Reprod Nutr Dev (1990) 30, 717-722

Original article

Exogenous and endogenous contribution to nitrogen fluxes in the digestive tract of pigs fed a casein diet. I. Contributions of nitrogen from the exocrine pancreatic secretion and the bile

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(Received 20 February 1990; accepted 2 October 1990)

Summary – The aim of the present work was to study the endogenous contribution of the exocrine pancreatic and biliary secretions to the total endogenous nitrogen production in the pig. Three growing Large White pigs weighing 45 ± 2.5 kg were fitted with permanent fistulae in the pancreatic duct, the bile duct and the duodenum. They were adapted to a semi-synthetic casein diet for 14 d before surgery. In a 7-d post-operative period and an 8-d experimental period, they were fed the same diet. Secretion rates were recorded, total nitrogen and TCA (trichloroacetic acid) insoluble nitrogen were determined in representative pancreatic juice and bile samples.

Daily pancreatic juice and bile flow rates were very similar: 1 850 and 1 820 ml, respectively. The amount of endogenous total nitrogen secreted in the intestinal lumen was 3.6 g per day: 1.9 g N through pancreatic secretion and 1.7 g N through bile secretion. Pancreatic nitrogen increased after meal intake, whilst the kinetics of nitrogen production in the bile were not affected. Throughout the experiment, the mean percentage of TCA insoluble nitrogen was 78.1% in pancreatic juice and 72.3% in bile.

endogenous nitrogen / pancreatic juice / bile / pig

Résumé — Contributions exogènes et endogènes aux flux d'azote dans le tube digestif des porcs nourris d'un régime à base de caséine. I. Contribution de la sécrétion pancréatique exocrine et de la bile. *La contribution des sécrétions pancréatique et biliaire à la production d'azote endogène a été étudiée chez le porc. Trois porcs Large White d'un poids moyen de 45 ± 2,5 kg ont été munis de fistules permanentes dans le canal pancréatique, le canal biliaire et le duodénum. Ils ont été adaptés à un régime semi-synthétique à base de caséine pendant 14 j avant l'intervention chirurgicale et ont reçu ce même régime au cours d'une période de récupération post-opératoire de 7 j et d'une période expérimentale de 8 j. L'azote total, l'azote insoluble dans l'acide trichloracétique (ATC) et les volumes sécrétés ont été déterminés pendant la période expérimentale.*

Les volumes quotidiens de suc pancréatique et de bile ont été respectivement de 1 850 et 1 820 ml. La quantité d'azote total sécrétée dans la lumière intestinale a été de 3,6 g/j : 1,9 g provenant de la sécrétion pancréatique et 1,7 g de la sécrétion biliaire. La quantité d'azote pancréatique augmente après la consommation du repas, tandis que celle produite par la sécrétion biliaire n'a pas été affectée. Au cours de la période expérimentale, le pourcentage moyen d'azote insoluble dans ATC a été de 78,1% dans le suc pancréatique et de 72,3% dans la bile.

azote endogène / suc pancréatique / bile / porc

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INTRODUCTION

During transit in the digestive tract, dietary proteins are submitted to various processes: hydrolysis by the enzymes of endogenous secretions and subsequent amino acid absorption, as well as degradation and utilisation by the intestinal microorganisms. The purpose of a series of studies was to study all these processes simultaneously in order to determine exogenous and endogenous contribution to nitrogen fluxes in pigs fed the same casein diet. Transit and digestion at the end of the small intestine were assessed by ileal digesta collection, according to Darcy *et al* (1980). The contribution of the large intestine was assessed by measurements performed in fæces. The contribution of pancreatic and biliary secretions to nitrogen fluxes in the digestive tract was measured according to Corring *et al* (1972) and Juste *et al* (1979). The appearance of nitrogen and amino acids in the efferent blood was studied by simultaneous measurement of porto-arterial concentration differences and portal blood flow rate (Rérat *et al*, 1980). In addition, using an isotopic labeling technique (^{15}N), we were able to dissociate the study of exogenous *vs* endogenous nitrogen digestion and absorption.

In this first paper we report data on the contribution of nitrogen from the exocrine pancreatic secretion and the bile. The experiment was carried out on the same animals fed a semi-synthetic casein diet.

