

Does the Concept of Nitrogen Efficiency Make Sense for Dairy Cattle?

M. Van Amburgh, A. Benoit and A. LaPierre
Department of Animal Science
Cornell University

Introduction

In many countries with a significant dairy industry, there is pressure to mitigate carbon and methane and to reduce fugitive nitrogen (N) from dairy production. Fugitive N consists of urinary and fecal N that was not utilized by the dairy cow for productive functions such as milk or tissues and fertilizer N that migrates to soil and plants roots or volatilizes away. Inherently, cattle will always excrete N through fecal and urinary losses; however, the potential to minimize and mitigate these losses through more precise estimations of both ruminal N requirements and post-ruminal amino acid (AA) requirements and supply is significant. Additionally, this precision can help reduce the societal pressure on the dairy industry. Over one-half of all ammonia emissions in the US are sourced from domesticated farm animals due to the presence of ureases in feces that mix with urine to convert urinary urea into volatilized products (Hristov et al., 2011). There is a direct effect of ammonia production and fine particulate matter in the air that can negatively impact human health (Wyer et al., 2022). Further, N has often been considered the primary cause of eutrophication in marine ecosystems (Liu et al., 2024) and reducing excretion would lead to increased water quality. Although still not fully quantified among all conditions and regions, nitrous oxide emissions from cattle manure range from 0.5 to 1.5 percent of total excretion. This means, for every 100 g of N excretion, nitrous oxide emissions are approximately 1 g, which is 273 times more potent as a greenhouse gas compared to carbon dioxide and about 10 times more potent than methane (Nichols et al., 2016; Rivera and Chara, 2021)

The concept of nitrogen efficiency in lactating dairy cattle has been proposed, published, and discussed for many years (Van Vuuren and Meijs, 1987; Dijkstra et al., 2013; Reed et al., 2015). Many concepts previously published on N efficiency focused on the amount of intake N transferred into milk N or productive N (maintenance, tissue, fetus, and milk). In most cases, improving N efficiency can be achieved by partitioning more nutrients to the mammary gland, which increases energy-corrected milk (ECM) yield following the same concept as feed efficiency and dilution of maintenance. As most practicing nutritionists know, it is difficult to change metabolizable energy (ME) supply and milk volume during established lactation or post-peak milk; however, modifications to the form and amount of N being fed are possible and can reduce the amount of excreted N in cattle diets. This approach does not significantly change N efficiency but reduces environmental impact.

Adhering to the classical definition of efficiency, N efficiency can be improved by creating an N deficiency from the diet, as the available N is a scarcer resource and is used where the demand is greatest. The problem with this approach is that high-

producing cattle cannot perform in a deficient state, which would result in the highest efficiencies, so we must work with optimum efficiencies, not maximum efficiencies. This has been discussed previously (Higgs and Van Amburgh, 2016; Van Amburgh and LaPierre, 2022), where the AA requirements were described based on ME supply anchoring the requirements to the energetic state of the animal, whether at fresh, peak, or post-peak milk yield. Most N efficiency calculations for cattle include the non-protein nitrogen (NPN) intake, which confuses the comparisons between ruminant and monogastric species. When evaluating the N efficiencies for pigs and chickens, only the efficiency of use of AA is considered, which makes the comparison biased by the NPN content of ruminant diets. This also suggests that N efficiency might be more variable for a ruminant on a total N basis due to the inability to account for NPN utilization and loss, as well as urea production and recycling.

