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Review: Impact of protein and energy supply on the fate of amino acids from absorption to milk protein in dairy cows

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*Making dairy farming more cost-effective and reducing nitrogen environmental pollution could be reached through a reduced input of dietary protein, provided productivity is not compromised. This could be achieved through balancing dairy rations for essential amino acids (**EAA**) rather than their aggregate, the metabolizable protein (**MP**). This review revisits the estimations of the major true protein secretions in dairy cows, milk protein yield (**MPY**), metabolic fecal protein (**MFP**), endogenous urinary loss and scurf and associated AA composition. The combined efficiency with which MP (**Eff_{MP}**) or EAA (**Eff_{AA}**) is used to support protein secretions is calculated as the sum of true protein secretions (MPY + MFP + scurf) divided by the net supply (adjusted to remove the endogenous urinary excretion: **MP_{adj}** and **AA_{adj}**). Using the proposed protein and AA secretions, Eff_{MP} and Eff_{AA} were predicted through meta-analyses (807 treatment means) and validated using an independent database (129 treatment means). The effects of MP_{adj} or AA_{adj}, plus digestible energy intake (**DEI**), days in milk (**DIM**) and parity (primiparous* v. *multiparous), were significant in all models. Models using (MP_{adj}, $MP_{adj} \times MP_{adj}$, DEI and DEI × DEI) or (MP_{adj}/DEI and MP_{adj}/DEI × MP_{adj}/DEI) had similar corrected Akaike's information criterion, but the model using MP_{adj}/DEI performed better in the validation database. A model that also included this ratio was, therefore, used to fitting equations to predict Eff_{AA}. These equations predicted well Eff_{AA} in the validation database except for Arg which had a strong slope bias. Predictions of MPY from predicted Eff_{MP} based on MP_{adj}/DEI, MP_{adj}/DEI × MP_{adj}/DEI, DIM and parity yielded a better fit than direct predictions of MPY based on MP_{adj}, $MP_{adj} \times MP_{adj}$, DEI, DIM and parity. Predictions of MPY based on each Eff_{AA} yielded fairly similar results among AA. It is proposed to ponder the mean of MPY predictions obtained from each Eff_{AA} by the lowest prediction to retain the potential limitation from AA with the shortest supply. Overall, the revisited estimations of endogenous urinary excretion and MFP, revised AA composition of protein secretions and inclusion of a variable combined Eff_{AA} (based on AA_{adj}/DEI, AA_{adj}/DEI × A_{adj}/DEI, DIM and parity) offer the potential to improve predictions of MPY, identify which AA are potentially in short supply and, therefore, improve the AA balance of dairy rations.*

Keywords: efficiency, ration, formulation, requirement, nitrogen

Implications

To improve the formulation of dairy rations, allowing a reduction of crude protein intake, feeding costs and nitrogen excretion into the environment, the current review proposed revisited estimations of daily true protein secretions in dairy cows and associated amino acid composition. A good prediction of milk protein yield was obtained using the predicted combined variable efficiency of utilization of absorbed amino acids based on the ratio of absorbed amino acids/digestible energy intake, days in milk and parity. This approach could help to identify which amino acids are in short supply and, therefore, improve the amino acid balance of dairy rations.

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Introduction

With an overall objective of increasing the sustainability of dairy farms, optimizing the efficiency of utilization of protein without compromising productivity becomes a must for dairy nutritionists. Emphasis is often put on the poor efficiency of utilization of N by dairy cows to produce milk protein (milk N/N intake) averaging, for example, 24.7 ± 4.1% and 27.7 ± 3.6% in 736 North American and 998 North

European treatment means, respectively (Huhtanen and Hristov, 2009). However, human-edible feed conversion efficiency (**heFCE**), calculated as the ratio of human-edible output/human-edible input, has been proposed to better evaluate the contribution of animal production to the human food chain (e.g. Wilkinson, 2011; Ertl *et al.*, 2015). In this context and considering protein, Wilkinson (2011) concluded that dairy cows were offering the most efficient animal production system in the United Kingdom; Ertl *et al.* (2015) reported heFCE for protein varying from 0.5 to slightly more than 2.0 in commercial dairy farms in Austria, whereas Broderick (2018) calculated heFCE varying between 1.4 and 2.1, depending of the production context from different countries. Therefore, dairy cows can make a valuable contribution to the human food chain with a high heFCE for protein.

Improving the overall efficiency of N utilization still remains, however, a target due to its dual impact on reducing both feeding cost and environmental impact. Dijkstra *et al.* (2013a) suggested that focusing on an optimal supply of rumen-degradable protein and optimizing the efficiency of utilization of absorbed amino acids (**AA**) for milk protein synthesis would be the potential strategies available for improving N efficiency. To improve AA recommendations for dairy rations, three major points need to be tackled: (1) quantify the net supply of AA; (2) assess the fate of absorbed AA (for which functions are they used for?) and (3) determine with which efficiency the absorbed AA are used to support the identified functions.

The current review focuses on the two latter points: revisiting true protein (**TP**) secretions and associated AA secretions and identifying the major factors affecting the efficiency of utilization of absorbed AA ($\mathbf{Eff_{AA}}$). We will also evaluate if predicted efficiency of utilization of metabolizable protein (**MP**) supply and Eff_{AA} are sufficiently robust to predict milk true protein yield (**MPY**). To simplify the review, only secretions and not accretions are included in AA demand: the cows are, therefore, considered at constant body weight (**BW**) and not in gestation.

Updates of true protein and amino acid secretions

To follow the fate of absorbed AA, the quantification of AA secreted into milk protein but also on AA 'lost' by the cow in endogenous secretions found in scurf, urine and feces is required. These endogenous AA losses as MPY remove AA irreversibly from the free AA pool. On a net basis, these exported AA need to be replaced on a timely basis by a minimal equivalent flow of digested AA: this predicted 'replacement' constitutes the basis of MP and AA recommendations which are calculated as the sum of secretions divided by an efficiency of utilization of absorbed MP or AA to support different identified secretions.

Scurf

True protein secretion. In most of the formulation models, the equation from Swanson (1977) predicting net crude protein (**CP**) requirement for scurf is used:

$$\text{CP scurf}_{\text{secretion}}(\text{g/day}) = 0.2 \times \text{BW}^{0.60} \quad (1)$$

Swanson's prediction was retained but adjusted to take into account that not all CP is TP:

$$\text{TP scurf}_{\text{secretion}}(\text{g/day}) = 0.2 \times \text{BW}^{0.60} \times 0.86 = 0.17 \times \text{BW}^{0.60} \quad (2)$$

where 0.86 represents the TP/CP ratio of scurf, based on its AA composition, detailed below, and total N content; here and throughout the text, BW is in kg.

Amino acids. The secretion of AA into scurf is obtained by multiplying TP $scurf_{secretion}$ by its AA composition, estimated using the head, hide, feet and tail composition reported by Williams (1978) and van Amburgh *et al.* (2015). The mean from these studies, corrected for incomplete recovery of AA with 24-h hydrolyses (Lapierre *et al.*, 2019), is reported on a TP basis in Table 1.

$$\text{AA scurf}_{\text{secretion}}(\text{g/day}) = 0.17 \times \text{BW}^{0.60} \times [\text{AA}_{\text{corr}-\text{Scurf}}]/100 \quad (3)$$

where ($AA_{corr–Scurf}$) is in g AA/100 g TP.

