SPECIAL GUEST EDITOR SECTION

In vivo Determination of Amino Acid Bioavailability in Humans and Model Animals

MALCOLM F. FULLER[1]
Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, United Kingdom
DANIEL TOMÉ
Unité INRA 914 Physiologie de la Nutrition et du Comportement Alimentaire, Institut National Agronomique Paris-Grignon, 16 Rue Claude Bernard, 75231 Paris, Cedex 05, France

Because the digestion of many dietary proteins is incomplete, and because there is a continuous (but variable) entry into the intestinal lumen of endogenous protein and amino acid nitrogen that is also subject to digestion, the fluxes of nitrogen, amino acids, and protein in the gut exhibit a rather complicated pattern. Methods to distinguish and quantitate the endogenous and dietary components of nitrogen and amino acids in ileal chyme or feces include the use of a protein-free diet, the enzyme-hydrolyzed protein method, different levels of protein intake, multiple regression methods, and stable-isotope labelling of endogenous or exogenous amino acids. Assessment of bioavailability can be made, with varying degrees of difficulty, in man directly but, for routine evaluation of foods, the use of model animals is attractive for several reasons, the main ones being cost and time. Various animals and birds have been proposed as models for man but, in determining their suitability as a model, their physiological, enzymological, and microbiological differences must be considered. Fecal or ileal digestibility measurements, as well as apparent and true nitrogen and amino acid digestibility measurements, have very different nutritional significance and can, thus, be used for different objectives. Measurements at the ileal level are critical for determining amino acid losses of both dietary and endogenous origin, whereas measurements at the fecal level are critical in assessing whole-body nitrogen losses. A complementary and still unresolved aspect is to take into account the recycling of intestinal nitrogen and bacterial amino acids to the body.

Information on the composition of foods and on the availability of dietary nutrients is required for the formulation of nutritionally adequate diets. Animals and humans require a diet supplying a well balanced pattern of indispensable amino acids as well as sufficient total nitrogen for the synthesis of dispensable amino acids and other substances. Protein usually represents 10–20% of the human diet, and the optimization of this protein fraction requires that the quantities of available nitrogen and indispensable amino acids are matched to those required by the organism. The nutrient composition of foods is obtained from chemical analysis, whereas the estimation of the availability of nitrogen and amino acids usually requires an in vivo assay.

Amino acids have been recognized as essential nutrients for over a century; for most of that time, however, estimates of dietary requirements have been expressed simply as total intakes, without regard for the fact that not all of the amino acid present in a food can be absorbed and utilized. Bioavailability is the term used, for amino acids as well as other nutrients, to express the proportion of the total amount of dietary amino acid that can be absorbed and utilized. It can be considered as having 3 components: digestibility, chemical integrity, and freedom from interference in metabolism.

The concept and measurement of digestible protein and amino acids for nonruminant species, including humans, has received a lot of attention. Most of the methods developed for the determination of nitrogen and amino acid bioavailability have focused on the intestinal absorption step, i.e., protein and amino acid digestibility, calculated as the percentage of amino acid intake that does not appear in digesta or feces. Bioavailability can also be estimated from the postprandial portal uptake of amino acids, or from the nutritional efficiency of protein utilization determined from nitrogen and amino acid retention.

In most instances, digestibility is the most important component of bioavailability, but the other aspects are also frequently important with specific foods and food processing methods. Assessment of bioavailability can be made, with varying degrees of difficulty, in man directly but, for routine evaluation of foods, the use of model animals is attractive for several reasons, the main ones being cost and time. Various animals and birds have been proposed as models for man but, in determining their suitability as a model, their physiological,

Guest edited as a special report on "Dietary Protein Quality For Humans" by Paul J. Moughan.

[1] Corresponding author's present address: 107 Quaker Path, Stony Brook, NY 11790; e-mail: malcolm689@aol.com.

enzymological, and microbiological differences must be considered.

The Digestive Processes

The processes that lead from the ingestion of food proteins to the intracellular utilization of amino acids are very different in ruminant and nonruminant animals. For this reason, only nonruminants are considered as potentially suitable models for digestion in man. Among nonruminants, the processes vary in their detail from one animal species to another but follow the same general sequence outlined below. More complete descriptions of the various aspects are given in several reviews (1–4).

Prehension and Selection

While all species have some ability to select parts of a food presented to them, food selection behavior differs greatly among species. Birds, for example, can use their beaks accurately to select specific particles for ingestion and reject others. Small rodents use their paws for this purpose. These behaviors are important when relatively coarsely processed foods are to be evaluated. Pigs, being larger, tend to consume their food without any selection, although they will reject large unpalatable fragments or unpalatable food in its entirety. Although food selection can be avoided by processes such as grinding, homogenizing, and pelletting, these processes may alter the native structure of the food being evaluated in such a way that the derived value may no longer apply to the original material.

Comminution

In animals, this is achieved almost entirely by the mouthparts (lips, tongue, cheeks, palate, and teeth) but, in birds, which lack teeth, the gizzard plays the major role. In humans, food may be extensively comminuted by various types of processing before being served, when knives and forks may further reduce the size of morsels conveyed to the mouth. Thereafter, as in animals, further comminution depends on the extent of chewing. The degree of comminution achieved may affect the measured nutrient bioavailability and is, thus, a factor in the choice of model species.

Hydration and Solubilization

For many food proteins, the initial phases of digestion involve mixture with ingested water or saliva, creating a medium in which digestive enzymes can diffuse and act on their substrates. In many plant foods, however, proteins are enclosed within cell walls that are resistant to mammalian and avian enzymes, and it may only be by the actions of microbial enzymes, especially in the more distal parts of the gastrointestinal (GI) tract, that such proteins are released. Thorough comminution, either by prior processing (mechanical, thermal, or both) or by the physiological mechanisms outlined above, is, therefore, necessary for their maximum utilization.

Digestion by Acid Pepsins

The mammalian stomach secretes HCl and pepsinogens, which are converted to pepsins by an autocatalytic process in which a peptide is cleaved from the *N*-terminal in the presence of HCl. In birds, HCl and pepsinogen are secreted by the proventriculus, a glandular structure at the lower end of the esophagus just above the gizzard. Residence time of digesta in the proventriculus is very limited, but the action of acid pepsin continues while food is ground in the gizzard, a very muscular organ in which ingested grit is retained to aid the process of grinding coarse food such as whole grains.

Digestion by Pancreatic Proteases

In both mammals and birds, the pancreas secretes a number of proteases, of which the principal activities are contributed by the endopeptidases trypsin, chymotrypsin, elastase, and the exopeptidases carboxypeptidase A and B. All of these proteases are secreted as inactive zymogens. The duodenal mucosa secretes enterokinase, which activates trypsinogen by cleaving a peptide from its *N*-terminal end; trypsin then activates trypsinogen and all the other pancreatic zymogens. The proportions of the major pancreatic enzymes are broadly similar among several species studied (5–7), although in avian pancreatic secretions, chymotrypsin rather than trypsin is the predominant enzyme.

Digestion by Intestinal Proteases

The oligopeptides released by the actions of pepsin and the pancreatic proteases are further hydrolyzed by enzymes of the small intestine, some expressed in the brush border and others in the cytoplasm of apical enterocytes. The brush border proteases include amino-oligopeptidase, which cleaves single amino acids from the *N*-terminal end of oligopeptides. Its activity is complemented by dipeptidyl-peptidase IV and aminopeptidase A. The end products of the aminopeptidases are di- and tripeptides that, together with free amino acids, are absorbed by a variety of transport mechanisms across the brush border membrane of villous enterocytes. Their hydrolysis to free amino acids is completed by cytoplasmic aminopeptidases, so that almost all amino acids entering the portal vein are in free form, although some quantities of peptides, and even of intact protein, may enter the portal circulation. The essential features of protein hydrolysis are broadly similar among all of the species being considered and, in general, enzymic sufficiency is not considered to be a limiting factor in amino acid bioavailability.

