

# Equations to predict nitrogen outputs in manure, urine and faeces from beef cattle fed diets with contrasting crude protein concentration

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#### 19 ABSTRACT

Accurately predicting nitrogen (N) outputs in manure, urine and faeces from beef cattle is 20 crucial for the realistic assessment of the environmental footprint of beef production and the 21 development of sustainable N mitigation strategies. This study aimed to develop and validate 22 equations for N outputs in manure, urine and faeces for animals under diets with contrasting 23 crude protein (CP) concentrations. Measurements from individual animals (n=570), including 24 25 bodyweight, feed intake and chemical composition, and N outputs were (i) analysed as a merged database and also (ii) split into three sub-sets, according to diet CP concentration (low 26 CP, 84 -143 g/kg dry matter, n=190; medium CP, 144-162 g/kg dry matter, n=190; high CP, 27 163-217 g/kg dry matter, n=190). Prediction equations were developed and validated using 28 residual maximum likelihood analysis and mean prediction error (MPE), respectively. In low 29 30 CP diets the lowest MPE for N outputs in manure, urine and faeces was 0.244, 0.594 and 0.263, respectively; diet CP-specific equations improved accuracy in certain occasions, by 4.9% and 31 18.3% for manure N output and faeces N output respectively, while a reduction by 5.7% in the 32 prediction accuracy for urinary N output was noticed. In medium CP diets the lowest MPE for 33 N outputs in manure, urine and faeces was 0.227, 0.391 and 0.394, respectively; diet CP-34 specific equations improved accuracy by 13.2%, 41.2% and 16.8% respectively. In high CP 35 diets the lowest MPE for N outputs in manure, urine and faeces was 0.120, 0.154 and 0.144, 36 respectively; diet CP-specific equations improved accuracy in certain occasions by 5.8%, 9.1% 37 38 and 6.3% respectively. This study demonstrated that for improved accuracy of N outputs in manure, urine and faeces from beef cattle, the use of dietary CP concentration is essential while 39 dietary starch, fat, and metabolisable energy concentrations can be used to further improve 40 accuracy. In beef cattle fed low N diets, using diet CP-specific equations improves prediction 41 accuracy when feed intake or dietary CP concentration is not known. However, in beef cattle 42 fed medium or high CP concentration diets, using equations that have been developed from 43

- 44 animals fed similar CP concentration diets, substantially improves the prediction accuracy of
- 45 N outputs in manure, urine and faeces in most cases.

# 46 Keywords

47 Nitrogen, efficiency, beef, urine, faeces, prediction

#### 48 **1. Introduction**

Environmental issues arising from nitrogen (N) excretion in beef production systems are a 49 concern for both the industry and for domestic and international regulation bodies, who 50 51 increasingly seek improved calculation methods in order to promote more accurate reporting of greenhouse gas (GHG) and ammonia (NH<sub>3</sub>) emission estimates, as well as to inform 52 mitigation strategies (European Commission, 2010; DEFRA, 2017). Beef cattle often retain 53 54 less than 20% of the total nutrients they ingest, with the rest being excreted mostly in faeces and urine as well as end products of various other metabolic processes (e.g. respiration, 55 56 gastrointestinal gases) (NASEM, 2016). As regards N, the amount retained in the body can be as low as 10% of the N intake, with an upper limit of 20% (Satter et al., 2002). Several studies 57 involving beef and dairy cattle have reported an average N use efficiency (NUE) of nearly 58 59 25%, with measured values being between 15% and 40% (Kohn et al., 2005; Huhtanen and Hristov, 2009; Calsamiglia et al., 2010). Nevertheless, a large fraction of N in growing and 60 finishing beef cattle rations comes from sources not suitable for human consumption, thereby 61 reducing competition for food and transforming low human nutritional quality forages, grains 62 and by-products into meat protein of higher value (Baber et al., 2018). 63

A large proportion of dietary N which is excreted in faeces and urine contributes to atmospheric 64 pollution and climate change by increasing volatilised NH<sub>3</sub> (an air quality concern) and nitrous 65 oxide (N<sub>2</sub>O; a potent GHG) emissions (Tamminga, 2006), eutrophication of terrestrial and 66 67 aquatic habitats through subsequent N deposition and leaching of nitrates to groundwater (NASEM, 2016; Uwizeye et al., 2020). However, faecal N is mostly present as organic 68 compounds which typically exhibit slower mineralisation rates (Muck and Steenhuis, 1982), 69 70 consequently producing less "reactive" N compared to urinary N; the latter is more labile and can have a more immediate impact on the environment as it swiftly cycles through it, taking 71 different reactive N forms (Galloway et al., 2003). Several studies conducted with beef cattle 72

have shown that 40-80% of non-retained N is excreted in urine, and this amount increases with 73 higher crude protein (CP) or rumen degradable protein (RDP) concentrations of the diet 74 (Archibeque et al., 2007; Vasconcelos et al., 2009; Erickson and Klopfenstein, 2010; Koenig 75 and Beauchemin, 2013a, b). Reynolds and Kristensen (2008) have also concluded that feeding 76 N above requirements increases NH<sub>3</sub> absorption and subsequent urea production in the liver, 77 therefore increasing urea excretion in urine. Most of the N excreted in urine is in the form of 78 79 urea, especially at higher N intakes, and urea is a substance rapidly converted to ammonium and carbon dioxide once exposed to the action of microbial urease enzymes (Varel et al., 1999; 80 81 Monteny et al., 2002). In feedlot operations, the production of ammonium and carbon dioxide might be greater than in pasture systems due to the high abundance of microbes (Cole et al., 82 2009) and the reduced infiltration of urine (Rotz and Oenema, 2006; Hristov et al., 2011) in 83 feedlot surfaces compared to soil. An amount of infiltrated N at pasture may be further reduced 84 by plant uptake to support growth (Petersen *et al.*, 1998), although excessive N infiltration will 85 still result in groundwater pollution (NASEM, 2016). 86

Previously published studies on N excretion from beef cattle, did not either incorporate the diet 87 chemical composition, nutrient digestibilities and energy values (Guo et al., 2004; Guo and 88 Zoccarato, 2005), or account for the different N amounts excreted in urine and faeces (Yan et 89 90 al., 2007) in their analyses. More recent studies have partitioned N excretion into urine and 91 faeces (Hirooka, 2010; Waldrip et al., 2013; Dong et al., 2014; Reed et al., 2015) with the latter 92 also using fibre and energy related predictors for the first time. A recent study from Angelidis et al. (2019) was the first to include a wider set of explanatory variables for the prediction of 93 N excretion in urine and faeces specifically by beef cattle, as well as the evaluation of NUE. 94 95 The evaluation of the previously published models and the developed ones in the study of Angelidis et al. (2019), has shown a degree of under-prediction in N outputs for animals at the 96 highest range of actual N excretion rates. The issue was partly, but not completely resolved 97

with their equations, highlighting the potential risk of underpredicting the impact that intensive 98 beef systems may have on atmospheric and water pollution. This could be attributed partly to 99 100 the fact that all evaluated models were created using N excretion data obtained at a lower range of N intake than for the measurements in which under-prediction was noticed. In conjunction 101 with the documented decrease in NUE with increasing dietary crude protein concentrations 102 (Waldrip et al., 2013; Dong et al., 2014; Angelidis et al., 2019), this finding emphasizes the 103 104 necessity to create prediction models, potentially highly influenced by diet CP content and N intake, based on a N excretion range applicable to the one that the models will be used for. 105

106 Therefore, the aim of our study was to (i) develop diet CP-specific prediction equations for N 107 output in total manure, faeces and urine from growing and finishing beef cattle which were fed 108 diets with different protein concentrations, and (ii) compare their prediction accuracy with that 109 of existing prediction equations.

# 110 **2. Materials and methods**

#### 111 *2.1 The database*

The database used in the present study was constructed by merging three datasets of individual 112 animal measurements from digestibility trials, conducted with beef cattle at Agri-Food and 113 Biosciences Institute (AFBI, UK; n=286) Hillsborough (Yan et al., 2007), Centre for Dairy 114 Research, University of Reading, UK (CEDAR; n=48) (Hammond et al., 2014; Hammond et 115 al., 2015) and Beltsville Agricultural Research Center (n=236), USDA ARS (Haaland et al., 116 117 1981; Tyrrell and Reynolds, 1988; Reynolds et al., 1991; Lapierre et al., 1992; Reynolds et al., 1992). All digestibility trials involved animals housed in individual stalls where feed intake 118 and total collection of faeces and (acidified) urine was taking place over 5-7 days and the mean 119 daily value for each measured parameter was used in the dataset. Composite samples were then 120 analysed for N content by the macro Kjeldahl method (AOAC, 1995). The resulting database 121 contained 570 observations from individual animals that included at least the following 122

parameters: animal body weight (BW, kg); diet total forage content (TF, g/100g DM); diet 123 concentrations of crude protein (CP, g/kg DM), N (g/kg DM) and metabolisable energy (ME, 124 MJ/kg DM); intakes of dry matter (DMI, kg/d); and outputs (g/d) of N in manure (MNO), urine 125 (UNO) or faeces (FNO). Where available, the following diet concentration parameters were 126 also included in the database: neutral-detergent fibre (NDF, g/kg DM), acid-detergent fibre 127 (ADF, g/kg DM), ether extract (EE, g/kg DM), starch (ST, g/kg DM), ash (g/kg DM), organic 128 129 matter (OM, g/kg DM), gross energy (GE, MJ/kg DM). The mean values, standard deviation, number of observations, and minimum and maximum values for each parameter in the database 130 131 are presented in Table 1 and a brief description is given in the supplementary material (Appendix; Summary of the data used). There was a wide variation in animal traits and 132 production characteristics in this database, such as the animal breed (including Holstein, 133 Hereford x Angus, Angus, and others), BW (153-631 kg), production stage (growing,  $\leq$ 350 kg 134 BW; and finishing, >350 kg BW), TF (20-100% of total DM), and various diet ingredients. All 135 abbreviations used in this manuscript are introduced at their first instance in the text and also 136 provided as a list following the Conclusion section. 137

# 138 2.2 Statistical analysis

The equations for the prediction of N excretion in manure, urine and faeces were produced 139 using linear and multiple regression models in Genstat 17th edition (VSN International, 2015). 140 The prediction equations were developed using residual maximum likelihood analysis, so that 141 142 the potential random effects of experiment ID, animal ID, and treatment ID, experiment location and animal production stage (growing or finishing) were accounted for (Robinson, 143 1987; Searle et al., 1992). The linear regression equations developed included MNO (g/d), 144 UNO (g/d) and FNO (g/d) as response variables and (i) DMI, NI, BW in single linear 145 relationships (Table 2), and (ii) DMI, NI, BW, TF, CP, NDF, ADF, ST, EE, ME in multiple 146 linear relationships (Table 2), as explanatory variables. These two distinct approaches aimed 147

to produce both (i) simple models for easier application in a commercial farm environment, 148 where accurate feed intake measurements are challenging while BW is readily avilable and can 149 serve as a proxy for DMI (because heavier animals consume more food), as well as (ii) higher 150 complexity models with an improved prediction accuracy, to be used where relevant predictors 151 are available (e.g. research environment). The method used in the present study to develop the 152 prediction equations has been previously used in several studies (Stergiadis et al., 2015a; 153 154 Stergiadis et al., 2015b; Stergiadis et al., 2016). In brief, the optimum random model developed for each response variable was built by fitting the same fixed effect model and the prospective 155 156 models of the random variation. The observed changes in deviance was the driver of whether to include a random factor in the model or not; and eventually the optimum random model 157 included the individual experiment ID, animal ID, treatment ID. The Wald statistic was used 158 in order to evaluate the significance of the various explanatory variables used in the single and 159 multiple linear regressions. In the current study, the predictors comprising the prediction 160 equations were statistically significant (P<0.05) according to the Wald statistic. The residual 161 diagnostics of the final model were evaluated using normality plots. An approximate  $R^2$ 162 (pseudo correlation coefficient; squared correlation of the response and the fitted values) was 163 generated to represent the proportion of variability explained. 164

In a recent study, Angelidis et al. (2019) showed that literature equations tend to under-predict 165 N outputs in manure, urine and faeces, in animals with N excretions close to the highest end of 166 the range. In order to provide an insight into how the prediction accuracy in specific N outputs 167 ranges may be improved by using equations developed for animals with N intakes 168 corresponding to those outputs, the database was split into three sub-sets, according to the diet 169 170 CP concentrations (low CP, 84 -143 g/kg DM, n=190; medium CP, 144-162 g/kg DM, n=190; high CP, 163-217 g/kg DM, n=190), in line with the Agriculture and Horticulture Development 171 Board (AHDB) recommendations for growing and finishing beef cattle dietary protein (AHDB, 172

173 2016). Three additional sets of equations for the prediction of MNO (g/d), UNO (g/d) and FNO
174 (g/d) were developed, using the methods and explanatory variables described for the merged
175 digestibility trials database (Tables 3, 4 and 5; for low CP, medium CP and high CP,
176 respectively).

An external validation was performed to assess the prediction accuracy of all equations developed in the current study as well as of those previously published in literature. For this purpose, the literature database developed in a previously published study (Angelidis *et al.*, 2019) was used. Furthermore, this external database was also divided into three sub-sets representing low, medium and high dietary CP concentration (using the same range as described above). Evaluations were performed using the mean-square prediction error (MSPE) method:

$$MSPE = 1/n \Sigma (P-A)^2$$

where P and A are the predicted and actual values respectively, and n represents the number of
pairs of P and A values compared. Mean prediction error (MPE) was calculated to describe the
prediction accuracy, using the following formula:

188  $MPE = \sqrt{(MSPE) / (\Sigma A/n)}$ 

The quantification of agreement between actual and predicted values was derived from a Lin's 189 Concordance Correlation Coefficient (Rc) analysis (Lawrence, 1989), with the results 190 presented in Table 4 (for the equations produced from the merged digestibility trials database 191 192 and the previously published models) and Table 5 (for the equations developed from the merged digestibility trials sub-sets). For the graphic representation of the agreement between 193 predicted and actual values of MNO, UNO and FNO, Bland – Altman plots were used (Altman 194 195 and Bland, 1983) including (i) equations presented previously from other authors, (ii) equations developed in the current study using the same explanatory variables, (iii) equations developed 196 in the current study with higher prediction accuracy than the existing ones and (iv) equations 197

developed in the current study from the partitions of the merged digestibility trials database.Rc with 95% confidence interval are also presented in the same graphs.

A total of 129 new equations, of which 51 were developed by the entire merged digestibility 200 trials database, 24 by the low CP sub-set, 18 by the medium CP sub-set and 36 by the high CP 201 sub-set, were validated against the literature database developed in the study by (Angelidis et 202 al., 2019) and its corresponding sub-sets. The models developed by the merged digestibility 203 204 trials database were initially validated against the entire literature database (Table A2 for the prediction of MNO, UNO and FNO; Eq. 1a-1p, 2a-2s and 3a-3p, respectively), while the 205 206 models developed from the sub-sets were validated against the corresponding CP sub-sets of the external validation database (Table A3 for the prediction of MNO, UNO and FNO; Eq. 4a-207 4i, 7a-7g and 10a-10p; Eq. 5a-5g, 8a-8f and 11a-11k; Eq. 6a-6h, 9a-9e and 12a-12i, 208 209 respectively). In order to assess the potential benefit in prediction accuracy by using the models developed by corresponding sub-sets, the equations resulting from the entire merged 210 digestibility trials database that included exactly the same predictors as the ones resulting from 211 the sub-sets, were validated against the same external validation data (Table A4 for the 212 prediction of MNO, UNO and FNO; Eq. 4a-4i, 7a-7g and 10a-10p; Eq. 5a-5g, 8a-8f and 11a-213 11k; Eq. 6a-6h, 9a-9e and 12a-12i, respectively). Finally, 23 external equations presented in 214 the appendix (Table A1), for the prediction of MNO (Eq. E1-E9; Yan et al. (2007); Reed et al. 215 (2015)), UNO (Eq. E10-E17; Hirooka (2010); Reed et al. (2015)); Waldrip et al. (2013); Dong 216 217 et al. (2014)) and FNO (Eq. E18-E23; Hirooka (2010); Reed et al. (2015)); Waldrip et al. (2013); Dong et al. (2014)), were validated against the literature database developed in the 218 study by Angelidis et al. (2019) (Table A2 for the prediction of MNO, UNO and FNO; Eq. E1-219 E9, E10-E17 and E18-E23, respectively). 220

221 **3. Results** 

*3.1 Prediction of N outputs using the entire merged digestibility trials database* 

The effects of DMI, CP, ME, TF, ADF, NDF, NI, ME, ST, EE, BW for the prediction of MNO, 223 were significant according to the Wald statistic (Table 2; Eq. 1a-1p). MNO was positively 224 correlated to DMI, CP, TF, ADF, NDF, ST and negatively correlated to EE and ME. When 225 DMI and CP were used as predictors (Eq. 1b) the prediction accuracy was higher compared to 226 using DMI alone in a single linear model (Eq. 1a), while adding ME to the former model, 227 further reduced MPE (Eq. 1c). The use of NI instead of DMI as a sole predictor for the 228 229 prediction of MNO (Eq. 1g), produced a lower MPE. Furthermore, the model including NI as the primary predictor and ME, ADF and ST as secondary predictors (Eq. 1j) showed the lowest 230 231 MPE for the prediction of MNO. When BW was used as sole predictor (Eq. 11), the prediction accuracy was low compared to the linear models using either DMI or NI as sole predictors. 232 However, by using BW and CP in a multilinear model the MPE was slightly reduced (Eq. 1m), 233 taking its lowest value when ME was added to the above model (Eq. 1n). 234

For the prediction of UNO, the effects of DMI, CP, TF, ADF, ME, EE, ADF, ST, NI, BW were 235 significant according to the Wald statistic (Table 2; Eq. 2a-2s). UNO was positively correlated 236 to DMI and CP, and negatively correlated to ME and EE. Prediction accuracy was higher when 237 CP was used in combination with DMI for the prediction of UNO (Eq. 2b), compared to using 238 DMI as sole predictor (Eq. 2a). The combination of DMI, CP and ADF produced a better MPE 239 (Eq. 2d), while the addition of either EE or ST to the above model (Eq. 2g and 2h, respectively), 240 further increased the prediction accuracy. The model including DMI as the primary predictor 241 and CP, ADF, ST and ME as secondary predictors (Eq. 2i), produced the lowest MPE for the 242 prediction of UNO. Using NI as a predictor in a single linear model (Eq. 2j) produced a better 243 MPE compared to the respective single linear model with DMI. Furthermore, including ST and 244 EE as secondary predictors (Eq. 2n) improved the prediction accuracy, yet only slightly. 245 Equations including BW as the primary predictor and several secondary predictors in single 246 and multiple linear models (Eq. 20-2s) had similar MPEs, with the model including BW as a 247

sole predictor (Eq. 20) having a higher MPE compared to the previous single linear models
with either DMI or NI, and the addition of CP to the above model (Eq. 2p) only marginally
improving the prediction accuracy.

