

Current Status of Amino Acid Requirement Models for Lactating Dairy Cows

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Abstract

The lactating dairy cow is fairly inefficient at converting dietary nitrogen to milk protein. When cows are fed to National Research Council (NRC, 2001) requirements, they convert approximately 25% of dietary nitrogen to milk protein. There may be some inherent limitations to the efficiency that can be achieved by a ruminant; however, examination of the NRC model with respect to available data suggest several areas where requirement or supply predictions could be improved. Ruminally degradable protein (**RDP**) requirements appear to be too high. Reducing these requirements would allow reductions in dietary protein and improved animal efficiency. Amino acid (**AA**) flow to the small intestine appears to be predicted with good accuracy provided diets are within current NRC requirements for RDP. Absorption of AA from the gut lumen is not constant across AA within an ingredient or across ingredients as assumed by NRC. Thus, absorbed AA may be predicted with bias. The gut tissues as represented in the portal-drained viscera and the liver remove approximately 2/3 of the available AA on a daily basis, and this removal is dependent on supply. Variable use by these maintenance tissues violates the NRC assumption of fixed maintenance use and introduces a significant bias in our current predictions. Mammary AA removal is highly regulated and counters supply, i.e. as supply declines, transport activity increases and vice versa. Variable transport activity with respect to AA supply

violates the NRC assumption of fixed conversion efficiencies for absorbed AA and introduces bias into predictions. Thus, our ability to successfully balance diets for adequate AA while minimizing dietary nitrogen inputs is currently hampered by a lack of data with respect to AA digestibility in the small intestine and poor representations of postabsorptive use of AA. Amino acid digestibility coefficients for individual ingredients are needed to address the first challenge and an alternate representation of postabsorptive use and recycling of AA is required to address the second challenge.

Introduction

Production per cow continues to increase annually (APHIS, 2002). Much of this improvement is associated with genetic gain. However, management and nutrition must keep pace with genetic gain to allow expression of full genetic merit. Certainly, there is constant economic pressure to identify nutritional and management factors that may be limiting cow performance and correct those deficiencies.

In many areas of the country, including the mid-Atlantic region, environmental pressure has also increased. The Chesapeake Bay Watershed is an environmentally sensitive area with large concentrations of livestock (Golleshon et al., 2001). Considerable emphasis is being placed on reducing nitrogen (**N**) and phosphorus influx into the watershed. A significant component of these efforts

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is related to nutritional management of production species, including the dairy cow.

Nitrogen required by the ruminant to synthesize products and maintain the animal is derived from the protein synthesized by microbes in the rumen and from undegraded feed sources. As compared to other species, the dairy cow appears to be fairly inefficient at converting dietary N to milk N (Figure 1). However, this may simply reflect our lack of knowledge in the area. Animals in the U.S. are generally fed to requirements as set by NRC (2001). As these requirements reflect our knowledge base, the fact that current N efficiencies are low is not necessarily indicative of an inherent limitation in the species relative to N efficiency. It is possible our requirements are set too high relative to the real needs of the cow, causing us to overfeed N.

Over the past 100 years, investigators have derived dose-response relationships between animal performance and dietary N inputs. Such work has demonstrated that requirements must be considered relative to both ruminal and postabsorptive needs. Ruminal requirements reflect the needs of the microbes inhabiting the rumen and the postabsorptive requirements reflect the animal needs. Thus, the overall N needs of the animal are comprised of the N available in the rumen for microbial use, and the N or protein that escapes the rumen in the form of undigested feed protein and as microbial protein and becomes available for animal use.

These requirements historically have not reflected the need for specific AA by ruminal microbes or by the cow. The most recent release of the NRC (2001) provides predictions of absorbed essential AA and suggestions regarding the quantity of absorbed lysine and methionine required to meet animal needs, and thus reflects some progress in the area.

This review will summarize some key components of that progress and highlight some deficiencies in our current knowledge base and in our requirement system. As identification of deficiencies in our prediction systems is critical to making further progress, more time will be spent discussing these. This should not be construed as a condemnation of our current systems. They have served the industry well and continue to do so today. But to improve these systems for the future, it is critical to understand the deficiencies. Additionally, such knowledge helps users of the current system recognize the potential limitations of the system so they can avoid making mistakes when using the system.

Ruminal Nitrogen Metabolism and Requirements

Mammalian species do not possess the ability to digest and absorb structural fiber, e.g. fiber insoluble in acid detergent. However, ruminants possess a large population of microbes that reside in the rumen that are able to ferment fiber as well as other nutrients. As the end-products of fermentation represent approximately half of the energy supply of the animal, this is a critical component of their digestive system. Another product of this microbial growth is microbial protein which represents approximately half of the N absorbed from the digestive tract of the ruminant. Thus, microbial growth and metabolism are an essential component of ruminant metabolism, and the nutritional needs of these microbes must be considered when designing diets if cows are to achieve their genetic potential for production. In general, this equates to maximizing or attempting to maximize microbial growth as that will result in the greatest nutrient supply (energy and protein) to the animal when significant forage is included in the diet. Greater energy densities can be achieved on low or zero forage diets, but such diets are inconsistent with long-term cow health in a dairy production setting.

