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Relation between *in vitro* and *in vivo* assessment of amino acid availability

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Summary — Even though the availability of dietary amino acids is the result of integrated phenomena of digestion, absorption and transport, it may be mainly affected by the stage of luminal digestion. In this case, amino acid availability could be predicted by an *in vitro* method designed to reproduce *in vivo* proteolysis conditions. In order to check this hypothesis, the essential amino acid (EAA) profiles of digesta collected at 8 intervals during a 24-h *in vitro* enzymatic proteolysis of casein and rapeseed proteins were compared to the pattern of appearance of dietary EAA in portal vein of pigs fed the same proteins, determined at each hour over a 8-h postprandial period by coupling blood flow rate with porto-arterial differences in plasma EAA concentrations. Comparisons of *in vitro* and *in vivo* data first bore on overall EAA profiles measured at each interval, and then on the individual kinetics of each EAA. Regarding total profiles, the highest correlations for casein (r : 0.80—0.98) were found when comparing EAA patterns determined during the first half of *in vitro* digestion and *in vivo* absorption periods. Similar *r* values were obtained with rapeseed proteins, but over longer periods of measurement. Concerning individual kinetics, the highest correspondences were found with rapeseed proteins, with 5 out of 9 EAA (methionine, isoleucine, leucine, phenylalanine and arginine) having their *in vitro* sequence of release significantly correlated with their *in vivo* sequence of absorption. With casein, correlations were significant for threonine, valine, isoleucine and leucine. These results suggest that sequential hydrolysis in the digestive tract, as reproduced by the *in vitro* technique, is a key determinant of amino acid appearance in the portal blood to a degree varying with the protein source and with the nature of the amino acid.

amino acids — availability — digestion — absorption — pig

Résumé — Relation entre l'estimation *in vitro* et *in vivo* de la biodisponibilité des acides aminés. *Bien que la disponibilité des acides aminés alimentaires résulte de phénomènes imbriqués de digestion, d'absorption et de transport, elle est probablement surtout affectée par l'étape de digestion luminale. Dans ce cas, la disponibilité des acides aminés pourrait être prédite à l'aide d'une méthode* in vitro *conçue pour reproduire les conditions de protéolyse* in vivo. *Dans le but de vérifier cette hypothèse, nous avons comparé les profils d'acides aminés essentiels (AAE) de digestats de caséine et de protéines de colza, recueillis à 8 périodes lors d'une protéolyse enzymatique* in vitro *de 24 h, aux profils d'apparition des AAE dans la veine porte de porcs ayant ingéré les mêmes protéines. Ces profils ont été déterminés pour chaque heure au cours d'une période postprandiale de 8 h, en couplant le débit sanguin aux différences porto-artérielles dans les*

concentrations d'AAE plasmatiques. Les comparaisons des données in vitro *et* in vivo *ont d'abord porté sur les profils complets d'AAE mesurés à chaque intervalle, puis sur les cinétiques individuelles de chacun des AAE. Avec les profils complets, les plus fortes corrélations (r : 0,80—0,98) pour la caséine ont été obtenues en comparant les profils d'AAE mesurés pendant la première moitié des périodes d'observation* in vitro *et* in vivo. *Des coefficients similaires ont été obtenus avec les protéines de colza, mais sur de plus longues périodes. En ce qui a trait aux cinétiques individuelles, les plus fortes correspondances ont été notées avec les protéines de colza, la séquence de libération* in vitro *étant significativement reliée à la séquence d'absorption* in vivo *pour 5 des 9 AAE (méthionine, isoleucine, leucine, phénylalanine et arginine). Avec la caséine, les corrélations ont été significatives pour la thréonine, la valine, l'isoleucine et la leucine. Ces résultats suggèrent que l'hydrolyse séquentielle dans le tractus digestif, telle que reproduite par la technique* in vitro, *est un facteur clé de l'apparition des acides aminés dans la veine porte, à un niveau dépendant de l'origine de la source protéique et de la nature de l'acide aminé.*

acides aminés — disponibilité — digestion — absorption — porc

INTRODUCTION

The availability of amino acids is a primary determinant of protein nutritional quality, but it is not readily measurable in an *in vivo* situation. According to Tanksley and Knabe (1985) its availability not only implies that a nutrient is digested and absorbed, but also that it is available at cellular sites for synthesis reactions. Furthermore, for dietary amino acids, availability has a time-related dimension, since it is concerned with the simultaneous presence of all essential amino acids for an optimal protein synthesis in the organism (Geiger, 1947).