An efficiency of use of less than one demonstrates that not all N is allocated to a productive use, thus, the difference is what we cannot recover as milk or tissue. Higgs (2014) discussed this during CNCPS v7 development, where it was recognized that after accounting for rumen N requirements and all supplies of AA, the overall efficiency of use of the essential AA (EAA) on a metabolizable protein (MP) basis was 0.73 or 73%. This is reasonably high compared with pigs and chickens and suggests that the difference in efficiency in N utilization by lactating dairy cattle is the metabolism and fate of the NPN. The overall N efficiency when NPN is included is approximately 48% lower or essentially one-half of what we observe on farms or in research studies. In addition, even if we knew the non-essential AA (NEAA) requirements, it is unlikely that the efficiency of the use of EAA could be increased in high-producing lactating dairy cattle because we need to operate at an optimum efficiency, not a maximum. Additionally, we need to recognize that the NEAA is required for optimum energy metabolism and is used for many functions unrelated to N output, including energy substrates not captured in net protein yield. Thus, the opportunity to improve overall N efficiency is to reduce the amount of N excreted in the urine because that demonstrates overfeeding of nutrients to the cow that cannot be used for productive functions, ultimately contributing to excess excretion into the environment.

It is also critical to recognize other end-products of metabolism that can confound our estimations of N utilization, mainly when we apply a reductionist approach to N metabolism where the utilization of N appears intuitive but might not produce a direct outcome. For example, when considering the EAA requirements of lactation in cattle, many calculations are made solely based on the EAA requirements for milk protein synthesis and yield (NASEM, 2021; Lapierre et al., 2005). This is partially true, as EAA, although essential and contributing to the production of milk protein, is functional in the mammary gland outside of milk protein synthesis. Lactose and fat synthesis are EAA-intensive processes that also involve metabolic regulation through protein and enzyme synthesis. When discussing EAA requirements, all metabolic processes in the mammary gland for the yields of milk and milk components are protein synthetic pathways (Bionaz and Looor, 2008; Mu et al., 2021; Osario et al., 2016; Palmquist and Harvatine, 2020) and calculating efficiencies of use for EAA must incorporate all uses of the EAA integrated with diet energy allowable productivity – from a CNCPS perspective this is ME. The study

of Higgs et al. (2023) and review by Reed et al. (2014) suggested the concept of relating N efficiency to the ME supply and energy status of the animal and expands on many of the approaches previously used to improve N efficiency. When considering available ME, all available energy sources, including carbohydrates, lipids, and proteins, should be well described. In the context of nitrogenous compounds, such as AA, many EAA and NEAA are glucogenic, providing necessary precursors for cellular energetics and metabolism. Discrete predictions for EAA supplies have been commonplace in our diet formulation systems, yet, until discrete NEAA supply is fully described, MP will still be used as a proxy of total AA sufficiency, for both protein synthesis and energy metabolism.

Further, the recognition that some EAA can be utilized as signaling mechanisms and are required for the synthesis of most end-products should be prioritized in future models to optimize our predictions for metabolic demand. For example, Li et al. (2019) observed that lysine (Lys) enhanced fatty acid binding protein (FABP) and sterol regulatory element binding protein (SREBP) in bovine mammary epithelial cells, which are vital regulators of milk fat synthesis. Lysine is also part of many carrier proteins that support the synthesis of milk fatty acids (FA). Thus, some Lys will never be available for milk protein synthesis due to these other metabolic demands. This does not mean the mammary gland inefficiently uses Lys or other AA, rather the mammary gland has a requirement for these AA for other metabolic pathways such as lactose and fat synthesis. Ultimately, this leads to efficiencies that are optimums as the cattle cannot operate at a deficiency of EAA or NEAA while partitioning all nutrients to ECM yield. Additionally, the efficiency of use of energy for milk synthesis is improved when balancing for AA. For example, Table 1 shows actual treatment outputs from cows fed a basal diet not balanced for AA and actual treatment outputs from cows fed a diet balanced for Methionine (Met), Lys and Histidine (His). The basal and AA-balanced diets were formulated iso-calorically, but the cows fed the AA-balanced diet had 1.7 kg/d greater ECM yield, suggesting that balancing for AA improved the efficiency of energy use by partitioning more nutrients to the mammary gland for increased milk component synthesis. If calculated correctly, the supply of EAA from escape feed, bacteria, protozoa, and endogenous protein is accounted for to meet the needs for productive functions and can be incorporated into the concept of productive N. This condenses the N for productive purposes into one metric, which can be used to evaluate the diet formulation for N intake relative to the productive N requirements. Consideration of ruminal N requirements is paramount in leveraging a ruminant's ability to optimize productivity. It is likely that we will not make significant improvements to post-ruminal N efficiency without considering the N demand for efficient microbial growth, as the fermentation of carbohydrates and proliferation of microbial supply led to a larger source of AA and energy for cattle.

