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Intestinal nitrogen flow, total nitrogen and ^{15}N balance in pigs labelled intravenously with ^{15}N-leucine and fed a meat meal diet

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ABSTRACT

Three pigs of about 33 kg body weight, each fitted with re-entrant cannulas into the duodenum and ileum and with bladder catheters were used for the study. One of the pigs was infused intravenously with ^{15}N-leucine for 8 days. Total N passage, ^{15}N nitrogen passage through the duodenum and ileum, and N balance were determined. The mean amount of total N passing the duodenum exceeded the ingested N by 12%, and that passing the ileum accounted for 27% of N intake. Of the infused ^{15}N, 67.4% was found in the urine, faeces, digesta and carcass after slaughter. Taking the recovered amount of ^{15}N as 100%, 20.4% was found in urine, 0.6% in faeces, 8.2% in digesta and 70.8% in tissues. It was concluded that ^{15}N retention from intravenously administrated leucine was much higher than from ^{15}N in urea given with feed, as estimated previously.

KEY WORDS: pig, nitrogen, absorption, secretion, flow, balance

INTRODUCTION

In previous papers (Krawielitzki et al., 1990, 1992; Żebrowska et. al., 1992) the passage of dietary and endogenous nitrogen along the different sections of the

digestive tract (GIT) of pigs given a soyabean meal diet were studied. The results showed that the duodenal and ileal flow of total N amounted to about 118 and 29% of intake, respectively, and that the relative proportion of endogenous-N to total-N rose from 13 to 35 and 39% in duodenal and ileal digesta and faeces, respectively.

Soyabean meal protein is known to be a protein highly digested in the small intestine of pigs. The flow of dietary protein and the proportion of endogenous to dietary nitrogen in duodenal and ileal digesta may differ when protein of low digestibility is given.

The aim of the present experiment was to measure the flow of total and endogenous nitrogen through the duodenum and ileum of pigs fed a meat meal diet of low digestibility, and to evaluate continuous intravenous infusion L-^{15}N leucine as a method of determining endogenous N secretion into the GIT.

MATERIAL AND METHODS

Animals

Three litter mate gilts of about 33 kg were fitted with re-entrant cannulas into the duodenum and distal ileum, and with bladder catheters. The animals were kept individually in metabolic cages permitting quantitative collection of faeces, urine, duodenal and ileal digesta. The pigs were fed a diet consisting of wheat, meat meal, starch supplemented with mineral mixture containing 18% crude protein (Table 1) in two equal portions of 600 g each at 7.00 and 19.00 h; the feed was mixed with water (1:2 w/v) immediately before feeding.

TABLE 1

Composition of the diet (g/kg)	
Wheat	426
Meat meal	185
Starch	366
Mineral mixture	23
Dry matter	884
In DM:	
crude protein	181.3
crude fibre	17.6
ether extract	11.9
ash	78.3
N-free extractives	710.9

Experimental design

The experiment was carried out according to a procedure similar as to that described by Krawielitzki et al.(1990). After cannulation the animals were allowed a 10-day adaptation period to the experimental diet and management conditions. The catheter into the jugular vein of pig no. 1 was then inserted followed by a further 2-day recovery period.

The ^{15}N labelled leucine (96.2 at % ^{15}N excess) solution containing 0.635 mg ^{15}N/ml was infused continuously into the jugular vein of animal no. 1, at a rate of 15 ml/h. The infusion started on the first day of the 5-day balance period and was continued for the subsequent 3-days of digesta collection. The amount of ^{15}N infused amounted to 0.303 mg ^{15}N/kg body weight/h. During the last 3 days of infusing ^{15}N-labelled leucine, the digesta from the duodenum and ileum of pigs no. 1, 2 and 3 were collected quantitatively, sampled and exchanged according to the scheme given in Figure 1. Urine and faeces were collected, measured and sampled from all the pigs during the whole experiment.

