DETERMINATION OF THE APPARENT ILEAL DIGESTIBILITY OF PROTEIN AND AMINO ACIDS IN FEEDSTUFFS AND MIXED DIETS FOR GROWIG-FINSHING PIGS WITH THE MOBILE NYLON BAG TECHNIQUE*

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Summary

A series of experiments was conducted to determine the influence of various pepsin-HCL pretreatment factor, hereby the factors of duration of washing for the retrieved bags, inherent to the mobile nylon bag technique (MNBT), on apparent ileal digestibility of crude protein (AIDCP) and apparent ileal digestibility of dry matter (AIDDM). At last, the AIDCP and apparent ileal digestibility of amino acids (AIDAA) in maize, barley, wheat, rapeseed meal, cottonseed meal and three mixed diets were determined with the MNBT and ileo-rectal anastomis pigs (IRAT). For the MNBT techniques, bag measuring 25 × 40 MM and containing 0.75 g feedstuff samples, after pre-digestion *in vitro*, were introduced into the ileo-rectal anastomis pigs (IRAT) gastrointestinal tract through a duodenal cannula and recovered in the ileal digesta between 6 and 12 h. later. 1. The apparent ileal digestibility of dry matter (AIDDM) and crude protein (AIDCP) of the tested samples, with the exception of fish meal, determined by MNBT were not affected by the different pepsin-HCL pretreatment times *in vitro* between 2.5 h. and 4 h. 2. There was no significant ($p > 0.05$) difference of the AIDCP and AIDDM of maize determined by the MNBT among different pepsin concentration (0.03%, 0.07% and 0.1%) treatment *in vitro*. 3. The AIDCP determined with the MNBT was affected by the washed and unwashed recovered bags from the ileal digesta. 4. The AIDCP and AID amino acids (AIDAA) of maize, barley, wheat, rapeseed meal, soya-bean meal, cottonseed meal and three mixed diets from the MNBT, with a solution of 0.01N HCL (PH 2) and 0.1% of pepsin concentration, a pepsin-HCL pretreatment time *in vitro* or 4h. and a washing time of the recovered bag from the ileal digesta compared well with those from the IRAT. The linear regression analysis showed a significant correlation ($p < 0.01$) of AIDCP and AIDDA between the IRAT and MNBT.

(**Key Words** : Pig, Digestibility, Amino Acid, Mobile Nylon Bag)

Introduction

The determination of digestibility by conventional methods requires a large quantity of feedstuffs, a number of animals, and considerable expenditure on equipment and manpower. Fourthermore, analysis of faecal or ileal residues provides little information on the degradation of the components of individual feeds due to the number of feed constituents often necessary to provide a nutritionally-adequate diet and being contaminated by endogenous and bacterial material during digestion.

Recent work has shown that the digestion of feed sample contained in nylon bags and passed through the gastrointestinal tract of pigs can be a means of a rapid determination of feed quality (Sauer et al., 1989 and Leibholz, 1991). However, Wuensche et al. (1987) reported that AIDCP was more varible than those obtained by the lieal convention technique, which was also found by Sauer et al. (1983) who used faecal mobile nylon bag technique. Therefore, the present study was undertaken to obtain more detailed insight into the factors that affect the ileal digestibility of dry matter (DM) and CP as determined by the MNBT and compare the accuracy of the MNBT with the IRAT with the IRAT in the determination of AIDCP and AIDAA.

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Experimental design

A series of experiments was conducted to determine the influence of various pepsin-HCL pretreatment factors, hereby the factors of duration of washing for the retrieved bags, inherent to the MNBT, on the AIDCP and AIDDM. The factors were the pepsin concentration, duration of the pepsin HCL-pretreatment and duration of washing the retrieved bags. At last, the AIDCP and AIDAA of maize, barley, wheat, rapeseed meal (RSM), cottonseed meal (CSM) and three mixed diets (M1, M2 and M3) were determined with the MNBT and IRAT.