Microbial N Requirements

To maximize microbial growth, mixed ruminal microbes appear to require peptides, free AA (Argyle and Baldwin, 1989), and ammonia (Roffler and Satter, 1975). All 3 of these entities can be generated via protein degradation, which occurs in an ordered process (protein - peptides - AA - ammonia) via microbial activity (Figure 2). As peptides, AA, and ammonia are all generated from ruminally degradable N, one can aggregate the requirements for the 3 entities into a single RDP or ruminally degradable N requirement. In taking this approach, one must recognize that the apparent RDP requirement will reflect the most limiting nutrient of the 3 N classes (peptides, AA, or ammonia). Thus, there is potential for improved ruminal N efficiency if the individual requirements are better reflected in requirement systems so that feeding programs can be devised to meet each requirement independently.

The RDP supply is assessed using duodenally cannulated animals and can be estimated from the solubility and degradation extent of dietary proteins. The standard for the latter, as set by NRC (2001), is the *in situ* or *in sacco* technique. The challenge with this method is that it requires the use of a ruminally cannulated animal and has significant cost. Samples cannot be assessed in real-time, and thus, it is not always possible to determine RDP content prior to ingredient use. Expected mean values have been derived from existing literature and tabulated (NRC, 2001). These values are commonly used in formulation, although they do not necessarily reflect the true value of the ingredient being fed.

As most ruminal microbes can synthesize all 20 primary AA, the requirement for AA appears to reflect a need for amino acid N rather than a given AA, *per se* (Atasoglu et al., 2003). Work in the 1970's suggested that branch-chain AA (leucine, isoleucine, and valine) or their keto-acid precursors could limit microbial growth (Bryant, 1973).

Addition of branched chain keto-acids that can be converted to branched-chain amino acids were observed to increase fiber digestion, microbial protein production, and microbial growth efficiencies (Russell and Sniffen, 1984). However, subsequent work was equivocal and a comprehensive study at the Univ. of Illinois showed no significant effects on a broad range of biological processes (Klusmeyer et al., 1987).

Recent work with methionine analogs has hinted at a potential methionine or methionine metabolite requirement (Noftsger et al., 2005). But, statistically significant responses have not been observed in all studies (Noftsger et al., 2003). Responses to other AA have not been consistently observed. Therefore, excepting a potential role for methionine analogs in supporting microbial growth, it appears that given an adequate supply of total AA, mixed microbial populations can synthesize the mix of AA needed to support growth and metabolism.

In addition to total AA supply, some populations of microbes appear to require AA in peptide form. Addition of peptides to ruminal fluid has been observed to increase fiber digestion, microbial protein production, and microbial growth efficiencies (Russell and Sniffen, 1984). *In vitro* work has demonstrated responses to individual peptides (Argyle and Baldwin, 1989); however, *in vivo* concentrations of peptides appear to be much greater than those required to maximize growth (Chen et al., 1987). Given the huge number of potential peptides that could exist, it is a daunting task to test all of them for growth stimulation effects, and thus, one cannot rule out the potential for discovery of one or more peptides that would stimulate microbial growth rates in the rumen. However, based on current knowledge, the known AA and peptide requirements of mixed ruminal microbes appear to be much lower than prevailing ruminal concentrations of these substrates when typical dairy diets are fed.

Work by several investigators has clearly identified a requirement for ammonia by mixed ruminal populations and further work associated this requirement with the fiber digesting bacterial population (Bryant, 1973). Thus, failure to meet minimal ammonia requirements could compromise microbial yield and fiber digestibility, leading to a loss in animal productivity. The work of Satter and colleagues (Satter and Slyter, 1974; Roffler and Satter, 1975; Satter and Roffler, 1975) demonstrated that this requirement was met at a ruminal ammonia concentration of approximately 5 mg/dl, which occurred at a dietary crude protein (CP) level of 14%. This requirement could be met by provision of ruminal degradable protein or of non-protein N (NPN) sources such as urea. However, provision of the latter in amounts that resulted in ammonia concentrations greater than 5 mg/dl would not result in incorporation of NPN into microbial protein, i.e. it would be absorbed as ammonia and excreted in urine as urea.

Ruminal ammonia can also be derived from urea that has been transferred from blood to the rumen. The balance of absorption of ammonia from the rumen to blood and blood urea to the rumen determines whether the ruminal NPN balance is positive (ammonia N absorption exceeds urea N influx) or negative (urea N influx exceeds ammonia N absorption). The balance of N across the rumen wall has been observed to be 0 when ruminal ammonia concentrations were approximately 9.5 mg/dl (Remond et al., 2002), and thus, a net influx of N occurs from blood at concentration below that range. This helps buffer ruminal ammonia concentrations, helping prevent a deficiency when low protein diets are fed.

Although ruminal ammonia requirements of 5 mg/dl are well supported by the work of the Satter group, Klusmeyer et al. (1990) observed no loss of fiber digestibility or animal performance when diets with 11% CP were fed to lactating cows, even though ruminal ammonia concentrations were as low

as 2 mg/dl. Thus, there are apparently certain dietary conditions that will support maximal microbial growth and fiber digestibility at ruminal ammonia concentrations much less than the commonly accepted requirement. Undoubtedly, movement of blood urea into the digestive tract is a key component of this ability. Such a mechanism is not represented in the NRC model (NRC, 2001), and thus, it cannot be used to design diets, such as those of Klusmeyer et al. (1990) for use in a production setting.