The pigs were killed at the end of the experiment and samples of blood, digesta and tissue of different GIT sections, including the liver and pancreas, were taken for total N and ^{15}N measurement.

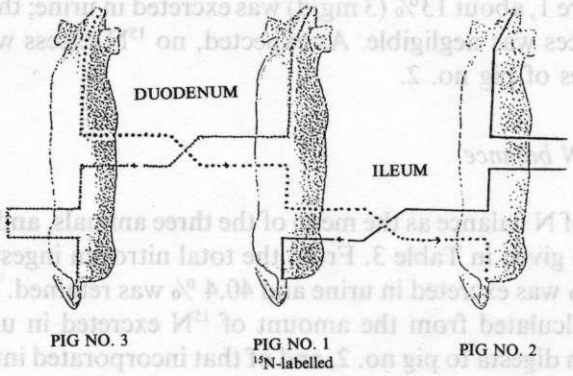

Figure 1. Scheme of digesta exchange between animals

Sample collection and preparation

Urine was collected into vessels containing 5% sulphuric acid, measured and sampled every 12 h. Collected faeces were preserved with chloroform and also sampled every 12 h. The digesta passing through the duodenal and ileal cannulas were measured every hour and 5% aliquots were pooled for 6 h periods.

All samples were kept at 0^0C during collection and then stored at -25^0C until analysis.

Total nitrogen in all samples was estimated by the Kjeldahl method. ^{15}N was determined by emission spectrometry using a NOI-6e FAN ^{15}N analyzer (Fischer Analysen Istrumente, Leipzig).

RESULTS

Total N and ^{15}N excretion in urine and faeces

Daily urinary N excretion in the pigs was similar during the balance and the digesta exchange periods and ranged from 16.2 to 17.0 g/day. The mean amounts of N (4.2 vs 4.4 g/d) excreted in the faeces were also similar in both periods (Table 2).

Of the amount of ^{15}N infused daily into pig no. 1 during the digesta exchange period, about 16% was excreted in urine, 0.7% in faeces, and about 10.5% was transferred with digesta to the other pig. The course of ^{15}N excess in the urine of animal no. 1 increased exponentially and reached a plateau value at about 0.22 ^{15}N at % excess after 4 days. From the ^{15}N (23.8 mg/d) introduced into the duodenum of pig no. 3 with the digesta taken from pig no. 1, according the scheme in Figure 1, about 13% (3 mg/d) was excreted in urine; the amount of ^{15}N excreted in faeces was negligible. As expected, no ^{15}N excess was found in the urine and faeces of pig no. 2.

Total N and ^{15}N balance

The results of N balance as the mean of the three animals, and the ^{15}N balance of pig no. 1 are given in Table 3. From the total nitrogen ingested, 87.8 % was digested, 47.6% was excreted in urine and 40.4 % was retained. The ^{15}N balance of pig no. 1 calculated from the amount of ^{15}N excreted in urine and faeces, transferred with digesta to pig no. 2, and of that incorporated into tissues during the 8 days of experiment, shows a low recovery of ^{15}N, as only 67.5% of the given ^{15}N amount was found in all these fractions.

Flow of digesta, total N and ^{15}N through the duodenum and ileum

Daily amounts of digesta and total N passing through the duodenum during the 3 days of the digesta exchange period were similar in all pigs and ranged from 13.4 to 15.1 kg/d and from 38.6 to 39.7 g N/d, respectively; the amount of N exceeded the amount of N ingested by 12 %. The amount of nitrogen passing through the ileum ranged from 9.6 to 9.1 g/d, and accounted for about 27 % of

TABLE 2

Daily N and ^{15}N excretion in urine and faeces during the balance and the exchange period (mean ± SD)

