KEY ROLES OF AMINO ACIDS IN COW PERFORMANCE AND METABOLISM – CONSIDERATIONS FOR DEFINING AMINO ACID REQUIREMENTS

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INTRODUCTION

Traditionally, maintenance protein requirement (rqt) included integumental, endogenous urinary and metabolic fecal protein loss; NRC (2001) has added a rqt for duodenal endogenous protein. Most of the current models used to balance dairy rations for metabolizable protein (MP; e.g. NRC (2001), CNCPS (Fox et al., 2004), van Duinkerken et al. (2011)) base their estimation of maintenance MP rqt on an excellent but relatively old review from Swanson (1977). However, recently rqt for metabolic fecal protein (MFP) has been revisited in Norfor (2011) and Systali (Sauvant et al., 2015) as well as rqt for endogenous urinary in Systali (Sauvant et al., 2015). Regarding individual amino acid (AA) rqt, the NRC (2001) 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" (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 rqt based on empirical relationships between observed milk protein concentration or yield relative to the proportion of the AA in MP supply.

In contrast, other models (e.g. CNCPS (Fox et al., 2004), AminoCow (Evonik AG Industries, Hanau, Germany)) have adopted a factorial approach. Estimation of AA rqt using the factorial approach can be viewed as a 3-step procedure: 1) identify and quantify the quantity of true protein (TP) excreted out of the cow daily or protein accretion as body weight gain or conceptus: the "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. As our knowledge on AA metabolism has increased over the last decades, it becomes appropriate to use this knowledge to update the factorial estimation of MP and AA rqt of high producing dairy cows. Therefore the current review aims to integrate knowledge developed in recent years on AA metabolism to update the estimation of MP and AA rqt to allow a better usage of the factorial approach to improve the formulation of rations. Discussion will be limited to essential AA (EAA) and simplified to a lactating, mature, non-gestating cow without changes in body weight (BW, expressed in kg through the text) and composition. Numerical examples will be given using a 700 kg cow producing 45 kg milk/d at 3.2% CP (3.0% TP) and with a daily dry matter intake (DMI, expressed in kg/d through the text) of 27 kg.

EXPORTED PROTEINS

Metabolic fecal loss is by far the largest component of the maintenance rqt, representing 67 and 87% of maintenance rqt in our example cow using NRC (2001) and CNCPS (Fox et al., 2004) models, respectively (Table 1). However, its estimation is based on DMI and as such, does not truly represent what is meant by maintenance. Therefore, we suggest using the term “non-productive functions” proposed by Sauvant et al. (2015) to refer to this group of functions inherent to the biology of the cow but not supporting productive functions as growth, gestation or milk production.

Integumental proteins include loss and growth of hair, scurf and scales rubbed from the skin surface, along with some N containing compounds in skin secretions. They represent less than 2 % of MP maintenance rqt (Table 1) and as such have received limited attention further than Swanson (1977). There is not enough information to update this estimation, which will be retained: integumental exported proteins were estimated at 0.20 g CP/$BW^{0.60}$ per day (Swanson, 1977). To transfer this relation into MP rqt, we need, however, to convert it to TP and divide it by the efficiency with which the MP supply is being used to support the protein function. Although the efficiency of converting MP supply to exported proteins is variable depending on the supply of MP relative to rqt (this will be discussed in a following section), the value of 0.67 is commonly used. This value seems to correctly represent the biology when MP supply is in a relative equilibrium with the rqt and will be used, when needed, to present the changes proposed to the estimation of the MP rqt. Therefore, in the current model, **daily MP rqt (g MP/d) for integumental proteins = 0.26 g MP × $BW^{0.60}$**, using a TP/CP ratio of 0.859 (based on AA composition: see next section) and an efficiency factor of 0.67 (0.20 × 0.859 / 0.67).

