Development and evaluation of a mechanistic model of post-absorptive nitrogen partitioning in lactating goats

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Summary

Understanding and controlling nitrogen (N) utilization efficiency in dairy goats is desirable to maximize farmers profits while minimizing N environmental pollution. A mechanistic dynamic model was developed and validated as a research tool that can support more flexible decision systems. This model provides a framework to learn to manage N under different diets and lactation stages for dairy goats.

Abstract

Context: Goats contribute to global warming through emission of nitrous oxide from urine and feces. To reduce nitrogen (N) excretion, improvements of N efficiency of goats is necessary.

Aims: The present study develops and evaluate a dynamic mechanistic research-oriented model that explicitly represents N partition into feces, urine, and milk in dairy goats fed total mixed rations.

Methods: Data from five N balance dairy goats' experiments were used to develop a mechanistic dynamic model of post-absorptive N partition. Various representations considering either mass action or Michaelis-Menten kinetics of N usage for milk was proposed.

Key results: The data for fecal and urine N responses were best fit by a straight line; whereas, data for milk N responses were best fit by curvilinear saturating curve. The model with curvilinear saturating curve had more precise parameter estimates with predicted N excretion in feces (15.6 g/d), urine (15.4 g/d) and milk N output (11.7 g/d) very close to the observed values; 15.31 gN/d in feces, 18.78 gN/d in urine and 12.24 gN/d in milk. Independent data set with twelve studies were used to evaluate the model. The model tends to under-predict fecal N outflow at lower N intake and urinary N outflow at higher N intake level with lowest mean bias for milk N outflow.

Conclusions: The final chosen model was adequate to represent N fecal, urinary and milk outflows in dairy goats.

Implications: The model provides a mechanistic description of N usage, useful to frame and test hypotheses of physiological regulation of N use by goats, focus on a more efficient transfer of dietary N into milk, reducing the N excretion in feces and urine.

Additional keywords: Protein, efficiency, allocation, regulation

Introduction

The world food economy is increasingly being driven by the shift of human diets towards animal-based products such as meat, milk and dairy (FAO 2015). Within the top five live animals in production, goats come in third place after cattle and sheep, at 1,006 million head (FAO 2015). In meeting the increased animal food demand, the overall efficiency of milk and meat production must be increased to support closer to optimal trade-offs between access to food by humans, negative effects on the environment per unit of product, and the economic success of the livestock enterprise. However, controlling the efficiency of animal production requires understanding of nutrient (e.g. nitrogen (N)) intake and use by the animals. Profit maximization by farmers requires a flexible ration formulation framework that adjusts protein supply periodically according to market price variations of high protein ingredients and milk protein. Yet, such flexible system must accurately represent goat milk protein responses to varying N intake. Therefore, understanding of N partition must precede such a system. Although numerous studies on N partition have been conducted (Kebreab *et al.*, 2002), and feeding systems have been developed for dairy cattle (NRC, 2001; AFRC 1993; INRA, 2018), less progress has been made with dairy goats comparatively. The Small Ruminant Nutrition System (Tedeschi *et al.*, 2010) adopted a constant N efficiency value

69 0.64 for milk, as suggested by the INRA (1989). This can be problematic for practical ration
70 formulation because predicted performance losses and gains at varying levels of N intake
71 could be biased, and the extent of such bias will entail financial expense from costly protein
72 sources to guarantee performance levels or lower output of valuable milk protein from
73 underfeeding N. It is documented that this efficiency varies in lactating cows according to
74 diet and animal's potential (Kebreab *et al.*, 2002; INRA, 2018) and recently, INRA (2018)
75 proposed an approximation where protein incorporation into milk depends non-linearly on
76 dietary supply of truly digestible protein (g PDI/kg DM) about a pivot value of 0.66 in goats.
77 This model describes empirically metabolizable protein inputs and outputs and has the
78 potential to be readily applied to diet formulation in the field in order to optimize N use.
79 However, explicitly representing other physiological processes that largely impact N
80 economy and productivity in goats such as recycling, body growth and the overall dynamics
81 of N allocation to these functions in relation to milk N incorporation throughout lactation,
82 provides for a longer-term research framework that can support even more flexible decision
83 systems. The aim of this study was to develop and evaluate a dynamic mechanistic research-
84 oriented model that explicitly represents N partition into feces, urine, and milk in dairy goats
85 fed total mixed rations.

86 **Material and methods**

87 The experimental procedures carried out were approved by the Committee on Animal Use
88 and Care at the Universitat Politècnica de Valencia in Spain. Animals were cared for by
89 trained personnel and managed in accordance with the Spanish guidelines for experimental
90 animal protection (Royal Decree No. 1201 2005) and the European Convention for the

Protection of Vertebrates used for Experimental and other Scientific Purposes (European Directive 86/609).