Table 1 *Amino acid (AA) composition of protein secretions used in the calculation of efficiency of utilization of AA in lactating dairy cows*

	g AAcorr / 100 g CP[1]	g AAcorr /100 g TP[1]				g AAcalc/ 100 g TP[2]
AA	Duodenal endogenous	Microbial	Scurf	Whole empty body	Metabolic fecal	Milk
Ala	4.69	7.38	9.17	8.59	6.32	3.59
Arg	4.61	5.47	9.60	8.20	5.90	3.74
Asx	4.75	13.39	8.39	9.61	7.56	8.14
Cys	2.58	2.09	2.70	1.74	3.31	0.93
Glx	11.31	14.98	14.69	15.76	15.67	22.55
Gly	5.11	6.26	21.08	14.46	8.45	2.04
His	2.90	2.21	1.75	3.04	3.54	2.92
Ile	4.09	6.99	2.96	3.69	5.39	6.18
Leu	7.67	9.23	6.93	8.27	9.19	10.56
Lys	6.23	9.44	5.64	7.90	7.61	8.82
Met	1.26	2.63	1.40	2.37	1.73	3.03
Phe	3.98	6.30	3.61	4.41	5.28	5.26
Pro	4.64	4.27	12.35	9.80	8.43	10.33
Ser	5.24	5.40	6.45	5.73	7.72	6.71
Thr	5.18	6.23	4.01	4.84	7.36	4.62
Trp	1.29	1.37	0.73	1.05	1.79	1.65
Tyr	3.62	5.94	2.62	3.08	4.65	5.83
Val	5.29	6.88	4.66	5.15	7.01	6.90

[1]g AA_{corr}: AA composition corrected to account for incomplete recovery of AA with 24-h hydrolysis; TP = true protein.
[2]g AA_{calc}: AA composition calculated from the primary structure of the reference protein of each family; see text for details.

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Endogenous urinary

True protein secretion. Most formulation models predict endogenous urinary daily protein losses according to Swanson (1977), at 2.75 g/$BW^{0.50}$. To better quantify the AA required to cover this loss, a literature review was conducted to quantify the composition of urinary N. Force is to admit that literature is scarce on that domain in dairy cattle (Dijkstra *et al.*, 2013b). The major N metabolites in endogenous urinary N losses are: urea synthesized from endogenous sources, endogenous purine derivative (**PD**), creatinine and creatine, hippuric acid and 3-methyl-His. Therefore, endogenous N urinary loss is not a protein secretion per se. From studies with low CP dietary intake, daily excretion of endogenous urea has been quantified as 10 mg N/BW per day (Hutchinson and Morris, 1936; Biddle *et al.*, 1975; Marini and Van Amburgh, 2005; Wickersham *et al.*, 2008a and 2008b). To predict creatinine excretion, a database using exclusive dairy breeds, with growing and mature animals (111 treatment means from 24 publications from 1979 to 2015: Supplementary Material S1) was built. Urinary excretion of creatinine was regressed to 25.5 mg creatinine/BW per day, representing 9.46 ± 0.157 mg N/BW per day. Creatine excretion was evaluated as 0.37 that of creatinine (Blaxter and Wood, 1951; Nehring *et al.*, 1965; Bristow *et al.*, 1992). Urinary excretion of endogenous PD was assumed to average 27.1 mg N/$BW^{0.75}$ per day (483 μmol/$BW^{0.75}$: reviews from Tas and Susenbeth, 2007; Fujihara and Shem, 2011). Daily urinary excretion of 3-methyl-His (μmol) was evaluated at $50.4 + 3.54 \times$ BW (Harris and Milne, 1981).

Using the database from Spek *et al.* (2013), the 'measured' endogenous urinary N excretion was calculated as non-urea urinary excretion plus endogenous urea (predicted as described above) minus estimation of PD derived from absorbed microbial protein (Chen and Gomes, 1992). The sum of predictions described above represented 54% of the 'measured' endogenous urinary N excretion. As previously mentioned, hippuric acid is another N metabolite excreted in urine. Hippuric acid is formed in the liver to detoxify benzoic acid originating from rumen fermentation of dietary phenolic compounds. Although this excretion cannot be purely defined as 'endogenous', it has probably been included in previous predictions of endogenous urinary N excretion. When determined, it averaged 25.7% of non-urea N urinary excretion (Nehring *et al.*, 1965; Bristow *et al.*, 1992; Kool *et al.*, 2006). Including hippuric acid to the 'endogenous' urinary excretion, the calculated *v.* 'measured' values from Spek's database were not different (29.3 ± 4.5 *v.* 33.4 ± 15.4 g N/day), but there was a strong slope bias. The potential hippuric acid excretion was best related, in the database, to the proportion of urea N in urinary N excretion. Using this relationship to evaluate hippuric acid excretion, the calculated endogenous urinary N excretion averaged 33.2 ± 11.2 g N/day compared with 33.4 g N/day for the 84 treatment means 'measured' as described above. Although smaller, there remained a slope bias that could not be corrected, indicating an important gap in our knowledge on the composition of urinary N excretion. Besides hippuric acid, most of the estimations of urinary excretion were based on BW, and the sum of endogenous urinary N excretions was expressed relative to BW, averaging 53 mg N/BW (or 0.053 g N/BW) per day. Using a totally different approach, the prediction of daily endogenous urinary N loss averages 50 mg N/BW in the formulation model of the Institut National de Recherche Agronomique (INRA, 2018), very similar to our prediction and roughly twice as large as the Swanson (1977) prediction. As these compounds are expressed in g N/day, there is no need to convert from CP to TP. Therefore,

$$\text{TP endogenous urinary}_{\text{secretion}}(\text{g/day}) = 0.33 \times \text{BW} \quad (4)$$

where 0.33 is derived from 0.053×6.25.

Amino acid composition. The reason for revisiting endogenous urinary excretion was to identify which AA were upstream of these urinary excretions. After the examination of metabolic pathways yielding each of these urinary excreted compounds, only endogenous urea and 3-methyl-His excretions require a direct input of essential AA (**EAA**), if we exclude Arg from true EAA. Indeed, endogenous PD are synthesized from Asp, Gln and Gly; creatine and creatinine from Arg and Gly (it requires S-adenosyl Met, but as for other metabolic pathways, this does not represent a net Met requirement); and hippuric acid is synthesized from Gly. Endogenous urea excretion is assumed to have a revisited whole empty body AA composition (Williams, 1978; Rohr and Lebzien, 1991; Ainslie *et al.*, 1993; Van Amburgh *et al.*, 2015); the mean of these studies, corrected for incomplete recovery of AA with 24-h hydrolyses, is reported on a TP basis in Table 1. Therefore, to determine AA secretion in endogenous urinary N output, we need first to calculate endogenous urea excretion,

$$\text{TP endogenous urinary-urea}_{\text{secretion}}(\text{g/day}) = 0.0625 \times \text{BW}, \quad (5)$$

where 0.0625 was derived from 0.010 g N/day × 6.25; and multiply this secretion by the corresponding AA_{corr} composition, assumed to be that of whole empty body (Table 1).

$$\text{AA endogenous urinary}_{\text{secretion}}(\text{g/day}) = 0.0625 \times \text{BW} \times \left[\text{AA}_{\text{corr-WholeEmptyBody}}\right]/100 \quad (6)$$

where ($AA_{corr-WholeEmptyBody}$) is in g AA/100 g TP.

To complete the estimation of His excretion in endogenous urinary loss, 3-methyl His urinary excretion, as described above (mg His/day = 7.82 + 0.55 × BW), needs to be added. And finally, to complete the estimation of contribution of Arg to urinary N excretion, we need to include its contribution to creatinine and creatine, that is, 0.052 × BW g Arg/day.

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Metabolic fecal protein secretion

True protein secretion. This is certainly protein secretion with the largest discrepancy in its prediction varying, for example, between 337 and 621 g/day for a cow eating 23 kg/day of a 16.5% CP diet when predicted with five formulation models (Lapierre *et al.*, 2018). Reasons for this high discrepancy are inherent to the difficulty in performing its measurements and to the ambiguous definition of MFP. First, the determination of MFP in ruminants cannot be done as simply as in monogastrics where MFP is measured in animals fed an N-free diet. Predictions of MFP in ruminants have been based on regressing intake of digestible CP on CP intake with the negative intercept estimated as MFP, averaging, for example, 34, 33 and 29 g CP/kg DM intake (**DMI**) in earlier studies (Holter and Reid, 1959; Waldo and Glenn, 1984) comparable to 32 and 27 g CP/kg DMI reported in more recent studies (Jonker *et al.*, 1998; Kauffman and St-Pierre, 2001). In a meta-analysis using 65 growing-finishing cattle studies (291 treatment means) and 43 dairy cow studies (164 treatment means), Marini *et al.* (2008) obtained an intercept of 30 g CP/kg DMI when ignoring the multidimensionality of the relationship with other parameters. Predictions of MFP have also been calculated subtracting predicted undigested feed N from fecal N when animals were fed low CP diets (29.4 g CP/kg DMI; Swanson, 1977). However, it has been demonstrated that MFP losses are related more closely to feces output than to feed intake (Swanson, 1977): for this reason, some formulation models have based their estimation of MFP on indigestible DM (CNCPS; Fox *et al.*, 2004) or the outflow of organic matter from the digestive tract (NorFor, 2011; INRA, 2018). However, because of the uncertainty related to the estimation of DM digestibility, the National Research Council (2001) predicted MFP based on DMI.

