Protein Feeding and Balancing for Amino Acids in Lactating Dairy Cattle

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KEYWORDS

• Amino acids • Lactation • Metabolizable protein • Microbial protein • Limiting AA

KEY POINTS

- Amino acid (AA) nutrition of the dairy cow is complicated because of feeding 2 systems at the same time: one microbial and one mammalian.
- The cow must detoxify ammonia to urea; excess urea is secreted in urine.
- Several nutrition models can predict duodenal flow of protein and essential AA (EAA) with reasonable accuracy as well as the digestible flow of individual EAA leading to a prediction of metabolizable protein (MP).
- Metabolism of absorbed AA still has not been well characterized.
- All EAA can become limiting depending on the diet, but lysine, methionine, histidine, and leucine have been the most studied.
- Requirements for MP and AA for the lactating dairy cow have also not been well defined.
- Balancing for MP and AA should allow feeding of lower protein rations resulting in greater milk nitrogen efficiency and less environmental impact.
- AA balance for dairy cattle is still an evolving science.

INTRODUCTION

Nature has made the protein nutrition of the lactating dairy cow complicated. When dairy cows are fed, two systems are being fed: a microbial system that can use amino acid (AA) but whose basic requirement is for ammonia and nonprotein nitrogen (NPN),

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and a mammalian system that requires AA and must detoxify ammonia as in other mammalian species.

Interdependency of these systems complicates defining AA requirements and supply. First, the amount of microbial protein (MCP) must be determined, and then this amount must be separated from dietary AAs that escape rumen degradation. The sum of MCP and dietary AAs that escape rumen degradation and that flows to the small intestine is, after digestion in the small intestine, termed metabolizable protein (MP). The term MP is used to define the total AA available to the cow in support of all physiologic functions.

Studies that are needed to close gaps in the knowledge of these interactions, in order that cow MP requirements may be better defined, require costly and invasive techniques. Furthermore, animal studies are often of short duration, because both of the expense and the intensive labor needed to conduct such studies. This limitation should be considered when evaluating AA effects because in the short term, the animal may be able to use body protein to fulfill deficiencies. The body condition loss in early lactation is a good example. Conversely, the animal may respond to AA treatment in the short term but may adjust and show no response in the longer term.[1]

On a practical basis, dietary crude protein (CP) is often overfed to ensure a sufficient supply of all AA to support all biological functions. Balancing milking cow rations for AA can increase profitability by lowering protein cost and increasing production of milk and milk protein.[2,3] However, this does not occur in all circumstances.[4,5] Lowering CP intake by adequate balancing for AA will reduce urea excretion and therefore environmental pollution. Unfortunately, lowering the MP supply without regard to the AA composition of the MP can significantly reduce milk production.[3,5]

For a variety of reasons, there are many questions regarding the application of AA balancing. There is a need for specific field recommendations regarding the use of MP and AA concepts to achieve greater economy and efficiency of protein utilization.

The purpose of this article is to describe the current knowledge of protein and AA metabolism in lactating cows with an emphasis on information generated since National Research Council (NRC) 2001, discuss areas where the knowledge is incomplete, and suggest some recommendations to make AA balancing practical.

Amino Acids for Dairy Cows

Of the 20 AAs required to build proteins, 9 are considered essential because the cow cannot produce them: histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val). Although arginine (Arg) can be synthesized by the cow, it should be considered provisionally essential because it can become limiting under conditions of high production or disease.[6]

The nonessential AAs (NEAA) are also required for protein synthesis, but in addition to dietary sources, they are all synthesized by tissues from other AAs, both essential and nonessential.[7] In addition to dietary sources of protein that escape rumen degradation, rumen bacteria produce both NEAA and essential AA (EAA). The AAs of rumen microbes are well-digested and reasonably well-balanced relative to the AA needs of the cow, whereas both the digestibility and the balance of dietary AA can vary considerably.

AMINO ACID FLOW TO THE SMALL INTESTINE

The flow of AA arriving at the duodenum with the potential to be digested and absorbed originates from 3 sources:

- MCP leaving the rumen
- Undegraded portion of feed protein
- Endogenous protein secreted by gut tissue

Microbial Protein Synthesis in the Rumen

The MCP provides most of the MP (including both EAA and NEAA), is lowest in cost, and has an AA distribution much like milk (**Table 1**). However, MCP is deficient relative to milk protein in His and Met. Providing a rumen environment that produces the optimum amount of MCP should be a major goal when balancing dairy rations.

The factors known to impact the amount of MCP production have been reviewed,[7,8] and little additional knowledge has been added since these studies. Briefly, the factors exerting the greatest influence on MCP synthesis are as follows:

- Presence of an adequate rumen fiber mat to provide a good microbial environment as well as to provide rumination and buffering
- Providing adequate fermentable organic matter; the amount of organic matter (ie, neutral detergent fiber [NDF], non-fiber carbohydrate [NFC], and true protein) that is fermented will determine the amount of MCP produced
- Maintaining a level of degradable protein that provides sufficient free rumen AA and ammonia concentration.[9]

The MCP has 2 sources: bacteria and protozoa. Protozoa contribute varying percentages of the MCP ranging from 5% to 20%.[10,11] Previously it was thought little protozoal protein reached the abomasum because protozoa were preferentially held in the rumen.

There are 3 sources of the free ammonia for rumen bacteria:

- Degraded feed protein
- Urea from saliva and from arterial blood passing directly through the rumen wall
- Ammonia that becomes available from microbial cell lysis.

Combinations of these processes along with the mixing of rumen contents provide stable levels of rumen ammonia, free AA, and peptides.[12] Stable ammonia levels along with multiple meals and different rates of carbohydrate degradation account for the modest success of timing protein and carbohydrate degradation, although in vitro studies suggest that the effect should be significant.[13]

Both the percentage of MCP that is true protein and the AA composition of that true protein have been the subject of debate.[7,14] Estimates between 50% and 80% of MCP as true protein are common. A review by Clark and colleagues[15] summarized the variation in reported AA composition. Some of this difference may be due to the contamination of bacterial protein with feed protein. Estimates of bacterial and protozoal AA composition are summarized in **Table 1** along with feed proteins.

