

Prediction of portal and hepatic blood flow from intake level data in cattle

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1 **INTERPRETIVE SUMMARY:**

2 **Prediction of portal and hepatic blood flow in cattle. Ellis et al.** Given the extent of variability in
3 post absorptive metabolism, there is growing interest in developing integrated post-absorptive
4 metabolism models for cattle. An integral part of linking a multi-organ post-absorptive model is
5 the prediction of nutrient flow between organs, and thus blood flow. This paper applied a
6 multivariate meta-analysis technique to simultaneously predict incoming and outgoing blood flows
7 to the liver. Prediction equations based on DMI performed well, and division of DMI into forage
8 and concentrate DMI improved blood flow predictions.

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10 **RUNNING HEAD: PREDICTION OF LIVER BLOOD FLOW IN CATTLE**

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12 **Prediction of portal and hepatic blood flow from intake level data in cattle**

13
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ABSTRACT

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There is growing interest in developing integrated post-absorptive metabolism models for dairy cattle. An integral part of linking a multi-organ post-absorptive model is the prediction of nutrient fluxes between organs, and thus blood flow. It was the purpose of this paper to use a multivariate meta-analysis approach to model portal blood flow (PORBF) and hepatic venous blood flow (HEPBF) simultaneously, with evaluation of hepatic arterial blood flow (ARTBF; $\text{ARTBF} = \text{HEPBF} - \text{PORBF}$) and $\text{PORBF}/\text{HEPBF}$ (%) as calculated values. The database used to develop equations consisted of 296 individual animal observations (lactating and dry dairy cows and beef cattle) and 55 treatments from 17 studies, and a separate evaluation database consisted of 34 treatment means (lactating dairy cows and beef cattle) from 9 studies obtained from the literature. Both databases had information on DMI, MEI, body weight and a basic description of the diet including crude protein intake and forage proportion of the diet (FP; %). Blood flow (L/h or L/kg $\text{BW}^{0.75}/\text{h}$) and either DMI or MEI (g or MJ/d or g or MJ/kg $\text{BW}^{0.75}/\text{d}$) with linear and quadratic fits were examined. Equations were developed using cow within experiment and experiment as random effects, and blood flow location as a repeated effect. Upon evaluation with the evaluation database, equations based on DMI typically resulted in lower root mean square prediction errors, expressed as a % of the observed mean (rMSPE%) and higher concordance correlation coefficient (CCC) values than equations based on MEI. Quadratic equation terms were frequently non-significant, and the quadratic equations did not out-perform their linear counterparts. The best performing blood flow equations were: $\text{PORBF (L/h)} = 202 (\pm 45.6) + 83.6 (\pm 3.11) \times \text{DMI (kg/d)}$ and $\text{HEPBF (L/h)} = 186 (\pm 45.4) + 103.8 (\pm 3.10) \times \text{DMI (kg/d)}$, with rMSPE% values of 17.5 and 16.6 and CCC values of 0.93 and 0.94, respectively. The residuals (predicted – observed) for $\text{PORBF}/\text{HEPBF}$ were significantly related to the forage % of the diet, and thus equations for

52 PORBF and HEPBF based on forage and concentrate DMI were developed: PORBF (L/h) = 210
53 (± 51.0) + 82.9 (± 6.43) \times Forage (kg DM/d) + 82.9 (± 6.04) \times Concentrate (kg DM/d), and
54 HEPBF (L/h) = 184 (± 50.6) + 92.6 (± 6.28) \times Forage (kg DM/d) + 114.2 (± 5.88) \times Concentrate
55 (kg DM/d), where rMSPE% values were 17.5 and 17.6 and CCC values were 0.93 and 0.94,
56 respectively. Division of DMI into forage and concentrate fractions improved the joint Bayesian
57 Information Criterion (BIC) value for PORBF and HEPBF (BIC = 6512 vs. 7303), as well as
58 slightly improved the rMSPE and CCC for ARTBF and PORBF/HEPBF. This was despite
59 minimal changes in PORBF and HEPBF predictions. Developed equations predicted blood flow
60 well, and could easily be used within a post absorptive model of nutrient metabolism. Results also
61 suggest different sensitivity of PORBF and HEPBF to the composition of DMI, and accounting
62 for this difference resulted in improved ARTBF predictions.

63

64 **Key words:** blood flow, portal, hepatic, cattle, meta-analysis, multivariate

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INTRODUCTION

67

68 The ability of current feed ration systems to predict the effects of metabolizable protein
69 supply on milk protein production and nitrogen excretion to the environment by dairy cattle is
70 limited by an oversimplified representation of post-absorptive metabolism (Lapierre et al., 2006).
71 Given the variability in post-absorptive metabolism, there is interest in developing integrated post-
72 absorptive models of metabolism (portal-drained viscera, liver, mammary gland, and other organs
73 or tissues) to replace current empirical feeding systems for cattle. Integration of such organ-based
74 models requires prediction of nutrient flow between organs, including prediction of hepatic arterial
75 (**ARTBF**), portal (**PORBF**) and hepatic venous (**HEPBF**) blood flows (**BF**). Across the liver, the

76 relative contribution of ARTBF and PORBF can have a significant effect on nutrient fluxes
77 through the organ (e.g. Barnes et al., 1986), warranting reliable prediction of these blood flows.
78 Nutrient concentration in PORBF is modified by the net absorption of nutrients following digestion
79 of feeds (or the net utilization of nutrients from arterial blood), while ARTBF nutrient
80 concentration is mainly the result of the residual balance between nutrient absorption, utilization,
81 endogenous synthesis, and mobilization from body tissues. Several attempts to model ARTBF,
82 PORBF and/or HEPBF in ruminants are present in the literature, but 1.) were conducted on sheep
83 (e.g. Vernet et al., 2009), 2.) use older meta-analysis techniques which exclude random effects
84 (e.g. Lescoat et al., 1996), or 3.) examined only one of the 3 blood flows of interest (e.g.
85 Huntington, 1984; Bermingham et al., 2008). Species differences in blood flow (e.g. between cattle
86 and sheep) have already been observed (Vernet et al., 2005; Bermingham et al., 2008), indicating
87 that cross-species application of blood flow equations may be poor. Equations developed using
88 older meta-analysis techniques may inherently contain prediction errors (St-Pierre, 2001; Sauvant
89 et al., 2008). A fully integrated post-absorptive model for cattle would require all 3 blood flows to
90 be estimated simultaneously. Therefore, a multivariate meta-analysis approach, simultaneously
91 fitting equations for ARTBF, PORBF and HEPBF, while accounting for the interrelationship
92 between BF, is warranted.

93 The purpose of this study was therefore to (1) investigate the simultaneous prediction of
94 ARTBF, HEPBF and PORBF for cattle via a multivariate meta-analysis on published studies,
95 considering DMI and MEI as driving variables, and (2) to compare these predictions to available
96 extant prediction equations on an evaluation database, in order to identify the most appropriate
97 prediction equations for use in future cattle metabolism models.

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MATERIALS AND METHODS

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Developmental Database

The database used for equation development is summarized in Table 1. It consisted of 17 studies with 296 individual animal means and 55 treatment means. Published experiments included: Reynolds et al. (1991; 1992a,b; 1993; 1994a,b; 1995a,b; 1998; 1999; 2001; 2003a,b), Caton et al. (2001), Hanigan et al. (2004), Maltby et al. (2005) and Røjen et al. (2011). Experiments covered both lactating and dry dairy cows and growing beef cattle (steers and heifers). Method of BF measurement was downstream dilution of para-aminohippuric acid (PAH) (Katz and Bergman, 1969) for all studies. Within studies, BF results were means of (between) 5 to 12 hourly measurements. All reported BF values are on a whole blood basis. Criteria for inclusion in the developmental database included availability of individual animal data and provision of information on both PORBF and HEPBF, DMI, metabolizable energy intake (**MEI**), BW and forage % (**FP**) in the diet. Within study, any treatments which were not nutritional were removed in order to minimize non-nutritional variation in the database.

Within the database, the average SD within treatment across the database (indicator of within treatment animal variability) was 135 L/h, 210 L/h, 177 L/h and 0.852 kg/d for ARTBF, PORBF, HEPBF and DMI, respectively, and the average SD of treatment means (indicator of variation across treatment means) was 152 L/h, 548 L/h, 673 L/h and 6.35 kg/d for ARTBF, PORBF, HEPBF and DMI, respectively. Preliminary analysis (not shown) revealed that within-treatment BF variation was significantly related to within-treatment DMI variation ($P < 0.01$).

Evaluation Database

The database used for equation evaluation is summarized in Table 2. It consisted of 9

123 studies with 34 treatment means extracted from the published literature (Wieghart et al., 1986;
124 Eiseman and Nienaber, 1990; Huntington et al., 1990; Guerino et al., 1991; Reynolds and Tyrrell,
125 1991; Casse et al., 1994; Eiseman and Huntington, 1994; Whitt et al., 1996; Alio et al., 2000), and
126 included both lactating dairy cows and beef cattle. Method of BF measurement for all studies was
127 downstream dilution of PAH (Katz and Bergman, 1969). Similar to the developmental database,
128 all reported BF values are whole blood. Criteria for inclusion in the database included published
129 studies with provision of information on PORBF, HEPBF, DMI, MEI, BW and FP. Having MEI
130 and simultaneous reporting of PORBF and HEPBF as inclusion criteria for the evaluation database
131 limited the number of potential studies which could be included, but ensured an equal comparison
132 between DMI and MEI, and PORBF and HEPBF based equations. Similar to the developmental
133 database, within study, any treatments which were not nutritional were removed in order to
134 minimize non-nutritional variation in the database.