Finally for the prediction of FNO, the effects of DMI, CP, ME, TF, ADF, NI, NDF, ST, EE, 251 BW were significant according to the Wald statistic (Table 2; Eq. 3a-3p). FNO was positively 252 correlated to DMI, CP, ST and EE and negatively correlated to ME, TF and ADF. The 253 254 prediction accuracy of the model using DMI as a sole predictor (Eq. 3a), was improved after the addition of CP in a multiple linear prediction model (Eq. 3b), with further improvement 255 256 after TF was added to the above model (Eq. 3d). The model including NI as a sole predictor (Eq. 3g), had better prediction accuracy than adding any other secondary predictor (Eq. 3h-3k). 257 Conversely, BW as a sole predictor (Eq. 31) resulted in a high MPE, but when CP, ME and EE 258 were added to the above model (Eq. 30) the MPE was reduced, taking the lowest value in the 259 equation including CP, ME and ST as secondary predictors (Eq. 3n). 260

261 3.2 Prediction of N outputs using the low dietary protein sub-set

The effects of DMI, CP, ME, ADF, NDF, NI, BW for the prediction of MNO, were significant according to the Wald statistic (Table 3; Eq. 4a-4i). MNO was positively correlated to DMI, CP, NDF, NI and BW and negatively correlated to ME and ADF. Prediction accuracy was improved when CP was used in combination with DMI for the prediction of MNO (Eq. 4b), compared to using DMI as sole predictor (Eq. 4a). The single linear model with NI (Eq. 4e) had the lowest MPE for the prediction of MNO.

Subsequently, for the prediction of UNO, the effects of DMI, CP, NI, BW, ME, TF were significant according to the Wald statistic (Table 3; Eq. 5a-5g). UNO was positively correlated to DMI, CP, NI, BW and TF and negatively correlated to ME. The multiple linear model including DMI and CP as predictors along with the single linear including NI as predictor (Eq. 5b and 5c, respectively) gave the best prediction accuracy among the group, yet the MPE valueswere high.

For the prediction of FNO, the effects of DMI, CP, ME, TF, NI, BW were significant according to the Wald statistic (Table 3; Eq. 6a-6h). MNO was positively correlated to DMI, CP, NI and BW and negatively correlated to ME and TF. The various models appeared to have similar prediction accuracy despite the primary predictor involved, with the exception of the multiple linear model including BW, CP and ME as predictors (Eq. 6h), which had a notably lower MPE.

# 280 *3.3 Prediction of N outputs using the medium dietary protein sub-set*

For the prediction of MNO, the effects of DMI, CP, ME, NI, ST, and BW were significant according to the Wald statistic (Table 4; Eq. 7a-7g). MNO was positively correlated to DMI, CP, NI, ST and BW and negatively correlated to ME. Prediction accuracy was improved when CP and ME was used in combination with DMI for the prediction of MNO (Eq. 7c), compared to using DMI as sole predictor (Eq. 7a). Similarly accurate was the single linear model with NI (Eq. 7d), while the model with BW as the primary predictor had low accuracy (Eq. 7g).

The effects of DMI, CP, NI, ADF, ST, and BW for the prediction of UNO were significant according to the Wald statistic (Table 4; Eq. 8a-8f). UNO was positively correlated to DMI, CP, NI, BW and TF and negatively correlated to ME. The single linear model including NI as predictor produced a low MPE (Eq. 8c), which was further improved after adding ADF and ST as secondary predictors (Eq. 8d).

For the prediction of FNO, the effects of DMI, NI, ME, BW, and CP were significant according to the Wald statistic (Table 4; Eq. 9a-9e). FNO was positively correlated to DMI, NI and BW and negatively correlated to CP and ME. The various models appeared to have similar prediction accuracy, as happened with the respective equations in the table 3 (Eq. 6a-6h), despite the primary predictor involved. In this case, the multiple linear model with NI and ME

as predictors, had the lowest MPE among the models (Eq. 9c).

298 *3.4 Prediction of N outputs using the high dietary protein sub-set* 

The effects of DMI, CP, TF, ME, EE, NI, ADF, and BW for the prediction of MNO were 299 significant according to the Wald statistic (Table 5; Eq. 10a-10p). MNO was positively 300 correlated to DMI, CP, NI and BW and negatively correlated to ME, EE and ADF. Prediction 301 302 accuracy observed while using CP in conjunction with DMI for the prediction of MNO (Eq. 10b) was better than using DMI as sole predictor (Eq. 10a). Furthermore, adding ME as 303 304 secondary predictor to the above model (Eq. 10e) further increased prediction accuracy. Similar results were observed in the model that included DMI as primary and CP, TF and ME as 305 secondary predictors (Eq. 10f). When NI was used in combination with either ME alone or ME 306 307 and TF (Eq. 10h and 10i) the MPE was in both cases lower compared to using NI as sole predictor (Eq. 10g). Furthermore, the substitution of TF with ADF in the above model produced 308 an equation with similar prediction accuracy. When BW was used as a sole predictor (Eq. 10l), 309 MPE appeared high in the single linear model, yet after the addition of CP and ME as secondary 310 predictors (Eq. 10n), the prediction accuracy was notably improved. 311

For the prediction of UNO, the effects of DMI, CP, ME, NI, NDF, EE, BW, and TF were 312 significant according to the Wald statistic (Table 5; Eq. 11a-11k). UNO was positively 313 correlated to DMI, CP, NI, BW and TF and negatively correlated to ME, NDF and EE. The 314 315 multiple linear model including DMI, CP and ME as predictors (Eq. 11c) displayed a notably lower MPE than using DMI as sole predictor (Eq. 11a). In the same manner, the multiple model 316 including NI and ME as predictors (Eq. 11e) was more accurate than the one using NI as sole 317 predictor (Eq. 11d). When BW was used as the primary predictor, only the model comprising 318 CP and ME as secondary predictors showed a low MPE (Eq. 11j), with the model using the 319 above predictors plus TF (Eq. 11k) showing similar, yet lower prediction accuracy. 320

Finally, for the prediction of FNO, the effects of DMI, CP, ME, TF, NI, and BW were 321 significant according to the Wald statistic (Table 5; Eq. 12a-12i). FNO was positively 322 correlated to DMI, CP, NI and BW and negatively correlated to ME. The model comprising 323 DMI, CP and ME (Eq. 12c) had the highest prediction accuracy among the group of equations 324 with DMI as primary predictor. When NI was used along with ME as a secondary predictor 325 (Eq. 12f), the MPE was lower than using NI as sole predictor (Eq. 12e), while the addition of 326 327 TF to the former model (Eq. 12g) did not improve the prediction accuracy. The model including BW as primary predictor (Eq. 12h) was only marginally improved when CP and ME were 328 329 added as secondary predictors (Eq. 12i), however both MPE values were high compared to the rest in the FNO group. 330

# 331 *3.5 External validation of the prediction equations*

For the prediction of MNO in the merged digestibility trials database, (Fig. 1; plots a-c), the 332 equation incorporating the most predictors (plot c), showed a higher Rc compared to both the 333 existing (plot a) and new (plot b) equations which used NI as sole predictor. The Rc values for 334 UNO and FNO (Fig.1; plots d-i) appeared similar. Rc and variation of the residual MNO, UNO 335 and FNO was reduced when new models developed in the present study, including additional 336 predictors (Eq. 1j, 2i and 3d), were applied. For the prediction of MNO, there was an over 337 prediction in the equations including NI as sole predictor (Eq. E3 and 1g), when actual MNO 338 was lower than 60 (g/d) and 67 (g/d), respectively; and under-prediction in the same equations, 339 340 when actual MNO was higher than 168 (g/d) and 170 (g/d), respectively. For the prediction of UNO, there was an over prediction in the equations including NI as sole predictor (Eq. E10 341 and 2j) when actual UNO was lower than 49 (g/d) and 50 (g/d), respectively; and an under-342 prediction in the same models when actual UNO was higher than 121 (g/d) and 109 (g/d), 343 respectively. In the case of FNO, there was no significant over- or under- prediction. 344

As regards the equations produced from the low CP sub-set compared to their identical ones 345 from the full database (Fig. 2; plots a-f), the equation produced from the sub-set for the 346 347 prediction of MNO showed a notably higher Rc compared to the identical equation produced from the full database, which also greatly over-predicted MNO, while the equations for the 348 prediction of UNO and FNO showed similar Rc values. In the case of UNO, there was an 349 under-prediction when actual UNO was higher than 93 (g/d) in both sets of equations. 350 351 Furthermore, equations developed from the medium CP sub-set, when compared to the identical ones from the merged digestibility trials database (Fig. 3; plots a-f) showed higher Rc 352 353 values for the prediction of MNO and FNO, with a similar variation across the zero line and no significant over- or under- prediction. For the prediction of UNO (Eq. 2j and 8c), there was 354 a small overprediction when actual UNO was lower than 50 (g/d) and 54 (g/d); and an 355 underprediction when actual UNO was higher than 99 (g/d) and 105 (g/d), respectively. Finally, 356 equations developed from the high CP sub-set (Fig. 4) had in all cases similar or higher Rc 357 values, when compared to their identical ones from the merged digestibility trials database. 358 Variation of the residual MNO was reduced when the medium CP sub-set equation was used, 359 while in the case of both UNO and FNO no significant differences were observed 360

# 361 **4. Discussion**

4.1 Prediction accuracy of equations developed using the merged digestibility trials database 362 The most accurate prediction of MNO was seen when NI, ME, ADF and ST were used as 363 predictors, and this equation may be used when such data are available. Yan et al. (2007) found 364 that adding predictors in a model already containing NI did not improve prediction accuracy, 365 while Angelidis et al. (2019) there found a 67% improvement in prediction accuracy (MPE 366 reduced from 0.440 to 0.162) when either dietary forage proportion, fibre concentration or 367 nutrient digestibility data were used as additional predictors. In contrast to Angelidis et al. 368 (2019), this study demonstrated that individually adding fibre or energy parameters in a model 369

already containing NI may not beneficial to prediction accuracy but when these are added 370 altogether, and in conjunction with ST the prediction accuracy may be increased up to 47% 371 (MPE reduced from 0.242 to 0.129). The use of DMI alone as a predictor for MNO was 372 expected to show low prediction accuracy, as it does not account for the level of dietary N. The 373 addition of either fibre or forage proportion did not further improve the model, similarly to Yan 374 et al. (2007), while the addition of ME improved the accuracy of combined model of DMI and 375 376 CP by 13% (MPE reduced from 0.305 to 0.270). Energy values, such as the readily available at commercial farms as measured GE or calculated ME, are known to improve MNO prediction 377 378 (Yan et al., 2007; Reed et al., 2015; Angelidis et al., 2019), as they are both and useful indicators of microbial CP synthesis in the rumen (Hespell and Bryant, 1979). According to 379 the Bland-Altman plots, the addition of diet energy and fibre concentration as predictors in 380 equations already including NI improved the MNO underprediction, which was observed when 381 NI was used as a sole predictor. Although NI is an accurate predictor for MNO, energy 382 parameters, when added, may explain more variation in the data as they are profoundly 383 affecting NUE (Angelidis et al., 2019). As feed intake cannot be accurately measured in 384 commercial farms, the readily available BW (which can serve as proxy for DMI because 385 heavier animals eat higher amounts of feed), was also evaluated in the current study; the best 386 performing model included BW, CP and ME as prediction, yet in the absence for DMI the 387 overall prediction accuracy was relatively low. 388

The most accurate model to predict UNO included DMI, CP, ADF, ST and ME. DMI as sole predictor showed low prediction accuracy, a finding consistent with previous studies (Dong *et al.*, 2014; Angelidis *et al.*, 2019). However, prediction of UNO had an overall low accuracy, while the most accurate model relies on predictors that may be available in a research environment, but are unlikely to be recorded on commercial farms. Addition of ST and EE in the present work further improved prediction accuracy of models already containing NI as sole

predictor. Dietary energy sources, such ST and EE may improve the energy supply in rumen 395 microorganisms and enhance microbial protein synthesis, instead of ammonia, and therefore 396 reduce UNO in the form of urea (Bach et al., 2005). It is known that the addition of 397 supplemental fat over 30 g/kg DM in the diet may disrupt ruminal fermentation and reduce the 398 digestibility of structural carbohydrates, however amounts of up to 60 g/kg DM can be 399 supplemented without problems, provided this is reached through a diet adaptation period 400 401 (Hess et al., 2008). Average fat intake was less than 30 g/kg DM in our database with a maximum of 63 g/kg DM, thus being unlikely that animals developed any adverse effects on 402 403 digestibility. Models that did not account for NI had poor prediction accuracy give the strong positive correlation between NI and UNO (Archibeque et al., 2001), and the fact that most 404 excess dietary N is excreted in urine rather than faeces (Varel et al., 1999). This is finding is in 405 406 line with recent studies (Waldrip et al., 2013; Dong et al., 2014; Reed et al., 2015; Angelidis et al., 2019) showing NI to be an essential predictor for UNO. The equation from Hirooka 407 (2010) was slightly more accurate when compared with its corresponding equation in the 408 present study, while the under-prediction at the higher end of the range of actual N excretion 409 as also observed by Angelidis et al. (2019), was common in both equations. The equation 410 including DMI, CP, ADF, ST and ME as predictors, resolved this issue and can be 411 recommended when such data are available. 412

Finally, FNO was accurately predicted when DMI, CP and TF were used together, yet excluding TF from the prediction model resulted in similar accuracy. When DMI is not available in practice, the equation including BW, CP, ME and ST could be used without compromising accuracy, although a detailed feed analysis is needed to obtain the additional parameters. The strong relationship between FNO and NI, as well as models of similar accuracy, were previously reported (Waldrip *et al.*, 2013; Dong *et al.*, 2014), . Microbial protein produced in the hindgut from the digestion of starch, increases N excretion through 420 faeces, contributing to lower apparent N digestibility and a shift in N excretion from faeces to 421 urine (Reynolds *et al.*, 2001); thus explaining the beneficial role of ST on increasing the 422 explained variation and prediction accuracy. In case of FNO prediction, there were no 423 significant over- or under-prediction issues.

424 *4.2 Equations performance on the low range of diet CP concentration* 

The equation using DMI or BW as sole predictors and the low CP sub-set for the prediction of 425 426 MNO, was markedly more accurate compared to the one produced from the merged digestibility trials database, when validated against the low CP range of the existing literature 427 428 database by Angelidis et al. (2019). This suggests that when diet N concentration is not known, it becomes important that the equations used have been developed from animals at low diet 429 CP. This is not necessary when diet CP is known because when CP was added to the above 430 models, the prediction accuracy of the equations from the merged digestibility was higher than 431 the diet CP-specific equations; and overall higher than the models without CP. The efficiency 432 of microbial CP synthesis depends on the rumen N availability and the energy supply to ruminal 433 microorganisms for growth, as mentioned above, and that explains why the addition of CP 434 benefits the prediction accuracy (Bach et al., 2005). Other combinations of NI, DMI or BW 435 with CP and ME, or with CP, ADF and NDF led to similar prediction accuracy among the 436 equations coming from both the merged database and the low CP dataset. However, adding 437 these additional predictors did not improve prediction of MNO, which reveals that predictors 438 439 describing diet and energy and fibre contents as not as important when we predict MNO from animals consuming low CP diets. Angelidis et al. (2019) have shown that the addition of dietary 440 forage proportion can improve prediction accuracy by up to 20% in models already including 441 DMI and CP, a fact demonstrated for the animals consuming low CP diets. 442

443 When DMI and BW were used as sole predictors for the prediction of UNO, prediction 444 accuracy was expected to be low as this has been previously observed (Dong *et al.*, 2014;

Angelidis et al., 2019). The combination of the above models with CP did not markedly 445 improve the prediction accuracy, even when diet CP-specific equations were used. NI appeared 446 to be the best sole predictor for the prediction of UNO, yet displaying an overall low accuracy 447 compared to the equations available in literature (Waldrip et al., 2013; Dong et al., 2014; 448 Angelidis et al., 2019). ME was statistically significant in a single case, yet it did not benefit 449 the accuracy, in the model including BW, CP and ME as predictors. Reynolds and Kristensen 450 451 (2008) have concluded that feeding N above requirements raises NH<sub>3</sub> absorption and subsequent urea production, therefore increasing N excretion in urine. However, in cases of 452 453 animals consuming low-CP diets, the excess dietary N is minimised and therefore ME does not play such an important role as a predictor as in animals at medium or high CP diets. Prediction 454 of UNO is still challenging in low CP diets, and this has not been resolved by using diet CP-455 specific equations or additional predictors. Incorporating the metabolisable protein as predictor 456 and accounting for all protein fractions reaching the duodenum (ruminally undegradable 457 protein, microbial protein and endogenous protein), may further improve prediction accuracy 458 of existing models but such data are scarce in literature. 459

Equations for the prediction of FNO including DMI either as sole predictor or in combination 460 with CP, ME and TF, showed similar accuracy among the databases, confirming that DMI is a 461 reliable sole predictor for FNO in low CP diets (Stergiadis et al., 2015a; Angelidis et al., 2019). 462 The models originating from the merged digestibility trials database including NI as sole 463 predictor or in combination with either ME or ME and TF, performed better than diet CP-464 specific equations for the prediction of FNO in animals under low-CP diets. However, the 465 combination of BW with CP and ME developed from the low CP sub-set, improved prediction 466 accuracy compared with its identical from the merged digestibility trials database, and its use 467 can be recommended in the common commercial situation that DMI is not available but these 468 predictors are. 469

#### 470 *4.3 Equations performance on the medium range of diet CP concentration*

Equations for the prediction of MNO using the medium CP sub-set, showed similar prediction 471 472 accuracy when they included DMI or BW as sole predictors, with their respective models from the merged digestibility trials database. In case of animals in medium CP diets, NI as a sole 473 predictor can be used for small improvements in the accuracy of prediction of MNO than using 474 DMI or BW, whendiet CP content is known. Incorporating additional predictors, such as CP, 475 476 ME and ST, in all cases improved accuracy compared to their respective equations from the merged digestibility trials database; thus highlighting that, in contrast with low CP diets, there 477 478 is a benefit of using diet-specific equations for animals in medium CP diets when these predictors are available. Several previous studies have suggested that dietary N concentration 479 is negatively correlated with NUE in beef (Yan et al., 2007; Waldrip et al., 2013; Dong et al., 480 2014). Therefore, using equations that have been developed using data from animals 481 consuming diets of different N concentrations than the animals the equations are used to assess 482 may deteriorate prediction accuracy; as the potential differences in kg N output per kg NI may 483 not be as effectively accounted for. 484

As in low CP diets and previous work (Angelidis et al., 2019), prediction equations for UNO 485 from animals consuming medium CP diets, developed by using either DMI, NI or BW as sole 486 predictors, showed low accuracy levels in all cases. The addition of CP as predictor benefited 487 the prediction accuracy, as previously shown Angelidis et al. (2019), yet no benefit was seen 488 489 by using diet-specific equations. Contrastingly, CP had no significant impact on the prediction accuracy of UNO when combined with BW, thus agreeing with Angelidis et al. (2019) that 490 feed intakes are essential for the prediction of UNO. In contrast to low CP diets, the diet-491 specific equation combining of NI, ADF and ST, exhibited the highest prediction accuracy for 492 UNO in animals under medium CP diets; thus revealing that energy and fibre variables, as well 493

494 as diet-specific equations, are becoming more efficient on improving prediction accuracy as495 diet CP contents increase.