Animal and diets

Six barrows with an initial live weight of 35 kg (Yorkshire × Chinese Black Pig) were ileorectomized (IRA) according to the procedure described by Yin et al. (1992a) and three of those pigs were also fitted with a simple duoedenal T-cannula according to Yin et al. (1991b). After surgery, the pigs were placed immmediatedly in metabolism cages and fasted one day. Following a period of 7 days recovery to normal appetite, feed was given at 2.5 times of the ME requirement for maintaince (425KJ $LW^{-0.75}$) as specified by NRC (1988).

Ten days after surgery, the pigs were fed a diet of 32% of wheat, 50% of barley, 15% of SBM and 5% of pre-mix of macro-mineal, trace elements and vitamin during the MNBT experiment.

The composition of the tested feedstuffs and mixed diets for the IRAT and MNBT experiment is shown in table 1. The complete single feedstuff samples of RSM, SBM, CSM were used for the MNBT.

Procedure for MNBT

Monofilament nylon bags (25 × 40 mm; pore size of 40 μm; Germany) were prepared as described by Borgmann et al. (1991), filled with 0.75 g ground (1 mm) experimental samples and sealed. The bags were grouped in blocks of six and placed in a beaker containing 75 ml of a solution made up of deionized water with 0.01N HCL and 0.1% of purified pepsin powder (pepsin, 5000EE). The beaker was placed in a water bath at 37℃ and agitated at a rate of 90 oscillations/min. for 4h.. Thereafter, the bags were removed from the beaker, washed with deionized water and frozen until required.

The thawed bags were inserted during a 5-min. period while the pigs were eating, six bags during the morning meal (08:00 to 08:30), six bags during the evening meal (18:00 to 18:30) and altogether 36 of the bags for each experimental feedstuff sample were inserted in each pig. When the bags were retrieved in the ileal digesta (within 12 h.), carefully isolated, washed with a clothe washer for 4 min. and frozen at -40℃ until analysis. Bags were discarded if they were accidentally washed with urine or if they were chewed or otherwised damaged.

Experiment 1. Study of the optimal pepsin-HCL pretreatment time *in vitro*

In this experiment, the effect of varying the duration of pepsin-HCL pretreatment was studied. Two of the pretreatment times of 2.5 h. and 4 h. were chosen. The concentration of pepsin in the preteratment solution was 0.1% at a pH of 2.0. The other procedures were the same as the above "Procedure for MNBT". The experiment was conducted over a 20-d period with a total 756 bags being placed in each pig, which represented 108 bags for each of the six feedstuffs pretreated at the two different times. The tested feedstuffs were the complete single feeds of maize b (bigger partical size, 1.4 mm), rice seed, rice seed b (bigger partical size, 1.4 mm), fish meal and RSM.

Experiment 2. Study of the optimal pepsin concentration

In the second experiment, the effect of pepsin activity on the AIDDM and AIDCP of maize with the MNBT was studied. Three of the pepsin concentrations of 0.03%, 0.07% and 0.1% were set, with a PH 2, a pepsin-HCL pretreatment time *in vitro* of 4 h. The other procedures were the same as the above "Procedure for MNBT". The experiment was conducted over a 5-d period with a total of 324 bags being placed in each pig. This resulted in a total of 108 bags for each of the pepsin concentration treatment.

Experiment 3. Study of the optimal washing time of the retrieved bags

In this experiment, the effect of varying the washing time of retrieved bags was studied. Washing times of 0 min., 4 min. and 8 min. were used for determining the AIDCP of maize with the MNBT. The experiment was conducted over 15-d period with a total of 324 bags being placed in each bag. This resulted in a total of 108 bags for each of three washing time treatment.

Experiment 4. Study of the accuracy of the MNBT

The AIDCP and AIDAA in all of the experimental feedstuffs and mixed diets listed in table 1 were respectively measured with the IRAT and MNBT. This experimental technique and procedure for the IRAT were the same as reported by Yin et al. (1992a). The MNBT procedure was the same as the above "Procedure for MNBT".