Amino Acid Metabolism by the Gastrointestinal (GI) Microflora

The endogenous proteases are not the only agents for the dissimilation of dietary proteins. The GI tract is the habitat for a very large number of microorganisms, each of which has its own range of enzyme activities. These activities include not only the hydrolysis of proteins, but the deamination and decarboxylation of amino acids as well. In addition, the microflora synthesize amino acids de novo. They derive

energy for these syntheses from the breakdown of a variety of substrates, but especially those such as nonstarch polysaccharides that are resistant to the digestive enzymes of the host. Finally, certain microflora possess ureolytic activity; the hydrolysis of urea secreted into the intestine allows urea nitrogen to be recycled both by microbial amino acid synthesis and by the uptake from the gut of ammonia. The ammonia is captured by a number of carriers, especially alanine, aspartate, and glutamate, from which, by transamination, it may be incorporated into most amino acids. This mechanism of urea recycling may be of value in conserving nitrogen (8).

Nitrogen and Amino Acid Metabolism in the Small and Large Intestines and Nutritional Significance of Digestibility Measured at Different Sites

Because the digestion of many dietary proteins is incomplete, and because there is a continuous (but variable) entry into the intestinal lumen of endogenous protein and amino acid nitrogen that is also subject to digestion, the fluxes of nitrogen, amino acids, and protein in the gut exhibit a rather complicated pattern. In humans, ingested dietary proteins (40–110 g/day), protein secreted into the gut (20–50 g/day), and secreted nonprotein nitrogen (urea and other nitrogen-containing molecules) are mixed in the lumen of the stomach and the small intestine and are subjected to transit, digestion, and absorption. The main part is transferred into the body by absorption across the intestinal mucosa, but a small part remains in the lumen and reaches the terminal ileum. This, along with other undigested luminal components, passes from the terminal ileum into the large intestine, and the whole is subjected to fermentation by the microflora.

The consequence of the activities of the gastrointestinal microflora is that, by the time digesta are excreted as feces, they consist largely of microbial protein (9–10). Fecal amino acid excretion is, thus, more a reflection of the activities of the gut microflora than of the amino acid absorption of the host. In the case of amino acids, therefore, the premise of the classical digestibility measurement (i.e., that the difference between the amount of a nutrient consumed and the amount excreted in feces represents the amount absorbed) is not valid. Compared with the amount of amino acid actually absorbed from the diet, the difference between intake and faecal output may be greater as a result of amino acid degradation by the microflora, or less by the addition of amino acids synthesized de novo by the microflora. Given that most of the nitrogen in feces is in microbial protein, much of it the product of de novo amino acid synthesis, estimation of the proportion of the dietary amino acid intake that is actually absorbed depends upon distinguishing those events that result in the absorption of dietary amino acids from the transformations brought about by the activities of the microflora, which are capable of both degrading amino acids and synthesising them de novo.

The microflora of the large intestine alter not only the amino acid composition of the digesta but the quantitative passage of nitrogenous compounds as a whole. For example, Zebrowska (11) showed that, when hydrolyzed casein was infused into the terminal ileum of pigs, little of the nitrogen was excreted in feces, but there was an increase in urinary nitrogen equivalent to 83–90% of the amount infused. In contrast, when the same amount of casein was given orally, a large proportion of the nitrogen was retained. Evidently, much of the amino acid nitrogen entering the caecum was absorbed from the large intestine but not in a form that could be utilized, that is, not as amino acids. It has also been shown that infusions of starch into the caecum increase the excretion of nitrogen in feces (12), with reduced urinary nitrogen excretion; this is evidence of microbial incorporation of endogenous nitrogen into biomass that is eventually excreted in the feces. From this brief overview, it is clear that any attempt to identify the proportion of a dietary amino acid absorbed during its transit of the gut must exclude the transformations in the large intestine of that fraction of dietary protein that escapes absorption in the proximal intestine. This has led over the past 40 years to the development of a variety of methods to estimate the digestibility of amino acids up to the point at which they enter the large intestine, a measure usually called ileal digestibility.

Depending on the amino acid and on the diet, digestibility values obtained by the fecal analysis method may overestimate (usually the case) or underestimate those obtained by the ileal analysis method (13). This can be attributed to the fact that the microflora in the large intestine have the capacity to deaminate amino acids and to use the carbon skeletons for energy. A part of the amino-derived nitrogen can be absorbed from the large intestine in the form of ammonia, transported to the liver, converted to urea, and excreted in the urine. In this case, apparent amino acid digestibilities obtained by the fecal analysis method are higher than those determined by the ileal analysis method. Cystine, threonine, and tryptophan usually disappear, to a large extent, in the large intestine (14–16). On the other hand, microbial net synthesis of methionine and, sometimes, of lysine, has been reported in some studies, resulting in lower fecal than ileal digestibility values (15, 17, 18). The anabolic activity of the intestinal microflora depends on diet composition, but between 50 and 90% of the total nitrogen in feces is of bacterial origin (18–20).

Under those conditions, fecal or ileal digestibility measurements, as well as apparent and true nitrogen and amino acid digestibility measurements, have very different nutritional significance and can, thus, be used for different objectives. Measurements at the ileal level are critical for determining amino acid losses of both dietary and endogenous origin, whereas measurements at the fecal level are critical in assessing whole-body nitrogen losses. A complementary and still unresolved aspect is to take into account the recycling of intestinal nitrogen and bacterial amino acids to the body. This aspect is discussed further below.

The Sampling of Ileal Effluents for Measurement of Endogenous and Dietary Nitrogen and Amino Acids

Metabolism by colonic bacteria implies that ileal outflow represents the critical parameter for determining both endogenous and dietary amino acid losses. For their measurement and characterization, it is necessary both to collect ileal effluents and to differentiate secreted from dietary nitrogen fractions.

The estimation of ileal digestibility requires some method to relate the flow of amino acids out of the ileum to the corresponding amino acid intake. The simplest method used with experimental animals (21) is to sample digesta from the terminal ileum of animals post mortem or, better, under terminal anaesthesia. This approach has the advantage of avoiding surgical intervention, but has several disadvantages. First, it does not allow the repeated measurements that help to reduce variability. Second, the sample obtained represents the digesta of only one short part of the feeding cycle and may, therefore, not be representative of 24 h flow. Third, especially with small animals, insufficient sample may be obtained from one animal, so that digesta from 2 or more animals may need to be combined to provide sufficient sample. In consequence of those disadvantages, several surgical approaches have been developed that allow digesta to be removed more or less continuously from the terminal ileum. The simplest of these is a T-cannula in the terminal ileum, through which some proportion (variable and uncontrolled) of the passing digesta flows by the natural propulsive forces of the intestine. Both the slaughter method and methods using T-cannulas (because only a proportion of the digesta flow is removed) require an indigestible marker to be added to the diet to measure flow. The principle of the marker is essentially that, if the digesta collected contain, e.g., 20% of the marker fed in the day, the collected digesta represent 20% of the daily flow. Note that calculation of the volume flow is not necessary for the calculation of apparent digestibility (AD), which can be derived from simple proportions according to the equation

$$AD = 1 - nM/Nm$$

where N and n are the concentrations [g/kg dry matter (DM)] of an amino acid in the diet and digesta, respectively, and M and m are the corresponding concentrations (g/kg DM) of the marker in the diet and digesta, respectively. However, it should be borne in mind that the marker makes up part of the dry matter in each sample. For the strictest accuracy, this should be taken into account.

Markers can be avoided by the use of a re-entrant cannula, by which all of the flow is collected and then sampled and returned via the distal part of the cannula. Re-entrant cannulas require constant attention and tend to block when the diet contains fibrous matter. This problem can be alleviated by grinding the diet very finely, but that, in turn, may mean that the diet as tested is not the same as the diet as normally eaten, for which the measured digestibility value may, therefore, be inappropriate. Markers are also avoided by an ileostomy or an ileorectal anastomosis, when the entire output can be collected, although it may still be desirable to add a marker as a check on the completeness of collection. One of the sequelae of ileostomy or ileorectal anastomosis is that the terminal ileum is modified to assume some of the functions of the absent (or dysfunctional) large intestine. The ileum becomes colonized to an extent that may be quite uncharacteristic of the ileum in an intact subject. The steerable postvalvular cannula has been developed in several variants to overcome many of the objections given above. First, it involves minimal interference with the function of the small intestine; second, it allows the unfractionated ileal effluent to be collected; third, it can be of large diameter to minimize the possibility of its being blocked by fibrous matter in the digesta; and fourth, when not in use, it allows a normal flow of digesta into the large intestine.