MATERIALS AND METHODS

Animals and diet

Three Large White female pigs were fitted with permanent fistulæ in the pancreatic duct, the bile duct and the duodenum (Corring *et al*, 1972; Juste *et al*, 1979) at a mean live weight of 45 ± 2.5 kg. Because these pigs were also used in a study in which endogenous nitrogen was labelled with ^{15}N L-leucine, they were fitted with a catheter in the carotid artery and jugular vein, and with a urinary probe. Results on labelling of endogenous nitrogen will be given in a separate paper. After surgery, the animals were housed in metabolism-type cages. They were adapted to a casein diet (15% total proteins) for 14 d before surgery. The diet, the composition of which is reported in table I, was given during a 7-d recovery period and an 8-d experimental period. The diet was provided in 2 daily meals of 800 g each diluted in water (1/1) at 9.00 and 16.00 h.

Pancreatic juice and bile samplings and analysis

At the end of surgery, pancreatic juice and bile were returned to the animals by 2 automatic de-

Table I. Composition of the diet.

Ingredients (%)	Purified wheat starch	72.3
	Hydrochloric casein	16.1
	Cellulose	6.0
	Peanut oil	2.0
	Minerals and vitamins	3.6
Chemical composition (*) (%)	Total nitrogen	2.37 ± 0.10

* Mean ± SEM of 3 determinations performed in the 2 laboratories.

vices which restituted the digestive secretion immediately at a rhythm mimicking normal secretion (Juste *et al,* 1983a). Using these devices, it was possible to measure the volume drained and to take representative and continuous samples of ≈ 4% of the juice collected. During the 8-d experimental period, pancreatic juice and bile were sampled every day and samples were pooled in 2-h fractions. Such an experimental pattern allowed a study to be made of the response of both digestive secretions to the 9.00 meal. Because the 16.00 meal was located during a collecting period (from 15.00 to 17.00 h) the present study would not permit any conclusion to be reached about the response of pancreatic and bile secretions to this second daily meal.

Total nitrogen (Kjeldahl method) was determined in each pancreatic juice and bile pooled sample according to the following procedure: 5 ml 10% TCA (trichloracetic acid) solution were added to 5 ml of pancreatic juice or bile. After centrifugation (5000 *g,* 15 min) the precipitate was mixed with 2 ml 10% TCA instead of 5% TCA in order to complete the extraction procedure, and centrifuged. Supernatants were pooled and the sediment isolated. Total nitrogen was determined in each fraction. All data were processed by Student's *t*-test, and results are given as means ± SEM.

RESULTS AND DISCUSSION

Pancreatic juice and bile flow rates

The mean volume of pancreatic juice secreted (fig 1) changed within 24 h. It increased significantly between the 2nd and the 4th h after the 9.00 meal ($P < 0.05$, relative to the 2 h period before feeding) and within the 6–8 period following it. Maxima values were ≈ 180 ml/2 h, whereas lowest values were obtained at the end of the nocturnal period and were ≈ 120 ml/2 h. The total volume of pancreactic juice seceted within 24 h was 1 850 ± 88 ml.

Bile flow rate (fig 2) was more stable over the 24 h period, mean values being ≈ 150 ml/2 h. The total volume of bile secreted per day was 1 820 ± 48 ml.

As far as the pancreatic secretion was concerned, the daily volume of juice secreted was in the range of values obtained by others (Partridge *et al,* 1982; Zebrowska *et al,* 1983; Corring *et al,* 1986; Langlois *et al,* 1987). The pancreatic juice flow rate was shown to be affected by the concentration of the diet (*ie,* liquid, solid, semi-synthetic or natural), being lower with a semi-synthetic diet (Partridge *et al,* 1982; Zebrowska *et al,* 1983).