However, the values obtained from the methods described above are not strictly a measure of loss of true protein from endogenous origin. Indeed, it was already raised at the beginning of the 1980s that these estimations of MFP included bacteria and bacterial debris (Swanson, 1982) and then the question 'Is the source of bacteria primarily waste N rather than a metabolic cost to the animal?' was raised (question from Trenkle: Swanson, 1982). Indeed, the metabolic demand for MFP should be only derived from endogenous secretions originating directly from AA (either from arterial supply and small intestinal digestion) and not from urea recycled into microbial protein. Therefore, NRC (2001), recognizing that a part of this fecal material contains undigested ruminal microbial CP, assumed that 50% of indigestible microbial protein appears in the feces and should be excluded from initial MFP prediction.

To improve the estimation of endogenous secretions through the gut in dairy cows, Ouellet *et al.* (2002 and 2010) adapted an isotopic dilution approach used in pigs (e.g. Lien *et al.*, 1997) and sheep (Sandek *et al.*, 2001). This approach allowed the development of a model delineating the contribution of undigested rumen bacteria synthesized from endogenous secretions or from urea to fecal N, thus allowing the exclusion of the latter from MFP. Because endogenous proteins have multiple origins (saliva, gastric juices, bile, pancreatic secretions, sloughed epithelial cells and mucin: Tamminga *et al.*, 1995), it is a challenge to determine the isotopic enrichment of the precursor pool when using a dilution approach. Values obtained using the enrichment of the mucosa as representative of endogenous secretions have been retained for this revision. Furthermore, the metabolic cost of the loss of undigested endogenous secretion across the upper gut should be measured at the ileum, because the endogenous secretions flowing out of the small intestine and disappearing across the hindgut do not result in absorbed AA. Endogenous fecal loss was, therefore, adjusted using a factor of 1.13, representing the ratio of ileal endogenous flow divided by fecal endogenous flow, measured in dairy cows (Ouellet *et al.*, 2007). Therefore, the prediction of endogenous ileal flow calculated using the enrichment of gut mucosa was retained as a basis for the estimation of MFP, averaging 14.9 g CP/kg DMI (Lapierre *et al.*, 2007).

Although MFP is calculated relative to DMI, as discussed above, it has been recognized that the driving force of MFP should be indigestible DM (Swanson, 1977). However, because of the uncertainties associated with estimating DM digestibility, we propose to still use DMI as a basis to predict MFP, but to include the neutral detergent fiber of the ration (**NDF**, %DM) based on Marini *et al.* (2008) regression of total tract digestibility of N *v.* N content of the diet. This inclusion will partially account for diet DM digestibility. The Marini equation also included a carbohydrate fermentation rate (fast, medium or none), but for practical purposes and to remove subjectivity, the average of three values was used to derive the final equation. Therefore, the equation of Marini *et al.* (2008) was adjusted to yield the value mentioned above at 14.9 g CP/kg DMI for cows fed diets at 36% NDF (in the rations in Ouellet *et al.*, 2002, 2007 and 2010). In addition, endogenous secretions occurring across the hindgut also create a demand on AA as demonstrated in pigs (Zhu *et al.*, 2003). Based on observations in sheep (Sandek *et al.*, 2001), this demand was estimated as 60% of N ileal flow of small intestinal endogenous secretion, the latter averaging 5.1 g CP/kg DMI in dairy cows (Ouellet *et al.*, 2007). Due to the scarcity of data on the exact origin of this hindgut N, it is assumed that half of this input originated from endogenous proteins and the other half from urea. Therefore, the estimation of MFP excretion (g CP/day) = $(11.62 + 0.134 \times \text{NDF}_{\%\text{DM}}) \times \text{DMI}$. Note that we keep the term metabolic fecal protein, although the small intestinal loss was truly predicted at the ileum. Based on its AA composition, detailed below, and N content, a ratio of 0.73 for TP/CP of MFP is calculated and

$$\text{TP MFP}_{\text{secretion}}(\text{g/day}) = (8.5 + 0.1 \times \text{NDF}_{\%\text{DM}}) \times \text{DMI} \quad (7)$$

where $\text{NDF}_{\%\text{DM}}$ is the percentage of NDF in the ration; here and throughouot the text, DMI is in kg/day.

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Amino acid composition. The AA composition of MFP is based on the AA composition of ruminal and abomasal isolates from Ørskov *et al.* (1986) and the endogenous flow at the ileum in pigs (Jansman *et al.*, 2002), assuming that 70% of MFP is from undigested endogenous duodenal flow and the remaining 30% from the intestine (Ouellet *et al.*, 2002 and 2010). The averaged composition corrected for incomplete recovery of AA with 24-h hydrolyses is reported on a TP basis in Table 1. Therefore, individual AA secretion in MFP is calculated as:

$$\text{AA MFP}_{\text{secretion}}(\text{g/day}) = [(8.5 + 0.1 \times \text{NDF}_{\%\text{DM}}) \times \text{DMI}] \times [\text{AA}_{\text{corr}-\text{MFP}}]/100 \quad (8)$$

where ($AA_{corr–MFP}$) is in g AA/100 g TP.

Milk

True protein secretion. Milk true protein secretion is certainly the most accurate measurement of export protein to make. The factor used to convert the measured milk N concentration into CP varies between 6.34 and 6.39. Based on the AA composition of milk protein, 6.34 would be the best factor (Karman and van Boekel, 1986; authors' calculations), but using different factors only has a limited impact on the estimation of MPY, smaller than 1%. Similar to other protein secretions, it has to be expressed as TP. If the TP/CP ratio is not known, NPN content of milk is assumed to be 4.9% (DePeters and Cant, 1992).

Amino acid composition. Although critical in the definition of AA requirements, milk AA composition has not been recently investigated. Indeed, early studies reported (1) that the EAA composition of milk produced from cows fed urea and ammonium N as the sole source of N differed by <3% from the EAA composition of milk from control cows (Syvaöja and Virtanen, 1965), and (2) that a change in the forage–grain ratio of the ration did not alter the AA composition of milk (Featherston *et al.*, 1964). From these observations, it has been assumed that the AA composition of milk protein is fairly constant, and this dogma has not been really challenged. Therefore, milk AA composition is still assumed to be constant, although this issue might need to be re-addressed with improved techniques to measure AA concentration. The same approach as that used in Swaisgood (1995) has been adopted to determine the AA composition of milk TP. Milk AA composition has been calculated based on the primary structure of reference protein of each family as detailed by Farrell *et al.* (2004). Based on the distribution of milk proteins reported in 15 manuscripts published between 1980 and 2012 (Supplementary Material S2), protein fractions in milk TP were assumed to be 82.4% casein (as a percentage of total protein: 35.2% αs1-casein; 7.6% αs2-casein; 30.9% β-casein; 8.7% κ-casein) and 17.6% whey (as a percentage of total protein: 3.7% α-lactalbumin; 10.5% β-lactoglobulin; 1.04% albumin; 1.64% IgG1; 0.21% IgG2; 0.04% IgA; 0.33% IgM; 0.21% lactoferrin). The AA composition of milk protein calculated using this procedure is presented in Table 1. Therefore, individual AA secretion in milk protein is calculated as:

$$\text{AA Milk}_{\text{secretion}}(\text{g/day}) = \text{MPY}(\text{g/day}) \times [\text{AA}_{\text{calc}-\text{Milk}}]/100 \quad (9)$$

where $AA_{calc-Milk}$ is in g/100 g TP.