135 The observed PORBF and HEPBF vs. DMI relationship for both the developmental and
136 evaluation databases are presented in Figure 1 and the distribution of FP across DMI in Figure 2.

137

138 *Equation Development*

139 To model the effect of DMI and MEI of cattle on ARTBF, PORBF and HEPBF, mixed
140 model analysis was performed. Linear and quadratic multivariate mixed model analysis was
141 conducted using the NLINMIX macro of SAS (NLMM 8.0 SAS; Moser, 2004; Littell et al., 2006),
142 with simultaneous parameterization of the response variables (PORBF, HEPBF) and
143 representation of the correlation between these variables via the repeated effects statement (Strathe
144 et al., 2010). For a recent example of NLMM code, see the appendix of Strathe et al. (2009).

145 Due to the high degree of error and low sensitivity of ARTBF to the driving variables, it

146 was difficult to obtain convergence of the multivariate model when ARTBF was modelled directly
 147 (not shown). This is likely because ARTBF is a comparatively small flow determined by difference
 148 experimentally (*in vivo*, observed ARTBF = observed HEPBF – observed PORBF). As a result,
 149 predicted ARTBF was determined by calculation of the difference between predicted PORBF and
 150 HEPBF. Similarly, PORBF/HEPBF (%) was evaluated as the ratio of predicted blood flows, and
 151 not modeled directly.

152 As the data were compiled from multiple studies, it was necessary to analyze not only the
 153 fixed effects of the dependent variables, but also the random effect of experiment as this accounts
 154 for differences between experiments such as physiological status of the animals, experimental
 155 design, measurement methods, techniques, and laboratory variation (St-Pierre, 2001; Sauvant et
 156 al, 2008). As it was desirable to examine the between animal variation in DMI and BW, the full
 157 model also included the random effect of cow nested within experiment.

158 The statistical model can be written as follows, where fixed and random effects are
 159 incorporated directly into parameters:

$$160 \quad Y_{ijk} = f(\emptyset_{ij}, \text{intake}_{ijk}) + e_{ijk}, \quad [1]$$

$$161 \quad \emptyset_{ij} = \begin{bmatrix} \emptyset_{1ij} \\ \emptyset_{2ij} \\ \vdots \\ \emptyset_{dij} \end{bmatrix} = \begin{bmatrix} B_{11} \cdot x_1 + B_{12} \cdot x_2 \\ B_{21} \cdot x_1 + B_{22} \cdot x_2 \\ \vdots \\ B_{d1} \cdot x_1 + B_{d2} \cdot x_2 \end{bmatrix} + \begin{bmatrix} b_i^{(1)} \\ b_i^{(2)} \\ \vdots \\ b_i^{(d)} \end{bmatrix} + \begin{bmatrix} b_{i,j}^{(1)} \\ b_{i,j}^{(2)} \\ \vdots \\ b_{i,j}^{(d)} \end{bmatrix}$$

162 In this equation, the function f is a linear or quadratic function of intake (DMI or MEI), with the
 163 parameter vector \emptyset_{ij} and model error e_{ijk} . The experiment and cow(experiment) random effects,
 164 $\{b_i\}$ and $\{b_{i,j}\}$, are assumed independent of each other and independent of within cow errors e_{ijk} .
 165 The B 's are the fixed effects influencing the curve parameters due to blood flow (PORBF,
 166 HEPBF), and are introduced via two dummy variables x_1 and x_2 , respectively.

167 Initial analysis revealed a potential ‘fan’ shape in the residuals, where residual variance increased
168 with the predicted BF value. In addition, within-treatment and across treatment BF variation
169 increased as BF and/or DMI increased ($P < 0.05$; data not shown). This may reflect the different
170 type of animals used at low and high DMI (beef cattle vs. dairy cows), milk yield or body reserve
171 mobilization, or the range of diets examined. To compensate, a variance weighting statement (**wt**)
172 was added to the NLMM macro model, $wt = 1/(\text{predicted value})^2$, which decreased variance weight
173 with increasing predicted BF value (see Strathe et al., 2009 for discussion).

174 The joint distribution of random effects was assumed to be multivariate normal and the
175 dual quasi-Newton technique was used for optimization with an adaptive Gaussian quadrature as
176 the integration method.

177

178 *Equation Evaluation*

179 Goodness of fit of the statistical model (inclusion/exclusion of random effects,
180 variance/covariance structure selection etc.) was evaluated using the Bayesian information
181 criterion (**BIC**) fit statistic (SAS Inst. Inc., Cary, NC), where lower values indicate better model
182 fit, and the value and significance of the fixed effect model parameters were tested against a P
183 value of 0.05.

184 Evaluation of newly developed and extant equations against the evaluation database were
185 performed via two methods. Firstly, root mean square prediction error (**rMSPE**) was performed,
186 where the mean square prediction error (**MSPE**) is calculated as:

$$187 \quad \text{MSPE} = \sum_{i=1}^n (O_i - P_i)^2 / n \quad [2]$$

188 where n is the total number of observations, O_i is the observed value, and P_i is the predicted value.

189 The rMSPE, expressed as a percentage of the observed mean, gives an estimate of the overall

190 prediction error. The rMSPE can also be decomposed into error in central tendency or mean bias
191 (ECT), error due to deviation of the regression slope from unity (ER) and error due to the
192 disturbance (random error) (ED) (Bibby and Toutenburg, 1977).

193 Secondly, concordance correlation coefficient analysis (CCC) was performed (Lin, 1989),
194 where CCC is calculated as:

$$195 \quad CCC = r \times C_b \quad [3]$$

196 where r is the Pearson correlation coefficient and C_b is a bias correction factor. The r
197 variable gives a measure of precision, while C_b is a measure of accuracy. Associated CCC variables
198 (used in calculation of C_b) are v , which provides a measure of scale shift, and u , which provides a
199 measure of location shift relative to the scale. The v value indicates the change in standard
200 deviation, if any, between predicted and observed values. A v value greater than 1.0 indicates larger
201 variance in the predicted data compared to observed, while a v value less than 1.0 indicates a
202 smaller variance in the predicted data compared to observed. A positive u value indicates over-
203 prediction, while a negative u value indicates under-prediction.

204

205 **RESULTS AND DISCUSSION**

206

207 ***Low vs. High Intake***

208 Visual inspection of the data revealed two potential clusters within the databases,
209 representing a cluster of ‘lower-intake’ and ‘higher-intake’ data (Figure 1). These intake groups
210 are confounded with animal type, and also represent clusters of studies, where the low intake group
211 comprised all beef cattle data and the high intake group comprised all dairy cow data. As a result,
212 analysis was initially performed by separating the data (by studies) into low and high intake groups

213 (or, alternatively, animal type) (Table 1), and analysing for statistical differences between intake-
214 group parameter estimates. In sheep, Vernet et al. (2005; 2009) suggested that BF responses to
215 DMI or MEI differed based on the level of intake. Additionally, physiological status may have an
216 effect on BF. A major difference between the data of Vernet et al. (2005; 2009) and the current
217 data (aside from species) is, however, that in the current database level of intake did not fall far
218 below maintenance requirements (Table 1). In this study, the average multiple of maintenance
219 feeding level was 1.31 (± 0.378) for the low-intake and 2.65 (± 0.749) for the high-intake groups,
220 compared to 0.5 and 1.3 in the study of Vernet et al. (2009), respectively. Separation of studies
221 into two intake groups in the current dataset did not result in significantly different parameter
222 estimates between low- and high-intake groups ($P > 0.09$) (Table 3). As a result, separate equations
223 for the low intake and high intake groups (or animal type) are not reported, and equations reported
224 were fit to the full database.

225

226 *Linear and Quadratic Blood Flow Equations*

227 Results of linear and quadratic curve fitting to the BF development database are presented
228 in Table 3. Equations were fit to data with BF units of L/h combined with DMI or MEI units of
229 kg/d or MJ/d, or with BF units of L/kg BW^{0.75}/h combined with DMI or MEI units of kg/kg
230 BW^{0.75}/d or MJ/kg BW^{0.75}/d. Scaling relative to BW was also examined, but resulted in no
231 improvements over BW^{0.75}, and is not reported. Model structure (random effects, variance-
232 covariance structures, variance weighting) was optimized to ensure convergence and to minimize
233 the joint BIC value. Joint BIC values represent the BIC for PORBF and HEPBF combined, which
234 were fit simultaneously. The significance of parameter estimates (vs. zero) are reported, as well as
235 the P -value for testing the low vs. high intake parameter estimates against each other, via

236 CONTRAST statements in SAS (Table 3). This division into low and high intake groups was not
237 performed for quadratic equations due mainly to lack of convergence, but also because a quadratic
238 fit should inherently capture changes in the slope of the relationship across intake level. In support
239 of the findings that parameter estimates did not differ significantly between low and high intake
240 groups, fitting quadratic equations to the database resulted in similar or marginally better joint BIC
241 values, and the quadratic parameter estimates were not always significant (Table 3). Lack of
242 significance of the quadratic parameter indicates potential over-parametrization of the model or
243 that the relationship was linear within the range of data available. When BF was expressed in units
244 of L/h, the negative quadratic parameter was significant for HEPBF, but not for PORBF (driving
245 variable of DMI or MEI). When BF was expressed in units of L/kg BW^{0.75}/h, the quadratic
246 parameter was only significant for PORBF with DMI as a driving variable. Linear equation
247 parameters (slope and intercept) were always significant ($P < 0.01$).

