

## Effect of essential and non-essential amino acid addition to a nitrogen-free diet on endogenous ileal amino acid flow in growing pigs

P. DOLEŠOVÁ, S. NITRAYOVÁ, P. PATRÁŠ, J. HEGER

Animal Production Research Centre, Institute of Nutrition, Nitra, Slovak Republic

**ABSTRACT:** Pigs fitted with ileal T-cannula in the terminal ileum were used to study the effect of synthetic amino acids (AA) added to a nitrogen-free diet on endogenous losses. We compared the obtained data with data from other research centres in the world that are focusing on these problems. In the present experiment, we used three synthetic diets – a nitrogen-free (NF) diet, a diet with added essential AA (E), a diet with the same amounts of essential AA and a mixture of non-essential AA (E+N) added. The diets, apart from the amount of AA, had the same composition. Chromium oxide was used as an indigestible marker. The supplementation of the NF diet with sulphur AA, threonine and tryptophan resulted in a reduction of the endogenous flow of all AA except for cysteine. However, the difference between the NF and E diet was significant only in the case of proline. The effect of the non-essential AA addition to diet E was negligible, even though the endogenous flow of most AA tended to decrease. In comparison with NF diet, the endogenous flow of total N in diets E and E+N was reduced by about 30%. This reduction was mainly due to the decreased endogenous flow of proline, which accounted for 59% in diet E and 55% in diet E+N. Proline was the most abundant AA in all diets, followed by glycine and glutamic acid. The sum of proline and glycine in diets NF, E and E+N amounted to 59, 44, and 46% of total AA flow, respectively. In contrast, the proportion of the sum of essential AA of total AA flow in diets NF, E and E+N was 21, 28 and 27%, respectively. Our data on the endogenous AA and N flow are in the range of previously published values.

**Keywords:** pigs; ileal digestibility; amino acids; endogenous losses

Standardised ileal digestibility (SID) in pigs is now routinely used as a criterion of amino acid (AA) availability. The values for SID are calculated by deducing ileal AA outflow corrected for basal endogenous AA losses from dietary AA intake. The basal endogenous AA losses are defined as the quantities of AA found in the terminal ileum which are independent of diet (Stein et al., 2007). It is assumed that they represent the minimum inevitable AA losses, originating mainly from digestive secretions, sloughed-off epithelial cells and mucin. The basal AA losses are mostly estimated using an N-free diet (De Lange et al., 1989; Mariscal-Landin et al., 1995) since all N-containing compounds present

in the gastrointestinal tract must be of endogenous origin under these conditions. The N-free method is criticized mainly because of its non-physiological nature, associated with the mobilization of body protein reserves during protein deprivation (Hess and Sève, 1999). When feeding an N-free diet for a prolonged period of time, very high ileal flows of proline and glycine were observed (Wünsche et al., 1987; Furuya and Kaji, 1992; Stein et al., 1999). De Lange et al. (1989) suggested that the high ileal flow of proline might be the result of catabolism of glutamine found in large quantities in muscle protein. Indeed, De Lange et al. (1989) and Leterme et al. (1996) observed a significant decrease in the

endogenous proline loss in pigs fed N-free diets that were given an AA mixture intravenously in order to reduce the muscle protein breakdown.

The main purpose of muscle protein catabolism at protein deprivation is to provide AA required for processes associated with maintenance. However, the AA pattern of muscle protein is considerably different from that of maintenance and the AA supplied in excess must be deaminated and their N excreted. Studies on rats (Yoshida and Moritoki, 1974; Yokogoshi and Yoshida, 1976) and pigs (Lubaszewska et al., 1973; Fuller et al., 1989) showed that the AA most limiting for maintenance are sulphur AA, threonine and possibly tryptophan and that their addition to an N-free diet significantly reduced N excretion. As suggested by Křížová et al. (2001), non-essential AA might be the next-limiting additive as their addition to an N-free diet supplemented with sulphur AA, threonine and tryptophan substantially increased N retention.

It is possible that the addition of AA which are the most-limiting for maintenance to an N-free diet may improve the re-utilization of AA released by protein breakdown, thus reducing the ileal losses of proline and other AA. To test this hypothesis, an experiment was carried out to study the effect of small supplements of essential and nonessential AA to an N-free diet on endogenous N and AA flow in the terminal ileum of growing pigs.