496 For the prediction of FNO, single or multiple linear models with DMI or BW as the main predictors, show similar and high MPE values in all cases; thus being in line with previous 497 work (Angelidis et al., 2019). This contrasts the finding for low CP diets and reveals that the 498 need for additional predictors for FNO increases at diets with medium CP. The use of NI with 499 500 ME improved prediction accuracy compared to the aforementioned models, especially when developed from the medium CP sub-set compared to the merged digestibility trials database. 501 502 Therefore it is likely that the improvement in the prediction of MNO when using diet CPspecific equations in animals consuming medium CP diets, mainly comes from the 503 improvement in the prediction of UNO, and at a lesser extent FNO, as the prediction error of 504 505 the latter was still relatively high.

# 506 4.4 Equations performance on the high range of diet CP concentration

Diet-specific equations for the prediction of MNO, produced from the high CP sub-set, 507 demonstrated in most cases higher prediction accuracy when compared to their merged 508 digestibility trials database respective models. This finding emphasizes the need to use diet CP-509 specific equations in animals with increased NI (which are expected to have the highest N 510 outputs; (Yan et al., 2007; Waldrip et al., 2013; Dong et al., 2014)) in most cases. The addition 511 of CP to the model including DMI as a sole predictor increased prediction accuracy, while the 512 513 subsequent addition of TF did not affect it, which is in line with Yan et al. (2007). Further adding ME as secondary predictor offered a even higher accuracy for the prediction of MNO, 514 thus further highlighting that CP and ME are key predictors for MNO, which is in line with 515 Angelidis et al. (2019). When DMI, CP and ME are the only available predictors, then diet-516 specific equations are preferable. However, if TF or EE are also available the prediction 517 accuracy can be maximised if the equations from the merged database are used. This may also 518

demonstrate that for animals in extreme diet CP contents prediction accuracy is more likely to maximise by using more predictors rather than diet CP-specific equations; although the dietspecific equations can be used in cases that less predictors are available. Equations using BW as the main predictor did show a good prediction capacity only when CP and ME were added to produce a multiple linear prediction model; a need which to be higher with increasing dietary N.

525 In line with the findings for the low- and medium- CP datasets and previous work (Angelidis et al., 2019), the single linear model using DMI or BW as the predictor and the model 526 comprised of DMI or BW and CP, did not show good prediction accuracy for UNO. 527 Conversely, the addition of ME to these models significantly increased the prediction accuracy, 528 and the model developed from the high CP sub-set was slightly more accurate. As seen in the 529 case of MNO from animals at high CP diets, more complex models, also including fibre data, 530 were more accurate when developed from the merged digestibility trials database rather than 531 its high CP sub-set, with the combination of NI, ME and NDF predicting UNO with the highest 532 accuracy. The accurate prediction of UNO remains challenging and it seems that a combination 533 of diet CP-specific equations, when DMI (or BW), CP and ME are available, or higher 534 availability of predictors are necessary to maximise prediction accuracy; although the 535 prediction accuracy of UNO has not appeared higher than 0.208 in any of the sub-sets in this 536 study or previous work (Angelidis et al., 2019). The fact that the influence of diet is higher on 537 UNO than FNO (Vasconcelos et al., 2009; Erickson and Klopfenstein, 2010) is possibly among 538 the main reasons why UNO prediction is far more challenging than FNO or MNO (which partly 539 consists of FNO). 540

541 Similar prediction accuracy was noticed when different combinations including DMI as the 542 main and CP, ME and TF as the secondary predictors were produced using either the merged 543 digestibility trials database or its high protein sub-set. Similarly, to animals under medium

diets, the model including DMI, CP and ME showed the highest prediction accuracy for the 544 prediction of FNO in the highest range of feed protein concentration. Differences in prediction 545 accuracy among the models developed from the two databases, were seen only in the case of 546 models using NI as the main predictors, favouring the specific equations developed by using 547 the high CP sub-set. Therefore, there is no need for diet-specific equations for the prediction 548 of FNO in animals at high CP diets when DMI, CP and ME are known but the prediction 549 550 accuracy will be favoured by diet-specific equations when ME is not available. The improvement in the prediction of MNO when using diet CP-specific equations in animals with 551 552 high diet CP concentration comes from the improvement in the prediction of FNO and UNO collectively, as the prediction both parts of N outputs is benefited. Finally, in contrast with 553 animals at low-CP diets, using BW as the main predictor, cannot predict FNO satisfactorily. 554

# 555 **5. Conclusions**

The equations developed in the current study using a large database explore the relationships 556 between N output in manure, urine and faeces from beef cattle with various dietary factors and 557 animal body weight. This study confirmed previous results that for a higher prediction accuracy 558 of nitrogen outputs, recording and using dietary nitrogen concentration is essential while 559 energy-related parameters (dietary starch, fibre, fat, metabolisable energy) can further improve 560 the accuracy of prediction models across the spectra of dieary protein concentrations. Diet 561 crude protein-specific equation improved prediction accuracy of nitrogen outputs in several 562 occasions across the spectra of diet protein concentration (84-217 g/kg dry matter) and this was 563 more pronounced in diets with crude contents over 143 g/kg dry matter; and in particular when 564 feed intake and diet nitrogen, and energy concetrations were available. However, the accurate 565 prediction of urine nitrogen outputs, remains a challenge and it is likely predictors that account 566 for protein utilisation (metabolisale protein, undegradable protein) could improve prediction 567 accuracy in future. 568

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573 **Abbreviations** 

ADF: Acid detergent fibre; AFBI: Agri-Food and Biosciences Institute; BW: animal body 574 weight; CEDAR: The Centre for Dairy Research; CP: Crude protein; DM: Dry matter; DMI: 575 Dry Matter intake; EE: Ether extract; FNO: Faecal nitrogen output; GE: Gross energy; GHG: 576 Green House Gases; ID: Identity; LinCCC: Lin's concordance correlation coefficient; ME: 577 Metabolisable energy; MNO: Manure nitrogen output; MPE: Mean prediction error; MSPE: 578 Mean squared prediction error; N: Nitrogen; N2O: Nitrous oxide; NDF: Neutral detergent 579 580 fibre; NH3: Ammonia; NI: Nitrogen intake; NUE: Nitrogen Use Efficiency; OM: Organic matter; RDP: Rumen-Degradable Protein; ST: Starch; TF: Total forage; UNO: Urinary 581 nitrogen output. 582

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#### **Figure captions**

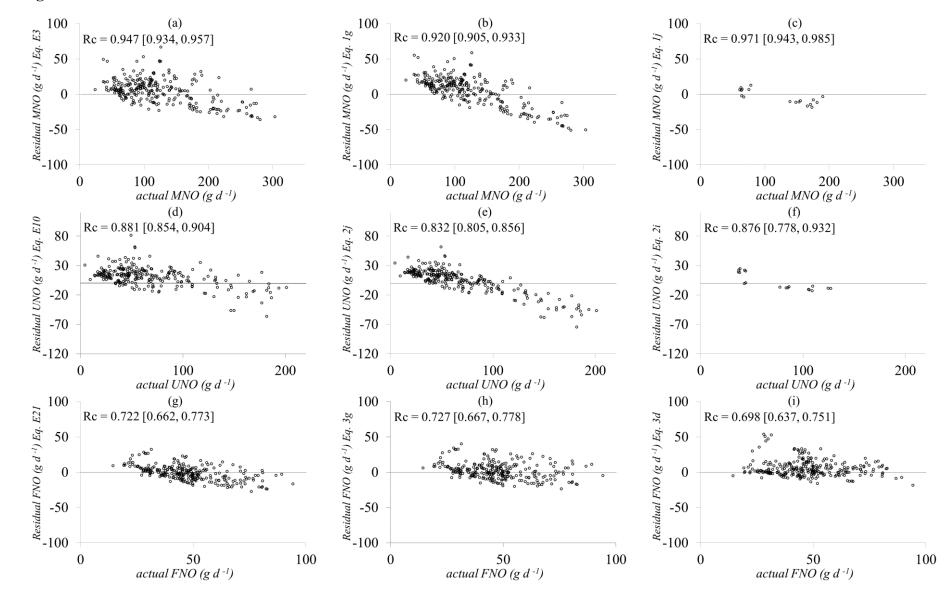
**Figure 1** Bland-Altman plots showing the agreement between actual manure nitrogen output (MNO), urine nitrogen output (UNO) and faeces nitrogen output (FNO) and predicted from equations shown by Yan *et al.* (2007), Hirooka (2010) and Dong *et al.* (2014) (panels a, d and g, respectively) or developed in the current study by using exactly the same variables (panels b, e and h, respectively) or newly introduced ones (panels c, f and i). In order to predict (i) MNO, (ii) UNO and (iii) FNO, the following were used as predictors: (i) nitrogen intake (NI) for panels a, b, d, e, g and h (ii) NI, metabolisable energy (ME), acid detergent fibre (ADF) and starch (ST) for panel c, (iii) dry matter intake (DMI), crude protein (CP), ADF, ST, ME for panel f and (iv) DMI, CP and forage proportion for panel i. Prediction equations are shown in Table A1 (for panels a, d and g), Table 2 (for panels b, c, e, f, h and i). Residual represents the difference between predicted minus actual value. Rc is Lin's concordance correlation coefficient with 95% confidence interval given in square brackets.

**Figure 2** Bland-Altman plots showing the agreement between actual manure nitrogen (N) output (MNO), urine N output (UNO) and faeces N output (FNO) and predicted from equations developed in the current study by using either the merged animal trials database (panels a, c and e, respectively) or the low crude protein sub-set (panels b, d and f). In order to predict (i) MNO, (ii) UNO and (iii) FNO, the following were used as predictors: (i) dry matter intake (DMI), crude protein (CP), acid detergent fibre and neutral detergent fibre for panels a and b, (ii) DMI and CP for panels c and d and (iii) bodyweight, CP and metabolisable energy for panels e and f. Prediction equations are shown in Table 2 (for panels a, c and e) and Table 3 (for panels b, d and f). Residual represents the difference between predicted minus actual value. Rc is Lin's concordance correlation coefficient with 95% confidence interval given in square brackets.

**Figure 3.** Bland-Altman plots showing the agreement between actual manure nitrogen (N) output (MNO), urine N output (UNO) and faeces N output (FNO) and predicted from equations developed in the current study by using either the merged animal trials database (panels a, c and e, respectively) or the medium crude protein sub-set (panels b, d and f). In order to predict (i) MNO, (ii) UNO and (iii) FNO, the following were used as predictors: (i) dry matter intake (DMI), crude protein (CP) and metabolisable energy (ME) for panels a and b, (ii) N intake (NI) for panels c and d and (iii) NI and ME for panels e and f. Prediction equations are shown in Table 2 (for panels a, c and e) and Table 4 (for panels b, d and f). Residual represents the difference between predicted minus actual value. Rc is Lin's concordance correlation coefficient with 95% confidence interval given in square brackets.

**Figure 4.** Bland-Altman plots showing the agreement between actual manure nitrogen (N) output (MNO), urine N output (UNO) and faeces N output (FNO) and predicted from equations developed in the current study by using either the the merged animal trials database (panels a, c and e, respectively) or the high crude protein sub-set (panels b, d and f). In order to predict (i) MNO, (ii) UNO and (iii) FNO, the following were used as predictors: (i<sub>a</sub>), N intake (NI), metabolisable energy (ME), acid detergent fibre (ADF) and starch for panel a, (i<sub>b</sub>) NI, ME and ADF for panel b, (ii) dry matter intake, crude protein and ME for panels c and d, (iii) NI and ME for panels e and f. Prediction equations are shown in Table 2 (for panels a, c and e) and Table 5 (for panels b, d and f). Residual represents the difference between predicted minus actual value. Rc is Lin's concordance correlation coefficient with 95% confidence interval given in square brackets.

Figure 1



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Figure 2

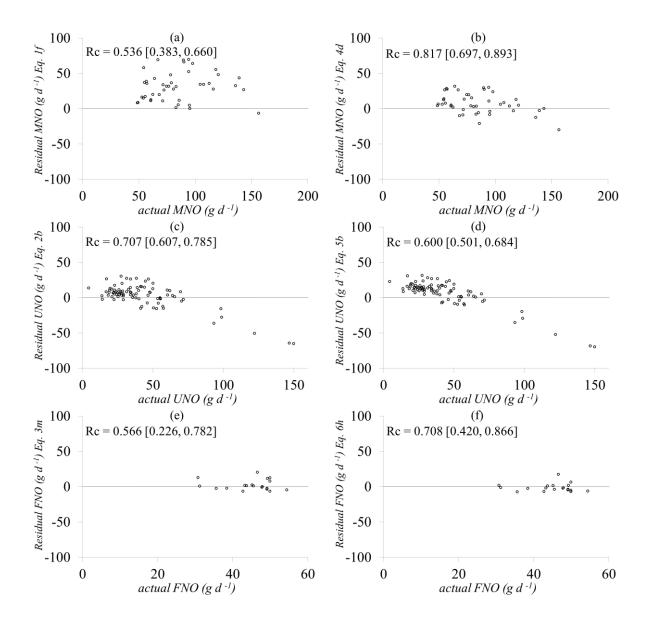


Figure 3

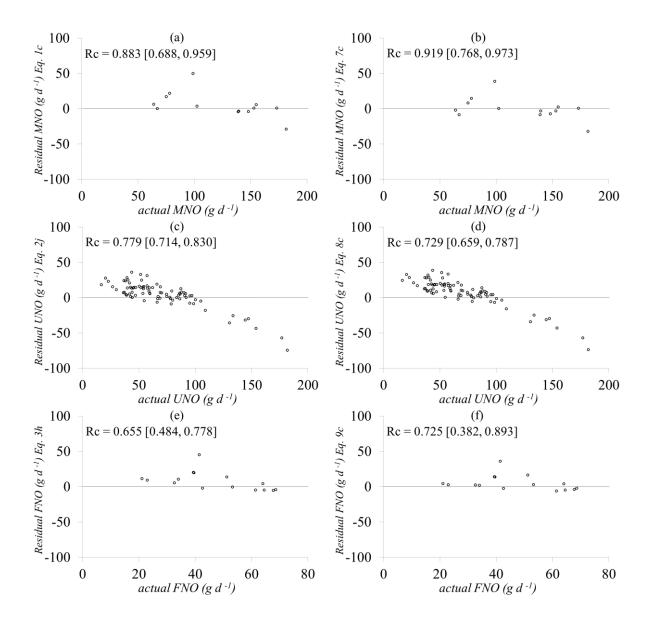
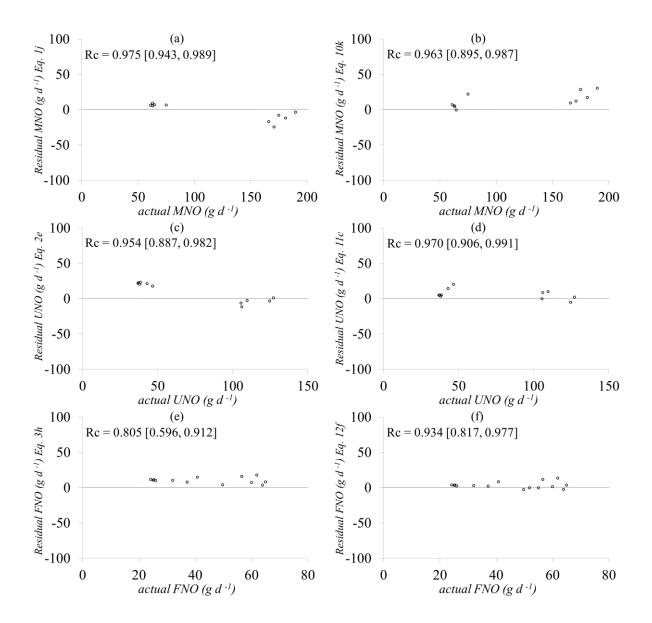