248 Equations based on MEI generally had lower BIC values compared to equations based on
249 DMI (Table 3), indicating better model fit. Conclusions on BF units cannot be made based on BIC,
250 as BIC values are scaled by the units.

251

252 *DMI and MEI Based Equation Evaluation*

253 Equations developed were tested on an independent evaluation database (described in
254 Table 2) to compare prediction precision and accuracy. Although the evaluation database may be
255 considered somewhat small relative to the size of the development database, it does represent a
256 complete dataset, where all variables predicted and evaluated were reported in the publications.
257 Results are presented in Table 4 for PORBF, HEPBF, ARTBF (predicted by difference) and
258 PORBF/HEPBF (% , ratio of predicted blood flows) for each equation.

259 Comparing DMI to MEI as the driving variable, DMI typically resulted in slightly better
260 predictions based on rMSPE and CCC results, except for PORBF/HEPBF (Table 4). This could
261 be the result of added variation or error due to MEI determination. However, Han et al. (2002) also
262 suggested that portal BF responded primarily to bulk fill rather than nutrient supply. Reynolds et
263 al. (1991) suggested that in addition to ME consumed, ME density of the diet affected PORBF and
264 HEPBF via effects of forage content on gut fill and subsequent effects on gut mass and the work
265 of digestion, which may also explain the better relationship for splanchnic blood flow and DMI.
266 Vernet et al. (2009) found a similar lack of improvement with MEI over DMI in predicting BF in
267 sheep. Therefore, it is likely that this observation has a physiological basis rather than being error
268 related.

269 Comparing linear to quadratic equations, predictions were similar but slightly improved
270 with the linear equations (Table 4). As many of the quadratic parameter estimates were not
271 significant, this is not a surprising result.

272 Comparing L/h and L/kg BW^{0.75}/h as units for BF, CCC results were in general slightly
273 improved when L/h was used and rMSPE results were in general slightly improved when L/kg
274 BW^{0.75}/h was used (Table 4). Scaling with BW^{0.75} reduced the contribution of non-random error
275 sources (ECT, ER) to the rMSPE total, indicating improved predictions compared with scaling
276 without BW^{0.75}. However for CCC, BW^{0.75} scaling reduced the total CCC via a decrease in C_b ,
277 despite a slight increase in r . This difference in results is likely due to differences in division of
278 error within rMSPE and CCC calculations (for a discussion see Ellis et al., 2010). Scaling by
279 BW^{0.75} is presumed to extend the range of data the equations may be applicable on, and thus was
280 of interest when combining dairy and beef data, but it may also introduce additional variation due
281 to BW measurement (difficulty getting a precise scale number, variation in gut fill contribution to

282 BW, etc.). For whichever reason, these results indicate that scaling by $BW^{0.75}$ may not improve
283 predictions of blood flow over units of L/h, as performance between the equations was similar.

284 Predictions for PORBF and HEPBF, as evaluated by rMSPE and CCC analysis, were
285 typically very good, with CCC values greater than 0.84 and rMSPE values less than 19% (Table
286 4). The best predictions of PORBF and HEPBF when blood flow was expressed in L/h, were the
287 linear equations with DMI as the driving variable (P1 and H1 equations; rMSPE = 17.5 and 16.6%,
288 CCC = 0.93 and 0.94, respectively). Similarly, when PORBF and HEPBF were expressed relative
289 to $BW^{0.75}$, the linear DMI equations resulted in slightly better predictions (P5 and H5 equations;
290 rMSPE = 15.4 and 14.9%, CCC = 0.87 and 0.90, respectively). However, in general predictions
291 were similar and good across all equations with only minor differences.

292 Residual analysis was conducted on the seemingly best performing equations (linear, DMI;
293 L/h and L/kg $BW^{0.75}$ /h), and is displayed in Figure 3. Residuals plotted against predicted BF
294 (Figure 3) did not reveal any significant trends in the data ($P > 0.05$), nor for the most part when
295 plotted against the driving variable DMI (kg/d or g/kg $BW^{0.75}$ /d; $P > 0.05$), with the exception of
296 residual ARTBF (L/h), where $P = 0.04$ (residual ARTBF (L/h) = $40.2 (\pm 29.2) - 6.4 (\pm 2.83) \times$
297 DMI(kg/d); graphs not shown). The residuals were also plotted against the forage proportion (FP,
298 %) of the diet, and while the regression was not significant for ARTBF, PORBF or HEPBF ($P >$
299 0.05), it was significant for PORBF/HEPBF (%) ($P = 0.03$ and 0.03, for L/h and L/kg $BW^{0.75}$ /h
300 equations, respectively; Figure 4). As the result of the FP pattern in the residuals, the FP of the diet
301 was considered as an additional driving variable. The results of separating forage and concentrate
302 DMI is outlined in the following section.

303

304 ***Separating Forage and Concentrate DMI***

305 To further examine the potential effect of the FP of the diet, DMI was separated into forage
306 and (starch-rich) concentrate components (kg/d) in the developmental database, and new equations
307 were parameterized for PORBF and HEPBF, with ARTBF again calculated by difference.
308 Equations developed are presented in Table 5.

309 When testing the PORBF forage and concentrate slopes against each other, the difference
310 between parameter estimates was non-significant (Table 5), indicating no difference in effect of
311 type of DMI on PORBF. However, testing HEPBF forage and concentrate slope parameters against
312 each other revealed a significant difference, the slope for concentrate being higher (Table 5). This
313 result suggests a higher sensitivity of HEPBF to FP or energy intake compared to PORBF. In
314 support of this, the slope of MEI based equations was also generally higher for HEPBF than for
315 PORBF (Table 3). This may reflect an increased absorption and liver metabolism of propionate
316 and other VFA with an increasing concentrate proportion in diet DM (Huntington, 1990).

317 Dividing DMI into forage and concentrate components resulted in improved joint BIC
318 values (Table 3 vs. Table 5), slightly improved ARTBF and PORBF/HEPBF predictions, and
319 similar PORBF and HEPBF predictions to equations based on total DMI (Table 4 vs. Table 6).

320 Interpretation of these FP equations is challenging. For PORBF, it appears forage and
321 concentrate DMI do not differ in their magnitude of effect on BF (similar parameter estimates).
322 This may, however, be the compound result of two opposing mechanisms: forage DMI may
323 stimulate BF less than concentrate DMI due to lower energy content and digestibility, but this may
324 be countered by a higher bulk fill value which is stimulatory to BF (Reynolds et al., 1991).

325 In contrast, it appears that HEPBF may be more sensitive to concentrate (or energy intake)
326 than to forage intake (significantly different parameter estimates), suggesting that total liver BF is

327 still more heavily regulated by energy status and absorption of VFA and other components of ME
328 than gut fill. Vernet et al. (2009) made similar observations in sheep.

329 While these differences did not greatly alter PORBF and HEPBF predictions, prediction of
330 the calculated ARTBF and PORBF/HEPBF were both improved. This suggests that while DMI
331 alone may predict PORBF or HEPBF adequately, differences between them (ARTBF) may be
332 better predicted with consideration of the diet FP. While Vernet et al. (2009) did not examine
333 residuals of arterial/venous BF against FP, they did observe a significant relationship between the
334 residuals and OM digestibility, suggesting again that BF depend on both bulk and the nutrient
335 density of the diet. In order to better understand these effects, an examination of the regulation of
336 liver BF is required.

337

338 *Blood flow regulation through splanchnic tissues*

339 Blood flow through the portal vein (PORBF), the main blood supply to the liver, is
340 regulated by the portal drained viscera (**PDV**) which is responsible for nutrient uptake and delivery
341 to the post-absorptive environment, as opposed to being controlled by the liver (Lautt, 2009). Bulk
342 fill as well as nutrient delivery to the animal impact this flow (e.g. see Reynolds et al., 1991)
343 through regulation by intrinsic and extrinsic mechanisms. Intrinsic mechanisms include local
344 metabolic control (response to oxygen supply and demand), myogenic control (transmural
345 pressure), local reflexes (presence of lumen contents) and locally produced vasoactive substances
346 (e.g. gastrin, secretin, cholecystokinin) (Lautt, 1996; Lautt, 2009). The extrinsic factors include
347 sympathetic innervation, circulating vasoactive substances and systemic haemodynamic changes
348 (Lautt, 1996; Lautt, 2009). Hepatic arterial blood flow (ARTBF), while regulated by local tissue
349 oxidation levels in other organs, is also not regulated by the liver (Lobley et al., 2000). Instead, it

350 appears that ARTBF regulation is linked to PORBF, ensuring the liver receives a constant total
351 blood flow relative to liver mass (Lautt, 1996; Lautt, 2009). This appears to be regulated via a
352 continuous release of adenosine into the space of Mall, independent of oxygen supply or demand,
353 followed by removal through both ARTBF and PORBF. Adenosine itself is a powerful vasodilator
354 (Lautt, 2009). If PORBF is reduced, the local concentration of adenosine increases, stimulating
355 arterial vasodilation and increased ARTBF to remove the adenosine. On the other hand, when
356 PORBF is high, e.g. during peak absorption of nutrients from the rumen, this may cause a reduction
357 in ARTBF due to a decrease in local adenosine concentrations. This process is referred to as the
358 hepatic arterial buffer response. In this respect, the liver does not drive either of the incoming blood
359 flows; PORBF is driven by the PDV, and ARTBF is driven, inversely, by PORBF. However, the
360 liver can have significant indirect regulatory effects on incoming BF, via mechanisms impacting
361 BF to splanchnic organs that drain into the PORBF. As well, longer-term effects on BF can be
362 mediated by changes in liver mass. For a full review of liver BF regulation, see Lautt (2009).