The ruminal requirements for N have been discussed extensively over the last 30 years (Russell et al., 1992; Chen et al., 1987; Wallace, 1996; Firkins et al., 2007). There are other considerations around the idea of N efficiency, as the cow has two separate metabolic systems operating in synchrony. Rumen microbes require N, in the form of amino or ammonia N sourced from either the diet, protein degradation, or hepatic urea recycling secreted by the salivary glands (Van Kessel and Russel, 1996; Firkins et al., 2007; Hackman and Firkins, 2015). Generally, ruminal N requirements have been

reasonably well described in the CNCPS and applied in a manner that is consistent with good diet formulation practice. However, as the industry looks to reduce protein feeding in cattle, more discrete metabolic demands tend to elucidate themselves as sources of N become scarcer in the rumen. One such demand is the obligate requirement of fibrolytic bacteria for branch chain volatile fatty acids (BCVFA).

Nearly all diet formulation systems that utilize CNCPS provide a rumen ammonia value expressed as a percent of requirement, which is calculated based on the ammonia N requirement of rumen bacteria to digest the amount of fermentable carbohydrates (fCHO). Bacteria have an N requirement to ferment the feed and grow. In a simple approach, bacteria range from 9 to 10% N; thus, to digest 100 grams of fCHO, the model would calculate 0.5 grams bacteria/gram fCHO/h, resulting in 50 grams of bacteria/h. If the bacteria are 10% N, the requirement for growth would be 5 grams of N. If this is scaled up to the total fCHO intake, considering the rate of passage and digestion, the result is calculated by the pool size of fCHO, the grams of bacteria produced, and the N required to allow it to happen. Most of the bacteria in the rumen require either ammonia or amino N to synthesize AA for growth and metabolism (Russell et al., 1992). As characterized in the CNCPS (Russell et al., 1992; Chen et al., 1987), the amylolytic bacteria (starch and sugar utilizers) have an enhancement in growth dependent on the concentration of dietary peptides in the rumen. One of the factors that have impeded the ability to formulate at the lower rumen degradable protein (RDP) is the consideration for more discrete nutrient needs, including BCVFA needs for fibrolytic bacteria (Allison, 1969; Firkins, 2023; LaPierre and Van Amburgh, 2023). As such, a sub-model has been developed that will allow users of the CNCPS to predict any ruminal shortcomings of BCVFA, which, when implemented, should allow for the formulation of diets with lower and more targeted N intake. There has been little focus on the precision of predicting the ruminal N requirements and the minimum N supply in the diet that should be fed and still meet the requirements of the rumen microbes for maximum fCHO digestion and growth. Further refinements of these BCVFA predictions will look to predict what dietary strategies promote the most efficient production of microbial protein in the rumen.

Another consideration when estimating and managing the efficiency of N use in dairy cattle is the amount of ammonia produced, diffused to the mesentery and portal blood, and converted to urea in the liver. This is a continuous process and is a function of the total intake of N in the diet and the pool size of urea N in the blood. On average, dairy cattle intake from about 600 to 870 grams of N per day in a modern dairy diet. Of this, 50% to 70% will be converted to ammonia in the rumen, of which a significant portion will diffuse out of the rumen and be converted to urea in the liver (Reynolds and Kristensen, 2008; Recktenwald et al., 2014). Of the urea production, the amount recycled back to the gastrointestinal tract will range from 40% to 60% in high producing cattle in the range of most N intakes, with the balance being excreted in the milk and urine (Reynolds and Kristensen, 2008). The efficiency of use of the recycled N is a probability function based on the amount of consumed N relative to the demand from fCHO digestion. Given that urea recycling is essentially a function of the relatively constant plasma pool size, the greater the intake of N, the lower the probability of recycled N being captured by bacteria and moving into the AA pool; thus, the efficiency is reduced, and more urea is excreted in

