1   **Modeling amino acid requirements in dairy cows**

2

3   ***Interpretive Summary:*** *Higgs et al.* Improved predictions of the net and optimum AA supply

4   to dairy cows in ration formulation systems could provide an opportunity to balance diets closer

5   to animal requirements and improve nutrient utilization. Predictions of AA supply in a dynamic

6   version of the Cornell Net Carbohydrate and Protein System (CNCPS) were refined by

7   modeling endogenous N transactions along the entire gastrointestinal tract including

8   incorporation into microbial N supply. A strong relationship was observed when the efficiency

9   of AA use was regressed against AA supply relative to ME suggesting expressing AA supply

10   relative to energy could improve predictions of AA utilization.

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12   **Version 7 of the Cornell Net Carbohydrate and Protein System: III. Endogenous nitrogen**

13   **and amino acid requirements**

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15   **R. J. Higgs,* D. R. Ouellet,† H. Lapierre,† E. A. Collao-Saenz,‡ H. R. Kingi,§ and M. E.**

16   **Van Amburgh*[1]**

17   *Department of Animal Science, Cornell University, Ithaca, NY 14853

18   †Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC,

19   Canada J1M 0C8

20   ‡ Department of Animal Science, Universidade Federal de Goiás, Jataí-GO, Brazil

21   § Department of Economics, Cornell University, Ithaca, NY 14853

[1] Corresponding author: mev1@cornell.edu

ABSTRACT

Improved predictions of the net and optimum AA supply to dairy cattle in ration formulation models like the Cornell Net Carbohydrate and Protein System (CNCPS) would provide an opportunity to balance diets closer to animal requirements and improve nutrient utilization. Predictions of net AA supply in a dynamic version of the CNCPS were refined by modeling endogenous N (EN) transactions along the entire gastrointestinal tract (GIT) including incorporation of EN into microbial N supply. Studies that used isotopic enrichment of total N from 15N-Leu infusion to mark endogenous components were used to develop the model. Predictions were close to measured data at the duodenum, ileum and in the feces. Incorporation of EN into microbial N and the original source of EN at various points in the GIT and in the feces were also accurately predicted. The optimum AA supply was determined using a dataset of published studies that infused AA post-ruminally. A logistic model was used to estimate AA requirements for the physiological processes quantified by the model. The optimum AA supply to maximize AA use and minimize wastage was determined where the third derivative of the logistic model was 0. The optimum AA supply was described for all essential AA. Requirements for Met (5.7% EAA) and Lys (15.1 % EAA) were similar to previous recommendations, indicating the model was within the range of current data. A log-logistic relationship was observed when the efficiency of AA use was regressed against AA supply relative to ME supply but no relationship was found when AA supply was expressed relative to MP. Considering AA supply relative to energy could improve predictions of AA utilization and indicates the relationships are not separate and linear, but integrated, and depend on the profile of nutrients consumed.

Key Words: CNCPS, amino acid, endogenous nitrogen, requirement, dairy cattle

INTRODUCTION

46   An improved understanding of both the net and optimum supply of AA to a dairy cow can

47   provide an opportunity to supply AA closer to animal requirements and reduce total protein

48   feeding while still maintaining high levels of production (Haque et al., 2012). This strategy can

49   reduce feed costs and lower the environmental impact of dairy production (Higgs et al., 2012).

50   Amino acids flowing to the duodenum encompass proteins originating from three major

51   sources: undegraded feed, microbial and endogenous proteins (Lapierre et al., 2006).

52   Combined, these fractions represent the gross AA supply, potentially available to the animal

53   after digestion. However, quantification of the endogenous N (**EN**) fraction, and its

54   contribution to the microbial pool of AA, is needed to establish the net AA supply (Ouellet et

55   al., 2002). The contribution of EN to the duodenal AA flow, either as free proteins or

56   incorporated into microbial protein, represents a recycling of previously absorbed AA that

57   cannot be considered new supply, but is significant (Lapierre et al., 2006). Currently, the

58   prediction of AA supply in the Cornell Net Carbohydrate and Protein System v6.5 (**CNCPS**)

59   is the sum of AA from feed and bacteria that escape the rumen and are digested in the small

60   intestine and does not consider endogenous AA or protozoa (O'Connor et al., 1993).

61   Incorporating both endogenous AA and protozoa into the CNCPS could refine and possibly

62   improve predictions of the net supply of AA to the animal.

63

64   Requirements in the CNCPS are first calculated individually by quantifying different

65   physiological processes, including their AA composition, then dividing these by a transfer

66   coefficient (efficiency of use): the results of each physiological process are summed to give

67   total AA requirements (O'Connor et al., 1993). Previous versions of the CNCPS have assumed

68   the protein and AA requirements for maintenance are the sum of scurf, endogenous urinary and

69 metabolic fecal N (Fox et al., 2004). Metabolic fecal N (**MFN**) has been estimated using

70 regression techniques in the previous versions of CNCPS and the NRC (2001) using the work

71 of Swanson (1977). Fox et al. (2004) suggested these calculations might have shortcomings

72 due to the contribution of microbial N from hind gut fermentation to total fecal N. The

73 regression techniques used would consider fecal microbial N as EN. Therefore, the protein or

74 AA requirement for maintenance estimated by a model using these predictions might be over-

75 estimated. The assumption used when considering MFN in the maintenance requirement of an

76 animal is that for the protein to be a true requirement and net loss, it needs to be excreted.

77 However, considerably more EN is secreted into the rumen of dairy cows than escapes in the

78 free form or is incorporated in bacteria (Ouellet et al., 2002; Marini et al., 2008; Ouellet et al.,

79 2010a). This means the balance is degraded in the rumen and the N absorbed as ammonia.

80 Once degraded, essential AA are lost for the animal and can only be replaced by absorbed AA,

81 originating, on a net basis, from RUP or microbial protein (**MCP**). Therefore, it makes sense

82 to consider all protein secreted into the gastrointestinal tract (**GIT**) which is not recovered in

83 the small intestine a maintenance requirement, not just what appears in the feces.

84

85   The objectives of this study were to replace current predictions of MFN with estimations of

86 EN transactions (AA, excludes urea) through the entire GIT in a dynamic version of the

87 CNCPS (Higgs and Van Amburgh CHO and PRO and Higgs and Van Amburgh microbial

88 growth). In doing this, the net supply of AA to the small intestine from all sources can be

89 refined and the shortcomings of the current predictions improved. A second objective was to

90 evaluate the efficiency of transfer of AA to milk and maintenance using the predicted net

91 supply and requirements of the new model. Interactions between protein and energy play an

92 important role in determining how an animal will utilize absorbed AA and it has been

93 recommended they be considered together (Hanigan et al., 1998; Lobley, 2007). Current

4

94   European models are incorporating energy supply or DMI in the estimation of the efficiency

95   of MP use (NorFor, 2011; Van Duinkerken et al., 2011; Sauvant et al., 2015). These

96   interactions were investigated in determining the optimum AA requirements for this revised

97   version of the model.