The estimation of endogenous urinary MP rqt used by most models is also based on Swanson (1977) estimating daily CP losses at 2.75 g/$BW^{0.50,}$ which divided by an efficiency of 0.67 yields a rqt of 4.1 MP g/$BW^{0.50}$ (the TP/CP ratio is not taken into account). However, it was not clear which AA composition should be assigned to this exported “protein” (in fact what is exported is N-containing compounds, derived from AA, but not protein anymore). The CNCPS (Fox et al., 2004) proposed using the AA composition of the empty body. In an attempt to better define the AA composition of this exported “protein”, a literature review was conducted to quantify the composition of urinary-N. Force is to admit that literature is scarce on that domain (Dijkstra et al., 2013). The major N-fractions in urine that contributed to the non-productive functions are: endogenous urea, endogenous purine derivative (PD), creatinine and creatine, hippuric acid and 3-methyl-His (considered because of its potential demand on His). 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 b). To estimate creatinine excretion, we built a database using exclusively dairy breeds, growing or lactating (141 treatment means from 27 publications from 1979 to 2015): urinary excretion of creatinine averaged 9.46 mg N/BW per day (25.5 mg creatinine/BW). 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 estimated to average 27.1 mg N/$BW^{0.75}$ per day (483 µmol: reviews from Tas and Susenbeth, 2007; Fujihara and Shem, 2011). Daily urinary excretion of 3-methyl-His (µmol) was estimated as 50.5 + 3.54 × 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 minus estimation of PD from absorbed MCP plus endogenous urea, estimated as described above. The sum of the estimates described above represented 54% of the "measured" endogenous urinary N excretion. Another important N-fraction excreted in urine is hippuric acid. 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 estimates of endogenous urinary 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). With this addition to the "endogenous" urinary excretion, the estimated and measured values were in a similar range (29.7 vs. 33.4 g N/d, for estimated vs. measured, respectively), 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 the hippuric acid excretion, the estimated endogenous urinary N excretion averaged 33.2 g N/d compared with 33.4 g N/d for the 84 treatment means "measured" as described above. There was a less important slope bias, but there is not enough information to correct it, indicating an important gap in our knowledge on the composition of urinary-N excretion. The BW range in this database is too small to find a significant relationship between the endogenous urinary-N excretion and BW. However, as beside hippuric acid, most of the estimations were based on BW, the endogenous urinary-N excretion was expressed relative to BW and averaged 53 mg N/BW. Interestingly, using a totally different approach, in the new French model, Systali, the daily endogenous urinary-N loss averaged 50 mg N/BW (Sauvant et al., 2015). As these compounds are 1) expressed in g N/d, there is no need to convert from CP to TP; 2) end-product of metabolism, we consider, as Sauvant et al. (2015), that an efficiency of 1.0 should be used. Therefore, in the current model, **daily MP rqt (g MP/d) for endogenous urinary loss = 0.33 g MP × BW** (0.053 × 6.25 × 1).

The estimations of rqt to cover MFP are probably those varying the most between models: Sauvant et al. (2015) reported that, using cow characteristics of their database "Bovidig_PDI", estimates of MFP rqt (g MP/d) varied among models:18.2 ± 3.4 (NorFor, 2011), 19.8 ± 3.7 (Sauvant et al., 2015), 23.1 ± 5.4 (Van Duinkerken et al., 2011), 23.5 ± 0.5 (NRC, 2001) and 38.0 ± 8.9 (CNCPS, Fox et al., 2004). The lowest SD observed for NRC (2001) is due to the fact that this estimation is solely based on DMI whereas the other models included, one way or the other, an estimation of the digestibility along the gastro-intestinal tract. One striking observation in the estimation of MFP rqt in NRC (2001) and CNCPS (Fox et al., 2004), is that for both models, there has been no conversion of the estimation of CP excretion in feces into TP and also, no utilization of an efficiency factor to obtain the final estimate of MP rqt. In fact, the definition of what is included in MFP has been somewhat vague and changing over the years. Our belief is that MFP loss should include all endogenous proteins secreted along the gastro-intestinal tract and not digested in the small intestine. Endogenous proteins can flow to