TABLE 1. INGREDIENT COMPOSITION AND PROXIMATE ANALYSIS (G KG^{-1}DM) OF THE DIETS[a]

	Maize	wheat	Barley	RSM	SBM	CSM	M1*	M2*	M3*
Ingredient									
Maize	970.0	–	–	–	–	–	544.0	450.0	615.0
Wheat	–	977.0	–	–		–	–	–	–
Barley	–	–	977.0	–	–	–	–	–	–
Rapeseed meal	–	–		277.0	–	–		–	200.0
Soya-bean meal	–		–		324.0	–	111.0	115.0	–
Cotton seed meal	–	–	–	–	–	402.0	–	–	
Fish meal	–	–	–	–	–	–	40.0	20.0	12.0
Wheat bran	–	–	–	–	–	–	290.0	400.0	160.0
L-lysine		–	–	–	–	–	1.5	1.0	1.0
Sucrose	–	–		50.0	30.0	25.0		–	–
Maize starch	–	–	–	629.0	581.0	550.0	–	–	–
Cellulose	–	–	–	20.0	42.0	–	–	–	–
$Ca_3(PO_4)_2$	25.0	18.0	18.0	19.0	18.0	18.0	9.5	10.0	10.0
Others**	5.0	5.0	5.0	5.0	5.0	5.0	4.0	4.0	4.0
Chemical analysis									
DM	805	900	880	893	889	936	864	893	706
CP	105	180	123	125	150	150	182	177	183
GE(MJ kg^{-1})	17	19	18	21	22	19	20	19	19
ADF	42	–	–	121	100	143	131	142	171
NDF	186	–	–	160	141	170	320	350	204
ADL	8	–	–	80	60	85	75	87	92
Aspartic acid	7.0	14.1	4.7	5.3	16.5	13.2	14.4	12.7	16.6
Threonine	3.9	7.3	2.6	3.4	5.0	4.6	5.9	5.8	6.9
Serine	5.0	6.5	3.4	3.0	5.4	4.9	5.2	5.9	7.7
Glutamic acid	21.1	19.3	17.4	17.3	5.9	29.1	30.0	30.4	32.0
Glycine	4.2	8.2	4.0	4.2	6.5	6.3	7.8	7.7	8.6
Alanine	9.2	9.9	8.9	4.0	6.5	6.3	9.6	9.4	11.4
Valine	4.6	7.4	6.7	4.8	7.6	6.5	8.4	8.2	9.7
Methionine	3.0	2.8	6.0	2.7	2.8	3.2	3.2	3.0	3.0
Isoleucine	4.1	5.9	5.3	5.3	5.7	5.5	6.5	6.7	7.1
Leucine	11.9	11.3	10.1	6.6	12.0	10.2	15.0	14.6	17.7
Phenylalanine	6.1	7.9	7.0	7.9	7.7	8.9	8.8	11.3	9.3
Tyrsine	2.4	3.9	3.9	1.9	2.9	2.9	3.7	3.5	3.8
Proline	13.2	20.0	20.0	1.8	2.1	2.0	10.8	9.9	9.7
Histidine	5.2	6.9	6.2	3.1	5.1	4.9	5.7	6.0	5.7
Lysine	2.6	7.5	6.7	4.9	8.1	5.7	8.6	6.9	8.5
Arginine	4.5	4.4	4.3	5.7	9.9	12.2	9.5	9.3	9.5

[a] Complete of single feedstuffs of RSM, SBM and CSM, withought supplement anything, were used for the MNBT.

* Mixed diet.

** Sodium chlorde and pre-mix. of trace elements and vitamin.

Analytical and statisitical procedures

At the end of the experiments, sample of ileal digesta and the retrieved bag were dried in a vacum oven at 104℃, and then ground through a 0.8 mm mesh screen and mixed, before sample were taken for analyses. DM and CP analyses were performed according to A.O.A.C. (1975). The cell wall constituents (NDF, ADF and ADL) analyses was performed according to Van Soest and Wine (1967). Amino acid analysis was performed by derivatisation with o-phthalaldehyde-3-mercaptopionic acid detection by fluorescence using a Shimadzu LC-4A HPLC, after hydrolysis of the protein in 6N HCL in sealed evacuated tubes at 110℃ for 24 h.

Data were subjected to analyses of variance to test for significant differences of the apparent ileal digestibility between the MNBT and IRAT according to t-test (Snedecor and Cocharan, 1967), and differences among different pepsin concentration treatments and washing times were determined by the Student-Newman Keuls multiple range test (Snedecor and Cochran, 1967), Regression analyses were calculated according to Enstein et al. (1977).