363 Based on the empirical blood flow prediction equations developed in the present work, it
364 is possible that stimulation of PORBF by concentrate (energy) intake is countered by a depression
365 in PORBF by a lower forage intake (bulk fill), resulting in similar forage and concentrate
366 parameters for PORBF prediction across a range of FP. When FP was low and total DMI alone
367 was the driving variable, PORBF/HEPBF was over predicted ($P < 0.05$) and as a result ARTBF
368 slightly under predicted (non-significant; Figure 4). This makes sense as ARTBF is calculated as
369 HEPBF – PORBF. At a low FP, over-prediction of PORBF/HEPBF could be due to over prediction
370 of PORBF and/or under prediction of HEPBF. Examination of the (albeit non-significant) slope
371 terms in Figure 4, suggest that both are occurring to some extent.

372 Since parameterization with separate forage and concentrate DMI resulted in similar
373 parameters for PORBF, if these results reflect *in vivo* observations, it suggests that for low FP
374 diets, HEPBF is under-represented by using total DMI because of an under-represented ARTBF
375 contribution. This suggests that while total blood flow through the liver is sensitive to energy
376 intake (and thus different forage and concentrate parameters for HEPBF), factors reducing PORBF
377 relative to the local adenosine concentration (in this case, FP or bulk fill) may drive an increase in
378 ARTBF to compensate. Thus, separating forage and concentrate DMI captures this effect of
379 ARTBF, without directly modeling ARTBF.

380 When interpreting these results, it should be noted that while DMI varied within all studies,
381 FP did not. Although the equations were parameterized on kg/d of forage and concentrate, in the
382 developmental database 5 of 17 studies specifically examined FP effects, and 4 of 9 studies in the
383 evaluation database examined FP effects. The distribution of FP across DMI is illustrated in Figure
384 2. Therefore, the forage + concentrate equations require examination on an additional database
385 with additional variation in FP to ensure it is not only an artifact of the data used.

386

387 ***Equations Based on Diet Chemical Composition***

388 Although one of the main purposes of this paper was to compare DMI and MEI as the
389 major drivers of PORBF and HEPBF in a multivariate analysis, CP and NDF content of the diet
390 were also available in the development databases. Therefore, initially, development of equations
391 based on CP or NDF intake (kg/d or g/kg BW^{0.75}/d) were also considered. However, while these
392 equations had BIC values comparable to the forage + concentrate DMI equations (joint BIC values
393 were: 6413 for CP (kg/d), 6534 for NDF (kg/d), 1391 for CP (g/kg BW^{0.75}/d), and 1491 for NDF
394 (g/kg BW^{0.75}/d) based equations), their rMSPE and CCC values were worse than those of DMI

395 and MEI (for e.g., CP (kg/d) predicting PORBF (L/h) resulted in $rMSPE\% = 37.8$, $CCC = 0.63$
396 and HEPBF (L/h) $rMSPE\% = 37.9$ and $CCC = 0.69$, on the evaluation database). As a result, these
397 equations were not pursued further. However, equations developed considering multiple chemical
398 components of the diet may be considered in the future, in particular given the relationship
399 observed here with FP.

400

401 *Comparison with Extant Blood Flow Equations*

402 To compare predictions of the newly developed blood flow equations to extant equations,
403 several equations were selected from the literature and applied to the evaluation database. The
404 equations of Lescoat et al. (1996) were not included, as the evaluation database used here shared
405 data with the developmental database used by Lescoat et al. (1996), resulting in unsurprisingly
406 good blood flow predictions by these equations (not shown). Although the equations of Vernet et
407 al. (2009) were developed on sheep, it represented an interesting challenge to include their
408 equations for comparison on cattle data.

409 Extant equation evaluations are presented in Table 7. Of the equations evaluated, the
410 PORBF equation of Huntington et al. (1984) based on MEI performed comparably to the newly
411 developed PORBF equations in terms of $rMSPE$ and CCC analysis. These equations (Huntington
412 et al., 1984) were developed on beef and dairy heifer data. The linear PORBF equation of
413 Bermingham et al. (2008) performed adequately, with slightly more bias (over prediction) and
414 lower CCC values. However, similar to the results found in the current study, the quadratic
415 equation for PORBF by Bermingham et al. (2008) did not improve predictions over their linear
416 equation. These equations were developed on a combination of sheep and cattle data.

417 The sheep equations of Vernet et al. (2009) also tended to over predict PORBF, HEPBF
418 and ARTBF, expressed relative to BW, likely illustrating a species difference. Of the 3 sets of
419 extant equations, only those of Vernet et al. (2009) allowed calculation and evaluation of ARTBF
420 and PORBF/HEPBF. Both the Vernet et al. (2009) above maintenance and above + below linear
421 equations tended to under predict the mean PORBF/HEPBF. Interestingly, the Vernet et al. (2009)
422 sheep equations also showed a relationship between the PORBF/HEPBF residual and the FP of
423 the diet (Figure 5) with a trend similar to that in the equations derived in the present study (Figure
424 4), and therefore seems to support the separation of forage and concentrate parameters.

425

426

CONCLUSIONS

427

428 Equations developed herein represent advancement over current PORBF, HEPBF, ARTBF
429 and PORBF/HEPBF prediction equations available in the literature for cattle. In the present
430 analysis, a more advanced meta-analysis technique was used, allowing simultaneous predictions
431 of multiple blood flows, as well as providing new equations which separate forage DMI from
432 concentrate DMI, resulting in improvements in ARTBF and PORBF/HEPBF predictions. All
433 PORBF and HEPBF equations performed well when evaluated on an evaluation database. These
434 equations can be applied within a post-absorptive model of cattle metabolism, in order to predict
435 nutrient fluxes to and from the liver, but should be further evaluated on additional data obtained
436 under a wider range of conditions.

437

438

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445

446

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585

586 **Table 1.** Summary of the blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) developmental database¹.

Variable	<u>All data</u>		<u>Beef cattle (Low-intake group)</u>				<u>Dairy cow (High-intake group)</u>			
	Mean	SD ²	Mean	SD ²	MIN ³	MAX ⁴	Mean	SD ²	MIN ³	MAX ⁴
DMI (kg/d)	11.8	6.58	5.5	1.11	3.0	8.3	17.3	3.84	7.9	25.1
MEI (MJ/d)	126.4	74.91	60.6	12.74	37.4	97.6	192.8	48.03	81.4	295.1
CP (kg/d)	1.8	1.03	0.9	0.25	0.5	1.4	2.6	0.81	1.0	4.6
NDF (kg/d)	3.7	2.44	1.3	0.58	0.6	3.3	5.9	1.13	3.3	9.0
BW (kg)	510	140.3	412	84.4	251	598	637	85.2	487	878
DMI (g/kg BW ^{0.75} /d)	96.3	46.93	61.0	13.94	42.6	104.1	142.2	32.85	57.0	202.6
MEI (MJ/kg BW ^{0.75} /d)	1.01	0.515	0.68	0.175	0.49	1.13	1.55	0.423	0.56	2.26
MEI (Multiple of MN ⁵)	1.81	0.850	1.31	0.378	0.86	2.27	2.65	0.749	0.90	3.90
Forage Proportion (%)	44	18.0	41	23.3	25	75	47	10.3	35	66
ARTBF (L/h)	234	206.4	91	64.2	3	437	359	207.1	18	1089
PORBF (L/h)	1188	586.6	650	126.9	382	986	1655	398.6	762	2887
HEPBF (L/h)	1409	708.7	736	138.3	428	1019	1992	431.0	929	3208
ARTBF (L/kg BW ^{0.75} /h)	1.8	1.52	1.0	0.69	0.0	4.1	2.8	1.66	0.5	8.8
PORBF (L/kg BW ^{0.75} /h)	10.0	3.88	7.2	1.57	4.6	14.4	13.5	3.00	5.6	19.4
HEPBF (L/kg BW ^{0.75} /h)	11.7	4.79	8.2	1.69	5.5	14.7	16.2	3.57	6.9	21.6
PORBF/HEPBF (%)	86	9.3	88	7.7	57	100	85	9.2	59	100
n (data points)	296	-	137	-	-	-	159	-	-	-
n (treatments)	55	-	22	-	-	-	33	-	-	-
n (studies)	17	-	7	-	-	-	10	-	-	-

587 ¹ Mean & SD reported are based on 'n (data points)'.

588 ² Standard deviation.

589 ³ Minimum value in database.

590 ⁴ Maximum value in database.

591 ⁵MN – maintenance energy requirement.

592 **Table 2.** Summary of the blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) evaluation database¹.

Variable	Mean	SD ²	MIN ³	MAX ⁴
DMI (kg/d)	8.4	4.71	4.3	21.8
MEI (MJ/d)	90.4	43.59	51.1	231.5
CP (kg/d)	1.3	0.89	0.7	4.0
BW (kg)	387	97.5	198	538
DMI (g/kg BW ^{0.75} /d)	94.0	36.91	57.1	204.6
MEI (MJ/kg BW ^{0.75} /d)	1.02	0.346	0.62	2.17
MEI (Multiple of MN ⁵)	1.99	0.613	1.16	3.94
Forage Proportion (%)	42	22.0	10	100
ARTBF (L/h)	165	137.9	26	563
PORBF (L/h)	832	369.3	336	1992
HEPBF (L/h)	996	495.9	400	2524
ARTBF (L/kg BW ^{0.75} /h)	1.8	1.24	0.3	5.3
PORBF (L/kg BW ^{0.75} /h)	9.4	2.95	6.4	18.7
HEPBF (L/kg BW ^{0.75} /h)	11.2	3.96	7.5	23.7
PORBF/HEPBF (%)	85	5.9	76	97
n (data points)	34			
n (treatments)	34			
n (studies)	9			

593

594 ¹ Mean & SD reported are based on 'n (data points)'.

