Use of the mobile nylon bag technique for determination of apparent ileal digestibilities of crude protein and amino acids in feedstuffs for pigs

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ABSTRACT: Three digestibility experiments were conducted to evaluate the potential of determination of apparent ileal digestibilities (AID) of crude protein (CP) and amino acids (AA) by the mobile nylon bag technique (MNBT) using 21 feedstuffs and three mixed diets. In two conventional digestibility experiments (Exp. 1 and 2), AID were determined using in total 10 barrows (BW 35 kg) fitted with simple T-cannulas at the terminal ileum. For the MNBT studies, four pigs were fitted with a simple T-cannula at the proximal duodenum and a Post-Valve T-Caecum (PVTC) cannula at the terminal ileum. The MNBT studies included the feedstuffs (n = 10) from Exp. 1 and 2 as well as 14 further feedstuffs and mixed diets in which AID coefficients had been determined in previous trials. For each feedstuff 60 nylon bags were used. *In vitro* digestion of the bags was carried out in pepsin-HCl solution with 450 IU pepsin/l at pH 2.0 and 37°C for 4 h. In the 28-day *in vivo* experiment, 15 nylon bags per pig and day were inserted through the duodenal cannula and collected through the PVTC cannula after passage through the small intestine. Coefficients of AID were calculated based on the disappearance of CP and AA from the nylon bags during the *in vitro* and *in vivo* phase. In comparison with AID determined by the conventional method, AID of CP was on average 2.4% lower, whereas AID of lysine was on average 8.5% higher when determined by the MNBT. There was no significant ($P > 0.05$) correlation between AID coefficients of CP and AA determined by the conventional method and the MNBT, when all feedstuffs were taken into account. However, in cereals (n = 11), the correlation between AID coefficients determined by both methods was significant ($P < 0.05$) for CP (r = 0.61) and some AA (r ranging between 0.62 and 0.72). In conclusion, the potential of the MNBT to determine AID of CP and AA is rather limited. Differences in coefficients of AID of CP and AA were attributed to several factors such as diffusion of sample particles or endogenous protein through the nylon bags as well as to the presence of anti-nutritional factors (e.g. in legume seeds and oilseed meals).

Keywords: mobile nylon bag technique (MNBT); ileal digestibility; crude protein; amino acids; pigs

There is a general agreement that in pigs ileal rather than faecal digestibility measurements represent more accurate estimates of AA bioavailabilities since AA are only absorbed proximal to the distal ileum (Mosenthin et al., 2000). Ileal digestibilities are conventionally determined by collecting digesta of pigs fitted with intestinal cannulas at the terminal ileum or being surgically modified according to the ileal rectal anastomosis technique (Zimmermann and Mosenthin, 2002). These trials are costly, time consuming, and require relatively large quantities of feed and experimental animals. For these reasons, Sauer et al. (1983) introduced a more feasible alternative, the so called 'mobile nylon bag technique' (MNBT).

The MNBT is a combined *in vitro/in vivo* technique to determine nutrient and energy digestibilities in pigs. In the first step, the gastric digestion of feed samples which are sealed in nylon bags is simulated under standardized *in vitro* conditions. In the second step, after being inserted via a cannula into the duodenum, these pre-digested samples pass through the animal's digestive tract with digestive enzymes penetrating into the nylon bag. The nylon bags are retrieved either in faeces for the determination of total tract digestibility (Sauer et al., 1983) or at the terminal ileum, by means of an ileal cannula, for determining ileal digestibility (Yin et al., 2002). The portion of the feed sample that escaped the nylon bag during both the *in vitro* and the *in vivo* phase is considered to be digested.

In comparison with conventional digestibility studies, the major benefits of the MNBT are that only small amounts of feed and few animals are necessary (Petry and Handlos, 1978). Moreover, various feedstuffs can be tested at a time in one animal (Petry and Handlos, 1978), and single dietary components as well as complete diets can be used, even if palatability is low (Sauer et al., 1983).

Sauer et al. (1983) concluded that the MNBT represents a promising approach for the rapid determination of protein digestibilities in pigs. However, subsequent studies showed that the MNBT, as originally introduced by Sauer et al. (1983), is not always suitable for predicting *in vivo* digestibilities of protein and amino acids (Garcia-Martinez et al., 1988; Sauer et al., 1989; de Lange et al., 1991; Thacker and Qiao, 2001). Thus, some authors (Cherian et al., 1989; Thacker and Qiao, 2001; Yin et al., 2002) modified the MNBT as originally described by Sauer et al. (1983) by altering various factors such as sample and particle size of the assay feedstuff, *in vitro* conditions (e.g. gastric pH, pepsin activity) or handling of the retrieved nylon bags in order to improve the accuracy of this method in comparison with the conventional method for determining digestibilities.

Only few studies have been carried out so far in which the MNBT was used for the determination of ileal crude protein (CP) and amino acids (AA) in feedstuffs frequently used in pig nutrition. Thus, the objective of the present study was to measure ileal CP and AA in different grains, protein supplements of plant and animal origin and in mixed diets. The results obtained with the MNBT were compared with those determined in conventional digestibility trials to assess if the MNBT is suitable to estimate ileal CP and AA digestibilities in a variety of different feedstuffs and diets.

MATERIAL AND METHODS

Conventional digestibility studies

Apparent ileal digestibilities of CP and AA of different feedstuffs were determined in two conventional digestibility studies carried out at the Department of Agricultural, Food and Nutritional Science at the University of Alberta in Edmonton, Canada. In each experiment, five Yorkshire × Landrace barrows with an average initial body weight (BW) of approximately 35 kg were fitted with a simple T-cannula at the terminal ileum according to the procedures described by Sauer et al. (1983) and Mosenthin (1987). After surgery, the pigs were individually placed in stainless steel metabolism crates.