98

MATERIALS AND METHODS

Modeling endogenous AA losses in the gut

101   Predictions of EN losses into the GIT were modeled mechanistically to capture the various

102   transactions along the GIT and between microbial pools. Gross EN to the forestomach and

103   intestines were estimated according to Ouellet et al. (2002) and Ouellet et al. (2010a) which

104   were subsequently partitioned into individual components (Table 1) using estimates reported

105   in Egan et al. (1984). The studies by Ouellet and co-workers directly estimate EN using $_{15}$N-

106   Leu intra-venous infusion for 8 d, in cows with rumen, duodenal and ileal cannulas. Using this

107   technique, different labelled precursor pools are available to represent the different sites of EN

108   production which have different isotopic enrichments. In dairy cattle, the enrichment of milk

109   probably provides a good representation of protein secretions whereas the rumen or intestinal

110   mucosa represents the pool contributing to EN through cell desquamation (Ouellet et al., 2002).

111   Enrichment of the rumen mucosa was used to estimate the contribution of EN to MCP as EN

112   contributions to the rumen would largely be from desquamation (Egan et al., 1984). Free EN

113   at the duodenum was assumed to be best represented by the 'combined' precursor pool, the

114   average of rumen mucosa and milk (Ouellet et al., 2010a), due to the contribution of both

115   sloughed cells and secretions into the abomasum and duodenum upstream of the cannula. Data

116   using a 'combined' precursor pool are not presented in Ouellet et al. (2002). Therefore, the

117   relative difference between the estimates using 'combined' and 'mucosa' precursor pools

5

118 (combined = 60% of mucosa) presented in Ouellet et al. (2010a) were used to calculate

119 estimates of EN flows with a combined pool from Ouellet et al. (2002). Endogenous secretions

120 early in the small intestine were assumed to be largely digested. Therefore, EN measured at the

121 ileum and in the feces would predominantly be from sloughed keratinized cells with poor

122 digestibility and would be best represented by the mucosa precursor pool. Endogenous

123 contributions are reasonably consistent among diets when expressed relative to DMI or OMI

124 (Tamminga et al., 1995; Ouellet et al., 2002; Marini et al., 2008; Ouellet et al., 2010a). Thus,

125 the model expresses each component as g EN per kg DMI. Quantitative estimates of fluxes to

126 and from the various pools in the model were estimated by setting the kinetic parameters and

127 digestibility coefficients in the model to align predictions at various points in the gut to

128 measured data (Ouellet et al., 2002 and 2010a). A summary of the model inputs used to estimate

129 the EN transactions are in Table 1.

130

131   Table 1 somewhere here

132

133   Endogenous N in the rumen has three potential fates: 1) degradation to ammonia; 2)

134 incorporation into MCP; 3) escape from the rumen. Degradation and passage are estimated

135 using the kinetic relationships described in Higgs and Van Amburgh (CHO and PRO) where

136 free EN is assumed to flow in the liquid phase. Incorporation into MCP is estimated using two

137 extensions of the microbial model described in Higgs and Van Amburgh (microbial growth).

138 The first (Figure 1) is used to replicate the enrichment of ^{15}N in MCP after an 8 d intra-venous

139 infusion of ^{15}N-Leu for the purpose of estimating the kinetic and digestibility coefficients of

140 EN secretions. The studies of Ouellet exclude the transfer of ^{15}N from recycled urea, but it is

141 still possible for $^{15}NH_3$ to be produced in the rumen by fiber bacteria (**FB**), non-fiber bacteria

142 (**NFB**) and protozoa (**PZ**) and incorporated into microbial protein. The model assumes if EN

143 is degraded to NH_3, the AA are lost to the animal, and are only recoverable if incorporated into

144 microbial protein intact. Therefore, the second model (Figure 2) uses the inputs estimated from

145 the first model but excludes transfers through the NH_3 pool and is used to estimate true EN AA

146 uptake by the microbes and subsequent endogenous AA recovery.

147

148   Transactions in the first model (Figure 1) begin with labeled EN (**LEN**) that is degraded

149 (LEN to R) and enters the peptide and free AA (**PAA**) pool in the rumen (LEN PAA R). From

150 there, the LEN can escape (LEN PAA Escape), be degraded to NH_3 (LEN PAA Deg) or be

151 taken up as PAA by NFB (LEN PAA Uptake NFB) or PZ (LEN PAA Engulfment). Protozoa

152 either incorporate the LEN (PZ LEN Engulfed Incorporated), excrete it as PAA (PZ LEN

153 Engulfed excreted as PAA), or excrete it as NH_3 (PZ LEN Engulfed excreted as NH_3). Labelled

154 PZ can escape the rumen (PZ Cell LEN Escape) or lyse (PZ Cell LEN Lysis). Protozoal

155 excretion of PAA, NH_3 and lysis has the effect of transferring EN through numerous rumen N

156 pools and also allows FB to be enriched through the labeled NH_3 pool (NH_3 LEN R) which can

157 also escape (FB Cell LEN Escape). Enrichment of MCP through the NH_3 pool is not considered

158 available for recovery given the AA itself has been degraded. Therefore, these same

159 transactions are considered in second model with LEN transfers through the NH3 excluded

160 (LEN*; Figure 2).

161

162   Figure 1 somewhere here

163   Figure 2 somewhere here

164

165   Each individual source of EN can be quantified within the model, as either free EN, or

166 incorporated in MCP, from the initial transfer into the gut to its final fate. An AA profile is

167 applied to each component using the profiles in Table 2. Microbial AA of endogenous origin

7

168   are not considered new supply and are subtracted off digested microbial AA using the profile

169   of the original source (Table 2). Endogenous AA in MCP are assumed to be evenly distributed

170   through the cell N and digestion is relative to the digestion of total MCP. Free EN can be

171   recovered if digested in the small intestine; otherwise the AA are either fermented in the

172   hindgut or excreted in the feces and are considered lost. Overall, losses occur from degradation

173   and absorption as NH_3 in the rumen and large intestine, or excretion in the feces. The

174   requirement for endogenous AA can, therefore, be calculated as total entry into the gut less

175   recovery in the small intestine.

176

177   Table 2 somewhere here

178

179   ***Estimating total AA requirements***

180   Amino acid net requirements (**AAnR**) estimated in the CNCPS v6.5 include those for milk,

181   growth, reserves, pregnancy, scurf, metabolic urinary losses and endogenous losses in the

182   gastrointestinal tract (GIT). Endogenous N losses in the GIT in this model are calculated as

183   described above whereas the other requirements, including AA profiles, are estimated

184   according to Fox et al. (2004). Amino acids used for other processes not accounted for by the

185   model (**AAO**) can be calculated as the difference between predicted AA supply (**AAS**) and

186   AAnR. The term often used to estimate AAnR relative to AAS is 'efficiency of use' which can

187   vary depending on AA supply relative to other nutrients and the physiological state of the

188   animal (Hanigan et al., 1998; Doepel et al., 2004). In order to balance a diet in which individual

189   EAA supply is not excessive, but also not limiting, estimates of AAO relative to AAnR are

190   required. In this model, the approach used to generate these estimates was similar to the study

191   of Doepel et al. (2004). Briefly, a dataset was constructed of studies that infused AA into the

192   abomasum, duodenum, or intravenously (Table 3). Infusion studies were used so that the

8

193   addition of AA above the basal diet was known and limited the reliance on model predictions

194   (Doepel et al., 2004). The final dataset included 41 publications, 51 experiments and 218

195   treatment means. Descriptive statistics for the dataset are in Table 4. Dietary and animal

196   characteristics reported in the publications were entered into the CNCPS. When limited

197   information was reported on the chemical composition of the dietary components, the reported

198   information was used, and uncertain values were predicted using an extension of the method

199   described in Higgs et al. (2015). Briefly, it was assumed that the feeds used in different

200   treatments in the same study had the same chemical composition. The procedure optimized

201   each chemical component in each feed to be within a likely range, to be internally consistent

202   (chemical components sum to 100% DM) and to allow the compiled diet to match the reported

203   composition. As described previously, infused AA were assumed to be 100% digested (Doepel

204   et al., 2004). Once compiled, each treatment was evaluated through the model to estimate AAS

205   and AAnR for each of the 10 EAA.