As the ultimate measure of amino acid availability at cellular level is not yet feasible, it is of interest to evaluate the fate of dietary amino acids at various steps of their arrival in the organism, and to examine the most determinant processes. A possible *in vivo* procedure is the rather elaborate method of Rérat *et al.* (1979) in the pig, which allows qualitative and quantitative measurement of the kinetics of appearance of dietary amino acids in the portal bloodstream, just before their distribution to the liver and to the other sites of protein synthesis. At that point, the measure of amino acid availability is already the result of many imbricated phenomena of digestion, absorption and transport. Of these, the sequence and form of release of amino acids during luminal digestion process might be two of the key factors. If such were the case, amino acid availability could be conveniently studied and partly predicted by an *in vitro* method imitating *in vivo* proteolysis conditions.

Savoie and Gauthier (1986) have developed such a technique, where the luminal hydrolysis of proteins is reproduced in a pepsin-pancreatin digestion cell, and where the end-products of proteolysis are continuously collected as they are released from the substrates.

In order to check to which extent the step of luminal proteolysis is a determinant factor of amino acid availability, the amino acid patterns of digesta collected at various intervals during an *in vitro* enzymatic proteolysis of casein and rapeseed proteins (Savoie *et al.*, 1988) were compared to the pattern of appearance of dietary amino acids in portal vein of pigs fed the same proteins (Galibois *et al.*, in press). Special attention was paid to peptide-bound amino acids

found in the *in vitro* digesta, as it was shown that peptide absorption is an important mechanism for assimilation of dietary protein (Adibi and Kim, 1981).

MATERIALS AND METHODS

Protein sources and amino acids (AA)

The casein and rapeseed protein sources were from the same origin and batches for both *in vitro* and *in vivo* studies. Their composition is described in Table I. For calculation of correlation between *in vitro* and *in vivo* data, the following 9 essential amino acids (EAA) were used : threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine and arginine. (Tryptophan was not measured in blood samples and thus was not included in the calculations).

Table I. Amino acid composition of experimental proteins [1].

Amino acid	*Casein* [2]	*Rapeseed proteins* [3]
	(mg/100 mg amino acids)	
THR	4.12	4.86
VAL	5.85	5.35
MET	2.31	1.75
ILE	4.56	4.10
LEU	8.97	8.01
PHE	4.86	4.43
HIS	2.71	3.12
LYS	7.52	6.98
ARG	3.47	6.84
TRP	1.38	1.10
ASP	7.09	7.69
SER	5.52	4.86
GLU	20.74	18.13
PRO	10.51	6.65
GLY	1.70	5.44
ALA	2.90	4.96
CYS	0.55	2.81
TYR	5.24	2.91

[1] As determined by ion-exchange chromatography (Amino Acid Analyser 6300, Beckman, Palo Alto, CA), following a 24-h in vacuo hydrolysis in 6 N HCl.

[2] Na caseinate, UCCCP, France. % protein (N x 6.25) = 86.6 %.

[3] Rapeseed proteins concentrate 00 Tandem, CETIOM, France.
% protein (N x 6.25) = 51.2 %.

In vitro *data*

The *in vitro* experimental procedure reproducing *in vivo* luminal proteolysis conditions has already been described in detail (Savoie *et al.*, 1988). Briefly, after a 30-min pepsin predigestion, the protein sources (250-mg aliquots) were submitted in 4 replicates to a 24-h pancreatin hydrolysis in dialysis tubes (molecular weight cut-off of the membrane : 1000 Da), with the continuous removal of digestion products by circulation of a phosphate buffer. Digesta were collected at 3-h intervals, and analyzed for content of peptides (isolated by fractionation with copper-Sephadex G-25 columns) and total AA. All aminoacidograms were determined using an amino acid autoanalyser (Bekman, model 6300, Palo Alto, CA). Tables II and III give the mean EAA compositions of the digesta, either for total amino acids collected or for the peptide fractions only. In this last case, values are given only for the first 4 intervals (up to 12 h of digestion), as the feeble quantity of material collected thereafter did not permit a reliable separation of peptides from free AA fraction.