Figure 4



## Tables

| <b>Table 1</b> Description of the data derived from the merged digestibility trials database, used to predict N excretion, |
|--|
| including body weight, diet chemical composition and energy concentrations, nutrient and energy intakes and N              |
| outputs in manure, urine and faeces.   |

| Parameters assessed                       | Mean ±SD          | Min   | Max   | CV   | n   |
|---|-------------------|-------|-------|------|-----|
| Animal data                               |                   |       |       |      |     |
| Bodyweight (kg)                           | $379 \pm 105.9$   | 153   | 631   | 0.28 | 570 |
| Diet chemical composition (g/kg DM)       |                   |       |       |      |     |
| Total forage (g/100g DM)                  | $56.6 \pm 25.40$  | 20.00 | 100.0 | 0.45 | 570 |
| OM  | 940.5 ±21.88      | 862.6 | 971.9 | 0.02 | 284 |
| СР  | 153.6 ±24.67      | 84.90 | 217.3 | 0.16 | 570 |
| Ν   | 24.57 ±3.948      | 13.60 | 34.80 | 0.16 | 570 |
| EE  | 28.44 ±9.863      | 6.700 | 63.20 | 0.35 | 284 |
| NDF                                       | 298.6 ±93.20      | 174.6 | 655.4 | 0.31 | 284 |
| ADF                                       | 157.7 ±76.17      | 75.00 | 367.2 | 0.48 | 284 |
| Starch                                    | $427 \pm 158.8$   | 23.50 | 641.1 | 0.37 | 284 |
| Ash                                       | 59.5 ±21.87       | 28.10 | 137.4 | 0.37 | 284 |
| Diet energy concentration (MJ/kg DM)      |                   |       |       |      |     |
| GE  | $18.4 \pm 0.510$  | 17.00 | 19.70 | 0.03 | 284 |
| ME  | $11.5 \pm 1.190$  | 6.500 | 14.40 | 0.10 | 564 |
| Nutrient (kg/d) and energy (MJ/d) intakes |                   |       |       |      |     |
| DM intake                                 | $6.36 \pm 2.006$  | 2.090 | 11.30 | 0.32 | 570 |
| OM intake                                 | $5.58 \pm 1.902$  | 1.990 | 10.70 | 0.34 | 284 |
| CP intake                                 | $0.97 \pm 0.332$  | 0.270 | 1.970 | 0.34 | 570 |
| N intake (g/d)                            | 155.4 ±53.14      | 43.40 | 316.1 | 0.34 | 570 |
| NDF intake                                | 1.83 ±0.969       | 0.420 | 5.320 | 0.53 | 284 |
| ADF intake                                | $0.99 \pm 0.687$  | 0.190 | 3.480 | 0.69 | 284 |
| Starch intake                             | $2.42 \pm 1.174$  | 0.180 | 6.050 | 0.49 | 284 |
| GE intake                                 | 109.3 ±36.99      | 39.50 | 210.1 | 0.34 | 284 |
| ME intake                                 | 73.0 ±24.38       | 26.80 | 137.9 | 0.33 | 564 |
| Diet digestibility                        |                   |       |       |      |     |
| N apparent digestibility (g/kg)           | $677.5 \pm 66.52$ | 354.2 | 814.3 | 0.10 | 570 |
| Nitrogen output and retention (g/d)       |                   |       |       |      |     |
| Manure N output                           | 123.3 ±42.37      | 26.90 | 261.4 | 0.34 | 570 |
| Urine N output                            | 74.1 ±29.71       | 11.90 | 179.2 | 0.40 | 566 |
| Faeces N output                           | $49.8 \pm 18.09$  | 11.00 | 105.3 | 0.36 | 570 |

N = nitrogen; SD = standard deviation; Min = minimum value observed; Max = maximum value observed; CV = coefficient of variation; n = number of observations; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; GE = gross energy; ME = metabolisable energy.

|       |   | Equations <sup>a</sup>  | n   | $\mathbb{R}^2$ | MPE Eq.                  |
|-------|---|---|-----|----------------|--------------------------|
| MNO = | 22.28(7.364)                              | + 15.64 <sub>(0.742)</sub> DMI  | 570 | 0.92           | 0.460 (1a)               |
|       | $-92.42_{(8.632)}$                        | $+ \ 16.61_{(0.491)} \ DMI + 0.704_{(0.0317)} \ CP$   | 570 | 0.94           | 0.305 (1b)               |
|       | -42.60(10.382)                            | $+ \ 16.60_{(0.463)} \ DMI + 0.759_{(0.0294)} \ CP - 5.048_{(0.5811)} \ ME$   | 564 | 0.94           | 0.270 (1c)               |
|       | $-97.54_{(9.017)}$                        | $+\ 16.62_{(0.505)}\ DMI + 0.691_{(0.0322)}\ CP + 11.38_{(3.901)}\ TF$  | 570 | 0.94           | 0.303 (1d)               |
|       | $-94.90_{(12.508)}$                       | $+ \ 16.32_{(0.798)} \ DMI + 4.183_{(0.3640)} \ CP + 0.073_{(0.0245)} \ ADF$  | 284 | 0.93           | 0.336 (1e)               |
|       | $-108.4_{(13.37)}$                        | $+\ 18.28_{(0.872)}\ DMI + 0.642_{(0.0679)}\ CP + 0.068_{(0.0422)}\ ADF + 0.019_{(0.0271)}\ NDF$  | 278 | 0.96           | 0.297 (1f)               |
|       | $17.34_{(6.208)}$                         | + 0.673 <sub>(0.0167)</sub> NI  | 570 | 0.94           | 0.242 (1g)               |
|       | 9.299(6.9250)                             | $+\ 0.668_{(0.0171)}\ NI + 13.89_{(3.750)}\ TF$   | 570 | 0.94           | 0.241 (1h)               |
|       | 82.79(8.538)                              | $+\ 0.691_{(0.0148)}\ NI - 5.922_{(0.5297)}\ ME$  | 564 | 0.94           | 0.317 (1i)               |
|       | 60.60(19.802)                             | $+\ 0.697_{(0.0229)}\ NI - 6.564_{(1.1004)}\ ME + 0.074_{(0.0350)}\ ADF + 0.044_{(0.0166)}\ ST$   | 278 | 0.94           | 0.129 (1j)               |
|       | $19.71_{(8.253)}$                         | $+\ 0.709_{(0.0241)}\ NI - 0.250_{(0.1128)}\ EE$  | 284 | 0.94           | 0.262 (1k)               |
|       | 73.14(7.646)                              | + 0.134 <sub>(0.0166)</sub> BW  | 570 | 0.88           | 0.569 (11)               |
|       | $-33.50_{(10.633)}$                       | $+ 0.166_{(0.0146)} BW + 0.615_{(0.0516)} CP$   | 570 | 0.89           | 0.472(1m                 |
|       | 39.71(11.775)                             | $+ 0.187_{(0.0099)} BW + 0.782_{(0.0485)} CP - 9.203_{(0.9168)} ME$   | 564 | 0.89           | 0.456 (1n)               |
|       | $-57.33_{(11.096)}$                       | $+\ 0.169_{(0.0145)}\ BW + 0.593_{(0.0500)}\ CP + 42.16_{(6.824)}\ TF$  | 570 | 0.88           | 0.541 (10)               |
|       | 13.37(13.759)                             | $+\ 0.182_{(0.0118)}\ BW + 0.750_{(0.0490)}\ CP - 7.787_{(0.9782)}\ ME + 27.02_{(6.722)}\ TF$   | 564 | 0.88           | 0.484 (1p)               |
| UNO = | 25.40(7.484)                              | + 7.254 <sub>(0.6927)</sub> DMI   | 566 | 0.84           | 0.695 (2a)               |
|       | $-78.14_{(8.864)}$                        | $+ 8.287_{(0.4863)} DMI + 0.630_{(0.0305)} CP$  | 566 | 0.86           | 0.536 (2b)               |
|       | $-83.60_{(9.223)}$                        | $+ 8.222_{(0.5006)} DMI + 0.617_{(0.0308)} CP - 12.75_{(3.759)} TF$   | 566 | 0.86           | 0.555 (2c)               |
|       | $-86.54_{(12.136)}$                       | $+7.792_{(0.7806)}$ DMI $+3.976_{(0.3405)}$ CP $+0.079_{(0.0204)}$ ADF  | 280 | 0.86           | 0.489 (2d)               |
|       | $-29.13_{(15.673)}$                       | $+7.287_{(0.7887)}$ DMI $+4.258_{(0.3471)}$ CP $-4.246_{(0.9276)}$ ME   | 278 | 0.87           | 0.616 (2e)               |
|       | $-74.01_{(11.642)}$                       | $+\ 8.471_{(0.7485)}\ DMI + 4.145_{(0.3456)}\ CP - 0.313_{(0.1179)}\ EE$  | 280 | 0.86           | 0.504 (2f)               |
|       | $-83.48_{(11.819)}$                       | $+7.987_{(0.7702)}DMI + 4.207_{(0.3409)}CP + 0.076_{(0.0199)}ADF - 0.321_{(0.1160)}EE$  | 280 | 0.86           | 0.476 (2g)               |
|       |   | $+ 7.601_{(0.7515)} DMI + 4.216_{(0.3358)} CP + 0.154_{(0.0359)} ADF + 0.049_{(0.0188)} ST + 7.140_{(0.7707)} DMI + 4.437_{(0.3472)} CP + 0.104_{(0.0400)} ADF + 0.048_{(0.0188)} ST - 2.428$ |     |                | 0.443 (2h)               |
|       | 5 172                                     | 3.428 <sub>(1.1796)</sub> ME  |     |                | 0.289 (2i)               |
|       |   | $+ 0.426_{(0.0181)}$ NI   |     |                | 0.424 (2j)               |
|       |   | $+ 0.419_{(0.0181)}$ NI $+ 17.58_{(4.058)}$ TF  |     |                | 0.474 (2k)               |
|       |   | $+ 0.429_{(0.0180)}$ NI $- 1.582_{(0.6178)}$ ME   |     |                | 0.518 (21)               |
|       |   | $+ 0.431_{(0.0266)}$ NI $- 0.072_{(0.0203)}$ ADF  |     |                | 0.460 (2m)               |
|       |   | + 0.450 <sub>(0.0274)</sub> NI – 0.025 <sub>(0.0115)</sub> ST – 0.238 <sub>(0.1153)</sub> EE  |     |                | 0.381 (2n)               |
|       | 42.45(6.473)                              | $+ 0.080_{(0.0135)}$ BW   |     |                | 0.837 (20)               |
|       | -62.64 <sub>(7.981)</sub>                 | $+ 0.110_{(0.0113)}$ BW $+ 0.611_{(0.0357)}$ CP   |     |                | 0.714 (2p)               |
|       | -25.93(9.910)                             | $+ 0.117_{(0.0111)}$ BW $+ 0.668_{(0.0357)}$ CP $- 4.172_{(0.6957)}$ ME   |     |                | 0.769 (2q)               |
|       | $-75.82_{(8.316)}$<br>$-43.52_{(11.263)}$ | $+ 0.112_{(0.0112)} BW + 0.592_{(0.0351)} CP + 25.02_{(4.421)} TF + 0.115_{(0.0111)} BW + 0.643_{(0.0366)} CP - 3.154_{(0.7550)} ME + 16.70_{(4.737)} TF$                                     |     |                | 0.772 (2r)<br>0.799 (2s) |
|       |   |   |     |                |                          |
| FNO = |   | + 8.507 <sub>(0.2184)</sub> DMI   |     |                | 0.311 (3a)               |
|       |   | $+\ 8.614_{(0.2081)}\ DMI + 0.076_{(0.0132)}\ CP$   |     |                | 0.278 (3b)               |
|       | 13.71(3.511)                              | $+\ 8.405_{(0.1969)}\ DMI + 0.111_{(0.0125)}\ CP - 2.958_{(0.2451)}\ ME$  |     |                | 0.323 (3c)               |
|       | $-18.01_{(2.762)}$                        | $+\ 8.584_{(0.2076)}\ DMI + 0.073_{(0.0132)}\ CP - 3.645_{(1.6253)}\ TF$  |     |                | 0.273 (3d)               |
|       | 17.61(3.916)                              | $+\ 8.441_{(0.1971)}\ DMI + 0.116_{(0.0127)}\ CP - 3.828_{(1.6730)}\ TF - 3.185_{(0.2641)}\ ME$   |     |                | 0.321 (3e)               |
|       | 30.41(6.374)                              | $+\ 8.472_{(0.2254)}\ DMI + 0.760_{(0.1068)}\ CP - 0.033_{(0.0083)}\ ADF - 3.935_{(0.3932)}\ ME$  |     |                | 0.297 (3f)               |
|       | $10.44_{(1.979)}$                         | + 0.256 <sub>(0.0102)</sub> NI  |     |                | 0.280 (3g)               |
|       | 65.57 <sub>(3.588)</sub>                  | $+ 0.261_{(0.0086)} \text{ NI} - 4.824_{(0.2886)} \text{ ME}$   | 564 | 0.94           | 0.421 (3h)               |
|       |   |   |     |                |                          |

**Table 2** Single and multiple linear prediction of nitrogen excretion in manure, urine and faeces using (i) intakes of feed, nutrients, or body weight, diet chemical composition, energy concentrations, and forage proportion and (ii) data representing feed protein concentrations across the whole available crude protein range.

| $72.81_{(4.277)} + 0.267_{(0.0085)}  NI - 5.149_{(0.3057)}  ME - 6.976_{(2.2088)}  TF$   | 564 0.94 0.418 (3i) |
|--|---------------------|
| $71.41_{(7.645)} + 0.267_{(0.0108)} \text{ NI} - 4.908_{(0.4533)} \text{ ME} - 0.024_{(0.0111)} \text{ ADF}$   | 278 0.94 0.413 (3j) |
| $-10.96_{(12.067)} + 0.291_{(0.0108)} \text{ NI} - 4.711_{(0.3841)} \text{ ME} + 0.093_{(0.1153)} \text{ NDF} + 0.090_{(0.0122)} \text{ ST} + 0.202_{(0.0474)} \text{ EE}$ | 278 0.96 0.353 (3k) |
|  |                     |
| $31.80_{(2.511)} + 0.051_{(0.0053)} \mathrm{BW}$   | 570 0.87 0.417 (31) |
| $66.51_{(6.030)} + 0.063_{(0.0067)} BW + 0.117_{(0.0234)} CP - 4.955_{(0.4375)} ME$  | 564 0.88 0.418 (3m) |
| $54.09_{(9.503)} + 0.056_{(0.0059)}  BW + 1.606_{(0.2247)}  CP - 7.058_{(0.6138)}  ME + 0.038_{(0.0095)}  ST$  | 278 0.87 0.273 (3n) |
| $60.30_{(8.721)} + 0.047_{(0.0060)} \ BW + 1.380_{(0.2393)} \ CP - 5.812_{(0.5337)} \ ME + 0.178_{(0.0741)} \ EE$  | 278 0.86 0.361 (30) |
| $86.83_{(11.587)} + 0.055_{(0.0060)} BW + 1.320_{(0.2348)} CP - 6.845_{(0.5973)} ME - 0.039_{(0.0105)} NDF$  | 278 0.86 0.406 (3p) |

n = number of observations;  $R^2 =$  pseudo correlation coefficient; MPE = mean prediction error; Eq. = equation; MNO = manure nitrogen output; DMI = dry matter intake; CP = diet crude protein concentration; ME = diet metabolisable energy concentration; TF = diet total forage; ADF = diet acid-detergent fibre concentration; NDF = diet neutral-detergent fibre concentration; NI = nitrogen intake; ST = diet starch concentration; EE = diet ether extract concentration; BW = body weight; UNO = urine nitrogen output; FNO = faeces nitrogen output

<sup>a</sup> Units: g/d for MNO, UNO, FNO, NI; kg/d for DMI; g/kg DM for CP, ADF, NDF, ST, EE; MJ/kg DM for ME; g/100g DM for TF. The effect of all explanatory variables was significant according to the Wald statistic (Fpr < 0.05). The random effects of the individual experiment, animal, treatment and growth stage were accounted for all predicted variables. The random factors were chosen according to changes in deviance during the development of the random model. <sup>b</sup> MPE derived from an external validation (details presented in Table A2).

