THE NEXT GENERATION OF PROTEIN AND AMINO ACID REQUIREMENT MODELS

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INTRODUCTION

Providing sufficient, high-quality protein to the world is a universal challenge. The global population is projected to exceed 9 billion people by 2050 (U.S. Census Bureau, 2008), rendering substantial increases in food energy and protein demand inevitable.

Protein is an expensive dietary nutrient representing approximately 42% of the cost of a lactating cow ration in the United States (US; (St-Pierre, 2012). The reduction of dietary protein could result in decreased demand for high-protein ingredients, reduced price of those ingredients, reduced land use change, and diversion of existing cropland use to higher-yielding crops, such as maize instead of oilseeds (Lambin and Meyfroidt, 2011). Nitrogen (N) excretion by cattle is also a source of waste N contributing to environmental pollution (Uuml et al., 2001, Agle et al., 2008). In a survey carried out on 103 large-scale dairies across the US (613 ± 46 cows; 34.5 ± 0.3 kg of milk per cow per day), nutritionists reported feeding diets with 17.8 ± 0.1 % crude protein (CP; (Caraviello et al., 2006). A meta-analysis of 846 experimental diets found a similar mean diet CP content and identified that conversion efficiencies for dietary and metabolizable N (based on NRC, 2001) to milk protein averaged 24.6 % and 42.6 %, respectively (Hristov et al., 2004). Assuming the same intake and diet composition (22.1 kg/d DMI and 17.8 % CP), over a 10-month lactation, the 9 million dairy cattle in the US would excrete

1.3 million metric tons (mmt) of N per year (Livestock, Dairy, and Poultry Outlook: August 2012, LDPM-218, Dairy Economic Research Service, USDA).

Efficiency by which dietary N is converted to milk protein could be increased to approximately 29% if animals were fed to NRC (2001) requirements (approximately 16% CP). If a dietary protein conversion efficiency of 35% could be achieved through reduced dietary CP with no change in milk protein output, excreted N could be reduced to 0.51 mmt per year (nearly 39%).

Some of the potential gains in efficiency require a better representation of amino acid (AA) requirements. Using an aggregation of individual AA requirements as metabolizable protein (MP) will inherently result in overfeeding of most AA to ensure that the limiting ones are provided in adequate quantities. Challenges with the existing NRC model include the assumption that efficiencies of use of AA are fixed (Arriola Apelo et al., 2014); calculation of AA requirements in terms of MP supply; biased estimates of feed and microbial passage to the small intestine (White et al., 2017a); a poor representation of the relationship between energy and protein supply and milk protein output (Hanigan et al., 1998a); and double-accounting for losses of AA associated with milk protein, growth, and maintenance. A more powerful prediction scheme that more accurately represents animal biology, such as the one we are developing, could manifest N efficiencies of 35% or greater in lactating cattle.

The question that will be discussed is whether current knowledge is adequate to construct a model that will allow diets to be constructed to achieve 35% efficiency. Achieving such a goal requires better models given the 29% efficiency that can be achieved with the NRC 2001 model.

FIXED AA USE EFFICIENCIES AND THE IDEAL PROTEIN CONCEPT

The first limiting nutrient and AA concept is based on a hypothesis which has become known as the Law of the Minimum. Sprengel (1828) formulated this concept based on plant growth responses to soil minerals. The original thesis stated that a nutrient can limit plant growth, and when limiting, growth will be proportional to supply. This is strongly supported by volumes of data over the past 175 years. However, Von Liebig (see Paris, 1992 for a translation) subsequently restated and expanded the hypothesis indicating that if a nutrient was limiting growth, responses to other nutrients could not occur (von Liebig, 1862). Mitchell and Block (1946) used von Liebig's extension of Sprengel's thesis to develop the concept of the order of limiting AA, which was described using the analogy of a water barrel with broken staves. Based on this formulation, if any nutrient is limiting milk production, then only the addition of that nutrient to the diet will result in a positive milk yield response, e.g. the single-limiting nutrient paradigm. The ideal protein concept loosely aligns with this framework in that it is assumed there is an ideal AA profile that should be provided to an animal and that profile will remain largely fixed as production levels change.