206

207   Table 3 somewhere here

208   Table 4 somewhere here

209

210 A logistic model with three parameters was used to fit the data which was previously shown to

211 give the most appropriate fit (Doepel et al., 2004). The selected model has the form

212

213     $y = \dfrac{\theta_1}{1 + \theta_2 e^{\theta_3(x)}}$ [1]

214

215 where y is the AAnR (g/d), x is the predicted AAS (g/d), $\theta_{1\text{-}3}$ are the model parameters used to

216 described the sigmoidal shape of the curve. The optimum supply of AA was considered to be

217   the point on the curve where the rate of change in the ratio of AAnR:AAS was the most rapid,

218   or, in other words, the rate at which cows were changing the way they managed additional AAS

219   was most rapid (Figures 3 and 4). This can be calculated when the third derivative of the logistic

220   model is zero. The third derivative has the form

221

222     $\dfrac{d^3y}{dx^3} = -\theta_1\theta_2\theta_3{}^3 e^{\theta_3(x)} \dfrac{1-4\theta_2 e^{\theta_3(x)} + \theta_2{}^2 e^{2\theta_3(x)}}{(1+\theta_2 e^{\theta_3(x)})^4}$ [2]

223

224   and the zero point of interest is calculated using the equation

225

226     $x = \dfrac{1}{\theta_3}\log\left(\dfrac{2-\sqrt{3}}{\theta_2}\right)$ [3]

227

228   where x is considered the optimum AAS for the dataset used. By substituting x into equation

229   [1], and dividing y (AAnR) by x (AAS) the optimum ratio of AAnR to AAS can be calculated,

230   and indirectly, the optimum level of additional AA for other functions not considered by the

231   model. When balancing a diet, the total required supply (g AA/d) can be calculated by dividing

232   AAnR by the optimum ratio of AAnR to AAS. The same calculations were also performed for

233   MP.

234

235   The relationship between ratio of AAnR and AAS and AA supply relative to other nutrient

236   supplies (g AA/ Mcal ME and g AA/ 100g MP) was also investigated. A log-logistic model

237   with three parameters was used to fit this relationship with the form

238

239     $y = \theta_1 - \log\left(1 + \theta_2 e^{-\theta_3(x)}\right)$ [4]

241 where y is the ratio of AAnR to AAS, x is the AA supply expressed relative to Mcal of total

242 ME supply or relative to 100 g MP, and $\theta_{1\text{-}3}$ are the model parameters used to describe the

243 shape of the curve. The optimum supply of a given EAA relative to ME or MP can then be

244 found by rearranging formula [4] and solving for x using the AAnR:AAS (y) previously

245 calculated.

$$x = \frac{-1}{\theta_3} log \left(\frac{e^{\theta_1 - y} - 1}{\theta_2} \right) \qquad [5]$$

249 Given the information presented by studies published in the literature is typically limited

250 compared to the inputs required by the CNCPS, a large number of assumptions had to be made.

251 To limit the influence of potential input errors, points were weighted on the likelihood of being

252 an outlier. The scheme used was the Tukey Biweight and was implemented according to

253 Motulsky and Christopoulos (2004). Data analysis was performed using the non-linear

254 modelling function in SAS (2010).

<p style="text-align:center">RESULTS AND DISCUSSION</p>

257 *Endogenous N flows*

258 The mechanistic framework developed in Higgs and Van Amburgh (CHO and PRO) and

259 Higgs and Van Amburgh (microbial growth) enabled EN to be modeled in all parts of the GIT

260 including the microbial transactions in the rumen and large intestine in a manner that reconciled

261 entrance and disposal of both N and AA by compartment. Model estimates compared to

262 measurements taken from multi-cannulated animals in the studies of Ouellet and coworkers are

263 in Table 5. Model predicted flows of EN at the duodenum were similar to measured values.

264 The greatest difference was observed in the prediction of microbial EN in the 'Inoc' and

265 'Formic' treatments (Ouellet et al., 2010a). The model assumes microbes do not differentiate

266 between the original source of N in the rumen with uptake being based on the relative

267 availability of each source (Marini et al., 2008). Silages fed in the 'Inoc' and 'Formic'

268 treatments had higher levels of soluble protein than the hay treatment (Martineau et al., 2007)

269 which increased the availability of feed N in the rumen relative to EN and resulted in lower

270 predicted microbial uptake of EN. The rate of CHO digestion in the rumen also impacts

271 predictions of EN uptake through its effect on microbial growth (see Higgs and Van Amburgh

272 microbial growth). Therefore, more accurate estimates of the CHO digestion kinetics could

273 improve the present model predictions. Although differences in EN secretion into the foregut

274 among dietary treatments has been observed (Ouellet et al., 2010a), the mechanism of action

275 is still unclear (Larsen et al., 2000). Therefore, expressing EN secretion relative to DMI seemed

276 appropriate until the factors involved are better understood. Further down the GIT, estimates

277 were similar to measured values at the terminal ileum and in the feces (Table 5).

278

279 Total EN transactions through each compartment in the model for the 'Hay' treatment in

280 Ouellet et al. (2010a) are summarized in Figure . These data were generated using the model

281 in Figure 2 where EN transfers through the NH_3 pool were excluded. The 'Hay' treatment was

282 chosen given the close agreement between model and measured values. Total EN secretions

283 into the GIT were 135.4 g/d of which 46.4 g/d was recovered as absorbed AA from intestinal

284 digestion originating from either the forestomach or the small intestine. The EN contribution

285 to total rumen N supply was 90 g/d and demonstrates an additional method by which the cow

286 provides nutrients to support the microbial population. Previous versions of the CNCPS, based

287 on the data from Russell et al. (1992), indicate dietary provision of peptides was necessary to

288 enhance the fermentation and growth of NFB. However, the data from Ouellet et al. (2010a)

289 as described in this model demonstrate that large quantities of peptides are provided to the

290 microbes in the form of EN reducing the reliance on dietary sources. Endogenous N not

291 recovered (89.0 g/d) was considered lost by the animal and is part of the maintenance

292 requirements for protein. Of the 89.0 g/d lost, 31.8 g/d appeared in the feces and 57.2 g/d was

293 degraded in the GIT to NH_3. The total estimated requirement (89.0 g/d) when expressed relative

294 to DMI is 5.1 g EN/ kg DMI which, interestingly, is similar to current model (CNCPS v6.5,

295 Van Amburgh et al. 2015) estimates of MFN for the same diet (5.0 g MFN/kg DMI).

296

297   Figure somewhere here

298   Table 5 somewhere here

299

300 ***Amino acid requirements***

301   Requirements for each individual EAA in the CNCPS v6.5 are predicted from the net

302 protein required for each physiological process, quantified by the model (maintenance,

303 lactation, pregnancy, growth) multiplied by the AA composition of each process and

304 subsequently divided by the efficiency of transfer to that process to give the total AA

305 requirement (O'Connor et al., 1993; Fox et al., 2004). The efficiency of transfer could also be

306 considered as the additional usage of each AA relative to the requirements quantified by the