In vivo *data*

A more complete description of the *in vivo* experience is available in Galibois *et al.* (in press). In short, 6 pigs, fitted with permanent catheters in the portal vein and in the carotid artery as well as with an electromagnetic flow probe around the portal vein, successively received three 800-g test meals containing either 12 % rapeseed proteins or 12 % or 24 % casein. (In the present paper, only data relating to the 12 % protein diets will be used.) Blood samples were collected and portal blood flow rate recorded during a postprandial period of 8 h. For each 1-h interval, the quantity of AA absorbed was determined by coupling blood flow rate with porto-arterial differences in plasma amino acid concentration. Tables IV and V report these AA patterns.

Table II. Total EAA composition of each 3-h digesta collected over the 24-h *in vitro* enzymatic proteolysis.

A. Casein

Digestion interval (h)	*THR*	*VAL*	*MET*	*ILE*	*LEU (mg)*	*PHE*	*HIS*	*LYS*	*ARG*
0— 3	1.01[1]	1.58	0.95	1.19	3.58	1.92	0.81	3.13	1.94
3— 6	0.91	1.40	0.70	1.08	2.34	1.24	0.71	1.79	1.00
6— 9	0.59	0.95	0.42	0.70	1.32	0.69	0.42	0.95	0.41
9—12	0.49	0.70	0.30	0.49	0.80	0.43	0.27	0.63	0.19
12—15	0.21	0.34	0.11	0.25	0.26	0.16	0.11	0.22	0.09
15—18	0.23	0.35	0.09	0.26	0.26	0.17	0.11	0.16	0.13
18—21	0.18	0.24	0.06	0.20	0.19	0.12	0.09	0.11	0.07
21—24	0.14	0.17	0.04	0.14	0.13	0.02	0.07	0.10	0.20

B. Rapeseed proteins

Digestions interval (h)	*THR*	*VAL*	*MET*	*ILE*	*LEU (mg)*	*PHE*	*HIS*	*LYS*	*ARG*
0— 3	0.77	0.90	0.42	0.73	1.50	1.19	0.51	1.02	1.36
3— 6	0.83	1.04	0.58	0.82	1.61	1.05	0.51	1.22	1.27
6— 9	0.64	0.81	0.42	0.59	1.19	0.66	0.50	1.10	0.82
9—12	0.44	0.56	0.27	0.38	0.74	0.35	0.28	0.82	0.46
12—15	0.34	0.42	0.15	0.29	0.43	0.18	0.21	0.57	0.42
15—18	0.28	0.30	0.09	0.22	0.28	0.11	0.15	0.49	0.33
18—21	0.21	0.24	0.07	0.17	0.21	0.09	0.09	0.35	0.24
21—24	0.21	0.22	0.07	0.15	0.20	0.03	0.11	0.29	0.27

[1] Each value is the mean of 4 replicates.

Statistics

All statistical analyses were performed with the statistical analysis system (SAS Institute, Cary, NC). The levels of correspondence between *in vitro* and *in vivo* AA profiles were calculated with Pearson's test of correlation.

The first analyses of correlation were made using the whole of essential amino acids. The patterns of EAA collected at each *in vitro* interval (rows; Tables II and III) were compared to the patterns of EAA appearing in portal blood at each *in vivo* interval (rows; Table IV). First, all EAA collected *in vitro* (mixture of free AA and of low molecular weight peptides) were comprised. In a second analysis of correlation, only EAA from the *in vitro* peptide fraction were compared to the *in vivo* EAA profiles.

