What is the True Supply of Amino Acids for a Dairy Cow?¹

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ABSTRACT

Improving the prediction of milk protein yield relies on knowledge of both protein supply and requirement. Definition of protein/amino acid supply in ruminants is a challenging task, due to feedstuff variety and variability and to the remodeling of nutrient intake by the rumen microflora. The questions arise, therefore, how and where should we measure the real supply of AA in the dairy cow? This review will follow the downstream flow of AA from duodenum to peripheral tissue delivery, with a glance at the efficiency of transfer into milk protein. Duodenal AA flow comprises rumen undegradable feed, microbial protein, and endogenous secretions. Most attention has been directed toward definition of the first two contributions but the latter fraction can represent as much as 20% of duodenal flow. More information is needed on what factors affect its magnitude and overall impact. Once digested, AA are absorbed into the portal vein. The ratio of portal absorption to small intestinal apparent digestion varies among essential AA, from 0.43 (threonine) to 0.76 (phenylalanine), due to the contributions of preduodenal endogenous secretions to the digestive flow, non-reabsorption of endogenous secretions and gut oxidation of AA. Few data are available on these phenomena in dairy cows but the evidence indicates that they alter the profile of AA available for anabolic purposes. Recent comparisons of estimated duodenal flux and measured portal flux have prompted a revisit of the NRC (2001) approach to estimate AA flows at the duodenum. Changes to the model are proposed that yield predictions that better fit the current knowledge of AA metabolism across the gut. After absorption, AA flow first to the liver where substantial and differential net removal occurs, varying

from zero for the branched-chain AA to 50% of portal absorption for phenylalanine. This process alters the pattern of net supply to the mammary gland. Overall, intermediary metabolism of AA between the duodenum and the mammary gland biologically explains the decreased efficiency of the transfer of absorbed AA into milk protein as maximal yield is approached. Therefore, variable, rather than fixed, factors for transfer efficiencies must be incorporated into future predictive models. **Key words:** dairy cow, amino acid, gut, portal absorption

INTRODUCTION

To optimize milk production, requirements must be determined and matched with dietary supply. Such information requires studies in which the supply of both energy and protein to the cow is accurately known. But where and how do we measure this supply? This review will focus on the supply of protein and more specifically, the base units, the individual AA. In nonruminant nutrition, it is well recognized that supply and requirements must be expressed for individual AA rather than their aggregate (total protein). The biological consequence of such an approach has positive economic impacts and single AA are routinely added to commercial diets to improve animal performance and farm income in swine and poultry production.

In nonruminants, intake represents supply and any deficiency can be corrected with the simple addition of any individual AA directly to the diet. Ruminants are different: their capacity to use forages that are indigestible for the nonruminant relies on the presence of microorganisms in the rumen, reticulum, and omasum to digest such feedstuffs. Unfortunately, this ability incurs a toll. First, during passage through the reticulorumen, dietary ingredients are partially digested and reused for microbial growth. Therefore, nutrients delivered for absorption differ from those present in the diet making prediction of nutrient supply to the animal difficult. Second, because free AA are rapidly degraded in the rumen environment (Velle et al., 1997; Volden

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et al., 1998), simple addition of an AA to the diet is not an efficient option to increase AA flow at the duodenum. Instead, the AA must either be coated with a form of protection against the microorganisms (but still allow digestibility in the small intestine) or be presented in chemical forms that are more resistant to ruminal degradation or more readily absorbed through the rumen wall (e.g., hydroxy analogues).

Over the last few years, there has been renewed interest in strategies to improve the efficiency of N use in dairy cows without reducing productivity. Increased efficiency of N use is best achieved by reducing the total amount of CP fed to the animal. Such a reduction, however, must be made cautiously but can be accomplished if appropriate supplies of the critical AA are provided. This raises a number of crucial questions. How do we measure the supply of individual AA? What is the true supply of AA? In ruminants, it is clear that AA intake is a meaningless way to express the real supply of AA to the animal. Should we then look at duodenal supply or perhaps small intestinal disappearance? Is either really net supply? Would it be better to measure AA appearing in the portal circulation that drains the whole gut? Answering these challenging questions requires knowledge of AA metabolism between intake and absorption. Recognizing and understanding the complex interactions of gut AA metabolism will enable the development of better predictive models to formulate diets that contain less total CP but are better balanced to meet AA requirements. Such an approach will improve the efficiency of conversion of diet N into milk protein.

This review will focus on specific aspects of present knowledge and suggest improvements to current prediction models taking into account gut metabolism of AA. For simplicity and to reflect the paucity of data on nonessential AA flows in dairy cows, this review will focus on essential AA (EAA), even though data for these are also scarce. Nonetheless, future models will also need to consider metabolism of nonessential AA as these are extensively used by gut tissues to maintain integrity and functionality.

DUODENAL FLOW

A first measurement of protein supply to the ruminant is protein flow at the entrance of the small intestine. This measurement is performed through placement of a cannula at the proximal duodenum, with the challenge to obtain representative samples coupled with a good estimate of DM flow (Harmon and Richards, 1997). Duodenal flow of CP encompasses 3 major fractions: RUP, microbial crude protein (MCP), and endogenous protein. The contribution of each fraction to the

total flow is directly related to the diet composition and DM intake and varies widely, with the MCP fraction usually supplying the majority of the proteins (Clark et al., 1992). The origin of these proteins will directly affect estimation of the net supply from the diet. Although AA absorbed from RUP plus most of that derived from MCP may be considered as a new supply of AA to the animal, the endogenous fraction mainly constitutes a recycling of previously absorbed AA used to build body proteins that are returned into the lumen of the gut prior to the duodenum. Endogenous proteins originate from various sources, including mucoproteins, saliva, sloughed epithelial cells, and enzyme secretions into the abomasum (Tamminga et al., 1995).