	Pig No 1 (^{15}N labelled)		Pig No 2		Pig No 3	
	Urine	Faeces	Urine	Faeces	Urine	Faeces
Balance period (5d)						
N amount (g/d)	16.2 ± 1.2	4.4 ± 1.6	16.5 ± 1.9	3.4 ± 0.9	17.0 ± 1.0	4.9 ± 1.9
^{15}N excess (at. %)	0-0.22	0-0.04	0	0	0	0
Exchange period (3d)						
N amount (g/d)	16.6 ± 2.5	4.7 ± 3.3	16.2 ± 2.2	3.8 ± 2.5	16.2 ± 0.2	4.7 ± 0.8
^{15}N excess (at. %)	0.22 ± 0.01	0.03 ± 0.05	0	0	0.03	0.03
^{15}N amount (mg/d)	36.8 ± 7.3	1.5 ± 0.9	0	0	3.0 ± 1.6	1.5 ± 1.0

TABLE 3

Total N balance (mean of 3 pigs, 5 days) and ^{15}N balance of pig No 1 after 8 days

	Total N		^{15}N		
	g/d	% of itake	mg/8d	% of infused	% of recovery
N intake	34.88	100.0			
^{15}N infusion (pig 1 only)	0.23		1820	100	
Excretion					
in urine	16.6 ± 0.32	47.6	250	13.7	20.4
in faeces	4.23 ± 0.62	12.2	7	0.4	0.6
N retention	14.06	40.4			
N absorption	30.62	87.8			
^{15}N content					
in duodenum digesta			76	4.2	6.2
in ileum digesta			16	0.9	1.3
^{15}N in samples after slaughter					
GIT content			9	0.5	0.7
blood			27	1.5	2.2
liver, pancreas,gut tissues			188	10.3	15.3
carcass			653	35.9	53.3
Recovery			1226	67.4	100.0

nitrogen intake. The daily pattern of digesta, total N and ^{15}N flow through the duodenum is shown in Figure 2.

Mean ileal flow varied from 2.0 to 2.6 kg digesta/day and from 9.1 to 9.6 g N/day (27% of the average N intake). The mean daily amount of ^{15}N passing through the duodenum and ileum of pig no. 1 was 23.8 mg and 5.0 mg, respectively. The ileal digesta of pig no. 1 was enriched in ^{15}N due to secretion of