the duodenum either as free proteins or incorporated into rumen-synthesized microorganisms. However, endogenous proteins should exclude bacteria-N synthesized from urea, as utilization of urea does not impose a demand on AA per se. With this concept in mind, the estimation of the ileal flow of endogenous protein in dairy cows obtained by Ouellet et al. (2.39 g N/kg DMI: Ouellet et al., 2002 and 2010) plus an estimation of the endogenous secretion into the large intestine excreted in feces would adequately represent the MFP. Endogenous secretion into the large intestine excreted in feces was estimated to be equivalent to 0.6 times the endogenous small intestinal flow passing at the ileum, based on observations in sheep (Sandek et al., 2001): it is assumed that half of the endogenous flow secreted in the large intestine originates from urea and half from AA. Endogenous proteins flowing to the ileum from small intestinal secretion were estimated at 0.81 g N/d (Ouellet et al., 2007). However, these results are limited and expressed relative to DMI. It has been clearly shown that MFP is better related to the indigestible DM than the DMI (Swanson, 1977). In an attempt to account for diet digestibility, the equation published by Marini et al. (2008) where the intercept of the relation between total tract digested N and dietary N was interpreted as the metabolic fecal N was used: this equation includes NDF (%OM in the diet) and the carbohydrate fermentation rate. However, this MFP estimation includes undigested bacteria protein synthesized from urea-N: therefore, to only account for undigested bacteria synthesized from endogenous AA-N, the intercept and the slope were scaled to yield MFP estimates obtained by Ouellet et al. (2002 and 2010) with an average carbohydrate fermentation rate. Overall the **MP rqt (g MP/d) for MFP = [12.7 + 0.15 × NDF (%DM)] × DMI**, using a TP/CP ratio of 0.732 (based on AA composition of MFP: see next section) and an efficiency factor of 0.67.

As previously discussed (e.g. Lapierre et al., 2007), we consider that there is no need to include the duodenal flow of endogenous proteins into the rqt, as long as they are also excluded from the net supply of MP and AA. Indeed, these proteins are mainly synthesized from AA provided by the arterial supply, and as such the fraction of that flow which is digested into the small intestine does not represent a rqt as it is returned blood circulation. Therefore, only the portion of that flow reaching the ileum undigested represents a rqt and is already included in the MFP fraction. As well, this flow does not represent a new input of MP and AA into the cow and needs to be removed from the duodenal flow to estimate the true net supply of MP and AA. Based on 12 studies conducted between 1980 and 2013, the duodenal endogenous protein flow was re-evaluated to be (g CP/d)= 96.1 + 7.54 × DMI (Lapierre et al., 2016).

In summary, in the current model, using an efficiency of 0.67, **MP rqt for non-productive functions (g MP/d) = [0.26 g MP × $BW^{0.60}$] + [0.33 × BW] + [(12.7 + 0.15 × NDF (%DM)) × DMI]**, with BW and DMI in kg. Supply of MP needed to cover milk protein secretion is relatively straightforward. Milk protein yield (MPY) is easily measured and should be expressed as TP. Using an average efficiency of 0.67, **MP rqt for milk (g MP/d)= MPY_{TP} / 0.67**. The MP rqt for non-productive functions and milk MP rqt for the example cow are compared in Table 1: rqt are lower in the current model than in NRC or CNCPS but we have to keep in mind that the estimation of the duodenal flow of endogenous protein needs to be removed from the estimated MP supply.

Table 1. Comparison of metabolizable protein (MP) requirements (rqt; g/d) between different models[a].

Protein function	Model[b]	Exported CP[c]	TP/CP[c]	Exported TP	Efficiency	MP rqt
Integumental loss	NRC, CNCPS	10	ND[d]	ND	0.67	15
	Current	10	0.86	9	0.67	13
Endogenous	NRC, CNCPS	73	ND	ND	0.67	109
urinary	Current	232	1.0	232	1	232
Metabolic	NRC	631	ND	ND	ND	631
fecal protein	CNCPS	810	ND	ND	ND	810
	Current	444	0.73	324	0.67	484
Duodenal	NRC	257	0.5	128	0.67	191
endogenous	CNCPS	ND	ND	ND	ND	ND
	Current	-	-	-	-	-
Milk	NRC	-		1350	0.67	2015
	CNCPS	1440	0.93	1339	0.65	2060
	Current			1350	0.67	2015
Total MP rqt	NRC					2961
	CNCPS					2994
	Current					2746

[a]Based on cow averaging 700 kg, 27 kg/d DMI, 45 kg milk/d at 3.0% TP; NDF of the ration: 36% of DM.
[b]NRC (2001); CNCPS (Fox et al., 2004); current : as detailed in this paper.
[c]TP: true protein; CP: crude protein.
[d]ND: not determined.