As noted above, requirements for peptides, AA, and ammonia can be expressed in aggregate as a RDP or N requirement. The RDP requirements have been derived by NRC (2001) and found to generally range from 9.5 to 10.5% of dietary DM. It would appear that such a requirement range is clearly adequate given the accuracy in predicting microbial yield (Figure 3). However, they may also be excessive as there are few observations in the literature where diets less than 9.5% RDP were fed. For example, if the true requirement were 7% RDP, then any diets with greater than 7% RDP would result in equal microbial growth as RDP is not a limiting nutrient. The low protein diets of Klusmeyer et al. (1990) had predicted RDP contents of 6.6 and 5.7% of DM as calculated from NRC (2001), and these diets did not appear to compromise microbial flow to the small intestine or fiber digestibility as compared to a diet with 8.7% RDP. We have recently tested RDP contents ranging down to 7.6% RDP (571 g/day deficit) and found no significant effects of RDP on milk production at 8.8% RDP (280 g/day deficit) and only a trend for a reduction at 7.6% RDP. Thus, it would appear that microbial needs for RDP are clearly met with diets containing 8.8% predicted RDP, likely met with 7.6% RDP, and may be met under some cases at 5.5% predicted RDP. As reduction in RDP feeding could be achieved by reductions in CP feeding, there is significant room for improving the N efficiency of the dairy cow if the current requirement system more accurately reflected ruminal N metabolism.

Ruminally Undegraded Nitrogen

Accurate predictions of ruminally undegraded protein (**RUP**) and microbial protein flows to the small intestine are required to accurately predict duodenal flows of AA. Predictions of these flows have apparently improved over time (Figure 3) reflecting the steady increase in knowledge. Current predictions of RUP and microbial N flows have a prediction error of approximately 20 and 17%, respectively. These prediction errors appear to be partially offsetting as the prediction error for total duodenal flow is approximately 10%. Relative to absorbed protein supply, the latter error reflects the current status of our knowledge, and this is well within the expected biological variation for that measurement.

Unexplained variation in RUP flow presumably arises from the 2 key components of the system: 1) estimates of the intrinsic protein degradation rate for individual ingredients and 2) estimates of the rate of passage. Error in either of these estimates will result in biased estimates of RUP and RDP.

Passage rate equations were developed by NRC (2001) from existing data; however, the accuracy of the equations was not reported. Driving variables were reported as dry matter intake (**DMI**), the percentage of concentrate in the diet, the neutral detergent fiber (**NDF**) content, and the class of feed where the latter were wet forage, dry forage, or concentrate. The NRC committee (2001) recognized the potential affects of particle size and density, functional specific gravity, processing, and the rumen environment as potential factors influencing passage rate and cited the lack of data density relative to these effects as the limitation in considering them in prediction equations. As little data exists relative to these effects on passage rate, it is unlikely that significant improvement in passage rate prediction accuracy will occur in the near future.

Another contributing factor to RUP errors is our limited ability to evaluate the intrinsic rate of protein degradation in the rumen. In sacco techniques are laborious and difficult to standardize across evaluation runs. Some of the variation may be associated with differences in DMI. Bateman et al. (2005) developed a linear adjustment method that significantly improved predictions of non-microbial N flow at the duodenum. This adjustment method if used when reporting RUP values may improve the accuracy of the system.

Current RUP assessment methods also do not account for interactions among ingredients. It is not clear to what extent such interactions influence rates of degradation, but the amount of CP and RDP offered appears to influence RUP content. Klusmeyer et al. (1990) observed a reduction in RUP flow from 28 to 21% of intake N as dietary protein was reduced from 14.5 to 11.0% via replacement of soybean meal with ground corn. In both cases, observed RDP content (72 and 79%, respectively) was significantly greater than the constant 60% predicted for both diets by NRC (2001). Further gains in prediction accuracy for RUP flow will require more accurate methods of assessing both the intrinsic rate of degradation and the rate of passage.

Amino Acid Flow from the Rumen

As microbial protein and RUP represent greater than 80% of the total protein flow to the small intestine, accurate predictions of the flows of these proteins and the AA composition of them is required to predict AA flow from the rumen. Thus, predictions of these AA flows would be subject to the same errors noted above for the parent proteins. However, for diets that meet NRC (2001) requirements, the accuracy of predicting flow of AA to the small intestine appears to be quite good (NRC, 2001). This assessment is supported by the work of Pacheco and Lapierre (2004) that demonstrated reasonably good correlations among

essential AA predicted to be absorbed from the digestive tract by the NRC (2001) and the observed appearance of the same AA in the portal vein. Thus, it would appear that our ability to predict the supply of AA for diets with RDP contents greater than 9% is fairly good. However, for diets with RDP contents less than NRC requirements, predictions will likely be in error given the apparent over-prediction of RDP needs of microbes.