Using this approach for milk, there is no need to do any correction for potential loss due to an incomplete recovery with hydrolyses. Due to the high diversity of proteins included in other types of secretions, an approach similar to milk protein cannot be used for these former proteins; therefore, the only way to obtain their AA composition is by hydrolysis. To correctly sum the AA in protein secretions, we used corrected AA concentrations for all protein secretions and calculated AA concentrations for milk.

Efficiency of utilization of metabolizable protein and amino acids

Variable efficiency

It is recognized that the efficiency of utilization of MP (**Eff_{MP}**) to support MPY is not fixed. Indeed, the marginal recovery of abomasal casein infusions averaged 21%, ranging from −5% to 45% in seven studies (Hanigan *et al.*, 1998), far below the traditional fixed efficiency of lactation of 65% to 67%. More recently, the marginal recovery of 81 comparisons of MPY response to post-rumen casein infusions averaged 24%, and was negatively related to the MP balance of control treatment (Martineau *et al.*, 2017). A similar trend is observed when variation in MP supply is achieved through a dietary change. For example, Metcalf *et al.* (2008) reported that the efficiency of lactation decreased from 77% to 50% when MP supply varied from 25% below to 25% above requirements, the efficiency for maintenance requirement assumed to be fixed.

In addition to MP supply per se, energy supply also has an impact on Eff_{MP}. Increments in MPY have been observed in response to post-ruminal supply of energy, either as glucose (Vanhatalo *et al.*, 2003a; Nichols *et al.*, 2016), propionate (Raggio *et al.*, 2007) or dietary rumen-inert fat (Nichols *et al.*, 2018), although not always (e.g. Clark *et al.*, 1977; Vanhatalo *et al.*, 2003b). Obviously, MPY increment in response to increased post-rumen energy supply (no effect on MP supply) increased Eff_{MP}. Using 825 treatment means, Daniel *et al.* (2016) concluded that both MP and net energy of lactation (**NE_L**) supplies increased MPY, the effects being additive, as observed in most of the individual studies where the interaction was tested. Only the study of Brun-Lafleur *et al.* (2010) reported a protein × energy interaction with a very targeted experimental design.

Therefore, there was enough evidence of the good use of a variable Eff_{MP}, related to both MP and energy supplies. Currently, NorFor (2011) and the DVE/OEB

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(Van Duinkerken *et al*., 2011) system are using a fixed efficiency for maintenance and a variable efficiency for lactation. Although not estimating directly Eff_{MP}, INRA (2018) is using MP and NE_L supplies to predict MPY, thereby introducing a variable Eff_{MP} for non-productive and lactation functions. Introducing a variable Eff_{MP} in the formulation models was yielding a better prediction of MPY in response to variations in MP and/or energy supply than the use of a fixed Eff_{MP}, still in use in most current North American models (Lapierre *et al*., 2018).

Combined efficiency

Based on the observed metabolism of AA across tissues, it has been proposed to use a single Eff_{AA}, different for each AA but identical for all the protein functions (Lapierre *et al*., 2007). The reason for this suggestion is that EAA catabolism does not occur at the site of protein synthesis or protein secretion but does occur in the organ(s) where appropriate enzymes are present (Lobley and Lapierre, 2003). For example, mammary uptake of Group 2 AA (Ile, Leu, Lys and Val) is in excess of MPY and the excess increases with MP supply; in contrast, Group 1 AA (His, Met, Phe+Tyr and Trp) net mammary uptake is almost equivalent to their secretion into milk protein (Lapierre *et al*., 2012). Therefore, it is proposed to use a single Eff_{MP} and Eff_{AA} (one for each EAA) for all the protein functions, except endogenous urinary loss. The latter represents end-products of metabolic pathways, and an efficiency of 1.0 should be used (Sauvant *et al*., 2015). Sauvant et *al*. (2015) reported that Eff_{MP} was better predicted when the same efficiency was assigned to all protein functions rather than a fixed efficiency for the non-productive functions and a variable efficiency for lactation.

With the objective of balancing dairy rations on an AA basis rather than MP basis, we developed equations to predict Eff_{MP} and Eff_{AA} in relation to MP or AA and energy supplies, which has not been done yet. Variable Eff_{AA} has already been proposed, but solely related to AA supply (Doepel *et al*., 2004).

Calculation of efficiency

MP supply and AA net digestible flow were calculated as the sum of digestible flow from rumen-undegraded protein (**RUP**) flow and microbial protein; endogenous duodenal flow was not included. The model of White *et al*. (2017) was used to predict RUP flows, with the AA composition of RUP assumed to be the AA composition of feed ingredients; the equation from Roman-Garcia *et al*. (2016) was used to predict microbial N, with an adjustment proposed by Myers *et al*. (2018), converted to microbial true protein assuming 16% N, and using the TP/CP (82.4%) ratio and the AA composition from Sok *et al*. (2017).

Using the supplies and secretions described above, the combined Eff_{MP} or Eff_{AA} was calculated as follows:

$$Eff_{MP} = (TP\ scurf_{secretion} + TP\ MFP_{secretion} + MPY)/MP\ supply_{adj} \quad (10)$$

where $MP\ supply_{adj} = MP\ supply - TP\ endogenous\ urinary_{secretion}$

and

$$Eff_{AA} = (AA\ scurf_{secretion} + AA\ MFP_{secretion} + AA\ Milk_{secretion})/AA\ net\ digestible\ flow_{adj} \quad (11)$$

where $AA\ net\ digestible\ flow_{adj} = AA\ net\ digestible\ flow - AA\ endogenous\ urinary_{secretion}$. In the text, $MP\ supply_{adj}$ will be referred to as MP_{adj}, and $AA\ net\ digestible\ flow_{adj}$ as AA_{adj}.

Prediction of efficiency

Databases. The calculations described above were applied to two databases, one used for the development of models, and the second for their validation. The developmental database included 208 publications (807 treatment means) and was an extension of the database used by Roman-Garcia *et al*. (2016) with studies added to offer a wider range of AA supply. An independent validation database was also built, including 32 publications (129 treatment means). The summary statistics of developmental and validation databases are presented in Tables 2 and 3, respectively. Publications included in the developmental database and in the validation database are listed in Supplementary Material S3. In both databases, studies have been coded to look specifically at the increment of MP supply. The relationship between MPY and MP supply depicts the overall meta-design (Figure 1).

Statistics. Models predicting Eff_{MP} were developed using variables that were strong predictors of the sum of protein secretions when tested individually: MP_{adj}, digestible energy intake (**DEI**), days in milk (**DIM**) and parity (primiparous *v.* multiparous). Digestible energy was used because metabolizable energy requires the estimation of urinary N, unknown until the efficiency is predicted, and net energy requires, in addition, the quantification of MPY: both are unknown that we are trying to predict. Digestible energy was predicted based on nutrient digestibility (Daley *et al*., 2018; de Souza *et al*., 2018). Models tested the linear and quadratic effects of (1) MP_{adj}, (2) MP_{adj} and DEI and (3) MP_{adj}/DEI; DIM and parity were tested in all models.