In humans, intestinal effluents are obtained either from ileostomy patients (ileostomates) or in healthy volunteers by using nasointestinal tubes; these approaches are, however, not straightforward. They can be used as reference methods (22, 23) but are too demanding for routine food evaluation. An alternative is the use of model animals, and among the most commonly used models are the rat and pig. The growing pig appears to be a satisfactory model animal for determining protein digestion for the adult human. However, pig ileal amino-acid digestibility assays are labor-intensive and relatively expensive, and the rat may be a useful alternative model for quality control purposes (24–28). The rat is used for the determination of protein quality in human diets (29) and has been shown to be an accurate animal model for estimating the ileal digestibility of amino acids of protein sources of other animal species (27, 30). However, for the reasons outlined above, the rat may not be suitable for all foods, and some differences in digestibility have been observed between rats and pigs (24, 31, 32).

Endogenous Secretion and Methods of Distinguishing Endogenous and Dietary Amino Acids

Both direct and indirect methods have been proposed to distinguish and quantitate the endogenous and dietary components of nitrogen and amino acids in ileal chyme or feces (2, 33–36). These approaches include use of a protein-free diet; the enzyme-hydrolyzed protein method (37, 38); different levels of protein intake (39–41); or multiple regression methods, in which it is assumed that the quantity and amino acid composition of endogenous losses is constant and independent of diet (42, 43). Substantial advances in the ability to discriminate between exogenous (dietary) and endogenous nitrogen have been achieved, however, using stable isotopes (2, 34).

Endogenous amino acid losses were first estimated by feeding a protein-free diet, as in the measurement of net protein utilization (NPU) or biological value. However, the protein-free diet approach has been criticized as creating an unphysiological state that may result in a reduction in the

amount of gastric and pancreatic enzymes secreted (44, 45) and a general decrease in the rate of protein synthesis in the body and gut (46). The accumulating evidence suggests that the endogenous secretions are influenced by the amount and nature of protein in the diet (47) and by other factors, and that the amino acid losses observed under conditions of protein-free feeding are not appropriate as corrections to apply under conditions in which protein is given. This has led to the development of several methods by which endogenous amino acid losses may be measured in the presence of dietary protein.

Successful methods of distinguishing endogenous and dietary amino acids in digesta are based on one of the following approaches:

The Amino Acid Patterns of Diet and Digesta

In this approach, a protein with an amino acid pattern very different from that of the endogenous protein in ileal digesta (as estimated on a protein-free diet) is given in the diet. The amino acid component of the digesta is assumed to consist of a simple mixture of undigested dietary protein and endogenous protein so that, by solving a series of simultaneous equations, the proportion contributed by each can be calculated. The method rests on 2 assumptions: first, there is no selective digestion of dietary protein, i.e., that the undigested fraction has the same composition as that fed; and second, although the amount of endogenous protein may change with the diet, its composition does not. Neither of these assumptions has been proved.

Giving Peptides Rather Than Protein in the Diet

The peptide alimentation method, sometimes called the enzyme-hydrolyzed casein method (48, 49), involves giving dietary amino acids as enzymically hydrolyzed protein (usually casein) having peptides with a molecular weight of <5000 daltons (Da). Ileal digesta are separated by ultrafiltration to remove peptides smaller than 10 000 Da and free amino acids. The fraction >10 000 Da is assumed to represent the endogenous material. The main uncertainties with this method are the assumption that the endogenous contribution to ileal digesta is entirely in the form of proteins (or peptides >10 000 Da). It is also assumed that the effects of dietary protein on endogenous losses are exactly replicated by small peptides. The first assumption was tested by analyzing the peptide size distribution of ileal digesta from animals (pigs) given a protein-free diet. The proportion of peptides <10 000 Da was found to range from 14 (48) to 33%. Leterme et al. (50) found a value in the same range, 22%. The second assumption has been tested by comparing the effect on endogenous flow of giving peptides of different sizes. From evidence summarized by Moughan (4), it appears that peptide size has little effect. However, it should be noted that, in all of these experiments, purified proteins have been used. Whether the same would be true with complex protein mixtures in a food matrix remains to be determined.

Feeding Proteins Labeled with Homoarginine

By guanidinating food proteins with *O*-methylisourea, lysine residues are converted to homoarginine, an amino acid that does not occur naturally in proteins. Thus, undigested dietary protein recovered in digesta can be recognized, and then the endogenous component can be estimated by difference. The method relies on several assumptions, the most important being that the guanidinated protein is digested to the same extent as the native protein and, if less than 100% of lysine residues are guanidinated, that there is no disproportionate digestion of the labeled parts. Moreover, this method cannot be applied in humans.

Labeling Endogenous Protein

Labeling of intestinal endogenous secretion has been performed by infusion of labeled amino acids. In this approach, the body tissues are labeled by a prolonged administration of, usually, a ^{15}N-tracer (^{15}N-leucine; 50–55). The method has the undoubted advantage that, once the tissues are labeled, a succession of different diets can be given. As discussed by Fuller and Reeds (3), the results obtained are influenced by the choice of tracer and the tissue sample chosen to represent the labeling precursor. This method has been shown to be, in some cases, inappropriate for the assessment of endogenous nitrogen and amino acids (53, 56, 57). As a result of the rapid incorporation into endogenous secretions of amino acids from the diet, the isotopic enrichment of endogenous secretions may be diluted very soon after feeding by the rapid incorporation of dietary amino acids. Nevertheless, despite these shortcomings, the method has provided the most extensive and coherent body of information on the quantitative contribution of endogenous secretions to digesta outflow.

Using Labeled Diets

By giving diets that are isotopically labeled (usually with carbon or nitrogen), the endogenous flow is estimated from the dilution of the isotopic enrichment in the digesta. Because special methods are needed to produce labeled proteins (e.g., fertilizing plants with ^{15}N ammonium salts), the method is not suited to routine evaluation of true digestibility but serves to provide data on the contribution of endogenous amino acids to digesta in different dietary and other circumstances. It is, of course, assumed that, during the course of the experiment, there is no labeling of endogenous secretions by recycling of dietary amino acids. However, it has long been known that absorbed amino acids may be utilized by mucosal cells and, because these cells are continuously shed from the villous tips they may be expected to contain amino acids of immediate dietary origin. Furthermore, amino acids taken up from the gut lumen may also reappear very rapidly in pancreatic and possibly other digestive secretions, so that the method probably underestimates the true contribution of endogenous proteins to the ileal digesta. Labeled foodstuffs, including uniformly ^{15}N-labeled dietary protein or [^{15}N]-leucine-labeled dietary protein, have been used for dietary nitrogen and amino acid absorption studies (58–61). Recycling of the tracer into (endogenous) secretion has been questioned, but the final error on digestibility figures may be small (3, 54, 61, 62).

Dietary Nitrogen and Amino Acid Digestibility—Apparent versus True Digestibility—Human versus Animal—Nitrogen versus Individual Amino Acids

The earliest and simplest measurements of ileal digestibility were based on the total nitrogen and amino acid fluxes at the terminal ileum and are, thus, referred to as "apparent ileal digestibilty" because they do not differentiate between endogenous and dietary components. Apparent ileal amino acid digestibilities represent an improvement over the older values of AD based on fecal analysis, but the results obtained may be confounded by the influence of the diet on endogenous nitrogen secretion (63–65) and may lead to nonadditive values. Ileal digesta contain variable amounts of endogenous protein from digestive secretions, sloughed-off epithelial cells, and mucins. Adjustments for endogenous protein and amino acid recoveries, which may be influenced by the diet, allow for the determination of true ileal protein and amino acid digestibility values.