Twenty-four hours after surgery, the animals were fed 100 g of one of the assay diets (Tables 1 and 2). Feed allowance was increased by 200 g daily until a maximum of 1600 g per day was reached. Pigs were fed the diets twice daily at 08:00 and 20:00 h in meal form. They had free access to water. Chromic oxide was included in the diets (0.25%) as an indigestible marker.

Each experiment lasted for 35 days. After an adaptation period of 5 days to the assay diets, pigs were randomly allotted to the dietary treatments according to a 5 × 5 Latin Square design.

Ileal digesta were collected for 48 h. Starting at 08:30 h on day 6, samples were collected for 2 h, followed by a 2-h break. This procedure was carried out until 08.30 h on day 8 except for day 7, on which a single collection from 04:30 to 08:30 h was conducted followed by a 2-h break. After completion of digesta sampling, the animals were adapted to the new diet. All samples of ileal digesta were freeze-dried and stored at –20°C.

Table 1. Components (% as fed) and analyzed chemical composition (% dry matter) of the diets in Experiment 1 of the conventional digestibility study

	Diet				
	Rye cv. Kodiak	Rye cv. Musketeer	Sunflower meal	Fish meal	Meat-and-bone-meal
Components					
Rye (cv. Kodiak)	94.68	–	–	–	–
Rye (cv. Musketeer)	–	94.86	–	–	–
Sunflower meal	–	–	47.34	–	–
Fish meal	–	–	–	28.63	–
Meat-and-bone meal	–	–	–	–	34.05
Dextrose	–	–	10.00	10.00	10.00
Corn starch	–	–	37.96	57.70	52.40
Rapeseed oil	2.50	2.50	2.50	2.50	2.50
Trace elements and NaCl	0.50	0.50	0.50	0.50	0.50
Vitamins	0.20	0.20	0.20	0.20	0.20
Mineral premix	1.87	1.87	1.25	0.22	0.10
Cr_2O_3	0.25	0.25	0.25	0.25	0.25
Chemical composition					
Crude protein	9.2	9.8	17.9	17.0	17.8
Ether extract (HCl)	4.7	4.4	5.9	7.0	8.0
Crude ash	3.9	4.1	5.5	7.6	9.7
Acid detergent fiber	3.3	3.3	10.5	0.9	1.8

In Experiment 1, pigs were fed five different diets in total. Two assay diets were based on two varieties of rye, supplemented with rapeseed oil, minerals and vitamins (Table 1). The other diets contained sunflower meal, fish meal, or meat-and-bone meal as assay feed ingredients, each included in a semi-synthetic diet based on maize starch and dextrose. Apparent ileal CP and AA digestibilities were calculated according to the direct method.

In Experiment 2, pigs were fed five different diets in total. One assay diet was based on wheat, whereas equal amounts of four different varieties of oats were included in a wheat-based diet each (Table 2). Digestibility coefficients were determined by means of the difference method according to the following equation (van Leeuwen et al., 1987):

$$D_A = (D_D - D_B \times S_B)/S_A$$

where:

D_A = AID of the nutrient in the assay feedstuff (%)

D_D = AID of the nutrient in the assay diet (%)

D_B = AID of the nutrient in the basal feedstuff (%)

S_B = contribution level of the nutrient from the basal feedstuff to the assay diet (%)

S_A = contribution level of the nutrient from the assay feedstuff to the assay diet (%)

Mobile nylon bag technique studies

The MNBT studies were carried out at the Institute of Animal Physiology and Biochemistry at the National Institute of Animal Science in Foulum, Denmark. Four Yorkshire × Landrace barrows with an initial BW of 33 to 43 kg were fitted with a simple T-cannula at the proximal duodenum (Sauer et al., 1983) for the insertion of nylon bags, and a

Table 2. Components (% as fed) and analyzed chemical composition (% dry matter) of the diets in Experiment 2 of the conventional digestibility study

	Diet				
	wheat	oats			
		cv. Jasper	cv. Grizzly	cv. Foothills	cv. Calibre
Components					
Wheat	94.73	47.37	47.37	47.37	47.37
Oats (cv. Jasper)	–	47.39	–	–	–
Oats (cv. Grizzly)	–	–	47.39	–	–
Oats (cv. Foothills)	–	–	–	47.39	–
Oats (cv. Calibre)	–	–	–	–	47.39
Rapeseed oil	2.50	2.50	2.50	2.50	2.50
Trace elements and NaCl	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.20	0.20	0.20	0.20	0.20
Mineral premix	1.82	1.79	1.79	1.79	1.79
Cr_2O_3	0.25	0.25	0.25	0.25	0.25
Chemical composition					
Crude protein	15.1	14.7	15.0	13.8	13.8
Ether extract (HCl)	4.5	6.7	5.4	6.1	6.0
Crude ash	3.9	4.2	4.5	5.0	4.8
Acid detergent fiber	4.3	9.8	10.1	10.3	9.8

Post-Valve T-Caecum (PVTC) cannula at the terminal ileum (van Leeuwen et al., 1991) for their retrieval. After surgery, the pigs were individually housed in stainless steel metabolism crates. They were fed twice daily at 07:00 and 16:00 h 900 g of a cereal-soybean meal based diet (Table 3), mixed with water at a ratio of 1:1 (w/v). After feeding, the feeding troughs were filled with water for *ad libitum* consumption until the next feeding.

