

## What's New in the NRC for How We Formulate for Protein in Diets for Dairy Cattle?

Mark D. Hanigan<sup>1</sup>

*Department of Dairy Science  
Virginia Tech*

### Abstract

There were 4 major changes in the protein section of the revised dairy requirement system and a number of more subtle changes to remove inconsistencies and better reflect observed data. The 4 major changes were updates to the RUP/RDP system to remove bias in the predictions, a new microbial protein prediction reflecting the integrative nature of protein and carbohydrate supplies on microbial growth, a new milk protein prediction based entirely on the supply of 5 essential amino acids and energy supply, and adoption of variable efficiency of use of amino acids for maintenance. Other changes included updated predictions of endogenous and reproductive uses, removal of endogenous ruminal outflow from the metabolizable protein supply, correction of stoichiometry errors when converting between protein and free amino acids, and revised estimates of the amino acid composition of feed and body proteins. The revised model has an overall prediction accuracy for milk protein from greater than 25% to just under 15%, and removed the substantial mean and slope bias present in the old system. The use of integrative functions for microbial protein and milk protein force a change in the conceptual approach to defining amino acid, protein, and energy requirements for lactation. It is no longer possible to define a specific requirement for any of those nutrients; the model represents the responses to each, and prevailing economics

will determine the best overall mix of nutrients to use, within biological bounds.

### Changes in the New NASEM Dairy Model

There were a number of substantial changes in the nutrient recommendations model for dairy published by the National Academies of Science, Engineering, and Medicine (NASEM, 2021) as compared to the previous NRC (2001) published by the National Academies of Science (they changed their name). Other speakers have covered the energy, vitamins, and minerals changes. This talk focuses on changes in the protein system.

There were 4 major changes in the protein model and a number of other less-substantial, but still important updates. The major changes are revisions in the ruminally degraded (**RDP**) and undegraded protein (**RUP**) system, microbial growth predictions, the representation of nutrient use for milk protein, and the adoption of variable efficiency of use of amino acids (**AA**) and protein for maintenance and productive functions. Less substantial but still important changes included an updated feed library including the AA composition of feeds, removal of ruminal endogenous protein outflow from the metabolized protein (**MP**) supply, updated RUP digestibility values, correction of stoichiometric errors in conversion between protein and free amino acids throughout, updated

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<sup>1</sup>Contact at: 175 W. Campus Drive, Blacksburg, VA 24061, (540) 231-0967, Email: mhanigan@vt.edu.



amino acid composition of feed and animal proteins, and revised predictions of protein use for maintenance.

### *RDP/RUP and Microbial Protein Prediction Changes*

NRC (2001) predictions of RDP and RUP by were found to be biased with RUP overpredicted and RDP underpredicted for ingredients with greater RUP content (Hanigan et al., 2021). This bias was found to be associated both with passage rate predictions and *in situ* assessments of protein degradation rates. As there was no apparent way to adjust the *in situ* degradation rates, the committee chose to address the problem solely through an adjustment of the passage rates. The revised passage rates for concentrates declined from 6.69%/hr in the old model to 5.28%/hr in the new model. Forage passage rates declined from approximately 5.07%/hr in the old model (dependent on the forage class) to a 4.87%/hr for all forages in the new model. The result was a reduction in the RUP content of all feeds, but the shift was greater for concentrate feeds, particularly those with high RUP content. Because RDP is reciprocal to RUP, the RDP content of all feeds increased with a greater increase for concentrate feeds with high RUP content.

The microbial protein prediction was also revised and is now simultaneously dependent on both ruminally degraded carbohydrate and RDP:

$$\text{Microbial N (g/d)} = \frac{101 + (82.6 \times \text{RDP})}{\left(1 + \frac{0.0939}{\text{RDNDF}}\right) \times \left(1 + \frac{0.0274}{\text{RDSt}}\right)}$$

where RDP, ruminally degraded NDF (**RDNDF**) and ruminally degraded starch (**RDSt**) are all expressed as kg/day. With this equation, RDP, RDNDF, and RDSt are all considered simultaneously with RDP being the main driver, and there was no apparent plateau to the