The degree of correspondence between *in vitro* and *in vivo* values was also calculated for each EAA considered separately. For these analyses, the pattern of the 8 values sequentially measured *in vitro* for each EAA (columns; Table II) was compared to the pattern of the 8 values sequentially measured *in vivo* (columns; Table IV) for the same EAA. For the analyses bearing on the EAA from the peptide fractions (Table III), only values from the first 4 intervals were used.

Table III. EAA composition of peptide fraction in each 3-h digesta collected over the 24-h *in vitro* enzymatic proteolysis.

A. Casein

Digestion interval (h)	*THR*	*VAL*	*MET*	*ILE*	*LEU*	*PHE*	*HIS*	*LYS*	*ARG*
					(mg)				
0— 3	1.01[1]	1.14	0.48	0.83	1.54	0.60	0.43	1.06	0.33
3— 6	0.91	1.08	0.40	0.82	1.10	0.51	0.41	0.68	0.17
6— 9	0.59	0.84	0.31	0.62	0.70	0.36	0.26	0.42	0.11
9—12	0.36	0.53	0.18	0.39	0.39	0.24	0.13	0.21	0.06

B. Rapeseed proteins

Digestion interval (h)	*THR*	*VAL*	*MET*	*ILE*	*LEU*	*PHE*	*HIS*	*LYS*	*ARG*
					(mg)				
0— 3	0.58	0.53	0.26	0.34	0.53	0.26	0.28	0.50	0.44
3— 6	0.75	0.71	0.31	0.43	0.69	0.27	0.36	0.74	0.53
6— 9	0.51	0.52	0.13	0.33	0.49	0.17	0.29	0.57	0.34
9—12	0.39	0.33	0.10	0.23	0.34	0.13	0.20	0.46	0.23

[1] Each value is the mean of 4 replicates.

RESULTS

Correspondence between patterns of EAA from in vitro *digestion products and patterns of EAA released in portal blood*

Tables V and VI indicate the coefficients of correlation obtained when comparing *in vivo* profiles to free and peptide-bound AA collected *in vitro*. With casein (Table V), only the *in vitro* EAA pattern measured up to the fifth interval (from 0—3 h to 12—15 h) had significant correlations with all the *in vivo* patterns up to 7 h. The strongest correspondences involved the *in vivo* patterns of the first 4 postprandial intervals. With rapeseed proteins (Table VI), correspondences between *in vitro* and *in vivo* patterns were distributed differently. The EAA pattern of the first *in vitro* interval (0—3 h) was poorly correlated to the *in vivo* patterns. Contrarily to casein, all EAA patterns of the subsequent digesta had significant coefficients of correlation with most *in vivo* patterns. However, as with casein, no correspondence was noted between *in vitro* patterns and the patterns of EAA released in the portal blood of pigs from 7—8 h after the rapeseed protein meal.

When only the EAA released as peptides in the *in vitro* digesta were taken into account for calculation of correlation, the pattern of only one interval (0—3) presented some resemblance with the

Table IV. Amounts (mg) of EAA absorbed for each 1-h interval over the 8 h following the ingestion of 12% protein diets in the pig.

A. Casein diet

Postprandial interval (h)	*THR*	*VAL*	*MET*	*ILE*	*LEU*	*PHE*	*HIS*	*LYS*	*ARG*
0—1	804[1]	1 207	430	819	1 479	683	485	1 469	544
1—2	481	813	410	586	1 127	687	376	1 044	478
2—3	575	898	572	764	1 419	735	618	1 426	521
3—4	530	1 109	403	587	1 127	547	485	1 304	617
4—5	315	361	222	536	591	396	329	750	436
5—6	532	469	241	643	886	592	378	1 086	454
6—7	467	479	227	485	914	552	734	940	378
7—8	316	764	326	337	643	337	847	646	329

B. Rapeseed proteins diet

Postprandial interval (h)	*THR*	*VAL*	*MET*	*ILE*	*LEU*	*PHE*	*HIS*	*LYS*	*ARG*
0—1	657	750	402	604	1 037	602	507	1 075	984
1—2	683	741	511	740	1 172	546	609	1 038	853
2—3	815	818	362	635	960	411	404	857	682
3—4	668	976	259	465	842	382	287	984	682
4—5	623	934	182	435	756	395	379	788	642
5—6	631	1 020	248	508	945	484	543	1 058	651
6—7	611	898	242	373	701	378	562	851	634
7—8	359	394	145	256	422	227	652	579	481

[1] Each value is the mean of 6 repetitions.