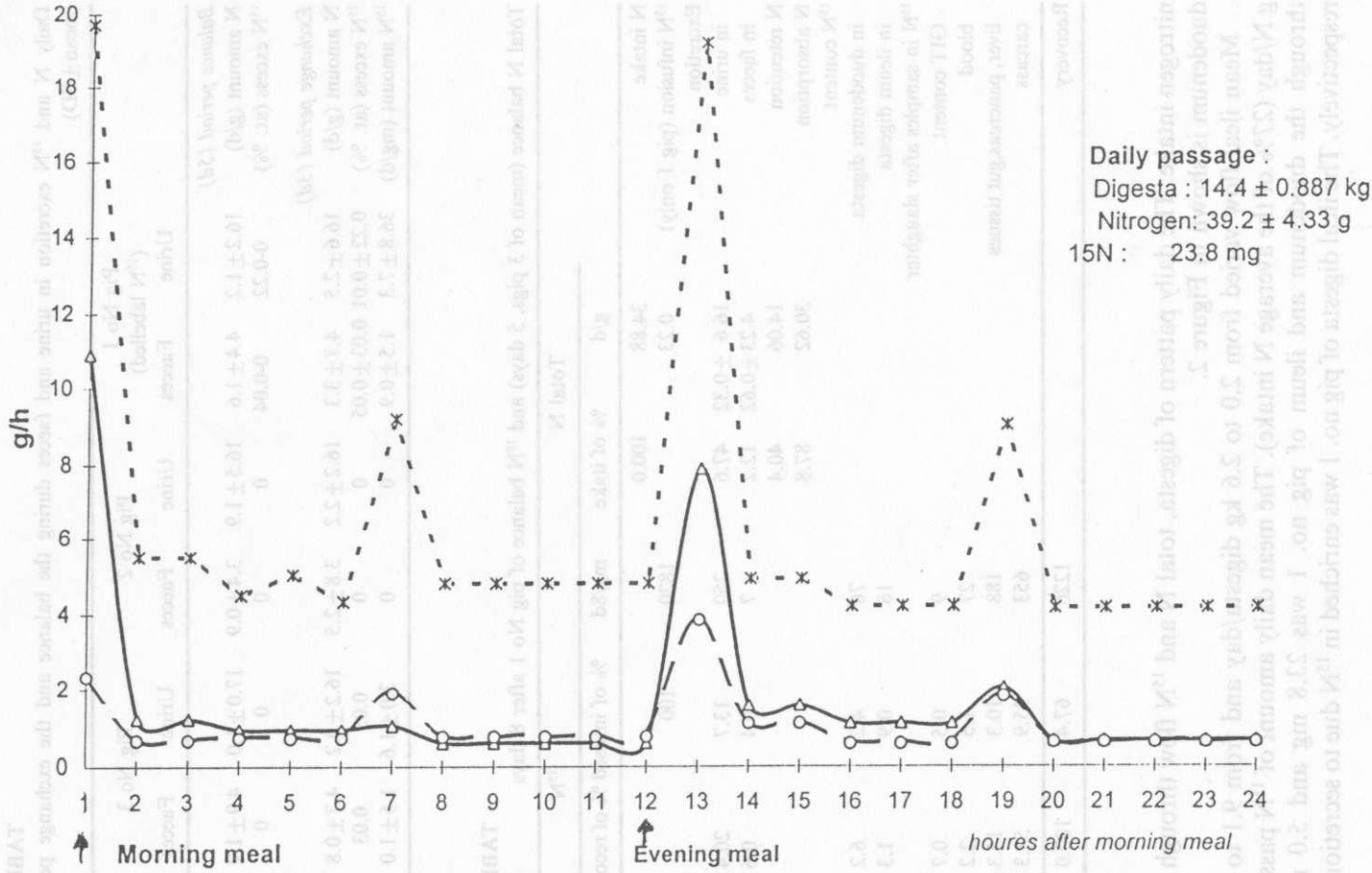

Figure 2. Transit rates of digesta, g/h x 10^2 (-*-); total N, gN/h (-△-); ^{15}N, g/h x 103 (-o-)

^{15}N-labelled nitrogen into the lumen of the small intestine. The daily variation of the transit rates of digesta, digesta N and ^{15}N is demonstrated in Figure 2. Immediately after feeding (at 7.00 and 19.00 h) the digesta flow rate reached a maximum followed by a second, smaller peak (0.9 to 1.1 kg/h) 7 h later. Excluding these maxima, the flow rate was relatively constant (0.4 to 0.6 kg/d). Digesta N and ^{15}N passage through the duodenum took a similar course.

DISCUSSION

Despite the pigs being fed different proteins and different ways of labelling with ^{15}N being used, the experimental data obtained supports the results of our previous study (Krawielitzki et al., 1990; Żebrowska et al., 1992) and are in agreement with other experiments; Żebrowska et al. (1982) and Souffrant et al. (1986) indicating its validity for growing pigs. In the present experiment, N retention, related to intake, was 10% lower in comparison to the soyabean meal diet of the previous experiment (Żebrowska et al., 1992) due to lower apparent digestibility and higher urinary N excretion.

In contrast to the high total N excretion (about 48% of the intake), urinary ^{15}N excretion was low (only 21% of ^{15}N recovered), suggesting that ^{15}N leucine was better utilized for body protein accretion than the dietary nitrogen. In fact, 72% of the recovered ^{15}N from ^{15}N-leucine was found in carcass fractions. As expected, after infusing ^{15}N-leucine, the ^{15}N incorporated into the carcass fractions doubled in comparison with feeding ^{15}N-labelled ammonium sulphate (about 34%) (Krawielitzki et al., 1991; Żebrowska et al., 1992).