The ideal protein concept is based on the assumption that there is a fixed, unique set of AA inputs that are required to maximize or optimize production. For that to be true, the efficiencies of use of absorbed AA must remain with respect to one another. For example, if the ideal protein target for methionine is half of that for histidine, doubling the need for methionine should exactly double the need for histidine. If this is true, then one can easily determine which nutrient is most limiting by calculate of the allowable milk yield from each AA, and this can be extended to energy and other required inputs. If the result of that calculation indicates that inadequate histidine is being provided, then one would predict a response to the addition of

histidine, and the same for any other nutrient that is apparently deficient. However, the transfer efficiency of absorbed AA to milk protein is not fixed. Because AA removal from blood is regulated in concert with needs for milk protein synthesis (Bequette et al., 2000), the efficiency of AA transfer from the gut to milk protein is variable. This complicates application of the ideal protein calculations and undermines the concept of a first limiting nutrient. If there are interactions among nutrients or among nutrients and the environment that affect efficiency, the predictions of which nutrient is first-limiting will be faulty.

Additive integration of signals arising from several AA, energy supply in the mammary cells, and hormonal concentrations at the cell surface to set rates of milk protein synthesis (Arriola Apelo et al., 2014, Castro et al., 2016a), and linkage between cell demands for AA and AA transport (Bequette et al., 2000) causes variable efficiency of transfer which undermines the ideal protein concept by potentially creating a range of inputs that can achieve similar efficiency. This also contributes to muted responses to AA as the efficiency declines as the supply increases. If provision of more than one nutrient or hormone can offset the loss or deficiency of another, there is almost an infinite number of combinations of AA, energy substrates, and hormonal concentrations that will result in the very same amount of milk. This concept is demonstrated *in vivo* by the work of Rius et al. (2010a). Addition of any single AA, while all others are held constant, will push milk protein synthesis higher regardless of which is perceived to be “first limiting” (Clark et al., 1978, Hanigan et al., 2000b). A recent report from Liu et al. (2017) clearly demonstrates that the response surface is complex and not well represented by the “Law of the Minimum” when applied to lactational responses to AA. Therefore, current protein and AA requirement models for lactation inappropriately represent the underlying biology, which leads to inflated prediction errors.

METABOLIZABLE PROTEIN SUPPLY AND REQUIREMENT PREDICTIONS

Metabolizable protein represents the true protein available to the cow that is absorbed from the intestine. Metabolizable protein includes digested microbial protein and protein escaping degradation in the rumen (RUP). The NRC (2001) included endogenous protein in this term, but that is not a correct representation as the endogenous protein was derived from absorbed microbial and ruminally undegraded feed protein.

Although we commonly state animal N requirements in terms of MP, the true requirements are for the specific AA resident in that protein. There is not protein requirement per se. Because there is diversity of AA composition in the absorbed protein, stating animal requirements in terms of MP inherently causes requirement over-prediction to compensate for variation in AA composition. This is perhaps most apparent when feeding diets constructed largely from maize products which are inherently low in lysine (Polan et al., 1991). Such a diet could be created to meet MP requirements, but animals may still respond to the addition of a protected lysine source or more protein that also provides lysine to the ration (Vyas and Erdman, 2009).

When these types of data plus other experiments in the literature are subjected to statistical analyses to derive MP requirements, the loss in milk production associated with a specific AA deficiency on some diets forces the statistical algorithm to solve to a higher MP requirement than would be necessary if all of the diets contained a perfect mix of AA. For example, pigs can achieve absorbed muscle protein deposition efficiencies of 85% when fed a diet perfectly matched to their AA requirements (Baker, 1996), as compared to 43% efficiency of conversion of MP to milk protein in lactating cows (Hristov et al., 2004). It is evident that MP requirements are greater than needed to compensate for variable AA supply in ruminants. Animals could successfully be fed a lower MP diet if the AA composition of that diet was better

matched to AA requirements; this was demonstrated by Haque et al. (2012) using diets with less than 13% CP. As the cost of ruminally undegraded protein (RUP) is generally 2 to 3-fold greater than the cost of ruminally degraded protein (RDP; (Knapp, 2009), being able to reduce dietary RUP is of great economic interest.

RUMINAL OUTFLOW OF MICROBIAL AND UNDEGRADED FEED PROTEIN

Errors in predicting milk yield responses to varying MP supply are partially driven by poor predictions of MP supply ((Roman-Garcia et al., 2016, White et al., 2016, White et al., 2017a, White et al., 2017b). The current NRC model over-predicts RUP flow on average by 40 g N/d for a typical animal, and the error increases as RUP flow increases. This indicates fundamental problems in model structure, which consequently contributes to the observed bias in predicting milk production.