response. Thus, there is not a natural point of inflection that can be used to set a requirement in classical terms (see the discussion below regarding the conceptual change). The addition or subtraction of RDP can be expected to alter microbial protein flow from the rumen regardless of the starting point. However, this may not be realized if the supply of RDNDF or RDSt are changed in a reciprocal direction, which may occur if starch or NDF are substituted for RDP in the diet. However, it is important to note that the half maximal responses to RDNDF and RDSt are well below normal feeding scenarios. If the diet contains 30% starch, the cow is eating 25 kg/day, and the starch degradability in the rumen is 70%, the RDSt load will be 5.25 kg, resulting in a ratio of 0.0274/RDSt of 0.005. The same is generally true for RDNDF and thus the 2 parenthetical expressions in the denominator essentially become just slightly greater than 1 each and still only slightly greater than 1 when multiplied together. Thus, the microbial N response is generally driven solely by RDP under normal feeding conditions, and the conversion of RDP to microbial CP is 51.6% when corrections are made for converting microbial N to CP and for kg to grams ( $[82.6 \times 1 \text{ kg RDP}] \times 6.25 / 1000 \text{ g/kg} = 0.516$ ).

The general recommendation for minimum RDP in the diet was set at 10% of DM, which is effectively slightly below the old recommendation given that RDP content is now predicted to be greater than in the old model. This recommendation was based on observations of reduced dry matter intake (**DMI**) when an RDP deficiency occurs; however, the point of that occurrence is not clearly defined, but it is known to occur at concentrations less than 10% of DM. From a practical standpoint, one could utilize pen intakes as a response criterium while reducing dietary RDP below 10% of DM to determine the true point of deficiency. This would be expected to result in a loss in microbial

N outflow at a rate of 51.6% of the reduction in RDP intake, but if RDP is expensive relative to RUP and general diet formulation space, the change may be economically advantageous. It certainly won't hurt the cow and it may make the animals more N efficient if dietary CP is also reduced and provided a decline in DMI is avoided.

Because total ruminal protein outflow was predicted without substantial bias previously, the overprediction of RUP in general in that model was offset by an underprediction in microbial protein which is also corrected in the revised model. Thus, the composition of the total protein outflow is altered, reflecting more contribution from microbial outflow and less from RUP, in general.

Microbial protein digestibility was revisited, but it was found to be no different than previous estimates and the proportion of non-metabolizable N was also the same. Intestinal digestibility of the RUP arising from individual ingredients was updated using primarily data from mobile bag-based experiments. Thus, estimates of MP supply are more precise in the NASEM (2021) model, and as noted above, there was a shift in the estimated supply due to removal of consideration of endogenous ruminal outflow as an MP source.

The protein supply changes combined with updated feed library values for the AA composition of the proteins resulted in ruminal AA outflow predictions that contained minimal bias and had greater precision than from NRC (2001) without requiring the empirical adjustments used in the prior work to address the substantial bias problems.

The protein and AA use model was completely updated. The general factorial scheme remains the same with predictions of

use for endogenous secretions in urine and feces, scurf loss, growth, reproduction, and lactation; however, the predictions of each were revisited and updated to reflect newer findings. The 2 major changes were the representation of use for lactation and the consideration of all 10 essential AA (EAA) for all postabsorptive uses.

Use of EAA and protein for milk protein is now integratively driven by the supply of 5 EAA (histidine, isoleucine, leucine, lysine, and methionine), digested energy (DE) arising from non-protein sources, digested NDF, and body weight. The prediction equation is:

$$\begin{aligned} \text{Milk Protein (g/d)} = & -97.0 + 1.68 \times \text{His} + 0.885 \times \text{Ile} + 0.466 \times \text{Leu} + 1.15 \times \text{Lys} \\ & + 1.84 \times \text{Met} + 0.077 \times \text{OthAA} - 0.00215 \times \sum_{a=1}^{N_{\text{EAA}}} \text{EAA}b_a^2 + 10.8 \times \text{DEInp} \\ & - 4.60 \times (\text{dNDF} - 17.06) - 0.420 \times (\text{BW} - 612) \end{aligned}$$

where His, Ile, Leu, Lys, and Met (lysine, isoleucine, leucine, lysine, and methionine, respectively) reflect the absorbed supplies of each (g/day) and OthAA represents the sum of the absorbed supply of the 15 other amino acids (g/day). DEInp is the non-protein digested energy intake (Mcal/day) and dNDF represents the dietary concentration of digested NDF

(% of DM).  $\sum_{a=1}^{N_{\text{EAA}}} \text{EAA}b_a^2$  represented the

sum of the squared supply of each of the EAA present in the equation (His, Ile, Leu, Lys, and Met) denoted by the subscript a.