Rumen Undegradable Protein

The other major source (~30%–45% of total AA flow to the duodenum) is feed protein that is not degraded in the rumen.[7] This proportion may be overestimated because of the presence of endogenous protein.

Overall, rumen undegradable protein (RUP) content of a feed is determined by:

- AA content of the feed protein
- Physical structure (folding) of the protein
- Amount of heating the protein has undergone.

Proteins with more cross-binding are more resistant to microbial degradation and tend to have a more compact physical structure. Proteins with more Lys and Arg residues are more susceptible to degradation because these residues are more easily attacked by bacterial enzymes. Proteins that have more tertiary folding are more

Table 1
Mean essential AA composition (% of CP) of milk, rumen microbes, bovine tissue, and various feedstuff

	AA										
Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	% EAA[a]
Milk[b]	3.3	2.8	5.7	9.9	7.9	3.0	5.0	4.1	1.4	6.6	49.7
Rumen bacteria[c]	5.1	2.0	5.7	8.1	7.9	2.6	5.1	5.8	—	6.2	48.5
Rumen protozoa[d,92]	3.5	1.5	5.5	6.7	8.2	1.7	4.7	4.4	—	5.2	42.4
Alfalfa hay, 18.1% CP[e]	4.2	1.9	3.9	6.7	4.8	1.3	4.6	4.0	1.4	5.0	37.8
Alfalfa silage, 19.3% CP	1.8	1.9	4.1	6.7	4.7	1.3	4.4	3.8	1.2	5.1	35.1
Corn silage, 8.2% CP	2.3	1.7	3.4	8.5	2.8	1.6	3.9	3.4	0.7	4.5	32.9
Grass silage, 18.8% CP	3.0	1.5	4.1	7.1	4.3	1.5	4.5	3.9	—	5.3	35.3
Grass pasture, 13.4% CP	4.1	1.9	4.0	7.4	4.9	1.6	4.8	4.1	2.1	5.2	40.1
Barley grain, 12.3% CP	4.9	2.2	3.4	6.8	3.6	3.6	5.1	3.3	1.2	4.8	39.0
Corn grain, 9.1% CP	4.8	2.9	3.4	12.0	3.0	2.0	4.9	3.6	0.8	4.6	41.9
Wheat grain, 11.9% CP	4.8	2.3	3.3	6.6	2.8	1.5	4.5	2.9	1.3	4.2	34.0
Corn distillers (distillers dried grains), 29.9% CP	4.3	2.7	3.7	11.7	2.8	2.0	4.9	3.7	0.8	4.9	41.3
Canola meal, 42.5% CP	6.4	2.9	4.2	6.8	5.9	2.1	4.2	4.5	1.5	5.4	43.7
Soybean meal, 53.3% CP	7.3	2.6	4.5	7.6	6.1	1.3	5.1	3.9	1.3	4.7	44.5
Blood meal, 93.4% CP	4.3	5.9	1.1	12.3	8.7	1.2	6.8	4.6	1.4	8.2	54.3
Pork meal, 59.1% CP	6.7	2.0	2.8	5.8	5.1	1.4	3.3	3.1	0.7	4.0	34.7
Poultry meal, 62.1% CP	6.6	2.2	3.8	7.0	5.9	1.9	3.9	3.9	1.0	4.7	41.0

[a] % of essential AAs of total.
[b] Data from Lapierre H, Lobley GE, Doepel L, et al. Triennial lactation symposium: mammary metabolism of amino acids in dairy cows. J Anim Sci 2012;90:1708–21.
[c] Data from Clark JH, Klusmeyer TH, Cameron MR. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J Dairy Sci 1992;75:2304–23.
[d] Data from Ibrahim EA, Ingalls JR. Microbial protein biosynthesis in the rumen. J Dairy Sci 1972;55:971–8.
[e] CP, % DM; all feed data from: AminoDat V5, 2013.

resistant to microbial degradation as are those proteins that have undergone more heating.[7] These factors are accounted for in estimates of CP rumen degradability as used by various nutritional models.

When proteins are heated, 3 physical processes occur:

- Compaction of the protein, making it more difficult for the microbes to attach
- AA residues bind to fiber of the feed, increasing the amount of RUP and reducing the overall digestibility of the protein
- Formation of Maillard products, which are indigestible.

Another factor that determines degradation is the physical form of the protein.[16] Proteins with smaller particle size are more degradable than larger particles within the same protein source. If the particle is not readily fermentable, the particle has the potential to pass out of the rumen more rapidly, reducing overall degradability. Any factors increasing the rate of passage reduce protein degradability.

Different systems have been developed for modeling the amount of a feed protein that is undegraded.[17] Models use 1, 3 (A, B, C), or 5 (A1, A2, B1, B2, C) protein fractions to calculate the protein degradability, with each feed ingredient having its own protein fractions.

Studies suggest there is little difference in EAA content between intact protein and RUP.[7,18,19] However, some data indicate there are significant differences in AA composition between the intact protein and the portion of protein that escapes degradation.[8] Most of this difference appears to be in Arg, Lys, and NEAA. However, for the feeds that are highly resistant to degradation, such as animal protein meals, there seems to be substantial AA differences between fractions.[20,21] In summary, most data indicate there are no major differences in AA distribution between the whole protein and the RUP fraction except for animal-based protein meals or those products that have been damaged, by heat or silages that have undergone extensive protein hydrolysis and/or which have been subject to aeration and secondary fermentation.

Endogenous Protein

The third source of duodenal AA flow is AA from endogenous secretion.[7] Endogenous protein includes salivary, gastric, pancreatic and bile secretions, and mucus and sloughed cells.[22] The amount of endogenous protein is currently thought to be related to the dry matter intake (DMI), although estimates of endogenous protein vary significantly (from −0.85 at low intakes to 8.5 g N/kg DMI at high intake[23]). There is only one study that has directly measured the AA content of pre-duodenal endogenous protein in cattle.[24] Because of limited data, some modelers ignore the AA contribution of endogenous protein to duodenal flow and consider that any AA from endogenous protein is part of the maintenance requirement.