Models were developed using the *rma.mv* function from the metafor package in R. Potential outlying and influential observations and studies were detected using the *rstandard*, the *rstudent* and the *cook.distance* functions in the metafor package. All relationships were graphed and evaluated using the *ggplot* function of the ggplot2 package for R (Wickham, 2016). Using the *rma.mv* function of metafor, the hierarchy of studies, as a random effect, was taken into account. For example, two or more different studies could be reported in the same experiment; therefore, data were fitted to a three-level mixed-effect meta-regression model. To weigh data by $\sqrt{N}$, the V argument was set to zero and the R argument was used to specify a known matrix,

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Table 2 *Summary statistics of the developmental database (n = 807) from studies conducted in lactating dairy cows*

Variable	Mean	SD	Minimum	Maximum
Days in milk	135	56.4	28	344
BW (kg)	606	49.7	479	788
Year of publication	2002	8.5	1974	2019
DM intake (kg/day)	21.1	3.53	9.1	31.8
MP supply (g/day)	2072	415.9	997	3393
MP_{adj} (kg/day)	1873	409.7	798	3157
DE intake (MJ/day)	275	47.9	120	421
MP_{adj}/DE intake (g/MJ)	6.8	0.88	4.4	9.6
Digestible flow of Arg (g/day)	111	25.3	50	181
Digestible flow of His (g/day)	50	11.3	22	103
Digestible flow of Ile (g/day)	122	23.1	62	191
Digestible flow of Leu (g/day)	190	42.6	86	396
Digestible flow of Lys (g/day)	159	30.1	79	248
Digestible flow of Met (g/day)	48	9.7	23	83
Digestible flow of Phe (g/day)	120	24.8	59	203
Digestible flow of Thr (g/day)	110	20.9	56	176
Digestible flow of Trp (g/day)	27	5.6	13	44
Digestible flow of Val (g/day)	130	25.3	65	225
Sum of protein secretions (g/day)	1198	235.1	512	1803
Milk true protein yield (g/day)	945	207.1	389	1522
Metabolic fecal true protein (g/day)	245	38.5	113	382
Scurf true protein (g/day)	8.0	0.39	6.9	9.4
Endogenous urinary true protein loss (g/day)	201	16.4	159	261
Efficiency of utilization of MP_{adj}	0.65	0.102	0.40	1.06
Efficiency of utilization of Arg	0.72	0.210	0.31	2.44
Efficiency of utilization of His	0.78	0.141	0.37	1.27
Efficiency of utilization of Ile	0.61	0.088	0.38	0.89
Efficiency of utilization of Leu	0.67	0.110	0.31	1.05
Efficiency of utilization of Lys	0.67	0.104	0.36	1.06
Efficiency of utilization of Met	0.71	0.113	0.37	1.10
Efficiency of utilization of Phe	0.54	0.084	0.31	0.81
Efficiency of utilization of Thr	0.58	0.079	0.36	0.85
Efficiency of utilization of Trp	0.77	0.125	0.42	1.26
Efficiency of utilization of Val	0.65	0.098	0.36	0.97

MP = metabolizable protein; MP_{adj} = metabolizable protein supply minus endogenous urinary loss; DE = digestible energy.

that is, diag(1/developmental_database$Nexp0.5), with an unknown multiplicative variance component which was then estimated by metafor (Viechtbauer, 2018). Unbiased estimates of fixed effects and valid estimates of SE were obtained using the *robust* function in the metafor package with publication as the clustering variable. The use of *robust* function does not change the weight matrix but only affects the way the variance–covariance matrix of the fixed effects and downstream SE and *P* values are computed (Viechtbauer, 2017a and 2017b). Model performance was evaluated using RMSE and root mean squared prediction error (**RMSPE**) for the development and validation databases as a percentage of the observed mean. Mean bias and slope bias, which are two MSE decomposition terms, were also computed and expressed as a percentage of MSE (Theil, 1966; Bibby and Toutenburg, 1978). Concordance correlation coefficients (**CCC**; Lin, 1989 and 1992) and the corrected Akaike's information criterion (**AICc**; Hurvich and Tsai, 1993) are also reported. Ideal models are those with RMSE closest to 0, CCC closest to 1, mean and slope biases closest to 0, and smallest AICc.

Factors affecting efficiency. We initially tested the models using MP_{adj} as a surrogate of individual AA_{adj} to delineate which model(s) would yield the best goodness of fit. Three models are reported in Table 4: Eff_{MP} as a function of MP_{adj} and its squared term (equation 12); the latter plus DEI and its squared term (equation 13); and the ratio of MP_{adj}/DEI and its squared term (equation 14); DIM and parity were significant in the three models. The inclusion of DEI, either as an independent term (equation 13) or as a ratio with MP_{adj} (equation 14), improved the goodness of fit compared with MP terms alone (Figure 2), as shown by a substantial reduction of AICc, a large decrease in slope bias

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Table 3 *Summary statistics of the validation database (n = 129) from studies conducted in lactating dairy cows*

Variable	Mean	SD	Minimum	Maximum
Days in milk	124	58.2	11	273
BW (kg)	598	59.8	442	704
Year of publication	2002	7.8	1990	2018
DM intake (kg/day)	21.6	3.66	10.8	29.8
MP supply (g/day)	2105	393.2	1142	3252
MP_{adj} (kg/day)	1908	384.1	943	3052
DE intake (MJ/day)	281	48.9	138	382
MP_{adj}/DE intake (g/MJ)	6.8	0.69	5.3	8.8
Digestible flow of Arg (g/day)	110	22.7	58	173
Digestible flow of His (g/day)	49	10.0	26	77
Digestible flow of Ile (g/day)	124	22.6	69	186
Digestible flow of Leu (g/day)	195	38.5	97	303
Digestible flow of Lys (g/day)	159	28.8	88	236
Digestible flow of Met (g/day)	48	8.1	25	70
Digestible flow of Phe (g/day)	121	22.9	65	190
Digestible flow of Thr (g/day)	112	20.4	62	170
Digestible flow of Trp (g/day)	27	5.4	15	45
Digestible flow of Val (g/day)	131	24.5	73	203
Sum of protein secretions (g/day)	1206	256.4	655	1674
Milk true protein yield (g/day)	948	225.0	499	1390
Metabolic fecal true protein (g/day)	251	39.1	147	342
Scurf true protein (g/day)	7.9	0.49	6.6	8.7
Endogenous urinary true protein loss (g/day)	198	19.8	146	233
Efficiency of utilization of MP_{adj}	0.64	0.079	0.41	0.82
Efficiency of utilization of Arg	0.70	0.144	0.39	1.18
Efficiency of utilization of His	0.77	0.100	0.50	1.03
Efficiency of utilization of Ile	0.60	0.074	0.37	0.78
Efficiency of utilization of Leu	0.65	0.090	0.42	0.84
Efficiency of utilization of Lys	0.66	0.081	0.41	0.89
Efficiency of utilization of Met	0.71	0.100	0.45	0.95
Efficiency of utilization of Phe	0.53	0.067	0.35	0.69
Efficiency of utilization of Thr	0.57	0.064	0.38	0.73
Efficiency of utilization of Trp	0.77	0.100	0.47	1.03
Efficiency of utilization of Val	0.65	0.080	0.41	0.84

MP = metabolizable protein; MP_{adj} = metabolizable protein supply minus endogenous urinary loss; DE = digestible energy.

in both the developmental and validation databases and an increased CCC (Table 4). The improvement of the relationship of Eff_{MP} with MP_{adj}/DEI compared with MP_{adj} can also be visually appreciated in Figure 3. This agrees with a better prediction of MPY when energy supply is included in the model than based only on MP supply (Doepel *et al.*, 2004) or with a final model which includes both protein and energy supplies (Daniel *et al.*, 2016). Although equation (13) yielded a slightly lower AICc than equation (14), in the validation database, the slope bias was larger with the former equation and CCC was lower. Currently, two European systems, the DVE/OEB (Van Duinkerken *et al.*, 2011) and NorFor (2011), are using the ratio of MP/NE_L available for milk to predict the efficiency of lactation and MPY; Sauvant *et al.* (2015) estimated that Eff_{MP} was better related to MP/DMI than MP/NE_L. Another main advantage of using the ratio is the practicality of transferring results to 'cows of the future' eating more and producing more than cows from the studies included in the current review. In theory, predictive equations should be used within the range of values of predictors used for their development. Even if cows in commercial farms are eating more than observations in the developmental database, the ratio MP_{adj}/DEI remains within the limits of current observations, whereas MP_{adj} and DEI of high-producing dairy cows are already higher than the maxima observed in the developmental database. Therefore, we decided to use MP_{adj}/DEI as the driving force of Eff_{MP} and apply the same concept to individual AA_{adj}. Results for Eff_{AA}, equations (15) to (24), are presented in Table 5. All estimates were highly significant ($P < 0.01$) for all variables except parity ($P < 0.05$). Globally, the trends were very similar for the estimation of Eff_{AA} compared with Eff_{MP}, that is, very low mean and slope bias in the developmental database and a mean bias of ±5% MSE in the validation database. In both databases, His and