Animal Requirements

Protein that passes from the rumen is digested to AA and peptides and mostly absorbed from the digestive tract, although evidence of absorption of peptides from the omasum exists (Matthews and Webb, 1995). After absorption, the AA are released into portal blood and eventually into general circulation where they are available for all tissues to utilize for maintenance and productive purposes.

Although the animal requires AA, they are not convenient to measure. An estimate of the aggregated AA content of the diet or a feed can be derived by measuring the N content with corrections for a NPN sources in the feed. This will yield no information relative to the AA composition of the feedstuff, but given the knowledge that feed AA generally have a mean N content of 16% allows one to estimate the overall AA content. In a similar manner, microbial protein that can be used by the animal is estimated from the total N content with corrections for NPN components. Absorbed protein that is utilizable by the animal is labeled metabolizable protein.

Current metabolizable protein requirements are calculated as additive functions of that required for maintenance, growth, reproduction, and lactation with minimal consideration of the AA composition required for each of those functions. Maintenance is assumed to remain fixed, regardless of the level of production. Growth, reproduction, and lactation

are derived by multiplying the protein deposited in product by a conversion factor to account for inefficiencies. The partial efficiency assumed by NRC (2001) for milk protein synthesis is 65%, which is significantly greater than the partial efficiency observed for lactating cows (Figure 4). So there are some intrinsic limitations in our current representations of post-absorption N metabolism. Exploration of these limitations provides a framework for future progress. As most of the limitations of our current systems revolve around the aggregation of individual AA requirements into a metabolizable protein requirement, the remainder of the discussion will focus mostly on AA metabolism.

Although some AA can be synthesized by post-absorptive tissues, 10 essential AA (EAA) cannot be synthesized in quantities adequate to supply needs. These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. And some of the nonessential AA can only be synthesized from EAA. Thus the EAA must be obtained from gut absorption in quantities adequate to support inherent losses associated with metabolism and net deposition in tissue and milk protein.

In conducting dose:response experiments to determine metabolizable protein requirements, a variety of different ingredients were used, and thus a range of dietary AA inputs occurred. This approach ensured that individual AA requirements are generally met as any deficiencies would have been observed as a deficiency in metabolizable protein. Of course, there may be dietary conditions where one or more EAA are not provided in adequate quantities, but this should be a rare occurrence.

Although there are probably limited opportunities for increasing production through manipulation of AA content on N sufficient diets, there is likely significant progress that can be

achieved in N efficiency by reducing the input of AA that are provided in excess of needs. This could be achieved by reducing the amount of protein in the diet and supplementing with the AA that are most limiting to production. Such an approach has been adopted by the swine and poultry industries. Identification of the limiting AA can be achieved by trial and error, but given that all 10 EAA must be considered and the large number of ingredients fed to ruminants, testing for limitations of each AA and combinations of them under all dietary conditions would be an overwhelming task. Thus, it is critical to develop an understanding of the metabolism of at least the EAA in order to derive a model that will allow predictions of limitations.

While AA absorption capacity in the intestine is not thought to be limiting, individual AA are digested with varying efficiencies, and the efficiency for any given AA varies by ingredient (Figure 5). Such variation is not reflected in the current NRC (2001), and data appear to be inadequate to derive robust prediction equations. Thus, the constant digestion coefficient assumed by NRC (2001) likely does not reflect all the variation for absorbed AA. Such variation may explain a portion of the variation in absorbed AA relative to predictions observed by Pacheco and Lapierre (2004).

Recent work has suggested that the portal-drained viscera (**PDV**) catabolizes the equivalent of 1/3 of the AA that are absorbed on a net basis from the gut lumen (Hanigan et al., 2004b). This catabolism occurs primarily from blood and is responsive to blood concentrations and, to a lesser extent, blood flow. Hepatic tissue has been found to catabolize another 1/3 of the absorptive supply, and as for PDV, this catabolism is responsive to blood concentrations and blood flow (Hanigan et al., 2004a). But, removal by the splanchnic tissues is not constant for all AA, and thus, the composition of the post-splanchnic AA is altered relative to absorbed AA (Figure 6). Thus, the activity of the splanchnic tissues (PDV plus liver) results in the

clearance of approximately 2/3 of the AA available to the post-absorptive tissues, and most of this clearance and use is derived from blood supplies rather than directly from the absorption stream of AA.

More importantly, these tissues represent the maintenance component of NRC (2001), and their AA clearance rates are not fixed as assumed in that model. As the absorbed supply of protein from the gut lumen increases, clearance of AA from blood also increases. This leads to significant bias in predicting AA availability for productive use in the current NRC (2001) as the maintenance component is not fixed as assumed in that system. Rather, it appears to be a variable component that is a function of the balance of AA supply and use for productive purposes.

Other work has demonstrated that the mammary AA transport activity for several EAA is adjusted to help buffer deficiencies or excesses in AA availability (Bequette et al., 2000). For example, when animals were made histidine deficient, they increased their histidine transport activity more than 40-fold. This buffered the drop in mammary histidine uptake that would have occurred due to declining arterial concentrations. At the same time, the transport activity for other EAA declined, thereby reducing their removal so that the composition of the EAA taken up by the udder remained very similar to the histidine sufficient state.

