a positive PDV flux of alanine, arginine, aspartic acid, glutamic acid and proline (Wu *et al.* 1994*a*). A high portal absorption coefficient for alanine (the PDV flux of alanine divided by the amount of alanine ingested × 100) reflects the synthesis of alanine by the PDV (Rérat *et al.* 1992; Prior & Gross, 1995).

It can be concluded that both *in vitro* and *in vivo* studies indicate that glutamine is metabolized to a great extent by gastrointestinal tissue to NH_3, glutamic acid, alanine and aspartic acid with ornithine, citrulline and proline being minor nitrogenous products. However, except for the neonatal pig, glutamine may be less essential as fuel for the pig intestine than for the rat intestine.

Arginine metabolism

Arginine is an important AA as it is a constituent of many proteins and involved in the synthesis of other biologically-important molecules, e.g. NO (a vasodilator, a neurotransmitter and a mediator of the immune response; Moncada & Higgs, 1993), creatine phosphate (important for skeletal muscle function; Reeds *et al.* 1993) and polyamines (essential for growth and differentiation of intestinal epithelial cells; Johnson, 1988). Enterocytes of rats have a high capacity for arginine catabolism, resulting in citrulline and ornithine, while the catabolism of arginine to polyamines is negligible (Blachier *et al.* 1991*a*). This is in line with the observation that there is a low rate of arginine appearance in the portal vein after lumen perfusion of arginine into the isolated rat jejunum (Windmueller & Spaeth, 1976).

As for rats, catabolism of arginine to citrulline (Blachier *et al.* 1991*b*) and NO (M'Rabet-Touil *et al.* 1993) has been demonstrated in isolated enterocytes of growing pigs. The synthesis of polyamines from arginine or glutamine is negligible in enterocytes isolated from newborn pigs (Blachier *et al.* 1992). However, in the pig, PDV flux of citrulline is not affected by levels of dietary arginine (Gross & Prior, 1995), and low portal absorption coefficients for arginine have not been reported in the pig. Furthermore, in the post-absorptive state a positive PDV flux of arginine has been reported (Wu *et al.* 1994*a*). This finding corresponds with the *in vitro* synthesis of arginine from glutamine by enterocytes isolated from 0- to 7-d-old pigs (Blachier *et al.* 1993; Wu *et al.* 1995) and the conversion of citrulline into arginine by enterocytes isolated from newborn and post-weaning pigs (Blachier *et al.* 1993). The higher capacity for *de novo* synthesis of arginine from citrulline in enterocytes isolated from newborn compared with those isolated from post-weaning pigs (Blachier *et al.* 1993) corresponds with a higher PDV flux of arginine in 14- to 29-d-old pigs *v.* 29- to 58-d-old pigs (Wu *et al.* 1994*a*).

It can be concluded that in contrast to the observations in the rat, both *in vitro* and *in vivo* studies indicate that catabolism of arginine is limited in the gastrointestinal tract of the pig.

Table 3. *Synthesis of urea (nmol/30 min per mg protein) from glutamine by pig enterocytes* in vitro

(Data taken from Wu, 1995) (Values are means with their standard errors)

Glutamine (mM)...	0	1		5	
Age (d)		Mean	SE	Mean	SE
0–21	nd	nd		nd	
29	nd	6·3	0·7	15·2	1·3
58	nd	7·9	0·8	16·5	1·4

nd, not detected.