Because MP is not directly considered in the milk protein equation, there is no MP requirement for lactation in the new model. Additionally, because each of the terms in the above equation are additive, the target level of milk protein output can be achieved by an infinite number of combinations of individual amino acids (AA), DEInp, and dNDF. Therefore, there are no specific lactational "requirements" for individual AA or for digested energy.

## The Concept of a Nutrient Requirement

In previous versions of the NRC Dairy Nutrient Requirement models, the model utilized fixed efficiencies of conversion of dietary nutrients to animal product (1989 and older). This conceptually allowed one to specify the amount of each nutrient that must be provided in the diet to achieve a target level of production; a nutrient requirement. This concept largely remained intact in the 2001 version of the model for conversion of absorbed or metabolized supplies of each nutrient to product, but for energy and protein, the conversion of dietary nutrients to absorbed nutrients was no longer fixed. Thus, requirements were specified in terms of metabolized supplies. The NASEM (2021) model specifies both energy and protein requirements or needs in net energy and net amino acid terms with variable efficiencies of conversion from diet to product in both cases.

The concept of a requirement is not terribly useful when it is reduced to a restatement of the target net product output and varying conversion efficiencies from diet to product. Under this construct, the amount “required” in the diet to achieve the target level of production is not fixed. It varies depending on what other nutrients are fed, and to a certain extent, the environment the animal is in and the animal’s physiological state. The answer to any question of whether a diet meets requirements for energy and amino acids is “It depends”. From a practical standpoint, consider NDF. The cow definitely “requires” fiber in her diet to maintain her health, and NDF is an excellent measure of dietary fiber. So how much NDF is required? It depends on how much the cow is eating and other factors in the diet, such as the amount of starch, the proportion of the NDF provided in forage form, and the length of the forage particles. Thus, we don’t have a specified requirement for NDF, but we do have a range

in NDF content that is generally recognized as adequate which guides daily ration formulation.

So how do we balance diets if we don’t have set targets for nutrient intakes or concentrations? It is easier than you might imagine. If you can roll your mind back to the point in nutrition class where you had finished studying nutrients and began to worry about how much of each feed should be fed, you can unburden yourself of all the classical nutrient requirement concepts. Animals don’t die if they are shorted a bit of energy or protein, they simply reduce production. Only with extended, severe depredation of those nutrients would they succumb. If we exclude that outcome given that it is outside of our production system goals, we can begin to think of the problem in terms of a response surface; how much milk or growth can we expect given the current mix of nutrients and how does that change, or not, when given another mix of nutrients.

For energy, the animal will increase production as energy intake increases. As the drive to produce milk to feed the offspring is greater than the drive to deposit energy in fat stores, one can observe almost constant increases in production output as energy intake increases. But the animal does not have unlimited capacity to make milk, and thus as energy intake increases, a portion of the energy starts to be diverted to body fat stores, and the fractional amount diverted increases as energy intake moves further and further above maintenance use. But, although not unlimited, the capacity to synthesize milk is very high in modern animals, and thus milk production continues to increase as energy intake increases. If you don’t believe that, put a pen of cows on an 85% grain diet. They will produce much more milk than before the diet switch, and they will also gain a lot of body fat. Of course, they will also experience acidosis and subsequent health problems. So,

it is not the capacity to produce milk that is generally holding the animal back. It is the supply of energy and cow health maintenance. And when we have adequate forage in the diet to keep the cow healthy, we also limit her intake of energy due to gut fill and lower digested energy in the forages as compared to grains.

Protein, or more specifically, the amino acids present in the protein are also required to make milk, but the efficiency of conversion from diet to milk protein varies considerably. When diets are fed with low protein concentrations, the animal will sense the relative deficit and reduce catabolic losses in an attempt to preserve enough amino acids to maintain production. They will also recycle more urea N to the rumen where a fraction of it is captured in microbial protein and ultimately contributes to the MP supply. But like, energy, there is not a switch that can be thrown to fully shut off catabolism. Catabolism will be reduced when faced with a deficiency, but so will productive use, i.e. milk protein and growth. But the latter have a higher priority and thus the relative loss in production is generally less than the relative decline in catabolism. The animal will become more efficient at utilizing the available amino acids for production.