307 model. Such processes include oxidation across tissues, anaplerotic requirements, synthesis of

308 non-essential AA, gluconeogenesis etc. (Lapierre et al., 2005; Lapierre et al., 2006; Lemosquet

309 et al., 2010; Lobley, 2007). The apparent efficiency of AA use for any given diet can be

310 calculated by dividing model predicted AAnR by AAS, which can be variable, and typically

311 decreases as AAS increases relative to AAnR (Hanigan et al., 1998; Doepel et al., 2004); the

312 apparent efficiency has also been reported to decrease when the ratio of MP relative to energy

313 supply increases (NorFor, 2011; van Duinkerken et al., 2011). The ability of cows to direct AA

13

314   to other uses demonstrates the interactions among different nutrients and is an example of the

315   metabolic flexibility that allows productivity to be maintained across a wide range of nutrient

316   inputs and supply (Lobley, 2007). The pertinent question for diet formulation is: what level of

317   additional AA supply above the predicted requirements for milk protein synthesis, body protein

318   requirements and other metabolic needs will maximize productivity and minimize AA

319   wastage? The answer to this question is going to differ among models as supply and

320   requirements are calculated in different ways. For example, changing the maintenance

321   requirements from using MFN as in previous version of the CNCPS to estimating AA loss

322   through the GIT using isotopic enrichment techniques considers 9 different sources of EN, each

323   with a different AA profile (Table 2), and so it would be expected that AA requirements among

324   models will be different.

325

326   The optimum supply of EAA in this study was defined where the logistic curve was

327   approaching a plateau most rapidly. This point was defined by Doepel et al. (2004) as an

328   optimal supply and is similar to the break-point in the segmented linear model used in the NRC

329   (2001). Previous versions of the CNCPS have treated different physiological functions

330   separately with the original values coming from a range of sources outlined in O'Connor et al.

331   (1993). Lapierre et al. (2007) suggested using a single efficiency of use to calculate total AA

332   requirement for maintenance and milk production. Amino acid catabolism is linked to the tissue

333   where enzymes are present to ensure catabolic pathways (Lobley and Lapierre, 2003) and is

334   not linked to the site of protein secretion. For example, there is no catabolism of Phe in the

335   mammary gland, independently of the Phe supply (Lemosquet et al., 2010); Phe is almost

336   exclusively removed by the liver (Lapierre et al., 2005). Recommendations for fixed

337   efficiencies of use of individual AA were presented by Lapierre et al. (2007) and have been

338   implemented in the most recent update of the CNCPS (v6.5; Van Amburgh et al., 2015). Model

14

339   parameters and the fit summary for the logistic model used to make the calculations in this

340   study are in Table 6. The variation explained in the present study by the logistic model was

341   similar to Doepel et al. (2004). Examples of model fit and optimum supply for Met and Lys

342   are in Figures 3 and Figure . The optimum ratio of model predicted AAnR to AAS for each AA

343   and MP are in Table 6. As explained, it is difficult to compare the ratio of AAnR:AAS among

344   studies due to the different approach models used to calculate AAnR. However, it is possible

345   to compare the optimum AAS expressed as % EAA and also in g/d relative to the study of

346   Doepel et al. (2004) given the similarities in the datasets. The required supply and balance of

347   EAA in the current study compared with Doepel et al. (2004) are fairly similar despite the

348   differences in the models used to estimate supply.

349

350   Table 6 somewhere here

351   Figure 3 somewhere here

352   Figure somewhere here

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354   This concept is important when discussing how high the efficiency of use of an AA should

355   be. Does a high efficiency describe efficient use of an AA or does it describe a relative

356   deficiency of this particular AA? On a general basis, the higher the efficiency the more

357   restricted the supply is relative to the requirement. As the AA becomes less 'limiting' up to an

358   optimum, the amount of milk, milk protein or energy corrected milk increases until a plateau

359   is achieved, and any AA supplied beyond that point is not utilized with the same efficiency for

360   the expected outcome (Rulquin et al., 1993; Schwab, 1996). Thus, a high efficiency of use

361   more likely reflects an under-supply of AA relative to the available energy, other AA or both.

362

Interactions between AA supply and energy

The impact of energy supply on the utilization of AA was investigated by regressing the ratio of AAnR and AAS (efficiency of use) against AA supply relative to total ME and total MP supply. No relationship was found when AA were expressed relative to MP, but a log-logistic relationship was observed when AA supply was expressed relative to ME. The optimum supply of each EAA relative to ME was determined by using the optimum ratio of AAnR to AAS calculated in the previous analysis and solving for *x* using the log-logistic model Eq. [5]. Examples of the log-logistic fit and optimum supply relative to ME for Met and Lys are in Figures 3 and 4, respectively. The model parameters, summary of fit and optimum AA supply relative to ME for all 10 EAA are in Table 7. Using the proportional approach, recommendations for AA balancing are made with the ratio of the AA relative to total MP supply. This approach has been successful in establishing Met and Lys requirements from dose response studies (Rulquin et al., 1993; Schwab, 1996; NRC, 2001). The studies used to estimate these requirements are unique in that they isolate the response to the increased supply of Lys, Met or both, from post-ruminal infusion or rumen-protected AA, but holding all other dietary variables constant. The data used in the present study was different in that 81% of the treatments simultaneously infused more than 1 AA with the average number of AA infused > 8. Interestingly, the optimum supply of Met and Lys estimated in this study was 15.1% and 5.7% of EAA, respectively, which is similar to results found in other studies that used different approaches (Schwab et al., 1992b; Rulquin et al., 1993; Schwab, 1996). Under these circumstances, no relationship was observed between the 'efficiency' of AA use when AA supply was expressed relative to MP supply but a strong relationship was observed when AA were expressed relative to ME supply which is in agreement the findings of Van Straalen et al. (1994), whereas Sauvant et al. (2015) used the ratio of AA supply to DMI. This approach is consistent with the relationship between energy intake and endocrine signals to regulate

16

388   nutrient partitioning of absorbed nutrients (Bauman and Currie, 1980, McGuire et al., 1995,

389   Bequette et al., 1998, Arriola Apelo et al., 2014). Protein synthesis within the mammary gland

390   is dependent on the energy supply and the associated signals necessary to direct nutrients to

391   the mammary gland for uptake (Lemosquet et al., 2010, Mackle et al., 2000). Therefore, when

392   balancing rations, it might be more appropriate to consider AA supply relative to ME which is

393   the approach used in swine (NRC, 2012). Establishing requirements for monogastrics is less

394   complicated than in ruminants as the net AA supply is more easily determined (Lapierre et al.,

395   2006). Following the monogastric example, the predicted Lys requirement for a lactating sow

396   in the NRC (2012) model is 2.72 g Lys/Mcal ME which is 11% lower than the 3.03 g Lys/Mcal

397   ME calculated in this study for dairy cows. The recommended ratios for each EAA relative to

398   ME and relative to Lys are compared in Table 7: out of the 10 EAA, 5 differ by more than

399   25%. As improvements are made to the predictions of net AA supply in dairy cows,

400   consideration of the approach used to balance AA in other species where AA supply is more

401   easily determined could provide opportunities to improve productivity and the efficiency of

402   nutrient use.

