

Is It Needed to Balance a Dairy Ration for Metabolizable Protein If It Is Balanced for Essential Amino Acids and RDP?

H. Lapierre¹, R. Martineau¹, S. Binggeli², D. Pellerin², and D. R. Ouellet¹

¹Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, J1M 0C8; ²Department of Animal Science, Université Laval, QC, Canada, G1V 0A6

INTRODUCTION

Metabolizable protein (MP) supply is a better predictor of milk protein yield (MPY) than crude protein (CP) intake (Huhtanen and Hristov, 2009; Lapierre et al., 2012). Therefore, balancing diets based on MP rather than CP is a first step in improving the efficiency of utilization of nitrogen (N). However, MP supply is an aggregate of individual amino acids (AA): 20 AA are used to synthesize all the proteins in the body, including tissues, hormone, enzymes, milk, etc. Of these 20 AA, the carbon skeleton of 9 of them cannot be synthesized by the animal and should therefore be provided by the digested protein. These essential AA (EAA) include histidine (His), isoleucine, leucine, lysine (Lys), methionine (Met), phenylalanine, threonine, tryptophan, and valine. Ten can be synthesized by the cow and have been categorized as the non-EAA (NEAA): alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine. One AA, arginine, is often classified as an EAA: although it can be synthesized in substantial amount by the cow, its synthesis might be not sufficient to cover the need of a high producing dairy cow (NRC, 2001).

Would we gain in efficiency, if rather than balancing for total MP supply, we were balancing for individual EAA supply? Just like if playing Scrabble, you could ask for specific letters rather than random letters, you would then need fewer letters to write the desired words and would have less “excess” letters with nothing to do with. The cow has to eliminate the AA in excess: these represent an expensive fraction of the ration and furthermore, their removal is done through synthesis of urea, which is excreted in urine and has a negative impact on the environment.

IS IT THE MP OR THE AA SUPPLY WHICH DRIVES MIK PROTEIN YIELD?

From experiments where the supply of a single AA or a group of AA has been changed, it is obvious that changes in MP supply are poorly associated with changes in MPY. Table 1 summarizes a few studies where the

supply of MP has been changed but the MPY has not been affected due to differences in the individual AA supply (they are identified in ***bold-italic*** in Table 1). For example, in Doepel and Lapierre (2010; reference 1 in Table 1), infusion of NEAA to either the control diet (compared with NEAA) or to the EAA infusion (compared with TAA) did not affect MPY, despite the increment in MP supply. Admittedly, the variation of the ratios of NEAA to MP supply was exacerbated by infusions in this study compared to what can be observed with only dietary treatments. However, ratios of NEAA to MP supply can vary substantially between diets. For example, from the database used by Doepel et al. (2004) where the digestive flows of EAA (excluding tryptophan) had been estimated with NRC (2001), the proportion of total EAA relative to MP supply in control treatments varied from 42 to 48% (mean 45.4%). So, a ration supplying 3,000 g/d of MP could provide 1,260 to 1,440 g of EAA, from which the pattern of EAA is also probably affected by the protein source and its AA composition. In Lee et al. (2012; reference 2 in Table 1) and Giallongo et al. (2016; reference 3 in Table 1), inclusion of rumen protected His, Lys, and Met allowed a decrease of 450 g/d of MP supply while maintaining MPY. In Haque et al. (2015; reference 4 in Table 1), when comparing low MP supply plus EAA (MPD+EAA) vs. high MP supply including NEAA infusion (MPA+NEAA), increasing MP supply by 17% had no effect on MPY. So, clearly, it is the supply of EAA and not total MP which is driving MPY. With sufficient knowledge, rations could therefore be balanced for individual EAA and if the requirement of each EAA is met, then, there should be no need to balance further for MP.

In fact, this is currently what is being done in pig nutrition, where they can prepare synthetic diets including large proportions of EAA. This has even lead to a shortage of total N for de novo synthesis of NEAA, which can be alleviated through supply of non protein-N (NPN) in the diet (e.g. Mansilla et al., 2017). Such a shortage of NPN should not happen in dairy rations as rumen degradable



Table 1. Effect of metabolizable protein (MP) and amino acid (AA) supply on milk protein yield (MPY). Bold-italic print indicates the supply of MP was changed but the MPY was not affected due to differences in the individual AA supply.