Table V. Correlation between EAA profiles of the *in vitro* digesta of casein and profiles of EAA absorbed *in vivo* following the casein meal in the pig.

in vitro / in vivo *(h)*	*0—3 h*	*3—6 h*	*6—9 h*	*9—12 h*	*12—15 h*	*15—18 h*	*18—21 h*	*21—24 h*
				(r [1])				
0—1	0.83 **	0.92 ***	0.94 ***	0.93 ***	0.77 **	0.61	0.58	0.29
1—2	0.93 ***	0.98 ***	0.95 ***	0.88 ***	0.65 *	0.48	0.44	0.13
2—3	0.91 ***	0.93 ***	0.90 ***	0.82 **	0.56	0.34	0.33	0.04
3—4	0.81 **	0.86 **	0.84 **	0.81 **	0.67 *	0.50	0.43	0.33
4—5	0.81 **	0.76 *	0.65 *	0.54	0.36	0.20	0.18	0.23
5—6	0.85 **	0.83 **	0.75 *	0.68 *	0.45	0.27	0.28	0.11
6—7	0.74 *	0.74 *	0.67 *	0.58	0.30	0.13	0.17	− 0.05
7—8	0.25	0.34	0.40	0.39	0.34	0.22	0.22	0.04

[1] Coefficient of correlation; $n = 9$.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table VI. Correlation between EAA profiles of the *in vitro* digesta of rapeseed proteins and profiles of EAA absorbed in vivo following the ingestion of rapeseed proteins meal in the pig.

in vitro / in vivo (h)	0—3 h	3—6 h	6—9 h	9—12 h	12—15 h	15—18 h	18—21 h	21—24 h
				(r)				
0—1	0.81 **	0.91 ***	0.95 ***	0.90 ***	0.91 ***	0.86 **	0.85 **	0.82 **
1—2	0.69 *	0.86 **	0.94 ***	0.91 ***	0.85 **	0.76 *	0.76 *	0.73 *
2—3	0.56	0.72 *	0.83 **	0.87 **	0.88 **	0.81 **	0.84 **	0.82 **
3—4	0.55	0.71 *	0.84 **	0.90 ***	0.94 ***	0.88 ***	0.92 ***	0.84 **
4—5	0.56	0.69 *	0.79 **	0.82 **	0.88 ***	0.81 **	0.84 **	0.81 **
5—6	0.53	0.71 *	0.86 **	0.91 ***	0.90 ***	0.83 **	0.84 **	0.76 *
6—7	0.42	0.57	0.73 *	0.78 **	0.86 **	0.82 **	0.82 **	0.80 **
7—8	0.16	0.28	0.41	0.41	0.54	0.56	0.48	0.56

Statistical significance as in Table V.

EAA patterns measured *in vivo* in the portal blood of pigs, in the case of casein (Table VII). On the other hand, with rapeseed proteins (Table VIII), strong correlations were noted for all *in vitro* intervals. Particularly, peptides of the *in vitro* digesta collected between 0 and 3 h had a EAA composition which was very similar to those measured *in vivo* from 2—7 h after the meal, while low correlations had been observed when EAA from total digestion products of this *in vitro* interval were considered.

Correspondence between the kinetics of release in the in vitro *digesta and the kinetics of release in portal blood for EAA considered individually*

Results of the analyses of correlation performed for single EAA are presented in Table IX. With the casein diet, the *in vivo* kinetics of absorption of threonine, valine, isoleucine and leucine were significantly correlated to their *in vitro* kinetics of release in the total digestion products of

Table VII. Correlation between profiles of EAA found in peptide fractions of the *in vitro* digesta of casein and profiles of EAA absorbed *in vivo* following the ingestion of casein meal in the pig.

in vitro / in vivo (h)	0—3 h	3—6 h	6—9 h	9—12 h
		(r)		
0—1	0.87 **	0.75 *	0.66	0.59
1—2	0.76 *	0.62	0.52	0.46
2—3	0.74 *	0.56	0.44	0.36
3—4	0.71 *	0.57	0.49	0.44
4—5	0.45	0.28	0.16	0.10
5—6	0.63	0.44	0.30	0.22
6—7	0.60	0.36	0.20	0.11
7—8	0.40	0.29	0.26	0.21

Statistical significance as in Table V.