Results

Experiment 1

Table 2 shows that except fish meal and rice seed b, there were no significant ($p > 0.05$) differences of AIDDM and of other feedstuffs of maize, maize b, rice seed and RSM between the different pepsin-HCL pretreatment times (2.5 h vs 4 h). Similar to the AIDDM, there were no significant ($p > 0.05$) differences of AIDCP in all of the tested feedstuffs between the different pretreatment times, except that of fish meal.

Experiment 2 and 3

The influence of altering pepsin concentration and the duration of washing the retrieved bags on AIDDM and AIDCP of maize determined with the MNBT are shown in table 3. and 4, respectively. As the pepsin concentration increased, the AIDDM and AIDCP did not increase significantly ($p > 0.05$). The absence of washing the retrieved bags (0 min.) resulted in a lower AIDCP, but there was on significant difference of the washing times

TABLE 2. THE EFFECT OF PEPSIN PRETREATNENT TIME *IN VITRO* ON THE LIEAL DIGESTIBILITIES (%) OF DM AND CP DETERMINED WITH THE MNBT[a]

		Maize	Rice seed	Fish meal	Maize[b]	Rice seed[b]	RSM
DM	2.5h	88.4	76.7	84.4	75.2	75.2	83.9
	4.0h	87.8	78.4	92.8	83.7	77.5	83.0
	SE[1]	1.35	0.59	1.08	1.40	0.52	0.83
	Sig[2]	NS	NS	**	NS	*	NS
CP	2.5h	70.6	88.9	86.7	71.3	88.2	65.6
	4.0h	72.1	93.9	97.5	72.8	86.9	65.8
	SE	0.60	0.41	0.87	0.67	0.45	0.53
	Sig	NS	NS	**	NS	NS	NS

[a] All of the digestibilities determined usind a pepsin concentration of 0.1%, a pH of 2.0 and a washing time of the retrieved bag of 4 min.

[b] 1.4 mm of the partical size.

[1] Standard error of the means.

[2] Significant different between the pepsin pretreatment times of 2.5 h. and 4. 0h. *in vitro*.

NS = not significant difference ($p > 0.05$).

* = highly significant difference ($p < 0.05$).

** = significant difference ($p < 0.01$).

TABLE 3. THE EFFECT OF THE PEPSIN CONCENTRATION ON THE ILEAL DIGESTIBILITIES (%) OF DM AND CP IN MAIZE DETERMINED WITH THE MNBT[a]

	0.30%*	0.07%*	0.10%*	SE[1]
DM	91.8[b]	90.6[b]	89.6[b]	0.52
CP	69.2[b]	72.5[b]	71.6[b]	0.09

[a] All of the digestibilities determined using a pH of 2.0, a washing time of the retrieved bags of 4 min. and pepsin pretreatment time of 4h. *in vitro*.

* Pepsin concentration in the solution.

[b] Not significant difference ($p > 0.05$).

[1] Standard error of the means.

TABLE 4. THE EFFECT OF THE WASHING TIMES OF THE RETRIEVED BAG ON THE ILEAL DIGESTIBILITY (%) OF CP IN MAIZE DETERMINED WITH THE MNBT

	0 min.*	4 min.*	8 min.*	SE[1]
CP	65.8[c]	70.1[b]	74.2[b]	2.21

All of the digestibility determined using a pepsin concentration of 0.1%, a pH of 2.0 and a Pepsin pretreatment time of 4h. *in vitro*.

* Washing time of the retrieved bag.

[1] Standard error of the means.

[b, c] Significant difference ($p < 0.05$).

TABLE 5. PRESENTATION OF THE ILEAL DIGESTIBILITIES (%) OF CP AND AA ACCORDING TO IRAT TO MNBT AND TO A REGRESSIVE CALCULATION (CMNBT) OF THE FEEDSTUFFS AND DIETS[a]