The MNBT studies included 10 feedstuffs assayed in experiments 1 and 2 of the conventional digestibility studies. Additionally, another set of 14 feedstuffs was used which included four varieties of wheat, four varieties of peas, three rapeseed meals of different origin and three mixed feeds. Apparent ileal digestibilities of CP and AA of these 14 feedstuffs had been previously determined in conventional trials (Just et al., 1985; Fan, 1994). The feedstuffs that were assayed (Table 4) had been previously used in the conventional digestibility studies.

For the *in vitro* part of the study, samples of 0.8 to 1.0 g of each feedstuff were ground to 1 mm and filled into nylon bags (25 × 40 mm, pore size 48 µm, Nitex mononylon tissue No. 3-270-53 ASTM, Thompson and Co. Ltd. Montreal, Canada) and afterwards sealed by means of a heat sealer. Sixty nylon bags per feedstuff were prepared, 15 for each animal. According to procedures described by Cherian et al. (1988), nylon bags were incubated for 4 h in a pepsin-HCL-solution with 450 IU pepsin/l at pH 2.0 and 37°C. After the incubation, the bags were dipped into deionized water for 1 min, subsequently air-dried for 10 min on a mesh sieve, and stored at –18°C in plastic boxes until to be used for the following *in vivo* studies.

The *in vivo* part of the study lasted for 28 days and was carried out with four pigs. In total, 15 nylon bags per pig and day were introduced into the duodenum twenty min after the morning feeding. Each of two nylon bags was inserted via the duodenal T-cannula at 15-min intervals until the application of 15 bags was finished. For their retrieval, the PVTC-cannula was opened 3 h after the application of the first bag. Nylon bags were separated from

Table 3. Components and analyzed chemical composition of the basal diet fed to pigs in the MNBT study

Item	(%)
Components (% as fed)	
Barley	50.0
Wheat	20.4
Soybean meal	24.0
Fat	2.0
Molasses	1.0
$CaPO_4$	1.2
$CaCO_3$	0.8
NaCl	0.4
Vitamins and minerals	0.2
Chemical composition (% dry matter)	
Gross energy (MJ/kg)	18.9
Crude protein	21.1
Ether extract	5.5
Crude fiber	5.1
Ash	5.0
Starch	41.7
Sugars	2.0
N-free extracts[1]	63.3

MNBT – Mobile Nylon Bag Technique; [1]calculated

caecal digesta, subsequently cleaned from adherent digesta using a pincer, and thereafter immediately frozen at –23°C.

Chemical Analyses

Prior to the chemical analysis of nylon bag contents, bags were freeze-dried, and their surface was cleaned using a brush. After weighing, they were cut open and contents were pooled within animal and feedstuff. Feed, digesta, and nylon bag contents were analyzed for proximate nutrients according to the procedures of VDLUFA (Naumann and Bassler, 1976). Dry matter content of the freeze-dried feed sample in the nylon bag was determined at 103°C in an air-drying oven. Analysis of Cr_2O_3 was conducted according to the method described by Fenton and Fenton (1979). The AA contents of feed and digesta samples were determined at the Department of Animal Science, University of Alberta, Edmonton, Canada, using the method of Jones and Gilligan (1983). AA analysis of nylon bag contents was conducted at IS Research Institute for Experimental Animal Physiology and Animal Nutrition, Wahlsted, Germany, according to the VDLUFA standards (Naumann and Bassler, 1976).

Table 4. Assay feedstuffs used in conventional digestibility studies

Cereals	Protein supplements	Complete diets
Rye (cv. Kodiak)	Peas (cv. Titan)[1]	Mixed diet DK 11[3]
Rye (cv. Musketeer)	Peas (cv. Princess)[1]	Mixed diet DK 13[3]
Wheat (mixed wheat)	Peas (cv. Tipu)[1]	Mixed diet DK 17[3]
Oats (cv. Calibre)	Peas (cv. Trapper)[1]	
Oats (cv. Foothills)	Rapeseed meal (cv. Russel)[1]	
Oats (cv. Grizzly)	Sunflower meal	
Oats (cv. Jasper)	Fishmeal	
Wheat (cv. Columbus)[1]	Meat-and-bone meal	
Wheat (cv. Kyle)[1]	Rapeseed meal (cv. Nipawin)[1]	
Wheat (cv. Neepawa)[1]	Rapeseed meal[2]	
Wheat (cv. Katepawa)[1]		

[1]AID determined in conventional studies (Fan, 1994)
[2]AID determined in conventional studies
[3]AID determined in conventional studies (Just et al., 1985)

Statistical Analyses

Data were subjected to the analysis of variance using the GLM procedure of SAS (SAS, 1999). Animal represented the experimental unit. The model used in experiments 1 and 2 was as follows:

$$Y_{ijk} = \mu + T_j + P_j + A_j + \varepsilon_{ijk}$$

where:

Y_{ijk} = dependent variable
μ = overall mean
T_j = fixed effect of treatment
P_j = fixed effect of period
A_j = fixed effect of animal
ε_{ijk} = residuals

The model used in experiment 3 was as follows:

$$Y_{ijk} = \mu + T_j + A_j + \varepsilon_{ijk}$$

where:

Y_{ijk} = dependent variable
μ = overall mean
T_j = fixed effect of treatment
A_j = fixed effect of animal
ε_{ijk} = residuals

An alpha level of 0.05 was used for determination of statistical significance.