Variable AA extraction efficiencies by mammary tissue result in variable rates of return of AA to general circulation where they are subject to clearance by the splanchnic tissues. This explains the variable efficiency of N use for milk production as summarized by Hanigan et al. (1998) and is consistent with the apparent linkage of post-splanchnic AA supply and mammary AA use (Bequette et al., 2003). But, it also violates the assumption of a constant efficiency of conversion of AA to product used in construction of the NRC (2001) model.

Work examining the relationships between AA supplied to the mammary and milk protein production has demonstrated that the oft-used first-limiting AA model of milk protein synthesis explains very little variation in milk protein synthesis. Clark et al. (1978) demonstrated that multiple AA could be limiting at the same time. This observation was consistent with the work of Hanigan et al. (2000), indicating that representations of protein synthesis as a function of the first-limiting AA explained very little of the observed variation in a large data set including AA infusions. The data set contained a number of observations where a single AA was infused which introduced independent variation. The first-limiting model appears to work when used with data derived from protein infusion or dietary protein manipulation experiments. The lack of independent variation in AA supply imposed by infusion of complete proteins does not allow identification of the problem. Thus, this approach may appear to work for dietary manipulations, but bias is being introduced into those predictions which reduces model reliability. A more accurate representation of the process is needed for use in ration balancing software.

In summary, predictions of AA flow at the duodenum have improved in accuracy tremendously over the past 20 years and are likely adequate for field use. Additional improvements in supply predictions could be achieved by accounting for interactions among nutrients and ingredients. Absorption of AA from the intestinal tract varies by AA and ingredient, and this variation is not currently represented in our prediction systems. The splanchnic tissues catabolize significant quantities of AA and thus represent a major source of AA loss. Mammary tissue has the ability to change its expression of AA transport capacity to match its needs for AA. Our requirement models need to be updated to account for variable absorption rates of AA, variable use of AA by splanchnic tissues, and variable efficiency of use by mammary tissue. In the absence of such updates, our ability to design

diets to maximize milk production and N efficiency will be hampered.

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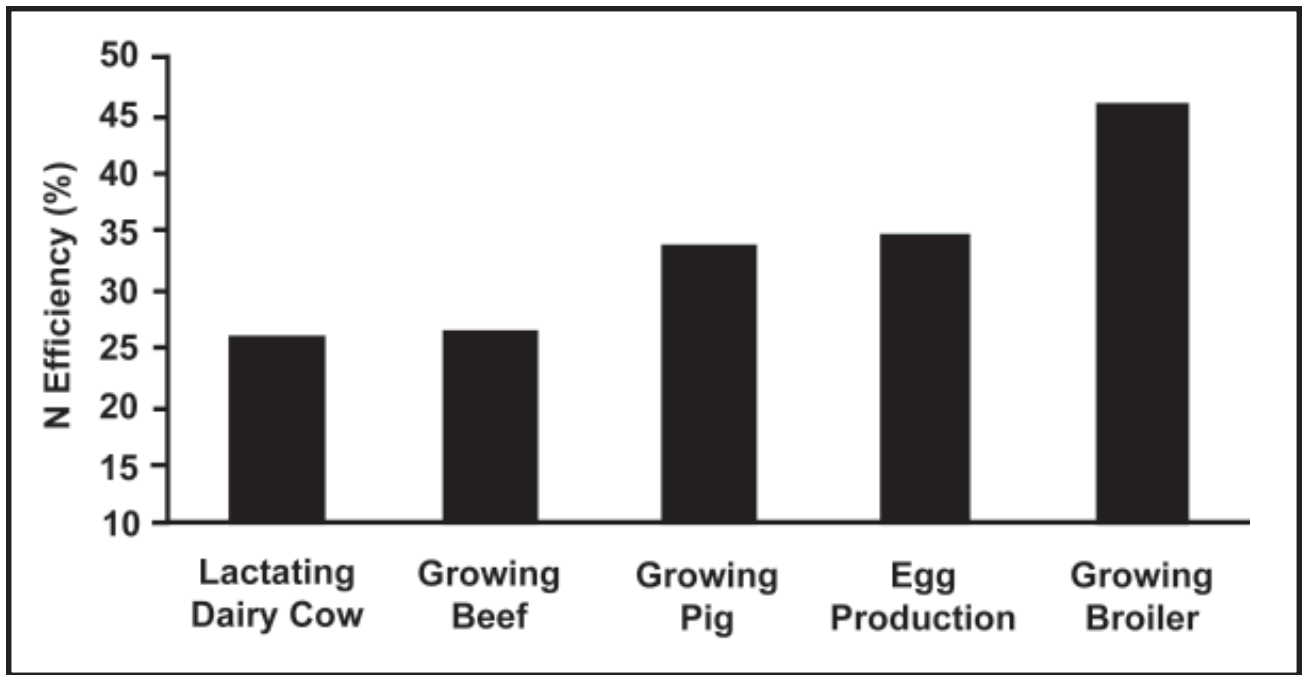


Figure 1. Efficiencies of conversion of dietary nitrogen (N) to product N (meat, milk, and eggs) in various species. Adapted from Bequette et al. (2003).