Therefore, determination of the true net supply of protein (and of AA) flowing to the duodenum requires that the contribution from preduodenum endogenous proteins be subtracted from measured total duodenal flow. In practice, however, a number of simplifications are usually adopted. Rumen microbial protein flow, due to its potentially large contribution and associated variation, has received much attention and different methods have been developed for measurement (see review in Broderick and Merchen, 1992). Consequently, in most studies where protein flow at the duodenum is measured, MCP is determined and RUP is calculated as the difference between total CP and MCP flows, with any contribution from endogenous protein ignored. Indeed, although it is critical to determine what fraction of duodenal flow represents recycled material, and thus accurately determine the real net supply, the endogenous fraction has received little attention in ruminants. This has not only been because of a lack of recognition of the relative importance of the endogenous contribution but also because of the technical challenges associated with its measurement. Even when allowance is made for endogenous inputs, these are often based on data from studies conducted in rather artificial conditions having little relevance to the feeding regimen of high-yielding dairy cows. Thus, NRC (2001) adopted an average value of 1.9 g of N/kg of DMI for the endogenous contribution to duodenal flow based on data obtained in studies using animals receiving either only intragastric infusions of volatile fatty acids (Ørskov et al., 1986) or fed diets with very low protein supply and degradability (Hart and Leibholz, 1990; Hannah et al., 1991; Lintzenich et al., 1995). Similarly, the French system uses a value equivalent to 1.7 g of N/kg of DMI (Vérité and Peyraud, 1989). More appropriate estimates of endogenous protein flows, pertinent to "real" situations, require use of tracer techniques but, even with these, caution is needed. Endogenous secretions estimated from ^{15}N -urea infusion (Brandt et al., 1984; Leng and Nolan, 1984) include use of urea for microbial protein

Table 1. Amino acid composition (% of essential AA) of milk, microbial protein (MCP), 2 feed ingredients, and endogenous protein at the duodenum and from small intestine

Amino acid	Milk ¹	MCP ²	Soybean meal ¹	Corn gluten meal ¹	Abomasal isolate ³	Ileal endogenous protein ⁴
His	5.9	4.4	7.3	5.1	8.8	5.9
Ile	12.3	12.8	12.1	9.8	11.2	11.8
Leu	21.0	18.1	20.5	40.0	11.7	15.4
Lys	17.2	17.6	16.6	4.0	17.8	12.6
Met	5.9	5.8	3.8	5.6	3.7	3.6
Phe	10.8	11.3	13.8	15.2	11.2	10.7
Thr	9.6	13.0	10.4	8.1	15.9	18.9
Trp	3.2	3.0	3.3	1.3	4.6	4.5
Val	14.0	13.9	12.2	11.1	14.9	16.7

¹NRC, 2001.²Clark et al., 1992.³Ørskov et al., 1986, except Trp (Stein et al., 1999: from endogenous protein composition in lactating sows).⁴Jansman et al., 2002.

synthesis. Although this can be considered “endogenous,” the impact on AA availability will differ from endogenous protein arising from previously absorbed AA. In this review, only endogenous proteins that arise from direct use of AA will be considered and discussed.

Recently, Ouellet et al. (2002) used an 8-d infusion of ¹⁵N-leucine to develop a model in dairy cows to estimate the endogenous contribution to the duodenum, including free endogenous secretions and endogenous proteins incorporated into the MCP flow. This latter fraction averaged 2.1 g of N/kg of DMI, approximately equal to the free endogenous flow of 2.3 g of N/kg of DMI. Together (4.4 g of N/kg of DMI), these 2 fractions represented 15% of the total duodenal N flow, irrespective of the fiber content of the diet. In sheep, using a continuous abomasal infusion of ¹⁵N-labeled, grass meal-beer yeast suspension, the endogenous contribution averaged 12% of duodenal N flow (Van Bruchem et al., 1997). Another study in sheep, based on ¹⁵N-labeled digesta exchange between animals, reported endogenous N contributing between 3 and 12% of total N duodenal flow for diets ranging from 15 to 25% crude fiber content, respectively (Sandek et al., 2001). Furthermore, in a second study in lactating cows using an infusion of ¹⁵N-leucine (Ouellet et al., 2005), the endogenous contribution to duodenal N flow averaged 18% when silage was fed, and 20% when hay was offered, representing in this latter case, 5.9 g of N/kg of DMI. In dairy cows, using a mathematical approach including estimates of the composition of AA in each fraction, N from endogenous origin varied between <1% (Shabi et al., 2000) and 32% (Larsen et al., 2001) of total duodenal N flow.

With such a high potential contribution, the endogenous fraction can no longer be ignored and needs to be subtracted from measured duodenal flow to determine

the true net supply at the duodenum. However, with such a large imprecision in these evaluations, more research is required to determine what factors affect endogenous duodenal flows to obtain better estimation of the contribution in different feeding situations. In addition, these endogenous secretions will not only have an impact on the evaluation of the total duodenal N flow, but will also alter the pattern of the net supply of individual AA, due to the differences in AA composition between endogenous proteins, MCP, and RUP (Table 1). Although such differences are acknowledged, more information is needed on the AA profile of endogenous secretions at the duodenum. The best estimation available relies on just one study in which AA composition of abomasal isolates was measured in animals receiving only intragastric infusions of volatile fatty acids (Ørskov et al., 1986).

To follow how assessment of gut metabolism has an impact on the interpretation of measurements of AA availability, examination will be made of the biological meaning of flows observed in one study, where duodenal, ileal, fecal, and portal flows of EAA were measured in dairy cows (Berthiaume et al., 2001) with estimations for endogenous inputs based on data from dairy cows offered similar rations (Ouellet et al., 2002, 2005). Interpretation of the net flow of CP and individual AA are presented in Tables 2 and 3, respectively. In the text, we will follow the fate of one AA, lysine, because it is considered one of the limiting AA in typical North American diets. From measurements reported by Berthiaume et al. (2001), duodenal flow of lysine averaged 144 g/d, with 30 g estimated to be of endogenous origin. This means that the net supply of lysine arriving at the duodenum was, in fact, 114 g/d. So, right at the start, acknowledgment of the contribution of endogenous secretions changes the picture of what is supplied

Table 2. Flow of CP at different sites of measurement in dairy cows¹

Site	Flow, g/d
Intake	2,259
Duodenal	2,585
Endogenous duodenal ²	414
Net duodenal	2,172
Ileal	747
Apparent ileal digestible ³	1,838
Endogenous ileal ⁴	209
From undigested endogenous duodenal	104.5
From nonreabsorbed endogenous small intestine	104.5
True ileal digestible ⁵	1,943
Ileal digestible from net supply ⁶	1,634
Available, discounting the endogenous loss ⁷	1,425

¹Adapted from Berthiaume et al., 2001.

²Assuming 4.3 g of N per kg of DMI (from Ouellet et al., 2002).

³Duodenal – ileal.

⁴Assuming 28% of ileal CP flow, with half of it from undigested preduodenal secretions and the other half from non-reabsorbed endogenous secretions into the small intestine (from D. R. Ouellet, R. Berthiaume, G. Holtrop, G. E. Lobley, and H. Lapierre; Agriculture and Agri-Food Canada and Rowett Research Institute, UK; unpublished data).

⁵Apparent digestible + endogenous ileal from small intestine.

⁶True digestible – (endogenous duodenal – endogenous ileal from undigested preduodenal endogenous secretions).

⁷True ileal digestible from net supply – endogenous ileal.

to the cow! Furthermore, because the AA profile of the different duodenal fractions is not homogeneous, not only does the true duodenum flow decrease when the endogenous contribution is removed, but the proportion

of each AA to total EAA may increase (e.g., leucine) or decrease (e.g., threonine).