RESULTS

Conventional digestibility studies

Coefficients of AID of CP and AA determined in the conventional digestibility trial in Experiment 1 are presented in Table 5. Digestibility coefficients of CP and AA both in fish meal and meat-and-bone meal were similar and did not differ signifi-

Table 5. Apparent ileal digestibilities of crude protein and amino acids (%) determined in Experiment 1 of the conventional digestibility study

Item	Rye cv. Kodiak	Rye cv. Musketeer	Sunflower meal	Fish meal	Meat-and-bone-meal	SE
Crude protein	59.9^{c}	68.2^{b}	74.9^{a}	80.2^{a}	79.3^{a}	1.9
Indispensable amino acids						
Arginine	70.4^{b}	68.1^{b}	89.8^{a}	90.5^{a}	88.8^{a}	6.6
Histidine	71.1^{b}	65.4^{c}	83.7^{a}	86.0^{a}	86.8^{a}	2.0
Isoleucine	67.1^{b}	68.0^{b}	82.4^{a}	85.6^{a}	83.7^{a}	5.2
Leucine	68.8^{b}	69.1^{b}	81.7^{a}	86.1^{a}	85.5^{a}	4.5
Lysine	59.9^{c}	58.9^{c}	79.1^{b}	88.7^{a}	86.6^{a}	2.7
Methionine	79.1^{b}	78.1^{b}	93.3^{a}	90.6^{a}	90.3^{a}	4.0
Phenylalanine	72.7^{b}	73.9^{b}	83.6^{a}	85.7^{a}	86.1^{a}	3.5
Threonine	55.4^{b}	53.9^{b}	75.9^{a}	81.4^{a}	81.0^{a}	7.4
Valine	67.0^{b}	67.0^{b}	80.7^{a}	84.5^{a}	84.4^{a}	4.9
Dispensable amino acids						
Alanine	57.9^{c}	57.4^{c}	78.7^{b}	87.2^{a}	86.3^{a}	2.7
Aspartic acid	64.9^{b}	66.6^{b}	80.2^{a}	78.5^{a}	79.7^{a}	4.3
Cysteine	n.d.	n.d.	n.d.	n.d.	n.d.	
Glutamic acid	80.2^{b}	81.7^{ab}	88.3^{a}	86.0^{ab}	85.6^{ab}	2.9
Glycine	34.8^{c}	32.1^{c}	66.9^{b}	84.3^{a}	85.9^{a}	6.3
Serine	63.1^{b}	63.8^{b}	77.0^{a}	82.1^{a}	80.8^{a}	4.7

^{a,b,c}within a row, values with different superscript letters differ ($P < 0.05$)
n.d. = not determined

cantly ($P > 0.05$). In sunflower meal, AID of lysine, glycine and alanine were significantly ($P < 0.05$) lower than in fish meal and meat-and-bone meal. In both rye cultivars AID of CP and AA, except for glutamic acid in cv. Musketeer, were lower ($P < 0.05$) than in the other assay feedstuffs. Among rye cultivars, cv. Musketeer showed higher ($P < 0.05$) AID of CP as compared to cv. Kodiak (68.1 vs. 59.9%). In contrast, AID of histidine was higher ($P < 0.05$) in cv. Kodiak than in cv. Musketeer (71.1 vs. 65.4%).

Table 6 includes the ileal digestibilities of CP and AA determined in Experiment 2. The AID of CP in wheat was numerically higher ($P > 0.05$) in comparison with the different varieties of oats. However, as compared to oat cv. Calibre, AID of CP and glutamic acid in wheat was significantly ($P < 0.05$) higher.

Mobile nylon bag technique studies

The recovery rate of the nylon bags ($n = 1440$) amounted to 60%, ranging between 48 and 72% for individual pigs. The passage time of recovered nylon bags ($n = 868$), from the proximal duodenum to the terminal ileum, averaged 285 min (± 44); the minimum and maximum time needed for recovery ranged between 180 and 450 min, respectively.

Apparent ileal digestibilities of CP and AA

AID of CP and AA determined by the MNBT are presented in Table 7. For all feedstuffs, AID of AA were higher than those obtained for CP. For CP, the coefficients of variation in AID ranged from 1.9% in wheat up to 13% in oilseed meals. The highest

Table 6. Apparent ileal digestibilities of crude protein and amino acids (%) determined in Experiment 2 of the conventional digestibility study

Item	Wheat	Oats				
		cv. Calibre	cv. Foothills	cv. Grizzly	cv. Jasper	SE
Crude protein	80.1^{a}	52.7^{b}	62.0^{ab}	72.0^{ab}	68.7^{ab}	9.8
Indispensable amino acids						
Arginine	85.7	77.9	79.2	84.7	80.2	3.4
Histidine	88.6	71.1	72.4	80.7	68.7	8.6
Isoleucine	87.0	69.5	72.0	77.7	72.5	8.0
Leucine	87.9	72.8	76.3	80.3	74.6	7.0
Lysine	76.9	43.1	55.3	65.2	52.4	15.0
Methionine	92.4	79.3	81.9	85.0	81.2	6.5
Phenylalanine	90.1	76.4	79.2	83.0	77.8	6.0
Threonine	77.7	45.0	61.5	67.7	52.0	14.9
Valine	84.9	66.8	72.4	77.2	70.2	8.0
Dispensable amino acids						
Alanine	77.6	51.3	61.2	71.9	63.1	11.9
Aspartic acid	78.8	58.7	69.3	73.8	64.4	8.7
Cysteine	n.d.	n.d.	n.d.	n.d.	n.d.	
Glutamic acid	94.3^{a}	80.2^{b}	84.4^{ab}	85.8^{ab}	79.8^{b}	5.0
Glycine	70.2	44.1	61.3	64.1	53.2	12.0
Serine	85.6	60.4	70.5	73.9	64.8	11.9

^{a,b}within a row, values with different superscript letters differ ($P < 0.05$)

n.d. = not determined

Table 7. Comparison of apparent ileal digestibilities (%) of crude protein and amino acids determined by the conventional method (Conv.) and the Mobile Nylon Bag Technique (MNBT)