Reference ¹							
1	Treatment ²	Control	+NEAA	+EAA	+TAA		
	MP supply ³	1413	1661	1761	2061		
	MTPY ⁴	903	868	1031	1083		
2	Treatment	MPA	MPD	MPD+LM	MPD+LMH		
	MP supply	2656	2079	2152	2198		
	MTPY	1130	1010	1100	1140		
3	Treatment	MPA	MPD	MPD+M	MPD+L	MPD+H	MPD+MLH
	MP supply	3142	2638	2705	2685	2706	2762
	MTPY	1270	1130	1150	1170	1180	1230
4	Treatment	MPD+NEAA	MPD+EAA	MPA+NEAA	MPA+EAA		
	MP supply	2280	2263	2638	2634		
	MTPY	1067	1143	1130	1218		

¹1: Doepel and Lapierre, 2010; 2: Lee et al., 2012; 3: Giallongo et al., 2016; 4: Haque et al., 2015.
²NEAA: non-essential AA; EAA: essential AA; TAA: total AA; MPA: MP adequate diet; MPD: MP deficient diet; L: Lys; M: Met; H: His.
³MP supply: g/d (diet + infusion).
⁴MTPY: milk true protein yield, g/d.

protein (RDP) has to be supplied sufficiently to support microbial growth. Indeed, we always have to keep in mind that when balancing a dairy ration we have to feed first the rumen microflora with sufficient RDP and degradable energy and complement that microbial synthesis with appropriate rumen undegradable protein (RUP).

BALANCING RATIONS FOR INDIVIDUAL AA

General considerations

Most of the values available on AA composition of proteins are concentrations obtained after a 21-h or 24-h hydrolysis. It is however acknowledged that a period of 21 to 24 h for the hydrolysis of a protein is a compromise to reduce time and cost related to laboratory analysis. It has been known for a long time that acid-labile AA like Ser and Thr are partially destroyed after their release from the protein during a 24-h hydrolysis (Rees, 1946). On the other hand, because peptide bonds involving the branched-chain AA (BCAA) Ile, Leu and Val are difficult to cleave, a hydrolysis lasting 24 h is insufficient to release all the BCAA (Blackburn, 1968). The AA concentrations obtained with a 24-h hydrolysis are therefore lower than the true concentrations in the analyzed material. This missing portion needs to be quantified because in many models, milk AA composition used to determine

AA requirement is obtained from calculation based on the protein composition of milk and AA composition of each milk protein fraction based on its primary structure. So, when setting up a factorial approach to balance AA supply and requirement, it is not coherent to use in the same calculation, AA composition obtained with one method (24-h hydrolysis underestimating some AA) for all the supply and all the requirements except milk and use a theoretical true AA composition for milk, the major component of requirements. Based on multiple time hydrolyses, corrections factors have been proposed to estimate the true AA concentration of protein from concentrations obtained after a 24-h hydrolysis (Lapierre et al., 2016; Table 2).

The second point recognizes that complete hydrolysis of 1 kg of pure protein should yield ± 1.15 kg of free AA (this factor will vary depending of the AA composition of the protein). Indeed, when a peptide bond is cleaved, one molecule of water is added to each released AA. Therefore, the true protein (TP) content of a protein is not the sum of AA concentrations obtained after a 24-h hydrolysis relative to total CP. First the AA concentrations obtained after a 24-h hydrolysis need to be corrected as described above and their sum has to be related to the CP value multiplied by a factor of approximately 1.15.



Supply

The AA supply is determined using rumen sub-models which first determine duodenal protein flow comprising the microbial protein, the dietary protein which escapes rumen degradation (RUP), and the endogenous protein. Then either a regression approach (e.g. NRC, 2001) or a factorial approach, assigning an AA composition to each fraction (Rulquin et al., 1998), is used to predict duodenal AA flow. When an AA composition was assigned to the microbial fraction, two references were generally used: Le Hénaff (1991) and Clark et al. (1992). However, the microbial populations analyzed in these reviews included only the fluid-associated bacteria (not the particle-associated bacteria and the protozoa). A recent review proposed an AA composition of the microbial duodenal flow corrected for what is missing in a 24-h hydrolysis and including fluid-associated bacteria, particle-associated bacteria, and protozoa in a proportion of 33, 50, and 17%, respectively (Sok et al., 2017). The supply of EAA from microbial protein would increase from 3 (Met) to 30% (Phe) of what would have been predicted with the values of Clark et al. (1992). So far, the AA concentration in the RUP fraction is considered to be similar to the AA concentration of the feed ingredients. Although we know this is not strictly true (e.g. Maxin et al., 2013), there is not enough data to propose an adequate correction. Finally, it is also proposed that the endogenous flow should be removed from the net supply as these proteins are mainly synthesized from AA obtained from arterial supply and should not therefore be considered as a new input of AA to the cow (Lapierre et al., 2006).