- Endogenous protein contribution is estimated between 1%[8] and 15%[25] of total duodenal AA flow and everywhere in between.[10]
- Endogenous protein secretion in the duodenal flow does not represent a net supply because these AA have already been absorbed.

Protein in the Diet

The true protein content of the dietary CP depends on the type of feed. In refined protein meals, there is a high amount of true protein, typically greater than 88%. For forages other than corn silage, there is a high percentage of NPN, typically about 40% to 45% for grasses and up to 88% for alfalfa silages. Legumes usually contain more NPN

than true protein[19]; however, this does not normally present a problem because it is considered part of the degradable protein and, if matched with sufficient carbohydrate, makes for efficient production of bacterial protein.

The NPN sources found in feeds include the following:

- Free AA
- Urea
- Ammonia
- Amines
- Nitrates and nitrites
- Small peptides.

Depending on the microbial species present and the amount of fermentable organic matter, all NPN can contribute to AA synthesis.

- The AA composition of feed is genetically determined, and, therefore, tends to be conserved.

However, the genetically determined AA composition of the intact protein can be modified by processing (particularly heating), hydrolysis (such as occurs in silages or wet storage), and contamination by bacteria or mold (as happens under poor storage conditions). Heated products (such as blood meal, roasted soy beans, and dry distillers grain), byproduct feeds (ie, cookie meal and bakery waste), as well as poorly fermented silages are products whose AA content and availability can vary greatly[26] and should be tested for degradability, digestibility, and AA content.

Transformation of Dietary Protein in the Rumen

There are 3 fates for dietary CP once it reaches the rumen[7]:

- NPN fraction is quickly converted to rumen ammonia
- Degradable true protein (RDP) is fermented by some species of bacteria to produce CO_2, volatile fatty acids, ammonia, peptides, and free AA
- RUP fraction passes from the rumen into the omasum.

Recent studies[27,28] suggest that more peptide and free AA pass from the rumen to the omasum and perhaps to the small intestine than was previously estimated because these pass quickly with the liquid fraction. Excess ammonia can be absorbed directly from the rumen[12] or from the small intestine (33%–50% of ammonia absorption).[29]

Ammonia absorbed into portal blood flows to the liver where it is detoxified into urea. Urea produced by the liver is partly reintroduced into the gut, including the rumen, via saliva or directly from arterial blood through the gut wall, apparently as salvage mechanisms for N, or is removed by the kidneys to be excreted in urine.[23,30] Urine nitrogen is the primary source of ammonia emitted from manure.[31] The proportion of the urea produced, which is recycled to the rumen, is reduced as MP supply is increased[32] and as N is consumption is increased.[33]

Supply Summary

Despite all the factors affecting the fate of dietary protein in the rumen and sometimes contradictory research, the newer computer models have been able to capture the complexity of rumen metabolism and produce adequate estimates of MCP, RUP, and EAA flow to the small intestine across a wide range of diet types.[17]

POSTRUMEN AMINO ACID METABOLISM

Although there have been a limited number of studies on postruminal AA metabolism of the milking cow, and while this is still a "work in progress," this research promises to enhance the understanding of AA utilization toward anabolic functions, such as milk protein synthesis. A thorough understanding of AA metabolism will allow the updating of models to balance dairy rations for protein and AA and allow reduced N intake without penalizing milk yield. Because these models will be based on dairy cow physiology, they will therefore be robust in operation, especially in response to constantly changing feed ingredient composition.

Protein Digestion/Amino Acid Absorption

True gastric digestion of proteins that pass from the rumen begins in the abomasum with the addition of hydrochloric acid and gastric enzymes to initiate hydrolysis.[34] When the digesta reaches the small intestine, it is buffered back to near neutral pH and both pancreatic and intestinal digestive enzymes are added.

Ruminant digestion in the small intestine is analogous to that in the nonruminant, with pancreatic trypsin, chymotrypsin, and elastase beginning the breakdown, and carboxypeptidases A and B completing the intestinal digestion.[34]

The AA must be absorbed from the small intestine, principally from the jejunum by specific binding proteins.[35] Although limited research does show some uptake of AA by rumen tissue and/or the deaminated form of AA, this does not seem to be a significant source of blood AA.[36]

Assuming no growth of the gastrointestinal tissue (GIT), AAs removed, on a net basis, by the GIT have 2 major fates. A portion, which includes all AAs, is used directly for the synthesis of endogenous secretions; those AAs not digested and reabsorbed are excreted in the feces (fecal metabolic nitrogen). Another fraction that includes only some AA, most particularly the branched-chain AA and the NEAA, are catabolized by the GIT.[37] This catabolism seems to be blood concentration–dependent as more branched-chain AA are removed with higher concentrations.[6] Although some AAs used by the intestinal tissue originate from the intestinal lumen from protein digestion, most of the AAs used by the GIT originate from the arterial supply.[38]

Intestinal Amino Acid Digestibility

The digestibility of the individual AA that passes to the small intestine is another critical consideration. Globally, the digestibility of all AAs in protein that reaches the duodenum is approximately 80%.[39] Some models use this average, whereas other models use a coefficient of digestibility different for each feed ingredient, based on estimates made using the mobile bag technique.[40] This technique, however, provides rather wide estimates of AA digestion. Furthermore, it is evident that this varies considerably with both the protein source and the individual AA.[17,19] The digestibility of microbial true protein is also currently estimated at 80% by most of the models, but data are scarce on this important parameter.