From the different studies, it has become clear that the flow of endogenous protein from the ileum varies with the amount of protein given. It is also affected by the total amount of feed given and the presence in the diet of so-called antinutritional factors, such as trypsin inhibitors, certain lectins, nonstarch polysaccharides, and a whole range of substances found in specific plants. The estimates of endogenous amino acid loss made with protein-free semipurified diets are, therefore, minimal values; with normal diets, the losses are substantially higher. The correction of ileal digestibility from apparent to true requires values of endogenous amino acid losses appropriate to the protein intake and total amount of diet given. Some authors prefer to use a standard rather than a specific correction, and the resulting values are referred to as "standardized true ileal digestibility." Endogenous losses can, in principle, be considered either as part of the amino acid requirement or as a factor reducing digestibility. The advantage of correcting digestibility values is that it gives rise to a system in which the digestible amino acid concentrations of foods are additive; this cannot be achieved by using AD values. The important point is that, whatever correction is applied to apparent digestibility estimates, it must be consistent with the way that amino acid requirements are expressed.

The availability of dietary nitrogen and amino acids are, therefore, best estimated using true digestibility, and it is also agreed that, due to the presence of microbes that metabolize amino acids entering the large intestine, ileal measurement is

Table 1. True digestibility of dietary nitrogen and amino acids after the ingestion of milk or soy protein in healthy human volunteers

	Milk			Soy			Wheat
	Human[a]	Pig[b]	Rat[b]	Human[a]	Pig[b]	Rat[b]	Pig[c]
Aspartate + asparagine	94.3	98	96	93.2	97	95	
Serine	92.0	97	90	93.2	97	98	
Glutamate + glutamine	95.3	98	93	96.6	100	98	
Proline	96.1	—	—	92.8	—	—	
Glycine	91.6	90	86	90.1	90	87	
Alanine	95.9	96	97	92.3	96	95	
Tyrosine	99.3	99	100	96.8	97	99	
Threonine	93.4	95	94	89.0	91	92	92.1
Valine	95.9	98	97	92.5	96	96	92.5
Isoleucine	95.4	98	95	93.5	97	97	95.4
Leucine	95.1	99	99	93.3	96	95	95.4
Phenylalanine	95.6	98	100	95.5	96	97	95.3
Lysine	94.9	99	99	95.0	97	98	91.4
Histidine	94.9	99	96	91.7	95	93	93.9
Cysteine	—	89	99	—	85	95	
Methionine	—	100	98	—	97	98	
Arginine	—	98	98	—	98	99	
Avg. amino acid digestibility	95.3			93.8			95.1
Nitrogen digestibility	95.3			91.7			95.8

[a] Gaudichon et al. (ref. 77).
[b] Rutherfurd and Moughan (ref. 28).
[c] Jondreville et al. (ref. 43).

preferred to the fecal method as a means of determining the digestion and absorption of dietary amino acids in simple-stomached species (66, 67). True ileal digestibility is, thus, considered as the critical biological parameter for availability of the dietary indispensable amino acids because it represents the specific behavior of the dietary protein source, taking into account the effect of the particular food on endogenous nitrogen secretion. The determination of true ileal digestibility requires a means of estimating the contribution of endogenous sources to ileal amino acid outflow.

The quantities of amino acids entering the GI tract from the diet are augmented and, sometimes, exceeded by those entering from endogenous sources. These include not only salivary, gastric, biliary, pancreatic, and other secretions, but also the constitutive proteins of mucosal cells, especially those of the villi that are continuously shed from the villous tips at the end of their migration from the crypts of Lieberkühn. In addition, mucus is secreted from all parts of the GI mucosa. Two characteristics of mucus are particularly important in the present context. First, in keeping with its function of protecting the mucosa from hydrolytic attack, mucus is highly resistant to the digestive enzymes of the host; second, its amino acid composition is markedly different from the generality of body proteins, being distinguished by high proportions of proline, serine, glycine, and threonine. Mucus also contains significant amounts of amino sugars that, although not contributing to amino acids entering the lumen, add to intestinal nitrogen flux.

Much of the endogenous nitrogen that enters the gut lumen is later reabsorbed. By the use of ^{15}N-labeling, Krawielitski et al. (67) estimated that, in growing pigs, approximately 90% of the endogenous nitrogen entering the gut was reabsorbed proximal to the terminal ileum. The proportion that is not reabsorbed but enters the large intestine is, like undigested dietary protein, exposed to the wider range of enzyme activities expressed by the resident flora. However, as described above, little if any of the nitrogen released by these activities is absorbed as amino acids. In addition, the presence of endogenous nitrogen secretions means that it is necessary to discriminate between exogenous (dietary) and endogenous nitrogen.

In human subjects adapted to 0.8–1.0 g protein/kg/day with a standard occidental diet, the total nitrogen flux at the terminal ileum entering the colon, which is made up of luminal dietary and endogenous nitrogen products that have not been absorbed in the small intestine, is estimated as 2–4 g N/day. Of this total nitrogen fraction, the luminal dietary products that have not been absorbed in the small intestine are estimated as 0.6–1.2 g N/day, consisting of a dietary amino-acid fraction (0.5–0.8 g N/day) and a (usually lower) dietary-derived non-amino-acid fraction (0.1–0.4 g N/day). Values for dietary nitrogen and amino acid ileal digestibilities of different protein sources in humans and in animal models are given in Table 1. In healthy humans, the true ileal digestibility of dietary protein nitrogen varies, depending on the type of protein (ranging, e.g., from 51% for raw egg to 94% for milk protein), but it is only slightly sensitive to variations in gastric emptying rate and intestinal transit for standard diets (58–60, 62, 69–76). It ranges from 90 to 95% for most of the traditional sources.

Regarding the dietary amino acid fraction, it is also questionable whether protein (overall nitrogen) digestibility is a good proxy for individual ileal amino acid digestibility because some studies have reported modest ranges of variation of individual amino acid digestibility around the value for nitrogen digestibility (77). However, individual amino acid fluxes are still poorly documented, and no data are available in humans consuming high fiber-containing diets. Experiments in animals showed that, in some cases, there are substantial differences in true digestibility among amino acids (53, 61, 78). The digestibility of ^{15}N-amino acids determined in pig intestine varied from 82% (isoleucine) to 95% (methionine; 57). Experiments in pigs have also shown that true ileal digestibility for cysteine in peas was 13–16% lower than nitrogen digestibility, whereas for methionine it was 17–28 % higher (54). Differences in the true ileal digestibility of amino acids in human milk have also been demonstrated. Among the indispensable amino acids, digestibility ranged from 86% for threonine to 100% for methionine and tyrosine. This emphasizes the need to account for amino acid availability in establishing an amino acid requirement profile.

Nitrogen and Amino Acid Metabolism in the Distal Lumen

Three distal intestinal metabolic processes contribute indirectly to dietary nitrogen and amino acid efficiency because they can be modified by the nature of the diet: (*1*) the intestinal losses of indispensable amino acids of endogenous origin as a part of indispensable amino acid requirements; (*2*) the fecal excretion of nitrogen as a part of body nitrogen losses and requirement; and (*3*) the possible recycling to the body of intestinal nonprotein nitrogen and indispensable amino acids derived from bacterial metabolism.