Bateman et al. (2005) and Broderick et al. (2010) observed similar problems. Correlation analyses indicated the problem was associated with passage rate (Kp) estimates. The Kp equations used by the NRC (2001) were biased compared to Kp measurements from studies that used indigestible NDF as a marker (Krizsan et al., 2010). A recent study of Kp on forage-based diets also supports bias in prediction of particulate Kp (Gregorini et al., 2015). Attempts to address the problem by refitting the Kp equation or bias adjusting in situ determined degradation rate (Kd) estimates failed to generate any substantial gains in model performance (White et al., 2017a). As a point of comparison, a simple model predicting non-ammonia non-microbial N (NANMN) from N intake using a single slope and intercept had a lower RMSE than the NRC (2001) model even after re-derivation of model parameters. However, such a simple representation would fail to capture known effects of ingredients that are particularly susceptible or resistant to ruminal degradation, and thus do not help achieve our end goals (Cecava et al., 1988, Erasmus

et al., 1992, Cunningham et al., 1993, Erasmus et al., 1994, Abreu et al., 2004).

Despite the clear limitations in the ABC/Kd system used by the NRC, there does seem to be value in the fractionation scheme. Fitting digestion coefficients for each fraction within fairly broad feed categories, grasses, legumes, energy concentrates, plant protein sources, animal protein sources, etc. yielded a system that performed considerably better than the existing NRC system (Figure 1, White et al., 2017a).

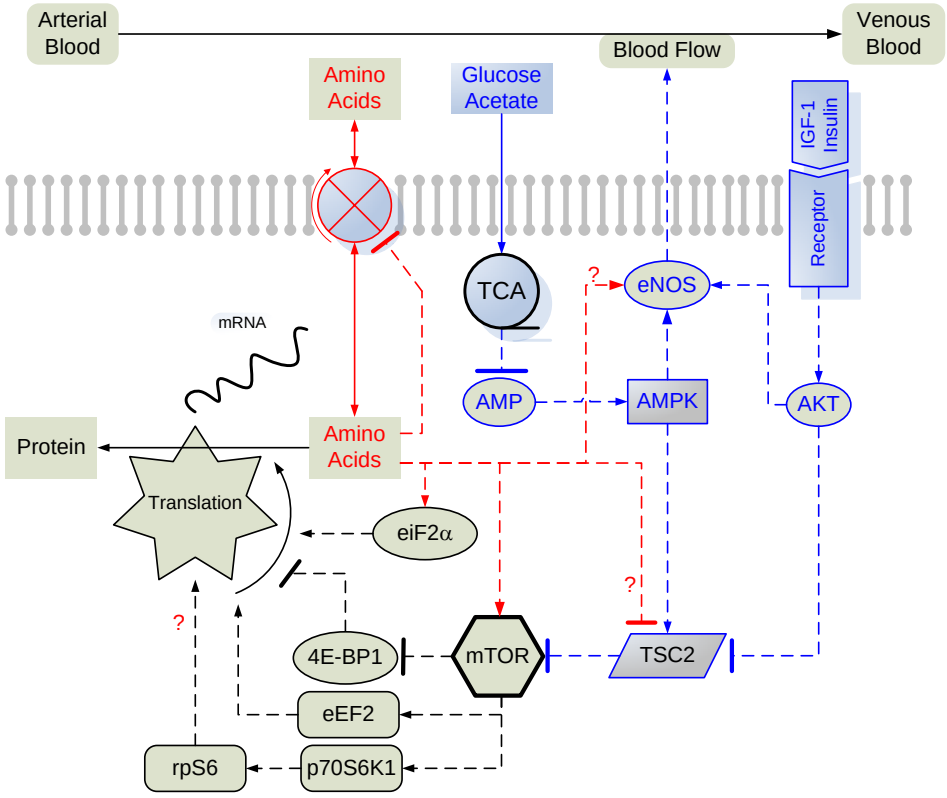

Figure 1 - A partial schematic of the regulation of protein synthesis by mammalian target of rapamycin (mTOR) and associated pathways. TCA=tricarboxylic acid cycle.

RUMINAL OUTFLOW OF AA AND ABSORPTION FROM THE INTESTINE

Predicting AA supply and requirements is a greater challenge for ruminant species than for monogastric species. Amino acid flow from the rumen is a function of the AA content of undigested feed protein, microbial protein, and sloughed digestive tract cells and secretions (NRC, 2001). Additionally, a significant amount of productive N lost to catabolism in the rumen and body can subsequently be recovered by recycling of urea into the digestive tract and recaptured in microbial protein (Reynolds and Kristensen, 2008). The difficulty of predicting each of these entities has greatly hampered our ability to derive AA requirements based on performance data, as is done with swine and poultry.