Hepatic Metabolism

The liver plays a role in the regulation of plasma AA concentrations. Whether the liver is the primary regulator or if it is only responding to blood concentrations is not defined at present. The liver serves as a major site of both deamination of excess AA and gluconeogenesis mainly from NEAA, as well as a site of production of required NEAA, although all tissues can synthesize NEAA. The nature of the regulation or the signals

that control the removal of AA from the liver are not understood at this time. Furthermore, data suggest that, in high-producing dairy cows, branched-chain AA and Lys are barely removed on a net basis, whereas His, Met, and Phe show the highest liver removal rate for the EAA.[41,42]

Mammary Metabolism

Most of the net supply of AA in the dairy cow is used by the mammary gland for protein secretion into milk. It is not unusual for cows to secrete more than 1.5 kg of protein in milk per day. Much new information has been developed regarding the use of AA for milk protein synthesis.

The direct source of milk protein AA is the free AA in blood arterial supply. These AAs are taken up by specific binding proteins, which are the same or analogous to those of the intestine.[43] Whether small peptides can be taken up as suggested by Bequette and colleagues[44] is still unproven.

It has long been known that milk protein synthesis is under the control of various hormones, including somatotropin, prolactin, insulin, and the locally produced insulin-like growth factors.[5] Recently, it has been observed that protein elongation is under the local control of the mammalian target of rapamycin (mTor) as part of protein transcription and subsequent activation of proteins 4E-BP1 and S6K1.[6,45] This complex has been shown to be activated by increased concentrations of Leu within the mammary cell, which led to suggestions that increasing Leu supply could result in increased milk protein production.[45]

It had been assumed that the percentage of AA in blood taken up by the mammary gland was constant, leading to the speculation that blood flow through the mammary gland was also constant. Both of these assumptions have been shown to be incorrect.

In a classic study, Bequette and colleagues[46] found that when blood His concentration was low, mammary blood flow increased as did the rate of extraction relative to supply of His, effectively decreasing the negative impact of low His arterial concentration on His supply and mitigating the impact on milk protein synthesis. Also, from a literature study, it was confirmed that although milk protein yield increased as duodenal flow of AA increased, there was a decreased efficiency of AA utilization that tended to plateau,[47] proving that AA secretion into milk protein was not a constant fraction of supply.

It has been reported in both swine[48] and dairy cows[39] that when plasma concentrations of EAA are increased, the mammary gland will take up greater quantities of these AAs in preference to NEAA. The clear implication is that the gland uses the EAA to produce NEAA that are nevertheless vital for milk protein production. This process would seem to be an unusual biological adaption as AA cannot be stored and energy is required for AA recycling and resynthesis, but this may offer flexibility in terms of energy source to the mammary gland.

- From these studies, it appears the mammary gland has the ability to control the use of AA by the amount and perhaps the pattern of AA in the blood.
- Whether this control is external or internal to the mammary gland is unknown.

Fates of Amino Acids in the Cow

Integration of all the previously described factors will lead to calculations such as those prepared by Lapierre and colleagues[39] as presented in **Table 2**. Presented is the flow of key EAA through the digestive tract. Although in reality these are calculations of what was observed from one experiment, the best use of these calculations is to develop statistical relationships to define requirements.

Table 2
Flow of selected essential AAs (g/d) at different sites of measurement in dairy cows

	Amino Acids					
Site	His	Ile	Leu	Lys	Met	Val
Duodenum	53	120	189	144	55	133
Endogenous duodenal[a]	15	19	20	30	6	25
Net duodenal	39	101	169	114	49	108
Ileal	24	38	61	37	19	55
Apparent ileal digestible[b]	30	82	128	107	36	78
Endogenous ileal[c]	7	11	14	16	4	15
From undigested endogenous duo	5	6	6	9	2	8
From nonreabsorbed endogenous from small intestine	3	5	8	7	3	8
True ileal digestible[d]	32	87	136	114	39	85
True ileal digestible from net supply[e]	22	73	122	93	35	68
Available, accounting for endogenous loss[f]	15	63	108	77	30	53
Portal absorption	22	51	80	60	24	40
Milk	15	33	54	45	15	37
Milk as % of duodenal flow	28	28	29	31	27	28
Milk as % of available	100	52	50	58	50	70

[a] Assuming 4.3 g of N per kg DMI and AA composition of abomasal isolate.[24]
[b] Duodenal–ileal, scurf protein, and endogenous urinary secretion.
[c] Assuming 28% of ileal CP flow, 1/2 from undigested.
[d] Apparent digestible + endogenous ileal from small intestine.
[e] True digestible − endogenous duodenal − endogenous ileal from undigested preduodenal endogenous.
[f] True ileal digestible from net supply − endogenous ileal.
Adapted from Lapierre H, Pacheco D, Bethiaume R, et al. What is the true supply of amino acids for a dairy cow? J Dairy Sci 2006;89(E Suppl):E1–14.

In particular, these calculations show 3 important traits of AA use by lactating cows:

- Conversion of duodenal AA into milk protein ~ 30%
- The true ileal digestible AA is only about 70% of that isolated at the duodenum
- Conversion of truly available AA into milk can range from 40% to 100%.

AMINO ACID REQUIREMENTS

The most surprising of all aspects of balancing dairy rations for AA is that actual requirements have not been clearly defined. It is this fact that has caused working nutritionists to question the value of AA balance for dairy cattle. In classical nutrition, requirements are established for various biological processes. These include the following:

- *Maintenance* (ie, AA needed to be replaced in already constructed proteins and excreted as metabolic fecal protein, scurf protein, and endogenous urinary secretion)
- *Growth* (ie, AA required for skeletal muscle and bone accretion)
- *Lactation* (ie, AA required for the production of milk protein)
- *Reproduction* (ie, AA required for the growth of the placenta and fetus).

There is no evidence that on a tissue level the AA requirement for maintenance is different between dairy cattle and other mammalian species. Likewise, the AA

requirements for growth and fetal growth have been well investigated in several species, and only minor differences between species have been observed. The large unknowns are the AA requirement for lactation and AA contents of metabolic fecal protein.