Ileal Losses of Endogenous Amino Acids

These losses appear to reach significant levels, as was first assessed in ileostomized subjects fed on protein-free diets (22, 79) and confirmed in healthy subjects receiving protein meals (76). In humans, the flux of endogenous nitrogen at the terminal ileum represents 1.6–2.2 g N/day and is constituted by an endogenous amino acid nitrogen fraction (0.6–1.0 g N/day) arising from luminal-secreted proteins and peptides, and a non-amino-acid nitrogen fraction (1.0–1.1 g N/day) that is mainly urea arising from hepatic recycling (77). The endogenous indispensable amino acids entering the colon and being further deaminated are considered to be an important pathway for indispensable amino acid losses, contributing to the maintenance requirement (Table 2). Their contribution to the daily requirement reaches dramatic levels for some amino acids, such as threonine, and this may explain some of the differences observed between amino acid requirement estimates made using different methods, depending on their

Table 2. Daily ileal endogenous amino acid losses in adult humans

Amino acids	Protein-free diet[a], mg/kg/day	Protein-containing meal[b], mg/kg/day
Aspartate + asparagine	—	8.7–13.5
Alanine	—	3.7–5.2
Glutamate + glutamine	—	6.1–11.5
Glycine	—	7.5–9.2
Proline	—	6–6.8
Serine	—	3.5–5.4
Isoleucine	1.7	3–3.7
Leucine	3.2	4.2–5.8
Valine	2.9	4.8–6
Lysine	3.9	2.7–5.5
Aromatic amino acids	3.9	4.8–7.1
Histidine	1.9	1.9–2.1
Threonine	4.2	5.6–6.8
Sulphur amino acids	1.8	—

[a] Fuller et al. (ref. 22).
[b] Gaudichon et al. (ref. 77).

ability to take these losses into account. Endogenous ileal amino acid excretion is higher in carnivorous animals (cats and dogs) than in omnivorous animals such as rats and pigs. Whereas the pattern of endogenous amino acid excretion was similar in rats and dogs, dogs excreted a significantly greater amount of nitrogen (1.91 vs 2.27 and 1.63 vs 4.12 g/kg dry matter intake for the protein-free and peptide alimentation method, respectively) and all amino acids, except for glycine, isoleucine, and leucine (80).

Fecal Excretion of Nitrogen

Endogenous and dietary amino acids, as well as urea, entering the colon are mainly deaminated. The nitrogen of the colonic ammonia pool is used either for microbial amino acid synthesis or reabsorbed through the ammonia/urea enterohepatic recycling. Some may be excreted in feces. Protein infused into the caecum of growing pigs has been demonstrated to be completely digested, but the absorbed nitrogen was almost fully recovered in urine, with no nitrogen retention, in contrast to the same protein given orally. Similarly, lysine infused into the caecum of growing pigs given a lysine-deficient diet did not improve nitrogen retention, whereas the same amount given orally did. Fecal losses—because they incorporate specific (i.e., diet-induced) nitrogen losses—represent an important parameter reflecting nutritional status in the longer term, as measured by nitrogen balance, with a complex diet. When referring to a mixed protein diet (i.e., human nutrition), these losses probably depend on several dietary parameters apart from the specific dietary protein (amount of food, total fibre, and type of fiber; 81). For these purposes, measurements in humans are recommended, but pig values can be used when human results are not available.

Microbial Activity in the Upper Digestive Tract

Although less abundant than in the caecum and colon, microorganisms also populate the upper digestive tract. Notwithstanding their smaller numbers, it has been shown in pigs that their metabolic activity is as high in the terminal ileum as in the large intestine (82, 83). The fermentative capabilities of the small intestinal microflora of the pig are demonstrated by the substantial digestion of various classes of nonstarch polysaccharides (34, 84–87), none of which can be degraded by mammalian enzymes. This is accompanied by the synthesis of microbial amino acids, as shown by Mason et al. (10), who reported an ileal diaminopimelic acid (DAPA) outflow of 23 mg/day, a quarter of the amount excreted in feces (95 mg/day). Poppe et al. (19) estimated from ileal DAPA flow of pigs given diets with different proteins that 25–55% of ileal protein was microbial. Similarly, Hennig et al. (88) estimated from the ileal excretion of D-alanine that, depending on diet, 19–47% of the nitrogen passing out of the ileum was in the form of microbial protein. Unfortunately, comparable information on other species is not available. These observations leave no doubt that "ileal amino acid digestibility" does not entirely exclude the activities of the GI microflora. The effect of microbial amino acid synthesis in the upper GI tract on estimates of amino acid digestibility depends on several factors and is still unclear. From evidence reviewed by Fuller (89), it appears that amino acids in microbial protein entering the colon are predominantly preformed, with only a small proportion being derived by de novo synthesis. The error introduced by the presence of an upper intestinal microflora is, therefore, expected to be correspondingly small.

Utilization of Microbial Amino Acids

A still unresolved issue with respect to protein and amino acid requirements concerns nitrogen and amino acid recycling by the distal intestine. Measurements of the recycling of urea have revealed the quantitative importance of nitrogen flux in the colon, estimated as 15 g/day in adult humans, which is equivalent to one third of the daily total body nitrogen flux and comparable to the daily dietary nitrogen supply (3). The absorption of indispensable amino acids synthesized by the GI microflora has been documented in rats, pigs, and humans (90, 93). In rats, this absorption is attributable to coprophagy (90), but in pigs (92, 94, 95) and humans (95, 96), there is direct absorption. Recent evidence in pigs (93, 94) points to the importance of the small intestine as the site of absorption, but the origin of the recycled nitrogen remains unclear; this is critical to establishing the nutritional importance of the phenomenon (3).

In the present context, the significance of the phenomenon is that the net absorption of amino acids from the GIT includes not only dietary and endogenous amino acids, but also amino acids synthesized de novo by the microflora. In a conventional ileal digestibility assay, this additional quantity would be ascribed to the diet and enhance its true value. Elucidating the magnitude of the microbial amino acid supply to the host is,

Table 3. Daily intake of amino acids, the amounts apparently absorbed from the small intestine, and the amounts appearing in the portal circulation in 50 kg pigs[a]

Amino acid	Intake, g/24 h	Uptake, g/24 h	Portal removal, g/24 h
Aspartate	12.9	12.0	22.3
Threonine	7.2	6.6	8.3
Serine	9.4	8.5	13.8
Glutamate	39.5	37.9	3.5
Proline	19.7	19.1	21.4
Glycine	3.4	2.8	10.3
Alanine	5.4	4.9	26.8
Valine	11.3	10.7	14.9
Isoleucine	9.0	8.4	10.8
Leucine	16.5	15.7	16.3
Tyrosine	6.6	6.0	10.7
Phenylalanine	9.5	9.2	9.8
Lysine	13.3	12.9	20.1
Histidine	4.9	4.8	6.1
Arginine	0.3	6.0	10.2
Cysteine	0.9	0.7	1.3
Methionine	4.2	4.1	5.8

[a] Data of Darcy-Vrillon et al. (ref.13).

therefore, critical to refining the accuracy of digestibility measurements, as well as for assessing its impact on the estimation of amino acid requirements.

Dynamic Aspects of Nitrogen and Amino Acid Metabolism

During the postprandial phase, dietary protein nitrogen and amino acids pass transiently through the metabolic pools of the body. Acute amino acid uptake and utilization during the postprandial phase is critical in terms of the deposition of dietary amino acids in the tissues, and assessment of the postprandial utilization of dietary proteins is an appropriate approach to determining dietary amino acid bioavailability and nutritional efficiency. The rate of amino acid uptake into the portal circulation reflects not only the rate of absorption by the enterocytes, but also the metabolic activity of the GI tissue. In Table 3, the amount of each amino acid consumed is compared with the amount apparently absorbed from the lumen of the small intestine (i.e., intake minus ileal outflow) and the amount appearing in the portal circulation. For all amino acids except glutamate and glutamine (which are an important energy source for enterocytes), a greater quantity appears in the portal circulation than is supplied by the diet. The difference represents, in part, the reabsorption of amino acids from endogenous secretions and also the metabolism of amino acids by the gut tissue itself.