Urea synthesis

It is generally accepted that urea is synthesized only in the liver of mammals. NH_3 produced from intestinal degradation of AA is released into the portal vein and taken up by the liver for ureagenesis. The synthesis of urea from NH_3 via the urea cycle involves five enzymes, carbamoyl phosphate synthase I (*EC* 6.3.4.16), ornithine carbamoyl transferase (*EC* 2.1.3.3), argininosuccinate synthase (*EC* 6.3.4.5), argininosuccinate lyase (*EC* 4.3.2.1) and arginase (*EC* 3.5.3.1). All these urea-cycle enzymes have been demonstrated in enterocytes isolated from pre- and post-weaning pigs (Blachier *et al.* 1993; Wu *et al.* 1994*b*; Wu, 1995). Recently Wu (1995) demonstrated the production of urea and ornithine from NH_3, glutamine and arginine in a dose-dependent manner in enterocytes from post-weaning pigs, while in enterocytes from newborn and sucking pigs there was no measurable synthesis of urea (Table 3). Although the extent of the activity of some urea-cycle enzymes is still debatable (Blachier *et al.* 1993; M'Rabet-Touil *et al.* 1993; Wu, 1995), it is clear that the rate of urea synthesis in incubated enterocytes is low compared with that of hepatocytes (Wu, 1995). *In vivo*, urea synthesis by gastrointestinal tissue has still to be demonstrated. The PDV flux of urea is slightly negative (Rérat & Buraczewska, 1986; Simoes Nunes *et al.* 1989; Fig. 1) or approaches zero (Malmlöf, 1987; Malmlöf *et al.* 1989; Malmlöf & Simoes Nunes, 1992; Wu *et al.* 1994*a*).

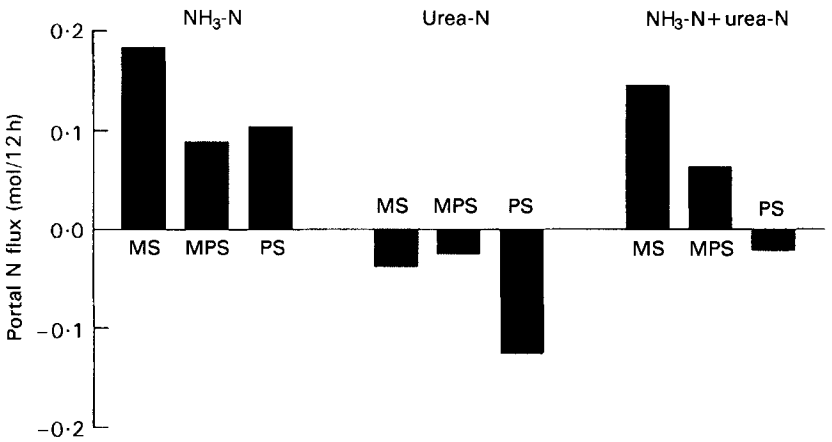

Fig. 1. Effects of non-resistant (maize starch; MS) and resistant starch (maize + potato starch (MPS) and potato starch (PS)) on portal-drained viscera (PDV) flux of urea- (which is transferred to the PDV) and ammonia-nitrogen (which is absorbed from the PDV) and the resulting ammonia- and urea-nitrogen balance (From J. van der Meulen, G. C. M. Bakker, J. G. M. Bakker, H. de Visser, A. W. Jongbloed and H. Everts, unpublished results).

Thus, the small intestine can be regarded as an organ with a urea cycle, but the rate of urea synthesis appears to be limited.

EFFECT OF DIET COMPOSITION ON NITROGEN ABSORPTION AND METABOLISM

Protein

There is a strong linear correlation between the PDV flux of total AA (TAA) at different time intervals after feeding and the amount of fishmeal protein ingested (Rérat *et al.* 1988*a*). This relationship is valid for individual essential AA (EAA) and some non-essential AA (NEAA; Rérat *et al.* 1988*a*). Although an increase in the amount of ingested fishmeal protein is associated with an increase in the PDV flux of AA, it results in a relative decrease in their coefficients of portal absorption (Rérat *et al.* 1988*a*). The slopes of the regression lines for PDV flux *v.* the amount ingested depend on the source of protein ingested (Rérat, 1988) and differ markedly from one AA to another (Rérat *et al.* 1988*a*). While the profile of EAA absorbed depends on the mixture of EAA ingested, the profile of the PDV flux of NEAA differs greatly from the mixture of NEAA ingested as a consequence of metabolism in the gastrointestinal tissue. Increasing the amount of protein ingested by adding NEAA results in an increase in the PDV flux of NEAA, but at the same time there is an increased PDV flux of NH_3 indicating increased metabolism of AA in gastrointestinal tissues (van der Meulen *et al.* 1996*b*). Feeding the same amount of protein but different EAA : NEAA values, the PDV flux of EAA *v.* NEAA is also affected (van der Meulen *et al.* 1995). Changing the composition of the NEAA, however, does not change PDV flux of the individual NEAA (Prior & Gross, 1995).