Determining Amino Acid Requirements for Lactation

Methods for determining lactation requirements could be

- To determine the amount of AAs that are secreted in milk protein, then make assumptions about digestibility and efficiency of utilization of AA.
- To measure the uptake of AA by the mammary gland compared with the amount of AA in the secreted milk protein.
- To assume that there is an "ideal protein" generally considered to have an AA composition like casein or MCP.

For Method 1, summing these variables provide estimates of each AA and MP needed for lactation. This method is simple, but there is no way to evaluate the accuracy of assumptions regarding digestibility and efficiency.

For Method 2, estimates for AA uptake versus output for various EAA are presented in **Table 3**. The uptake-to-output ratio has been suggested to represent the efficiency of use of AA[49] and is used in conjunction with milk protein AA composition to arrive at AA requirements. On average, some EAA are taken up in about the same ratio as they are exported (His, Met, and Phe + tyrosine).[50] Others (Arg, Leu, and Lys) are taken up greatly in excess of what is exported as protein, while the other EAA are taken up in somewhat intermediate quantities. Although the thinking behind this method is dated, it is still used by several models.

Arg is taken up in greatest quantity compared with output and is one reason some models have a large Arg requirement. The question is, does this uptake reflect requirements for synthesis of NEAA or regulatory proteins or is this simply "luxury consumption"? This model is subject to the same errors as the technique above.

Table 3
Mean ratio of uptake (U) of essential AAs across the mammary gland in relation to output (O) in milk protein

Variable	Mean Ratio	Standard Deviation	Minimum	Maximum
Milk true protein yield, g/d	794	155	370	1076
Metabolizable protein, g/d	1794	485	747	3619
Arg U:O[a]	2.45	0.60	0.88	4.18
His U:O	1.08	0.25	0.46	1.80
Ile U:O	1.41	0.20	1.01	1.96
Leu U:O	1.31	0.24	0.98	2.37
Lys U:O	1.33	0.25	0.60	2.09
Met U:O	0.96	0.11	0.59	1.18
Phe U:O	1.07	0.08	0.82	1.29
Thr U:O	1.19	0.18	0.87	1.58
Val U:O	1.49	0.27	0.85	2.22
Mammary plasma flow, L/d	14,160	2784	9384	23,976

[a] U:O = mammary AA uptake/AA secreted in milk protein.

From Lapierre H, Lobley GE, Doepel L, et al. Triennial lactation symposium: mammary metabolism of amino acids in dairy cows. J Anim Sci 2012;90:1708–21.

For Method 3, the ideal protein is fed to fulfill the MP requirement. The AA requirements are determined as the MP × the percentage of each AA in the ideal protein. This technique is also vulnerable to errors in the assumptions of digestion and transfer for MP as well as to the lack of knowledge of the exact MP requirement and whether the AA distribution affects the MP requirement.

Thus, although firm AA requirements have not been established, ranges of required EAA can be provided. It is assumptions of authors of models made from rather limited data that that produce differences in model requirements. In fact, there is speculation that given all the partial efficiencies of extraction and mammary blood flow that make up EAA requirements for lactation, it may be impossible to set "exact" requirements.[50]

Metabolizable Protein Requirement

Unfortunately, there is disagreement among models regarding MP requirements. As defined,[7] the MP requirement signifies the total supply of EAA and NEAA that a cow needs to support a defined level of production. Two meta-analysis studies[17,51] found that the NRC MP requirements[7] were not fulfilled in about one-third of the studies that were summarized for their meta-analysis, suggesting that the NRC MP requirements were too high. The unfulfilled MP requirements may also reflect the fact that NRC does not take into account the AA balance of the MP when computing requirements. The AminoCow model, which has significantly lower MP requirements,[51] assumes that MP has the exact amount of EAA that is "required" by the cow.[26]

Some of these differences in MP requirement may be due to different assumptions regarding the effects of AA composition on the MP needed to maintain production.

Another factor is the need for AA to contribute to gluconeogenesis. Bell[52] suggested that for the developing fetus and for cows in very early lactation, greater than 50% of the glucose is due to the conversion of AA. Thus, a high demand for use as a glucose precursor might need to be integrated into the AA and MP requirements at various stages, and probably in relation with energy supply.

It has been suggested that AA requirements should be expressed as grams per day on a factorial basis. In a recent study,[53] it was found that both milk protein yield and milk protein percentage were better related to grams of duodenal AA flow per day than for AA as a percentage of MP.

Nutritional programs do vary in their requirements (**Table 4**). Because of the large differences for some AA and MP, it is easy to think that one or more models are "wrong." The differences are a reflection of the way authors have looked at the data and of assumptions they have made in the development of their models. Because estimates of protein and EAA duodenal flows are close to measurements (**Table 5**), it appears the differences are largely due to the assumptions regarding postruminal metabolism. In fact, it is certain that all models are "wrong," but by using them and challenging them meaningful requirements may be developed.

Limiting Amino Acids

On a practical basis, to determine a limiting AA, there needs to be a response that can be measured as this AA is added. For lactation studies, milk protein yield is the appropriate response for AA limitation, utilization, and AA efficiency. The yield of milk protein represents both a volume and a percentage function. As pointed out,[51] milk protein percentage can increase with no increase in AA utilization for lactation if milk production is decreased; conversely, if yield is increased, but percentage milk protein decreased sharply, there may also be no change in AA utilization.