AMINO ACID COMPOSITION

Correction factors

Most of the values available on AA composition of proteins are concentrations obtained after a 21-h or 24-h hydrolysis; then the sum of these AA relative to the CP of the protein is assumed to represent the TP/CP ratio. In fact, these 2 assumptions are not correct. First, it is well recognized 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). Concentrations obtained with a 24-h hydrolysis are useful to rank or compare feed ingredients. However, when we want to link the digestive flow of AA obtained by hydrolysis with, for example, their net portal absorption measured as free AA into blood circulation, there might be discrepancy due to the low concentration of some AA obtained with the 24-h hydrolysis compared with their true concentration in the digestive flow (Pacheco et al., 2006). On the other hand, often, milk AA composition used in models to determine AA rqt is obtained from calculation based on the protein composition of milk and AA composition of each milk protein fraction based on its primary structure (e.g. CNCPCS, Fox et al., 2004). So, when setting up a factorial approach to balance AA supply and rqt, 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 rqt except milk and use a totally different theoretical approach for milk, the major component of the rqt, which is assumed to have the true AA composition.

To obtain the true amount of an AA in the protein in the original material, before the start of hydrolysis, a method of extrapolation with multiple hydrolysis times involving simultaneous release and decay has been proposed (Robel and Crane, 1972): they were using 5 times of hydrolysis, ranging from 4 to 141 h. This method was further explored with up to 19 times of hydrolysis, ranging from 2 to 141 h (e.g. Rutherfurd et al., 2008). The results obtained at 24 h were compared with the extrapolated values estimating the "true" AA composition of the protein. Others have compared the maximal AA concentration obtained after different times of hydrolysis ranging from 4 to 10 times with the 24-h value (e.g. Rowan et al., 1994). In addition, comparison has also been made with the theoretical value calculated based on the primary structure of the protein (e.g. lysozyme: Darragh et al., 1996; our unpublished work with bovine serum albumin). We have also conducted multiple hydrolysis times (13 times in triplicate, ranging from 2 to 168 h; unpublished) on 6 feeds. The ratio of the "true" concentration relative to the 24-h measurement was calculated combining the ratios of the maximal value, theoretical value and extrapolated value relative to 24-h measurement (Table 2).

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

AA	Missing in 24-h hydrolysis	MW[a] AAA / MW AA	global CF_{AA}[b]
Ala	1.05	0.798	0.84
Arg	1.03	0.897	0.93
Asx	1.03	0.865	0.89
Cys	1.23	0.850	1.05
Glx	1.06	0.878	0.93
Gly	1.09	0.761	0.83
His	1.02	0.884	0.90
Ile	1.12	0.863	0.97
Leu	1.07	0.863	0.92
Lys	1.06	0.877	0.93
Met	1.05	0.879	0.92
Phe	1.09	0.891	0.97
Pro	1.05	0.844	0.88
Ser	1.13	0.829	0.94
Thr	1.08	0.903	0.98
Trp	1.12	0.849	0.95
Tyr	1.08	0.912	0.98
Val	1.11	0.901	1.00

[a]MW: molecular weight.
[b]The global CF_{AA} is used to calculate the corrected AAA (AAAc) composition from the 24-h hydrolysis.

The second consideration relates to the chemistry of the protein. When a peptide bond in cleaved, one molecule of water is added to each released AA: complete hydrolysis of 1 kg of protein should yield ± 1.15 kg of free AA. Therefore, summing the AA concentrations obtained after a hydrolysis and expressing this result over total CP overestimates the TP/CP ratio by approximately 15%. The weight of each AA should be corrected by the ratio of the molecular weight (MW) of the AA without a molecule of water (anhydrous AA = AAA)/ MW of the AA. This approach is currently used in the NorFor system (2011). Multiplying the ratio of the AA missing because of the 24-h hydrolysis with the ratio the MW AAA/ MW AA yields a global correction factor for each AA. This global correction factor can be applied to 24-h hydrolysis concentrations to obtain the "true" corrected value of the AAA (AAAc). Although tedious, more work is needed to determine if the same factors are valid across proteins analyzed when building models (e.g. RUP, duodenal protein). Preliminary results from our laboratory indicate that the RUP fractions share the same factors than the feed ingredients.