We have recently adapted an approach used by Maxin et al. (2013) to assess the absorbed supply of each AA from individual dietary ingredients. The method makes use of a 2-h constant infusion of a ^{13}C labelled AA mixture derived from enriched algae to assess the entry rate of each AA. Data are interpreted using a 2-pool model representing slow and fast turnover pools. Fitting the model to the observed rise and fall of isotopic enrichment allows derivation of the rate of turnover of each AA in both pools, and thus the entry rates of each AA into the blood pool. The entry rate is the absorption rate for essential AA and absorption plus synthesis for non-essential and semi-essential AA. The contribution of each test ingredient to AA entry is derived by regressing protein consumed from each of the test ingredients on total entry rates. The AA infusate is introduced into the jugular vein and blood collection for isotope enrichment determinations occurs either upstream from the infusate line or in the contralateral jugular vein. Consequently, measurements can be made with minimal animal preparation.

Entry rate errors of measurement are approximately 10%. Application of this method should allow the generation of a table of AA bioavailabilities for all ingredients comparable to energy or CP

tables. Such a table would greatly improve our knowledge of AA supply from RUP and avoid the current in situ challenges with assigning AA composition to the residue proteins.

USE OF AA BY NON-MAMMARY *TISSUES*

Because AA uptake is a function of supply to the tissue and intracellular demand, any signals that increase protein synthesis will also stimulate AA uptake via increased affinity for the needed AA (Hanigan et al., 2000a, Hanigan et al., 2001, Hanigan et al., 2002, Castro et al., 2016b). However, AA transport is quite complicated, as more than 25 different AA transporters are expressed in epithelial cells and many interactions between AA occur (Calvert and Shennan, 1996, Calvert et al., 1998, Bröer, 2008, Shennan et al., 2008).

Although the primary driving force for AA uptake may be the balance of AA supply and demand, those forces can be modified by the relative supply of other AAs. For example, glutamine concentrations in blood are at least partially determined by metabolism in other tissues, and its transport into mammary tissue is Na-dependent (Calvert et al., 1998). Thus, glutamine can be concentrated within the cell and be exchanged for other AAs that are not Na-dependent. Glutamine also may play a role in maintenance of cell volume, which has been linked to rates of protein synthesis. The interactive influence of glutamine or other non-essential AA on transport of essential AA could be quite varied. It remains uncertain how large these influences are.

Because the tissue can adapt its AA extraction capacity to meet intracellular AA demand, the overall rate of protein synthesis and postabsorptive AA efficiency are functions of a combination of these regulators rather than dictated by the most limiting one. We are currently focusing on a mechanistic representation of the postabsorptive system that we believe will capture the nonlinear response behavior previously observed in the empirical equation of White et al. (under review). The approach utilizes tissue

clearance rates for each AA which can be calculated from arteriovenous difference data for those tissues which can be catheterized and by difference for the remaining tissues. An initial effort using this approach was described in Hanigan et al. (1998). Such a system should provide the basis for a more robust model of postabsorptive AA metabolism that can be used for future field application development.

MILK PROTEIN OUTPUT FROM ENERGY AND AA SUPPLIES

Work to define the mechanisms controlling mammary AA uptake and subsequent use for milk protein has progressed considerably over the past 15 years. Fairly robust mechanistic models of mammary metabolism capture the independent and additive effects of key essential AA, energy supply, and insulin (Hanigan et al., 2000a, Hanigan et al., 2001, Hanigan et al., 2002, Castro et al., 2016b).

A key component of mammary responses to substrate supply and hormonal signals is the intracellular regulation of protein synthesis through intracellular signaling pathways such as the mTOR, Akt, and AMPPK pathways. This signaling integrates information regarding the intracellular supply of several key AA (Appuhamy et al., 2011, Appuhamy et al., 2012), the supply of energy in the cell (Appuhamy et al., 2009), hormonal signals (such as those from insulin) indicating overall animal status (Appuhamy et al., 2011) and likely IGF-1 as well (

Figure 1). These signaling pathways ultimately regulate protein synthesis, thus tying rates of protein synthesis to substrate supply and energy state in the animal.

Observations at the tissue level have shown that liver and gut tissues appear to remove a constant fraction of AA from blood presented in each pass by that tissue. Because mammary tissue does not generally remove more than half of the AA presented to it, there is significant recycling to the gut and liver resulting in additional removal. This is magnified as AA supply increases

relative to energy supply, as the mammary tissue has the ability to change its removal of AA to meet its needs (Bequette et al., 2000). Thus, if mammary tissue is presented with adequate energy supply, it will be able to produce milk near its maximum potential by increasing its AA extraction efficiency. The same will happen if energy is held constant and AA supply is reduced.

Conversely, if the mammary tissue is presented with inadequate energy, it will reduce its use of AA and reduce extraction from blood (Hanigan et al., 2000a). In the former case, AA extraction efficiency is increased, fewer AA are recycled to the liver and gut, and fewer are catabolized. In the latter case, mammary AA extraction efficiency is decreased, more AA are recycled, and catabolism is increased. Consequently, assuming a constant post-absorptive AA efficiency for milk protein synthesis is imprecise.