Requirement

There are two approaches used to balance dairy rations for AA. In NRC (2001), the subcommittee agreed that “current knowledge is too limited, both for model construction and model evaluation, to put forth a model that quantifies AA requirements for dairy cattle” (i.e., in a factorial approach). They have therefore adopted a proportional approach, similar to the French system (Rulquin et al., 2001). This approach determines the AA requirement based on empirical relationships between observed milk protein concentration or yield relative to the proportion of the AA in MP supply. Although attempts have been made to estimate the requirement for all EAA (e.g. Rulquin et al., 2001; Doepel et al., 2004), current recommendations are proposed only for Lys and Met in NRC (2001), in % of each AA relative to total MP supply.

For the factorial approach, estimation of AA requirement requires to: 1) identify and determine the quantity of TP

Table 2. Correction factors proposed for individual amino acids (AA) to estimate the true AA concentration from concentrations obtained after a 24-h hydrolysis of a protein.

AA	Correction factor
Alanine	1.05
Arginine	1.03
Aspartate and asparagine	1.03
Cysteine	1.23
Glutamate and glutamine	1.06
Glycine	1.09
Histidine	1.02
Isoleucine	1.12
Leucine	1.07
Lysine	1.06
Methionine	1.05
Phenylalanine	1.09
Proline	1.05
Serine	1.13
Threonine	1.08
Tryptophan	1.12
Tyrosine	1.08
Valine	1.11

Adapted from Lapierre et al. (2016).

excreted out of the cow daily (milk, scurf, endogenous urinary and metabolic fecal protein) plus protein accretion as body weight gain or conceptus: these “exported” proteins need to be balanced by an exogenous supply; 2) determine the AA composition of these TP excretions or accretions; and 3) determine the efficiency of utilization of the digested AA to support the protein functions. For example, the CNCPS (Fox et al., 2004; Van Amburgh et al., 2015) and AminoCow (Evonik AG Industries, Hanau, Germany) use the factorial approach. A detailed review integrating knowledge developed in recent years on AA metabolism to update the estimation of MP and AA requirement has been recently presented (Lapierre et al., 2016). Briefly, TP exported for the non-productive functions, are: 1) **scurf** still based on Swanson (1977) but with the ratio of TP/CP = $0.17 \text{ g MP} \times \text{BW}^{0.60}$; 2) **endogenous urinary** excretion estimated at $0.33 \times \text{BW}$ and 3) **metabolic fecal protein** estimated at $[8.5 + 0.1 \times \text{NDF} (\% \text{DM})] \times \text{DMI}$. The TP exported are then



multiplied by their respective AA composition to yield the “exported” AA.

Then the AA exported need to be divided by an efficiency of utilization for each AA. As previously proposed (Lapierre et al., 2007) based on AA metabolism, a single “combined” efficiency should be used for the non-productive functions and the MPY, with an efficiency of 1.0 assigned to the endogenous urinary excretion, because these products are end-products of metabolic pathways. This is also what is proposed in the new French system Systali (Sauvant et al., 2015).

The combined efficiency is calculated as the ratio of exported AA in the TP divided by the digestive flow of AA from MP supply (with the endogenous urinary removed from both components). The biggest challenge is the determination of this efficiency. Traditionally, for the MP, it has been fixed around 0.67 (e.g. NRC, 2001) although it is well acknowledged that the efficiency increases at lower MP supply and decreases at higher MP supply (e.g. Metcalf et al., 2008). For individual AA, the CNCPS first used the ratio of milk AA output on mammary uptake as the efficiency of lactation (Fox et al., 2004). However, considering the distribution of enzymes for AA catabolism and the dominant role the liver plays in the catabolism of Group 1 AA (His, Met, Phe, Trp), they proposed a combined, but still fixed, efficiency of AA utilization which better reflects the utilization of AA by the cow (Table 3; Van Amburgh et al., 2015).