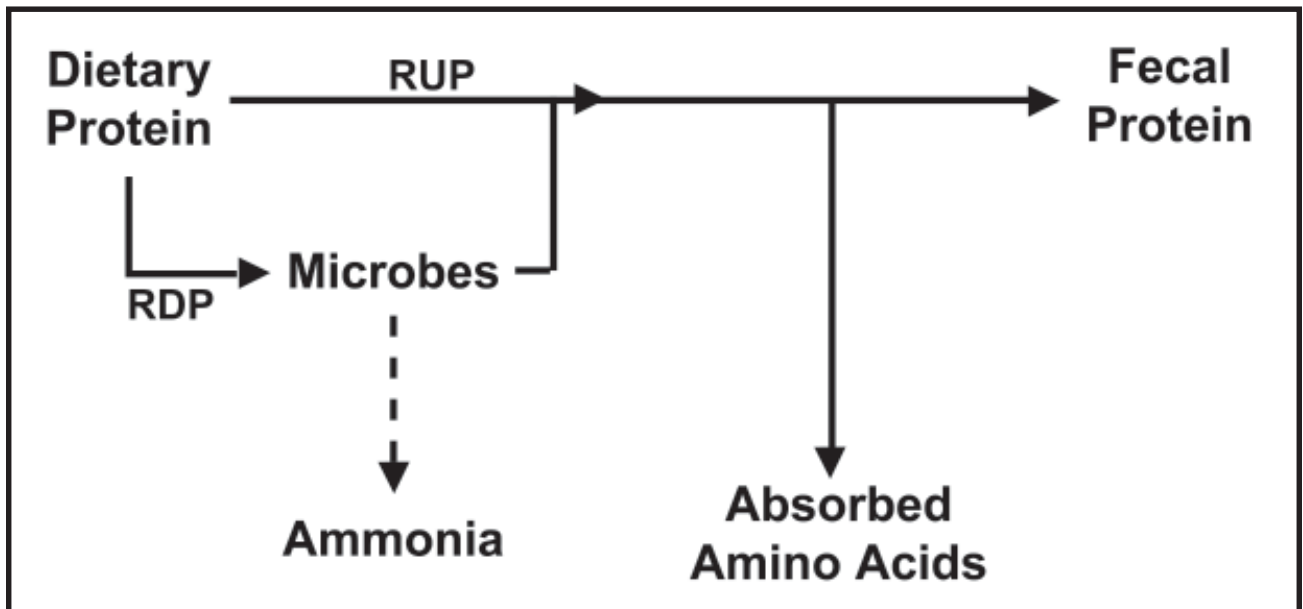


Figure 2. Schematic of protein and nitrogen flows in the digestive tract of cattle. RUP and RDP represent ruminally undegradable and degradable protein, respectively.