403

404   Figure 3 somewhere here

405   Figure 4 somewhere here

406   Table 7 somewhere here

407

408   **CONCLUSIONS**

409   Predictions of endogenous N transactions along the entire GIT have been incorporated into

410   a dynamic version of the CNCPS (v7). This has replaced metabolic fecal N used in previous

411   versions of the CNCPS in estimating AA requirements for maintenance. Model predictions for

412 endogenous N transactions along the GIT are close to measured data and have refined the

413 predictions of net AA supply to the animal. Using a logistic approach, efficiency of utilization

414 of individual AA to support the physiological processes was also quantified. The optimum

415 supply of Met and Lys relative to total EAA were similar to other studies. A log-logistic

416 relationship was also observed when the efficiency of AA use was regressed against AA supply

417 relative to ME. Expressing AA supply relative to energy could improve predictions of AA

418 utilization. Recommendations for each EAA are given in g AA/Mcal ME and also in a ratio

419 with Lys.

420

424

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671

672   **TABLES AND FIGURES**

673   **Table 1.** Endogenous contributions and digestion coefficients used to predict endogenous AA

674   flows in the models described in Figures 1 and 2[1]

Endogenous component	Secretion (g N/kg DMI)	Kd (%/hr)[3]	SID (%)[4]
Saliva	0.9	150	5
Rumen sloughed cells	4.3	150	5
Omasum/abomasum sloughed cells	0.3	0.0	70
Omasum/abomasum secretions	0.2	0.0	70
Pancreatic secretions	0.4	0.0	70
Bile	0.1	0.0	70
Small intestine sloughed cells[2]	0.7	75	50
Small intestine secretions[2]	0.7	75	50
Large intestine sloughed cells	0.3	150	N/A

675   [1]Back calculated to align predictions at various points in the gut to measured data (Ouellet et al.,

676   2002; Ouellet et al., 2010a).

677   [2]Includes secretions prior to the terminal ileum (Egan et al., 1984).

678   [3]Rate of microbial degradation in either the rumen or large intestine

679   [4]Small intestinal digestibility

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683 **Table 2.** Profiles of EAA as a percent of EAA-N, total EAA-N as a percent of AA-N, and AA-N as a percent of total N for endogenous

684 N components predicted by the model

Endogenous component	Met	Lys	Arg	Thr	Leu	Ile	Val	His	Phe	Trp	EAA-N[7]	AA-N
Saliva[1]	1.0	12.4	24.7	13.2	13.2	6.5	12.9	8.7	4.6	2.8	48.9	80.0
Rumen, omasum /abomasum, large intestine sloughed cells[2]	2.5	18.5	29.2	6.7	12.8	6.3	8.5	8.5	4.8	2.2	56.1	79.0
Omasum/abomasum secretions[3]	1.9	19.4	21.9	10.6	7.1	6.9	10.1	13.5	5.5	3.0	52.2	64.9
Pancreatic secretions[4]	2.0	16.2	18.0	10.6	13.0	7.7	12.4	12.6	5.0	2.6	51.3	94.3
Bile[5]	2.6	13.3	13.4	9.8	13.4	7.4	13.3	18.9	4.7	3.0	7.2	51.5
Small intestine sloughed cells and secretions[6]	1.9	14.3	23.7	13.2	9.8	7.5	11.8	9.5	5.4	3.0	39.2	72.9

685 [1]Salivary protein (Yisehak et al., 2012)

686 [2]Rumen epithelia (Larsen et al., 2000)

687 [3]Abomasal isolates (Ørskov et al., 1986)

688 [4]Pancreatic juice from Hamza (1976) reported by Larsen et al. (2000)

689 [5]Cow bile (Larsen et al., 2000)

690 [6]Ileal endogenous AA in pigs (Jansman et al., 2002)

691 [7]The contribution of NEAA-N to endogenous secretions is represented by the proportion AA-N not accounted for by total EAA-N.

692   **Table 3.** Studies included in the dataset to estimate AA requirements by the logistic approach

Aldrich et al., 1993	Köning et al., 1984
Bruckental et al., 1991	Lapierre et al., 2009
Cant et al., 1991	Lynch et al., 1991
Choung and Chamberlain, 1992a	Mackle et al., 1999a
Choung and Chamberlain, 1992b	Mackle et al., 1999b
Choung and Chamberlain, 1993	Metcalf et al., 1996
Choung and Chamberlain, 1995a	Pisulewski et al., 1996
Choung and Chamberlain, 1995b	Raggio et al., 2006
Choung and Chamberlain, 1995c	Relling and Reynolds, 2008
Clark et al., 1977	Rius et al., 2010
Cohick et al., 1986	Robinson et al., 2000
Doepel and Lapierre, 2010	Rogers et al., 1984
Doepel and Lapierre, 2011	Schwab et al., 1976
Griinari et al., 1997	Schwab et al., 1992a
Guinard and Rulquin, 1994	Schwab et al., 1992b
Guinard and Rulquin, 1995	Seymour et al., 1990
Guinard et al., 1994	Vanhatalo et al., 1999
Huhtanen et al., 1997	Varvikko et al., 1999
Kim et al., 1999	Vicini et al., 1988
Kim et al., 2000	Weekes et al., 2006
King et al., 1991	

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696 **Table 4.** Descriptive statistics of the dataset used to estimate optimum AA efficiency

	Mean	SD	Min	Max
Dry matter intake (kg/d)	18.0	3.1	11.0	27.6
Day in milk (d)	107	51	28	240
Body weight (kg)	551	55	487	733
Milk yield (kg/d)	26.3	5.85	10.7	40.0
Milk fat (%)	3.98	2.65	2.37	41.90
Milk true protein (%)	2.88	0.20	2.38	3.52
Fat yield (kg/d)	1.01	0.51	0.53	8.09
Milk true protein yield (kg/d)	0.76	0.16	0.32	1.11

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699 **Table 5.** Measured and model predicted endogenous N flows along the gut (g EN/kg DMI)

Endogenous flow	High forage[1] Study	High forage[1] Model	Low forage Study	Low forage Model	Hay Study	Hay Model	Formic Study	Formic Model	Inoc Study	Inoc Model	Average Study	Average Model
Total duodenum	3.4	3.8	3.7	3.6	4.9	4.8	4.3	4.1	4.7	4.1	4.2	4.1
Microbial	2.0	2.3	2.3	2.1	3.3	3.3	3.1	2.6	3.4	2.5	2.8	2.6
Free[2]	1.3	1.5	1.4	1.5	1.6	1.5	1.2	1.5	1.3	1.5	1.4	1.5
Total ileum[3]		2.0		2.0	2.1	2.3	2.4	2.2	2.9	2.1	2.5	2.2
Secreted in the forestomach[3]		1.3		1.3	1.3	1.6	1.8	1.5	1.8	1.5	1.6	1.5
Secreted in the small intestine		0.7		0.7	0.8	0.7	0.6	0.7	1.1	0.7	0.8	0.7
Fecal	1.8	2.0	2.0	1.9	2.4	2.3	2.1	2.1	2.5	2.1	2.1	2.1
Secreted in the forestomach	1.4	1.3	1.3	1.3	1.8	1.6	1.5	1.5	1.7	1.5	1.6	1.4
Secreted in the intestine[4]	0.4	0.7	0.6	0.6	0.6	0.7	0.6	0.7	0.8	0.7	0.6	0.7

700 [1]High forage and Low forage are from Ouellet et al. (2002); Hay, Formic and Inoc are from Ouellet et al. (2010b)

701 [2]Estimated using the combined precursor pool. All other data represent the mucosa precursor pool