The PDV flux of AA is affected by the physico-chemical structure of the AA subjected to absorption. After duodenal infusion of a solution of small peptides, obtained by mild hydrolysis of milk proteins, the appearance of AA in the portal vein is more rapid and the PDV flux of AA is higher than after duodenal infusion of an equivalent mixture of free AA (Rérat *et al.* 1988*b*).

Thus, the PDV flux of EAA depends on the mixture of EAA ingested, while the amount of protein ingested and the physico-chemical structure of the AA subjected to absorption determine the PDV flux of AA.

Digestible carbohydrates

In the extreme situation of omitting carbohydrates from the diet and feeding a pure protein meal, the PDV flux of α-NH_2-N increases (Deutz *et al.* 1995). The PDV flux of α-NH_2-N also increases when the PDV flux of glucose is almost halved by feeding maltitol instead of maltose as the carbohydrate source (Rérat *et al.* 1991). The kinetics of glucose absorption also affect the PDV flux of AA. We have shown that feeding pea (*Pisum sativum*) starch (slowly degraded) instead of maize starch (rapidly degraded) increases the PDV flux of AA, and especially that of EAA, from potato protein (Table 4). The PDV of AA may be changed as a consequence of the mutual inhibition of sugars and AA during intestinal absorption (Vinardell, 1990; Rérat *et al.* 1991). Because there are differences in metabolism along the small intestine (Kight & Fleming, 1995), a change in site of absorption associated with the presence of slowly- or rapidly-degraded carbohydrates might also affect the PDV flux of AA.

The previous discussion indicates that the PDV flux of AA is affected by the size of the PDV flux of glucose and the kinetics of glucose degradation.

Table 4. *Portal-drained viscera (PDV) flux and absorption coefficient of glucose, essential amino acids (EAA), non-essential amino acids (NEAA) and total amino acids (TAA) of diets with potato protein and 650 g maize (diet M) or native pea (*Pisum sativum*) starch (diet P)/kg* (Data from van der Meulen *et al.* 1996*a*)

	PDV flux (mmol)				Portal absorption* (%)			
	Diet M	Diet P	SED	Statistical significance of difference: *P*	Diet M	Diet P	SED	Statistical significance of difference: *P*
Glucose	1759	1265	183	0·05	97	72	10	0·05
EAA	183	225	13	0·04	80	96	4	0·02
NEAA	71	89	22	0·48	34	42	11	0·52
TAA	265	326	33	0·14	61	73	7	0·16

* Based on amounts ingested corrected for ileal digestibility.

Fermentable carbohydrates

After feeding fermentable carbohydrates (NSP or resistant starch), N required for bacterial growth in the gastrointestinal lumen has to be provided by non-digested dietary proteins, endogenous protein or urea. Urea enters the gastrointestinal tract via the digestive secretions and diffuses freely from the circulation into the stomach and small intestine (Mosenthin *et al.* 1992*a*). In contrast to observations in the rat (Rémésy & Demigné, 1989; Younes *et al.* 1995*a*,*b*), the flux of urea from the circulation into the gastrointestinal tract of the pig is not increased by feeding a high-fibre diet (Malmlöf, 1987; Malmlöf *et al.* 1989). After feeding a mixture of a digestible (maize) and a fermentable (potato) starch we also observed no change in urea transferred to the gastrointestinal tract but the PDV flux of NH_3 was decreased (Fig. 1). After feeding potato starch as the only carbohydrate source, the urea flux to the gastrointestinal tract was increased and the PDV flux of NH_3 was the same as that after feeding the mixture of maize and pea starch. This supports the suggestion of Mosenthin *et al.* (1992*b*) that in the pig the NH_3 PDV flux is of quantitatively more importance than the urea flux.