CONVERSION OF MP TO MILK PROTEIN

A portion of the problem in predicting MP responses is driven by the model assumption that the conversion of MP to milk protein, after subtraction of maintenance use, is a constant 65%. In a summary of literature data, Lapierre et al. (2007) found that the highest conversion efficiency was 43%. Efficiency decreased as milk protein output (and MP supply) increased. In a summary of publications that reported responses to post-ruminally infused casein, Hanigan et al. (1998b) found a similar maximal efficiency of conversion of about 45% with an average conversion efficiency of 22%. Whitelaw et al. (1986) abomasally infused casein at 3 different levels and observed responses at each level with efficiencies of conversion ranging from 40 for the first increment to 15% for the last increment.

If the model was altered to reduce the efficiency of MP use for milk protein synthesis, this could address the slope bias problem displayed in Figure 2, where each unit increase in MP supply resulted in an over-prediction of the change in milk protein output.

It may also introduce bias at the lower MP supply levels as the model predicts production at the lower input levels without bias. Introduction of mean bias associated with the change in MP efficiency would indicate a problem with estimates of the maintenance requirement.

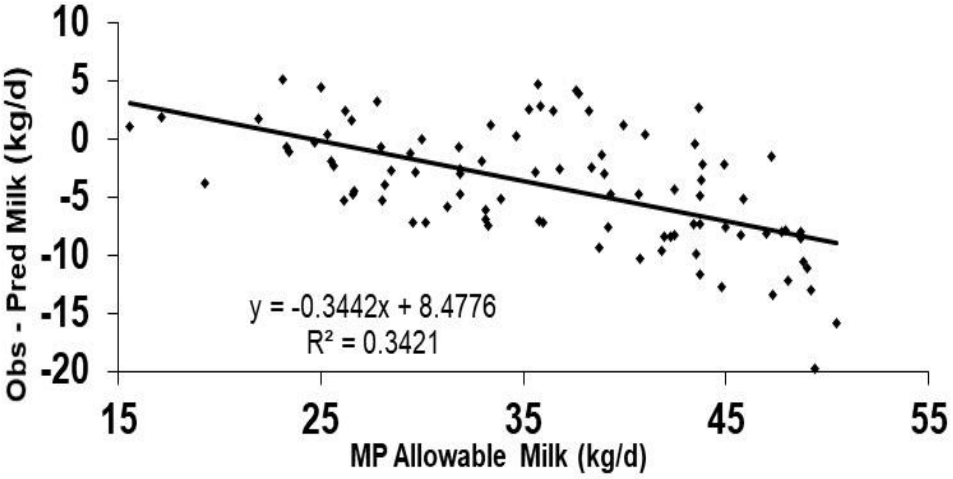

Figure 2 - Residual errors (Obs – Pred) associated with predictions of metabolizable protein (MP) allowable milk yields by the NRC (2001) model. Adapted from NRC (2001).

Although not apparent from

Figure 2, there is also nonlinear bias present in the system (Lapierre et al., 2007). Several efforts are underway to address this variable efficiency problem using empirical or semi-

mechanistic approaches. In the latter, case we can account for the fraction of each EAA that is removed by the portal-drained viscera, liver, mammary, and other tissues. The first 3 tissue beds have been extensively observed using arterio-venous difference techniques allowing construction of simple models of each which capture much of the variation. Use by other tissues is a small fraction of overall EAA use, and thus can be calculated by difference. Preliminary work on these approaches indicates the error of prediction for milk protein can be reduced from 25% to about 17% with equations that are driven by a combination of energy and several amino acids.

Aside from the lack of balancing rations for AAs to achieve greater efficiency, there are additional problems with the NRC (2001) MP requirement system equations. Obviously, one would expect the model to predict requirements at all levels of production with the same precision. For example, if the precision of the system at 25 L of milk/d is plus or minus 15%, then one should expect similar precision at 35 and 40 L of milk. Unfortunately, this is not the case. As demonstrated in

Figure 2, the model over-predicts the amount of MP allowable milk at high levels of production while predicting more accurately at lower levels. Thus, when using the model, one may need to balance for slightly greater amounts of MP in the diet if working with high-producing cows, assuming requirements for maintenance and gestation are correct.