Since the pioneering research of Rulquin and coworkers[54,55] and Schwab and coworkers[56,57] as well as the publication of NRC 2001, it has been fashionable to

Table 4
Requirement estimates for MP and metabolizable AAs by various nutrition programs

	Nutrition Program			
Requirement[a]	NRC[b]	AC[c]	CPM[d]	CNCPS[e]
g/d				
MP	2784	2047	2266	2759
Arg	—	78	136	167
His	—	57	44	52
Ile	—	111	120	129
Leu	—	200	185	206
Lys	200[f]	168	131	150
Met	67[g]	55	40	45
Phe	—	98	72	83
Thr	—	97	71	130
Trp	—	30	24	30
Val	—	129	133	146
Requirement, % MP				
Arg	—	3.81	6.00	6.05
His	—	3.03	1.94	1.88
Ile	—	5.42	5.30	4.68
Leu	—	9.77	8.16	7.47
Lys	7.2	8.21	5.78	5.44
Met	2.4	2.69	1.77	1.63
Phe	—	4.79	3.18	3.01
Thr	—	4.74	3.13	4.71
Trp	—	1.47	1.06	1.09
Val	—	6.30	5.87	5.29
Total EAA		50.23	42.19	41.25

[a] For this comparison it is assumed that the cow is a Holstein, 3rd Lactation, weight 650 kg, with a body score of 2.75 and an average daily gain of 136 g per day while producing 41 kg of milk with a 3.60% butter fat test and a 3.10% true milk protein at 180 days in milk.
[b] Data from NRC is National Research Council model.
[c] AC is AminoCow, Evonik Industries, Hanau, Germany.
[d] CPM is Cornell-Penn-Minor Model version 3.
[e] CNCPS is Cornell Net Carbohydrate-Protein System version 6.1.54.
[f] Calculated as NRC MP requirement × .072.
[g] Calculated as MP requirement × .024.

concentrate only on Lys and Met as limiting AA on North American–type diets with His as a limiting AA on grass silage diets.[58] Since then, it has been proven that His can be a limiting AA in North American diets, especially in diets with a low protein supply because MCP contributes more to total MP.[3] Because of obligate use by intestinal tissue as energy sources, the branched-chain AAs (Ile, Leu, and Val) have also been suggested as limiting at high levels of milk production. Less often Arg and glutamine have been proposed to be limiting. Much summarized research[51] has shown that the addition of rumen protected Met results in more production of milk protein, more or less confirming the supposition that Met is often a limiting AA in North American–type diets.

Less convincing is the effect of Lys in established lactation. Robinson[59] summarized studies where dietary Lys was increased and observed no effects. In other studies[60,61]

Table 5
Simplified comparison of commercially available models to predict protein and AA flows

	Observed		AC			AMTS			CPM			NRC		
Item	Mean	SE	Mean	SE	% Obs.[a]	Mean	SE	% Obs.	Mean	SE	% Obs.	Mean	SE	% Obs.
CP	3027	790	2945	769	97.3	3026	638	100	3148	633	104	2951	708	97.5
MCP[b]	1610	407	1605	499	99.7	1678	314	104.2	2050	415	127.3	1573	338	97.7
RUP	1480	614	1368	372	92.4	1348	409	91.1	1126	315	76.1	1415	416	95.6
Arg	122	38	123	33	100.8	152	37	124.6	160	38	131.1	116	28	95.1
His	61	20	59	18	96.7	66	18	108.2	69	20	113.1	56	16	91.8
Ile	119	36	127	34	106.7	126	28	105.9	134	30	112.6	120	27	100.8
Leu	230	79	220	64	95.7	219	60	95.2	224	61	97.4	226	62	98.3
Lys	157	48	161	45	102.5	164	40	104.5	178	43	113.4	160	38	101.9
Met	47	16	48	13	102.1	53	11	112.8	59	13	125.5	47	11	100
Phe	129	38	128	34	99.2	134	33	103.9	140	34	108.5	126	31	97.7
Thr	123	34	124	32	100.8	120	28	97.6	127	29	103.3	120	27	97.6
Val	141	45	145	37	102.8	147	36	104.3	155	38	109.9	138	32	97.9

Abbreviations: AC, AminoCow version 3.5.2; AMTS, Argricultural Modeling and Training Systems LLC, version 2.0.15; CPM, Cornell-Penn-Miner Dairy, version 3.01; NRC, National Research Council (2001). After Pacheco, Patton, Parys et al.[17]

[a] Predicted value as a percentage of observed value.

[b] Microbial crude protein.

in which Lys was deleted from a mixture of infused AA, production of milk and milk protein was reduced in the Lys-deficient infusion, confirming the importance given to Lys by NRC 2001. In contrast, Patton[51] could find no relationship between Lys supply and milk protein response to Met. Thus, the exact relation of Lys to AA deficiencies has yet to be established. However, increased Lys supply does appear important in very early lactation,[58,62] a time when microbial synthesis is reduced because of lower DMI, to drive output of both milk and milk protein.

Although His has long been established as a limiting AA on grass diets, this may be more related to diets with a high contribution of MCP relative to total MP supply (>70% from the authors' data) regardless of diet type.[2] When His was added to low MP diets, improved DMI and milk production without changing milk protein percentage were observed.[3] It has been demonstrated that blood His concentration is greatly reduced on low protein diets.[63] Thus, because of low concentration in MCP, His has the potential to be limiting on reduced MP diets.

Despite speculation based on mTor studies, addition or deletion of Leu to diets has resulted in no changes in milk protein yield.[60,64] Studies with branched-chain EAA also have been disappointing, generally having no effect on milk protein production.[60,64,65] It appears well established that Arg is not greatly limiting, because both a deletion[66] and an infusion experiment[65] have shown no response to Arg.

Studies with the other AA (Ile, Phe, Thr, Trp, Val, glutamine, and cysteine) have been inconclusive as to their potential to be limiting. However, any AA has the potential to be limiting depending on the amount and type of RUP and proportion of MCP.

In summary, limiting AA in typical North American diets appears to be as follows:

- Met—still the most likely to be limiting
- His—may be the most limiting on low MP diets
- Lys—appears to have good efficacy in early lactation; supplement studies in established lactation are disappointing.