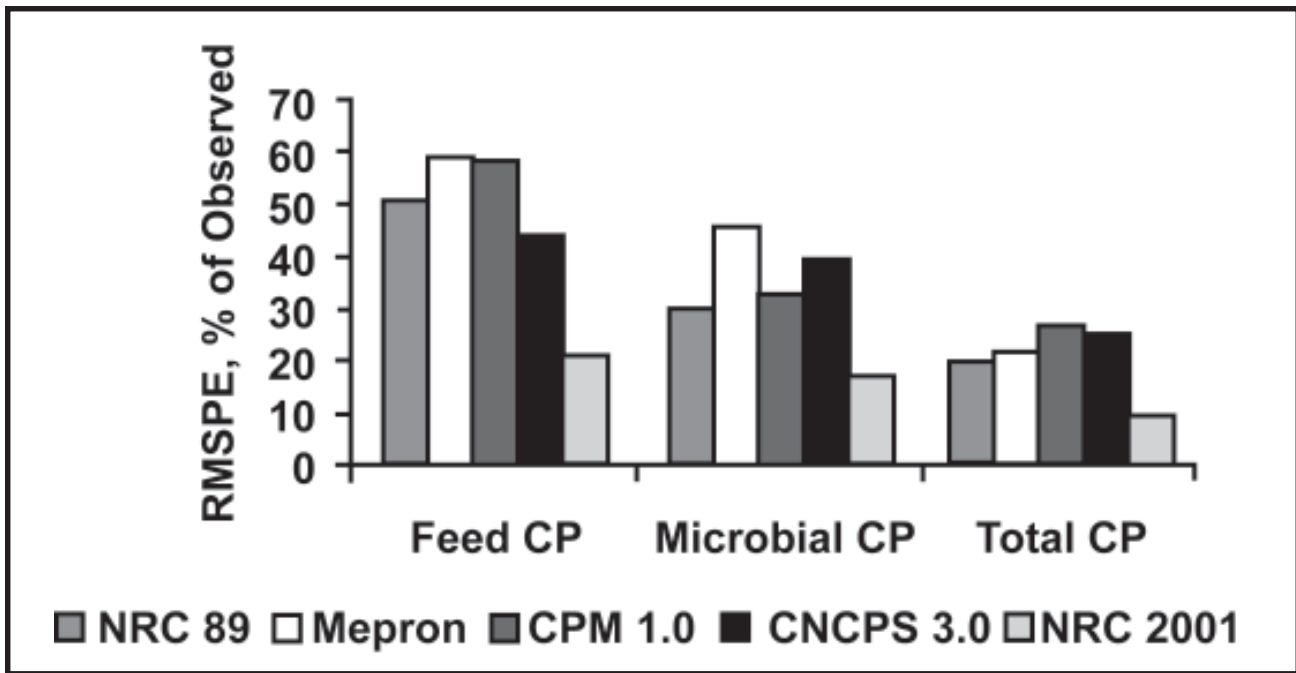


Figure 3. Prediction errors for several dairy models used in the industry. Entities predicted were total, microbial, and feed crude protein (CP) flows to the duodenum of lactating dairy cows. Errors are expressed as root mean square prediction errors (RMSPE). Adapted from Bateman et al. (2001) and NRC (2001). NRC = National Research Council, CPM = Cornell-Penn-Miner program, CNCPS = Cornell Net Carbohydrate and Protein System, and Mepron refers to a commercial model developed by Degussa, Inc (Parsippany, NJ).

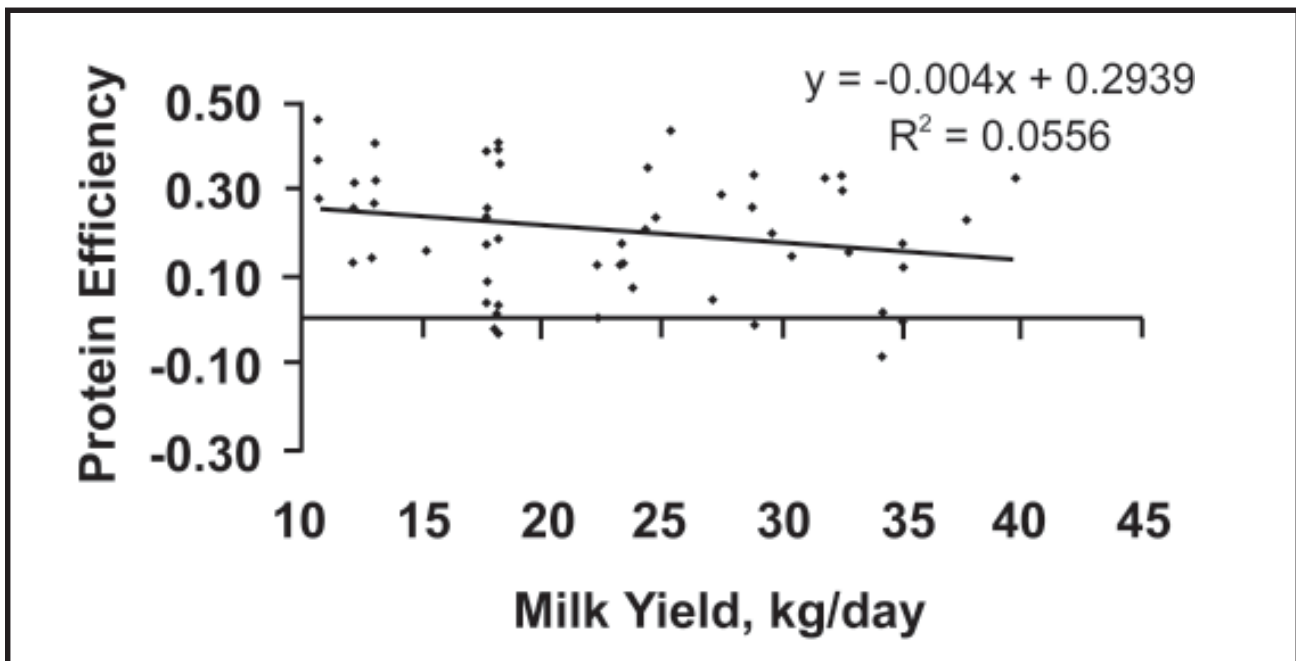


Figure 4. The efficiency of use of infused casein for milk protein synthesis. From Hanigan et al. (1998).