Variable efficiencies of utilization of AA for lactation (Doepel et al., 2004) and then variable combined efficiencies (Lapierre et al., 2007) based on AA supply were proposed. A variable efficiency between AA supply and

AA in exported protein represent that the efficiency of utilization of digested AA decreases with supply: this is represented in Figure 1, from a dietary treatments database (77 treatment comparisons from 35 publications, Martineau personal communication) built similarly to the database described in Martineau et al. (2016). Using the database of studies involving casein infusions (from Martineau et al., 2016) and the database involving dietary treatments, the combined efficiencies have been calculated and are presented in Table 3. They are very similar to the efficiencies obtained at 100% of optimal supply presented by Lapierre et al. (2007) except for Met. More recently, however, it became evident that not only AA supply but also energy supply is affecting the efficiency of utilization of AA. Indeed, European models estimate the efficiency of utilization of MP based on the ratio of MP supply on energy supply (Van Duinkerken et al., 2011) or the ratio of MP supply on dry matter intake (Sauvant et al., 2015). Accordingly, the ratio of AA supply on energy supply gave a better relationship with the efficiency of utilization of AA than the sole AA supply, both for the casein infusion database (Lapierre et al., 2016) and for the dietary treatment database (Figure 2: R² between efficiency and AA supply vs. AA/NE_L supply: Lys 51.3 vs. 61.7; Met 48.8 vs. 58.8, respectively). The relationships between AA in exported proteins and the ratio of AA/NE_L supply are presented in Figure 2 for Lys and Met. This approach would be similar to what is being used in pig nutrition. Also, rather than using the ratio of AA to energy supply, both variables could be used independently to predict the efficiency: the R² increased slightly for Met compared with the ratio (R² between efficiency and AA supply + NE_L supply: 61.9 and 60.3, for Lys and Met respectively).

Table 3. Combined efficiency of utilization of amino acids (AA).

AA	Mean ¹	SD ¹	Minimum ¹	Maximum ¹	CNC-2016 ²	CNCPS v6.5 ³
Histidine	0.85	0.16	0.46	1.33	0.82	0.76
Isoleucine	0.66	0.10	0.40	0.98	0.63	0.67
Leucine	0.67	0.12	0.38	0.97	0.67	0.61
Lysine	0.78	0.12	0.52	1.26	0.72	0.69
Methionine	0.84	0.13	0.49	1.23	0.78	0.66
Phenylalanine	0.58	0.09	0.35	0.86	0.57	0.57
Threonine	0.57	0.09	0.38	0.86	0.57	0.66
Valine	0.65	0.09	0.39	0.98	0.63	0.66

¹Estimated from a database involving dietary treatments (Martineau, personal communication), according to description detailed in the text.

²Estimated from the database of Martineau et al. (2016) with studies involving casein infusion, see Lapierre et al., Cornell Nutrition Conference, 2016 (CNC-2016).

³From Van Amburgh et al. (2015) and Lapierre et al. (2007).



ECONOMIC AND ENVIRONMENTAL IMPACTS

The economic and environmental impacts of balancing dairy rations based on the revised recommendations presented above and using the variable efficiency concept have been evaluated in 3 different Canadian contexts (Binggeli et al., 2017). Although the Canadian and American dairy contexts are very different, the trends observed with the Canadian system would re-

main similar in USA. The 3 Canadian contexts were the Maritimes, Central Canada, and the Prairies, with respective average milk production per cow per year of 8,608, 9,102 and 9,198 kg. The major crops used to balance the rations were, respectively, corn silage, alfalfa silage, mixed silage, grass hay, and barley or corn, for the Maritimes and Central Canada, and corn silage, barley silage, alfalfa silage, legume haylage, grass hay, and barley for the Prairies. They were supplemented