		Maize	Wheat	Barley	RSM	SBM	CSM	M1[b]	M2[b]	M3[b]
CP	IRAT	78.5	86.9	77.9	66.8	87.3	76.7	79.5	78.2	74.5
	MNBT	80.6	93.6	83.7	65.8	84.3	78.0	77.4	75.3	77.3
	SEM[1]	0.75	1.30	0.85	1.01	1.46	0.83	1.11	0.53	0.40
	Sig[2]	NS	NS	*	NS	NS	NS	NS	NS	NS
	CMNBT	79.1	87.5	81.1	69.5	81.5	77.4	77.0	75.6	76.9
THR	IRAT	79.0	91.4	79.3	68.2	84.8	74.2	69.6	71.4	62.5
	MNBT	80.3	94.8	79.9	73.3	84.7	77.2	71.6	71.0	75.5
	SEM	0.77	0.92	1.08	0.08	2.08	1.39	1.59	0.86	1.50
	Sig	NS	NS	NS	*	NS	NS	NS	NS	*
VAL	CMNBT	76.8	90.8	76.5	70.1	81.1	73.9	68.5	67.9	72.2
	IRAT	75.9	86.7	80.6	69.1	88.9	80.7	71.2	72.3	73.0
	MNBT	79.2	92.0	80.5	73.0	86.4	79.3	69.0	71.7	78.1
	SEM	1.79	1.84	1.85	2.07	0.33	1.01	1.57	1.58	2.05
	Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS
MET	CMNBT	78.0	87.3	78.9	73.4	83.2	78.4	70.5	72.5	77.2
	IRAT	90.1	84.9	78.9	81.9	89.1	68.8	83.6	82.8	75.0
	MNBT	93.5	85.0	75.9	87.7	88.9	68.1	94.3	78.8	79.0
	SEM	0.87	2.01	1.22	1.50	1.93	0.63	0.49	2.87	0.39
	Sig	NS	NS	NS	NS	NS	NS	*	NS	NS
ILE	CMNBT	87.3	82.8	77.9	84.2	84.8	73.8	87.7	79.5	79.6
	IRAT	83.1	85.4	78.1	72.2	89.2	71.5	73.1	74.8	72.6
	MNBT	84.1	92.6	86.4	74.3	89.0	73.0	70.6	63.8	75.4
	SEM	0.72	0.90	1.62	0.96	1.30	1.27	1.42	1.85	1.13
	Sig	NS	*	NS	NS	NS	NS	NS	*	NS
LEU	CMNBT	80.1	84.4	81.3	75.2	82.6	74.6	73.4	70.0	75.8
	IRAT	89.0	89.9	81.7	75.5	86.9	70.5	77.9	81.3	74.0
	MNBT	85.9	93.0	85.5	74.5	86.5	78.0	75.6	78.2	71.3
	SEM	1.39	1.01	1.60	1.40	0.79	0.76	2.08	2.53	1.73
	Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS
PHE	CMNBT	83.8	88.6	83.5	76.1	84.2	78.4	76.8	78.6	73.9
	IRAT	90.1	90.4	84.0	86.4	87.0	77.1	84.2	88.0	86.0
	MNBT	90.2	91.9	89.5	84.3	87.4	79.4	86.0	85.7	86.5
	SEM	0.90	0.69	1.44	0.65	1.32	0.68	1.61	0.97	1.47
	Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS
HIS	CMNBT	88.4	89.5	87.9	84.4	86.5	81.1	85.6	85.3	85.9
	IRAT	79.6	92.3	81.5	76.1	88.3	66.9	77.2	85.7	83.2
	MNBT	85.1	92.1	87.7	84.8	83.1	67.6	79.5	77.1	80.9
	SEM	0.40	0.83	1.93	0.55	1.57	1.90	0.88	1.81	0.59
	Sig	*	NS	NS	*	NS	NS	NS	NS	NS
LYS	CMNBT	83.0	87.2	84.5	82.8	81.8	72.5	79.6	78.2	80.4
	IRAT	81.1	88.4	73.7	68.3	85.7	64.9	74.4	76.6	85.9
	MNBT	79.6	92.8	75.5	75.6	88.1	69.6	77.0	78.7	86.3
	SEM	1.11	0.86	1.29	0.57	1.10	1.34	1.24	1.06	1.00
	Sig	NS	NS	NS	*	NS	NS	NS	NS	NS
	CMNBT	76.7	89.3	72.8	72.9	84.8	67.2	74.3	75.9	83.1

TABLE 5. (CONTINUED)