		Cereals	Wheat	Oats	Rye	Peas	Oilseed meals	Animal products[1]	Mixed diets
	n	11	5	4	2	4	4	2	3
CP	Conv.	70.4 ± 9.1	78.2 ± 2.4	63.9 ± 8.5	64.1 ± 5.9	74.2 ± 2.3	71.4 ± 3.7	79.8 ± 0.6	72.6 ± 7.0
	MNBT	76.1 ± 7.6	81.9 ± 1.9	74.7 ± 5.9	64.5 ± 4.4	80.5 ± 5.2	50.2 ± 13.0	64.6 ± 5.1	62.6 ± 8.7
	Sig.	**	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Indispensable amino acids									
ARG	Conv.	79.6 ± 5.5	81.8 ± 2.9	80.5 ± 3.0	69.3 ± 1.6	86.0 ± 3.5	86.2 ± 4.5	89.7 ± 4.1	88.7 ± 2.2
	MNBT	86.4 ± 6.0	87.7 ± 3.4	88.9 ± 6.6	78.0 ± 3.4	93.6 ± 1.8	74.1 ± 10.2	79.3 ± 1.2	82.9 ± 3.5
	Sig.	**	*	n.s.	*	**	n.s.	n.s.	n.s.
HIS	Conv.	76.1 ± 7.2	81.5 ± 5.2	73.2 ± 5.2	68.3 ± 4.0	74.8 ± 4.6	82.5 ± 4.2	86.3 ± 0.6	85.0 ± 2.3
	MNBT	85.4 ± 6.9	81.9 ± 7.3	82.8 ± 5.7	72.7 ± 3.1	86.9 ± 1.6	69.5 ± 6.0	79.5 ± 7.8	76.0 ± 4.8
	Sig.	n.s.	n.s.	*	n.s.	**	*	n.s.	*
ILE	Conv.	76.0 ± 6.5	81.8 ± 3.2	72.9 ± 3.4	67.6 ± 0.6	76.4 ± 2.2	77.5 ± 6.0	84.7 ± 1.3	80.5 ± 3.3
	MNBT	85.4 ± 6.9	88.8 ± 2.1	86.9 ± 4.2	73.9 ± 8.4	87.9 ± 2.3	67.3 ± 5.8	72.7 ± 9.1	76.6 ± 4.6
	Sig.	**	**	**	n.s.	**	n.s.	n.s.	n.s.
LEU	Conv.	78.1 ± 6.2	83.3 ± 2.9	76.0 ± 3.2	69.0 ± 0.2	77.0 ± 2.1	79.2 ± 5.1	85.8 ± 0.4	81.2 ± 3.0
	MNBT	85.9 ± 5.7	89.3 ± 1.5	85.5 ± 6.8	78.2 ± 0.8	88.0 ± 2.4	66.8 ± 8.4	77.3 ± 7.7	77.7 ± 3.8
	Sig.	**	**	*	**	**	*	n.s.	n.s.
LYS	Conv.	61.1 ± 9.1	67.6 ± 6.5	54.0 ± 9.1	59.4 ± 0.7	82.7 ± 1.6	77.3 ± 3.3	87.7 ± 1.5	84.7 ± 3.8
	MNBT	81.7 ± 5.3	82.3 ± 3.3	84.8 ± 4.9	74.2 ± 2.5	91.5 ± 1.2	70.5 ± 7.3	79.6 ± 4.1	80.0 ± 4.4
	Sig.	**	**	**	**	**	n.s.	n.s.	n.s.
MET	Conv.	81.5 ± 4.4	81.9 ± 6.2	81.0 ± 2.4	78.6 ± 0.7	71.8 ± 1.5	85.9 ± 6.2	90.5 ± 0.2	83.4 ± 2.9
	MNBT	88.3 ± 5.8	91.6 ± 2.2	89.3 ± 2.2	78.6 ± 5.2	86.5 ± 2.8	74.8 ± 4.4	83.3 ± 21.1	70.7 ± 12.4
	Sig.	**	*	**	n.s.	**	*	n.s.	n.s.
PHE	Conv.	81.3 ± 5.7	86.2 ± 2.6	79.1 ± 2.8	73.3 ± 0.8	72.9 ± 3.4	78.8 ± 5.4	85.9 ± 0.3	80.0 ± 1.5
	MNBT	85.0 ± 7.6	89.4 ± 1.6	84.9 ± 9.0	74.3 ± 0.7	88.2 ± 4.7	68.8 ± 8.5	76.2 ± 8.2	78.0 ± 4.4
	Sig.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
THR	Conv.	62.5 ± 9.9	70.5 ± 4.9	56.6 ± 10.0	54.7 ± 1.1	65.0 ± 2.8	71.4 ± 9.2	81.2 ± 0.3	70.0 ± 5.4
	MNBT	81.9 ± 5.4	83.8 ± 2.0	82.6 ± 5.1	75.5 ± 9.6	87.1 ± 2.1	66.2 ± 7.5	76.0 ± 6.8	75.6 ± 4.6
	Sig.	**	**	**	n.s.	**	n.s.	n.s.	n.s.
VAL	Conv.	73.5 ± 5.7	77.6 ± 4.5	71.7 ± 4.4	67.0 ± 0.0	72.1 ± 2.9	75.2 ± 6.4	84.5 ± 0.1	77.7 ± 2.9
	MNBT	84.0 ± 5.5	86.2 ± 3.2	85.2 ± 5.7	76.1 ± 3.3	85.5 ± 2.3	70.0 ± 7.4	73.5 ± 9.6	73.9 ± 3.5
	Sig.	**	**	**	*	**	n.s.	n.s.	n.s.