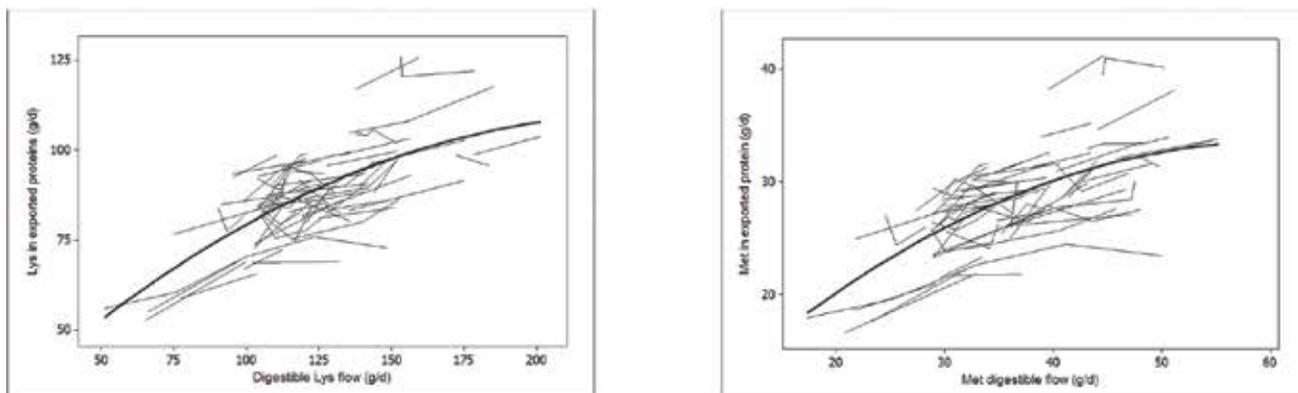


Figure 1. Relation between Lys and Met exported in true proteins and AA supply (both excluding endogenous urinary secretion).