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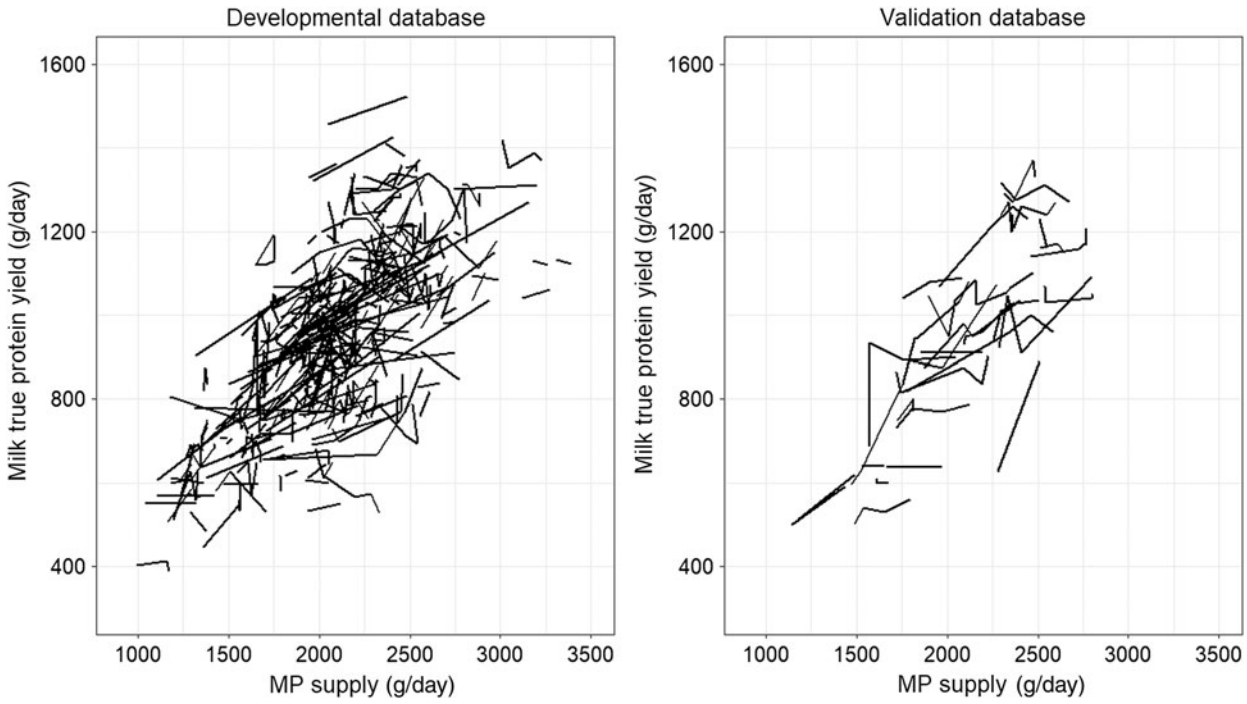

Figure 1 Relationship between milk true protein yield and metabolizable protein (MP) supply in lactating dairy cows in the developmental and validation databases.

Table 4 *Models of efficiency of utilization of metabolizable protein (MP)[1] in lactating dairy cows*

	Equation (12)			Equation (13)			Equation (14)		
Item[2]	Estimate	SE	*P* value	Estimate	SE	*P* value	Estimate	SE	*P* value
Intercept	135	8.8	<0.001	83	8.9	<0.001	190	12.0	<0.001
MP_{adj}	−44.5	8.31	<0.001	−62.7	7.84	<0.001			
$MP_{adj} \times MP_{adj}$	5.6	2.01	0.006	8.3	1.86	<0.001			
DEI				0.40	0.082	<0.001			
DEI × DEI				−0.00044	0.00014	0.002			
MP_{adj} /DEI							−26.7	3.43	<0.001
MP_{adj} /DEI × MP_{adj} /DEI							1.31	0.246	<0.001
DIM	−0.051	0.0223	0.02	−0.037	0.0111	<0.001	−0.035	0.0086	<0.001
Parity	−10.8	1.65	<0.001	−5.3	1.28	<0.001	−2.5	1.20	0.04
AICc	4662			4407			4452		
Developmental database ($n = 807$)									
Observed mean	65.1			65.1			65.1		
Predicted mean	64.9			65.1			65.1		
RMSE (% observed mean)	14.9			11.1			10.7		
Mean bias (% MSE)	0.1			0.0			0.0		
Slope bias (% MSE)	21.5			2.1			0.4		
CCC		0.55			0.70			0.71	
Validation database ($n = 129$)									
Observed mean	63.5			63.5			63.5		
Predicted mean	65.3			65.7			65.4		
RMSPE (% observed mean)	16.0			11.5			10.4		
Mean bias (% MSE)	3.0			8.8			8.0		
Slope bias (% MSE)	41.1			9.1			3.4		
CCC	0.27			0.51			0.58		

AICc = corrected Akaike's information criterion; CCC = concordance correlation coefficient; RMSPE = root mean squared prediction error.
[1]Efficiency of utilization of MP = (true protein secretion in milk + scurf + metabolic fecal protein)/MP_{adj} × 100.
[2]MP_{adj} (kg/d): metabolizable protein supply minus endogenous urinary loss; DEI (MJ/d): digestible energy intake; DIM: days in milk; parity (1 = primiparous; 0 = multiparous).

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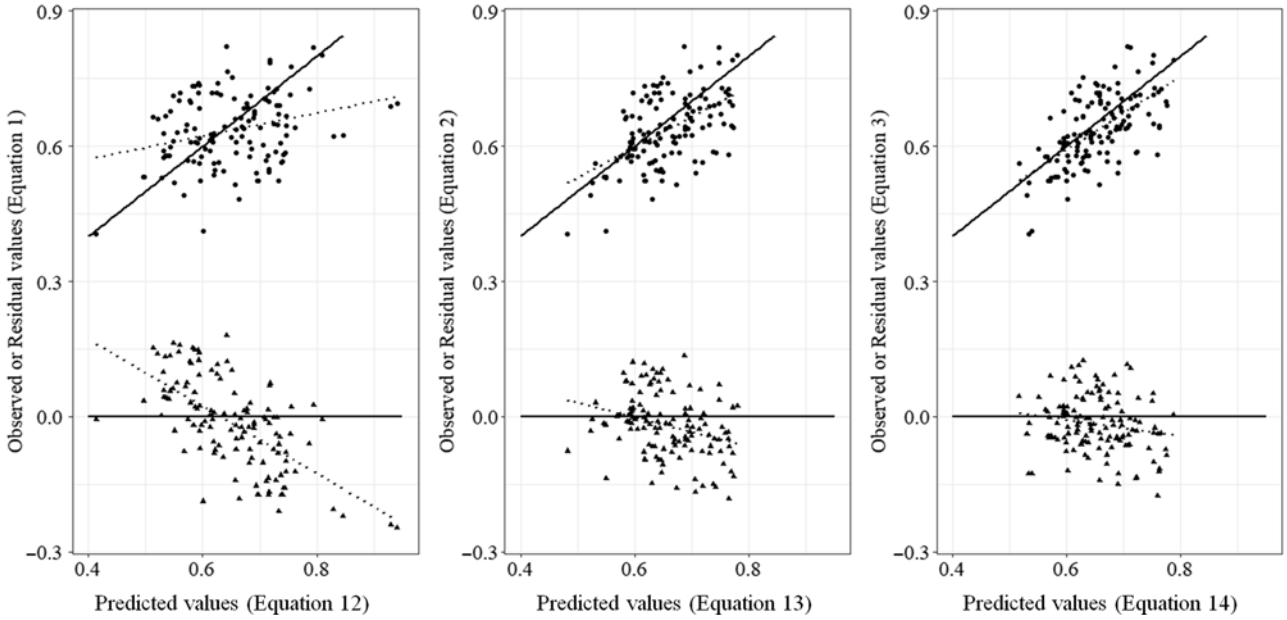

Figure 2 Observed (•) and residual (▲) values of efficiency of utilization of metabolizable protein in the function of efficiency predicted according to equations (12), (13) and (14) (see text for details of the equations) in lactating dairy cows in the validation database.