Table VIII. Correlation between profiles of EAA found in peptide fractions of the *in vitro* digesta of rapeseed proteins and profiles of EAA absorbed *in vivo* following the ingestion of rapeseed proteins meal in the pig.

in vitro / in vivo (h)	*0—3 h*	*3—6 h*	*6—9 h*	*9—12 h*
		(r)		
0—1	0.68 *	0.71 *	0.71 *	0.66
1—2	0.67 *	0.71 *	0.72 *	0.69 *
2—3	0.95 ***	0.94 ***	0.93 ***	0.93 ***
3—4	0.89 **	0.90 ***	0.91 ***	0.89 **
4—5	0.88 **	0.91 ***	0.88 **	0.84 **
5—6	0.80 **	0.85 **	0.90 ***	0.85 **
6—7	0.80 **	0.87 **	0.89 ***	0.83 **
7—8	0.31	0.48	0.52	0.44

Statistical significance as in Table V.

casein. For the 5 other EAA, correlations did not reach the 95 % significance level.

With rapeseed proteins, strong positive correlations were observed between both kinetics for methionine and arginine ($P < 0.001$), as for isoleucine ($P < 0.01$), leucine and phenylalanine ($P < 0.05$). Contrary to what had been noted with casein, the *in vivo* absorption kinetics of valine from rapeseed proteins presented no correspondence to its *in vitro* kinetics of release in total digestion products.

In order to test if the release of EAA in low molecular weight peptides rather than in total digestion products was a better indicator of their *in vivo* kinetics of release

Table IX. Correlation between the *in vitro* digestion kinetics of each EAA and its *in vivo* absorption kinetics for the 8 sampling intervals, according to protein nature.

EAA	*Casein*	*Rapeseed proteins*
	(r [1])	
THR	0.74 *	0.60
VAL	0.71 *	− 0.02
MET	0.64	0.93 ***
ILE	0.71 *	0.90 **
LEU	0.79 *	0.81 *
PHE	0.69	0.79 *
HIS	− 0.32	− 0.06
LYS	0.65	0.59
ARG	0.38	0.91 ***

[1] Coefficient of correlation; $n = 8$ (8 intervals *in vitro* and 8 intervals *in vivo*).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

in portal blood, correlations were calculated with the 4 first intervals using for the *in vitro* values only the EAA amounts found in peptide fractions. The lowest correspondences were found with casein and the highest with rapeseed proteins (Table X). With these, significant positive correlations were noted for phenylalanine, isoleucine and leucine. For these two latter EAA, the correspondence was about the same as when amounts in total digestion products (peptides and free AA) were used for calculations.

DISCUSSION

The availability of nutrients is the result of a complex physiological process of digestion and metabolism, and it is of interest to estimate the contribution of each step in this process. The aim of the present study was to determine the role of luminal digestion using an *in vitro* technique. The specific purpose was to verify to which extent the characteristics of *in vitro* hydrolysis of EAA from 2 different protein sources, casein and rapeseed concentrate, were indicative of their *in vivo* sequence of release in portal blood of pigs.

In a previous study (Galibois and Savoie, 1987), good correlations were already found when comparing EAA patterns of digestion products released after a 6-h *in vitro* proteolysis with qualitative plasma essential amino acid profiles, measured by porto-arterial differences at given intervals in the rat. These results were in agreement with the fact that this *in vitro* hydrolysis method, when used for relatively short-time proteolysis, allows collection of amino acids that are preferentially released by the action of digestive enzymes (Vachon *et al.*, 1983). The free amino acids and short peptides thus obtained are most likely those which are rapidly delivered to the enterocytes in an *in vivo* situation. A more elaborate comparative study was undertaken in the present work, where the almost complete *in vitro* proteolysis of proteins was carried out, with digesta

Table X. Correlation between the *in vitro* digestion kinetics as peptide of each EAA and its *in vivo* absorption kinetics, for the first 4 sampling intervals.