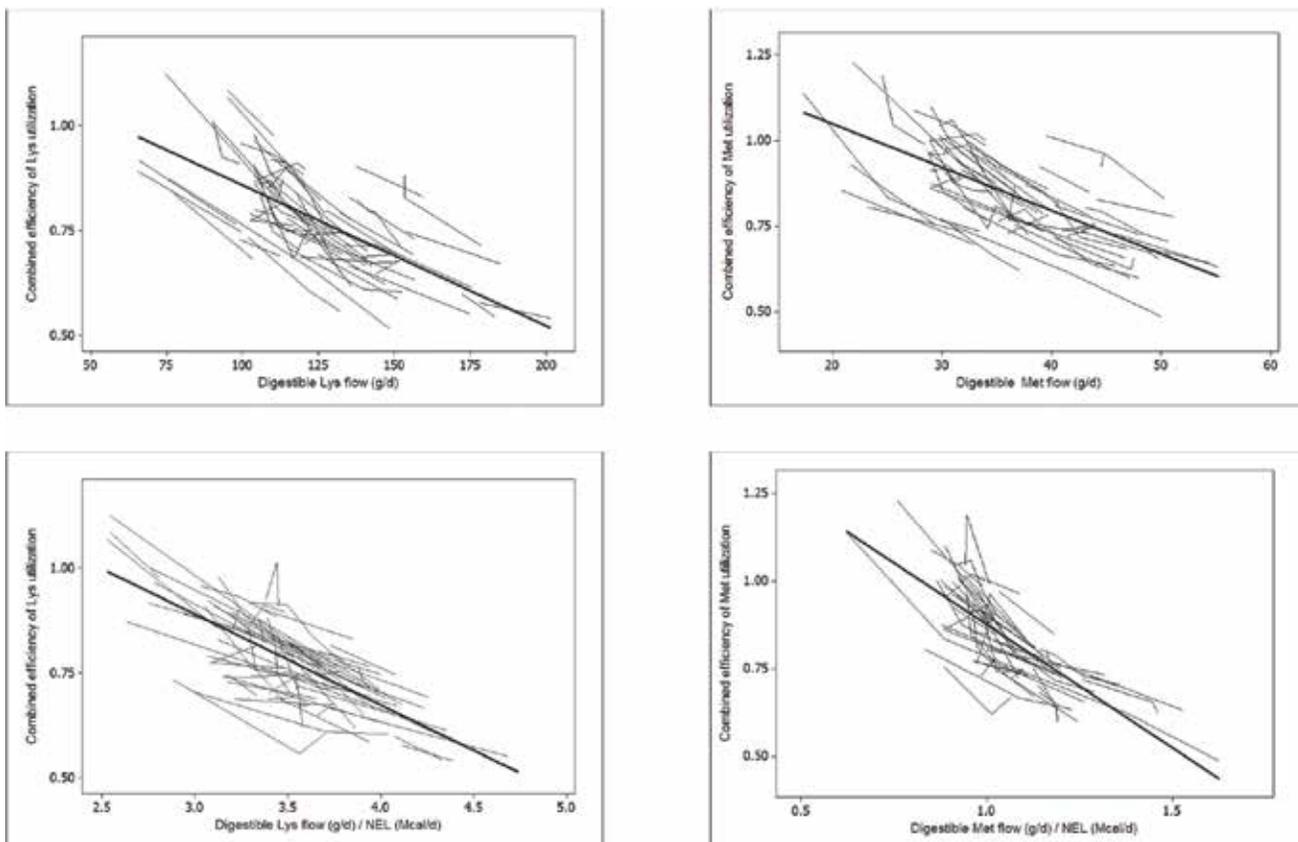


Figure 2. Combined efficiency of utilization of amino acids (AA) relative to digestible flow of AA or to the ratio of digestible flow of AA / NE_L intake, for Lys and Met (both excluding endogenous urinary secretion).

with a choice of five different protein concentrates and four energy concentrates. Data used for simulations were 2010–2014 average data from dairy herd improvement (DHI) and management group databases for the Maritimes and Central Canada, and from Alberta agriculture websites for the Prairies. N-CyCLES, an Excel-based linear programming whole farm model built using NRC (2001) recommendations was used (Pellerin et al., 2017). It was updated with revised AA requirements (maintenance and lactation: as presented above; growth and gestation: NRC beef cattle (2016); efficiency: as described above) and revised supply (microbial CP: White et al., 2016 and RUP: NRC, 2001). Assuming no effect on MPY, the net income (\$CAN per kg of fat- and protein-corrected milk, FPCM) was calculated with diets balanced only for MP using NRC (2001) or balanced with the revised recommendations only for the 3 most likely limiting AA, His, Lys, and Met. The net income (\$CAN per kg FPCM) increased from 0.079 to 0.102, 0.195 to 0.212, and from 0.210 to 0.229 when diets were balanced for individual AA rather than MP, for each region respectively. Also, the N balance (g N per kg FPCM) decreased from 12.2 to 11.9, 13.7 to 12.4, and 13.8 to 13.0) for each region respectively, implying an overall better usage of N on the farm.

Therefore, an AA factorisation model based on the parameters described above would have global positive impacts on dairy farms increasing net income and reducing total CP supply and N excretion, and this across a variety of feeding systems.

REFERENCES

- Binggeli, S., H. Lapierre, E. Charbonneau, D. R. Ouellet, and D. Pellerin. 2017. Economic and environmental impacts of revised amino acid recommendations on Canadian dairy farms. *J. Dairy Sci.* 100 (Suppl2):83-84.
- Blackburn, S. 1968. Amino acid determination. Marcel Dekker Inc., New York, NY.
- Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *J. Dairy Sci.* 75:2304-2323.
- Doepel, L. and H. Lapierre. 2010. Changes in production and mammary metabolism of dairy cows in response to essential and nonessential amino acid infusions. *J. Dairy Sci.* 93:3264-3274.
- Doepel, L., D. Pacheco, J. J. Kennelly, M. D. Hanigan, I. F. López, and H. Lapierre. 2004. Milk protein synthesis as a function of amino acid supply. *J. Dairy Sci.* 87:1279-1297.
- Fox, D. G., L. O. Tedeschi, T. P. Tytlutki, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. Tech.* 112:29-78.
- Giallongo, F., M. T. Harper, J. Oh, J. C. Lopes, H. Lapierre, R. A. Patton, C. Parys, I. Shinzato, and A. N. Hristov. 2016. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. *J. Dairy Sci.* 99:4437-4452.
- Haque, M. N., J. Guinard-Flament, P. Lambertson, C. Mustière, and S. Lemosquet. 2015. Changes in mammary metabolism in response to the provision of an ideal amino acid profile at 2 levels of metabolizable protein supply in dairy cows: Consequences on efficiency. *J. Dairy Sci.* 98:3951-3968.
- Huhtanen, P. and A. N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk n efficiency in dairy cows. *J. Dairy Sci.* 92:3222-3232.
- Lapierre, H., L. Doepel, D. Pacheco, and D. R. Ouellet. 2016. Updating the estimation of protein and amino acid requirements of dairy cows according to biology. Pages:56-68 In Southwest Nutrition Conference Proceedings.
- Lapierre, H., G. E. Lobley, L. Doepel, G. Raggio, H. Rulquin, and S. Lemosquet. 2012. Mammary metabolism of amino acids in dairy cows. *J. Anim. Sci.* 90:1708-1721.
- 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 Dpt. Anim. Science, Cornell University, NY.
- Lapierre, H., D. Pacheco, R. Berthiaume, D. R. Ouellet, C. G. Schwab, P. Dubreuil, G. Holtrop, and G. E. Lobley. 2006. What is the true supply of amino acids for a dairy cow? *J. Dairy Sci.* 89 Suppl 1:E1-14.
- Le Hénaff, L. 1991. Importance des acides aminés dans la nutrition des vaches laitières. in Département des Sciences de la vie et de l'environnement. PhD thesis. Université de Rennes, Rennes, France.
- Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.* 95:6042-6056.
- Mansilla, W. D., J. K. Htoo, and C. F. M. de Lange. 2017. Nitrogen from ammonia is as efficient as that from free amino acids or protein for improving growth performance of pigs fed diets deficient in nonessential amino acid nitrogen. *J. Anim. Sci.* 95:3093-3102.
- Martineau, R., D. R. Ouellet, E. Kebreab, and H. Lapierre. 2016. Casein infusion rate influences feed intake differently depending on metabolizable protein balance in dairy cows: A multilevel meta-analysis. *J. Dairy Sci.* 99:2748-2761.
- Maxin, G., D. R. Ouellet, and H. Lapierre. 2013. Ruminant degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distillers grains. *J. Dairy Sci.* 96:5151-5160.
- Metcalfe, J. A., R. J. Mansbridge, J. S. Blake, J. D. Oldham, and J. R. Newbold. 2008. The efficiency of conversion of metabolizable protein into milk true protein over a range of metabolizable protein intakes. *Animal* 2:1193-1202.
- NRC. 2001. Nutrient Requirements of dairy cattle. 7th rev. ed. The National Academies Press, Washington, DC.
- NRC. 2016. Nutrient requirements of beef cattle. 8th rev. ed. The National Academies Press, Washington, DC.
- Pellerin, D., E. Charbonneau, L. Fadul-Pacheco, O. Soucy, and M. A. Wattiaux. 2017. Economic effect of reducing nitrogen and phos-