We need to think of the feeding problem as a set of relationships between nutrients in the diet and production which are continuous throughout the biological range. There is a relationship between energy supply and milk energy output. There is also a relationship between protein supply and milk protein output, or more accurately a set of relationships among the supply of AA and milk protein output. And there is interplay between energy and protein/AA. Low absorbed protein/AA supply will limit the amount of milk protein that can be made which will limit lactose production (milk protein is a driver of lactose synthesis, and thus the tight link between the two components, and thus milk

energy output. The cow will then divert more energy to body fat and may reduce intake as the extra energy is sensed in the intake regulation center of the brain. In this case, the apparent efficiency of energy conversion to milk will decline as more energy is diverted to fat. The efficiency of conversion of absorbed protein to milk will also increase as the animal reduces catabolic losses in other tissues. Neither change is adequate to totally avoid a loss in production, but it mitigates the size of the loss by reducing catabolism and storage to a greater relative extent than production is decreased.

The problem seems relatively simple with only an energy and a protein response surface, but unfortunately, or perhaps fortunately, given the complexity requires expert help, i. e. professional nutritionists, the surface includes all 10 of the essential amino acids (EAA), some of the fatty acids, and likely many or all of the vitamins and minerals. We avoid having to consider the impact of vitamin and mineral responses by simply feeding at levels that are known to be adequate under normal circumstances. But that still leaves fiber, starch, possibly several fatty acids, and 10 EAA to worry about.

One needs to think of the problem as equivalent to climbing a mountain. There are many routes to move up the mountain, and only at the very peak does the route converge to a single point. Because the cows are operating at production levels less than the peak, there are a number of nutrient combinations that can be used to achieve the target production. Therefore, we don't have specific "requirements" for a nutrient, but we do have ranges of apparent adequacy, and those ranges may vary depending on what else is being fed, the environment, and the physiological state of the cow. Therefore, the new approach is to build a picture of the mountain (the model) that can be used to

define production at all combinations of dietary nutrients. The user simply selects the target level of production and manipulates the diet to derive a diet that will achieve the target, preferably at minimum cost and with minimum environmental impact while maintaining cow health. To the user, it should be irrelevant what the MP, NEL, or dietary fat contents are provided as long as they are in the healthy zone; all the user needs to know is whether the diet will support the target level of production.

This doesn't mean nutritionists are no longer needed. The models are far from perfect and they often lack biological bounds. For example, based strictly on the milk protein equation, it suggests that one could feed a very large amount of pure Lys as the sole nutrient and achieve target production levels. To make 3.3 lb (1500 g) of milk protein for a cow producing 110 lb/day at 3.0% protein, the cow would have to consume 7.27 lb (3300 g) of absorbed Lys. Obviously, this is not biologically possible for several reasons. The other EAA cannot be synthesized from Lys, and thus they would all be severely deficient and limit production. The majority of the Lys would be catabolized to supply energy needs, and the released ammonia N would likely overwhelm the liver, resulting in ammonia toxicity. Animals are able to sense an imbalance among the AA where one or a few AA are provided in great excess relative to the supply of the other AA. When this occurs, the intake center in the brain shuts down intake, forcing the animal to mobilize body protein which helps dilute out the imbalance in blood. So not only would the animal likely be killed by such a Lys intake, she also would not eat it and would still be deficient in the other EAA. So, despite not having a set lactational "requirement" for any of the nutrients, there are normal ranges for each that have been observed to be adequate to support target production levels. Nutritionists use a combination of this nutritional adequacy

knowledge, the model, and common, educated sense to design appropriate diets.

### **EAA Adequacy and Diet Formulation**

With respect to nutritional adequacy of the EAA, a clear boundary for the minimum supply of each of the EAA is the net amount of each exported in secretions and sequestered in body tissue (growth and reproduction) plus the minimum loss to inefficiency as the supply is passed through the system. Analysis of the literature suggested that the maximum, average efficiency of EAA use for maintenance and production across a large number of observations was between 60 and 75%, with the range for His, Ile, Leu, Lys, and Met being between 71 and 75%. These efficiencies can be used to calculate a target for absorbed supplies of each which provides some guidance on minimum needs. However, the literature clearly indicates that some substitution can occur with no loss in production. One could feed more of one EAA or of non-protein DE to offset an apparent deficit of another provided the deficit is not greater than the biologically achievable efficiency of use. The latter is not known with confidence, but the density of observations becomes much sparser when apparent efficiencies exceed 90%, and thus this seems a practical bound for diet formulation. One cannot try to operate all of the efficiencies at 90% as a production loss will definitely occur, but one could likely operate 1 or possibly 2 at 90% provided energy supply is high and one or more of the driving EAA (His, Ile, Leu, Lys, and Met) is operating at less than the target efficiency.