|       |                          | Equations <sup>a</sup>   | n   | $\mathbb{R}^2$ | MPE <sub>LL</sub> <sup>b</sup> | MPE <sub>AL</sub> <sup>b</sup> | Eq.          |
|-------|--------------------------|--|-----|----------------|--------------------------------|--------------------------------|--------------|
| MNO = | 10.05(6.191)             | + 15.03 <sub>(0.850)</sub> DMI   | 190 | 0.93           | 0.389                          | 0.540                          | (4a)         |
|       | $-51.06_{(14.100)}$      | $+\; 14.97_{(0.764)} \; DMI + 0.477_{(0.1028)}  CP$  | 190 | 0.93           | 0.282                          | 0.244                          | (4b)         |
|       | -30.02(12.917)           | $+\ 14.38_{(0.661)}\ DMI + 0.748_{(0.0919)}\ CP - 4.543_{(0.9507)}\ ME$                      | 188 | 0.94           | 0.398                          | 0.396                          | (4c)         |
|       | $-90.46_{(22.516)}$      | $+ 12.71_{(0.711)} DMI + 0.748_{(0.1596)} CP - 0.145_{(0.0586)} ADF \\ 0.125_{(0.0419)} NDF$ |     | 0.90           | 0.266                          | 0.472                          | (4d)         |
|       | $13.87_{(4.703)}$        | $+ 0.699_{(0.0316)}  NI$   | 190 | 0.94           | 0.232                          | 0.237                          | (4e)         |
|       | $72.34_{(10.128)}$       | $+\ 0.691_{(0.0278)}NI - 5.004_{(0.8378)}ME$   | 188 | 0.94           | 0.404                          | 0.429                          | (4f)         |
|       | 57.96(7.783)             | $+ 0.131_{(0.0162)} BW$  | 190 | 0.88           | 0.416                          | 0.526                          | (4g)         |
|       | $-6.121_{(12.9010)}$     | $+ 0.138_{(0.0160)}BW + 0.475_{(0.1387)}CP$  | 190 | 0.88           | 0.351                          | 0.341                          | (4h)         |
|       | 19.19(18.433)            | $+\ 0.150_{(0.0139)}  BW + 1.014_{(0.1348)}  CP - 8.773_{(1.3907)}  ME$                      | 188 | 0.91           | 0.470                          | 0.468                          | (4i)         |
| UNO = | $17.11_{(0.881)}$        | + 6.412 <sub>(0.7810)</sub> DMI  | 188 | 0.88           | 0.782                          | 0.991                          | (5a)         |
|       | $-48.87_{(12.530)}$      | $+ 6.428_{(0.7109)} DMI + 0.512_{(0.0907)} CP$   | 188 | 0.87           | 0.628                          | 0.594                          | (5b)         |
|       | $14.14_{(4.792)}$        | + 0.332 <sub>(0.0313)</sub> NI   | 188 | 0.88           | 0.637                          | 0.642                          | (5c)         |
|       | 27.69(5.087)             | + 0.080 <sub>(0.1044)</sub> BW   | 188 | 0.90           | 0.788                          | 0.973                          | (5d)         |
|       | $-48.08_{(12.746)}$      | $+ 0.089_{(0.0101)} BW + 0.560_{(0.0905)} CP$  | 188 | 0.89           | 0.682                          | 0.673                          | (5e)         |
|       | $-27.85_{(13.365)}$      | $+\ 0.098_{(0.0097)}\ BW + 0.772_{(0.0960)}\ CP - 4.499_{(0.9617)}\ ME$                      | 188 | 0.90           | 0.820                          | 0.793                          | (5f)         |
|       | $-66.42_{(13.700)}$      | $+\ 0.091_{(0.0010)}  BW + 0.599_{(0.0890)}  CP + 19.66_{(6.659)}  TF$                       | 188 | 0.88           | 0.772                          | 0.790                          | (5g)         |
| FNO = | -4.043(2.1234)           | + 8.258 <sub>(0.2981)</sub> DMI  | 190 | 0.94           | 0.364                          | 0.386                          | (6a)         |
|       | 11.44(5.545)             | $+7.993_{(0.2827)}$ DMI $+0.117_{(0.0392)}$ CP $-2.536_{(0.0109)}$ ME                        | 188 | 0.96           | 0.340                          | 0.354                          | (6b)         |
|       | 18.83(6.091)             | $+ 8.047_{(0.2758)} DMI + 0.1223_{(0.0387)} CP - 2.899_{(0.4210)} ME \\ 6.828_{(2.5161)} TF$ |     | 0.96           | 0.354                          | 0.354                          | (6c)         |
|       | 3 327 (2 5177)           | $+ 0.348_{(0.0166)}$ NI  |     | 0.96           |                                | 0.333                          | (6d)         |
|       | 50.86 <sub>(4.945)</sub> | $+ 0.340_{(0.0143)}$ NI $- 4.104_{(0.4157)}$ ME  |     |                | 0.375                          | 0.295                          | (6e)         |
|       | 57.99 <sub>(6.004)</sub> | $+ 0.343_{(0.0139)}$ NI $- 4.384_{(0.4386)}$ ME $- 6.705_{(2.9635)}$ TF                      |     | 0.95           |                                | 0.275                          | (6c)         |
|       | 33.20 <sub>(4.182)</sub> | $+ 0.044_{(0.0088)} BW$  |     |                | 0.341                          | 0.347                          | (6g)         |
|       |                          | $+ 0.053_{(0.0084)}$ BW $+ 0.290_{(0.0791)}$ CP $- 4.397_{(0.8380)}$ ME                      |     |                | 0.215                          | 0.263                          | (6g)<br>(6h) |

**Table 3** Single and multiple linear prediction of nitrogen excretion in manure, urine and faeces using intakes of feed, nutrient and energy, or body weight, diet chemical composition, energy concentrations for age proportion and (ii) data representing low feed protein concentrations.

n = number of observations;  $R^2$  = pseudo correlation coefficient;  $MPE_{LL}$  = mean prediction error derived from the validation of the above equations by using the low CP sub-set of the literature database;  $MPE_{AL}$  = mean prediction error derived from the validation of the identical Table 2 equations by using the low CP sub-set of the literature database; Eq. = equation; MNO = manure nitrogen output; DMI = dry matter intake; CP = diet crude protein concentration; ME = diet metabolisable energy concentration; ADF = diet acid-detergent fibre concentration; NDF = diet neutral-detergent fibre concentration; NI = nitrogen intake; BW = body weight; UNO = urine nitrogen output; TF = diet total forage; FNO = faeces nitrogen output <sup>a</sup> Units: g/d for MNO, UNO, FNO; kg/d for DMI; g/kg DM for CP, ADF, NDF; MJ/kg DM for ME; g/d for NI; g/100g DM for TF. The effect of all explanatory variables was significant according to the Wald statistic (Fpr < 0.05). The random effects of the individual experiment, animal and treatment were accounted for all predicted variables. The random factors were chosen according to changes in deviance during the development of the random model.

<sup>b</sup> MPE<sub>LL</sub> and MPE<sub>AL</sub> derived from an external validation (details presented in Table A3 and Table A4, respectively).

|       | uigestibility a               | nd (11) data representing medium feed protein concentrations.            |     |                |                                |                                |      |
|-------|-------------------------------|--|-----|----------------|--------------------------------|--------------------------------|------|
|       |                               | Equations <sup>a</sup>   | n   | $\mathbb{R}^2$ | MPE <sub>MM</sub> <sup>b</sup> | MPE <sub>AM</sub> <sup>b</sup> | Eq.  |
| MNO = | 9.452(5.7980)                 | $+ 17.96_{(0.803)} DMI$  | 190 | 0.94           | 0.338                          | 0.324                          | (7a) |
|       | $-205.9_{\left(39.14\right)}$ | $+\ 18.43_{(0.723)}\ DMI + 1.376_{(0.2470)}\ CP$                         | 190 | 0.93           | 0.245                          | 0.264                          | (7b) |
|       | $-151.7_{(38.85)}$            | $+\ 18.11_{(0.662)}\ DMI + 1.325_{(0.2281)}\ CP - 3.717_{(1.1480)}\ ME$  | 189 | 0.94           | 0.197                          | 0.227                          | (7c) |
|       | 5.472(5.3840)                 | + 0.753 <sub>(0.0304)</sub> NI   | 190 | 0.94           | 0.229                          | 0.239                          | (7d) |
|       | 51.28(15.045)                 | $+\ 0.739_{(0.0280)}\ NI - 3.662_{(1.1648)}\ ME$                         | 189 | 0.95           | 0.287                          | 0.322                          | (7e) |
|       | 98.17 <sub>(21.770)</sub>     | $+\ 0.643_{(0.0390)}\ NI - 9.935_{(1.9633)}\ ME + 0.094_{(0.0223)}\ ST$  | 80  | 0.93           | 0.272                          | NA                             | (7f) |
|       | 42.00(9.248)                  | $+ 0.219_{(0.0208)}  BW$   | 190 | 0.81           | 0.445                          | 0.443                          | (7g) |
| UNO = | $17.61_{(0.881)}$             | + 3.126 <sub>(0.7810)</sub> DMI  | 190 | 0.94           | 0.639                          | 0.455                          | (8a) |
|       | $-191.0_{(39.44)}$            | $+ 9.682_{(0.7419)} DMI + 1.328_{(0.2487)} CP$                           | 189 | 0.85           | 0.392                          | 0.391                          | (8b) |
|       | 13.60(5.681)                  | $+ 0.395_{(0.0319)} NI$  | 189 | 0.86           | 0.384                          | 0.364                          | (8c) |
|       | $-48.47_{(29.944)}$           | $+\ 0.359_{(0.0584)}  NI + 0.171_{(0.0795)}  ADF + 0.095_{(0.0405)}  ST$ | 80  | 0.76           | 0.230                          | NA                             | (8d) |
|       | 21.07(6.468)                  | $+ 0.142_{(0.0149)}  BW$   | 189 | 0.77           | 0.531                          | 0.541                          | (8e) |
|       | $-72.39_{(42.859)}$           | $+\ 0.140_{(0.0149)}  BW + 0.612_{(0.2795)}  CP$                         | 189 | 0.76           | 0.521                          | 0.530                          | (8f) |
| FNO = | $-7.151_{(2.2516)}$           | + 8.720 <sub>(0.3120)</sub> DMI  | 190 | 0.96           | 0.446                          | 0.459                          | (9a) |
|       |                               | + 0.356(0.0129) NI   | 190 | 0.96           | 0.407                          | 0.393                          | (9b) |
|       | 26.22(6.932)                  | $+ 0.350_{(0.0134)} \text{ NI} - 2.761_{(0.5293)} \text{ ME}$            | 189 | 0.96           | 0.328                          | 0.424                          | (9c) |
|       | 24.55(4.787)                  | $+ 0.068_{(0.0104)}  BW$   | 190 | 0.88           | 0.445                          | 0.435                          | (9d) |
|       | 179.0(27.65)                  | $+ 0.066_{(0.0098)} BW - 0.589_{(0.1694)} CP - 5.291_{(0.8655)} ME$      | 189 | 0.85           | 0.451                          | 0.394                          | (9e) |

**Table 4** Single and multiple linear prediction of nitrogen excretion in manure, urine and faeces using (i) intakes of feed, nutrient and energy, or body weight, diet chemical composition, energy concentrations forage proportion, and apparent total tract digestibility and (ii) data representing medium feed protein concentrations.

n = number of observations;  $R^2$  = pseudo correlation coefficient; MPE<sub>MM</sub> = mean prediction error derived from the validation of the above equations by using the medium CP sub-set of the literature database; MPE<sub>AM</sub> = mean prediction error derived from the validation of the identical Table 2 equations by using the medium CP sub-set of the literature database; Eq. = equation; MNO = manure nitrogen output; DMI = dry matter intake; CP = diet crude protein concentration; ME = diet metabolisable energy concentration; NA = Not applicable (no equations were developed with the exact same predictors from the merged database); NI = nitrogen intake; ST = diet starch concentration; BW = body weight; ADF = diet acid-detergent fibre concentration; UNO = urine nitrogen output; FNO = faeces nitrogen output

<sup>a</sup> Units: g/d for MNO, UNO, FNO; kg/d for DMI; g/kg DM for CP, ST, ADF; MJ/kg DM for ME; g/d for NI. The effect of all explanatory variables was significant according to the Wald statistic (Fpr < 0.05). The random effects of the individual experiment, animal and treatment were accounted for all predicted variables. The random factors were chosen according to changes in deviance during the development of the random model.

<sup>b</sup> MPE<sub>MM</sub> and MPE<sub>AM</sub> derived from an external validation (details presented in Table A3 and Table A4, respectively).

| total tract digestibility | , and (ii) data representing high feed protein concentrations.  |     |                |                                |                                |       |
|---------------------------|---|-----|----------------|--------------------------------|--------------------------------|-------|
|                           | Equations <sup>a</sup>  | n   | $\mathbb{R}^2$ | MPE <sub>HH</sub> <sup>b</sup> | MPE <sub>AH</sub> <sup>b</sup> | Eq.   |
| $MNO = 1.377_{(6.3161)}$  | + 22.42 <sub>(1.012)</sub> DMI  | 190 | 0.90           | 0.356                          | 0.389                          | (10a) |
| $-128.9_{(18.73)}$        | $+ 22.84_{(0.936)} DMI + 0.702_{(0.0992)} CP$   | 190 | 0.91           | 0.282                          | 0.315                          | (10b) |
| $-126.6_{(18.90)}$        | $+\ 23.01_{(0.971)}\ DMI + 0.621_{(0.1055)}\ CP + 18.44_{(7.459)}\ TF$                                  | 190 | 0.92           | 0.282                          | 0.320                          | (10c) |
| $-14.13_{(22.721)}$       | $\begin{array}{llllllllllllllllllllllllllllllllllll$  |     | 0.94           | 0.138                          | 0.120 <sup>c</sup>             | (10d) |
| $-34.26_{(21.375)}$       | $+\ 22.07_{(0.845)}\ DMI + 0.673_{(0.0857)}\ CP - 7.424_{(1.1252)}\ ME$                                 | 187 | 0.94           | 0.114                          | 0.130                          | (10e) |
| 3.775(36.6646)            | $_{0} + 22.07_{(1.278)} DMI + 0.671_{(0.1380)} CP - 0.457_{(0.1627)} EE \\ 9.172_{(1.6465)} ME$         |     | 0.95           | 0.156                          | 0.128 <sup>c</sup>             | (10f) |
| $0.970_{(5.5646)}$        | + 0.772 <sub>(0.0303)</sub> NI  | 190 | 0.91           | 0.201                          | 0.226                          | (10g) |
| 103.6(13.85)              | $+\ 0.754_{(0.0263)}  NI - 8.698_{(1.0859)}  ME$  | 187 | 0.94           | 0.113                          | 0.129                          | (10h) |
| 136.1(19.18)              | $+\ 0.759_{(0.0253)}\ NI - 10.62_{(1.375)}\ ME - 18.37_{(7.271)}\ TF$                                   | 187 | 0.94           | 0.142                          | 0.132 <sup>c</sup>             | (10i) |
| $-10.86_{(6.643)}$        | $+ 0.772_{(0.0318)}  NI + 18.99_{(7.071)}  TF$  | 190 | 0.92           | 0.199                          | 0.230                          | (10j) |
| 168.8(30.38)              | $+\ 0.816_{(0.0463)}\ NI - 0.127_{(0.0446)}\ ADF - 13.08_{(2.042)}\ ME$                                 | 111 | 0.95           | 0.132                          | 0.350 <sup>c</sup>             | (10k) |
| 51.72(7.424)              | + 0.249 <sub>(0.0190)</sub> BW  | 190 | 0.94           | 0.490                          | 0.554                          | (101) |
| $-35.94_{(25.843)}$       | $+\ 0.248_{(0.0187)}  BW + 0.483_{(0.1374)}  CP$  | 190 | 0.93           | 0.461                          | 0.497                          | (10m) |
| 123.9(28.36)              | $+\ 0.236_{(0.0172)}\ BW + 0.626_{(0.1207)}\ CP - 16.00_{(1.639)}\ ME$                                  | 187 | 0.92           | 0.241                          | 0.253                          | (10n) |
| 84.6(32.48)               | $\begin{array}{llllllllllllllllllllllllllllllllllll$  |     | 0.92           | 0.278                          | 0.310                          | (100) |
| $-175.2_{(40.38)}$        | $+\ 0.266_{(0.0281)}\ BW + 0.937_{(0.2251)}\ CP + 0.292_{(0.0537)}\ ADF$                                | 114 | 0.87           | 0.384                          | 0.416                          | (10p) |
| UNO = $2.814_{(5.758)}$   | + 13.87 <sub>(0.920)</sub> DMI  | 189 | 0.82           | 0.558                          | 0.605                          | (11a) |
| $-94.09_{(17.423)}$       | $+ \ 14.11_{(0.884)} \ DMI + 0.524_{(0.0920)} \ CP$   | 189 | 0.84           | 0.448                          | 0.497                          | (11b) |
| $-47.44_{(22.503)}$       | $+\ 13.75_{(0.886)}\ DMI + 0.523_{(0.0902)}\ CP - 3.889_{(1.1880)}\ ME$                                 | 187 | 0.86           | 0.140                          | 0.154                          | (11c) |
| 0.343(5.2566)             | $+ 0.490_{(0.0284)}  \mathrm{NI}$   | 189 | 0.85           | 0.328                          | 0.352                          | (11d) |
| 54.87(14.693)             | $+\ 0.482_{(0.0278)}  NI - 4.651_{(1.1608)}  ME$  | 187 | 0.86           | 0.155                          | 0.162                          | (11e) |
| $79.41_{(30.540)}$        | $+\ 0.569_{(0.0514)}  NI - 5.870_{(1.8754)}  ME - 0.075_{(0.0361)}  NDF$                                | 111 | 0.86           | 0.250                          | 0.135°                         | (11f) |
| 93.75(29.511)             | $+$ 0.572 $_{(0.0493)}$ NI $-$ 5.819 $_{(1.8253)}$ ME $-$ 0.074 $_{(0.0343)}$ NDF 0.528 $_{(0.1712)}EE$ |     | 0.87           | 0.248                          | 0.198 <sup>c</sup>             | (11g) |
| 25.75(5.350)              | $+ 0.180_{(0.0142)}  BW$  | 189 | 0.90           | 0.646                          | 0.707                          | (11h) |
| $-51.17_{(18.785)}$       | $+\ 0.178_{(0.0137)}  BW + 0.426_{(0.0999)}  CP$  | 189 | 0.89           | 0.554                          | 0.562                          | (11i) |
| 37.89(22.266)             | $+\ 0.183_{(0.0131)}\ BW + 0.468_{(0.0935)}\ CP - 8.656_{(1.2762)}\ ME$                                 | 187 | 0.88           | 0.204                          | 0.248                          | (11j) |
| 16.16(25.076)             | $\begin{array}{llllllllllllllllllllllllllllllllllll$  |     | 0.89           | 0.224                          | 0.314                          | (11k) |
| $FNO = -0.490_{(2.0148)}$ | + 8.445 <sub>(0.3215)</sub> DMI   | 190 | 0.96           | 0.298                          | 0.281                          | (12a) |
| $-26.35_{(6.378)}$        | $+\ 8.481_{(0.2991)}\ DMI + 0.141_{(0.0331)}\ CP$   | 190 | 0.96           | 0.276                          | 0.280                          | (12b) |
| 10.03(7.196)              | $+\ 8.499_{(0.2599)}\ DMI + 0.138_{(0.0290)}\ CP - 3.163_{(0.3949)}\ ME$                                | 187 | 0.97           | 0.135                          | 0.144                          | (12c) |
| $-21.69_{(6.056)}$        | $+\ 8.499_{(0.2613)}\ DMI + 0.082_{(0.0343)}\ CP + 9.501_{(2.1859)}\ TF$                                | 190 | 0.95           | 0.256                          | 0.264                          | (12d) |
| 1.578(1.9637)             | + 0.277 <sub>(0.0107)</sub> NI  | 190 | 0.94           | 0.256                          | 0.284                          | (12e) |
| 47.95(5.424)              | $+\ 0.272_{(0.0102)}\ NI - 3.987_{(0.4355)}\ ME$  | 187 | 0.96           | 0.152                          | 0.233                          | (12f) |
| 64.00(7.486)              | $+\ 0.276_{(0.0100)}\ NI - 4.974_{(0.5358)}\ ME - 8.793_{(2.8886)}\ TF$                                 | 187 | 0.96           | 0.189                          | 0.271                          | (12g) |
| 28.63(3.313)              | $+ 0.062_{(0.0082)}  BW$  | 190 | 0.92           | 0.382                          | 0.390                          | (12h) |
| 81.15(12.341)             | $+\ 0.053_{(0.0077)}\ BW + 0.171_{(0.0528)}\ CP - 7.135_{(0.6852)}\ ME$                                 | 187 | 0.88           | 0.348                          | 0.360                          | (12i) |

**Table 5** Single and multiple linear prediction of nitrogen excretion in manure, urine and faeces using intakes of feed, nutrient and energy, or body weight, diet chemical composition, energy concentrations and forage proportion, and apparent total tract digestibility, and (ii) data representing high feed protein concentrations.