702 [3]Only 2 cows had ileal canula (this is why the contributions from the forestomach are different compared with values reported with

703 fecal EN)

704 [4]Includes contributions from the large intestine

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707 **Table 6.** Model parameters, RMSE, R^2 and model outcomes for the log-logistic model fit between

708 predicted AA requirement and supply

AA	Model parameters			RMSE	R^2	AAnR:AAS[1]	g/d[2]	% EAA
	θ_1	θ_2	θ_3					
Arg	66.72	3.17	-0.03	3.31	0.79	0.55	96.4	10.2%
His	39.22	2.77	-0.05	2.47	0.76	0.70	43.9	4.5%
Ile	79.32	3.93	-0.03	4.85	0.74	0.61	102.7	10.8%
Leu	135.12	2.81	-0.01	8.52	0.72	0.67	158.3	17.1%
Lys	114.87	3.21	-0.02	7.33	0.72	0.62	145.1	15.1%
Met	39.23	2.49	-0.04	2.40	0.73	0.53	58.2	5.7%
Phe	69.30	3.52	-0.02	4.23	0.74	0.53	103.4	10.7%
Thr	69.54	3.50	-0.02	4.23	0.74	0.53	102.9	10.7%
Trp	20.74	4.42	-0.10	1.04	0.81	0.58	28.1	2.9%
Val	93.80	2.99	-0.02	6.10	0.68	0.62	118.8	12.4%
MP[3]	1625.35	3.67	-0.002	93.35	0.76	0.73	1751.8	N/A

709 [1] Optimum ratio of predicted AA requirement (AAnR) and supply (AAS)

710 [2] Optimum duodenal AA supply for the dataset used and presented in Table 4

711 [3] MP = Metabolizable protein

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Table 7. Model parameters and fit summary for the log-logistic relationship between AA requirement and supply as well as optimum supply of each EAA relative to ME and relative to Lys.

AA	Model parameters			R_2	RMSE	g AA/ Mcal ME	Lys:AA Dairy[1]	Lys:AA Swine[2]
	θ_1	θ_2	θ_3					
Arg	0.14	-0.88	0.47	0.80	0.05	2.04	1.49	1.85
His	0.19	-1.01	1.01	0.79	0.07	0.91	3.33	2.50
Ile	-0.53	-0.87	0.12	0.71	0.06	2.16	1.40	1.78
Leu	-0.27	-0.90	0.11	0.79	0.06	3.42	0.89	0.89
Lys	0.02	-0.89	0.23	0.73	0.06	3.03	1.00	1.00
Met	0.16	-0.97	1.01	0.75	0.06	1.14	2.66	3.71
Phe	0.09	-0.81	0.39	0.72	0.05	2.15	1.40	1.82
Thr	-0.53	-0.84	0.12	0.71	0.05	2.14	1.41	1.49
Trp	-0.21	-0.81	0.67	0.68	0.05	0.59	5.16	5.33
Val	-0.09	-0.88	0.22	0.75	0.06	2.48	1.22	1.15

718 [1] Optimum Lys:EAA ratio for the data set used and presented in Table 4
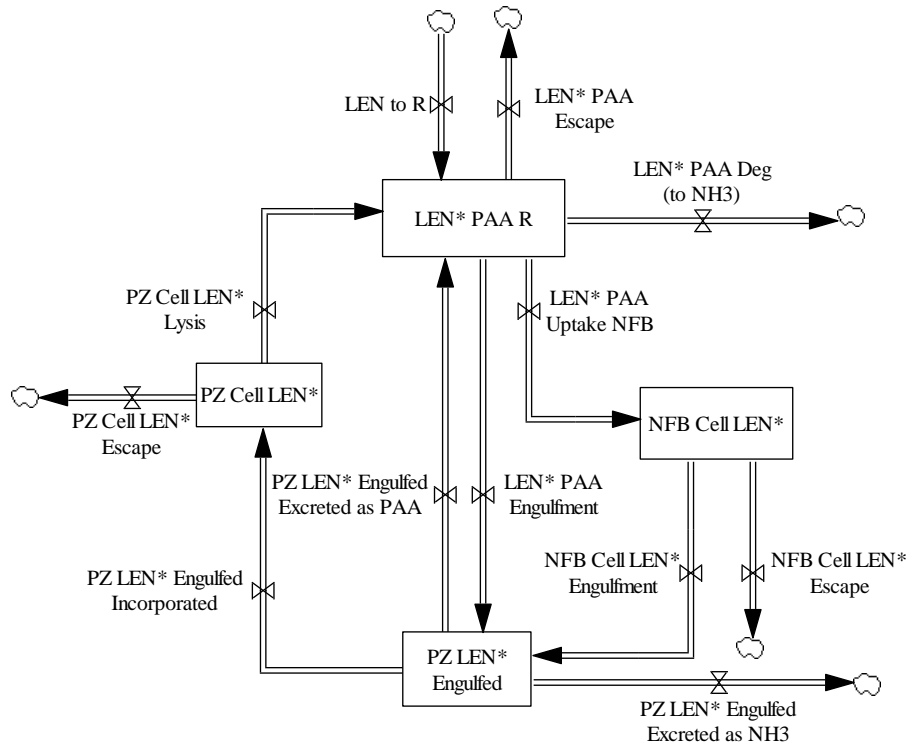
719 [2] Optimum Lys:EAA ratio for a lactating sow (NRC, 2012)

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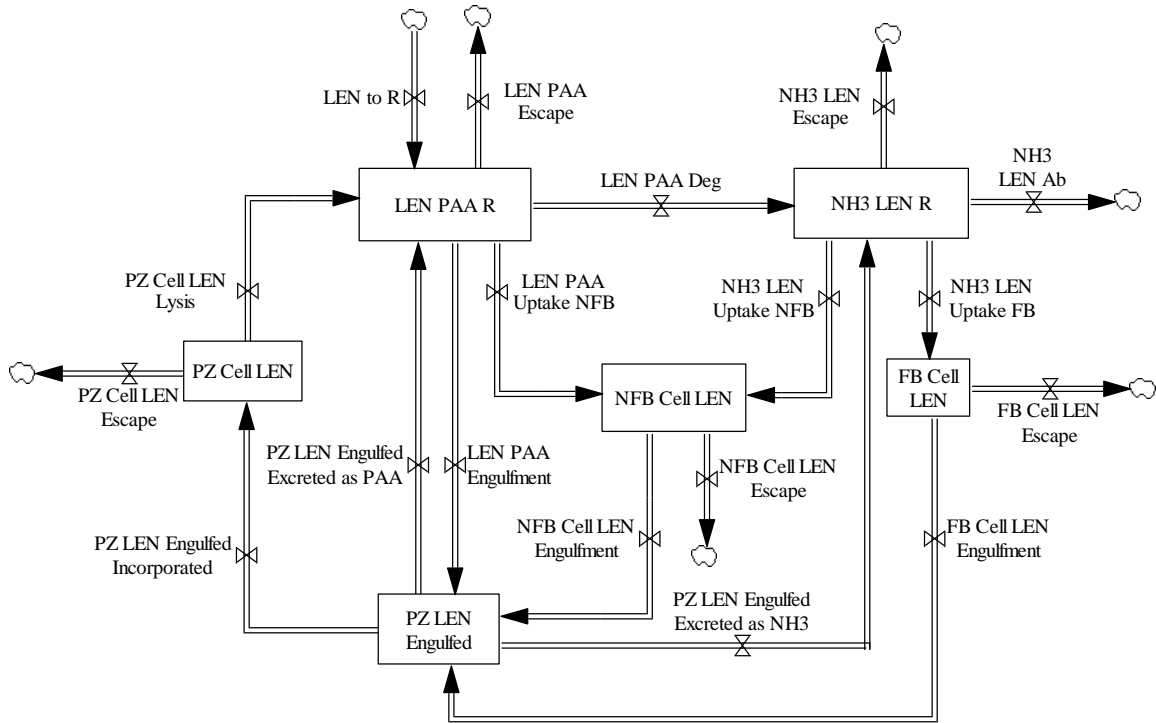
Figure 1. Schematic representation of the model used to predict the incorporation of labelled
endogenous N (LEN) into rumen microorganisms (see text for abbreviations)