Synopsis

Recent data in dairy cows suggest that endogenous contribution would represent between 15 and 20% of duodenal N flow and therefore can no longer be ignored. This endogenous contribution does not represent a net supply to the animal and as such, needs to be removed from the duodenal total protein flow to determine duodenal net supply from MCP and RUP.

DISAPPEARANCE IN THE SMALL INTESTINE

Even when estimated as the true net supply, duodenal flow does not represent what is available to the animal. The AA truly available to the animal are those digested in the small intestine. Apparent small intestinal disappearance (**SID**) is estimated by difference between duodenal and ileal flows. This should reflect the amount of AA disappearing from the digestive tract with the potential to be available to the animal, as absorption of AA occurs from the small intestine. Due to the difficulty of maintaining an ileal cannula (Harmon and Richards, 1997), there is limited information on ileal digestibility (e.g., Stern et al., 1985; Benchaar et al., 1994). Instead, digestibility has often been determined as the difference between duodenal and fecal flows. However, both dietary and endogenous proteins

Table 3. Flow of essential amino acids (g/d) at different sites of measurement in dairy cows¹

Site	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Duodenal	53	120	189	144	55	111	109	133
Endogenous duodenal ²	15	19	20	30	6	19	27	25
Net duodenal	39	101	169	114	49	92	82	108
Ileal	24	38	61	37	19	42	44	55
Apparent ileal digestible ³	30	82	128	107	36	70	65	78
Endogenous ileal ⁴	7	11	14	16	4	15	18	15
from undigested duodenum	5	6	6	9	2	6	8	8
from small intestine nonreabsorbed	3	5	8	7	3	9	10	8
True ileal digestible ⁵	32	87	136	114	39	79	75	85
True ileal digestible from net supply ⁶	22	73	122	93	35	65	56	68
Available, accounting for endogenous loss ⁷	15	63	108	77	30	51	38	53
Portal absorption	22	51	80	60	24	53	28	40
Milk	15	33	54	45	15	27	26	37

¹Adapted from Berthiaume et al., 2001.

²Assuming 4.3 g of N per kg of DMI (Ouellet et al., 2002), with the AA composition from abomasal isolate (Ørskov et al., 1986).

³Duodenal – ileal.

⁴Assuming 28% of ileal CP flow, with half of it from undigested preduodenal secretions and the other half from nonreabsorbed endogenous secretions into the small intestine (from Ouellet et al., 2005) with the AA composition from Jansman et al. (2002) for the intestinal endogenous proteins.

⁵Apparent digestible + endogenous ileal from small intestine.

⁶True digestible – (endogenous duodenal – endogenous ileal from undigested preduodenal endogenous secretions).

⁷True ileal digestible from net supply – endogenous ileal.

are metabolized by the hindgut microbial population (Darragh and Hodgkinson, 2000) and loss of AA through the large intestine is greater than the net gain of newly synthesized AA originating from microbial fermentation. Therefore, the apparent coefficient of digestibility of AA through the whole intestine (duodenum – feces) is higher than that in the small intestine (duodenum – ileum; Berthiaume et al., 2001), leading to an overestimation of the availability of the AA to the animal when digestibility across the whole intestine is used.

Although ileal digestibility might appear a more reliable measurement of AA availability, a closer examination reveals that several correction factors need to be applied to determine the true net supply to the animal. A proportion of the ileal digesta are of endogenous origin and this is not a negligible amount. For example, in dairy cows infused with ^{15}N -leucine, Ouellet and colleagues (D. R. Ouellet, R. Berthiaume, G. Holtrop, G. E. Lobley, and H. Lapierre; Agriculture and Agri-Food Canada and Rowett Research Institute, UK; unpublished data) observed that endogenous N losses at the ileum represented 28% of total ileal N flow. In sheep, the endogenous contribution to the ileum N flow comprised 32% (Sandek et al., 2001) and 48% of total ileal N flow (Van Bruchem et al., 1997). Therefore, estimation of true digestibility of protein and AA available to the animal varies greatly depending on whether, and how, these endogenous secretions are accounted for.

The presence of endogenous protein in ileal digesta is recognized in pig nutrition. Rather than apparent digestibility, standardized ileal digestibility, which accounts for endogenous secretion (Jansman et al., 2002), is successfully used to balance pig diets, because the relative endogenous contribution to total flow differs with the protein supply. However, in pigs, the relevant endogenous proteins are secreted between the input (intake) and the output (ileal cannula) of the digestibility measurement; that is, there is no endogenous contribution to the input. In contrast, for dairy cows, we have already seen that the input (i.e., duodenal flow) already contains endogenous secretions. As a result, part of the endogenous loss at the ileal cannula will originate from secretions already present at the input (duodenal cannula) that were not digested, as well as secretions that occurred between the input and the final measurement (i.e., of small intestinal origin). This means that in dairy cows, the ileal endogenous protein present at the ileum needs to be partitioned into that derived from undigested preduodenal endogenous secretions and that which originates from endogenous proteins secreted into the small intestine but not reabsorbed. A recent study in dairy cows indicated that both fractions were approximately equivalent (D. R. Ouellet, R. Berthiaume, G. Holtrop, G. E. Lobley, and H. Lapierre; Agri-

culture and Agri-Food Canada and Rowett Research Institute, UK; unpublished data). Therefore, in addition to the definition of the true digestibility calculated to account for secretions from the small intestine, as adopted in pigs, this introduces a consideration unique to the ruminants: the amount of AA truly digested in the small intestine does not represent the net supply to the animal. Digestion and absorption of the endogenous secretions present at the entrance of the duodenum will not provide a new supply to the animal. Instead, their absorption into blood circulation simply replaces AA previously extracted from arterial blood supply to support their synthesis. To determine the amount of AA absorbed from digested preduodenal endogenous secretions, both the endogenous contribution to the duodenal flow (see above) and the digestibility of this fraction need to be determined. In sheep, where labeled digesta were exchanged between animals, the digestibility of these secretions averaged 60% (Sandek et al., 2001), which is close to the estimation of 68% in dairy cows (Ouellet et al., 2002).