Studies have been undertaken to assess the acute postprandial utilization of dietary protein during the repletion phase of the diurnal cycle. The key steps concerning the fate of dietary nitrogen are considered to be: (*1*) the amount of nitrogen actually absorbed; (*2*) the amount that has been deaminated and recovered, mainly in the form of urea; and (*3*) the level of nitrogen retained in the body. The problems of measuring the postprandial utilization of dietary protein nitrogen in terms of ileal nitrogen digestibility and short-term retention of dietary protein nitrogen can be circumvented by the use of [^{15}N]-labeled proteins. This technique makes it possible to follow the metabolic fate of dietary nitrogen after its ingestion in humans (58, 74, 98–101). Methods based on digestibility and short-term protein retention are of interest when looking at the short-term utilization of dietary proteins, but few protein retention values are available in humans. Net postprandial protein utilization (NPPU) is calculated using true ileal digestibility and true [^{15}N]-labeled protein deamination parameters, and adding the dietary nitrogen collected in the urine and that retained in the body in the form of urea, as follows:

$$NPPU = [{}^{15}N_{absorbed} - ({}^{15}N_{ileal} + {}^{15}N_{body\ urea} + {}^{15}N_{urine})]/{}^{15}N_{ingested}$$

Using this approach, NPPU values of 75 and 71% were obtained for milk protein and soy protein, respectively, measured during the 8 h following ingestion of a standard meal by healthy human subjects. Furthermore, the relationship between protein characteristics and protein intake require additional study. This approach makes it possible to demonstrate differences in postprandial nitrogen retention according to the protein source, the protein fraction, and the nature of other nutrients in the meal (59, 101).

To improve our understanding of the acute phenomena following dietary nitrogen ingestion, and because of limited access to the compartments of interest in human experiments, a compartmental modeling approach can also be used. Compartmental modeling enables simulation of the distribution of exogenous nitrogen in the major body nitrogen pools (including those not experimentally monitored) based on experimental measurements. This tool also allows prediction of the future evolution of the system (23, 72, 73, 102, 103). This has been made possible by the development and validation of an 11-compartment model that makes a particular distinction between free and protein-bound amino acids in both the splanchnic and peripheral areas, in order to describe the cascade of transient metabolic processes that control the distribution of exogenous nitrogen throughout the body. The results obtained by modeling the pattern of dietary nitrogen distribution into the different body compartments after ingestion of a protein meal is a useful tool to better define protein quality in a period of protein gain, because it simulates the relative ability of a protein source to promote dietary nitrogen retention in different organs. It can also be used to discriminate between different nutritional conditions (type of protein ingested and energy content of the meal) and to

describe the processes involved in the differential metabolic utilization of various protein meals.

Chemical Integrity

Although digestibility is the major component of bioavailability, the chemical integrity of amino acids is also important. It has been known for some 50 years that, during heat processing of proteins, chemical reactions may result in some of the amino acids becoming at least partly nutritionally unavailable. This is especially true of lysine, of which the exposed ε-amino group is subject to reaction with carbohydrates in the food and formation of so-called early Maillard compounds. Although these conjugates may be released during digestion and absorbed, they have no nutritional value; they cannot be converted back to lysine but are excreted in urine. During the acid hydrolysis of conventional amino acid analysis, however, the Maillard reactions are largely reversed so that so-called "available lysine" is not distinguished from "unavailable" lysine. Although the term available lysine was originally used (104, 105) to describe just this aspect of bioavailability, it is perhaps less confusing to use the term "reactive lysine" (106). Methods of estimating the reactive lysine in foods use some detection reagent such as fluorodinitrobenzene (FDNB) that binds to the free ε-amino group of lysine, but not to those ε-amino groups that have undergone Maillard reactions. Heat-damaged proteins may be incompletely hydrolyzed in the upper digestive tract so that the ileal digestibility of amino acids may also be reduced. A complete assessment of bioavailability, particularly with foods that have been processed by heat and moisture, therefore involves estimating the digestibility of reactive lysine (106). Damage to food proteins can also be caused by chemical processing, especially by strong alkalis, that can result in the formation of cross-linked compounds, such as lysinoalanine. Damage by heat and chemical treatment is not limited to lysine, but may result in losses of bioavailability of other amino acids, especially methionine, cysteine, threonine, and tryptophan.

Freedom from Interference in Metabolism

In estimating the bioavailability of dietary proteins, it is rare that isolated proteins are evaluated. Most food protein sources, whether of animal or plant origin, contain not only a mixture of proteins but a host of other substances as well. The third aspect of bioavailability relates to the effects of those other substances that accompany food proteins on the estimate of bioavailability. Substances such as digestive enzyme inhibitors directly affect the bioavailability of the protein by their interference in the dynamics of digestion, but other food components, although not truly altering the bioavailability of the protein, may apparently do so by their influence on a bioassay such as the slope-ratio assay, described below, in which the animal's growth response may be affected by substances contained in the test food that are quite unconnected to the true bioavailability of amino acids. Such substances include alkaloids, goitrogens, hemagglutinins, and phytoestrogens.

Integrative Bioavailability Assays

A direct determination of ileal digestibility accounts for most of the variation in amino acid bioavailability and, for most food proteins, estimates of true ileal digestible amino acids provide the best practical basis for diet formulation. For proteins that have undergone heat or chemical processing, however, a bioassay may provide the best estimate of the overall amino acid bioavailability, integrating the effects of all 3 components. The preferred bioassay, usually conducted with model animals, is a slope-ratio assay in which the test amino acid source is compared with a pure standard. For example, to estimate the bioavailability of lysine in processed beans, a basal lysine-deficient diet is supplemented with graded quantities of either the beans or of pure lysine (that is assumed to be 100% available) in amounts equal to those provided by the beans. The responses to the supplements are measured as growth, nitrogen retention, or some other suitable criterion of lysine adequacy. The slopes of the 2 responses (which should both pass through the point of the basal diet) are compared, and the bioavailability of the lysine in the beans is estimated as the ratio of the two. It is important that the response to the beans should be attributable only to the bioavailable lysine they supply and not to the other nutrients that the beans supply. To ensure this, the increments of pure lysine must be accompanied by increments of carbohydrates, lipids, and other nutrients to simulate those provided by the beans. This includes the pattern of other amino acids. Such assays are, however, laborious, especially because a separate assay with a specially formulated diet is required for each amino acid, and several groups of animals are needed for each assay. For routine purposes, therefore, it is likely that assays of true ileal digestibility, coupled with in vitro determination of chemical integrity and of interfering dietary substances, will remain the procedure of choice.

References

(1) Longland, A.C. (1991) in *In vitro Digestion for Pigs and Poultry*, M.F. Fuller (Ed.), CABI, Wallingford, UK, pp 3–18

(2) Mariotti, F., Huneau, J.F., Mahé, S., & Tomé, D. (2000) *Curr. Opin. Clin. Nutr. Metab. Care* **3**, 45–50

(3) Fuller, M.F., & Reeds, P.J. (1998) *Annu. Rev. Nutr.* **18**, 385–411

(4) Moughan, P.J. (2003) *Nutr. Res. Rev*. **16**, 127–141

(5) Marchis-Mouren, G. (1965) *Soc. Chim. Biol. Bull*. **47**, 2207–2217

(6) Dal Borgo, G.A., Salman, J., Pubols, M.H., & McGinnis, J. (1968) *Proc. Soc. Exp. Biol. Med*. **129**, 877–881

(7) Zelikson, R., Eilam-Rubin, G., & Kulka, R.G. (1971) *J. Biol. Chem*. **246**, 6115–6120

(8) Jackson, A.A. (1995) *Proc. Nutr. Soc.* **54**, 535–547

(9) Mason, V.C., & Palmer, R. (1973) *Acta Agric. Scand.* **23**, 141–150

(10) Mason, V.C., Just, A., & Bech-Andersen, K.E. (1976) *Tierphysiol. Tierernähr. uttermittelk.* **36**, 310–324