the urine. This is where there is an opportunity to alter this formulation and N supply condition to increase the efficiency of the use of the recycled N. This requires having accurate DMI, appropriate and comprehensive feed chemistry, aNDFom and other fCHO digestibility, and appropriate “safety factors” to account for on-farm management like feed pushup, time budgets, and overcrowding at the feed bunk. Improved feed and feeding management reduce the need for “N safety factors” in the diet and this will increase the efficiency of use of recycled N for microbial protein yield.

One additional factor necessary to monitor the efficiency of N use is urinary N excretion predictions. Any N not utilized in the rumen or in post-ruminal metabolism is excreted in the urine, and although this number cannot be zero, it can be lower than what is observed in most dairy cattle. There are many diets being fed where urinary N excretion is over 200 grams and, in some situations, up to 280 grams per day. On a CP basis, that is 1.25 to 1.74 kg of protein being excreted. The opportunity is to lower urinary N so that it equals the amount of productive N (total protein used for all functions, converted to N basis) – 1:1 productive N to urinary N or achieve a ratio greater than 1 for productive N to urinary N (Table 1). In Table 1, Diets 1 and 2 were diets from Benoit et al (2021) and were the diets fed. Diet 3 is a formulated diet to show the potential to reduce N from the diet, especially rumen available N.

Achieving a 1:1 ratio in most herds results in at least a 40-gram reduction in urinary N excretion with a potential of up to 80 grams based on the starting point, the available alternative feeds, and our knowledge of how low we can formulate rumen N balance. Reducing the urinary N will reduce the potential for ammonia and nitrous oxide production, which are two targets for reduction in many countries around the world to improve air quality and global warming.

Table 1. Intake, production, and excretion parameters among three diets. A base diet and two diets modified from the base – one balanced for amino acids (AA) and increased nitrogen (N) efficiency through AA balancing. The final diet uses the AA balanced diet and reduces N intake by refining the formulation of the ruminal N supplies to reduce excretion. The days in milk are not equivalent among the diet comparisons between the base and modified diets, which explains part of the milk volume difference. For Diet 1, days in milk average 120, whereas for Diet 2 and 3, days in milk are 190.

Input/Output variables	<u>Diet 1</u> Base Diet	<u>Diet 2</u> Balanced for Amino Acids and N efficiency	<u>Diet 3</u> Balanced for reduced urinary N excretion
DMI, kg/d	26.4	26.7	26.6
Milk, kg/d	41.7	39.6	39.6
ECM, kg/d	44.4	46.1	45.9
Milk fat, %	4.10	4.72	4.70
Milk protein, %	3.10	3.37	3.35
ME Allow milk, kg	43.0	43.6	43.6
MP Allow. Milk, kg	43.4	47.3	45.4
Carbohydrate allowable bacteria, g	4,021	4,184	4,210
N allowable bacteria, g	5,019	4,646	4,668
N intake, g/d	684.9	683.5	668.9
Rumen NH ₃ balance, %	146	121	125
Rumen Degradable Protein, % DM	10.5	9.0	9.0
True RDP/Fermentable CHO, g/kg	199	188	178
Productive N, g/d	219	223	223.1
Urine N, g/d	221	215	192.6
Fecal N, g/d	257	258	256.1
Manure N, g/d	478	473	448.7
Productive N/Total N, %	32.0	32.6	33.5
Productive N/Urinary N, %	99.1	103.7	116.1
MP Supply, g/d	2888	3214	3131
MP Required, g/d	2820	2825	2840
MP Met, g/d	78.9	85.0	84.9
MP Met, g/Mcal	1.13	1.18	1.17
MP Lys, g/d	191.0	222.4	221.1
MP Lys, g/Mcal	2.74	3.20	3.05
MP His, g/d	76.0	88.9	86.7
MP His, g/Mcal	1.08	1.19	1.20