EAA	*Casein*	*Rapeseed proteins*
	(r [1])	
THR	0.58	− 0.10
VAL	− 0.13	− 0.88
MET	− 0.08	0.93
ILE	0.33	0.97 *
LEU	0.46	0.99 **
PHE	0.61	0.96 *
HIS	− 0.30	0.91
LYS	0.12	0.08
ARG	− 0.30	0.65

[1] Coefficient of correlation; n = 4.
* $P < 0.05$; ** $P < 0.01$.

obtained over 24 h. The *in vivo* method in the pig was more fitted for comparison to the *in vitro* technique, as both allowed qualitative and quantitative measurement of EAA profiles. In this work, 8 profiles sequentially measured in each method were compared. The discrepancy between the overall lengths of measurement periods in the 2 methods did not affect the adequacy of the comparison, since by the end of the sampling periods, comparable average values were found for EAA released in the *in vitro* digesta (> 95 % for casein and 88 % for rapeseed; Savoie *et al.*, 1988) and EAA released in portal blood (91 % for casein and 94 % for rapeseed; Galibois *et al.*, in press). In the *in vitro* technique, the speed of digestion is largely dependent upon the choice of enzymatic conditions and buffer flow rate, and duration of proteolysis is then only a relative value.

Some majors points arose from the assessment of the 2 methods. First, many strong correspondences were found for both types of proteins when comparing *in vitro* and *in vivo* EAA patterns. This very large number of highly significant correlations gave a first indication of the fact that luminal digestion is indeed a determinant step of EAA availability. In many cases, these nutrients tended to appear in the portal blood in about the same proportions as in the *in vitro* digesta. However, the agreements between *in vitro* and *in vivo* patterns were not necessarily distributed according to a time-parallel fashion, and varied with protein nature.

The fundamental difference between the two methods of measurement should be pointed out. During *in vitro* digestion, the protein substrate was only once in contact with the enzyme mixture and the kinetics of release was measured for each amino acid until the exhaustion of substrate. In the *in vivo* situation, the protein meal stayed in the stomach for a more or less long period, and was only gradually released into the duodenum. Then, contrary to the *in vitro* method, the substrate was progressively in contact with the enzymes. The rates of gastric emptying of the 2 diets were not measured in the *in vivo* study, but they were likely to be somewhat different, since protein nature as well as non-protein constituents may affect the length of stay of digesta in the stomach (Darcy, 1984).

As the digestion proceeded, there was a continuous mixing of the protein from the meal, and also a dilution with protein from endogenous source (Laplace *et al.*, 1985). As the measure of absorption was made in the portal vein, it was not possible to appreciate the difference between the amino acids arising from the proximal part and those from the distal part of the digestive tract.

With a rapidly hydrolysed protein such as casein (Savoie *et al.*, 1988), significative correlations appeared mostly between EAA patterns measured during the first *in vitro* and *in vivo* intervals. Later on, in the animal, the absorbed material became more and more diluted with amino acids from endogenous proteins, and displayed EAA compositions different from those of the last 3 *in vitro* periods, which accounted for only 15 % of the EAA in the original substrate. On the other hand, the rapeseed proteins were digested more gradually, as shown by the *in vitro* hydrolysis (Savoie *et al.*, 1988), and correspondences between *in vitro* and *in vivo* patterns were sustained over longer periods of measurement. A possible explanation could be that the various protein fractions of rapeseed were digested according to their different solubilities, and within similar kinetics in both methods.

Regarding the analyses involving the EAA from peptides in digestion products that were made to verify a preferential absorption of amino acids in the form of di- or tri-peptides suggested by some authors (Silk *et al.,* 1979; Adibi and Kim, 1981), this seemed from the present results as from previous ones (Galibois and Savoie, 1987) to be related to the protein nature. Many good correlations were found with rapeseed proteins and less with casein.