The daily bile volume was similar to that obtained in pigs fed a semi-synthetic diet with a low lipid content (Juste, 1982). How-

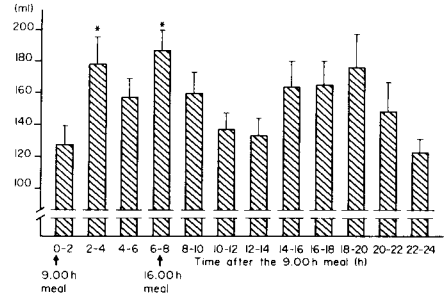

Fig 1. Exocrine pancreatic secretion flow rates (ml/2 h). Means ± SEM. 3 animals, $n = 24$, $\bar{x}$ = 1 850 ml/d. $^*P < 0.05$, relative to the 2-h period before the 9.00 h meal.

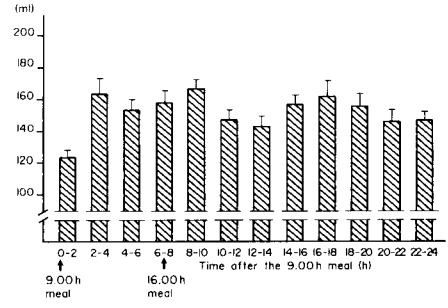

Fig 2. Bile secretion flow rates (ml/2 h). Means ± SEM. 3 animals, $n = 24$, $\bar{x}$ = 1 820 ml/d.

ever, it was shown that bile flow rate increased with a lipid-enriched diet (Aliev and Khamzatov, 1980; Juste *et al*, 1983b).

Nitrogen concentration and production

Total nitrogen concentration in the pancreatic juice did not significantly change over the 24 h, and varied from 0.77 to 1.22 mg/ml (fig 3). During the experimental period, the mean value was 1.01 ± 0.11 mg/ml.

The total nitrogen concentration in bile was more constant, varying from 0.88 to 1.10 mg/ml (fig 4). The mean value over the experimental period was 0.96 ± 0.02 mg/ml.

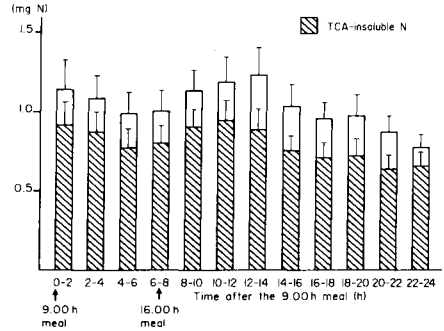

Fig 3. Total N and TCA insoluble N concentrations in pancreatic juice (mg N/ml). Means ± SEM. 3 animals, $n = 24$.

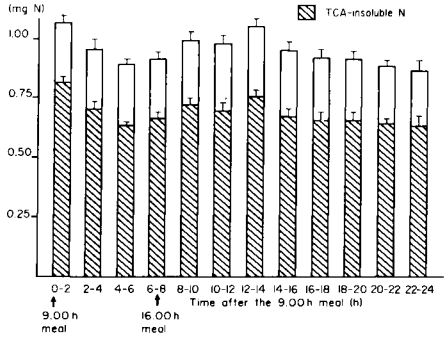

Fig 4. Total N and TCA insoluble N concentrations in bile (mg N/ml). Means ± SEM. 3 animals, $n = 24$.

Figure 5 shows that total nitrogen production in the pancreatic secretion was highest after intake of the 2 daily meals and reached 195 mg/2 h following the 9.00 meal. The basal production observed at the end of the nocturnal period was 95 mg/2 h. The daily production of total nitrogen secreted by the exocrine pancreas was 1 905 ± 218 mg.

The total nitrogen production in bile (fig 6) changed only a little within 24 h (130 to 165 mg/2 h). The daily production was 1 721 ± 70 mg N.

The amount of endogenous total nitrogen secreted through pancreatic and bile secretions into the intestinal lumen of a 45 kg live weight pig was 3.6 g per day, *ie* 22.6 g of total proteins (N x 6.25). This is the first time that such a result was obtained in the same animal.