THE MEANING AND PRACTICAL USE OF MILK UREA NITROGEN

One of the most active areas of research over the last decade has been the effect of excess protein on milk urea nitrogen (MUN). Whether from rumen ammonia or AA in RUP, excess protein results in higher urea production.[33] Higher urea production results in the loss of N in the urine, not only wasting an expensive resource (protein) but also causing greater environmental pollution.[67] Monitoring of MUN offers the potential to increase protein efficiency and to decrease feed costs.[68]

Because ammonia is toxic, it must be removed from the blood and converted to urea by the liver. The kidneys then filter urea into the urine. There is an energy cost to ureagenesis that must be borne by the animal.[69] Because urea is a small molecule and readily diffusible, blood urea nitrogen (BUN) or plasma urea nitrogen (PUN) is in rough equilibrium with MUN.[67] Studies indicate that MUN is related to BUN and PUN with correlations between 84% and 98%.[70] Equations for conversion of BUN or PUN have been proposed with these be most widely used:

$$\text{MUN mg/dL} = 0.620 \times \text{BUN mg/dL} + 4.75$$ [71]

$$\text{MUN mg/dL} = 1.176 \times \text{PUN mg/dL} - 3.76$$ [72]

Likewise, milk urea (MU) or MUN expressed as millimole per liter can be converted to milligram per deciliter by the following formula:

$$\text{MUN mg/dL} = 2.8 \times \text{MU mmol/L}$$

$$\text{MUN mg/dL} = 5.6 \times \text{MUN mmol/L}$$

Normal Milk Urea Nitrogen Values

Normal mean MUN values range from 10 to 15 mg/dL for herds in the United States, while individual cows within herds can range from 5 to 25 mg/dL. Herds on high protein pastures will often exhibit MUN from 17 to 22 mg/dL[73,74] without apparent loss of production, although another study reported loss of production at 17 mg/dL.[75] This wide range both across and within herds suggests that there are many factors that influence MUN and that there may be subpopulations within herds that are affected differently by the ration consumed.

Reproductive Concerns

Many practitioners are concerned about BUN or MUN for previously reported effects on reproductive efficiency.[76,77] However, more recent studies using more sophisticated statistical analyses have found little relationship of MUN to reproductive efficiency.[78,79] These authors found that MUN was confounded with loss of body condition in early lactation and insufficient energy in other stages of lactation. Guo and colleagues[80] found that MUN had no effect on conception rate across herds, but within a herd, a 10 mg/dL increase in MUN resulted in 2% to 4% loss in conception rate. Thus, although it may have an effect on reproduction, high MUN levels do not appear to be a large cause of reproductive failure; rather, high MUN levels may coincide with other factors that have a greater direct impact on reproductive efficiency.

Factors Affecting Milk Urea Nitrogen

Excess CP intake is largely responsible for high MUN,[67] with a 1% decrease in CP resulting in 1.1 mg/dL decrease in MUN. In addition, there are a multitude of other factors that can affect MUN positively, including body weight (BW), yield of fat corrected milk, DMI, and days in milk (DIM).[71] Breed differences are significant,[81] although breed effects become insignificant when BW enters the equation. Also, there are no effects of parity if BW is considered.[82] The effect of DIM is generally such that MUN is higher in the first 25 DIM and then drops to its lowest levels after peak lactation. After this, MUN tends to creep up as lactation progresses. Season also has an effect as MUN rises and falls with the mean monthly temperature.[83] Time of milking in relation to time of feeding and water intake and amount of urine production are other factors that are proposed as important in MUN concentration.[68] Unfortunately, MUN is sensitive to adjustments in analytical equipment and differences in equipment as well as changes in equipment calibration. Although within a given machine comparisons are perfectly valid, it is more problematic to compare values between machines and laboratories (Hristov and colleagues, unpublished data).

Ration Factors

As stated, protein intake in relation to milk output is the largest determinate, and by far the most important nutritional determinate of MUN. There is a small and inconsistent effect depending on the amount of RDP,[33,82] but if RDP is fed in excess, high MUN results. However, the same will occur with excess RUP. It has been suggested that protein balanced for AA (rather than CP, RDP, and RUP) lowers MUN.[72,84] Increasing readily digestible carbohydrates in the diet has been shown to decrease MUN.[85–87] It has been shown that corn can be more effective at reducing MUN than barley,[20]

presumably because of a longer fermentation time allowing capture of more ammonia N by rumen bacteria, although grain processing may have a greater effect than grain type. High sodium diets (13.5 g of Na/kg of dry matter [DM]) lowered MUN in milk by 1.7 mg/dL, but without decreasing urinary urea excretion[83] and may be a reflection of increased water intake and corresponding increased urine volume.

Recommended Milk Urea Nitrogen Levels

Recommendations for acceptable MUN levels are difficult to obtain, but they do seem to center around 11 mg/dL. Kalsheur and colleagues[88] found that RDP had to be 9.7% of DM and that MUN had to be less than 11.6 mg/dL before production declined. Nousiainen and colleagues[89] suggested that an MUN of 11.7 mg/dL reflected sufficient degradable protein. However, as they point out, this did not account for ruminally recycled N; therefore, MUN of 9 to 10 could be adequate. Data from Cyriac and colleagues[90] suggest RDP at 8.8% of DM and MUN at 12.4 mg/dL as the point at which milk yield begins to suffer. Although these recommendations differ slightly because of differences in nutritional balance, they do seem to indicate that with adequately balanced fiber, NFC, and AA, high milk production (>40 kg per head per day) can be obtained with CP levels near 15% to 16%, RDP levels around 9.0% to 10% of DM, with MUN in the 9 to 12 mg/dL range.

Use of Milk Urea Nitrogen Levels

Because of all the influencing factors described above, it has been suggested that MUN be monitored on a bulk tank basis and a baseline for the herd be established,[91] suggesting that changes in MUN are the most important factor to monitor. Although strictly correct, if MUN values are to be used to evaluate protein efficiency, more precise use of MUN should be encouraged. It must be recognized that MUN testing and interpretation as well as average herd MUN values have decreased during the past 5 years as nutritionists have adopted the use of MP and AA balance, as well as the pressure exerted by producers to lower ration cost as protein prices have soared.