AA Composition of the Protein Functions

In Table 3, the AA composition of each protein functions was revisited, based on the metabolic definition of each protein type and the global corrections factors of Table 2. Data are given for all AA, but rqt can only be established for the EAA, which are not synthesized by the cow. Therefore, Arg rqt will not be determined because there is substantial synthesis of Arg by the cow. The AA composition of the integumental proteins was estimated using the head, hide, feet and tail combined composition reported by Williams (1978) and van Amburgh et al. (2015). This is not totally accurate, but with the low contribution of this function to the total AA rqt, this was the best we could find. For the endogenous urinary excretion, the endogenous urea excretion is assumed to have the AA composition of the whole empty body (Williams, 1978; Rohr and Lebzien, 1991; Ainslie et al., 1993; Van Amburgh et al., 2015). The excretion of 3-methyl-His requires His whereas the excretion of the other N-fractions of the endogenous urinary does not require a direct input of EAA: PD are synthesized from Asp, Glu and Gly; creatine and creatinine from Arg and Gly (as many other metabolic functions, it requires S-adenosyl Met, but as for the other metabolic pathways this does not represent a net Met rqt); hippuric acid is synthesize from Gly. The AA composition of the 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 the MFP is from undigested duodenal flow and the remaining 30% from the intestine (Ouellet et al., 2002 and 2010). Milk AA composition is based on the primary structures of the different proteins in milk (Farrell Jr et al., 2004). The protein fractions in milk were distributed as 81.4% casein (as % of total protein: 34.5% α_{s1}-casein; 7.6% α_{s2}-casein; 29.9% β-casein; 9.4% κ-casein) and 18.6% whey (as % of total protein: 4.0% α-lactalbumin; 10.7% β-lactoglobulin; 1.15% albumin; 1.90% IgG; 0.29% IgA; 0.26% IgM; 0.29% lactoferrin), based on the means reported in 14 manuscripts published between 1986 and 2012.

Table 3. Amino acid (AA) composition of the different protein functions involved in the determination of metabolizable protein and AA requirement.

	Integumental		Endogenous urea-urinary		Metabolic fecal		Milk	
AA	g AAAc[a]/ 100 g CP[b]	g AAAc/ 100 gTP	g AAAc/ 100 g CP[b]	g AAAc/ 100 g TP	g AAAc/ 100 g CP[b]	g AAAc/ 100 g TP	g AA / 100 g TP[c]	g AAAc/ 100 gTP
Ala	6.27	7.30	5.93	6.84	3.67	5.01	3.63	2.89
Arg	7.14	8.31	6.16	7.09	3.73	5.09	3.73	3.35
Asx	6.22	7.25	7.20	8.29	4.76	6.51	8.20	7.09
Cys	2.09	2.44	1.36	1.57	2.18	2.98	0.98	0.83
Glx	11.04	12.86	11.97	13.79	10.01	13.68	22.43	19.68
Gly	13.52	15.75	9.37	10.80	4.61	6.29	2.04	1.55
His	1.25	1.46	2.20	2.53	1.96	2.68	2.90	2.56
Ile	2.19	2.55	2.76	3.18	3.39	4.63	6.17	5.32
Leu	5.18	6.04	6.25	7.20	5.84	7.98	10.50	9.06
Lys	4.18	4.87	5.92	6.82	4.80	6.56	8.81	7.72
Met	1.03	1.20	1.77	2.04	1.09	1.48	2.99	2.63
Phe	2.81	3.27	3.47	4.00	3.49	4.77	5.21	4.64
Pro	8.92	10.39	7.15	8.24	5.17	7.07	10.22	8.62
Ser	4.59	5.35	4.12	4.75	4.67	6.37	6.70	5.55
Thr	3.12	3.64	3.81	4.38	4.87	6.65	4.68	3.97
Trp	0.55	0.65	0.81	0.93	1.16	1.58	1.67	1.52
Tyr	2.06	2.40	2.45	2.83	3.11	4.25	5.82	5.25
Val	3.67	4.28	4.10	4.73	4.69	6.41	6.88	5.82
ratio TP/CP	0.859				0.732			

[a]AAAc: anhydrous AA corrected for the missing concentration with a 24-h hydrolysis (see table 2).
[b]Initial concentrations obtained after 24-h hydrolysis of the protein source; CP: crude protein.
[c]Initial concentrations obtained from the primary structure of the milk protein fractions; TP: true protein.