AMINO ACID REQUIREMENTS

Most of the progress that has been made in defining ruminant AA requirements has occurred through the use of catheterized and cannulated animals, allowing the provision of AA post-ruminally (for example Haque et al., 2012). However, this is very intensive and expensive work. To date we have amassed the greatest information on methionine and lysine with histidine results appearing more recently (Rulquin et al., 1993, Korhonen et al.,

2000, Noftsger and St-Pierre, 2003). For the remaining essential AA, we are far from the level of understanding that swine and poultry nutritionists have achieved and are unlikely to achieve that level of understanding in the near future.

Emerging isotope-based methods hold promise in allowing assessment of AA availability from individual ingredients in vivo which will allow construction of a database of true ingredient AA bioavailabilities. Also, there is now significant evidence that individual AA exert independent and additive effects on protein synthesis. As a result, new models must replace the concept of a single-limiting AA with fixed efficiencies of transfer from absorbed to milk protein. Data are now adequate to model the flow of AA from the gut lumen into mammary tissue, thus accommodating variable transfer efficiencies. This approach can also be used to assign losses to production, maintenance, and catabolic processes to avoid the double-accounting problem. Representing milk protein synthesis as an integrated function of the supply of energy and individual AA will provide a more precise biological representation of the effects of those substrates on milk protein output.

CONCLUSION

Rations can be balanced at levels well below 15% CP, probably even below 13%, if we are able to reliably match AA supply with true animal needs. However, current models of AA requirements used in field application programs appear to be incompatible with making such predictions. We are in the process of devising a new prediction scheme that will be a better representation of the biology, and thus should provide much greater accuracy allowing us to achieve N efficiencies of 35% or greater in lactating cattle. It is difficult to determine how much improvement in model accuracy and precisions will be gained by adoption of these changes, but the updated model framework will certainly provide a base for additional improvements and help guide future research efforts.

REFERENCES

Abreu, A., J. E. Carulla, C. E. Lascano, T. Diaz, M. Kreuzer, and H.-D. Hess. 2004. Effects of fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. J. Anim. Sci. 82(5):1392-1400.

Agle, M., A. N. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V. K. Vaddella. 2008. Effect of dietary protein level and degradability and energy density on ammonia losses from manure in dairy cows. Pages 324-324 in J. Dairy Sci. Vol. 91.

Appuhamy, J. A., N. A. Knoebel, W. A. Nayananjalie, J. Escobar, and M. D. Hanigan. 2012. Isoleucine and leucine independently regulate mtor signaling and protein synthesis in mac-t cells and bovine mammary tissue slices. J Nutr 142(3):484-491.

Appuhamy, J. A. D. R. N., A. L. Bell, W. A. D. Nayananjalie, J. Escobar, and M. D. Hanigan. 2011. Essential amino acids regulate both initiation and elongation of mrna translation independent of insulin in mac-t cells and bovine mammary tissue slices. The Journal of Nutrition 141(6):1209-1215.

Appuhamy, J. A. D. R. N., C. T. Bray, J. Escobar, and M. D. Hanigan. 2009. Effects of acetate and essential amino acids on protein synthesis signaling in bovine mammary epithelial cells in-vitro. J. Dairy Sci. 92(e-Suppl1):44.

Arriola Apelo, S. I., J. R. Knapp, and M. D. Hanigan. 2014. Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. J. Dairy Sci. 97(7):4000-4017.

Baker, D. H. 1996. Advances in amino acid nutrition and metabolism of swine and poultry. Pages 11-22 in.Nutrient management of food animals to enhance and protect the environment. E. T. Kornegay, ed. CRC Lewis Publishers, Boca Raton, FL.

Bateman, H., J. H. Clark, and M. R. Murphy. 2005. Development of a system to predict feed protein flow to the small intestine of cattle. J. Dairy Sci. 88:282-295.

Bequette, B. J., M. D. Hanigan, A. G. Calder, C. K. Reynolds, G. E. Lobley, and J. C. MacRae. 2000. Amino acid exchange by the mammary gland of lactating goats when histidine limits milk production. J. Dairy Sci. 83:765-775.

Broderick, G., P. Huhtanen, S. Ahvenjarvi, S. Reynal, and K. Shingfield. 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle - a meta-analysis. J. Dairy Sci. 93:3216-3230.

Bröer, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. Physiol. Rev. 88(1):249-286.

Calvert, D. T., T. G. Kim, J. J. Choung, C. Burns, and D. B. Shennan. 1998. Characteristics of l-glutamine transport by lactating mammary tissue. J. Dairy Res. 65(2):199-208.

Calvert, D. T. and D. B. Shennan. 1996. Evidence for an interaction between cationic and neutral amino acids at the blood-facing aspect of the lactating rat mammary epithelium. J. Dairy Res. 63:25-33.

Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large us commercial farms. J. Dairy Sci. 89(12):4723-4735.