In practice, MUN is monitored in groups of cows, but only after balancing the MP and EAA for each group, which requires first agreeing on goal MUN and second evaluating MUN for groups of cows across the lactation. The authors recommend examining groups of cows as follows:

Group	Range of MUN Recommended (mg/dL)	Acceptable (mg/dL)
Herd	10–12	9–13
Group 1 = 0–30 DIM	14–15	14–16.5
Group 2 = 31–60 DIM	10–11	9–12
Group 3 = 61–180 DIM	10–12	9–12
Group 4 = 181–270 DIM	10–12	9–13
Group 5 >271 DIM	10–13	9–14

The acceptable range depends on many factors including the feeding strategy. For herds fed a single total mixed ration (TMR), it has been found that the MUN runs 1.0 to 1.3 mg/dL higher. For herds not feeding a separate fresh group, it is found that during the first 10 days MUN trends 2–2.5 mg/dL higher than goal. The authors suspect this is from greater mobilization of body reserves, although overfeeding RDP is certainly a possibility. Likewise, for herds with a wide difference of BW within groups, it has been found that the MUN averages 0.25 to 0.50 mg/dL higher than typical.

Determining these numbers is relatively straightforward where there is test day data for individual cows. The caveat is that 7 of 10 cows should show the same trend (ie, higher or lower). That also means that there should be a minimum of 10 cows per group. For herds without monthly testing (generally larger herds) where the only data are monthly or daily bulk tank results, as long as the results are within the acceptable ranges and production consistent with nutritional balance, no further action need be taken. If the MUN are greater than the acceptable range, and MP and RDP are in balance, the first option is to increase starch and sugar in the diet. Often this results not only in lower MUN but also in greater milk production as well. If MUN are lower, more RDP is added and it is evaluated whether more milk is seen. If only the MUN goes up without additional milk yield, then previous RDP levels are reverted to. Obviously the challenge is to evaluate whether milk production goes up or not without individual tests of milk yield and MUN, but to the extent that a production trend is convincing, with the use of the above strategy. If a single TMR is fed, the only remedy is to lower or increase the CP depending on the situation.

If either the levels of peak milk production or the persistency of lactation are not satisfactory, then blood tests are done for BUN in groups of 10 cows to determine if poor protein utilization in a particular group or groups is contributing to this problem. The authors like to take 2 sets of samples within the same week at approximately the same time in relation to feeding and milking, adding the same precaution that at least 7 from 10 show the same trend.

Finally, and perhaps most importantly, MUN is a tool to help diagnose ration problems. It is not a replacement for nutritional knowledge and ration balance.

PRACTICAL APPLICATION

Obviously, the protein and AA nutrition of dairy cattle are too complicated to adequately meet the needs of the cow without the use of a model that takes into account the various fates of ingested protein, MCP, and endogenous protein and integrates this all into the cow metabolism. The well-known phrase, "All models are bad, but some are useful," applies here. Fortunately, most models at the disposal of the nutritionists are relatively accurate at predicting the duodenal flow of CP and individual AA[17] (see **Table 5** for a comparison of some models). This acceptable accuracy can be used to formulate more productive, economical, and less environmentally damaging rations. However, to do this will take work and dedication on the part of the user.

The authors recommend the following steps for formulating AA balanced rations:

- Commit to balancing all rations for MP and AA. CP is a term whose use in setting requirements has passed into history.
- Choose a model and learn to use it. The assumptions of the model should be sufficiently clear so that the user can understand what the model calculates and how the model calculates it. Likewise, there should be studies that validate the model. Although it may be preferable if studies are published in refereed journals, recommendations from friends and peers are also valuable.
- Collect the information that the model requires to produce accurate results. Do not assume all cow groups weigh the same. Do not assume that the default values will be sufficient for your feeds. Know what tests to request from the feed laboratory to maximize the performance of your chosen model.
- Measure DMI. Because DMI has such a huge effect on MCP and RUP, it is critical that actual DMI be used. In the work of Pacheco and colleagues,[17] the best model predicted the DMI within 1.6 kg only 60% of the time.

- Optimize MCP. The key to economic production of milk and milk protein is the maximization of NDF digestion coupled with optimization of nonfiber carbohydrate digestion. Because MCP increases marginally with each increment of NDF, NFC, and RDP, it is economically unwise to "maximize" MCP. The only way to optimize MCP is to rigorously track feed costs and to monitor the difference in income over feed costs that result from increasing MCP versus decreasing MCP and adding RUP and rumen protected EAA to meet model requirements.
- Meet projected AA deficiencies on a gram basis with either RUP or rumen-protected AA. The debate regarding setting the requirements as grams versus ratios will go on for some time. Ratios of one EAA versus another, percentages of DM, or percentage of MP might be useful guides, but cows eat pounds and grams of nutrients. When satisfying program requirements for EAA, it is recommended that they be met as grams per day.
- Believe in the cow. If the cows are producing a given quantity of milk and the model estimates they are deficient by X g of MP or a given AA is deficient by Y g, then it is obvious that the cow is receiving sufficient nutrients to produce this milk and milk protein. Body condition should be monitored to make sure that the nutrients are not coming from mobilization of body reserves. Given no body condition mobilization, in most cases, models will predict the correct flow within a reasonable margin of error if the correct model inputs have been entered. Although model inputs should be checked, what is plain is that the cow is consuming sufficient nutrients to produce the milk that she is making. What should be suspected is the requirements of the model are not correct. At least mentally, adjust the model with this in mind.
- Monitor the situation. If MP is lowered, or if AAs are added, check the response, not only the immediate response, but longer term as well. Check body condition and reproductive efficiency as well as production of milk and milk protein. Farmers and nutritionists often report both improved over the longer term with AA balance.

Experience indicates that with stable forages, production of greater than 41 kg of milk with greater than 3.15% true milk protein are possible with 15.0% to 16% CP in the ration when AA are balanced. When forages are of variable quality, the CP content of the ration may need to be increased to insure sufficient MP at all times. Maintaining sufficient energy is always critical for optimizing both milk yield and MCP production.

Like so many of the advancements in dairy nutrition, advances in AA balance will come from the field. Using AA balance and sharing results with colleagues are the best way to work out the best balance for your chosen model with your clients.

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