- phorus mass balance on Wisconsin and Québec dairy farms. *J. Dairy Sci.* In Press.
- Rees, M. W. 1946. The estimation of threonine and serine in proteins. *Biochem. J.* 40:632-640.
- Rulquin, H., J. Guinard, and R. Vérité. 1998. Variation of amino acid content in the small intestine digesta of cattle: Development of a prediction model. *Livest. Prod. Sci.* 53:1-13.
- Rulquin, H., R. Vérité, and J. Guinard-Flament. 2001. Acides aminés digestibles dans l'intestin. Le système AADI et les recommandations d'apport pour la vache laitière. *Prod. Anim.* 14:265-274.
- Sauvant, D., G. Cantalapiedra-Hijar, L. Delaby, J. B. Daniel, P. Faverdin, and P. Nozière. 2015. Actualisation des besoins protéiques des ruminants et détermination des réponses des femelles laitières aux apports de protéines digestibles dans l'intestin. *INRA Prod. Anim.* 28:347-368.
- Sok, M., D. R. Ouellet, J. L. Firkins, D. Pellerin, and H. Lapierre. 2017. Amino acid composition of rumen bacteria and protozoa in cattle. *J. Dairy Sci.* 100:5241-5249.
- Swanson, E. W. 1977. Factors for computing requirements of protein for maintenance of cattle. *J. Dairy Sci.* 60:1583-1593.
- Van Amburgh, M. E., E. A. Collao-Saenz, R. J. Higgs, D. A. Ross, E. B. Recktenwald, E. Raffrenato, L. E. Chase, T. R. Overton, J. K. Mills, and A. Foskolos. 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. *J. Dairy Sci.* 98:6361-6380.
- Van Duinkerken, G., M. C. Blok, A. Bannink, J. W. Cone, J. Dijkstra, A. M. Van Vuuren, and S. Tamminga. 2011. Update of the Dutch protein evaluation system for ruminants: The DVE/OEB2010 system. *J. Agric. Sci.* 149:351-367.
- White, R. R., Y. Roman-Garcia, and J. L. Firkins. 2016. Meta-analysis of post-ruminal microbial nitrogen flows in dairy cattle. II. Approaches to and implications of more mechanistic prediction. *J. Dairy Sci.* 99:7932-7944.