Castro, J. J., S. I. Arriola Apelo, J. A. Appuhamy, and M. D. Hanigan. 2016a. Development of a model describing regulation of casein synthesis by the mammalian target of rapamycin (mtor) signaling pathway in response to insulin, amino acids, and acetate. J. Dairy Sci. 99(8):6714-6736.

Castro, J. J., S. I. Arriola Apelo, and M. D. Hanigan. 2016b. Development of a model describing regulation of casein synthesis by

the mtor signaling pathway in response to insulin, amino acids and acetate. J. Dairy Sci. in press.

Cecava, M., N. Merchen, L. Berger, and G. Fahey. 1988. Effects of dietary energy level and protein source on site of digestion and duodenal nitrogen and amino acid flows in steers. J. Anim. Sci. 66(4):961-974.

Clark, R. M., P. T. Chandler, and C. S. Park. 1978. Limiting amino acids for milk protein synthesis by bovine mammary cells in culture. J Dairy Sci 61(4):408-413.

Cunningham, K., M. Cecava, and T. Johnson. 1993. Nutrient digestion, nitrogen, and amino acid flows in lactating cows fed soybean hulls in place of forage or concentrate. J. Dairy Sci. 76(11):3523-3535.

Erasmus, L., P. Botha, C. Cruywagen, and H. Meissner. 1994. Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs. J. Dairy Sci. 77(2):541-551.

Erasmus, L., P. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75(11):3056-3065.

Gregorini, P., P. Beukes, G. Waghorn, D. Pacheco, and M. D. Hanigan. 2015. Development of an improved representation of rumen digesta outflow in a mechanistic and dynamic model of a dairy cow, molly. Ecological Modelling 313:293-306.

Hanigan, M., J. Cant, D. Weakley, and J. Beckett. 1998a. An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. J. Dairy Sci. 81(12):3385-3401.

Hanigan, M., L. Crompton, B. Bequette, J. Mills, and J. France. 2002. Modelling mammary metabolism in the dairy cow to predict milk constituent yield, with emphasis on amino acid metabolism and milk protein production: Model evaluation. J. Theor. Biol. 217(3):311-330.

Hanigan, M., L. Crompton, J. Metcalf, and J. France. 2001. Modelling mammary metabolism in the dairy cow to predict milk constituent

yield, with emphasis on amino acid metabolism and milk protein production: Model construction. J. Theor. Biol. 213(2):223-239.

Hanigan, M. D., J. P. Cant, D. C. Weakley, and J. L. Beckett. 1998b. An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. J. Dairy Sci. 81(12):3385-3401.

Hanigan, M. D., J. France, L. A. Crompton, and B. J. Bequette. 2000a. Evaluation of a representation of the limiting amino acid theory for milk protein synthesis. Pages 127-144. CABI, Wallingford.

Hanigan, M. D., J. France, L. A. Crompton, B. J. Bequette, J. P. McNamara, and D. E. Beever. 2000b. Evaluation of a representation of the limiting amino acid theory for milk protein synthesis. Pages 127-144 in Modelling nutrient utilization in farm animals. CABI Publishing, Wallingford, UK.

Haque, M. N., H. Rulquin, A. Andrade, P. Faverdin, J. L. Peyraud, and S. Lemosquet. 2012. Milk protein synthesis in response to the provision of an "ideal" amino acid profile at 2 levels of metabolizable protein supply in dairy cows. J. Dairy Sci. 95(10):5876-5887.

Hristov, A. N., W. J. Price, and B. Shafii. 2004. A meta-analysis examining the relationship among dietary factors, dry matter intake, and milk and milk protein yield in dairy cows. J. Dairy Sci. 87(7):2184-2196.

Knapp, J. R. 2009. The good news: Feed costs have declined over the past year. Buckeye News.

Korhonen, M., A. Vanhatalo, T. Varvikko, and P. Huhtanen. 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. J. Dairy Sci. 83(11):2596-2608.

Krizsan, S., S. Ahvenjarvi, and P. Huhtanen. 2010. A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. J. Dairy Sci. 93:5890-5901.

Lambin, E. F. and P. Meyfroidt. 2011. Global land use change, economic

globalization, and the looming land scarcity. Proceedings of the National Academy of Sciences 108(9):3465-3472.

Lapierre, H., G. E. Lobley, D. R. Ouellet, L. Doepel, and D. Pacheco. 2007. Amino acid requirements for lactating dairy cows: Reconciling predictive models and biology. Pages 39-59 in Proc. Cornell Nutrition Conference for Feed Manufacturers. Dept. of Anim. Sci., Cornell Univ., New York.