 $\overline{n = number of observations}; R^2 = pseudo correlation coefficient; MPE_{HH} = mean prediction error derived from the validation of the above equations by using the highCP sub-set of the literature database; MPE_{AH} = mean prediction error derived from the validation of the identical Table 2 equations by using the high CP sub-set of the literature database; Eq. = equation; MNO = manure nitrogen output; DMI = dry matter intake; CP = diet crude protein concentration; TF = diet total forage; ME = diet metabolisable energy concentration; EE = diet ether extract concentration; NI = nitrogen intake; ADF = diet acid-detergent fibre concentration; BW = body weight; UNO = urine nitrogen output; FNO = faeces nitrogen output$ 

<sup>a</sup> Units: g/d for MNO, UNO, FNO; kg/d for DMI; g/kg DM for CP, ADF, NDF; g/100g DM for TF; MJ/kg DM for ME, EE; g/d for NI. The effect of all explanatory variables was significant according to the Wald statistic (Fpr < 0.05). The random effects of the individual experiment, animal and treatment were accounted for all predicted variables. The random factors were chosen according to changes in deviance during the development of the random model.

<sup>b</sup> MPE<sub>HH</sub> and MPE<sub>AH</sub> derived from an external validation (details presented in Table A3 and Table A4, respectively).

<sup>c</sup> Equations developed from the merged digestibility trials database to mimic 10d, 10f, 10i and 10k for the prediction of MNO and 11f and 11g for the prediction of UNO, have one or more predictors that was/were not significant according to the Wald statistic.

## Appendix

## Summary of the data used

A list of the mean, standard deviation, minimum and maximum observed values, coefficient of variation, number of observations for bodyweight, diet forage proportion, chemical composition and energy concentrations, nutrient and energy intakes, diet digestibility parameters, N outputs and NUE parameters is shown in Table 1. A high level of variation was observed among the variables used for the development of the prediction models. For example, the minimum and maximum bodyweight values differed by 478 kg, and the forage proportion in the diet ranged between 20% and 100% of the total DM. Maximum observed values regarding diet composition were up to 26 times higher (for ST) than minimum values, with maximum values of NDF, ADF and EE being more than 4, 5 and 9 times higher than their minimum values, respectively. Maximum ME concentration was 2.2 times higher than the minimum value. Highest intake values for DM and N were more than 5 and 7 times higher than their respective lower values, while maximum intakes of both GE and ME were nearly 5 times higher, compared to the lowest ones. The difference observed when comparing the highest and the lowest values for N output was 234.5, 167.3 and 94.3 g/d, for MNO, UNO and FNO respectively.

|     | Equations <sup>a</sup>                          | MPE <sup>b</sup> Eq. <sup>c</sup> |
|-----|---|-----------------------------------|
| MNO | =6.91 + 0.759 NI                                | 0.230 (E1)                        |
|     | 13.8 + 0.698 NI                                 | 0.235 (E2)                        |
|     | 0.775 NI  | 0.214 (E3)                        |
|     | 8.6 + 1.385 MBW                                 | 0.559 (E4)                        |
|     | -24.7 + 0.609 NI + 0.599 MBW                    | 0.260 (E5)                        |
|     | 15 + (0.55 + 0.032 NI/DMI) MBW                  | 0.455 (E6)                        |
|     | 26.4 + (0.071 + 0.523 NI/MEI) MBW               | 0.473 (E7)                        |
|     | -25.8 + 0.595 MBW + (0.579 + 0.058 FP) NI       | 0.261 (E8)                        |
|     | 11.50 + 0.65 NI – 4.47 ME + 1.77 CP + 0.432 MBW | 0.271 (E9)                        |
| UNO | =0.23 NI <sup>1.15</sup>                        | 0.330 (E10)                       |
|     | 6.8 + 0.405 NI                                  | 0.443 (E11)                       |
|     | -21.18 + 0.56 NI                                | 0.343 (E12)                       |
|     | -14.12 + 0.51 NI                                | 0.380 (E13)                       |
|     | -21.52 + 5.91 CP                                | 0.910 (E14)                       |
|     | -22 + 6.04 CP                                   | 0.907 (E15)                       |
|     | -3.93 + 0.62 NI - 3.72 DMI                      | 0.384 (E16)                       |
|     | -71.2 + 0.265 NI + 3.76 CP + 0.468 MBW          | 0.489 (E17)                       |
| FNO | =4.91 DMI <sup>1.21</sup>                       | 0.715 (E18)                       |
|     | 0.506 + 0.352 NI                                | 0.562 (E19)                       |
|     | 24.28 + 0.154 NI                                | 0.306 (E20)                       |
|     | 15.82 + 0.2 NI                                  | 0.286 (E21)                       |
|     | 30.91 + 1.165 CP                                | 0.508 (E22)                       |
|     | 19.68 + 1.81 CP                                 | 0.525 (E23)                       |

**Table A1** External equations validation using the literature database: Prediction of manure, urine, and faeces nitrogen output as presented by other authors.

MPE = mean prediction error; Eq. = equation; MNO = manure nitrogen output; NI = nitrogen intake; MBW = metabolic body weight (body weight<sup>0.75</sup>); DMI = dry matter intake; MEI = metabolisable energy intake; FP = diet forage proportion; ME = metabolisable energy; CP = diet crude protein concentration.

<sup>a</sup> Units: g/d for NI; kg for MBW; kg/d for DMI; MJ/d for MEI; kg/kg DM for FP; MJ/kg DM for ME; g/100g DM for CP.

<sup>b</sup> MPE derived from a validation against the literature database that was used to validate new equations developed in the current study.

<sup>c</sup> References: E1, E9, E11, E17, E19, (Reed *et al.*, 2015); E2 - E8, (Yan *et al.*, 2007); E10, E18, (Hirooka, 2010); E12, E14, E16, E20, E22, (Waldrip *et al.*, 2013); E13, E15, E21, E23, (Dong *et al.*, 2014).

|                   |              |        |       |       |       |      |       | Pre | dicted | – Actual |       |
|-------------------|--------------|--------|-------|-------|-------|------|-------|-----|--------|----------|-------|
| Eq. <sup>a</sup>  | Predicted    | Actual | $r^2$ | MPE   | SE    | Rc   | Mea   | n   | SD     | Min      | Max   |
| Manure nitrogen   | output (g/d) |        |       |       |       |      |       |     |        |          |       |
| (1a)              | 135.1        | 121.5  | 0.64  | 0.460 | 20.71 | 0.67 | 14.   | 18  | 37.19  | -133.8   | 93.55 |
| (1b)              | 125.5        | 121.5  | 0.84  | 0.305 | 18.94 | 0.88 | 6.13  | 35  | 24.82  | -73.92   | 96.54 |
| (1c)              | 120.0        | 121.5  | 0.84  | 0.270 | 13.80 | 0.86 | 11.9  | 98  | 17.86  | -35.07   | 49.89 |
| (1d)              | 122.9        | 121.5  | 0.84  | 0.303 | 17.96 | 0.88 | 3.14  | 47  | 25.03  | -75.93   | 95.74 |
| (1e)              | 129.7        | 121.5  | 0.79  | 0.336 | 19.84 | 0.84 | 12.   | 56  | 25.26  | -71.69   | 109.1 |
| (1f)              | 139.9        | 121.5  | 0.74  | 0.297 | 23.10 | 0.77 | 22.0  | )6  | 27.34  | -63.90   | 125.9 |
| (1g)              | 125.1        | 121.5  | 0.92  | 0.242 | 13.07 | 0.92 | 5.8   | 10  | 20.15  | -50.79   | 58.82 |
| (1h)              | 121.8        | 121.5  | 0.92  | 0.241 | 12.52 | 0.92 | 2.08  | 81  | 20.77  | -57.44   | 55.50 |
| (1i)              | 122.7        | 121.5  | 0.78  | 0.317 | 17.04 | 0.81 | 14.4  | 45  | 20.65  | -21.75   | 82.54 |
| (1j)              | 116.9        | 121.5  | 0.98  | 0.129 | 6.405 | 0.97 | -4.5  | 36  | 10.64  | -24.42   | 12.72 |
| (1k)              | 135.6        | 121.5  | 0.91  | 0.262 | 15.24 | 0.92 | 5.44  | 46  | 22.52  | -53.28   | 55.20 |
| (11)              | 121.8        | 121.5  | 0.41  | 0.569 | 12.66 | 0.33 | 0.19  | 94  | 50.72  | -177.9   | 105.8 |
| (1m)              | 111.3        | 121.5  | 0.63  | 0.472 | 18.62 | 0.63 | -8.8  | 59  | 40.05  | -133.5   | 95.72 |
| (1n)              | 100.6        | 121.5  | 0.42  | 0.456 | 19.38 | 0.59 | -0.68 | 80  | 29.43  | -72.64   | 48.44 |
| (10)              | 101.8        | 121.5  | 0.51  | 0.541 | 20.34 | 0.52 | -19.2 | 23  | 43.82  | -151.0   | 58.99 |
| (1p)              | 97.22        | 121.5  | 0.35  | 0.484 | 22.76 | 0.56 | -3.64 | 48  | 31.42  | -75.41   | 51.99 |
| (E1)              | 128.5        | 121.5  | 0.92  | 0.230 | 20.34 | 0.93 | 9.5   | 12  | 17.68  | -34.01   | 69.90 |
| (E2)              | 125.6        | 121.5  | 0.92  | 0.235 | 13.56 | 0.92 | 6.42  | 25  | 19.27  | -46.33   | 61.60 |
| (E3)              | 124.1        | 121.5  | 0.92  | 0.214 | 15.06 | 0.95 | 5.2   | 19  | 17.39  | -35.88   | 66.98 |
| (E4)              | 122.6        | 121.5  | 0.41  | 0.559 | 23.10 | 0.51 | 1.3   | 35  | 46.64  | -173.2   | 126.1 |
| (E5)              | 121.9        | 121.5  | 0.88  | 0.260 | 17.93 | 0.92 | 2.40  | )9  | 21.39  | -62.13   | 72.09 |
| (E6)              | 118.1        | 121.5  | 0.64  | 0.455 | 19.67 | 0.68 | -2.10 | 59  | 38.65  | -135.5   | 95.44 |
| (E7)              | 108.8        | 121.5  | 0.39  | 0.473 | 20.91 | 0.56 | 9.12  | 24  | 30.21  | -53.08   | 100.1 |
| (E8)              | 118.9        | 121.5  | 0.89  | 0.261 | 17.33 | 0.92 | -0.9  | 90  | 21.70  | -62.92   | 62.23 |
| (E9)              | 111.2        | 121.5  | 0.81  | 0.271 | 15.25 | 0.86 | 10.8  | 85  | 16.73  | -21.97   | 67.98 |
| Urine nitrogen ou | utput (g/d)  |        |       |       |       |      |       |     |        |          |       |
| (2a)              | 80.95        | 67.68  | 0.66  | 0.695 | 10.61 | 0.42 | -1.72 | 21  | 37.80  | -107.5   | 72.10 |
| (2b)              | 76.26        | 67.68  |       | 0.536 | 13.59 |      | -4.3  |     | 27.09  |          | 39.65 |
| (2c)              | 73.61        | 67.68  | 0.80  |       | 13.07 |      | -7.00 |     |        | -80.81   | 39.97 |
| (2d)              | 77.30        | 67.68  | 0.85  | 0.489 | 12.58 |      | 7.49  |     | 24.56  | -46.75   | 50.30 |
| (2e)              | 74.64        | 67.68  | 0.56  |       | 17.54 |      | 4.7   |     | 28.47  | -52.16   | 44.84 |
| (2f)              | 80.47        | 67.68  | 0.81  | 0.504 | 13.71 | 0.82 | -4.3  |     | 25.57  | -51.20   | 44.77 |
| (2g)              | 78.60        | 67.68  | 0.88  | 0.476 | 12.39 |      | -3.4  |     | 25.51  | -49.06   | 44.81 |
| (2h)              | 91.00        | 67.68  |       | 0.443 | 7.902 |      | -9.00 |     | 23.63  | -50.99   | 25.88 |
| (2i)              | 83.76        | 67.68  |       | 0.289 | 3.799 |      | 6.5   |     |        |          | 24.71 |
| (2j)              | 79.72        | 67.68  |       | 0.424 | 9.841 |      | -1.9  |     |        | -56.71   | 44.32 |
| (2j)<br>(2k)      |              |        |       |       |       |      |       |     |        |          |       |
| (21)              | 75.81        | 67.68  |       | 0.474 | 10.87 |      | -5.89 |     |        | -65.94   | 51.05 |
| ()                | 76.45        | 07.08  | 0.72  | 0.518 | 14.56 | 0.77 | 7.00  | 59  | 24.06  | -35.47   | 51.61 |

**Table A2** External validation using the literature database, equations developed from the mergeddigestibility trials database and previously published equations (n=570)

| (2m)                         | 76.96        | 67.68    | 0.85   | 0.460  | 13.19     | 0.81    | 6.053      | 23.03   | -45.74   | 62.89   |  |  |  |  |
|------------------------------|--------------|----------|--------|--------|-----------|---------|------------|---------|----------|---------|--|--|--|--|
| (2n)                         | 102.3        | 67.68    | 0.97   | 0.381  | 6.688     | 0.90    | -15.17     | 16.86   | -42.16   | 3.476   |  |  |  |  |
| (20)                         | 72.28        | 67.68    | 0.44   | 0.837  | 8.991     | 0.25    | -7.757     | 45.43   | -127.3   | 61.75   |  |  |  |  |
| (2p)                         | 64.54        | 67.68    | 0.63   | 0.714  | 15.77     | 0.69    | -13.62     | 35.46   | -106.9   | 37.68   |  |  |  |  |
| (2q)                         | 52.06        | 67.68    | 0.18   | 0.769  | 13.57     | 0.56    | -3.169     | 34.30   | -68.97   | 29.54   |  |  |  |  |
| (2r)                         | 60.29        | 67.68    | 0.57   | 0.772  | 15.84     | 0.60    | -17.84     | 37.80   | -118.4   | 23.99   |  |  |  |  |
| (2s)                         | 51.63        | 67.68    | 0.11   | 0.799  | 12.96     | 0.54    | -3.483     | 35.71   | -53.42   | 32.59   |  |  |  |  |
| (E10)                        | 88.86        | 67.68    | 0.91   | 0.330  | 12.82     | 0.88    | 6.066      | 15.54   | -33.21   | 45.98   |  |  |  |  |
| (E11)                        | 77.62        | 67.68    | 0.91   | 0.443  | 9.350     | 0.82    | -3.958     | 23.32   | -60.83   | 43.15   |  |  |  |  |
| (E12)                        | 76.75        | 67.68    | 0.91   | 0.343  | 12.93     | 0.91    | -5.840     | 16.33   | -46.99   | 35.57   |  |  |  |  |
| (E13)                        | 75.07        | 67.68    | 0.90   | 0.380  | 11.77     | 0.89    | -7.199     | 18.04   | -53.42   | 36.05   |  |  |  |  |
| (E14)                        | 63.73        | 67.68    | 0.24   | 0.910  | 17.84     | 0.45    | -12.75     | 44.59   | -119.9   | 68.20   |  |  |  |  |
| (E15)                        | 65.12        | 67.68    | 0.25   | 0.907  | 18.24     | 0.46    | -11.30     | 44.54   | -118.4   | 69.90   |  |  |  |  |
| (E16)                        | 76.00        | 67.68    | 0.89   | 0.384  | 13.13     | 0.90    | -5.066     | 18.57   | -54.10   | 34.41   |  |  |  |  |
| (E17)                        | 66.84        | 67.68    | 0.87   | 0.489  | 13.03     | 0.90    | -13.76     | 22.61   | -75.82   | 17.33   |  |  |  |  |
|                              |              |          |        |        |           |         |            |         |          |         |  |  |  |  |
| Faeces nitrogen output (g/d) |              |          |        |        |           |         |            |         |          |         |  |  |  |  |
| (3a)                         | 61.08        | 47.84    | 0.75   | 0.311  | 10.27     | 0.64    | 7.397      | 10.30   | -26.09   | 37.37   |  |  |  |  |
| (3b)                         | 60.50        | 47.84    | 0.80   | 0.278  | 9.329     | 0.68    | 6.679      | 9.282   | -19.59   | 35.37   |  |  |  |  |
| (3c)                         | 56.73        | 47.84    | 0.74   | 0.323  | 7.967     | 0.72    | 10.84      | 7.756   | -0.421   | 28.17   |  |  |  |  |
| (3d)                         | 59.73        | 47.84    | 0.80   | 0.273  | 9.342     | 0.70    | 6.003      | 9.305   | -20.20   | 33.39   |  |  |  |  |
| (3e)                         | 57.31        | 47.84    | 0.78   | 0.321  | 7.451     | 0.70    | 11.41      | 7.296   | 1.913    | 27.88   |  |  |  |  |
| (3f)                         | 54.83        | 47.84    | 0.83   | 0.297  | 7.293     | 0.81    | 10.58      | 7.123   | 2.226    | 27.06   |  |  |  |  |
| (3g)                         | 55.12        | 47.84    | 0.74   | 0.280  | 10.13     | 0.73    | 2.069      | 10.33   | -19.23   | 26.07   |  |  |  |  |
| (3h)                         | 58.82        | 47.84    | 0.51   | 0.421  | 11.10     | 0.66    | 12.92      | 10.96   | -8.892   | 45.26   |  |  |  |  |
| (3i)                         | 59.89        | 47.84    | 0.58   | 0.418  | 10.66     | 0.63    | 13.99      | 10.39   | -5.788   | 44.62   |  |  |  |  |
| (3j)                         | 59.29        | 47.84    | 0.69   | 0.413  | 10.11     | 0.66    | 15.04      | 9.740   | -0.960   | 41.79   |  |  |  |  |
| (3k)                         | 69.44        | 47.84    | 0.15   | 0.353  | 5.206     | 0.94    | 7.630      | 4.685   | 2.023    | 11.79   |  |  |  |  |
| (31)                         | 50.54        | 47.84    | 0.48   | 0.417  | 5.461     | 0.37    | -0.220     | 15.67   | -50.50   | 25.29   |  |  |  |  |
| (3m)                         | 51.10        | 47.84    | 0.24   | 0.418  | 8.712     | 0.52    | 9.177      | 10.46   | -6.338   | 29.27   |  |  |  |  |
| (3n)                         | 39.58        | 47.84    | 0.76   | 0.273  | 3.096     | 0.46    | 5.630      | 9.169   | -9.144   | 12.00   |  |  |  |  |
| (30)                         | 45.05        | 47.84    | 0.03   | 0.361  | 3.831     | 0.50    | -0.951     | 8.025   | -9.767   | 7.747   |  |  |  |  |
| (3p)                         | 46.40        | 47.84    | 0.16   | 0.406  | 7.805     | 0.41    | 5.859      | 10.79   | -10.07   | 25.25   |  |  |  |  |
| (E18)                        | 81.77        | 47.84    | 0.75   | 0.715  | 15.20     | 0.36    | 26.26      | 16.64   | -0.912   | 70.15   |  |  |  |  |
| (E19)                        | 61.99        | 47.84    | 0.35   | 0.562  | 22.08     | 0.39    | 7.535      | 22.18   | -41.20   | 85.46   |  |  |  |  |
| (E20)                        | 51.21        | 47.84    | 0.74   | 0.306  | 6.107     | 0.67    | -0.727     | 10.87   | -25.27   | 21.60   |  |  |  |  |
| (E21)                        | 50.80        | 47.84    | 0.74   | 0.286  | 7.931     | 0.72    | -1.647     | 9.895   | -23.80   | 18.94   |  |  |  |  |
| (E22)                        | 47.71        | 47.84    | 0.06   | 0.508  | 4.488     | 0.14    | -2.680     | 18.58   | -43.61   | -40.50  |  |  |  |  |
| (E23)                        | 45.79        | 47.84    | 0.07   | 0.525  | 6.973     | 0.19    | -4.688     | 18.73   | -40.49   | 35.64   |  |  |  |  |
| Eq. = equation: $r^2 = cor$  | rrelation be | etween r | redict | ed and | actual va | lues: N | /PF – mean | nredict | ion erro | r: SF - |  |  |  |  |