To better integrate these concepts, we will continue to follow the fate of lysine across the gut, based on the study of Berthiaume et al. (2001; Table 3). The difference between duodenal (144 g/d) and ileal (37 g/d) flow yields an apparent SID of 107 g/d. Does this number represent the amount of lysine that really disappears from the small intestine? If we want to know the total amount of lysine that was truly digested in the small intestine (equivalent to true ileal digestibility in the pig), the endogenous contribution arising from intestinal non-reabsorbed endogenous secretion must be removed from the ileal flow; this is 7 g/d in our example. Therefore, the total amount of lysine digested increases to 114 g/d ($107 + 7$). Although this true ileal digestibility is used in pigs to determine AA availability, this concept cannot be directly applied in dairy cows. As previously discussed, the true ileal digestible supply includes a portion of endogenous proteins that was already present at the duodenum and digested in the small intestine; this fraction does not constitute a net supply to the animal. We have already determined that endogenous contribution to the duodenal N flow is 30 g/d of lysine. From Ouellet and colleagues (D. R. Ouellet, R. Berthiaume, G. Holtrop, G. E. Lobley, and H. Lapierre; Agriculture and Agri-Food Canada and Rowett Research Institute, UK; unpublished data), it was determined that 9 g of these secretions was still present at the ileum and therefore not digested. This means that 21 ($30 - 9$) g/d of the lysine that was digested was not a true net supply, but rather represents a recycling of endogenous proteins. Therefore, the true amount of lysine digested from net supply is now 93 ($114 - 21$) g/d. At this point, bear in mind the 16 g of lysine from

endogenous origin flowing at the ileum; this will be discussed in the next section.

It must also be remembered that endogenous secretions have an impact not only on the “total” AA but also on the profile of AA available for the cow. Analogous to the situation already discussed for the duodenal AA profile, the proportion of an individual AA relative to total EAA can be markedly altered when corrections for endogenous inputs are applied. In our example, this results in a relative increase for leucine while threonine decreased.

Synopsis

The small intestinal disappearance, either apparent or “true” (i.e., corrected for small intestinal endogenous contribution at the ileum) is not yet “the” measurement that will give the real net amount of AA supplied from MCP and RUP. The endogenous contribution to the duodenal flow digested in the small intestine needs to be removed from the “true” amount digested to indicate the correct net supply to the animal

PORTAL ABSORPTION

Given the complexity of measuring the correct net supply of the dairy cow, it might be argued that the availability of AA to the animal would be better measured simply as portal absorption (i.e., net appearance in the portal vein, the vein draining the whole gut). At first glance, this looks attractive as a definitive measurement of availability to the animal. However, it must be recognized that measurement of net portal absorption of AA reflects the net balance across the portal-drained viscera: the AA used to support the needs of the gut tissues will result in a lower measurement of net portal absorption compared with the net input from digestion across the small intestine.

One option to quantify how much AA are lost across the gut is to compare apparent SID with portal absorption. The first attempt to do this in sheep reported large variations in the ratio of portal absorption on SID between the various AA, from 19% for histidine to 69% for lysine, at a high protein intake (Tagari and Bergman, 1978). These findings suggest a substantial loss of AA across the gut. More recent data obtained in sheep and dairy cows report ratios of portal absorption to SID smaller than 1, but the “losses” for essential AA were of smaller magnitude, with an average recovery of apparent SID as portal absorption varying between 43% for threonine and 76% for phenylalanine (Table 4; MacRae et al., 1997; Berthiaume et al., 2001). But does this ratio really indicate AA catabolism/loss by gut tissues?

Table 4. Ratio of portal absorption to small intestinal digestibility: apparent, net, and available¹

AA	Apparent ²	Net ³	Available ⁴
His	0.75	1.02	1.49
Ile	0.62	0.69	0.81
Leu	0.62	0.65	0.74
Lys	0.55	0.64	0.77
Met	0.67	0.70	0.81
Phe	0.76	0.81	1.05
Thr	0.43	0.50	0.74
Val	0.51	0.59	0.76

¹Adapted from Berthiaume et al., 2001; Ouellet et al., 2002, 2005; small intestinal disappearance was replaced by mesenteric net flow for His.

²Apparent: duodenal – ileal.

³Net: apparent digestible – endogenous duodenal + endogenous ileal.

⁴Available: true ileal digestible from net supply – endogenous ileal.

Impact of Endogenous Secretions

From the previous discussion, it is known that SID encompasses digestion of endogenous proteins contributing to duodenal digesta but these will not increase portal flow of AA because most originate from AA supplied through the portal drainage. In the example of the lysine flow, this represented the 21 g/d recycled within an apparent SID of 107 g/d. Recognizing the endogenous contribution to the duodenal flow and its subsequent apparent digestion results in a maximal portal absorption:SID ratio of 80% [(107 – 21)/107] – this is without requirement for catabolism of AA to occur. Therefore, a simplistic analysis of the portal absorption:SID ratio would yield a large overestimate of AA loss across the gut.

The real loss of AA across the gut originates from 2 fractions: endogenous proteins still present at the ileum that will not be reabsorbed, and direct AA oxidation. As previously determined in the lysine example, at the ileum, 16 g/d were of endogenous origin, not reabsorbed in the small intestine, and therefore, lost in the hindgut or in the feces. Therefore, the maximal quantity of lysine that could reach the portal vein is the net quantity apparently digested corrected for the digested preduodenal endogenous secretions (114 – 21 = 93 g/d) minus these endogenous losses (16 g/d). This would give a portal absorption:SID ratio of 72% [(93 – 16)/107]. Accounting for losses of endogenous protein would then yield a potential maximal availability of 77 (93 – 16) g/d, higher than the reported portal absorption of 60 g/d. Ratios for portal absorption on apparent SID, on estimated net absorption, and on net availability are given in Table 4. For some AA, the ratio of portal absorption on net availability, including endogenous, is close to unity (even higher), but for other essential AA, the loss of endogenous proteins does not explain all the lack

of recovery between estimated availability and portal flow. Other losses must occur; indeed oxidation of some EAA by gut tissues has been reported.

Impact of AA Oxidation by Gut Tissues

Obviously, many assumptions have been made for these calculations, particularly as relates to the quantities and profile of AA as endogenous secretions. Nonetheless, the exercise suggests that there is minimal oxidation of some AA, such as histidine and phenylalanine, and more extensive catabolism of the branched-chain AA, lysine and methionine. Unfortunately, available data on AA oxidation across the gut in lactating dairy cows are as limited as the data on endogenous secretions. Nonetheless, in ruminants, leucine oxidation across the gut has been reported, including observations in dairy cows (see review, Lobley and Lapierre, 2003). In sheep, oxidation of methionine averaged 10% of portal absorption, but no oxidation was observed for lysine and phenylalanine (Lobley et al., 2003). A recent study in sheep reported that net appearance into the portal circulation in comparison with abomasal infusions of incremental amounts of casein had a slope different to unity for the branched-chained AA and lysine (El-Kadi et al., 2004), but not for other EAA. This would support substantial oxidation (between 43 and 51% of the incremental supply) of these AA by gut tissues.