		Maize	Wheat	Barley	RSM	SBM	CSM	M1[b]	M2[b]	M3[b]
ARG	IRAT	63.9	90.4	90.0	75.5	94.0	74.7	90.1	90.1	87.8
	MNBT	64.9	90.1	85.9	80.8	92.5	77.8	86.5	84.8	89.3
	SEM	1.36	1.34	0.96	0.65	0.56	0.48	1.02	1.25	1.02
	Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CMNBT	63.9	90.6	86.1	80.7	93.1	77.5	86.8	84.9	87.9
SER	IRAT	75.2	92.5	87.2	70.7	85.1	80.2	76.8	78.2	71.6
	MNBT	75.3	93.7	85.0	73.0	85.8	81.5	72.8	73.8	78.6
	SEM	0.78	0.52	1.10	0.61	1.18	0.98	1.75	1.33	1.71
	Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CMNBT	75.8	90.7	83.7	74.0	84.3	80.8	73.8	74.6	78.5
GLU	IRAT	79.0	91.4	95.2	81.6	89.1	84.2	87.0	75.6	84.6
	MNBT	80.1	94.2	93.8	79.9	91.4	86.7	80.2	78.4	83.0
	SEM	1.60	0.70	1.01	0.39	1.02	0.57	1.07	1.21	1.16
	Sig	NS	NS	NS	NS	NS	NS	*	NS	NS
	CMNBT	81.5	91.9	91.6	81.3	89.9	86.4	81.6	80.2	83.6
PRO	IRAT	90.5	74.2	75.1	68.8	84.3	70.8	74.7	86.8	86.0
	MNBT	91.6	79.0	72.8	71.2	75.9	69.0	70.9	78.6	80.0
	SEM	0.79	1.56	0.89	0.87	1.12	0.93	1.20	1.06	1.34
	Sig	NS	NS	NS	NS	*	NS	NS	*	NS
	CMNBT	91.6	81.1	76.0	74.7	78.6	72.8	74.4	80.8	82.0
GLY	IRAT	77.5	81.1	80.3	69.1	83.2	78.6	74.5	73.5	76.1
	MNBT	72.5	92.0	83.3	74.4	85.0	78.7	76.9	75.2	80.4
	SEM	1.48	1.27	1.04	0.5	1.44	0.67	1.22	0.89	1.58
	Sig	NS	NS	NS	*	NS	NS	NS	NS	NS
	CMNBT	72.7	85.6	79.8	74.0	81.0	76.8	75.6	74.5	77.9
ALA	IRAT	75.2	88.4	75.5	68.0	81.9	73.1	74.5	76.3	68.4
	MNBT	69.5	93.9	78.0	72.3	85.8	73.6	69.9	71.2	75.5
	SEM	1.63	0.99	1.85	0.73	1.61	0.83	1.63	0.84	1.89
	Sig	NS	NS	NS	*	NS	NS	NS	*	NS
	CMNBT	71.4	85.3	76.2	73.0	80.7	73.7	71.6	72.4	74.8
TYR	IRAT	87.9	88.7	71.4	80.0	82.8	80.2	50.9	68.9	78.3
	MNBT	85.8	90.5	75.4	82.3	78.9	80.3	65.5	70.8	86.6
	SEM	1.12	0.37	0.81	0.79	1.03	0.72	2.15	1.45	1.70
	Sig	NS	NS	NS	NS	NS	NS	*	NS	NS
	CMNBT	82.5	87.5	71.4	78.8	75.1	76.6	60.8	66.4	83.4
ASP	IRAT	80.4	89.2	76.1	67.9	88.9	83.9	77.7	75.8	70.4
	MNBT	73.2	94.5	93.1	72.5	89.4	81.5	78.7	76.7	81.0
	SEM	1.33	0.71	0.43	0.73	0.73	0.82	0.37	0.83	1.29
	Sig	NS	NS	*	NS	NS	NS	NS	NS	*
	CMNBT	74.7	85.5	84.8	74.3	82.9	78.9	77.5	76.4	78.6

[a] All of the digestibilites determined using a pepsin concentration of 0.1%, a Ph of 2, a pepsin pretreatment time of 4h. and a washing time of the retrieved bag of 4 min.

[b] Mixed diet.