The exocrine pancreatic secretion produced 1.9 g N per day, a value similar to those obtained by Zebrowska *et al* (1983) in pigs fed a semi-synthetic diet. The pancreatic nitrogen production was shown to depend on the nature of the dietary protein, but also on the concentration of the

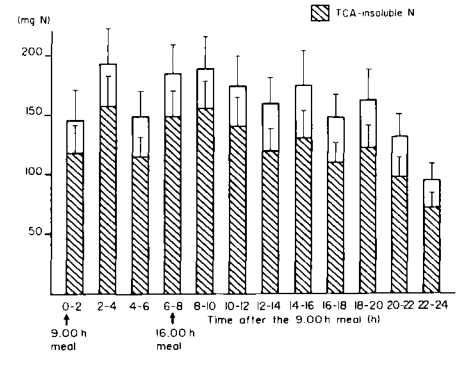

Fig 5. Total N and TCA insoluble N production in pancreatic juice (mg N/2 h). Means ± SEM. 3 animals, $n = 24$; $\bar{x} = 1\,905$ mg total N/d; $\bar{x} = 1\,486$ mg TCA insoluble N/d.

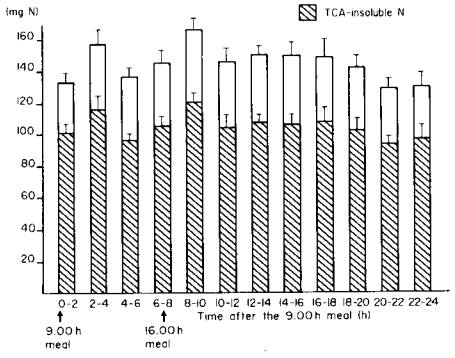

Fig 6. Total N and TCA insoluble N production in bile (mg N/2 h). Means ± SEM. 3 animals, n = 24; x = 1721 mg total N/d; $\bar{x}$ = 1 244 mg TCA insoluble N/d.

diet. It was lower in animals fed a semi-synthetic diet than in animals given a normal diet with similar amounts of nutrients (1.9 g *vs* 2.1 g according to Zebrowska *et al,* 1983; 1.1 g *vs* 1.5 g according to Partridge *et al,* 1982). The pancreatic endogenous total nitrogen production was 2.1 g per day in the pig fed heated soyabean (Corring *et al,* 1986) or 2.3 g per day when the diet contained wheat (Langlois *et al,* 1987). Furthermore, the present study shows that the amount of pancreatic nitrogen increased after meal intake, as reported earlier (Corring, 1980).

The daily bile secretion into the intestinal lumen included 1.7 g of endogenous nitrogen, which was close to the values reported by Sambrook (1981); the concentration of the diet did not appear to affect the daily production of endogenous nitrogen through bile secretion (Sambrook, 1981). In contrast to pancreatic secretion, meal intake did not affect the kinetics of nitrogen production in bile.

From results obtained by different authors, Low (1980) determined that the total endogenous nitrogen production in the digestive tract of a 40–50 kg live weight pig was ≈ 18 g per day. Taking into account this value, data in the present work showed that the exocrine pancreatic and biliary contribution to total endogenous nitrogen production was 20%. Moreover, exocrine pancreatic and bile nitrogen production represented ≈ 15% of ingested protein. The bile contribution (≈ 7%) was in the range of values reported by Juste (1982). The pancreatic contribution (≈ 8%) also corresponded to values reported by Juste (1982), but was lower than that determined by Corring (1975) in pigs fed a cereal-based diet.

The TCA insoluble form of nitrogen remained quite constant over 24 h and represented 75–82% in the pancreatic juice and 70–76% in bile. Throughout the experiment the mean percentage of TCA insoluble nitrogen was 78.1 in the pancreatic juice, higher than the value reported by Corring and Jung (1972), and 72.3 in bile. In the pig pancreatic juice we found (unpublished data) that digestive enzymes represented ≈ 90% of the TCA insoluble form of nitrogen. In man, the non-protein nitrogen mainly comes from amino-sugars and amides, especially urea, and also from mucoproteins which are TCA-soluble (Altman, 1961). Concerning bile secretion, data on the composition of TCA insoluble nitrogen are not available; but Altman (1961) reported that in man, it represented 71–78% of total nitrogen.

In conclusion, the present study shows that in the same pig fed a casein diet, daily pancreatic juice and bile flow rates were very similar, whilst the amount of endogenous total nitrogen secreted into the intestinal lumen was 3.6 g per day: 1.9 g through pancreatic secretion and 1.7 g through bile secretion. The mean percentage of TCA insoluble nitrogen was 78.1 in the pancreatic juice and 72.3 in bile.

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