595 ² Standard deviation.

596 ³ Minimum value in database.

597 ⁴ Maximum value in database.

598 ⁵MN – maintenance energy requirement.

599

600 **Table 3.** Summary of portal (PORBF) and hepatic venous blood flow (HEPBF) prediction equations based on DMI and MEI¹.

Response Variable	Driving Variable	Eqn	ID	Joint BIC	Int	SE	P	P (Intake Level) ²	Slope (Lin)	SE	P	P (Intake Level) ²	Slope (Quad)
<u>L/h</u>	<u>kg/d or MJ/d</u>												
PORBF	DMI	Linear	P1	7303	202	45.6	<0.01	0.98	83.6	3.11	<0.01	0.73	-
HEPBF	DMI		H1		186	45.4	<0.01	0.64	103.8	3.10	<0.01	0.90	-
PORBF	MEI	Linear	P2	6689	294	43.2	<0.01	0.19	6.8	0.26	<0.01	0.67	-
HEPBF	MEI		H2		264	42.8	<0.01	0.09	8.9	0.26	<0.01	0.96	-
PORBF	DMI	Quad	P3	7296	148	70.9	0.04	-	94.9	12.25	<0.01	-	-0.44
HEPBF	DMI		H3		72	69.9	0.31	-	129.3	12.03	<0.01	-	-1.03
PORBF	MEI	Quad	P4	6698	209	68.1	<0.01	-	8.3	1.07	<0.01	-	-0.01
HEPBF	MEI		H4		110	65.8	0.10	-	11.8	1.02	<0.01	-	-0.01
<u>L/kg BW^{0.75}/h</u>	<u>g or MJ/ kg BW^{0.75}/d</u>												
PORBF	DMI	Linear	P5	1548	2.10	0.417	<0.01	0.50	0.080	0.004	<0.01	0.88	-
HEPBF	DMI		H5		1.91	0.421	<0.01	0.17	0.100	0.004	<0.01	0.15	-
PORBF	MEI	Linear	P6	1337	2.80	0.286	<0.01	0.94	6.61	0.256	<0.01	0.81	-
HEPBF	MEI		H6		2.41	0.286	<0.01	0.43	8.71	0.258	<0.01	0.09	-
PORBF	DMI	Quad	P7	1543	0.58	0.728	0.43	-	0.119	0.016	<0.01	-	-0.0002
HEPBF	DMI		H8		0.84	0.769	0.27	-	0.128	0.018	<0.01	-	-0.0002
PORBF	MEI	Quad	P8	1327	1.53	0.690	0.04	-	9.26	1.465	<0.01	-	-1.09
HEPBF	MEI		H8		1.97	0.701	<0.01	-	9.53	1.499	<0.01	-	-0.30

601
602 ¹Abbreviations: Eqn = equation form, ID = equation name, BIC = Bayesian information criterion, Int = intercept.

603 ² Tested whether slope or intercept for data grouped into 'high' intake (dairy cow) differed from data grouped into 'low' intake (beef
604 cattle), via CONTRAST statements in SAS (data not shown).

605 **Table 4.** Summary of blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) predictions on the evaluation
606 database for ARTBF, PORBF and HEPBF locations, where ARTBF = predicted HEPBF – predicted PORBF, PORBF and HEPBF are
607 according to equations presented in Table 3, and PORBF/HEPBF = predicted PORBF/predicted HEPBF × 100.

Response Variable	Driving Variable	Eqn	ID	Pred Mean ¹	Pred SD ¹	rMSPE, % ²	ECT, % ³	ER, % ⁴	ED, % ⁵	CCC ⁶	r ⁷	C _b ⁸	v ⁹	u ¹⁰
<u>L/h</u>	<u>kg/d or MJ/d</u>													
ARTBF	DMI	linear		154	93.8	42.4	2.2	14.0	83.8	0.82	0.93	0.88	0.69	-0.09
PORBF			P1	907	388.3	17.5	27.0	9.0	64.1	0.93	0.98	0.95	1.07	0.20
HEPBF			H1	1061	482.1	16.6	15.5	1.1	83.4	0.94	0.99	0.95	0.99	0.13
PORBF/ HEPBF, %				86	1.7	6.6	5.7	0.2	94.1	0.18	0.52	0.34	0.30	0.42
ARTBF	MEI	linear		160	90.2	44.2	0.5	14.8	84.7	0.80	0.92	0.87	0.66	-0.05
PORBF			P2	909	292.0	17.6	27.7	13.8	58.5	0.90	0.95	0.95	0.80	0.24
HEPBF			H2	1068	382.2	18.7	14.8	19.5	65.7	0.91	0.96	0.95	0.78	0.17
PORBF/ HEPBF, %				86	2.4	6.5	3.2	0.3	96.5	0.24	0.68	0.35	0.41	0.27
ARTBF	DMI	quad		160	93.6	44.3	0.5	10.4	89.1	0.80	0.93	0.86	0.69	-0.05
PORBF			P3	908	391.1	17.7	26.7	10.0	63.2	0.93	0.98	0.95	1.07	0.20
HEPBF			H3	1067	484.3	17.3	17.0	1.5	81.4	0.94	0.99	0.95	0.99	0.15
PORBF/ HEPBF, %				86	2.2	6.9	2.8	3.3	93.9	0.13	0.65	0.20	0.38	0.27
ARTBF	MEI	quad		166	90.8	45.4	0.0	11.6	88.3	0.79	0.92	0.86	0.67	0.01
PORBF			P4	910	299.5	17.4	28.9	10.2	60.9	0.91	0.96	0.95	0.82	0.24
HEPBF			H4	1076	390.0	19.0	17.6	14.8	67.6	0.91	0.96	0.95	0.80	0.18
PORBF/ HEPBF, %				85	2.8	6.7	0.9	4.2	95.0	0.22	0.78	0.28	0.48	0.13
<u>L/kg BW^{0.75}/h</u>	<u>g or MJ/ kg BW^{0.75}/d</u>													
ARTBF	DMI	linear		1.7	0.73	43.3	2.0	10.2	87.8	0.70	0.87	0.80	0.60	-0.12
PORBF			P5	9.6	2.91	15.4	1.6	6.2	92.2	0.87	1.00	0.88	1.00	0.06
HEPBF			H5	11.3	3.64	14.9	0.2	0.5	99.3	0.90	1.00	0.90	0.93	0.02
PORBF/ HEPBF, %				86	1.3	6.6	1.2	0.9	97.9	0.14	0.43	0.32	0.23	0.22

ARTBF	MEI	linear		1.8	0.72	45.0	0.3	7.5	92.2	0.67	0.87	0.77	0.59	-0.05
PORBF			P6	9.6	2.25	14.4	0.7	6.2	93.1	0.86	0.97	0.89	0.78	0.05
HEPBF			H6	11.3	2.97	15.6	0.2	10.3	89.6	0.87	0.96	0.91	0.76	0.02
PORBF/ HEPBF, %				85	1.8	6.6	0.0	0.0	99.9	0.16	0.56	0.29	0.31	0.02
ARTBF	DMI	quad		1.7	0.80	41.5	3.2	6.7	90.0	0.74	0.91	0.81	0.65	-0.14
PORBF			P7	9.8	2.62	15.9	5.7	0.5	93.8	0.85	0.99	0.87	0.90	0.13
HEPBF			H7	11.5	3.41	15.5	1.6	0.2	98.1	0.89	0.99	0.90	0.87	0.06
PORBF/ HEPBF, %				86	1.9	6.4	4.6	1.5	93.9	0.24	0.56	0.43	0.32	0.35
ARTBF	MEI	quad		1.6	0.84	43.7	4.2	2.0	93.8	0.72	0.92	0.78	0.69	-0.16
PORBF			P8	9.7	2.14	15.4	3.7	8.4	87.9	0.84	0.95	0.88	0.74	0.11
HEPBF			H8	11.4	2.96	15.7	0.4	10.0	89.6	0.87	0.96	0.90	0.76	0.03
PORBF/ HEPBF, %				86	2.4	6.6	5.6	0.4	94.0	0.23	0.67	0.35	0.41	0.36

608

609 ¹ Where: observed means \pm SD: ARTBF, PORBF, HEPBF (L/h): 165 ± 137.9 , 832 ± 369.3 , 996 ± 495.9 ; ARTBF, PORBF, HEPBF
610 (L/kg BW^{0.75}/h): 1.8 ± 1.24 , 9.4 ± 2.95 , 11.2 ± 3.96 ; PORBF/HEPBF (%): 85 ± 5.9 , respectively.

611 ²Root mean square prediction error, % of observed mean.

612 ³Error due to mean bias, as a % of total MSPE.

613 ⁴Error due to regression, as a % of total MSPE.

614 ⁵Error due to disturbance, as a % of total MSPE.

615 ⁶Condordance correlation coefficient, where $CCC = r \times C_b$.

616 ⁷Pearson correlation coefficient.

617 ⁸Bias correction factor.

618 ⁹Scale shift.

619 ¹⁰Location shift relative to the scale.