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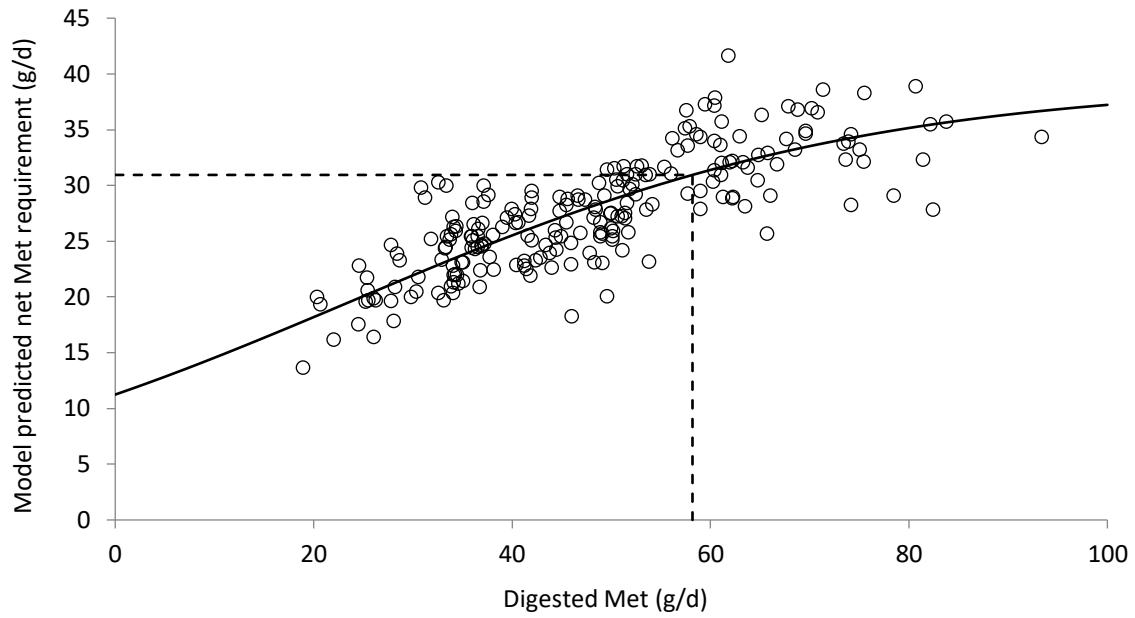725

Figure 2. Schematic representation of the model used to predict the incorporation of labelled

endogenous N, excluding transaction through the NH_3 pool (LEN*), into rumen microorganisms

(see text for abbreviations)

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Figure 3. Logistic fit of model predicted net Met requirement and Met supply. The dashed line

represents the optimum ratio of Met requirement and Met supply and is calculated by setting the

third derivative of the logistic equation to 0. For this dataset, the optimum level of digested Met

is 58.2 g/d (X axis) which corresponds to a model predicted requirement of 30.8 g/d (Y axis)
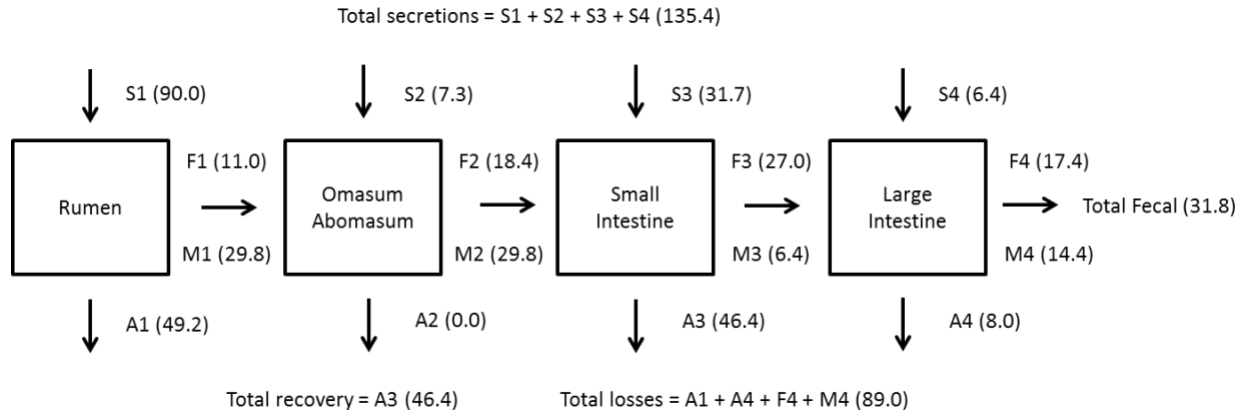
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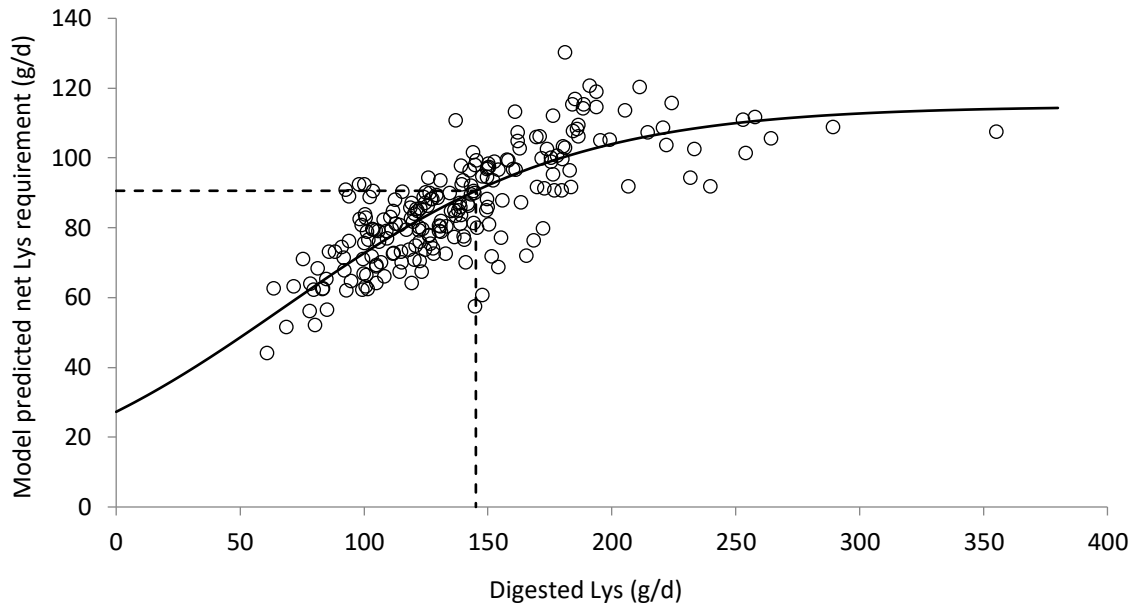
Figure 4. Logistic fit of model predicted net Lys requirement and Lys supply. The dashed line

represents the optimum ratio of Lys requirement and Lys supply and is calculated by setting the

third derivative of the logistic equation to 0. For this dataset, the optimum level of digested Lys

is 145.1 g/d (X axis) which corresponds to a model predicted requirement of 90.0 g/d (Y axis)