The few data available do not allow identification of all factors that would affect the magnitude of AA oxidation but it becomes increasingly evident that this is not a fixed absolute amount. For example, in dairy cows, leucine oxidation across the gut increased with MP supply and averaged 20 and 30% of portal absorption at low vs. high MP supply, representing 31 vs. 38% of whole-body leucine oxidation (Lapierre et al., 2002). In sheep, diet quality, quantity, and the presence of parasites affected leucine oxidation across the gut (see review: Lobley and Lapierre, 2003). All these measurements were made with labeled leucine infused into the systemic circulation; that is, the leucine used for oxidation was from an arterial source. In pigs, leucine used for oxidation by the gut originated from both luminal and arterial supply (Van der Schoor et al., 2001). Oxidation from the lumen source includes oxidation of the AA by the gut cells during passage to the blood circulation but may also involve oxidation by gut microorganisms. On the other hand, in pigs, lysine oxidation across the gut was sensitive to lysine supply but there was no oxidation of the AA from the systemic circulation (van Goudoever et al., 2000), as also observed in sheep, but enteral lysine could be catabolized by the porcine digestive tract. Indirect evidence also indicates that oxidation might be related to supply to the gut. In dairy cows

in which AA were infused into a jugular vein, resulting in a substantial increase in arterial concentrations of AA, net portal appearance decreased for most of the EAA except phenylalanine and threonine (Berthiaume et al., 2002). This result indicates greater uptake by gut tissues, probably related to the incremental total inflow. Similarly, the linear relationship between portal recovery of the branched-chained AA and lysine in sheep receiving abomasal infusion of incremental amount of casein, with slopes lower than unity, strongly indicates that these AA were oxidized in proportion to supply at the gut (El-Kadi et al., 2004).

Examination of our "case study" for lysine flow in dairy cows presents a mixed message about oxidation of lysine by the gut. In the example we used, portal absorption (60 g/d) was lower than the net available after endogenous losses were accounted (77 g/d), suggesting a potential lysine oxidation of 17 g/d. This would represent 15% of the total amount digested, in agreement with studies in pigs (van Goudoever et al., 2000) and sheep (El-Kadi et al., 2004) in which oxidation from the lumen side was reported. On the other hand, there was a substantial difference between mesenteric absorption and SID of lysine found by Berthiaume et al. (2001), suggesting a measured SID higher than expected; more recent data (R. Berthiaume and H. Lapierre; unpublished data) yielded close agreement between net available (after endogenous losses were accounted) and portal absorption. This result would suggest limited oxidation of lysine by the gut, as observed by Lobley et al. (2003).

To understand why the gut may utilize AA, we have to consider the many separate functions that the different sections of the digestive tract perform. The gut is not a homogeneous tissue and AA are absorbed only from one section—the small intestine—and this is the only part in positive AA balance. The other sections (forestomachs and hindgut) are in negative AA balance (Lobley et al., 2003; Rémond et al., 2003) and, to maintain their integrity and function, utilize AA from the arterial supply; that is, those that have already been absorbed and circulated around the body. Indeed, use of AA by the whole gut is dominated by arterial sources; 80% of gross use was from this source in sheep (MacRae et al., 1997b).

It is not clear to what extent this removal by the fore and hind regions of the gut is obligate or regulated. Although the need to support endogenous secretion is relatively easy to understand, why should the gut directly oxidize some AA, particularly methionine and perhaps lysine, that may be limiting to support other body processes, including lactation? Certain AA such as the branched-chain AA and lysine are catabolized by nonhepatic tissues (Goodwin et al., 1987; DeSantiago

et al., 1998; Pink et al., 2003); perhaps the digestive tract needs to contribute to whole body oxidation. Alternatively, these AA may perform metabolic functions linked to their oxidation. For example, lysine degradative pathways produce glutamate, an important energy source for the gut cells (Windmueller and Spaeth, 1980) and a precursor for de novo synthesis of arginine and proline (Ball, 2002). Methionine metabolism is linked to provision of 1-carbon units, as S-adenosylmethionine, and nucleic acid biosynthesis, important functions for proliferative cells, such as those of the gut epithelium. Leucine is the best EAA for transamination reactions.

Absorption of peptide-bound AA into the blood circulation has remained a controversial issue over recent years. This issue will not be addressed in depth in the current review, because for several studies in which peptides have been measured, the inclusion of absorption as peptides yields results that do not fit well with the integration of N metabolism across the gut. For example, N absorption into blood circulation (ammonia and AA) should not exceed entry of N in the gut [urea recycling plus digested N; in cow, see for example, Delgado-Elorduy et al. (2002) and Tagari et al. (2004)]. Even accurately measuring veno-arterial differences of individual AA across the gut presents a severe technical challenge and we believe that the greater complexity of direct peptide veno-arterial measurement with actual techniques is at the limit, or beyond, the sensitivity of the current methods available. In addition, in the following section, it will be shown that predicted digestible AA does not appear to account for all free AA absorbed into portal circulation, so the requirement for extra amino acid absorption in peptide form looks relatively small.

Synopsis

Gut metabolism substantially and differentially alters the availability of AA supplied from MCP and RUP. Based on the ratio of portal absorption to true net digestibility, endogenous loss and oxidation of EAA averaged 35% of the net digestible amount, with losses varying from 25% for phenylalanine to 55% for threonine. This is an important new area that requires further investigations, because losses of AA through the 2 routes of endogenous proteins and gut oxidation will need to be incorporated into future predictive models to define the AA demands of the dairy cow.

LINK BETWEEN PREDICTED DIGESTIBLE AA AND PORTAL ABSORPTION

Most of the predictive models of milk protein output estimate MP or digestible AA, and from there, using a fixed coefficient of transfer, predict milk protein output.

The transfer of AA supply to milk output is not constant (Doepel et al., 2004). With increased protein supply, although more AA are transferred to milk, there is also a marked increase in the amount catabolized. This increase in oxidation of AA can occur in many tissues of the body. For example, gut catabolism of certain AA is elevated (isoleucine, leucine, lysine, and valine: see this review), whereas fractional hepatic removal also increases markedly for other AA (histidine, methionine, phenylalanine, and threonine: Hanigan, 2005; Lapierre et al., 2005) and the mammary uptake to milk output ratio for other AA is higher (isoleucine, leucine, lysine, and valine; Guinard and Rulquin, 1994; Raggio et al., 2004). The partition of the extra AA supply between milk protein production and catabolism determines the efficiency of conversion, which is altered as the cow approaches its maximal output. Mechanisms that might underlie this regulation of AA use by the dairy cow have been discussed elsewhere (Lobley and Lapierre, 2003; Hanigan, 2005; Lapierre et al., 2005). Nonetheless, a model of milk protein yield based on digestible supply as the first input, followed by inclusion of metabolism of AA across the gut, the liver, and the mammary gland should predict a decreased return of milk protein as supply increases because removal of AA across these tissues is related to inflow (Hanigan, 2005; Lapierre et al., 2005). The first step of this approach is to link SID with portal absorption.