620

621 **Table 5.** Summary of portal (PORBF) and hepatic venous (HEPBF) blood flow prediction equations based on DMI divided into
 622 forage (F) and concentrate (C) intake¹.

Response Variable	Driving Variable	Eqn	ID	Joint BIC	Int	SE	<i>P</i>	Slope (F)	SE	<i>P</i>	Slope (C)	SE	<i>P</i>	<i>P</i> (F vs. C) ²
<u>L/h</u>	<u>kg/d</u>													
PORBF	DMI ³	Linear	P9	6512	210	51.0	<0.01	82.9	6.43	<0.01	82.9	6.04	<0.01	1.00
HEPBF			H9		184	50.6	<0.01	92.6	6.28	<0.01	114.2	5.88	<0.01	0.03
<u>L/kg BW^{0.75}/h</u>	<u>g/kg BW^{0.75}/d</u>													
PORBF	DMI ³	Linear	P10	1365	2.16	0.467	<0.01	0.08	0.006	<0.01	0.08	0.006	<0.01	0.41
HEPBF			H10		1.91	0.468	<0.01	0.09	0.006	<0.01	0.11	0.006	<0.01	0.01

623
 624 ¹Abbreviations: Eqn = equation form, ID = equation name, BIC = Bayesian information criterion, Int = intercept.

625 ² Tested whether the forage (F) and concentrate (C) slopes differed from each other, performed via CONTRAST statements in SAS.

626 ³Separated into forage DMI (kg/d) + concentrate DMI (kg/d).

627 **Table 6.** Summary of blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) predictions based on
628 separated forage + concentrate DMI, on the evaluation database for ARTBF, PORBF and HEPBF locations, where ARTBF =
629 predicted HEPBF – predicted PORBF, PORBF and HEPBF predictions are according to equations presented in Table 5, and
630 PORBF/HEPBF = predicted PORBF/predicted HEPBF × 100.

Response Variable	Driving Variable	Eqn	ID	Pred Mean ¹	Pred SD ¹	rMSPE, % ²	ECT, % ³	ER, % ⁴	ED, % ⁵	CCC ⁶	r^7	C_b^8	v^9	u^{10}
<u>L/h</u>	<u>kg/d</u>													
ARTBF	DMI ¹¹	linear	-	160	105.1	41.3	0.6	3.9	95.5	0.84	0.97	0.87	0.77	-0.04
PORBF			P9	909	385.0	17.5	28.4	7.6	63.9	0.93	0.98	0.95	1.06	0.21
HEPBF			H9	1069	485.0	17.6	17.0	1.7	81.3	0.94	0.99	0.95	0.99	0.15
PORBF/ HEPBF %				86	3.3	6.2	4.1	1.0	94.9	0.40	0.84	0.48	0.57	0.24
<u>L/kg BW^{0.75}/h</u>	<u>g or MJ/ kg BW^{0.75}/d</u>													
ARTBF	DMI ¹¹	linear	-	1.7	0.80	42.1	1.1	5.3	93.6	0.73	0.91	0.80	0.66	-0.08
PORBF			P10	9.7	2.91	15.7	3.2	6.2	90.6	0.87	1.00	0.87	1.00	0.09
HEPBF			H10	11.4	3.64	15.5	1.2	0.6	98.2	0.89	1.00	0.90	0.93	0.05
PORBF/ HEPBF %				85	2.9	6.2	0.8	0.5	98.7	0.36	0.80	0.44	0.51	0.11

631

632 ¹ Where: observed means ± SD: ARTBF, PORBF, HEPBF (L/h): 165 ± 137.9, 832 ± 369.3, 996 ± 495.9; ARTBF, PORBF, HEPBF
633 (L/kg BW^{0.75}/h): 1.8 ± 1.24, 9.4 ± 2.95, 11.2 ± 3.96; PORBF/HEPBF (%): 85 ± 5.9, respectively.

634 ²Root mean square prediction error, % of observed mean.

635 ³Error due to mean bias, as a % of total MSPE.

636 ⁴Error due to regression, as a % of total MSPE.

637 ⁵Error due to disturbance, as a % of total MSPE.

638 ⁶Condordance correlation coefficient, where $CCC = r \times C_b$.

639 ⁷Pearson correlation coefficient.

640 ⁸Bias correction factor.

641 ⁹Scale shift.

642 ¹⁰Location shift relative to the scale.

643 ¹¹Separated into forage DMI (kg/d) + concentrate DMI (kg/d).

644 **Table 7.** Blood flow (hepatic arterial - ARTBF; portal venous - PORBF; hepatic venous - HEPBF) predictions by extant equations on
 645 the evaluation database for ARTBF, PORBF and HEPBF locations.

Source	Response Variable	Driving Variable	Eqn	Pred Mean ¹	Pred SD ¹	rMSPE, % ²	ECT, % ³	ER, % ⁴	ED, % ⁵	CCC ⁶	r^7	C_b^8	v^9	u^{10}
Vernet et al. (2009) (above MN)	<u>L/kg BW/h</u>	<u>g/kg BW/d</u>												
	ARTBF	DMI	Lin	0.6	0.17	65.1	57.4	1.0	41.6	0.48	0.64	0.75	0.65	0.96
	PORBF	DMI	Lin	2.6	0.45	26.6	62.2	1.4	36.4	0.59	0.71	0.84	0.73	0.84
	HEPBF	DMI	Lin	3.2	0.62	29.9	72.4	1.5	26.1	0.61	0.69	0.88	0.76	0.91
	PORBF/ HEPBF	DMI	Lin ¹¹	81	1.2	7.7	29.6	1.9	68.5	0.11	0.30	0.37	0.22	-1.32
Vernet et al. (2009) (above + below MN)	<u>L/kg BW/h</u>	<u>g/kg BW/d</u>												
	ARTBF	DMI	Quad	0.6	0.15	66.5	60.1	3.5	36.4	0.45	0.58	0.77	0.58	1.07
	PORBF	DMI	Quad	2.7	0.64	32.4	70.6	3.1	26.3	0.58	0.70	0.82	1.02	0.92
	HEPBF	DMI	Quad	3.3	0.78	34.8	79.8	0.7	19.6	0.59	0.67	0.88	0.96	0.99
	PORBF/ HEPBF	DMI	Lin ¹¹	81	0.1	7.7	22.9	9.1	68.0	0.01	0.03	0.36	0.02	-4.03
Birmingham et al. (2008)	<u>L/kg BW/h</u>	<u>g/kg BW/d</u>												
	PORBF	DMI	Lin	2.4	0.51	20.5	38.6	0.0	61.4	0.74	0.88	0.84	0.82	0.48
	PORBF	DMI	Quad	2.7	1.21	45.4	35.5	51.2	13.4	0.57	0.69	0.82	1.94	0.67
Huntington (1984)	PORBF, L/h	MEI, MJ/d	Lin	876	249.1	18.6	8.3	39.6	52.1	0.88	0.92	0.95	0.68	0.15

646
 647 ¹ Where: observed means \pm SD: ARTBF, PORBF, HEPBF (L/h): 165 ± 137.9 , 832 ± 369.3 , 996 ± 495.9 ; ARTBF, PORBF, HEPBF
 648 (L/kg BW/h): 0.4 ± 0.26 , 2.1 ± 0.63 , 2.5 ± 0.83 ; ARTBF, PORBF, HEPBF (L/kg BW^{0.75}/h): 1.8 ± 1.24 , 9.4 ± 2.95 , 11.2 ± 3.96 ;
 649 PORBF/HEPBF (%): 85 ± 5.9 , respectively.

650 ²Root mean square prediction error, % of observed mean.

651 ³Error due to mean bias, as a % of total MSPE.

652 ⁴Error due to regression, as a % of total MSPE.

653 ⁵Error due to disturbance, as a % of total MSPE.

654 ⁶Condordance correlation coefficient, where $CCC = r \times C_b$.