EFFICIENCY

Efficiency of Utilization of MP

For comparison purpose between NRC (2001), CNCPS and our new estimates, MP rqt in the section above has been calculated using an efficiency of 0.67. However, it is well recognized that the efficiency of utilization of MP varies with MP supply relative to rqt (Doepel et al., 2004, Metcalf et al., 2008). Indeed, Sauvant et al. (2015) proposed a variation of the efficiency around a pivot of 0.67; however the variation was not related solely to the MP supply but to the ratio of MP supply/DMI. In this section, we will have a closer look at the variation of the efficiency using the database developed in Martineau et al. (2016) reporting studies where casein was infused post-ruminally in lactating dairy cows. Diet characteristics have been estimated using NRC (2001). As previously proposed (Lapierre et al., 2007) based on AA metabolism, a single “combined” efficiency will be used for the non-productive functions and the milk protein yield. Also, as proposed in the new French system Systali (Sauvant et al., 2015), an efficiency of 1.0 is assigned to the endogenous urinary excretion, these products being end-products

of metabolic pathways. The combined efficiency is calculated as the ratio of exported TP divided by MP supply (with the endogenous urinary removed from both components). In this database, the combined efficiency averaged 0.64 ± 0.13 (range 0.35 to 1.01): the mean agrees with the usual average efficiency used but the range clearly indicates how this efficiency is indeed variable. The relationship between total MP supply and total exported TP, with the endogenous urinary rqt removed from both components, was first examined. As expected for a variable efficiency, the relationship has a significant quadratic component ($P<0.05$; Figure 1A). We then studied the linear relationship between the combined efficiency and either MP supply, MP/NE_L supplies or MP supply/DMI. When study was not included into the equation, both MP/ NE_L supplies (adj. R^2=50%) and MP supply/DMI (R^2=60%) had a better linear relation with the combined efficiency than MP supply alone (R^2=31%). The quadratic component was significant but only slightly improved the equations for MP/NE_L supplies (R^2=55%) or MP/DMI (R^2=63%), but it was not significant for MP supply alone. The similar pattern of the relation between the combined efficiency and the ratios MP/ NE_L supplies or MP supply/DMI certainly holds due to the high correlation (r=0.98) between these 2 parameters. Because, biologically, energy supply should have more relevance to AA utilization than DMI per se, we prefer to use the MP/NE_L supplies ratio. In addition, with the ratio MP/NE_L, the intercepts and the slopes were very similar with and without study included into the linear model, indicating more robustness for the linear than quadratic model. Therefore, using the dietary characteristics estimated with NRC (2001), in this database, the combined efficiency of utilization of MP supply to support exported TP linearly declines ($P<0.001$) with an increment of the ratio MP/NE_L supplies: efficiency = 1.06 (±0.04) - 0.0078 (±0.0007) × MP/NE_L supplies (g/Mcal). This relation needs to be validated with a larger database, but the essence of the relation will remain.

Figure 1A.

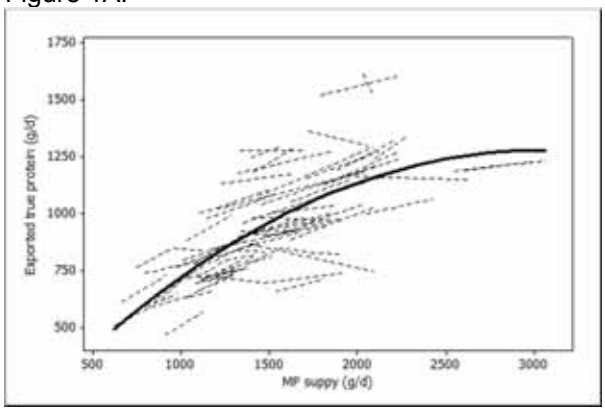

Figure 1B.