 $Eq. = equation; r^2 = correlation between predicted and actual values; MPE = mean prediction error; SE = standard error; Rc = Lin's concordance correlation coefficient; SD = standard deviation; Min = minimum value observed; Max = maximum value observed.$ 

<sup>a</sup> Equations are presented in Table 2 (Eq. 1a-1p, 2a-2s, 3a-3p) and Table A1 (Eq. E1-E23).

|                   |              |        | ~     |       |       |      |        | Predicted – Actual |        |      |  |
|-------------------|--------------|--------|-------|-------|-------|------|--------|--------------------|--------|------|--|
| Eq. <sup>a</sup>  | Predicted    | Actual | $r^2$ | MPE   | SE    | Rc   | Mean   | SD                 | Min    | Max  |  |
| Manure nitrogen o | output (g/d) |        |       |       |       |      |        |                    |        |      |  |
| Low CP group      |              |        |       |       |       |      |        |                    |        |      |  |
| (4a)              | 110.8        | 86.78  | 0.73  | 0.389 | 13.17 | 0.61 | 24.42  | 15.77              | -29.68 | 61.5 |  |
| (4b)              | 99.16        | 86.78  | 0.81  | 0.282 | 11.84 | 0.79 | 14.47  | 13.41              | -35.08 | 41.3 |  |
| (4c)              | 107.9        | 86.78  | 0.59  | 0.398 | 13.80 | 0.60 | 16.65  | 20.34              | -38.36 | 48.8 |  |
| (4d)              | 90.12        | 86.78  | 0.73  | 0.266 | 13.25 | 0.82 | 7.610  | 14.30              | -29.63 | 31.9 |  |
| (4e)              | 93.39        | 86.78  | 0.85  | 0.232 | 10.37 | 0.86 | 9.982  | 11.92              | -23.30 | 39.0 |  |
| (4f)              | 112.1        | 86.78  | 0.63  | 0.404 | 14.11 | 0.59 | 20.38  | 19.36              | -18.48 | 62.1 |  |
| (4g)              | 102.9        | 86.78  | 0.53  | 0.416 | 8.700 | 0.41 | 15.90  | 23.35              | -66.08 | 46.9 |  |
| (4h)              | 90.94        | 86.78  | 0.60  | 0.351 | 9.894 | 0.61 | 5.682  | 21.15              | -71.14 | 33.1 |  |
| (4i)              | 84.10        | 86.78  | 0.27  | 0.470 | 18.36 | 0.45 | -9.222 | 27.28              | -75.63 | 37.0 |  |
| Medium CP group   |              |        |       |       |       |      |        |                    |        |      |  |
| (7a)              | 145.6        | 120.1  | 0.77  | 0.338 | 18.29 | 0.73 | 24.97  | 21.71              | -24.98 | 96.' |  |
| (7b)              | 120.4        | 120.1  | 0.78  | 0.245 | 18.64 | 0.90 | -0.369 | 20.29              | -39.83 | 62.: |  |
| (7c)              | 121.6        | 120.1  | 0.86  | 0.197 | 14.83 | 0.92 | 0.145  | 15.93              | -31.97 | 39.  |  |
| (7d)              | 129.5        | 120.1  | 0.86  | 0.229 | 13.64 | 0.88 | 8.937  | 17.80              | -27.23 | 54.  |  |
| (7e)              | 130.4        | 120.1  | 0.72  | 0.287 | 19.54 | 0.81 | 9.444  | 22.17              | -17.17 | 74.  |  |
| (7f)              | 114.7        | 120.1  | 0.76  | 0.272 | 15.74 | 0.82 | -4.363 | 22.16              | -31.73 | 33.  |  |
| (7g)              | 124.2        | 120.1  | 0.38  | 0.445 | 21.82 | 0.54 | 4.280  | 35.97              | -86.16 | 122  |  |
| High CP group     |              |        |       |       |       |      |        |                    |        |      |  |
| (10a)             | 166.3        | 155.1  | 0.74  | 0.356 | 30.62 | 0.83 | 13.14  | 36.26              | -91.10 | 102  |  |
| (10b)             | 163.6        | 155.1  | 0.83  | 0.282 | 25.74 | 0.89 | 10.89  | 28.97              | -52.53 | 101  |  |
| (10c)             | 158.3        | 155.1  | 0.83  | 0.282 | 25.66 | 0.90 | 5.238  | 29.55              | -60.66 | 95.  |  |
| (10d)             | 133.8        | 155.1  | 0.98  | 0.138 | 9.738 | 0.95 | 15.99  | 9.545              | 5.966  | 31.  |  |
| (10e)             | 129.0        | 155.1  | 0.98  | 0.114 | 9.093 | 0.97 | 11.14  | 8.808              | -2.505 | 26.  |  |
| (10f)             | 156.8        | 155.1  | 0.94  | 0.156 | 8.834 | 0.91 | -3.220 | 15.51              | -17.97 | 20.  |  |
| (10g)             | 156.9        | 155.1  | 0.92  | 0.201 | 17.04 | 0.95 | 3.799  | 21.23              | -35.85 | 67.  |  |
| (10h)             | 129.7        | 155.1  | 0.98  | 0.113 | 7.786 | 0.97 | 12.08  | 8.116              | -3.240 | 25.  |  |
| (10i)             | 135.2        | 155.1  | 0.98  | 0.142 | 8.930 | 0.94 | 17.75  | 8.472              | 7.471  | 33.  |  |
| (10j)             | 150.7        | 155.1  | 0.93  | 0.199 | 16.12 | 0.95 | -2.717 | 21.24              | -43.91 | 57.  |  |
| (10k)             | 134.7        | 155.1  | 0.99  | 0.132 | 8.321 | 0.96 | 13.83  | 10.54              | -0.208 | 30.  |  |
| (101)             | 144.3        | 155.1  | 0.52  | 0.490 | 24.28 | 0.55 | -13.84 | 52.45              | -154.6 | 66.  |  |
| (10m)             | 140.9        | 155.1  | 0.60  | 0.461 | 23.76 | 0.61 | -16.96 | 48.94              | -129.2 | 54.  |  |
| (10n)             | 108.6        | 155.1  | 0.85  | 0.241 | 14.35 | 0.78 | 21.88  | 14.45              | -7.844 | 30.  |  |
| (100)             | 103.3        | 155.1  | 0.76  | 0.278 | 17.64 | 0.77 | 17.52  | 18.71              | -18.84 | 30.  |  |
| (10p)             | 142.7        | 155 1  | 0.68  | 0.384 | 27.04 | 0.77 | -8.303 | 39 24              | -106.7 | 48.  |  |

**Table A3** External validation using the literature database, partitioned in 3 groups according to their CP concentrationand equations developed from the merged digestibility trials database, representing low (n=190), medium (n=190) and high (n=190) protein concentrations

*Urine nitrogen output (g/d)* 

Low CP group

|               | (5a)                 | 60.11 | 42.68 | 0.50 | 0.782 | 7.675  | 0.36  | 17.58  | 20.21 | -64.82 | 57.88 |  |
|---------------|----------------------|-------|-------|------|-------|--------|-------|--------|-------|--------|-------|--|
|               | (5b)                 | 47.70 | 42.68 | 0.60 | 0.628 | 8.686  | 0.60  | 7.034  | 17.97 | -69.44 | 31.74 |  |
|               | (5c)                 | 51.90 | 42.68 | 0.65 | 0.637 | 7.561  | 0.55  | 10.82  | 17.89 | -68.40 | 40.88 |  |
|               | (5d)                 | 55.21 | 42.68 | 0.38 | 0.788 | 6.072  | 0.27  | 12.53  | 22.70 | -85.42 | 46.86 |  |
|               | (5e)                 | 41.05 | 42.68 | 0.47 | 0.682 | 9.082  | 0.53  | 0.440  | 20.23 | -89.93 | 24.29 |  |
|               | (5f)                 | 39.34 | 42.68 | 0.31 | 0.820 | 12.28  | 0.41  | -9.001 | 24.02 | -70.11 | 30.20 |  |
|               | (5g)                 | 38.11 | 42.68 | 0.30 | 0.772 | 10.92  | 0.42  | -2.673 | 22.41 | -100.7 | 29.05 |  |
| Mediu         | m CP group           |       |       |      |       |        |       |        |       |        |       |  |
|               | (8a)                 | 41.31 | 70.20 | 0.68 | 0.639 | 3.768  | 0.18  | -29.19 | 28.07 | -130.7 | 12.40 |  |
|               | (8b)                 | 62.45 | 70.20 | 0.74 | 0.392 | 11.91  | 0.77  | -8.843 | 17.80 | -83.19 | 27.40 |  |
|               | (8c)                 | 78.61 | 70.20 | 0.79 | 0.384 | 8.739  | 0.73  | 7.966  | 18.71 | -73.60 | 39.03 |  |
|               | (8d)                 | 80.98 | 70.20 | 0.86 | 0.230 | 5.557  | 0.87  | 1.853  | 9.191 | -13.81 | 20.71 |  |
|               | (8e)                 | 74.58 | 70.20 | 0.45 | 0.531 | 13.46  | 0.55  | 3.730  | 25.44 | -95.22 | 36.25 |  |
|               | (8f)                 | 63.23 | 70.20 | 0.49 | 0.521 | 13.67  | 0.58  | -7.707 | 24.54 | -104.0 | 24.64 |  |
| High CP group |                      |       |       |      |       |        |       |        |       |        |       |  |
|               | (11a)                | 104.9 | 90.66 | 0.57 | 0.558 | 21.31  | 0.68  | 7.708  | 33.32 | -69.31 | 93.92 |  |
|               | (11b)                | 102.7 | 90.66 | 0.72 | 0.448 | 20.24  | 0.81  | 6.918  | 26.90 | -49.19 | 83.81 |  |
|               | (11c)                | 80.62 | 90.66 | 0.97 | 0.140 | 6.571  | 0.97  | 6.438  | 6.916 | -4.840 | 20.42 |  |
|               | (11d)                | 99.35 | 90.66 | 0.87 | 0.328 | 13.91  | 0.89  | 4.586  | 20.31 | -39.36 | 73.13 |  |
|               | (11e)                | 81.81 | 90.66 | 0.97 | 0.155 | 6.115  | 0.96  | 7.897  | 7.605 | -3.756 | 23.08 |  |
|               | (11f)                | 85.75 | 90.66 | 0.94 | 0.250 | 12.09  | 0.92  | 11.83  | 13.43 | -5.371 | 32.32 |  |
|               | (11g)                | 107.4 | 90.66 | 0.89 | 0.248 | 8.898  | 0.87  | 4.718  | 13.64 | -12.65 | 22.98 |  |
|               | (11h)                | 92.57 | 90.66 | 0.40 | 0.646 | 20.17  | 0.50  | -3.701 | 40.46 | -105.5 | 76.31 |  |
|               | (11i)                | 89.59 | 90.66 | 0.58 | 0.554 | 19.44  | 0.65  | -5.430 | 34.77 | -91.50 | 66.89 |  |
|               | (11j)                | 64.80 | 90.66 | 0.94 | 0.204 | 7.210  | 0.87  | 12.06  | 6.584 | -1.916 | 19.39 |  |
|               | (11k)                | 61.73 | 90.66 | 0.90 | 0.224 | 8.789  | 0.88  | 9.660  | 8.173 | -8.290 | 14.29 |  |
| Faece         | s nitrogen output (g | /d)   |       |      |       |        |       |        |       |        |       |  |
|               | P group              | ,     |       |      |       |        |       |        |       |        |       |  |
| 2017 0        | (6a)                 | 51.34 | 43.80 | 0.42 | 0.364 | 10.74  | 0.54  | 7.540  | 10.90 | -9.785 | 55.49 |  |
|               | (6b)                 | 56.27 |       |      | 0.340 | 5.712  |       | 11.55  | 6.185 | -0.863 | 18.34 |  |
|               | (6c)                 | 57.68 |       |      | 0.354 | 5.473  |       | 12.96  | 5.621 | 1.965  | 20.44 |  |
|               | (6d)                 | 42.90 |       |      | 0.341 | 10.91  | 0.58  | -0.901 |       | -19.03 | 45.92 |  |
|               | (6e)                 | 52.65 |       |      | 0.375 |        | 0.50  | 7.926  | 9.041 | -5.537 | 28.56 |  |
|               | (6f)                 | 54.11 |       |      | 0.353 |        | 0.47  | 9.385  |       | -2.459 | 27.52 |  |
|               | (6g)                 | 48.46 |       |      | 0.341 | 3.741  | 0.29  | 4.512  |       | -22.74 | 25.60 |  |
|               | (6g)<br>(6h)         | 43.69 |       |      | 0.215 | 5.949  |       | -1.033 |       | -6.941 | 17.57 |  |
|               | ()                   | .2.07 | .2.00 | 0.00 | 0.210 | 2.2.17 | 5.7 1 | 1.555  | 202   | 0.7 11 |       |  |

Mediun . (

| um CP group |       |            |       |            |       |              |       |
|-------------|-------|------------|-------|------------|-------|--------------|-------|
| (9a)        | 58.95 | 48.23 0.42 | 0.446 | 13.92 0.54 | 10.21 | 14.33 -12.75 | 54.75 |
| (9b)        | 51.20 | 48.23 0.37 | 0.407 | 13.18 0.60 | 2.806 | 14.15 -20.49 | 46.46 |
| (9c)        | 52.42 | 48.23 0.59 | 0.328 | 10.79 0.73 | 5.469 | 10.96 -5.929 | 36.16 |
| (9d)        | 50.16 | 48.23 0.15 | 0.445 | 8.197 0.33 | 1.667 | 14.65 -28.82 | 30.50 |
| (9e)        | 60.74 | 48.23 0.39 | 0.451 | 8.584 0.38 | 13.79 | 12.48 -2.280 | 41.31 |

| High CP group | пıgn | Сr | group |
|---------------|------|----|-------|
|---------------|------|----|-------|

| (12a) | 61.64 | 52.09 | 0.73 | 0.298 | 10.34 | 0.82 | 5.742  | 10.46 | -23.09 | 38.63 |
|-------|-------|-------|------|-------|-------|------|--------|-------|--------|-------|
| (12a) | 01.04 | 52.07 | 0.75 | 0.270 | 10.54 | 0.02 | 5.742  | 10.40 | 25.07  | 50.05 |
| (12b) | 60.99 | 52.09 | 0.77 | 0.276 | 9.878 | 0.84 | 5.566  | 9.841 | -15.57 | 33.92 |
| (12c) | 48.43 | 52.09 | 0.96 | 0.135 | 3.650 | 0.94 | 4.782  | 3.657 | -0.733 | 13.65 |
| (12d) | 58.16 | 52.09 | 0.77 | 0.256 | 9.480 | 0.87 | 2.569  | 9.523 | -19.36 | 30.89 |
| (12e) | 57.55 | 52.09 | 0.79 | 0.256 | 9.784 | 0.87 | 3.408  | 9.727 | -14.87 | 29.00 |
| (12f) | 47.80 | 52.09 | 0.93 | 0.152 | 4.878 | 0.93 | 4.147  | 4.699 | -2.441 | 13.67 |
| (12g) | 50.42 | 52.09 | 0.94 | 0.189 | 4.874 | 0.89 | 6.771  | 5.164 | 0.420  | 18.09 |
| (12h) | 51.56 | 52.09 | 0.55 | 0.382 | 5.918 | 0.55 | -1.833 | 14.34 | -49.32 | 21.70 |
| (12i) | 43.99 | 52.09 | 0.47 | 0.348 | 6.147 | 0.47 | 8.404  | 9.224 | -5.633 | 16.62 |