References

- Allison, M.J. 1969. Biosynthesis of Amino Acids by Ruminant Microorganisms, *Journal of Animal Science*, Volume 29, Issue 5, November 1969, Pages 797–807, <https://doi.org/10.2527/jas1969.295797x>
- Bionaz M, Looor JJ. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics*. 9:366. doi: 10.1186/1471-2164-9-366.
- Benoit, A. C., P. A. LaPierre, G. D. Mechor, D. M. Barbano, and M. E. Van Amburgh. 2021. Effect of Increasing Monensin Concentration on the Performance of Lactating Dairy Cows Fed Contemporary Diets Proc. Cornell Nutr. Conf. <https://hdl.handle.net/1813/110235>
- Chen, G., J. B. Russell, and C. J. Sniffen. 1987. A Procedure for Measuring Peptides in Rumen Fluid and Evidence that Peptide Uptake Can Be a Rate-limiting Step in Ruminant Protein Degradation. *J. Dairy Sci.* 70:1211-1219.
- Dijkstra, J. *et al.* (2013). Challenges in ruminant nutrition: towards minimal nitrogen losses in cattle. In: Oltjen, J.W., Kebreab, E., Lapierre, H. (eds) Energy and protein metabolism and nutrition in sustainable animal production. Energy and protein metabolism and nutrition in sustainable animal production, vol 134. Wageningen Academic Publishers, Wageningen. https://doi.org/10.3920/978-90-8686-781-3_3
- Firkins JL, Mitchell KE, White AF. 2024. Invited Review: Role for isoacids in dairy nutrition. *Applied Anim. Sci.* 40:466-477.
- Firkins, J.L., Z. Yu, M. Morrison. 2007. Ruminant Nitrogen Metabolism: Perspectives for Integration of Microbiology and Nutrition for Dairy. *J Dairy Sci.* 90:E1-E16.
- Hackmann T.J., J. L. Firkins 2015. Maximizing efficiency of rumen microbial protein production. *Front Microbiol.* 6:465. doi: 10.3389/fmicb.2015.00465.
- Higgs, R. J. 2014. Development of a dynamic rumen and gastro-intestinal model in the Cornell Net Carbohydrate and Protein System to predict the nutrient supply and requirements of dairy cattle. PhD Dissertation, Cornell University, Ithaca, NY.
- Higgs, R. J. and M. E. Van Amburgh. 2016. Evolution of the CNCPS-Development of V7. Pages 125-146 in Proc. Cornell Nutrition Conference. Cornell University, Syracuse, NY.
- Higgs, R. J., L.E. Chase, C.G. Schwab, B. Sloan, D. Luchini, P.A. LaPierre, and M. E. Van Amburgh. 2023. Balancing dairy cattle diets for rumen nitrogen, methionine, or all essential amino acids relative to metabolizable energy. *J. Dairy Science* 106:1826-1836.
- Hristov A.N. 2011. Contribution of ammonia emitted from livestock to atmospheric PM_{2.5} in the United States. *J. Dairy Sci.*, 94:3130-3136
- Lapierre, H. et al. 2005. The route of absorbed nitrogen into milk protein. *Animal Science* 80: 10-22.
- LaPierre, P. Andrew and M.E. Van Amburgh. 2023. Taking the Stink Out of Branched Chain VFAs Capturing Targeted Nutrient Feeding through Modeling Proc. Cornell Nutr. Conf. <https://hdl.handle.net/1813/113588>
- Li N, Zhao F, Wei CJ, Liang MY, Zhang N, Wang CM, et al. Function of SREBP1 in the milk fat synthesis of dairy cow mammary epithelial cells. *Int J Mol Sci.* (2014) 15:16998–7013. doi: 10.3390/ijms150916998