To provide guidance to users, the software generates a table (6.3) which lists the target efficiencies and absorbed supply of each of the EAA given the target production and the predicted supply and efficiencies for each given the diet. One can quickly compare

across the EAA to determine which deviates more (plus or minus) from the target efficiencies and supply and consider possibly adjusting the diet to either improve production or to improve efficiency. Which of the EAA should be added to the diet is not strictly determined by the ranking of efficiencies, but the probability of an EAA limiting production should increase as the relative efficiency increases. So, it makes sense to add those with the greatest efficiencies, but the cost of the additions should also be considered. Because the response per unit of input decreases as the total EAA supply increases (caused by the negative EAA<sub>b2</sub> term), it also makes sense to remove EAA provided in excess based on efficiency and supply targets. This will help improve efficiency of the other EAA. Although the greatest chance of success may be associated with the addition of the EAA with the highest efficiency, another EAA that has lower efficiency but is much cheaper than one with higher efficiency may offer the best economic choice, and the model suggests that would be a logical approach, within reason.

If decisions on dietary supplementation are to be based at least partially on economics, it is important to consider the cost of the addition and the expected return in milk protein output. The slope coefficients for each of the nutrients provide the information needed to make such an assessment. Milk protein is currently worth \$2.30/lb, which equates to \$0.50/100 g. Each gram of additional absorbed Met is predicted to yield 1.84 g of milk protein. To achieve 100 g of extra protein would require 54 g of absorbed Met. To break even, the absorbed Met supply cannot cost more than \$0.01/g which equates to \$4.54/lb and \$8407/ton ( $\$0.50/54 \text{ g Met} * 454 \text{ g/lb} * 2000 \text{ lb/ton}$ ). Making the same calculation for Lys reveals that 87 g of absorbed Lys would be required and the Lys cannot cost more than \$0.006/g or \$5,220/ton. Leu would have to be even cheaper as gaining a 100 g/day

improvement would require 215 g of absorbed Leu, and the cost could be no more than \$0.002/g or \$2,112/ton. To estimate the value of a protein source, one would have to sum the values of the 5 EAA plus the value of OthAA provided by the source.

The same calculation can be undertaken for the value of DE. An additional 9.26 Mcal of DE<sub>Inp</sub> would be required to increase milk protein by 100 g/day if dNDF is held constant. The cost of the non-protein DE would have to be less than \$0.054/Mcal to break even. The group at Ohio State estimates the cost of NEL quarterly from ingredient prices using the Sesame software. The most recent estimate was \$0.068/Mcal of NEL as compared to a 5 year average of \$0.08/Mcal. One must convert this to DE by considering the efficiencies of conversions from DE to ME and finally to NEL which are listed in Table 4.1 of the NASEM 2021 software. Working backward, the efficiency of conversion of ME to NEL is 66%, thus the 5-year average cost per Mcal of ME would be \$0.051 ( $\$0.08/\text{Mcal NEL} * 0.64 \text{ NEL/ME}$ ). The DE to ME conversion is more variable than ME to NEL, but for the 100 DIM example diet that is provided with the software, the conversion efficiency is 87.3%. Thus, the cost of a unit of DE in the diet is approximately \$0.045/Mcal. As protein in the diet is generally more expensive per kg of diet fed, the cost of DE<sub>Inp</sub> may be somewhat less than \$0.045, but even at \$0.045, it is profitable to add energy to the diet, if the cows will consume it, as the cost of adding energy is well below the breakeven cost for milk protein.

Unfortunately, our knowledge of the responses of body frame and reserves gain has not progressed to the point of being able to accurately predict responses given nutrient supplies, and thus we cannot make similar calculations of the cost and benefit of added frame or reserves gain with respect to individual

nutrients. Perhaps this will be addressed in the next version of the Dairy Requirements System.

### Summary

The new NASEM 2021 model contains a number of changes in the protein system that greatly improved accuracy and precision. The bias in RUP and RDP was addressed. A better representation of microbial growth was derived. The digestibility of the RUP in ingredients was updated with more accurate estimates. A number of inconsistencies in calculations of AA supply and use was addressed. Maintenance predictions were improved, and a nutrient response equation for milk protein was added. These changes greatly improved accuracy and precision of the model, but they also necessitate replacement of the concept of nutrient requirements with one of nutritional adequacy and marginal production and economic responses to varying nutrient supplies. It is no longer acceptable to think of the EAA in terms of an order of limitation as laid out by Mitchell and Block (1946). That construct was based on an assumption of fixed efficiencies of conversion of dietary or absorbed AA to product (von Liebig, 1862). Such an assumption has been conclusively demonstrated to be false for AA in ruminants.

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