Recovery of the administered ^{15}N was lower (about 67% only) in the present experiment compared with that (76%) in the former study (Żebrowska et al., 1992). This may be due to analytical problems in measuring very low ^{15}N concentrations by emission spectrometry. It can be assumed that not all of the ^{15}N in carcass and other samples was detected, especially because the amount of infused ^{15}N (16.5 mg ^{15}N/kg $BW^{0.75}$/d) was much lower than the orally administered amount (145.2 mg ^{15}N/kg $BW^{0.75}$/d) in the previous experiment.

The ^{15}N isotope dilution technique in combination with other experimental methods is suitable for studies of N absorption and secretion in different sections of the gut because it discriminates between endogenous and exogenous nitrogen in the intestinal digesta, as shown by Souffrant et al. (1986), Krawielitzki et al. (1990 a, b), Bartelt et al. (1994). Recent studies by van Leeuwen et al. (1994) who compared different methods of administering ^{15}N in the context of their effect on the enrichment of body fractions have shown that ^{15}N enrichment in the TCA soluble fractions of blood and of muscle did not differ significantly after supplying the same amount of ^{15}N-labelled leucine by infusion or orally. In

relation to digestibility, both methods (classical calculation of true digestibility value on the basis of ^{15}N enrichment of the TCA soluble plasma fraction and a new alternative method) also resulted in similar true digestibility values. However, significant differences have been found in the enrichment of digesta depending on the manner of labelling. After oral ^{15}N administration, the enrichment of the ileal digesta and duodenal tissue was significantly enhanced as compared to the values after infusion of ^{15}N. As discussed by van Leeuwen et al. (1994), this should be taken into account in calculating the endogenous portion of protein.

In the present experiment, in contrast to daily N passage, total flow of ^{15}N through the ileum was also much lower (about 50%) than in the previous study where the ^{15}N compound was given orally. The high incorporation rate of ^{15}N-leucine into body tissues was probably responsible for the low ^{15}N flow.

It is difficult to explain the appearance of the second peak of digesta and nitrogen passage through the duodenum at 7 h after feeding (Fig. 2) A similar phenomenon was also found in a study on sheep where a second peak of total and ^{15}N appeared in digesta and blood after administering ^{15}N-labelled urea into the rumen (Kowalczyk et al., 1982).

Summarizing this and previous studies (Żebrowska et al., 1992) it can be concluded that the type of protein (soyabean meal or meat meal) had a small influence on the digesta and digesta N passage through the gut. The amount of digesta and nitrogen N transit through the upper small intestine showed a clear diurnal rhythm and high dependence on the feeding schedule; ^{15}N nitrogen retention from intravenously administered leucine was much higher than from urea given with feed.

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STRESZCZENIE

Przepływ azotu przez jelito cienkie i bilans azotu ogólnego i ^{15}N u świń żywionych mączką mięsną po dożylnym podaniu ^{15}N leucyny

Doświadczenie przeprowadzono na 3 loszkach o masie ciała około 33 kg z kaniulami mostkowymi do dwunastnicy i jelita biodrowego oraz kateterem do pęcherza. Jednej loszce podawano dożylnie ^{15}N leucynę przez 8 dni. Oznaczano przepływ N ogólnego oraz ^{15}N przez dwunastnicę i jelito biodrowe oraz ich bilans. Średnia dobowa ilość azotu ogólnego w treści dwunastnicy przewyższała ilość azotu pobranego z paszą o 12%, natomiast przechodząca z treścią przez jelito biodrowe stanowiła 27% azotu pobranego z paszą. Ilość ^{15}N w moczu i kale oraz oznaczona w treści przewodu pokarmowego i tuszy stanowiła 67,4% ^{15}N podanego.

Przyjmując odzyskaną ilość ^{15}N za 100%, azot ^{15}N w moczu stanowił 20,4, w kale 0,6, w treści przewodu pokarmowego 8,2, a w tuszy 70,8%. Retencja ^{15}N z leucyny podanej dożylnie była większa niż z ^{15}N mocznika podanego z paszą w poprzednim doświadczeniu.