(11) Zebrowska, T. (1973) *Rocz. Nauk Roln.* **B95**, 85–90
(12) Just, A., Jørgensen, H., & Fernandez, J.A. (1981) *Br. J. Nutr.* **46**, 209–219
(13) Darcy-Vrillon, B., Souffrant, W.B., Laplace, J.P., Rérat, A., Corring, T., Vaugelade, P., Gebhardt, G., & Köhler, R. (1991) *Reprod. Nutr. Dev.* **31**, 561–573
(14) Zebrowska, T., Buraczewska, L., Pastuszewska, B., Chamberlain, A.G., & Buraczewski, S., (1978) *Roczn. Nauk Roln*. **B99**, 75–83
(15) Sauer, W.C., Cichon, R., & Misir, R., (1982) *J. Anim. Sci*. **54**, 292–301
(16) Mosenthin, R., Sauer, W.C., & Ahrens, F. (1994) *J. Nutr.* **124**, 1222–1229
(17) Tanksley, Jr, T.D., & Knabe, D.A. (1982) *Feedstuffs* **54**, 16–21
(18) Sauer, W.C., Mosenthin, R., Ahrens, F., & den Hartog, L.A. (1991) *J. Anim. Sci*. **69**, 4070–4077
(19) Poppe, S., Meier, H., Bennke, H.J., & Stüwe, E. (1983) *Arch. Tierernähr.* **33**, 743–748
(20) Kreuzer, M., Roth, F.X., & Kirchgessner, M. (1989) *Landwirtsch. Forsch*. **42**, 72–92
(21) Payne, W.L., Combs, G.F., Kifer, R.R., & Snyder, D.G. (1968) *Fed. Proc.* **27**, 1199–1203
(22) Fuller, M.F., Milne, A., Harris, C.I., Reid, T.M.S., & Keenan, R. (1994) *Am. J. Clin. Nutr.* **59**, 70–73
(23) Fouillet, H., Bos, C., Gaudichon, C., & Tomé, D. (2002) *J. Nutr*. **132**, 3208S–3218S
(24) Moughan, P.J., Smith, W.C., & James, K.A.C. (1984) *N. Z. J. Agric. Res*. **27**, 509–512
(25) Moughan, P.J., Smith, W.C., & Kies, A.K. (1987) *N. Z. J. Agri. Res*. **30**, 59–66
(26) Picard, M., Bertrand, S., Duron, M., & Maillard, R. (1984) *Proceedings of the 4th European Symposium of Poultry Nutrition,* Tours, France, World's Poultry Science Association, Tours, France, pp 165–168
(27) Donkoh, A., Moughan, P.J., & Smith,W.C. (1994) *Anim. Feed Sci. Technol*. **49**, 57–71
(28) Rutherfurd, S.M., & Moughan, P.J. (2003) *J. Anim. Physiol. Anim. Nutr*. **87**, 292–300
(29) FAO/WHO (1990) *Report of a Joint FAO/WHO Expert Consultation on Protein Quality Evaluation*, Food and Agriculture Organization, Rome, Italy
(30) Pearson, G., Moughan, P.J., Dong, G.Z., & Morel, P.C.H. (1999) *Anim. Feed Sci. Technol*. **79**, 301–307
(31) Batterham, E.S. (1983) in *Proceedings of the 2nd Symposium of the International Network of Feed Information Centres*, G.E. Robards & R.G. Packham (Eds), Farnham Royal, Commonwealth Agricultural Bureaux, Slough, UK, pp 219–294
(32) Huisman, J., van der Poel, A.F.B., & Beynen, A.C. (1991) in *Proceedings of the Vth International Symposium on Digestive Physiology in Pigs*, M.W.A. Vestegen, J. Huisman, & L.A. den Hartog (Eds), Pudoc, Wageningen, The Netherlands, pp 108–113
(33) Moughan, P.J., Souffrant, W.B., & Hodgkinson, S.M. (1998) *Arch. Anim. Nutr*. **51**, 237–252
(34) Gdala, J., Buraczewska, L., Jansman, A.M.J., Wasilewko, J., & van Leeuwen, P. (1994) in *Proceedings of 6th International Symposium on Digestive Physiology in Pigs (EAAP Publication No. 80)*, W.B. Souffrant & H. Hagemeister (Eds), Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Dummerstorf, Germany, pp 93–96
(35) Souffrant, W.B., Rérat, A., Laplace, J.-P., Darcy-Vrillon, B., Kohler, R., Corring, T., & Gebhardt, G. (1993) *Reprod. Nutr. Develop.* **33**, 373–382
(36) Jansman, A.J.M., Smink, W., van Leeuwen, P., & Rademacher, M. (2002) *Anim. Feed Sci. Technol*. **98**, 49–60
(37) Moughan, P.J., Darragh, A.J., Smith, W.C., & Butts, C.A. (1990) *J. Sci. Food Agri*. **52**, 13–21
(38) Butts, C.A., Moughan, P.J., & Smith, W.C. (1991) *J. Sci. Food Agric.* **55**, 175–187
(39) Hess, V., & Sève, B. (1999) *J. Anim. Sci*. **77**, 3281–3288
(40) Donkoh, A., Moughan, P.J., & Morel, P.C.H. (1995) *J. Sci. Food Agric*. **67**, 359–366
(41) Stein, H.H., Trottier, N.L., Bellaver, C., & Easter, R.A. (1999) *J. Anim. Sci*. **77**, 1180–1187
(42) Baglieri, A., Mahé, S., Benamouzig, R., Savoie, L., & Tomé, D. (1995) *J. Nutr.* **125**, 1894–1903
(43) Jondreville, C., van den Broecke, J., Gatel, F., Grosjean, F., Van Cauwenberghe, S., & Sève, B. (2001) *Anim. Res*. **50**, 119–134
(44) Fauconneau, G., & Michel, M.C. (1970) in *Mammalian Protein Metabolism*, Vol. 4, H.N. Munro (Ed.), Academic Press Inc., London, UK, pp 481–522
(45) Schneeman, B.O. (1982) in *Physiologie Digestive Chez le Porc*, J.P. Laplace, T. Corring, & A. Rérat (Eds), Institut National de la Recherche Agronomique, Paris, France, pp 125–131
(46) Millward, D.J., Garlick, P.J., James, W.P.T., Sender, P.M., & Waterlow, J.C. (1976) in *Protein Metabolism and Nutrition*, D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale, & H. Swan (Eds), Butterworth's, London, UK, pp 49–69
(47) De Lange C.F.M., Sauer, W.C., & Souffrant, W.B. (1989) *J. Anim. Sci.* **67**, 755–762
(48) Moughan, P.J., & Schuttert, G. (1991) *J. Nutr*. **121**, 1570–1574
(49) Butts, C.A., Moughan, P.J., Smith, W.C., & Carr, D.H. (1993) *J. Sci. Food. Agric.* **61**, 31–40
(50) Leterme, P., Thewis, A., Francois, E., van Leeuwen, P., Wathelet, B., & Huisman, J. (1996) *J. Nutr*. **126**, 2188–2198
(51) Gaudichon, C., Mahé, S., Laurent, C., Meaugeais, P., & Krempf, M. (1996) *Eur. J. Clin. Nutr.* **50**, 261–268
(52) Leterme, P., & Sève, B. (1998) *J. Nutr*. **128**, 2526–2527
(53) Hess, V., Thibault, J.N., & Sève, B. (1998) *J. Nutr*. **128**, 1969–1977
(54) Hess, V., Ganier, P., Thibault, J.N., & Sève, B. (2000*) Br. J. Nutr*. **83**, 123–130
(55) Lien, K.A., Sauer, W.C., Mosenthin, R., Souffrant, W.B., & Dugan, M.E. (1997) *J. Anim. Sci.* **75**, 148–158
(56) Lien, K.A., Sauer, W.C., & Dugan, M.E. (1997) *J. Anim. Sci*. **75**, 159–169
(57) Leterme, P., Sève, B., & Thewis, A. (1998) *J. Nutr*. **128**, 1961–1968
(58) Tomé, D., & Bos, C. (2000) *J. Nutr*. **130**, 1868S–1873S
(59) Gaudichon, C., Mahé, S., Benamouzig, R., Luengo, C., Fouillet, H., Dare, S., Van Oycke, M., Ferriere, F., Rautureau, J., & Tomé, D. (1999) *J. Nutr*. **129**, 890–895