- Liu, X., Beusen, A.H.W., van Grinsven, H.J.M. *et al.* Impact of groundwater nitrogen legacy on water quality. *Nat Sustain* 7, 891–900 (2024). <https://doi.org/10.1038/s41893-024-01369-9>
- Mu T, Hu H, Ma Y, Feng X, Zhang J and Gu Y (2021) Regulation of Key Genes for Milk Fat Synthesis in Ruminants. *Front. Nutr.* 8:765147. doi: 10.3389/fnut.2021.765147
- National Academies of Sciences, Engineering and Medicine. 2021. Nutrient Requirements of Dairy Cattle. Eighth Revised Edition, National Academy Press, Washington, D.C.
- Kristopher L. Nichols, Stephen J. Del Grosso, Justin D. Derner, Ronald F. Follett, Shawn L. Archibeque, Catherine E. Stewart, Keith H. Paustian. 2016. Nitrous oxide and methane fluxes from cattle excrement on C3 pasture and C4-dominated shortgrass steppe. *Agric., Ecosystems & Environ.* 225:104-115,
- Osorio, J. S., J. Lohakare, and M. Bionaz. 2016. Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and post-transcriptional regulation. *Physiol Genomics* 48: 231–256.
- Palmquist, D.L., Harvatine, K.J. (2020). Origin of Fatty Acids and Influence of Nutritional Factors on Milk Fat. In: McSweeney, P.L.H., Fox, P.F., O'Mahony, J.A. (eds) *Advanced Dairy Chemistry, Volume 2*. Springer, Cham. https://doi.org/10.1007/978-3-030-48686-0_2
- Recktenwald, E. B., D. A. Ross, S. W. Fessenden, C. J. Wall, and M. E. Van Amburgh. 2014. Urea-N recycling in lactating dairy cows fed diets with 2 different levels of dietary crude protein and starch with or without monensin. *J. Dairy Sci.* 97: 1611-1622.
- Reed, K. F., D. P. Casper, J. France, E. Kebreab. 2015. Prediction of nitrogen efficiency in dairy cattle: a review. 2015. *CABI Reviews*. <https://doi.org/10.1079/PAVSNNR201510001>
- Reed, K.F., L.E. Moraes, D.P. Casper, E. Kebreab. 2015. Predicting nitrogen excretion from cattle. *J. Dairy Sci.* 98:3025-3035. <https://doi.org/10.3168/jds.2014-8397>.
- 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 (Suppl.):E293–E305.
- Rivera, Julián Esteban and Chará Julian. 2021. CH4 and N2O Emissions From Cattle Excreta: A Review of Main Drivers and Mitigation Strategies in Grazing Systems *Frontiers in Sustainable Food Systems* 5. URL=<https://www.frontiersin.org/journals/sustainable-food-systems/articles/10.3389/fsufs.2021.657936>
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70: 3551-3561
- Van Amburgh, M. E. and P. A. LaPierre. 2022. The Cornell Net Carbohydrate and Protein System Version 7: What is Taking So Long? *Proc. Cornell Nutr. Conf.* <https://hdl.handle.net/1813/111878>
- Van Kessel, J. S. and J. B. Russell. 1996. The effect of amino nitrogen on the energetics of ruminal bacteria and its impact on energy spilling. *J. Dairy Sci.* 79:1237-43. doi: 10.3168/jds.S0022-0302(96)76476-7.
- Van Vuuren, A.M., Meijjs, J.A.C. (1987). Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows. In:

Van Der Meer, H.G., Unwin, R.J., Van Dijk, T.A., Ennik, G.C. (eds) Animal Manure on Grassland and Fodder Crops. Fertilizer or Waste? Developments in Plant and Soil Sciences, vol 30. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-3659-1_2

Wallace, R.J. 1996. Ruminant Microbial Metabolism of Peptides and Amino Acids. *J. Nutr.*, 126:1326S - 1334S.

Wyer, Katie E., David B. Kelleghan, Victoria Blanes-Vidal, Günther Schaubberger, Thomas P. Curran. 2022. Ammonia emissions from agriculture and their contribution to fine particulate matter: A review of implications for human health. *J. of Environ Management.* 323: 116285,