Prediction of Digestible AA

Due to the scarcity of studies in which both SID and portal absorption of AA were measured simultaneously, we decided to use an existing subrumen model predicting digestible flow of EAA and link these estimations with measurements of portal absorption of AA.

Duodenal flow and intestinal digestibility, as discussed in this review, apply for total digesta and do not partition between individual feed ingredients. As such, they cannot be used to predict AA supply from different diets. Instead, to predict digestible AA to the dairy cow, sophisticated subrumen models have been developed to estimate duodenal protein flow based on characteristics of feed ingredients appropriate to each model [NRC, 2001; Cornell Net Carbohydrate and Protein System (CNCPS) in Fox et al., 2004; Vérité and Peyraud, 1989]. Despite the acknowledged endogenous contribution to the duodenal protein flow, for modeling purposes, certain models do not include this fraction into their estimation of the duodenal protein flow (CNCPS, Fox et al., 2004). Duodenal flow of individual AA is then determined with either a factorial approach attributing an AA composition to each of the fractions (Vérité and Peyraud, 1989; CNCPS in Fox et al., 2004) or through

Table 5. NRC (2001)-predicted duodenal amino acid flows vs. actual flows from studies reported by Bateman et al. (2001)

Amino acid	Measured duodenal flow (g/d)	Predicted duodenal flow (g/d)	RMSPE ¹ (g/d)	% Mean bias	% Slope bias	% Random noise
Arg	145.3	125.7	27.9	49.7	9.1	41.2
His	71.0	60.3	14.3	56.4	21.4	22.3
Ile	155.1	132.4	30.0	57.4	4.8	37.8
Leu	291.1	241.7	64.0	59.5	0.1	40.4
Lys	197.9	175.2	41.5	29.9	25.2	44.9
Met	55.5	51.5	14.0	8.1	14.5	77.4
Phe	159.5	136.1	28.1	69.8	5.4	24.9
Thr	150.7	132.6	28.5	40.0	4.3	55.6
Val	182.2	149.8	40.2	64.8	0.7	34.5

¹RMSPE = Root mean square predicted error.

the development of regression equations yielding the proportion of the AA relative to total EAA in the duodenal flow (NRC, 2001). In such models, conversion from protein duodenal flow to MP or from AA duodenal flow to digestible AA is achieved by multiplying the flow presented at the duodenum by the digestibility of each fraction.

As discussed previously, the difficulty of direct measurement of true intestinal digestibility of feed ingredients has led to the development of an indirect technique, the mobile bag technique. Feed ingredients, after incubation in porous bags suspended in the rumen, are transferred into other small porous bags preincubated, or not, with pepsin to mimic gastric digestion and then inserted into the small intestine via a duodenal cannula and eventually recovered from an ileal cannula or the feces. Using this mobile bag technique, there was no difference in recovery at the ileum or the feces, as long as the feed was preincubated in the rumen (Prestløkken and Rise, 2003), except for mature forages (Hvelplund and Weisbjerg, 2000). Feces could thus be used as the end-point, alleviating the need to maintain a patent ileal cannula (NRC, 2001). After washing the bags, it is assumed that there are no residual endogenous proteins, and therefore, disappearance from the bag is assumed to be the true digestibility. Using these feed digestibilities, coupled with a value for microbial digestibility plus another for endogenous secretions (when considered), models now predict the supply of digestible or metabolizable AA to the cow (French system: Vérité and Peyraud, 1989; CNCPS: Fox et al., 2004; NRC, 2001).

Link with Portal Absorption

The first step in our approach was to decide which model predicting digestible AA should be used. Bateman et al. (2001) had already tested several models designed for North American conditions [NRC, 1989;

CNCPS, version 3; Cornell Penn Miner Dairy (version 1.0); and Mepron Dairy Ration Evaluator (version 1.1 from the Degussa Corporation, Ridgefield Park, NJ)] and reported that no single model could be identified as better in predicting flow of individual EAA to the duodenum. To complete the picture in terms of models currently available, we simulated the same publications included in Bateman et al. (2001) using the latest version of NRC (2001), and concluded that this model underestimates the mean flow of EAA to the duodenum (Table 5). These results are similar to those presented by the authors of the original study, who also reported significant proportions of the prediction errors were associated with systematic bias (30 to 40%) for all the models tested.

However, it has been acknowledged that the use of mean values from generic databases could be a contributor to this bias. To use individual data generated for each cow, and assuming that the accurate knowledge of the feed characterization would reduce systematic errors in the estimation of duodenal flows of EAA, we used studies conducted by our group where net portal absorption of individual AA was measured (8 treatments: Berthiaume et al., 2002, 2006; H. Lapierre, unpublished data; Raggio et al., 2004). Net portal absorption was measured from plasma flow (estimated from downstream dilution of *para*-amino hippurate) and veno-arterial concentration differences. Relevant ingredient (including CNCPS fractions and AA analysis) and animal information was entered into CNCPS (v. 5.0.34) and NRC (2001) to compare the estimated digestible flows of AA with measurements of portal absorption. Given the current state of knowledge of AA use by the gut (see above), it became possible to double-check that estimation of digestible AA flows and measurement of portal absorption matched sufficiently to account for possible loss of EAA by gut tissue metabolism.

Based on concepts previously explained in this review, the digestible flow of AA predicted by NRC (2001)

Table 6. Amino acid amounts (g/d) and profiles (percentage of essential AA) from measured net portal absorption, or from estimation of digestible AA by the NRC 2001 (corrected to remove endogenous contribution) and CNCPS (v.5.0.34) models

AA	Net portal absorption measured		NRC estimation		CNCPS estimation	
	Mean $\pm$ SD	% of EAA	Mean $\pm$ SD	% of EAA	Mean $\pm$ SD	% of EAA
Arg ¹	89 $\pm$ 16.2	11.2	77 $\pm$ 11.0	10.0	115 $\pm$ 14.3	12.9
His	43 $\pm$ 10.6	5.4	37 $\pm$ 7.6	4.8	52 $\pm$ 7.4	5.9
Ile	83 $\pm$ 13.9	10.5	80 $\pm$ 9.6	10.5	94 $\pm$ 9.8	10.6
Leu	147 $\pm$ 31.1	18.6	161 $\pm$ 23.1	20.8	157 $\pm$ 19.5	17.6
Lys	119 $\pm$ 20.9	15.1	111 $\pm$ 17.3	14.3	129 $\pm$ 16.1	14.5
Met	41 $\pm$ 8.7	5.2	32 $\pm$ 4.9	4.3	44 $\pm$ 4.8	4.9
Phe	106 $\pm$ 23.4	13.4	86 $\pm$ 12.8	11.1	95 $\pm$ 11.2	10.6
Thr	69 $\pm$ 15.3	8.8	80 $\pm$ 10.6	10.5	93 $\pm$ 10.8	10.4
Val	93 $\pm$ 18.9	11.7	99 $\pm$ 14.8	12.8	113 $\pm$ 14.0	12.7