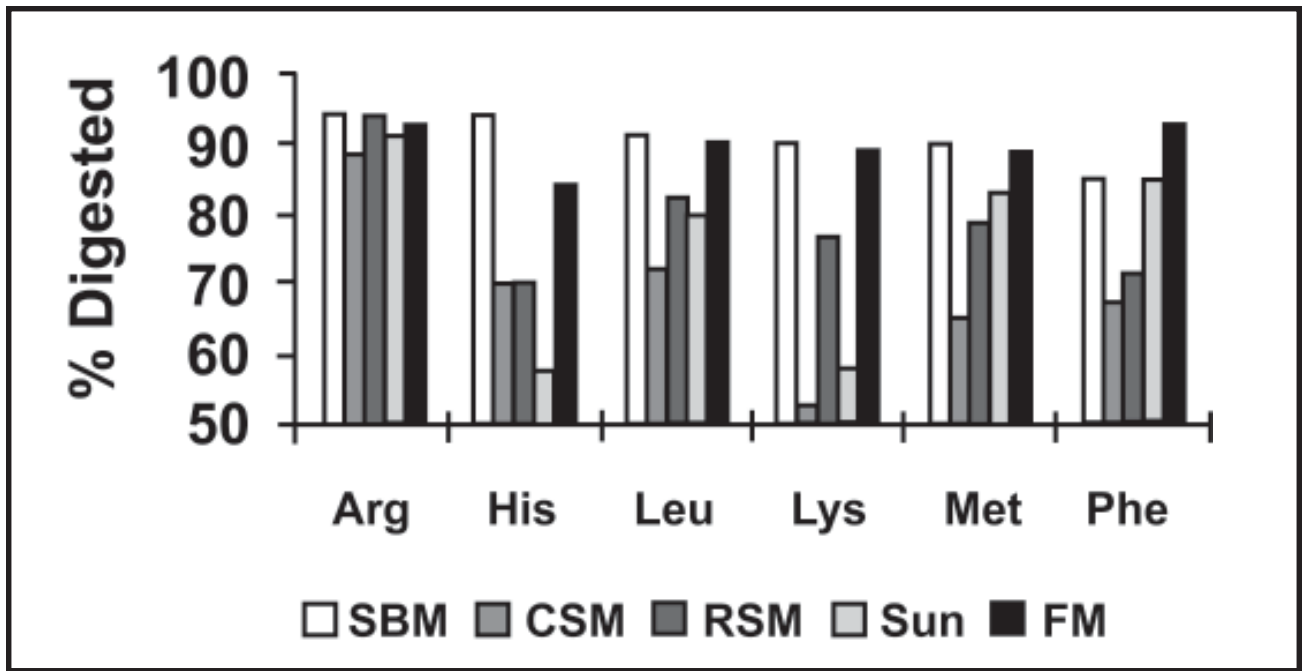


Figure 5. Amino acid digestion of various ingredients in the small intestine. Adapted from Hvelplund and Hesselholt (1987). Arg = arginine, His = histidine, Leu = leucine, Lys = lysine, met = methionine, and Phe + phenylalanine. SBM = soybean meal, CSM = cottonseed meal, RSM = rapeseed meal, Sun = sunflower meal, and FM = fishmeal.

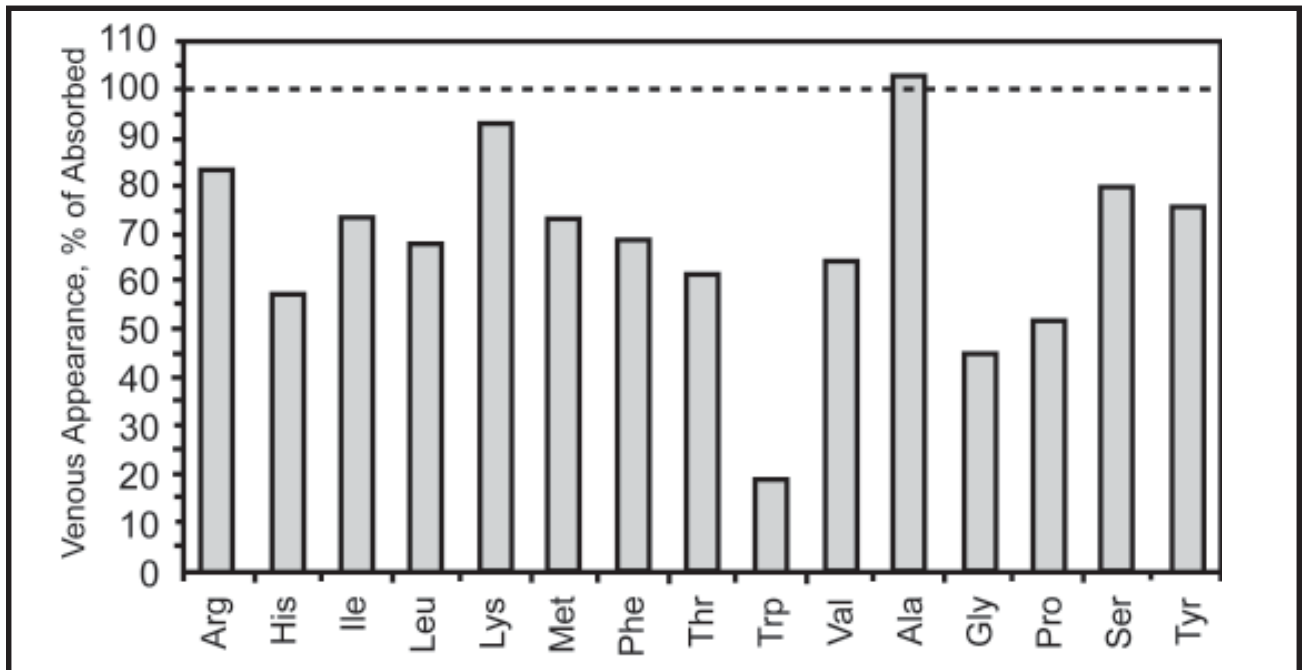


Figure 6. Hepatic vein amino acid appearance as a percentage of that absorbed from the digestive tract. From Hanigan (2005). Arg = arginine, His = histidine, ILE = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = Threonine, Trp = tryptophan, Val = valine, Ala = alanine, Gly = glycine, Pro = proline, Ser = serine, and Tyr = tyrosine.