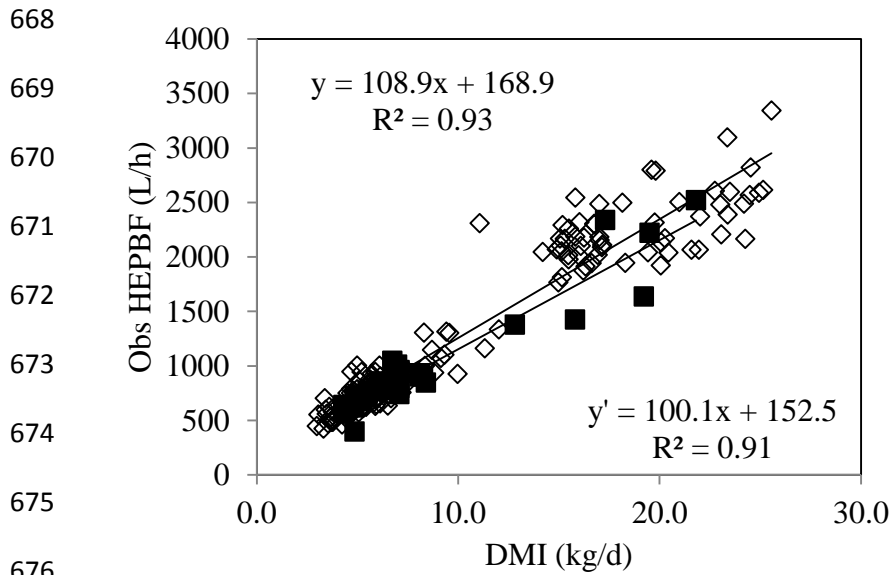
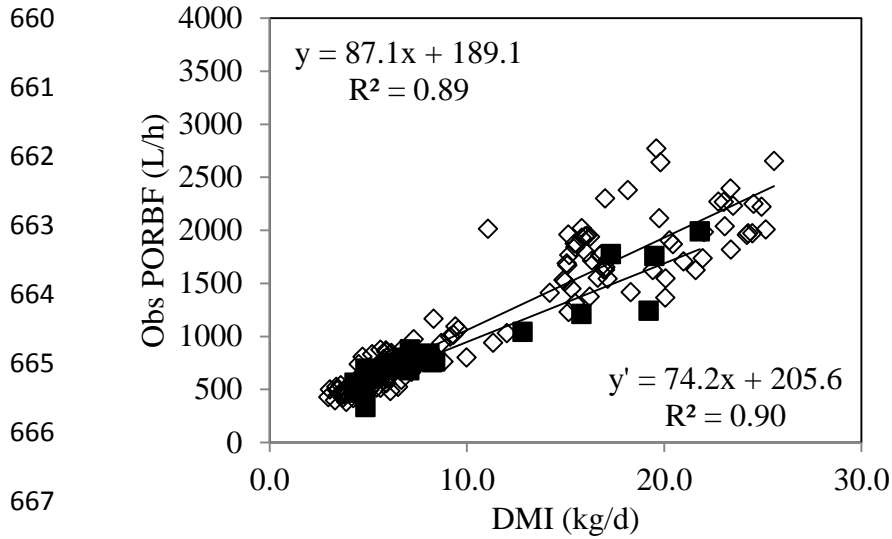
655 ⁷Pearson correlation coefficient.

656 ⁸Bias correction factor.

657 ⁹Scale shift.

658 ¹⁰Location shift relative to the scale.

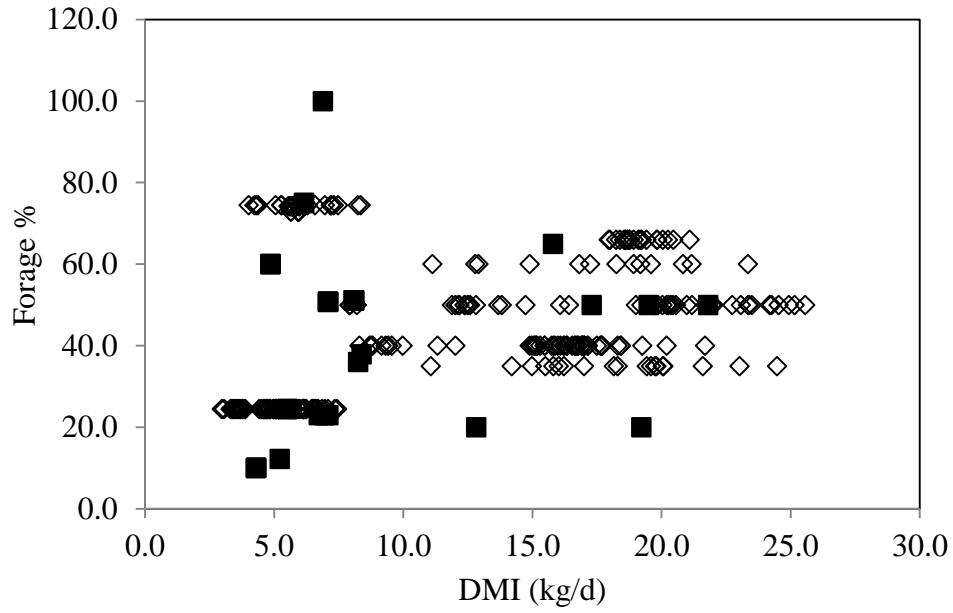
659 ¹¹ PORBF/HEPBF % = (100 – Arterial/venous % linear prediction equation from Vernet et al. (2009)).



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678 **Figure 1.** Observed portal blood flow (PORBF; top) and hepatic blood flow (HEPBF; bottom)
 679 vs. DMI (kg/d) for the developmental database (\diamond , y) and the evaluation database (\blacksquare , y').

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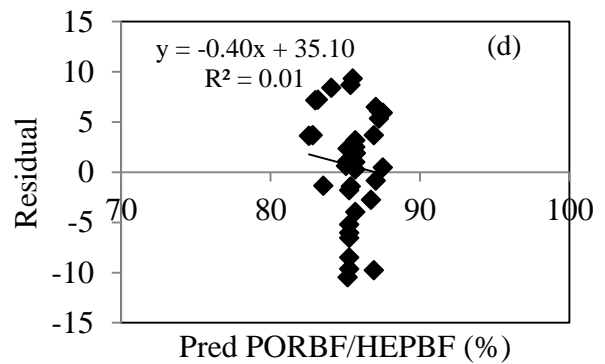
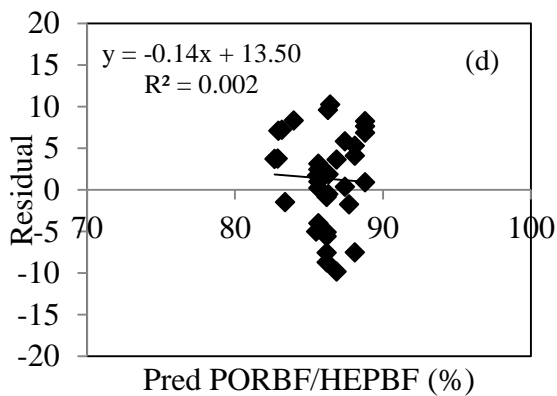
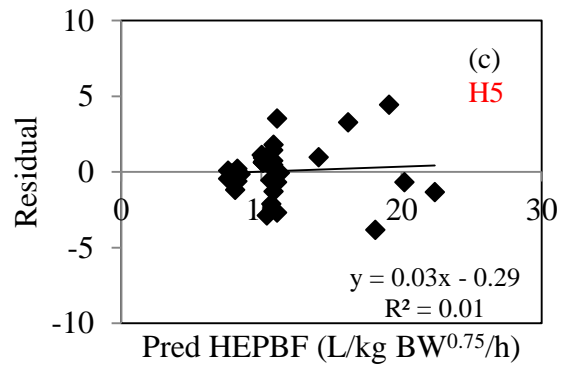
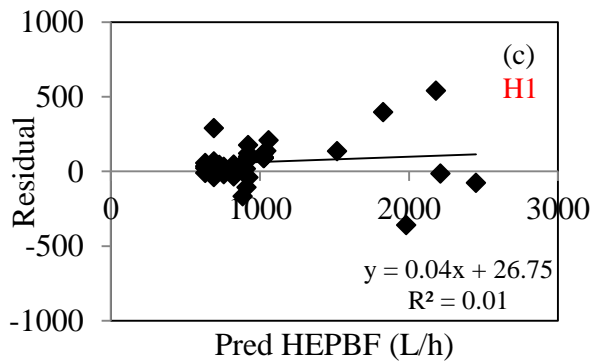
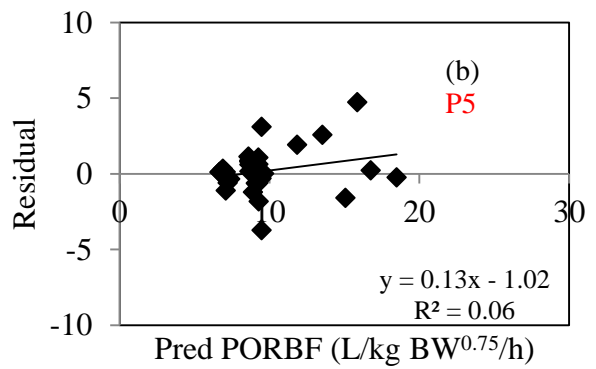
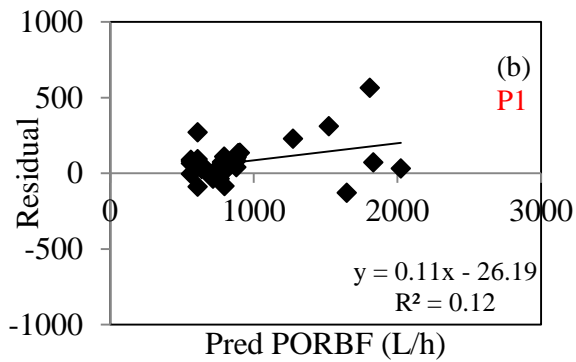
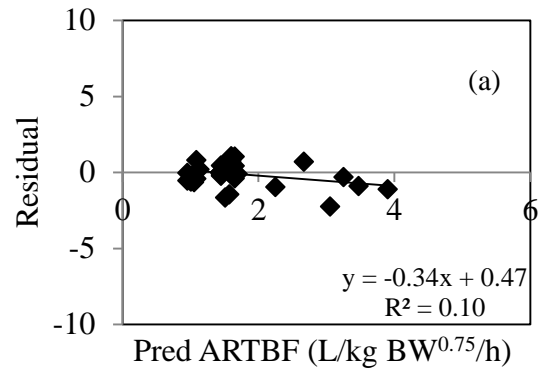
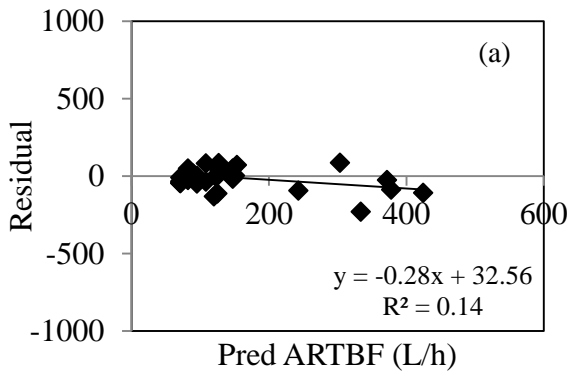


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682 **Figure 2.** Distribution of forage % across DMI (kg/d) for the developmental (◇) and evaluation
 683 (■) databases.