AID of CP was determined in wheat (81.9%) and peas (80.5%), whereas lower digestibilities were obtained for oats (74.7%), rye (64.5%) and feedstuffs of animal origin (64.6%). AID of CP was lowest in oilseed meals (50.2%).

The AID of lysine was highest in peas (91.5%), whereas in oats, wheat and feedstuffs of animal origin digestibilities amounted to 84.8, 82.3, and 79.6%, respectively. In rye and oilseed meals AID of lysine obtained by the MNBT was consider-

Table 7 to be continued

		Cereals	Wheat	Oats	Rye	Peas	Oilseed meals	Animal products[1]	Mixed diets
	n	11	5	4	2	4	4	2	3
Dispensable amino acids									
ALA	Conv.	65.4 ± 8.0	71.4 ± 4.2	61.9 ± 8.5	57.7 ± 0.4	70.8 ± 1.9	76.2 ± 3.7	86.8 ± 0.6	75.1 ± 3.3
	MNBT	81.1 ± 5.2	83.5 ± 1.6	82.1 ± 5.5	72.9 ± 0.8	86.2 ± 2.3	67.4 ± 7.1	77.3 ± 1.5	75.6 ± 3.9
	Sig.	**	**	**	**	**	n.s.	n.s.	n.s.
ASP	Conv.	68.5 ± 5.5	71.2 ± 5.1	66.6 ± 6.5	65.8 ± 1.2	77.7 ± 2.7	74.0 ± 7.7	79.1 ± 0.8	77.0 ± 4.6
	MNBT	79.9 ± 6.3	80.8 ± 2.6	80.2 ± 8.9	77.0 ± 10.3	88.4 ± 1.7	68.2 ± 7.7	76.0 ± 5.2	77.7 ± 4.0
	Sig.	**	**	*	n.s.	**	n.s.	n.s.	n.s.
CYS	Conv.	–	76.4 ± 4.2	–	–	61.8 ± 1.0	–	–	70.6 ± 7.6
	MNBT	–	84.0 ± 2.9	–	–	74.5 ± 7.0	–	70.8 ± 11.3	65.7 ± 4.9
	Sig.	–	*	–	–	*	–	–	n.s.
GLU	Conv.	86.3 ± 5.4	91.5 ± 2.0	82.6 ± 3.0	81.0 ± 1.1	81.7 ± 2.7	85.2 ± 4.2	85.8 ± 0.3	86.5 ± 1.4
	MNBT	89.3 ± 7.7	94.3 ± 1.2	85.0 ± 10.7	84.8 ± 1.0	90.2 ± 1.1	75.6 ± 6.3	77.4 ± 3.3	83.5 ± 3.9
	Sig.	n.s.	*	n.s.	*	**	*	n.s.	n.s.
GLY	Conv.	57.0 ± 14.0	67.6 ± 3.2	35.7 ± 9.0	33.5 ± 1.9	62.3 ± 3.4	71.7 ± 12.0	85.1 ± 1.1	67.6 ± 1.5
	MNBT	77.5 ± 7.9	81.9 ± 1.4	79.3 ± 5.9	63.2 ± 0.4	84.0 ± 3.1	63.6 ± 7.2	77.6 ± 3.0	73.3 ± 4.3
	Sig.	**	**	**	**	**	n.s.	n.s.	n.s.
SER	Conv.	73.3 ± 9.1	81.9 ± 2.6	67.4 ± 6.0	63.5 ± 0.5	71.8 ± 2.1	74.2 ± 8.2	81.5 ± 0.9	67.0 ± 5.9
	MNBT	85.4 ± 5.2	89.3 ± 1.1	84.7 ± 3.8	76.8 ± 1.1	87.6 ± 2.2	66.2 ± 8.9	76.3 ± 2.9	77.0 ± 4.7
	Sig.	**	**	**	**	**	n.s.	n.s.	n.s.

CP = crude protein, Conv. = conventional method, Sig. = significance
[1]feedstuffs of animal origin
*$P < 0.05$, **$P < 0.01$
n.s. = not significant ($P > 0.05$)

ably lower amounting to 74.2 and 68.2%, respectively.

DISCUSSION

Comparison of ileal digestibilities of crude protein and amino acids determined by the conventional method and the MNBT

Wheat, oats, and rye were grouped as cereals (Table 7). In cereals and peas, AID of CP and AA determined by the MNBT were significantly ($P <$ 0.05) higher in most cases compared to values obtained by the conventional method (Table 7). In cereals, differences between digestibility coefficients were highly significant ($P < 0.01$) for most AA except for histidine, phenylalanine and glutamic acid. In particular, AID of lysine, threonine and glycine were considerably lower, amounting to 20.6%, 19.4% and 20.5%, respectively, when determined by the conventional method. It is generally accepted that certain amino acids such as threonine and glycine substantially contribute to ileal AA recoveries due to their enrichment with endogenous protein (Ravindran et al., 2004). Presumably, these endogenous secretions do not or not completely penetrate into the nylon bags, thus resulting in higher AID coefficients when determined by the MNBT. Close agreement ($P > 0.05$) between digestibility coefficients determined by the MNBT or conventional studies was obtained in rye for AID of CP

(64.5% vs. 64.1%), methionine (78.6% vs. 78.6%) and phenylalanine (74.3% vs. 73.3%), and in wheat for AID of histidine (81.9% vs. 81.5%).