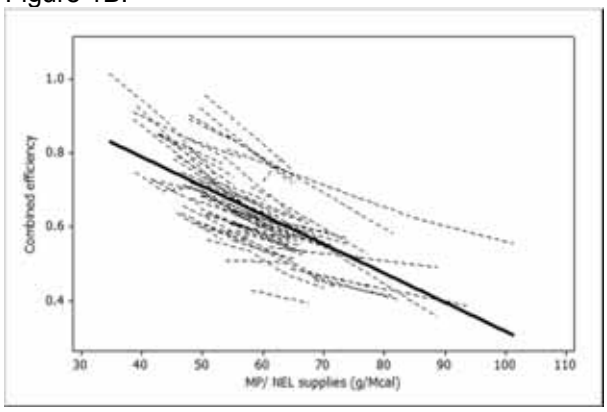

Figure 1. Relation between: *Panel A*: exported true protein and MP supply (both excluding endogenous urinary secretion); *Panel B*: Combined efficiency of utilization of MP and the ratio MP/NE_L supplies. See text for explanation.

Efficiency of Utilization of AA

The idea of using a combined efficiency for the utilization of MP was strongly suggested by AA metabolism. Indeed, examining AA metabolism in dairy cows, general trends were observed: Group 1 AA (His, Met, Phe+Tyr) are mainly catabolized by the liver and very little, if any, catabolism occurs in the peripheral tissues including the mammary gland; at the opposite, Group 2 AA (Ile, Leu, Lys and Val) are barely removed by the liver on a net basis but are catabolized by the gut, peripheral and mammary tissues (Lapierre et al., 2012). Because of the anatomical localization of the enzymes responsible of AA catabolism, the catabolism of AA does not occur at the site of the protein synthesis, certainly not for those exported proteins. For example, there was clearly no mammary catabolism of Phe even under excess supply (Lemosquet et al., 2010). Therefore, there is no biological reason to assign an efficiency of utilization for Phe, or other Group1 AA, different for non-productive functions and lactation, as its removal occurs at one site in the body, in the liver. Therefore, using a similar approach to that used for MP, the efficiency of utilization of EAA was also evaluated. The same database and assumptions as for the examination of MP efficiency were used. The exported AA were estimated from exported proteins × AA composition presented in Table 3 whereas the supply were estimated from the digestive flow (NRC, 2001); as for MP, the endogenous urinary rqt was subtracted from both components. The mean efficiency observed and variations are detailed in Table 4. The high maximal efficiencies observed might be related to the type of studies included in this database, where the control treatment was MP-deficient. However, the efficiencies higher than 1 noted for His and Met might be related to underestimation of their respective digestive flows. The averaged efficiencies observed for each AA are in the same range as those adopted by CNCPS v6.5 (van Amburgh et al., 2015) and derived from Lapierre et al. (2007).

Table 4. Combined efficiency of utilization of AA[a]

AA	Mean	SD	Min	Max	CNCPS v6.5[b]
Arg	0.56	0.21	0.30	0.89	0.58
His	0.82	0.22	0.41	1.34	0.76
Ile	0.63	0.18	0.35	0.93	0.67
Leu	0.67	0.21	0.35	0.99	0.61
Lys	0.72	0.18	0.38	1.05	0.69
Met	0.78	0.20	0.40	1.22	0.66
Phe	0.57	0.19	0.31	0.84	0.57
Thr	0.57	0.16	0.33	0.81	0.66
Val	0.63	0.17	0.35	0.92	0.66

[a]Estimated from the database of Martineau et al. (2016) according to description detailed in the text.
[b]From Van Amburgh et al. (2015) and Lapierre et al. (2007).

Results for Lys and Met are detailed to illustrate the variation of their efficiency. In fact, efficiency for these individual AA follows pretty much the same pattern as for MP. The AA exported are quadratically related to the supply (Figure 2). The combined efficiency is linearly better related to AA/NE_L (R^2: Lys=45 and Met=47%) supply than to AA supply by itself (R^2: Lys=25 and Met=29%). However, at the difference of MP, the

relationship between efficiency of utilization and the ratio of AA/NE_L supply has a significant quadaratic component, which slightly increases the R^2 to 49 and 52% for Lys and Met, respectively (Figure 3). However, in the range of the majority of the observations, the linear and quadratic relations yielded quite similar efficiencies. As for MP efficency, these relations need to be assessed with recent estimates of AA supply and validated with a larger database, but the essence of the relations will remain.