From these observations about the correspondence between *in vitro* and *in vivo* EAA profiles, the idea that the *in vivo* pattern of absorption as measured in portal circulation depends directly on the *in vitro* pattern of release of digestion products should be modified. It is in fact difficult, on the only basis of *in vitro* hydrolysis, to predict the site of release in the digestive tract and the conditions of saturation of active transport mechanisms.

The second type of comparison of *in vitro* and *in vivo* data reported here, concerning individual essential amino acids, was related to the sequential release over given time periods. This kind of analysis allowed the determination of which amino acids would be the least affected by endogenous dilution, absorption and transport across the enterocytes, and would appear in the portal blood at a rate similar to their *in vitro* kinetics of release.

In many instances, the sequence of release of EAA during the *in vitro* proteolysis was a good predictor of their *in vivo* sequence of appearance in portal vein. But an important effect of the protein nature was once more evidenced. The highest correlation levels were observed with rapeseed proteins, using either total EAA or only peptide fractions. The lack of correlation in certain cases was most likely indicative of the fact that some EAA, although well released during *in vitro* proteolysis, may have their *in vivo* absorption delayed due to competition for transport sites. It is well known, in particular, that free amino acids of the same class share the same systems of active transport (Munck, 1981). With rapeseed proteins, this could have reduced the absorption of valine, to the benefit of that of isoleucine and leucine. In the same way, the absorption of arginine could have been enhanced to the expense of lysine. However, with casein, such possible competition was less evident, and again no beneficial effect of the peptide form was evidenced for this protein. It is then important not to oversimplify the statement according to which amino acids are better absorbed as peptides than as free amino acids. The various compositions of the peptides derived from the primary structure of the protein from which they originate could induce specific rates of absorption.

Another explanation for the lack of correlation between *in vitro* hydrolysis and *in vivo* release in portal blood could be the existence of a larger metabolism in the intestinal wall, which is a very active tissue (Christensen, 1982; McNurlan and Garlick, 1984). For instance, a large part of histidine released in portal blood may come from the hydrolysis of endogenous blood dipeptides such as carnosine, that would take place in the intestinal tissue (Rérat *et al.,* 1988). As this kind of phenomenon could not be quantified, the comparison of the *in vitro* rate of release and *in vivo* sequence of absorption of dietary histidine is made difficult.

In spite of the fact that some details are still obscure, it is nevertheless interesting to note from this work that the strongest correspondences were consistently obtained with the EAA of rapeseed proteins, which were generally released

much more gradually than those of casein during the *in vitro* digestion. A gradual rate of hydrolysis might thus be a good indicator of an efficient absorption and bioavailability of amino acids, as in this case transport across the enterocyte would not be a limiting step.

To our knowledge, no other study has established the agreement between the form and kinetics of release of EAA during their enzymatic hydrolysis, and their kinetics of appearance in blood circulation. Our results suggest that the sequence of hydrolysis in the digestive tract, and not only the form of release, is a key determinant of amino acid bioavailability, varying to a degree with the protein source and with the nature of the amino acid. These results, however, also give rise to many questions. For instance, the advantage of a rapid proteolysis in the case of casein as compared to a more gradual one with rapeseed could be examined in the perspective of nutritional quality. A possible adaptation of enterocyte metabolism to the nature of dietary protein could also be investigated. Finally, interesting studies could be carried out on the comparative nutritional effects of different peptide mixtures as derived from the hydrolysis of various dietary proteins.

In conclusion, it can be stated that the *in vitro* hydrolysis technique with dialysis seems a practical and useful method for the study and prediction of availability of dietary amino acids. In its present form, this technique may present some drawbacks : for instance, it cannot take into account the varying rate of gastric emptying observed *in vivo* for different proteins (Laplace, 1979), and the important role that peptidases from the intestinal brush-border and from enterocytes play in the digestion of proteins (Silk *et al.*, 1985). Nevertheless, it permits the luminal digestion step to be isolated, and the kinetics of nitrogen and amino acid digestibility to be readily measured.

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