Differences between AID coefficients determined by means of the conventional method and the MNBT were extremely high for some AA in oats, ranging between 2% (glutamic acid) and 44% (glycine). AID of lysine and threonine differed by 31% and 26%, respectively, between both methods. These differences could be due, at least in part, to relatively high contents of dietary acid-detergent fibre (ADF) in the oat-based diets (Table 2) which, in turn, might have increased the endogenous protein secretion into the small intestine. According to Bergner et al. (1975), high concentrations of ADF may result in a decreased AA digestibility due to the adsorption of AA to fibre compounds during passage through the intestinal tract. Schneeman et al. (1982) reported that higher dietary fibre contents resulted in an increased intestinal secretion of mucus in rats. It can be assumed that AID coefficients are not confounded by interactions between fibre components in the feed and endogenous AA secretions when determined by the MNBT. Thus, digestibilities of CP and AA determined by the MNBT are higher than the values obtained by the conventional method.

In rye, differences in AID determined by both methods ranged between 0% (methionine) and 30% (glycine). Lower digestibility coefficients determined in rye by means of the conventional technique may be attributed, at least in part, to the presence of soluble non-starch polysaccharides (NSP) such as arabinoxylans, galactans or β-glucans, which have been reported to increase digesta viscosity, thus limiting the diffusion between digestive enzymes and nutrients, finally decreasing the rate of the absorption of AA in the small intestine (Ikegami et al., 1990; Serena and Bach Knudsen, 2007). According to Englyst et al. (1989), rye contains higher levels of soluble NSP (4.6%, DM basis), whereas wheat, for example, contains only 2.4% of soluble NSP (DM basis).

In agreement with the results obtained for cereals, AID of CP in peas were also higher if measured by means of the MNBT in comparison with AID coefficients determined in conventional studies (80.5% vs. 74.2%). These differences, however, failed to be significant ($P > 0.05$), whereas for most of the AA the AID determined by the MNBT were significantly ($P < 0.01$) higher in comparison with values determined in the conventional digestibility trials. Differences in AID between both methods, ranging between 6.3 (CP) and 22% (threonine), may be attributed to the presence of anti-nutritional factors (ANF) such as protease-inhibitors or tannins in peas (Yin et al., 2002). As shown in several reports (Mariscal-Landín et al., 2002; Święch et al., 2004), protease-inhibitors or tannins may have a negative effect on AID of CP and AA in pigs by inhibiting the activity of digestive enzymes or by increasing the secretion of endogenous protein. According to Yin et al. (2002), the potential impact of ANF on digestibility coefficients is not sufficiently taken into account when the MNBT is applied, thus resulting in higher values of AID of CP and AA in feedstuffs with high concentrations of ANF, which was confirmed by the results of this study.

In oilseed products, feedstuffs of animal origin and mixed diets, AID of CP and AA determined by the MNBT were lower as compared to the conventional digestibility studies. In most cases, however, differences were not significant ($P > 0.05$), presumably due to a high variation within dietary treatments.

After retrieval of the nylon bags, considerable swelling of the samples of oilseed products was observed. After further inspection, it was recognized that substantial digestion had only occurred in the peripheral bounds of the bag contents. Thus, in oilseed products, the lower digestibility coefficients determined by the MNBT may be attributed to the high bulking capacity of the samples which, according to Petry and Tiews (1972), might have limited the penetration of digestive enzymes into the nylon bags, thus resulting in decreased digestibilities (Leibholz, 1991).

For feedstuffs of animal origin, no differences ($P > 0.05$) between AID determined by both techniques were obtained. However, the values were numerically higher (3–15%) when determined in conventional digestibility experiments. These differences might be due to a high content of ash both in meat-and-bone meal (26%, DM basis) and fishmeal (22%, DM basis), which might have increased pH values due to a higher buffering capacity in the nylon bags. As a result, the activity of pepsin during the *in vitro* pre-digestion might have been limited. According to Blank et al. (1999), a high buffering capacity exerts negative effects on AID of CP and AA in pigs. Similar observations were reported by Cherian et al. (1988).

In the mixed diets, a comparison of digestibility coefficients for both methods revealed inconsistent

Table 8. Linear relationship between apparent ileal digestibilities of crude protein and amino acids in feedstuffs determined with the conventional method (y) and the Mobile Nylon Bag Technique (MNBT, x) (n = 24)

	Intercept	Slope	r[1]	RSD[2]	Significance
Crude protein	61.5	0.26	0.24	15.0	n.s.
Indispensable amino acids					
Arginine	75.0	0.09	0.13	6.1	n.s.
Histidine	89.0	–0.13	0.15	6.9	n.s.
Isoleucine	74.0	0.04	0.07	5.7	n.s.
Leucine	71.0	0.09	0.16	5.3	n.s.
Lysine	68.7	0.04	0.04	13.0	n.s.
Methionine	95.4	–0.17	0.26	6.4	n.s.
Phenylalanine	66.0	0.16	0.26	5.6	n.s.
Threonine	74.0	–0.10	0.09	9.7	n.s.
Valine	78.0	–0.03	0.05	5.8	n.s.
Dispensable amino acids					
Alanine	84.5	–0.16	0.15	8.7	n.s.
Aspartic acid	62.0	0.13	0.17	6.6	n.s.
Glutamic acid	70.8	0.17	0.33	4.3	n.s.
Glycine	40.8	0.31	0.20	13.4	n.s.
Serine	58.8	0.19	0.24	7.4	n.s.

[1]correlation coefficient
[2]residual standard deviation
n.s. = not significant (P > 0.05)

results. The AID of CP determined by the MNBT was 10% lower (P > 0.05) compared to the conventional digestibility trials, whereas AID of AA were 2–13% lower (P > 0.05) when determined by the MNBT. However, AID of threonine, glycine and serine were 6–10% higher (P > 0.05) when determined conventionally. The AID of alanine (75.6% vs. 75.1%) and aspartic acid (77.7% vs. 77.0%) showed close agreement (P > 0.05) between both methods. Differences in AID between both methods were significant (P < 0.05) for histidine only (9%).