[1] standard error of the means.

[2] significant difference between the IRAT and MNBT.

NS = not significant ($p < 0.05$).

* = significant difference ($p < 0.05$).

between 4 min. and 8 min.

Experiment 4

Table 5 shows that except AIDCP of barley, AID of threonine of RSM and M3 methionine of M1, isoleucine of wheat and M2, histidine of RSM and maize, lysine of RSM, aspartic acids of barley and M3, proline of SBM and M2, tyrosine of M1, glutamic acid of M1 glycine of RSM and alanine of RSM and M2, there were no significant ($p > 0.05$) differences in AIDCP and AIDAA between the IRAT and MNBT. The corrected data calculated for the MNBT from the regression equation listed in table 6 are similar to those from IRAT. Table 6 shows that for CP and all the amino acids the linear regressions of values determined by IRAT and MNBT were sigificant ($p < 0.05$ or $p < 0.01$) accounting for between 50 to 90 per cent of the variance.

TABLE 6. LINEAR RELATIONSHIP BETWEEN THE ILEAL DIGESTIBILITEIS OF CRUDE PROTEIN (CP) AND AMINO ACIDS (AA) DETERMINED WITH THE IRAT (Y) AND WITH THE MNBT (X)

	Intercept	Slope	r^2	n
CP	21.1	0.72	0.77**	9
Threonine	−7.65	1.06	0.79**	9
Valine	8.24	0.89	0.81**	9
Methionine	32.5	0.58	0.66**	9
Isoleucine	33.5	0.56	0.67**	9
Leucine	15.6	0.80	0.70**	9
Phenylalanine	9.48	0.88	0.66**	9
Histidine	19.7	0.75	0.50*	9
Arginine	−12.5	1.15	0.90**	9
Aspartic acid	12.4	0.82	0.59*	9
Serine	7.70	0.9	0.78**	9
Glutamic acid	14.1	0.83	0.76*	9
Glycine	16.6	0.77	0.76**	9
Alanine	28.8	0.61	0.64**	9
Proline	15.6	0.87	0.64**	9
Tyrosine	−21.6	1.22	0.78**	9

* $p < 0.05$.
** $p < 0.01$.

Discussion

AIDCP and AIDAA determined using the IRAT for all of the feedstuffs and mixed diets agree well with the previously published values (Sauer and Ozimek, 1986; Bock et al., 1989; Yang, 1989; Knabe et al., 1989; Yin et al., 1991a; Yin et al., 1992b). These data presented to provide a reference point with which to evaluate the potential of the MNBT to pretreatment conditions and feedstuffs and diets.

Choice of the pepsin-HCL pretreatment condition and especially to set standard conditions is very important for the MNBT (Cherian et al., 1988; Wuensche et al., 1987). Wilson and Leibolz (1981) reported that normal retention time of digesta in stomach is 2-3 h., which means the average time is 2.5 h. The present study reveals that, except the animal protein feedstuffs of fish meal, it was enough to incubate the bag for 2.5 h. to obtain a higher AIDDM and AIDCP for the cereals and RSM, compared with those determined by the conventional ileal digesta collection methods (Schroeder et al., 1989, Yang, 1989 and Yin et al., 1991b). However the AID was not significantly increased as the pepsin-HCL pretreatment time had reached 4 h.. In considering this point, we agree with the suggestion of Cherian et al. (1988) that the pepsin-HCL pretreatment time *in vitro* to be set 4 h. for practice analysis.

As the pepsin concentration increased, the AIDDM and AIDCP of maize did not increase. This result indicated that effect of altering pepsin activity on DM and CP digestibility for cereals is small, although Cherian et al. (1988) reported that it did in determination of the faecal CP digestibility of soyabean and Canola meal. But for security, it is also necessary to make a 0.1% of pepsin powder (pepsin, 5,000 EE) in the pretreatment solution, according to the normal pepsin activity in the stomach of pigs.