## MATERIAL AND METHODS

### Animals and procedures

All experimental procedures were reviewed and approved by the Animal Care Committee of the Research Institute of Animal Production. Six Large White gilts (mean initial body weight 47.3 kg) were fitted with a simple T-cannula at the terminal ileum

Table 1. Composition of experimental diets (g/kg, air dry)

	NF	E	E+N
Maize starch	739.00	735.92	710.02
Sucrose	100.00	100.00	100.00
Cellulose	50.00	50.00	50.00
Sunflower oil	50.00	50.00	50.00
Mineral mixture <sup>1</sup>	55.00	55.00	55.00
Vitamin and micromineral premix <sup>2</sup>	3.00	3.00	3.00
DL-methionine		0.27	0.27
L-cysteine		1.06	1.06
L-threonine		1.45	1.45
L-tryptophan		0.30	0.30
L-glutamic acid			10.36
Glycine			7.77
L-proline			5.18
L-serine			2.59
Chromic oxide	3.00	3.00	3.00
Total N (analysed)	0.94	1.29	4.78

<sup>1</sup>provided the following per kg of diet: monocalcium phosphate 31 g; limestone 15 g; salt 4.4 g; KCl 3.8 g; MgO 0.8 g

<sup>2</sup>provided the following per kg of diet: retinol 1.2 mg; cholecalciferol 25 mg;  $\alpha$ -tocopherol 10 mg; menadione 0.2 mg; riboflavin 4 mg; pyridoxine 2.5 mg; D-pantothenic acid 10 mg; niacin 20 mg; folic acid 0.5 mg; biotin 0.1 mg; cyanocobalamin 30  $\mu$ g; choline 500 mg; Fe 92 mg; Zn 103 mg; Mn 40 mg; Cu 19 mg; Co 0.5 mg; Se 0.16 mg

and housed in individual metabolism cages. After a recovery period of 14 days, the pigs were weighed and assigned randomly to three dietary treatments using a replicated 3 × 3 Latin square design. Each experimental period consisted of a 6-day preliminary period followed by a 24-hour collection period. During the collection period, samples of ileal digesta were collected into plastic bags attached to the cannula barrel in 60 minutes intervals. The samples were acidified with 6 M H<sub>2</sub>SO<sub>4</sub> to pH 3.5 to eliminate the enzymatic activity and stored at –20°C. At the end of the experiment, samples of digesta and faeces were pooled, freeze-dried, ground to pass a 1 mm screen and stored for subsequent analysis. The pigs were weighed at the beginning and end of each period.

### Diets and feeding

The basal N-free diet (NF) contained starch, sucrose, cellulose and sunflower oil and was supplemented with mineral and vitamin mixtures (Table 1). The diet contained 0.09% N coming from starch. To the basal diet, small quantities of essential AA and a combination of essential and non-essential AA were added at the expense of starch to form diets E and E+N, respectively. Sulphur amino acids, threonine and tryptophan were used in diet E since they are presumably limiting under maintenance conditions. The diet E+N contained the same amounts of essential AA and a mixture of non-essential AA. Total N concentration of diets E and E+N was 0.13 and 0.48%, respectively. The diets contained chromium oxide as an indigestible marker. The pigs were fed twice daily at 6.00 and 16.00 hours in two equal meals at a daily rate of 80 g/kg<sup>0.75</sup>. Water was available *ad libitum*.

### Calculation and statistical analysis

The endogenous amino acid flow was calculated according to the following formula:

$$EAAF = AA_i \times Cr_d / Cr_i$$

where:

EAAF = endogenous AA flow in g/kg DM intake

AA<sub>i</sub> = concentration of an AA in ileal digesta (g/kg DM)

Cr<sub>d</sub>, Cr<sub>i</sub> = concentrations of chromium (g/kg DM) in the diet and ileal digesta, respectively

Data were subjected to one-way ANOVA using Statgraphic Plus package (version 3.1., Statistical Graphics Corp., Rockville, MD, USA). When a significant value for the treatment effect was observed, the differences between means were assessed using Fisher's LSD procedure.

### Chemical analysis

Dry matter (DM) and total N of diets and digesta were analyzed in accordance with AOAC (1990) standard procedures. The amino acid composition of diets and digesta was analysed by ion-exchange chromatography according to the Directive of the Slovak Commission 98/64ES (1988). Chromic oxide was determined by atomic absorption spectrometry as described by Williams et al. (1962).

## RESULTS AND DISCUSSION

The supplementation of the NF diet with sulphur AA, threonine and tryptophan resulted in a reduction of the endogenous flow of all AA except for cysteine. However, the difference between the NF and E diets was significant only in the case of proline (Table 2). The effect of the addition of non-essential amino acids to diet E was negligible, even though the endogenous flow of most AA tended to decrease. In comparison with diet NF, the endogenous flow of total N in diets E and E+N was reduced by about 30%. This reduction was mainly due to the decreased endogenous flow of proline which accounted for 59% in diet E and 55% in diet E+N. Proline was the most abundant AA in all diets, followed by glycine and glutamic acid. The sum of proline and glycine in diets NF, E and E+N amounted to 59, 44, and 46% of total AA flow, respectively. In contrast, the proportion of the sum of essential AA in total AA flow in diets NF, E and E+N was 21, 28 and 27%, respectively.