The PDV flux of α-NH_2-N is reduced when the amount of fermentable carbohydrates in the diet increases, as has been shown by Giusi-Perier *et al.* (1989) who compared diets containing 60 and 160 g cellulose/kg and by Malmlöf (1987) who examined diets with and without straw. However, ileal digestibility of AA and N also is reduced by fermentable carbohydrates in the diet (den Hartog *et al.* 1988; Sauer *et al.* 1991; Lenis *et al.* 1996). Taking the change in ileal AA digestibility into account, the addition of purified neutral dietary fibre does not affect the portal absorption coefficient of ileally-digested AA (Lenis *et al.* 1996).

Thus, although the urea flux to the gastrointestinal tract may increase after feeding fermentable carbohydrates, the decrease in PDV flux of NH_3 appears to be more important. The PDV flux of ileally-digested AA may not be affected after feeding fermentable carbohydrates.

Lectins

As with the addition of a purified neutral dietary fibre (Lenis *et al.* 1996), adding Phaseolus beans with a high lectin content does not affect the PDV flux of ileally-digested AA (A. J. M. Jansman, unpublished results).

Portal-drained viscera flux of amino acids v. *ileally-digested amino acids*

Usually, the portal absorption coefficient of AA is not equal to 100 % because some of the protein remains undigested. When the ileal digestibility of AA is taken into account, however, a portal absorption coefficient of 100 % for ileally-digested AA is still not achieved (for example, see Table 4). This is because some of the AA are metabolized by the PDV. Furthermore, in ruminants (Webb *et al.* 1992) and rats (Galibois *et al.* 1991) peptide-bound AA (PBAA) contribute to the PDV flux of α-NH_2-N. PDV flux of PBAA may increase the portal absorption coefficient, but there have been no reports to suggest that this occurs in pigs. Recently, we have measured free AA in non-hydrolysed samples, and free AA + PBAA (excluding methionine, cystine, tryptophan, serine and arginine) in hydrolysed samples of arterial and portal plasma after removal of residual protein (> 1800 Da) by gel-permeation chromatography. In both arterial and portal plasma a significant proportion of isoleucine, leucine, alanine, glycine and proline appears to be present as PBAA and the portal–arterial concentration difference of TAA for hydrolysed samples is higher than for non-hydrolysed samples (van der Meulen *et al.* 1996*c*). This indicates that in the pig, also, PBAA may contribute to the PDV flux of AA.

CONCLUSIONS

The metabolism of proteins in gastrointestinal tissues of the pig is very active, and glutamine in particular is metabolized to NH_3, glutamic acid, alanine, aspartic acid, ornithine and citrulline. Small amounts of urea, also, may be synthesized by the small intestine. Compared with the rat, glutamine may be less essential as fuel for the gastrointestinal tract in pigs and catabolism of arginine is limited.

The PDV flux of EAA depends on the mixture of AA ingested, while the amount of protein ingested and the physico-chemical structure of the AA determine the PDV flux of AA. The PDV flux of AA is affected by the kinetics and magnitude of glucose absorption. Feeding fermentable carbohydrates decreases the PDV flux of NH_3 and increases secondarily the urea flux to the gastrointestinal tract, while the portal absorption of ileal-digested AA is not affected. Free AA, as well as peptide-bound AA may contribute to the PDV flux of α-NH_2-N in the pig.

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