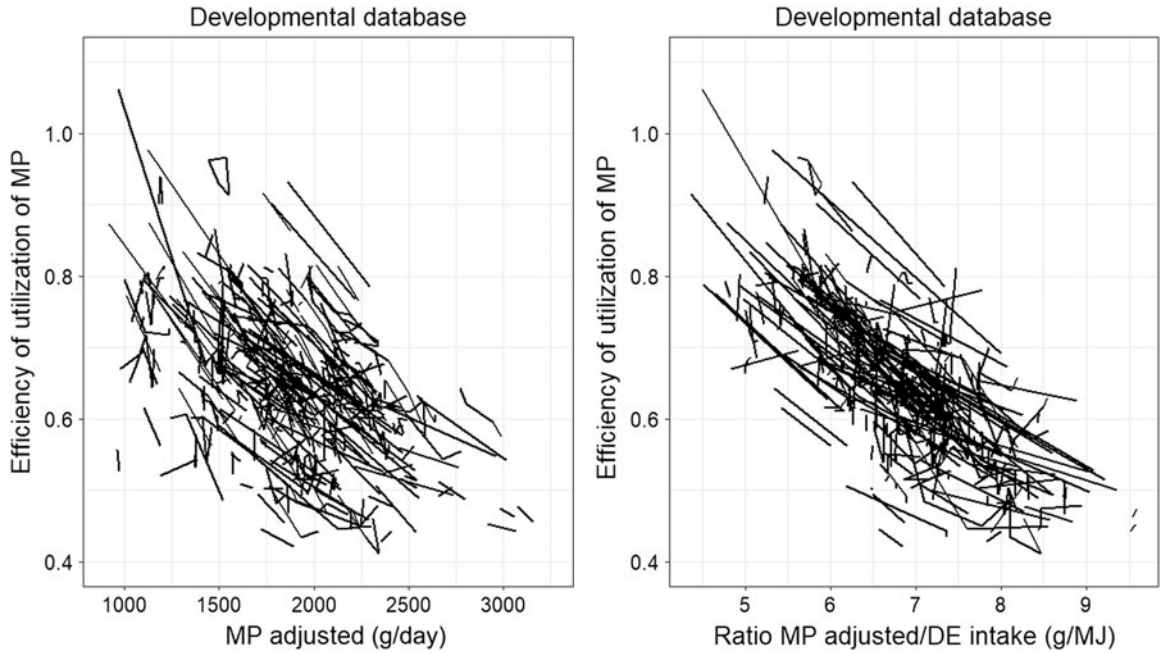

Figure 3 Relationship between efficiency of utilization of metabolizable protein (MP) and MP adjusted (MP supply minus endogenous urinary loss) or the ratio of MP adjusted/digestible energy (DE) intake in lactating dairy cows in the developmental database. Efficiency is calculated as (true protein in milk + scurf + metabolic fecal)/MP adjusted.

Trp were used with the highest efficiency, and Phe and Thr with the lowest. Values are in the same range as previously reported for combined efficiency (Lapierre *et al.*, 2007; Van Amburgh *et al.*, 2015) except for Arg being much higher and Lys and Met being lower. The large difference for Arg is due to the change in the prediction of AA in endogenous urinary loss: the current proposition involves an important loss of Arg related to creatine and creatinine urinary excretion decreasing substantially Arg_{adj}. Also, Arg displayed high CCC but had a large slope bias in the validation database, probably related to the uncertainty of its true supply due to unknown and unaccounted supply from *de novo* synthesis. In fact, for this reason, although given for a comparison, the current estimates for Arg should not be used. It is also important to note that the current estimates could only be used to predict efficiency until the minimal predicted efficiency is reached according to the quadratic function: after that threshold ratio of AAadj/DEI, the function will not apply.

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Table 5 *Models of efficiency of utilization of individual amino acids (AA)[1] in lactating dairy cows*

AA	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Item[2]	Equation (15)	Equation (16)	Equation (17)	Equation (18)	Equation (19)	Equation (20)	Equation (21)	Equation (22)	Equation (23)	Equation (24)
Intercept	254***	207***	170***	172***	183***	178***	134***	167***	227***	181***
AA_{adj}/DEI	−1030***	−1009***	−355***	−204***	−285***	−811***	−240***	−404***	−2258***	−53***
AA_{adj}/DEI × AA_{adj}/DEI	1331***	1650***	265***	83***	155***	1256***	146***	346***	7754***	243***
DIM	−0.039***	−0.037***	−0.035***	−0.037***	−0.038***	−0.042***	−0.028***	−0.027***	−0.041***	−0.035***
Parity	−2.0$	−2.6*	−2.4*	−2.6*	−2.7*	−3.4*	−2.0*	−2.2*	−2.9*	−2.7*
Developmental database ($n = 807$)										
Observed mean	71.8	78.1	60.6	67.2	66.6	70.9	54.0	58.0	76.6	65.4
Predicted mean	72.1	78.1	60.6	67.3	66.6	70.9	53.9	58.0	76.5	65.2
RMSE (% observed mean)	12.3	10.3	11.0	10.9	10.9	11.4	10.5	9.7	10.7	10.6
Mean bias (% MSE)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Slope bias (% MSE)	1.4	0.2	0.6	0.2	0.8	1.3	0.6	0.6	0.8	0.4
CCC	0.91	0.81	0.62	0.72	0.70	0.69	0.71	0.68	0.75	0.68
Validation database ($n = 129$)										
Observed mean	70.1	77.3	59.6	64.8	66.0	70.7	53.2	57.0	77.2	64.5
Predicted mean	72.3	79.7	61.4	67.0	67.9	72.8	54.8	58.5	79.2	66.3
RMSPE (% observed mean)	11.4	10.0	10.4	10.6	10.7	11.4	10.2	9.1	10.1	10.2
Mean bias (% MSE)	7.4	9.6	7.8	9.6	8.0	7.2	8.3	7.8	6.8	7.5
Slope bias (% MSE)	27.9	4.6	2.2	0.9	2.4	0.0	1.8	3.4	6.0	2.2
CCC	0.87	0.67	0.55	0.64	0.51	0.55	0.60	0.60	0.66	0.58

CCC = concordance correlation coefficient; RMSPE = root mean squared prediction error.
[1]Efficiency of utilization of AA = (AA secretion in milk + scurf + metabolic fecal protein)/AA_{adj} × 100.
[2]AA_{adj} (g/d): net flow of digestible AA supply minus endogenous urinary loss; DEI (MJ/d): digestible energy intake; DIM: days in milk; parity (1 = primiparous; 0 = multiparous); all estimates were significant: $P < 0.01$ for all parameters except parity where $P < 0.05$.
Degree of significance = $^{\$}P \leq 0.10$; $^{*}P \leq 0.05$; $^{***}P \leq 0.001$.

Table 6 *Predicted milk true protein yield (MPY, g/d) based on estimates of the efficiency of utilization of metabolizable protein (Eff_{MP}) or predicted directly in lactating dairy cows*

	Eff_{MP} estimation based on equation[1]			Predicted MPY from a direct equation (27)[2]
	Equation (12)	Equation (13)	Equation (14)	
Developmental database ($n = 807$)				
Observed mean (g/day)	945	945	945	945
Predicted mean (g/day)	925	943	947	943
RMSE (% observed mean)	18.8	13.6	13.1	13.7
Mean bias (% MSE)	1.3	0.0	0.0	0.0
Slope bias (% MSE)	0.0	3.2	1.5	4.5
CCC	0.43	0.74	0.80	0.73
Validation database ($n = 129$)				
Observed mean (g/day)	948	948	948	948
Predicted mean (g/day)	956	976	980	972
RMSPE (% observed mean)	18.8	13.6	12.8	13.6
Mean bias (% MSE)	0.2	4.8	6.9	3.5
Slope bias (% MSE)	11.3	16.3	0.1	22.8
CCC	0.45	0.76	0.83	0.76

CCC = concordance correlation coefficient; RMSPE = root mean squared prediction error.
[1]Equations detailed in the text; MPY = $Eff_{MP} \times MP_{adj}$ – (scurf true protein + metabolic fecal true protein); MP_{adj} (kg/d): metabolizable protein supply minus endogenous urinary loss.
[2]Equation detailed in the text.