Correlation between apparent ileal digestibilities of crude protein and amino acids determined by the conventional method and the MNBT

For linear regression analyses, AID of CP and AA were pooled for all assay feedstuffs. As shown in Table 8, there was no significant (P > 0.05) relationship between the coefficients of AID of CP and AA determined by the conventional method and the MNBT. As summarized in Table 9, only in cereals, AID of CP, arginine, isoleucine, leucine, phenylalanine, glycine and serine obtained by the conventional method and the MNBT were significantly (P < 0.05) correlated (r = 0.61–0.72). Thus, it can be concluded that the AID of CP and AA in feedstuffs of different origin but also in mixed diets cannot be accurately estimated by the MNBT, which is in accordance with Yin et al. (2002).

Possible sources of inaccuracies in the use of the MNBT

In conventional digestibility studies, AID of a particular nutrient is expressed as the difference between the level of the nutrient in the assay diet and in ileal digesta. In contrast, measurements of AID by the MNBT are based on the non-digested residue of the nylon bag content. Thus, AID of a nutrient is defined as the portion of a nutrient that

Table 9. Linear relationship between apparent ileal digestibilities of crude protein and amino acids in cereals determined with the conventional method (y) and the Mobile Nylon Bag Technique (MNBT, x) (n = 11)

	Intercept	Slope	r^1	RSD[2]	Significance
Crude protein	14.9	0.73	0.61	7.6	*
Indispensable amino acids					
Arginine	22.4	0.65	0.72	4.0	*
Histidine	59.5	0.21	0.20	7.4	n.s.
Isoleucine	25.6	0.59	0.62	5.4	*
Leucine	13.8	0.75	0.69	4.8	*
Lysine	66.5	–0.07	0.04	3.6	n.s.
Methionine	56.0	0.29	0.37	3.7	n.s.
Phenylalanine	38.7	0.50	0.67	4.4	*
Threonine	19.6	0.53	0.28	10.0	n.s.
Valine	27.9	0.54	0.53	5.1	n.s.
Dispensable amino acids					
Alanine	13.7	0.64	0.42	7.6	n.s.
Aspartic acid	70.6	–0.03	0.03	5.7	n.s.
Glutamic acid	52.7	0.38	0.53	4.9	n.s.
Glycine	–40.6	1.26	0.71	10.3	*
Serine	–23.5	1.13	0.65	7.3	*

[1]correlation coefficient
[2]residual standard deviation
*$P < 0.05$
n.s. = not significant ($P > 0.05$)

has escaped the nylon bag during the *in vitro* and *in vivo* digestion procedures. According to Sauer et al. (1989), *in vivo* AID may be overestimated by MNBT because during the *in vitro* incubation, compounds that are soluble in pepsin or HCl may escape the nylon bags. Furthermore, as discussed by Schadereit et al. (1992), soluble compounds containing nitrogen may be removed from the interior of the bags if the nylon bags are cleaned before chemical analysis.

As suggested by Schadereit et al. (1992) and Viljoen et al. (1997), the migration of intestinal content originating from feed or endogenous sources may negatively affect the accuracy of determination of ileal digestibilities by the MNBT. According to Schadereit et al. (1992), the portion of endogenous N in total N in the nylon bags may range between 25% and 70%, depending on the feedstuff under investigation.

In conventional studies, the ileal CP and AA digestibilities may be affected by the presence of ANF such as NSP, pectins and high levels of dietary fibre. For example, viscous indigestible polysaccharides have been reported to increase the digesta viscosity (Ikegami et al., 1990). Furthermore, the sloughing of mucosa cells may be stimulated by high levels of dietary fibre (Viljoen et al., 1997). However, the impact of ANF or dietary fibre is eliminated when AID is determined by means of the MNBT because a direct contact between feed and the intestinal wall is inhibited. All these factors may account for differences in AID between conventional methods and the MNBT. In agreement with Yin et al. (2002), it may be concluded that AID is not accurately estimated by the MNBT in feedstuffs containing ANF (e.g. legume seeds, oilseed meals), high levels of NSP or fibre (e.g. rye, oats) or high bulking capacity (e.g. rapeseed meal).

Furthermore, the accuracy of the estimation of AID by the MNBT may be affected by sample size, particle size, pH value and pepsin concentration during the *in vitro* digestion and by the pore size of the nylon bags (Cherian et al., 1989; Yin et al., 2002). In the present study, the sample size was 0.8–1.0 g per nylon bag, feed samples were ground to a particle size of 1 mm and the pore size of the nylon bags was 48 µm. Cherian et al. (1989) investigated the effects of varying factors of the MNBT in order to alleviate the discrepancies between AID in soybean meal determined by the MNBT and the conventional method. In this study, the closest agreement between both methods was achieved when the samples were ground using a screen size of 0.5 mm, the nylon bag pore size of either 48 or 63 µm, and the sample size of 0.5 g. Furthermore, Yin et al. (2002) observed the closest agreement between the conventional method and the MNBT when the sample size was 0.75 g, feed was ground through a 1.0-mm mesh screen and the bags were washed for 2 min after retrieval from digesta.

In conclusion, the potential of the MNBT, as described in the present study, is rather limited to estimate AID of CP and AA in pigs. Several factors may negatively affect the accuracy of the MNBT, such as the rate of diffusion of sample particles or endogenous proteins through the nylon bag as well as the presence of ANF in several feedstuffs (e.g. legume seeds or oilseed meals). Further studies should be directed to improve the accuracy of the MNBT for different feedstuffs for pigs.

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