Liu, G. M., M. D. Hanigan, X. Y. Lin, K. Zhao, F. G. Jiang, R. R. White, Y. Wang, Z. Y. Hu, and Z. H. Wang. 2017. Methionine, leucine, isoleucine, or threonine effects on mammary cell signaling and pup growth in lactating mice. J. Dairy Sci. 100(5):4038-4050.

Maxin, G., D. R. Ouellet, and H. Lapierre. 2013. Effect of substitution of soybean meal by canola meal or distillers grains in dairy rations on amino acid and glucose availability. J. Dairy Sci. 96(12):7806-7817.

Mitchell, H. H. and R. J. Block. 1946. Some relationships between the amino acid contents of proteins and their nutritive values for the rat. J. Biol. Chem. 163(3):599-620.

Noftsger, S. and N. R. St-Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci. 86(3):958-969.

NRC. 2001. Nutrient requirements of dairy cattle, 7th rev. Ed. National Academy Press, Washington, D.C.

Paris, Q. 1992. The von liebig hypothesis. Am. J. Agric. Econ. 74(4):1019-1028.

Polan, C. E., K. A. Cummins, C. J. Sniffen, T. V. Muscato, J. L. Vicini, B. A. Crooker, J. H. Clark, D. G. Johnson, and D. E. Otterby. 1991. Responses of dairy cows to supplemental rumen-protected forms of methionine and lysine. J. Dairy Sci. 74:2997-3013.

Reynolds, C. K. and N. B. Kristensen. 2008. Nitrogen recycling through

the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. J. Anim Sci. 86(14_suppl):E293-305.

Roman-Garcia, Y., R. R. White, and J. L. Firkins. 2016. Meta-analysis of postruminal microbial nitrogen flows in dairy cattle. I. Derivation of equations. J. Dairy Sci. 99(10):7918-7931.

Rulquin, H., P. M. Pisulewski, R. Verite, and J. Guinard. 1993. Milk production and composition as a function of postruminal lysine and methionine supply: A nutrient-response approach. Live.Prod.Sci. 37:69-90.

Shennan, D. B., I. D. Millar, and D. T. Calvert. 2008. Mammary-tissue amino acid transport systems. Proc. Nutr. Soc. 56(1A):177-191.

Sprengel, C. 1828. Von den substanzen der ackerkrume und des untergrundes (about the substances in the plow layer and the subsoil). Journal für Technische und Ökonomische Chemie 3:42-99.

St-Pierre, N. 2012. The costs of nutrients, comparison of feedstuffs prices and the current dairy situation. Buckeye News.

U.S. Census Bureau. 2008. Total midyear population for the world: 1950-2050. U.S. Census Bureau, Washington, D.C.

Uuml, D. R. LLING, H. MENZI, KR, Ouml, T. F. BER, A. NEFTEL, F. SUTTER, P. LISCHER, and M. KREUZER. 2001. Emissions of ammonia, nitrous oxide and methane from different types of dairy manure during storage as affected by dietary protein content. The Journal of Agricultural Science 137(02):235-250.

von Liebig, J. 1862. Die chemie in ihrer anwendung auf agricultur und physiologie. Vol. II. 7. Aufl ed. Friedrich Vieweg, Braunschweig.

Vyas, D. and R. A. Erdman. 2009. Meta-analysis of milk protein yield responses to lysine and methionine supplementation. J. Dairy Sci. 92(10):5011-5018.

White, R. R., Y. Roman-Garcia, and J. L. Firkins. 2016. Meta-analysis of

postruminal microbial nitrogen flows in dairy cattle. Ii. Approaches to and implications of more mechanistic prediction. J. Dairy Sci. 99(10):7932-7944.

White, R. R., Y. Roman-Garcia, J. L. Firkins, P. Kononoff, M. J. VandeHaar, H. Tran, T. McGill, R. Garnett, and M. D. Hanigan. 2017a. Evaluation of the national research council (2001) dairy model and derivation of new prediction equations. 2. Rumen degradable and undegradable protein. J. Dairy Sci. 100(5):3611-3627.

White, R. R., Y. Roman-Garcia, J. L. Firkins, M. J. VandeHaar, L. E. Armentano, W. P. Weiss, T. McGill, R. Garnett, and M. D. Hanigan. 2017b. Evaluation of the national research council (2001) dairy model and derivation of new prediction equations. 1. Digestibility of fiber, fat, protein, and nonfiber carbohydrate. J. Dairy Sci. 100(5):3591-3610.

Whitelaw, F. G., J. S. Milne, E. R. Orskov, and J. S. Smith. 1986. The nitrogen and energy metabolism of lactating cows given abomasal infusions of casein. Br. J. Nutr. 55(3):537-556.