These threshold ratios are in AA_{adj}/DEI (g/MJ): 0.31, 0.67, 1.23, 0.92, 0.32, 0.82, 0.58, 0.15 and 0.73 for His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val, respectively. In the validation database, only one treatment mean was higher than the threshold ratio for His at 0.32, whereas the ratios were all lower than the threshold values in the validation database.

Efficiency of utilization and prediction of milk true protein yield. The ultimate goal in the estimation of Eff_{MP} and Eff_{AA} is the prediction of MPY which was calculated either as:

$$\text{MPY} = \text{MP}_{\text{adj}} \times \text{Eff}_{\text{MP}} - (\text{TP scurf}_{\text{secretion}} + \text{TPMFP}_{\text{secretion}}) \quad (25)$$

or

$$\text{MPY} = (\text{AA}_{\text{adj}} \times \text{Eff}_{\text{AA}} - (\text{AA scurf}_{\text{secretion}} + \text{AA MFP}_{\text{secretion}}))/[\text{AA}_{\text{calc}-\text{Milk}}] \times 100 \quad (26)$$

First it appeared clearly that the prediction of Eff_{MP} based solely on MP_{adj} (equation 12) predicts MPY with a low CCC in both databases (Table 6). As observed for the prediction of the efficiencies themselves, adding DEI to MP into the prediction equations greatly improved the predictions of MPY. Adding DEI as an independent variable (equation 13) improved CCC; slope bias contributed to a greater proportion of the total prediction error, but the total error decreased in the validation database (Table 6). Finally, the equation, including the ratio MP_{adj}/DEI, provided the best goodness of fit in the two databases, with the highest CCC. For a comparison, a model was developed to predict the sum of protein secretions (MPY + MFP + scurf) directly from MP_{adj}, $MP_{adj} \times MP_{adj}$, DEI, DIM and parity; MPY was then calculated as predicted protein secretions minus (MFP + scurf). The equation is:

$$\begin{aligned}\text{Protein secretions} = {} & 160(\pm 79) + 381(\pm 74) \times \text{MP}_{\text{adj}} \\ & - 74(\pm 19) \times \text{MP}_{\text{adj}} \times \text{MP}_{\text{adj}} \\ & + 2.49(\pm 0.22) \times \text{DEI} - 0.6(\pm 0.1) \\ & \times \text{DIM} - 100(\pm 21) \\ & \times \text{parity}(1 = \text{primiparous}, \\ & \qquad 0 = \text{multiparous})\end{aligned} \quad (27)$$

The squared term of DEI was not significant ($P = 0.93$). Predictions from this model are detailed in Table 6 and are not as good as those obtained when Eff_{MP} was predicted with equation (14). Moreover, a strong slope bias was observed in the validation database. Inclusion of the ratio MP_{adj}/NE_L supply in predictive models of Eff_{MP} to predict MPY also yielded the best predictions when different formulation models were compared (Lapierre *et al.*, 2018). Therefore, predicted Eff_{AA} from equations (15) to (24), for each EAA, were used to predict MPY (Table 7). All AA are yielding fairly similar predictions of MPY except Arg. A comparison between observed and predicted MPY was also made using several combinations to explore the impact of individual Eff_{AA}. Five combinations, all excluding predictions from Arg, are presented in Table 7, which are: the lowest of MPY predicted from Eff_{AA} (**MinAA**), the mean of nine MPY predicted from Eff_{AA} (**MeanAA**), the mean of MinAA and MeanAA (**MinMeanAA**) and the mean of estimations from His, Lys and Met (**HLM**). All predictions provided good fitness with

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Table 7 *Prediction of milk true protein yield (MPY, g/day) based on estimates of the efficiency of utilization of amino acids (Eff_{AA}) in lactating dairy cows*

	Based on Eff_{AA} of individual AA[1]										Combination[2]				
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Min	Mean	Max	HLM	Average (min, mean)
Developmental database ($n = 807$)															
Observed mean (g/day)	945	945	945	945	945	945	945	945	945	945	945	945	945	945	945
Predicted mean (g/day)	952	948	946	949	947	945	946	957	946	937	931	947	965	947	939
RMSE (% observed mean)	17.1	13.1	13.0	13.2	13.0	13.0	12.9	13.2	13.0	13.0	13.0	13.0	13.3	12.9	12.9
Mean bias (% MSE)	0.2	0.0	0.0	0.1	0.0	0.0	0.0	1.0	0.0	0.5	1.4	0.0	2.6	0.0	0.3
Slope bias (% MSE)	11.8	2.0	0.6	1.6	0.7	0.5	1.1	2.4	0.9	0.6	0.8	1.0	1.7	0.9	0.9
CCC	0.68	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.79	0.80	0.80
Validation database ($n = 129$)															
Observed mean (g/day)	948	948	948	948	948	948	948	948	948	948	948	948	948	948	948
Predicted mean (g/day)	976	983	978	984	981	978	980	990	976	968	966	980	995	981	973
RMSPE (% observed mean)	14.4	12.8	12.7	12.8	12.8	12.8	12.7	13.1	12.7	12.5	12.4	12.7	13.4	12.8	12.6
Mean bias (% MSE)	4.2	8.2	6.4	9.0	7.3	6.3	7.2	11.6	5.4	3.0	2.4	7.0	13.8	7.3	4.5
Slope bias (% MSE)	0.5	0.0	0.3	0.1	0.1	0.6	0.1	0.0	0.1	0.3	0.4	0.1	0.0	0.2	0.3
CCC	0.79	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.84	0.83	0.82	0.83	0.83

CCC = concordance correlation coefficient; RMSPE = root mean squared prediction error.
[1]MPY = ($Eff_{aa} \times AA_{adj}$ – (AA in scurf + AA in metabolic fecal))/concentration of AA in milk × 100; AA_{adj} (g/d): net flow of digestible AA supply minus endogenous urinary loss.
[2]Min: minimum predicted MPY; mean: average predicted MPY; max: maximum predicted MPY; HLM: average predicted MPY from His, Lys and Met; Arg predicted MPY excluded from all the combinations.

observed MPY, and this is probably one of the limitations of the database and current work actually available. Although we extended the database from Roman-Garcia *et al.* (2016), trying to increase the number of studies where the supply of only one AA was changed at the time, in most of the studies variations of AA supply were achieved through a change in protein supply which affected simultaneously the supply of all AA. MinAA might be too severe, as the efficiency of a single AA might be maximized if only this one is in short supply (e.g. Lapierre and Ouellet, 2015). Until we have further development, MinMeanAA might be a prudent option as it ponders an average predicted MPY with the prediction from AA most probably in shortest supply. By doing so, it would certainly be fortuitous to verify which AA is yielding the lowest prediction as this might provide a tool to identify the AA with the shortest supply relative to estimated requirements.

Conclusion

The development of a factorial approach to balance dairy rations for individual EAA is moving forward with improvement of the quantification of proteins exported out of the animal and their respective AA composition. The major net utilization of EAA supports secretion into MFP and MPY, with a limited contribution to endogenous urinary and scurf secretions. To this net utilization, an inefficiency (100 – efficiency) needs to be added: Eff_{MP} and Eff_{AA} are positively related to energy supply and negatively related to MP_{adj} or AA_{adj}. And finally, the predictions of Eff_{MP} and Eff_{AA} can be used successfully to predict MPY. Although the concepts derived in the current study can probably be extended to most of the models used to balance dairy rations, it has to be noted that the current figures only apply when using the assumptions as presented. Also, although protein accretion from growth in cows from first parity is acknowledged because parity (primiparous *v.* multiparous) was included in the model, other changes in protein mass, either through gain or loss of BW or gestation, were not accounted for. Finally, studies where the supply of a single EAA is changed incrementally are currently lacking to really fine-tune our estimations of individual EAA recommendations in dairy rations.

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Declaration of interest

There is no potential conflicts of interest.

Ethics statement

None.

Software and data repository resources

None.

Supplementary material

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