 $Eq. = equation; r^2 = correlation between predicted and actual values; MPE = mean prediction error; SE = standard error; Rc = Lin's concordance correlation coefficient; SD = standard deviation; Min = minimum value observed; Max = maximum value observed.$ 

<sup>a</sup> Equations are presented in Table 3 (Eq. 4a-4i, 5a-5g, 6a-6h), Table 4 (Eq. 7a-7g, 8a-8f, 9a-9e) and Table 5 (Eq. 10a-10p, 11a-11k, 12a-12i).

|                  |                             |           |        |       |       |       |      | Predicted – Actual |       |        |       |  |
|------------------|-----------------------------|-----------|--------|-------|-------|-------|------|--------------------|-------|--------|-------|--|
| Eq. <sup>a</sup> | Equiv. Eq.                  | Predicted | Actual | $r^2$ | MPE   | SE    | Rc   | Mean               | SD    | Min    | Max   |  |
| Manure           | e nitrogen out <sub>l</sub> | out (g/d) |        |       |       |       |      |                    |       |        |       |  |
| Low CF           | 9 group                     |           |        |       |       |       |      |                    |       |        |       |  |
| (1a)             | (4a)                        | 127.2     | 86.78  | 0.73  | 0.540 | 13.70 | 0.42 | 40.75              | 15.75 | -11.90 | 78.0  |  |
| (1b)             | (4b)                        | 92.60     | 86.78  | 0.81  | 0.244 | 13.51 | 0.86 | 8.775              | 13.74 | -34.65 | 41.6  |  |
| (1c)             | (4c)                        | 107.7     | 86.78  | 0.60  | 0.396 | 15.13 | 0.62 | 16.99              | 20.05 | -35.07 | 49.6  |  |
| (1f)             | (4d)                        | 115.4     | 86.78  | 0.68  | 0.472 | 20.39 | 0.54 | 31.26              | 20.25 | -6.334 | 69.7  |  |
| (1g)             | (4e)                        | 93.84     | 86.78  | 0.85  | 0.237 | 9.974 | 0.85 | 10.30              | 12.09 | -25.09 | 39.0  |  |
| (1i)             | (4f)                        | 112.6     | 86.78  | 0.56  | 0.429 | 15.82 | 0.55 | 20.74              | 20.97 | -18.33 | 66.2  |  |
| (11)             | (4g)                        | 119.2     | 86.78  | 0.53  | 0.526 | 8.913 | 0.27 | 32.17              | 23.23 | -49.61 | 62.9  |  |
| (1m)             | (4h)                        | 87.84     | 86.78  | 0.60  | 0.341 | 12.18 | 0.69 | 3.067              | 20.10 | -70.72 | 35.4  |  |
| (1n)             | (4i)                        | 88.15     | 86.78  | 0.24  | 0.468 | 18.26 | 0.44 | -5.536             | 27.77 | -72.64 | 42.4  |  |
| Medium           | ı CP group                  |           |        |       |       |       |      |                    |       |        |       |  |
| (1a)             | (7a)                        | 140.8     | 120.1  | 0.77  | 0.324 | 15.92 | 0.74 | 20.28              | 22.62 | -37.44 | 93.5  |  |
| (1b)             | (7b)                        | 129.0     | 120.1  | 0.80  | 0.264 | 16.36 | 0.85 | 8.357              | 20.79 | -40.84 | 76.6  |  |
| (1c)             | (7c)                        | 126.6     | 120.1  | 0.82  | 0.227 | 15.64 | 0.88 | 5.158              | 17.99 | -28.93 | 49.8  |  |
| (1g)             | (7d)                        | 128.2     | 120.1  | 0.86  | 0.239 | 12.19 | 0.86 | 7.654              | 19.36 | -34.57 | 55.4  |  |
| (1i)             | (7e)                        | 129.4     | 120.1  | 0.64  | 0.322 | 22.08 | 0.77 | 7.907              | 25.28 | -21.75 | 82.5  |  |
| $(NA^*)$         | (7f)                        |           |        |       |       |       |      |                    |       |        |       |  |
| (11)             | (7g)                        | 119.4     | 120.1  | 0.65  | 0.336 | 11.20 | 0.59 | 2.276              | 29.03 | -52.91 | 37.9  |  |
| High Cl          | P group                     |           |        |       |       |       |      |                    |       |        |       |  |
| (1a)             | (10a)                       | 137.3     | 155.1  | 0.74  | 0.389 | 21.36 | 0.72 | -16.41             | 41.01 | -133.9 | 54.6  |  |
| (1b)             | (10b)                       | 154.8     | 155.1  | 0.82  | 0.315 | 20.26 | 0.84 | 1.498              | 34.24 | -73.92 | 96.5  |  |
| (1d)             | (10c)                       | 150.7     | 155.1  | 0.82  | 0.320 | 20.17 | 0.83 | -2.807             | 34.81 | -75.93 | 95.7  |  |
| (**)             | (10d)                       | 130.0     | 155.1  | 0.98  | 0.120 | 6.884 | 0.96 | 12.18              | 12.18 | -7.825 | 27.7  |  |
| (1c)             | (10e)                       | 130.3     | 155.1  | 0.99  | 0.130 | 5.972 | 0.95 | 12.31              | 11.87 | -10.08 | 26.5  |  |
| (**)             | (10f)                       | 164.8     | 155.1  | 0.96  | 0.128 | 7.674 | 0.94 | 4.810              | 12.49 | -8.629 | 25.0  |  |
| (1g)             | (10g)                       | 153.2     | 155.1  | 0.92  | 0.226 | 14.85 | 0.92 | -0.160             | 25.04 | -50.79 | 58.8  |  |
| (1i)             | (10h)                       | 129.9     | 155.1  | 0.98  | 0.129 | 8.145 | 0.96 | 12.44              | 10.42 | -6.630 | 29.6  |  |
| (**)             | (10i)                       | 131.9     | 155.1  | 0.98  | 0.132 | 9.013 | 0.95 | 14.50              | 9.204 | -2.209 | 31.4  |  |
| (1h)             | (10j)                       | 148.4     | 155.1  | 0.93  | 0.230 | 14.00 | 0.91 | -5.199             | 25.53 | -57.44 | 51.3  |  |
| (**)             | (10k)                       | 129.0     | 155.1  | 0.95  | 0.350 | 13.84 | 0.75 | -48.11             | 17.57 | -81.14 | -21.4 |  |
| (11)             | (101)                       | 122.9     | 155.1  | 0.52  | 0.554 | 13.05 | 0.29 | -36.01             | 59.59 | -177.9 | 58.0  |  |
| (1m)             | (10m)                       | 136.1     | 155.1  | 0.56  | 0.497 | 19.37 | 0.48 | -22.28             | 53.73 | -133.5 | 56.7  |  |
| (1n)             | (10n)                       | 112.3     | 155.1  | 0.88  | 0.253 | 10.20 | 0.68 | 25.28              | 16.06 | 0.021  | 35.9  |  |
| (1p)             | (100)                       | 107.1     | 155.1  | 0.72  | 0.310 | 13.98 | 0.64 | 20.50              | 21.54 | -12.01 | 36.1  |  |
| (**)             | (10p)                       | 138.5     | 155 1  | 0.68  | 0.416 | 17.11 | 0.60 | -12.55             | 46 17 | -118.4 | 55.4  |  |

**Table A4** External validation using the literature database, partitioned in 3 groups according to their CP concentrationand equations developed from the merged digestibility trials database (n=570)

Urine nitrogen output (g/d)

| Low CP g | group |       |       |      |       |       |      |       |              |       |
|----------|-------|-------|-------|------|-------|-------|------|-------|--------------|-------|
| (2a)     | (5a)  | 74.05 | 42.68 | 0.50 | 0.991 | 8.683 | 0.25 | 31.53 | 19.70 -47.61 | 72.10 |

| (2b)     | (5b)             | 43.32   | 42.68 | 0.60 | 0.594 | 10.99 | 0.71 | 3.136  | 16.88 | -64.78 | 30.63  |
|----------|------------------|---------|-------|------|-------|-------|------|--------|-------|--------|--------|
| (2j)     | (5c)             | 53.66   | 42.68 | 0.65 | 0.642 | 9.708 | 0.61 | 13.03  | 16.48 | -58.23 | 44.32  |
| (20)     | (5d)             | 70.12   | 42.68 | 0.38 | 0.973 | 6.106 | 0.17 | 27.44  | 22.69 | -70.45 | 61.75  |
| (2p)     | (5e)             | 39.00   | 42.68 | 0.48 | 0.673 | 10.50 | 0.58 | -1.444 | 19.59 | -88.80 | 24.59  |
| (2q)     | (5f)             | 40.62   | 42.68 | 0.35 | 0.793 | 11.47 | 0.43 | -7.793 | 23.53 | -68.97 | 29.54  |
| (2r)     | (5g)             | 37.89   | 42.68 | 0.27 | 0.790 | 12.65 | 0.44 | -3.032 | 22.74 | -101.1 | 33.09  |
| Mediur   | m CP group       |         |       |      |       |       |      |        |       |        |        |
| (2a)     | (8a)             | 80.39   | 70.20 | 0.68 | 0.455 | 8.744 | 0.59 | 9.514  | 22.36 | -78.78 | 49.54  |
| (2b)     | (8b)             | 70.15   | 70.20 | 0.74 | 0.391 | 9.435 | 0.73 | -0.911 | 19.72 | -82.97 | 37.10  |
| (2j)     | (8c)             | 75.43   | 70.20 | 0.79 | 0.364 | 9.444 | 0.78 | 4.743  | 17.88 | -74.40 | 36.17  |
| $(NA^*)$ | (8d)             |         |       |      |       |       |      |        |       |        |        |
| (20)     | (8e)             | 72.66   | 70.20 | 0.45 | 0.541 | 7.598 | 0.37 | 2.033  | 27.98 | -102.4 | 36.12  |
| (2p)     | (8f)             | 61.55   | 70.20 | 0.50 | 0.530 | 10.99 | 0.50 | -9.280 | 25.47 | -108.2 | 19.57  |
| High C   | CP group         |         |       |      |       |       |      |        |       |        |        |
| (2a)     | (11a)            | 78.76   | 90.66 | 0.57 | 0.605 | 11.14 | 0.42 | -15.29 | 39.06 | -107.5 | 49.53  |
| (2b)     | (11b)            | 94.68   | 90.66 | 0.67 | 0.497 | 16.79 | 0.71 | 1.931  | 31.48 | -74.48 | 85.94  |
| (2e)     | (11c)            | 81.16   | 90.66 | 0.98 | 0.154 | 4.932 | 0.95 | 6.942  | 8.660 | -5.547 | 19.79  |
| (2j)     | (11d)            | 91.26   | 90.66 | 0.87 | 0.352 | 12.10 | 0.85 | -2.972 | 22.91 | -53.68 | 62.02  |
| (21)     | (11e)            | 76.01   | 90.66 | 0.96 | 0.162 | 6.611 | 0.96 | 2.252  | 9.823 | -10.80 | 21.34  |
| (**)     | (11f)            | 77.24   | 90.66 | 0.97 | 0.135 | 5.947 | 0.97 | 3.329  | 7.700 | -7.385 | 18.29  |
| (**)     | (11g)            | 100.4   | 90.66 | 0.93 | 0.198 | 7.074 | 0.91 | -2.279 | 11.65 | -13.59 | 15.63  |
| (20)     | (11h)            | 72.34   | 90.66 | 0.40 | 0.707 | 9.022 | 0.23 | -22.46 | 45.08 | -127.3 | 38.28  |
| (2p)     | (11i)            | 85.42   | 90.66 | 0.59 | 0.562 | 16.25 | 0.59 | -8.066 | 36.10 | -106.9 | 47.58  |
| (2q)     | (11j)            | 66.03   | 90.66 | 0.92 | 0.248 | 5.022 | 0.74 | 12.76  | 10.76 | -8.014 | 20.15  |
| (2s)     | (11k)            | 62.47   | 90.66 | 0.80 | 0.314 | 7.361 | 0.71 | 9.535  | 13.65 | -15.16 | 19.77  |
| _        |                  |         |       |      |       |       |      |        |       |        |        |
|          | s nitrogen outpu | t (g/d) |       |      |       |       |      |        |       |        |        |
| Low C.   | P group          |         |       |      |       |       |      |        |       |        |        |
| (3a)     | (6a)             | 52.98   | 43.80 | 0.42 | 0.386 | 11.07 | 0.50 | 9.181  | 11.17 | -8.022 | 58.10  |
| (3c)     | (6b)             | 56.37   | 43.80 | 0.75 | 0.354 | 5.909 | 0.42 | 11.65  | 6.626 | -1.228 | 19.98  |
| (3e)     | (6c)             | 56.99   | 43.80 | 0.74 | 0.354 | 5.661 | 0.39 | 12.27  | 6.188 | 0.179  | 19.50  |
| (3g)     | (6d)             | 39.50   | 43.80 | 0.35 | 0.333 | 8.015 | 0.54 | -4.297 |       | -23.42 | 34.28  |
| (3h)     | (6e)             | 48.72   | 43.80 | 0.60 | 0.295 | 8.136 | 0.59 | 3.996  | 8.480 | -5.788 | 26.68  |
| (3i)     | (6f)             | 50.13   | 43.80 | 0.61 | 0.271 | 7.142 | 0.58 | 5.406  | 7.272 | -8.892 | 25.67  |
| (31)     | (6g)             | 49.18   | 43.80 | 0.25 | 0.347 | 4.262 | 0.31 | 5.233  | 9.822 | -22.85 | 27.04  |
| (3m)     | (6h)             | 47.23   | 43.80 | 0.40 | 0.263 | 7.573 | 0.57 | 2.510  | 7.375 | -6.368 | 20.52  |
| Mediur   | m CP group       |         |       |      |       |       |      |        |       |        |        |
| (3a)     | (9a)             | 60.42   | 48.23 | 0.42 | 0.459 | 13.58 | 0.51 | 11.69  | 14.08 | -11.58 | 55.55  |
| (3g)     | (9b)             | 52.55   | 48.23 | 0.37 | 0.393 | 9.472 | 0.56 | 4.199  | 12.47 | -17.54 | 40.31  |
| (3h)     | (9c)             | 54.94   | 48.23 | 0.38 | 0.424 | 12.20 | 0.54 | 7.994  | 13.53 | -5.132 | 45.26  |
| (31)     | (9d)             | 50.77   | 48.23 | 0.15 | 0.435 | 6.073 | 0.27 | 2.368  | 14.40 | -25.69 | -16.72 |
| (3m)     | (9e)             | 49.43   | 48.23 | 0.33 | 0.394 | 8.616 | 0.52 | 2.478  | 13.04 | 30.25  | 29.27  |

High CP group

| (3a) | (12a) | 58.51 | 52.09 | 0.73 | 0.281 | 10.42 | 0.84 | 2.588  | 10.51 | -26.09 | 35.64 |
|------|-------|-------|-------|------|-------|-------|------|--------|-------|--------|-------|
| (3b) | (12b) | 60.42 | 52.09 | 0.76 | 0.280 | 10.05 | 0.84 | 4.716  | 10.03 | -19.59 | 35.55 |
| (3c) | (12c) | 49.05 | 52.09 | 0.96 | 0.144 | 3.708 | 0.93 | 5.400  | 3.599 | -0.421 | 14.48 |
| (3d) | (12d) | 59.14 | 52.09 | 0.77 | 0.264 | 9.793 | 0.86 | 3.449  | 9.800 | -20.20 | 33.54 |
| (3g) | (12e) | 62.03 | 52.09 | 0.79 | 0.284 | 9.018 | 0.82 | 8.049  | 9.045 | -10.44 | 32.18 |
| (3h) | (12f) | 54.05 | 52.09 | 0.93 | 0.233 | 4.368 | 0.81 | 10.40  | 4.218 | 3.706  | 17.96 |
| (3i) | (12g) | 56.26 | 52.09 | 0.94 | 0.271 | 4.525 | 0.76 | 12.60  | 4.478 | 6.445  | 22.25 |
| (31) | (12h) | 50.57 | 52.09 | 0.55 | 0.390 | 4.844 | 0.47 | -2.680 | 15.05 | -50.49 | 21.51 |
| (3m) | (12i) | 46.87 | 52.09 | 0.65 | 0.360 | 5.285 | 0.48 | 11.29  | 7.640 | -1.606 | 18.24 |

Eq. = equation;  $r^2$  = correlation between predicted and actual values; MPE = mean prediction error; SE = standard error; Rc = Lin's concordance correlation coefficient; SD = standard deviation; Min = minimum value observed; Max = maximum value observed.

<sup>a</sup> Equations are presented in Table 2 (Eq. 1a-1p, 2a-2s, 3a-3p), Table 3 (Eq. 4a-4i, 5a-5g, 6a-6h), Table 4 (Eq. 7a-7h, 8a-8f, 9a-9e) and Table 5 (Eq. 10a-10p, 11a-11k, 12a-12i).

\* Equations using the same predictors and the merged digestibility trials database were not available and could not be produced in that case.

\*\* Equations using the same predictors and the merged digestibility trials database were produced in order to assist the comparison