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Total secretions = S1 + S2 + S3 + S4 (135.4)

S1 (90.0)   S2 (7.3)   S3 (31.7)   S4 (6.4)

| Rumen | F1 (11.0) | Omasum Abomasum | F2 (18.4) | Small Intestine | F3 (27.0) | Large Intestine | F4 (17.4) → Total Fecal (31.8) |
| | M1 (29.8) | | M2 (29.8) | | M3 (6.4) | | M4 (14.4) |

A1 (49.2)   A2 (0.0)   A3 (46.4)   A4 (8.0)

Total recovery = A3 (46.4)   Total losses = A1 + A4 + F4 + M4 (89.0)

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748 **Figure 5.** Model predicted endogenous transactions (g endogenous N/d) by compartment for the

749 hay treatment presented in Ouellet et al. (2010a). S1-S4 are the endogenous secretions into the

750 gut; F1-F4 are the flows of free endogenous N; M1-M4 are the flow of endogenous N in

751 bacteria; A1-A4 is the endogenous N absorption at different sites. Recovery is only possible in

752 the small intestine (A3) where the N can be absorbed as AA

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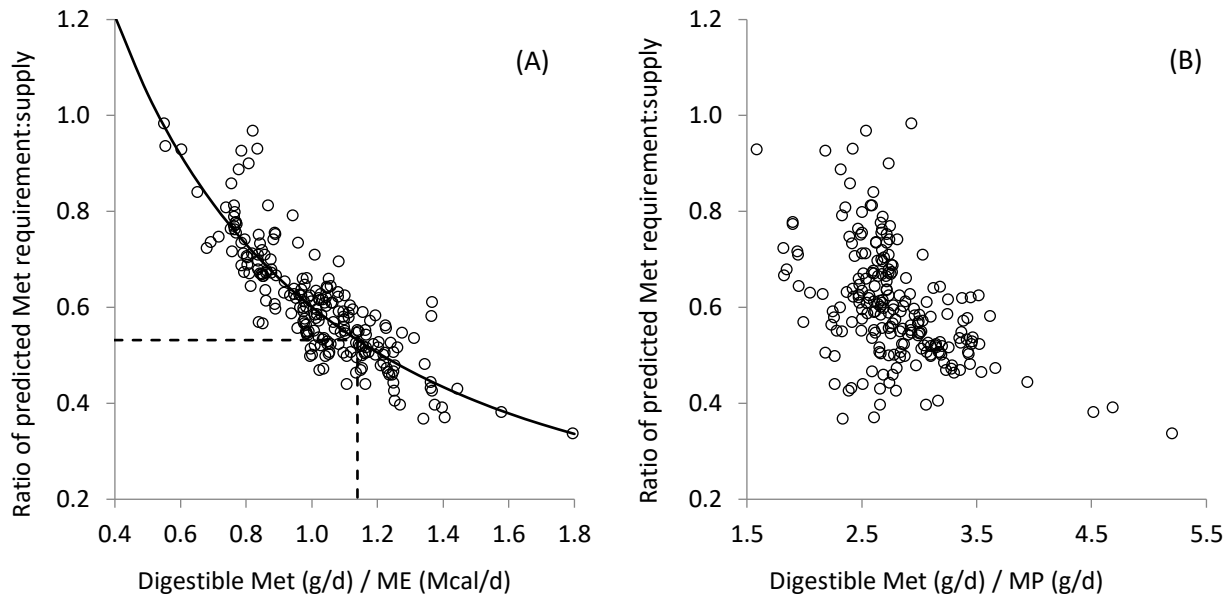
Figure 3. Relationship between model predicted Met net requirement:supply and Met supply relative to ME (A) or MP (B). The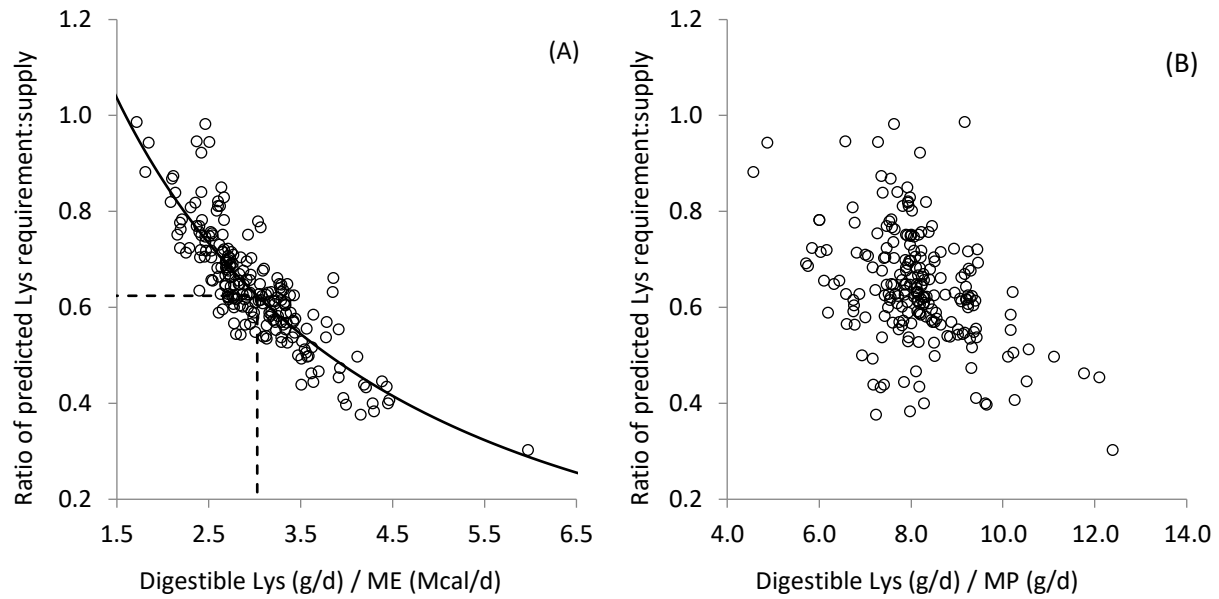 dashed line in (A) represents the Met supply at the optimum ratio of model predicted Met requirement and supply and is calculated by rearranging the log-logistic equation and solving for X. The optimum ratio of Met requirement:supply is 0.53 (Y axis) and the optimum supply is 1.14 g/Mcal ME (X axis). No significant relationship was obtained in (B)

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Figure 4. Relationship between model predicted Lys net requirement:supply and Lys supply relative to ME (A) or MP (B). The dashed line in (A) represents the Lys supply at the optimum ratio of model predicted Lys requirement and supply and is calculated by rearranging the log-logistic equation and solving for X. The optimum ratio of Met requirement:supply is 0.62 (Y axis) and the optimum supply is 3.03 g/Mcal ME (X axis). No significant relationship was obtained in (B)