The majority of nylon bags that were removed from the experiment were discarded due to being washed in the urine otherwise damaged and not because of an excessively long passage time. The mean transit time required for the nylon bags to pass from duodenum to the anus was 9 h., which means passage time was within 6-12 h.. This value was 26.3 h. shorter than that reported by Cherian et al. (1988) with a faecal MNBT, but 6.5 h. longer than that reported by Leibholz (1991). This difference are probably due to the differences in cannulation methods and bag collection sites. Leibholz (1991) collected the bags from the ileum T cannula with forceps as they appeared, while we picking-up directly from the digesta. A longer retention time of the ileum digesta of the IRA pigs than that of ileo-caecal pigs was also found in a previous study of Yin et al. (1992a). The advantage to collection of the bag at the ileal cannula is from the small intestine, but it is difficult to collect the bag directly from the ileal cannula and it is not possible to collect bags from the ileal-caecal re-entrant cannulae, according to our previous observation. However, this shortage or the impossibility of collection of bags from the ileum can be overcome by the present IRAT.

Washing the retrieved bags is necessary, because there are some digesta on the outside of the bag cloth, which does not originate from the sample in the bag. The present study indicated that the absence of washing of the bag resulted in a dramatically lower AIDCP in comparison with the other treatment. Now the question arises also from the washing, that washing of the bage allows loss of unabsorbed nutrients and endogenous nitrogen, majority leading to higher digestibility values, although there is also a course of the infiltration of the bag residues with some endogenous nitrogen of -4 to -14, according to the study of Schadereit et al. (1991). So the digestibility of CP or AA is either the apparent or the true digestibility. This may be well explained by the higher digestibility of CP and AA with the MNBT than that of IRAT.

The AIDDM, AIDCP and AIDAA with the MNBT were seldomly determined as far as we know. Leibholz (1991) and Sauer et al. (1989) obtained the same ileal bag ADCP for soya-bean meal as that determined with the ileal T cannula or faecal analyses methods respectively, which is in agreenemt with the present results for SBM. The present results also show that almost all of the AIDCP and AIDAA for most of the tested feedstuffs and mixed diets were a little higher than the corresponding data of IRAT, even for the RSM, CSM, barley, wheat and mixed diets with a higher fibre contents. Borgmann et al. (1991) also reported that all of the ileal bag ADCP of the experimental feedstuffs were higher than those of the IRAT, including the highest fibre content feedstuffs of two kinds lupin feeds. But Leibholz (1991) and Sauer et al. (1989) obtained a lower lieal bag ADCP or a faecal ADCP compared with the ileal cannulae method or the faecal analysis method, respectively with the higher fibre content feedstuffs of linseed meal, coconut meal, sunflower meal, cottonseed meal, barley and wheat. The lower ileal or faecal bag digestibility was not sound in physiology, according to the above discussion. Leibholz (1991) explained that, at first this could have been largely owing to the short retention time of the bags in the small intestine which would not have allowed sufficient time for the action of the degestive enzymes, and this must have a major reason for the discrepancy, as the digestibility of all protein sources in the whole tract and the retention time of the bags was similar to that obtained by total faecal collection. However, this shortage can be just overcome by the usage of the IRAT used by this study, because a much longer retention time of the bag in the intestine was observed with the IRAT. Second explanation of the lower digestibility of protein in nylon bags is that the sample is not completely digested in the intestine. That is why the samples in the nylon bag was only 0.75 g weighted by our experiments, which was 0.25 g less than that used by Sauer et al. (1989), Cherian et al. (1988), and Leibholz (1991), although the size of the bags used by us was the same as those used by the above authors.

A higher AIDCP and AIDAA than the IRAT can be also explainted by other reasons such as loss of feed particles (1 mm against 2 mm for the IRAT pigs or other convertial method pigs) and the solubilization of nitrogenous compounds which are not digested *in vivo*. Walter et al. (1981) found that increasing fineness of grind will improve ileal and faecal digestibility of CP and AA in soughum by growing pigs.

In conclusion, the overall results of these experiments present a detailed insight into some of the maxium optimization of ileal MNBT. Although values obtained using a pH of 2.0, a pepsin concentration of 0.1%, a pretreatment time of 4 h. *in vitro* and a washing time of the retrieved bags of 4 min. are a little higher than those obtained with IRAT how the values arevery similar when corrected with the established regressive equation. Under these conditions, the ileal MNBT would appear to have considerable portential for use in the rapid determination of AIDCP and AIDAA of the feedstuffs for swine.

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