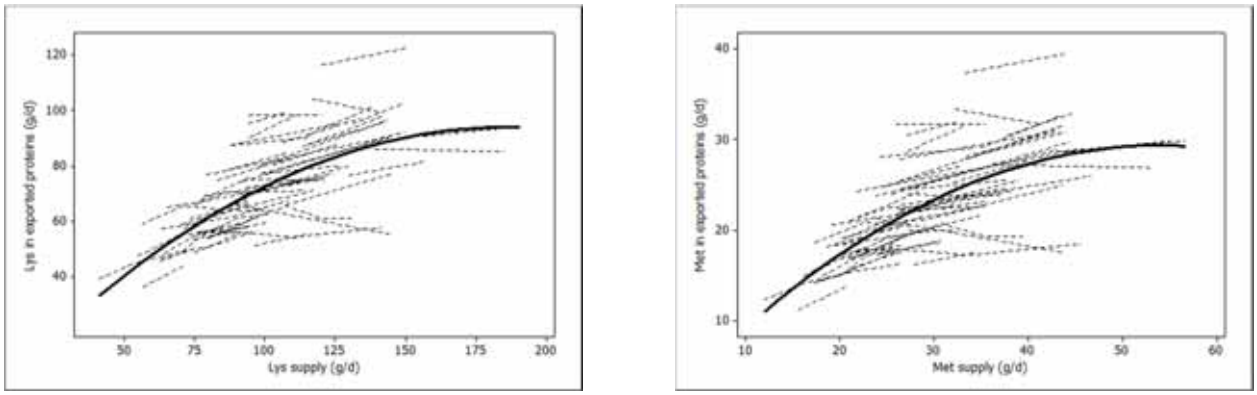

Figure 2. Relation between Lys and Met exported in true proteins and AA supply (both excluding endogenous urinary secretion).

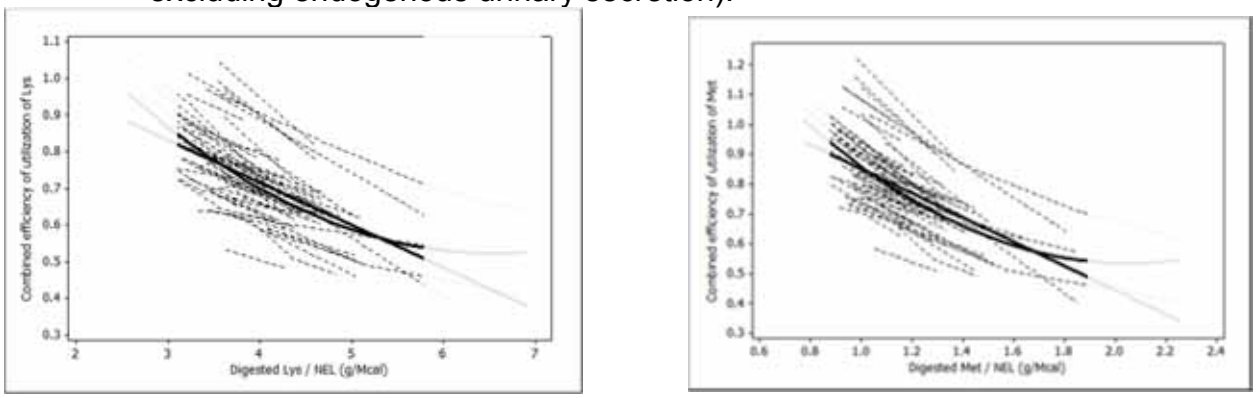

Figure 3. Combined efficiency of utilization of Lys and Met and the ratio AA/NE_L supplies. See text for explanation.

CONCLUSION

Overall, a better knowledge of AA metabolism has improved quantification of the daily amount of exported AA, either as non-productive functions or milk protein production. In addition, knowledge of AA metabolism has suggested 1) using a combined efficiency for these functions (except endogenous urinary excretion) and 2) a using a variable efficiency to convert these exported AA into requirements. Although it was first suggested that the efficiency of MP or individual AA was related to their digestive flow, it seems that the ratio of MP or AA supply to NE_L supply is better related to the efficiency: as the ratio AA/NE_L supplies increases, the efficiency decreases. In a whole model, optimization of the efficiency of the different EAA should allow a better estimation of the rqt and a better prediction of milk protein yield under known supply.

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