¹Arginine is included in the essential AA in the NRC and Cornell Net Carbohydrate and Protein System (CNCPS) models.

was corrected by subtracting the contribution of endogenous preduodenal secretion, using the estimation of the NRC model and AA composition of abomasal isolate (Table 1), whereas the CNCPS model did not include any endogenous contribution to the duodenal flow. All subsequent references to NRC (2001) predicted duodenal digestible AA flow are corrected for this endogenous contribution; that is, the values presented would now represent only the contribution from RUP and MCP to duodenal flow. Results of this exercise showed that digestible supply of EAA estimated by CNCPS were numerically higher (by 5 to 40%) than those obtained from the NRC model with the exception of leucine, which represented only 92% of the NRC value (Table 6). Analysis of correspondence indicated that AA profiles; that is, the relative amount of each EAA to the total EAA, were not significantly different between portal measurement and NRC or CNCPS estimations ($P > 0.05$, χ^2 test, 16 df), although there appeared to be a trend for the NRC model to give a higher relative proportion for leucine than CNCPS. In contrast, CNCPS estimates for the relative proportions of arginine and histidine tended to be higher than those from NRC.

Overall, the estimates from both CNCPS and NRC were reasonably close to the net portal absorption measured in our experiments. This close match is encouraging considering that the methodologies used to obtain the values of digestible supply and net portal absorption of AA are completely different. However, one would also expect digestible flows to be higher than the portal absorption, due to the loss of endogenous secretions and oxidation by gut tissues (Table 3).

Although the CNCPS yielded higher estimates of digestible AA flows than NRC, this did not indicate which model gave the better fit against our portal measurements. With the assumption that digestible supply of individual AA is directly related to the corresponding

net portal absorption, we applied the same assessment protocol as that used by Bateman et al. (2001), but here we used the portal absorption as the value modeled from the digestible supply data. Although neither NRC nor CNCPS were designed for estimation of net portal absorption, we would expect a degree of association between the model estimates (particularly as all the ingredient data and feed intake were known) and our measurements of portal absorption. We also assumed that errors in determining the true digestible flow would be equivalent between models. From this comparison, NRC gave a better prediction of net portal absorption from our experiments, as based on a lower root mean prediction error (Table 7). However, the partitioning of the prediction error indicated a systematic underprediction (mean bias). This then raised the obvious question: are NRC estimates underpredictions or are our net portal absorptions overpredictions? We have no clear answer to this question, but the close agreement in our studies between the post-liver supply of AA from group I (i.e., histidine, methionine, and phenylalanine; Mephram, 1982) and milk AA output would suggest that our blood measurements did not suffer from a systematic overprediction.

Based on the results presented in Tables 5, 6, and 7, NRC (2001) appeared to underpredict the flows, certainly for the AA where the slope was greater than unity (histidine, methionine, and phenylalanine) but also for other AA with a slope close to, or lower than, unity (isoleucine, leucine, lysine, threonine, and valine), recognizing the use of these AA across the gut (see above). A revisit of the NRC (2001) equations was then undertaken in an effort to improve the predictive ability of the model taking into account criticisms and suggested refinements of the model.

First, the use of combined data sets from lactating and growing cattle to generate equations for duodenal

Table 7. Analysis of the root mean square prediction error (RMSPE) and decomposition of prediction error using the portal absorption as the value being modeled from the digestible AA flow

Model ¹	Amino acid	Slope	RMSPE	% Mean bias	% Slope bias	% Random noise
NRC	His	1.14 (0.03)	4.21	66	14	20
	Ile	1.02 (0.05)	11.03	5	1	94
	Leu	0.92 (0.05)	31.07	32	2	68
	Lys	1.07 (0.03)	9.27	48	1	51
	Met	1.28 (0.05)	8.27	82	9	9
	Phe	1.24 (0.04)	18.73	74	12	14
	Thr	0.87 (0.04)	17.26	67	3	30
	Val	0.94 (0.03)	15.31	36	4	60
CNCPS	His	0.82 (0.05)	11.79	66	0	34
	Ile	0.87 (0.06)	18.94	36	17	47
	Leu	0.93 (0.07)	31.25	10	10	80
	Lys	0.92 (0.04)	17.30	37	0	63
	Met	0.95 (0.06)	5.94	19	10	71
	Phe	1.13 (0.06)	18.71	36	14	49
	Thr	0.75 (0.04)	25.52	82	0	18
	Val	0.82 (0.04)	24.86	70	1	29

¹NRC = NRC (2001) model; CNCPS = Cornell Net Carbohydrate and Protein System model v.5.0.34.

flows of protein and AA has been criticized (Alderman et al., 2001). Indeed, 22% of the observations used to generate the equations for duodenal flow of total EAA and the proportional of individual EAA are from growing cattle. Because the steer data cluster at low levels of EAA from RUP and MCP, and knowing the impact of intake on rumen fermentation, this point needed to be taken into account. Second, St-Pierre (2001) suggested that the data should be weighted based on the error associated with the measurements in each study. Therefore, the equations were reformulated using only the cow data and data were weighted as recommended. This yielded equations that increased the duodenal flow only by 2% (Tables 8 and 9). The equation to determine duodenal flux of total essential AA (Table 8), however, has a better fit with biological events: a coefficient of 0.95 (vs. 0.86) with the RUP fraction indicates almost a total recovery of EAA from the RUP fraction. A coefficient of 0.40 (vs. 0.43) with MCP is in agreement with MCP containing 80% of true protein and an EAA ratio on total AA lower than 50%. Finally, the intercept 60 g/d of EAA could be considered an estimate of endogenous protein and would scale to 120 g/d of total AA or 240 g/d of total endogenous CP (true protein, or AA, content of endogenous protein estimated at 50% - NRC, 200). This latter value would yield 28 g/d of N or, at an estimated intake of 20 kg of DMI, 1.9 g of N/kg of DMI, in line with the actual value used in the NRC model (2001). However, for individual AA, these improvements were still insufficient to account for AA loss across the gut, when comparing digestible AA flow with portal net absorption.

The next step was to increase the forage RUP digestibility from 60–70% to 88% (Hvelplund et al., 1992).