Although the endogenous ileal AA flow in pigs is commonly estimated using an N-free diet, this method is considered non-physiological since it leads to the degradation of body protein in order to provide AA for processes associated with maintenance. The most striking anomaly in feeding an N-free diet is the high excretion of proline (and of glycine to a lesser extent) into the ileum (Taverner et al., 1981; Wünsche et al., 1987; Furuya and Kaji, 1992; Stein et al., 1999). This phenomenon is as-

Table 2. Endogenous ileal flow of amino acids and of total N (g/kg dry matter intake)

Amino acid	Diet			Pooled SEM
	NF	E	E+N	
Arginine	0.54	0.33	0.32	0.08
Histidine	0.16	0.14	0.14	0.02
Isoleucine	0.22	0.20	0.19	0.16
Leucine	0.38	0.36	0.33	0.03
Lysine	0.24	0.20	0.19	0.03
Methionine	0.07	0.06	0.06	0.01
Phenylalanine	0.26	0.21	0.22	0.04
Threonine	0.48	0.45	0.42	0.03
Valine	0.32	0.30	0.28	0.02
Alanine	0.43	0.33	0.32	0.06
Aspartic acid	0.61	0.53	0.49	0.04
Cysteine	0.13	0.14	0.14	0.01
Glutamic acid	0.62	0.54	0.52	0.05
Glycine	1.37	1.00	0.94	0.19
Proline	6.10	2.53	2.73	0.94
Serine	0.40	0.34	0.33	0.03
Thyroxine	0.37	0.28	0.32	0.03
Total AA	12.69	7.94	7.94	1.54
Total N	2.34	1.62	1.75	0.26

<sup>a,b</sup>means within a row followed by different superscript are significantly different ( $P < 0.05$ )

sumed to be due to the release of large quantities of glutamine from skeletal muscle and its transformation to glutamate, citrulline and proline in the digestive tract. Owing to the low activity of the proline-degrading enzyme proline oxidase, proline may accumulate in enterocytes and diffuse into the lumen of the gut (De Lange et al., 1989; Mariscal-Landin et al., 1995; Stein et al., 1999). De Lange et al. (1989) and Leterme et al. (1996) found a significant decrease in proline endogenous loss in pigs consuming an N-free diet after the intravenous administration of a mixture of crystalline AA. These results suggest that the attainment of positive N balance reduces the concentration of circulating glutamine, thereby reducing the proline concentration in ileal digesta.

The working hypothesis of the present experiment was that the muscle protein catabolism in pigs fed an N-free diet can be reduced by improving

the re-utilization of AA present in the AA pool. It has been repeatedly demonstrated that small supplements of sulphur amino acids and threonine to an N-free diet significantly reduce urinary N excretion and improve N retention (Yokogoshi and Yoshida, 1976, 1981) and therefore these AA are the most limiting under maintenance conditions. Tryptophan (Fuller et al., 1989) and non-essential AA (Křížová et al., 2001) may be the next limiting. The present results support this hypothesis because the supplements of sulphur AA, threonine and tryptophan significantly reduced the endogenous proline loss. The addition of non-essential N does not seem to be necessary. It is interesting to note that the AA supply used in the present experiment was considerably lower than the amounts administered parenterally as reported by De Lange et al. (1989). The daily AA intake from diet E was about 4.4 g while the pigs of approximately the same body

weight in the experiment conducted by De Lange et al. (1989) were infused with 208 g of AA daily. In spite of this difference, the effect on endogenous N and AA losses was similar. Even though some authors reported an increased endogenous N flow in pigs fed purified AA diets in comparison with the N-free diet (Chung and Baker, 1992; Jansman et al., 2002), no such an effect was observed in the present study, presumably due to the low level of AA intake. The obvious advantage of the method using an AA-supplemented N-free diet in comparison with the parenteral infusion is its simplicity. However, further research is needed to optimise the composition of the AA mixture as well as its inclusion rate in order to make the resulting basal endogenous AA losses equal to minimum obligatory losses as proposed by Hess and Sève (1999).

Our data on endogenous AA and N flow are within the range of values published earlier. Jansman et al. (2002) compared various methods of estimating the endogenous N loss in pigs and found values ranging from 1.68 to 2.75 g/kg DM intake. A similar range (1.6–2.8 g/kg DM intake) was reported by De Lange et al. (1989). Of the particular amino acids, exceptionally high variability was observed in the case of proline, especially when N-free diets were fed. The values ranged from 0.25 (Hess and Sève, 1999) up to 6.9 g/kg DM intake (Furuya and Kaji, 1992). Our proline flow of 6.1 g/kg DM intake approaches the upper limit and is considerably higher than the average value of 1.31 given by Jansman et al. (2002). There are numerous factors affecting the endogenous flow of AA such as body weight (Leterme and Théwis, 2004), feed intake (Haydon et al., 1984), dietary fibre (Taverner et al., 1981) or duration of the feeding of N-free diet (Hodgkinson et al., 2000). The present results as well as those of Hess and Sève (1999) suggest that pig individuality may also be a significant source of variation. Therefore, it is recommended to estimate the endogenous AA flow on the same pigs on which the digestibility trials are carried out.

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*Corresponding Author*

Ing. Patricia Dolešová, Animal Production Research Centre, Institute of Nutrition, Hlohovská 2, 949 92 Nitra, Slovak Republic  
E-mail: dolesovapatricia@centrum.sk

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