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710 **Figure 3.** Residual (predicted – observed value) vs. predicted blood flow values for the linear
711 DMI based equations (Table 3) based on blood flow in L/h (left) or L/kg BW^{0.75}/h (right),
712 evaluated on the evaluation database for ARTBF (a), PORBF (b) HEPBF (c) and
713 PORBF/HEPBF % (d), and where ARTBF - hepatic arterial, PORBF - portal venous and HEPBF -
714 hepatic venous blood flows.

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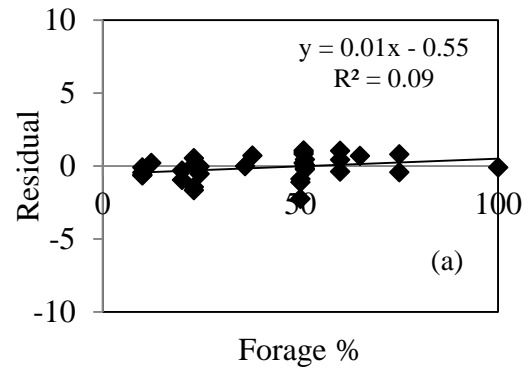
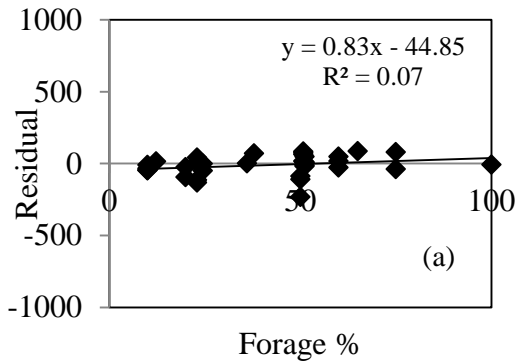
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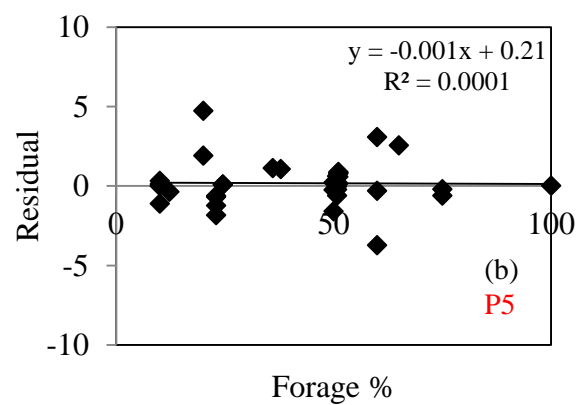
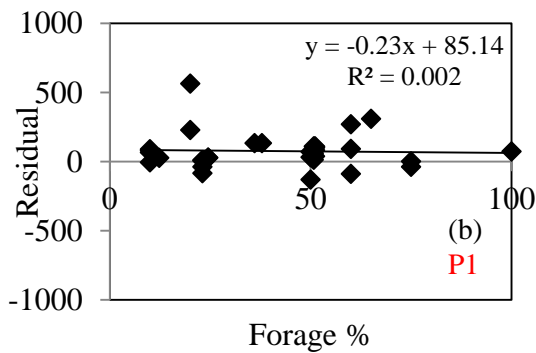
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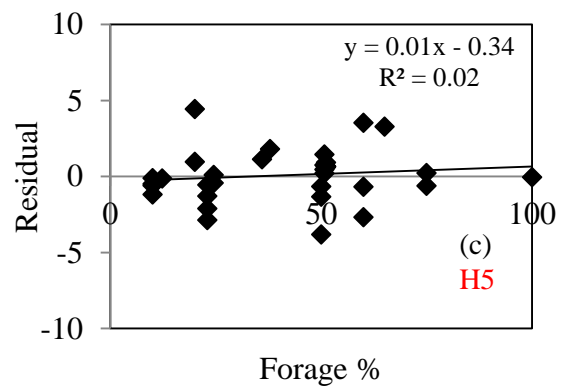
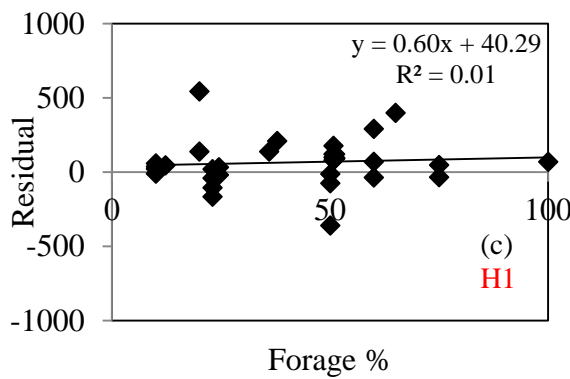
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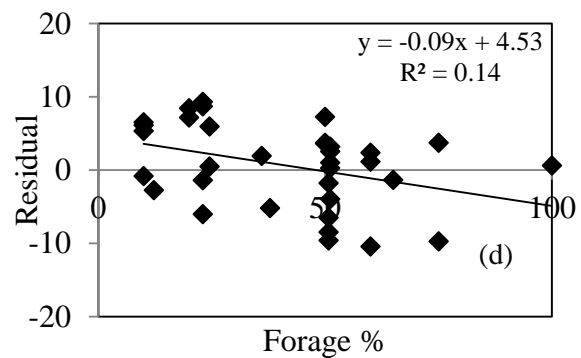
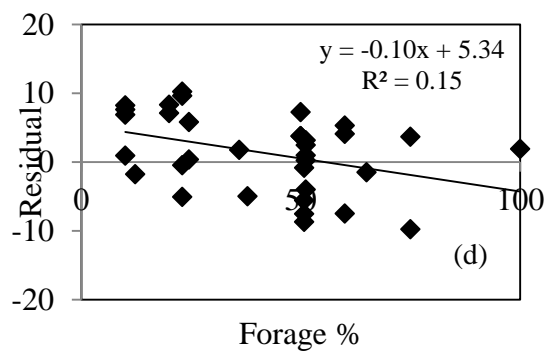
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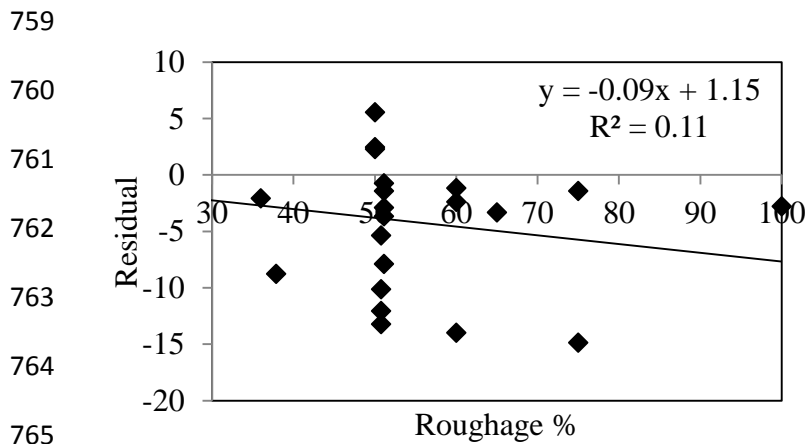
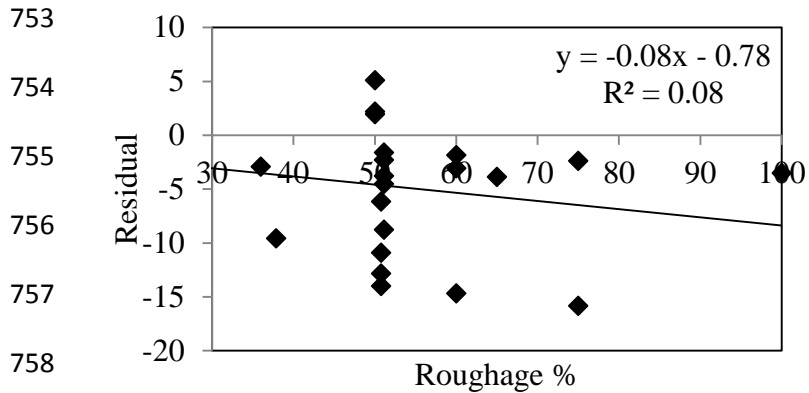
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747 **Figure 4.** Residual (predicted – observed value) vs. the forage proportion (%) of the diet for the
748 DMI based equations (Table 3) based on blood flow in L/h (left) or L/kg BW^{0.75}/h (right),
749 evaluated on the evaluation database for ARTBF (a), PORBF (b) HEPBF (c) and
750 PORBF/HEPBF (d), and where ARTBF - hepatic arterial, PORBF - portal venous and HEPBF -
751 hepatic venous blood flows.

752



766 **Figure 5.** Residual (predicted – observed value) PORBF/HEPBF (%) vs. the forage proportion
 767 (%) in the diet for the DMI based sheep equations of Vernet et al. (2009), for their above
 768 maintenance equation (linear) (Top), and above plus below maintenance equation (quadratic)
 769 (Bottom), evaluated on the evaluation database.

770