With this last adjustment, estimated digestible flows increased by an average of 9% (Table 9), and the slope of the portal measurements vs. these estimates also decreased (Table 10), indicating that the recovery of digestible AA flow into the portal circulation was not complete. Although this result would allow some potential loss of AA through gut metabolism, in agreement with the concepts presented earlier in this review, the magnitude of the losses still appear insufficient (e.g., losses of only 16% for leucine, and effectively no loss for lysine). However, the ranking of the AA in term of potential loss follows the same trend as previously discussed: minimal oxidation of phenylalanine and histidine, limited oxidation of lysine, substantial oxidation of the branched-chain AA, and a loss of threonine larger than for other AA due to its relatively high proportion in endogenous protein (Table 1). The slope was still greater than unity for methionine and phenylalanine. At this point there is no clear explanation of this observation, but we are currently investigating the preparation of the digesta samples before hydrolysis in the studies used to develop the equations.

The current exercise has improved the current NRC (2001) equations and appears to give reasonable fits compared with independent data sets based on portal absorption. Nonetheless, the predicted flows of digestible AA do still seem to underestimate net supply to the animal. For example, the estimated digestible flow of lysine increased from 111 to 120 g/d but is still low compared with the observed portal measurement of 119 g/d if we want to account for a possible loss of lysine at least through endogenous loss. These values may be even more underestimated if the observations made for endogenous contributions with silage-based diets in

Table 8. Comparison of NRC (2001) and proposed revised equations using the whole data set from NRC (2001) (steers and dairy cows) or using only publications with dairy cows¹

Amino acids	N	Observed	NRC (2001)	NRC revised equation
From whole database				
Total essential AA at duodenum (g)	199	921.6	914.5	
Arg		10.35	10.32	
His	199	5.01	5.00	
Ile	199	10.90	10.81	
Leu	199	20.21	20.14	
Lys	199	14.38	14.37	
Met	199	4.29	4.27	
Phe	199	11.29	11.28	
Thr	199	11.02	10.97	
Val	199	12.54	12.50	
From cow data only				
Total essential AA at duodenum (g)	155	1,099.3	1,079.6	1,098.5 ²
Arg		10.38	10.35	10.39
His	155	4.95	4.98	4.95
Ile	155	11.01	10.83	11.02
Leu	155	20.14	20.04	20.15
Lys	155	14.35	14.37	14.38
Met	155	4.29	4.25	4.28
Phe	155	11.37	11.31	11.39
Thr	155	11.04	10.97	11.00
Val	155	12.49	12.50	12.47

¹Arginine is included in the essential AA (EAA) in the NRC and Cornell Net Carbohydrate and Protein System models.

²Total EAA = 60.15 + 0.9515 RUP EAA + 0.4003 microbial CP.

Ouellet et al. (2002, 2005) also extend to rations used to develop the original NRC (2001) equations. One option could be to use a factorial approach in which AA flow at the duodenum would be estimated from the NRC (2001) estimations of MCP, RUP, and endogenous proteins and ascribing to each fraction an AA composition (e.g., endogenous: Ørskov et al., 1986; MCP: Clark et al., 1992; RUP: AA values of the feed ingredient in

NRC, 2001). Such an approach yielded higher estimated digestible flow of methionine, averaging 48 g/d compared with 35 g/d with the revised equation approach. These calculations now would supply more, rather than less, methionine than appears in the portal vein, which would allow for endogenous loss and limited oxidation by the digestive tract tissues, as observed in sheep (Lobley et al., 2003). Therefore, it is worthwhile to continue

Table 9. Amino acid flows (g/d) from measured net portal absorption, or estimated digestible AA with the revised equations of NRC (2001) and increment of the RUP digestibility of forage^{1,2}

AA	Digestible flow					
	Net portal absorption		Estimated with revised equations only		Estimated with revised equations only plus increment of RUP forage digestibility	
	Mean ± SD	% of EAA	Mean ± SD	% of EAA	Mean ± SD	% of EAA
Arg	89 ± 16.2	11.2	79 ± 11.3	10.0	84 ± 12.0	10.0
His	43 ± 10.6	5.4	38 ± 7.9	4.8	42 ± 8.1	4.9
Ile	83 ± 13.9	10.5	82 ± 9.1	10.5	87 ± 9.2	10.4
Leu	147 ± 31.1	18.6	164 ± 23.6	20.9	175 ± 23.8	20.8
Lys	119 ± 20.9	15.1	113 ± 18.7	14.4	120 ± 18.5	14.3
Met	41 ± 8.7	5.2	33 ± 4.9	4.3	35 ± 4.9	4.3
Phe	106 ± 23.4	13.4	88 ± 13.0	11.2	94 ± 13.2	11.2
Thr	69 ± 15.3	8.8	82 ± 10.7	10.4	87 ± 10.8	10.4
Val	93 ± 18.9	11.7	101 ± 15.3	12.8	109 ± 16.0	12.9

¹The digestible AA flows are corrected to remove the contribution from preduodenal endogenous secretions, estimated according to the NRC (2001) model.

²Arginine is included in the essential AA (EAA) in the NRC and Cornell Net Carbohydrate and Protein System models.

Table 10. Revised slopes of portal absorption being modeled from the digestible duodenal flow estimated with the revised NRC equations¹

AA	Slope
His	1.04 (0.03)
Ile	0.95 (0.05)
Leu	0.84 (0.04)
Lys	0.99 (0.03)
Met	1.18 (0.04)
Phe	1.14 (0.04)
Thr	0.80 (0.03)
Val	0.86 (0.03)

¹The digestible AA flows are corrected to remove the contribution from preduodenal endogenous secretions, estimated according to the NRC (2001) model.

considering the conceptual basis of the various predictive approaches and to evaluate which would better fit with measured values.

Synopsis

Integrating knowledge on AA metabolism will help build predictive models that should acknowledge a decreasing conversion of feed protein to milk protein as protein dietary supply is increased. Although all the needed information is not yet available, current knowledge can already help us to assess the validity of current models and direct us toward critical improvements.

CONCLUSIONS

Overall, there is not one perfect site to measure AA availability to dairy cows. At each point of measurement (duodenal flow, SID, portal absorption), gut metabolism has already exerted an influence. This fact needs to be recognized for a proper assessment of what the different measurements mean. Despite a known high contribution to whole-body protein kinetics, the quantification of the net impact of gut tissue metabolism on AA availability to peripheral tissues needs further refinement.

Although knowledge of factors affecting gut AA metabolism in dairy cows is slowly accumulating, there is already enough information available to signify the importance of this area. Therefore, future predictive models will have to integrate concepts and approaches relevant to this area improve their accuracy.

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