(60) Evenepoel, P., Geypens, B., Luypaerts, A., Hiele, M., Ghoos, Y., & Rutgeerts, P. (1998) *J. Nutr*. **128**, 1716–1722
(61) Leterme, P., Thewis, A., Francois, E., van Leeuwen, P., Wathelet, B., & Huisman, J. (1996) *J. Nutr*. **126**, 2188–2198
(62) Mariotti, F., Pueyo, M.E., Tomé, D., Bérot, S., Benamouzig, R., & Mahé, S. (2001) *J. Nutr*. **131**, 1706–1713
(63) Mosenthin, R., Sauer, W.C., Blank, R., Huisman, J., & Fan, M.Z. (2000) *Livest. Prod. Sci*. **64**, 265–280
(64) Williams, P.E.V. (1995) *Anim. Feed Sci. Technol*. **53**, 173–187
(65) Austic, R.E. (1983) in *Proceedings of the 2nd Symposium of the International Network of Feed Information Centres*, G.E. Robards & R.G. Packham (Eds), Commonwealth Agricultural Bureau, Slough, UK, pp 175–189
(66) Low, A.G. (1980) *J. Sci. Food Agric*. **31**, 1087–1130
(67) Rérat, A. (1981) *World Rev. Nutr. Diet*. **37**, 229–287
(68) Krawielitzki, K., Kreienbring, F., Zebrowska, T., Schadereit, R., & Kowalczyk, J. (1994) in *Proceedings of the 6th International Symposium on Digestive Physiology in Pigs*, W.B. Souffrant & H. Hagemeister (Eds), Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Dummerstorf, Germany, pp 79–82
(69) Lien, K.A., McBurney, M.I., Beyde, B.I., Thomson, A.B., & Sauer, W.C. (1996) *Am. J. Clin. Nutr*. **63**, 584–595
(70) Weber, E., & Ehrlein, H.J. (1998) *Dig. Dis. Sci*. **43**, 1141–1153
(71) Zhao, X.T., McCamish, M.A., Miller, R.H., Wang, L., & Lin, H.C. (1997) *J. Nutr*. **127**, 2350–2356
(72) Fouillet, H., Gaudichon, C., Mariotti, F., Bos, C., Huneau, J.F., & Tomé, D. (2001) *Am. J. Physiol. Endocrinol. Metab*. **281**, E248–E260
(73) Fouillet, H., Mariotti, F., Gaudichon, C., Bos, C., & Tomé, D. (2002) *J. Nutr*. **132**, 125–133
(74) Gausserès, N., Mahé, S., Benamouzig, R., Luengo, C., Drouet, H., Rautureau, J., & Tomé, D. (1996) *Br. J. Nutr*. **76**, 75–85
(75) Mariotti, F., Mahé, S., Luengo, C., Benamouzig, R., & Tomé, D. (2000b) *Am. J. Clin. Nutr*. **72**, 954–962
(76) Mariotti, F., Pueyo, M.E., Tomé, D., & Mahé, S. (2002) *Br. J. Nutr*. **87**, 315–323
(77) Gaudichon, C., Bos, C., Morens, C., Petzke, K., Mariotti, F., Everwand, J., Benamouzig, R., Daré, S., Tomé, D., & Metges, C.C. (2002) *Gastroenterology* **123**, 50–59
(78) Darcy-Vrillon, B., & Laplace, J.-P. (1984) *Ann. Zootech*. **33**, 43–64
(79) Rowan, A.M., Moughan, P.J., & Wilson, M.N. (1993) *J. Sci. Food Agric*. **61**, 439–442
(80) Hendriks, W.H., Sritharan, K., & Hodgkinson, S.M. (2002) *J. Anim. Physiol. Anim. Nutr*. **86**, 333–341
(81) Darragh, A.J., & Hodgkinson, S.M. (2000) *J. Nutr*. **130**, 1850S–1856S
(82) Jensen, B.B., & Jørgensen, H. (1995) *Appl. Environ. Microbiol.* **60**, 1897–1904
(83) Jensen, B.B. (1988) *Proceedings of the 4th Symposium on Digestive Physiology in the Pig*, L. Buraczewska, S. Buraczewski, B. Pastuzewska, & T. Zebrowska (Eds), Jablonna, Poland, pp 392–400
(84) Millard, P., & Chesson, A. (1984) *Br. J. Nutr.* **52**, 583–594
(85) Zhu, J.Q., Fowler, V.R., & Fuller, M.F. (1990) *Anim. Prod.* **50**, 531–539
(86) Gdala, J., & Buraczewska, L. (1997) in *Digestive Physiology in Pigs (EAAP Publication No. 88)*, J.P. Laplace, C. Fevrier, & A. Barbeau (Eds), INRA, Paris, France, pp 621–624
(87) Bach-Knudsen, K.E., & Canibe, N. (1997) in *Digestive Physiology in Pigs (EAAP Publication No. 88)*, J.P. Laplace, C. Fevrier, & A. Barbeau (Eds), INRA, Paris, France, pp 562–566
(88) Hennig, U., Schönhusen, U., & Souffrant, W.B. (1998) *Schriftenreihe-Forschungsinstitut fuer die Biologie landwirtschaftlicher Nutztiere* **11**, 121–122
(89) Fuller, M.F. (2003) in *9th International Symposium on Digestive Physiology in Pigs*, R.O. Ball (Ed.), University of Alberta, Edmonton, Canada, pp 183–198
(90) Torrallardona, D., Harris, C.I., Coates, M.E., & Fuller, M.F. (1996) *Br. J. Nutr.* **76**, 689–700
(91) Torrallardona, D., Harris, C.I., & Fuller, M.F. (1996) *Br. J. Nutr.* **76**, 701–709
(92) Torrallardona, D., Harris, C.I., & Fuller, M.F. (2003) *J. Nutr.* **133**, 1127–1131
(93) Torrallardona, D., Harris, C.I., & Fuller, M.F.(2003) *Am. J. Physiol.* **284**, E1177–E1180
(94) Torrallardona, D., Harris, C.I., Milne, E., & Fuller, M.F. (1994) in *Proceedings of 6th International Symposium on Digestive Physiology in Pigs (EAAP Publication No. 80)*, W.B. Souffrant & H. Hagemeister (Eds), Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Dummerstorf, Germany, pp 245–248
(95) Backes, G., Hennig, U., Petzke, K.J., Elsner, A., Junghans, P., Nürnberg, G., & Metges, C.C. (2002) *Am. J. Clin. Nutr*. **76**, 1317–1325
(96) Metges, C.C., El-Khoury, A.E., Henneman, L., Petzke, K.J., Grant, I., Bedri, S., Pereira, P.P., Ajami, A.M., Fuller, M.F., & Young, V.R. (1999a) *Am. J. Physiol*. **277**, E597–E607
(97) Metges, C.C., Petzke, K.J., El-Khoury, A.E., Henneman, L., Grant, I., Bedri, S., Regan, M.M., Fuller, M.F., & Young, V.R. (1999) *Am. J. Clin. Nutr*. **70**, 1046–1058
(98) Mahé, S., Huneau, J.F., Marteau, P., Thuillier, F., & Tomé, D. (1992) *Am. J. Clin. Nutr*. **56**, 410–416
(99) Gausserès, N., Mahé, S., Benamouzig, R., Luengo, C., Ferriere, F., Rautureau, J., & Tomé, D. (1997) *J. Nutr*. **127**, 1160–1165
(100) Bos, C., Mahé, S., Gaudichon, C., Benamouzig, R., Gausserès, N., Luengo, C., Ferriere, F., Rautureau, J., & Tomé, D. (1999) *Br. J. Nutr*. **81**, 221–226
(101) Mariotti, F., Mahé, S., Benamouzig, R., Luengo, C., Dare, S., Gaudichon, C., & Tomé, D. (1999) *J. Nutr*. **129**, 1992–1997
(102) Fouillet, H., Mariotti, F., Gaudichon, C., Bos, C., & Tomé, D. (2002) *J. Nutr*. **132**, 125–133
(103) Fouillet, H., Gaudichon, C., Bos, C., Mariotti, F., & Tomé, D. (2003) *Am. J. Physiol. Endocrinol. Metab*. **285**, E88–E97
(104) Carpenter, K.J., & Ellinger, G.M. (1955) *Biochem. J.* **61**, xi
(105) Carpenter, K.J. (1960) *Biochem. J.* **77**, 604–610
(106) Rutherfurd, S.M., Moughan, P.J., & Morel, P.C.H. (1997) *J. Agric. Food Chem.* **45**, 4378–4383