Data origin

Data from five N balance experiments (two unpublished) conducted at the Universitat Politècnica de Valencia were used to develop the model (López *et al.* 2014; Criscioni and Fernández 2016 and Ibáñez *et al.* 2016). These trials evaluated the response of lactating goats in terms of energy and N balance, apparent total tract digestibility and milk production, to supply of cereals and byproducts. The trial of López *et al.* (2014) studied the effect of replacing corn grain with citrus pulp, Criscioni and Fernández (2016) replaced oats with rice bran, Ibáñez *et al.* (2016) replaced barley grain with fibrous by products, and the other two unpublished studies replaced barley with orange pulp and mixed cereals with beet pulp, respectively. The trials encompassed a total of 104 multiparous Murciano-Granadina goats in mid or late lactation. The goats were fed 10 different total mixed diets with alfalfa hay and concentrate, and none of the trials were conducted in grazing conditions. The concentrate was mixed with alfalfa hay in a forage to concentrate ratio of 40:60. For each trial, total N intake and output of fecal, urinary, milk N were recorded. In addition, feed concentration of DM, CP, neutral detergent fiber (NDF), starch, ash and metabolizable energy (ME) were recorded.

In developing the conceptual model, a reference state was defined as a goat weighing 43 kg, producing 2.0 kg of milk/d, consuming 1.8 kg DM/d. Mixed diets ranged from 13 to 17% in CP, 1.5 to 46% in starch and 23 to 59% in NDF content on DM basis. Intake was *ad libitum* with diets offered at 110% of consumption on the preceding few days. Half the daily

ration was offered at 08:00 and half at 16:00 h, respectively. Goats had free access to water. A summary of the data used in the model development is given in Table 1.

When estimating models using data arising from multiple different studies, it is important to know if there is dependence of the effect of the independent variable X on the dependent variable Y, on the study effect. In other words, whether there is an interaction between X and the study effects, and hence whether the relationship between X and Y is consistent across studies. Furthermore, achieving as much balance as possible in a meta-design is critical to separate the effect of the study from the effect of X. Otherwise, the effect of all study related unidentified variables (e.g. lactation stage, diet, breed, management) would be confounded with the independent variable (Sauvant *et al.* 2008). Fig. 2 illustrates the relationship between N intake and fecal, urinary and milk N outputs. Visual assessment suggests that balance is far from perfect; however, it appears that the effect of N intake on the N outputs is consistent across studies, linear with similar slope for urinary and fecal N, and non-linear and saturating for milk N, except for trial C. This experiment was, therefore, withdrawn from the database. To account for the study effect, we have adjusted the individual measurements with respect to the study mean to remove variation among studies. Each residual was added to its corresponding Y predicted value to generate adjusted Y values.

The reason for choosing this manual approach to adjusting for study effects is because, to our knowledge, mixed model methodology is not readily available in the commercial differential equation solvers, and customarily programming the mixed effects equations in commercially available software (e.g. R or Matlab) would represent a major technical and financial challenge to overcome within our operational constraints.

Model building and description

The model consisted of a dynamic system of differential equations coded in Advanced Continuous Simulation Language (ACSLX version 3.1.4.2, Aegis Technologies Group, Huntsville, AL, USA). A four order Runge-Kutta method with an integration step size of 0.05 d was used for numerical integration, and the model was run until a steady state was achieved.

The model was conceptually based on the mechanistic model from Kebreab *et al.* (2002). It contains four N pools expressed in grams and represented by the abbreviation Q and depicted by a box, and the inflows and outflows to and from the pools are the flows in grams per day and are represented by arrows and denominated by the abbreviation F (Fig. 1 and Table 2). Therefore, the mass of Q will change with time depending on the magnitude of the fluxes, and the change is described by a differential equation of the form: $dQ/dt = F_{in} - F_{out}$.

We evaluated 3 versions of the same process model differing only in the type of kinetics (i.e. mass action vs saturation kinetics) to assess which would fit the data better. Hence, model 1 assumed mass action flow in feces, urine and milk with no intercept ($F = k \cdot Q$; k being the fractional rate constant). Model 2 is a mass action type flux in feces, urine and milk and allows for intercepts ($F = k \cdot Q + Pi$; where k and P*i* are the fractional rate constant and intercept for N excretion, respectively). Model 3 assumes mass action in feces and urine and a saturating flux (i.e. Michaelis-Menten) from plasma to milk [$F = V_{max} / (1 + (K_m/Q))$; where V_{max} is the maximal milk N incorporation and K_m is the affinity constant equal to N intake to reach ½ Vmax]. Table 2 describes all pools, fluxes and symbols used to develop the model.

To obtain initial values of the parameters to be used in the subsequent parameterization of the dynamic model in ACSLX (k_{PR_feces}, k_{P_urine}, k_{P_milk}, P_f, P_u, P_{milk}, V_{max}

and K_m), linear and non-linear regression was carried out first by minimization of least squares using the lm and nls functions of the Stats package of R (R Core Team, 2014). These regressions also allowed obtaining an estimate of the metabolic fecal nitrogen (MFN) (i.e. intercept value in regression of fecal N on N intake; P_f in Table 3) and endogenous urinary N (EUN) (i.e. intercept value in regression of urinary N on N absorbed; P_u in Table 3). Other parameter values (k_{R_PR}, k_{PR_P}, k_{P_R} and k_{P_PR}) were obtained from the literature and not estimated (Table 3).

Schematic representation of the model is shown in Fig. 1. Description of pools and the associated differential equations describing pool size change over time follow below and abbreviations are referenced in Table 2.

Rumen pool, Q_R (g N). The rumen pool includes microbial and ammonia N and has two inflows and two outflows. The inflows are the degradable N intake from the ration (F_{feed_R}) and the plasma urea N entry from the plasma pool into rumen through blood and saliva (F_{P_R}). The rumen undegraded protein from the diet (RUPd) was calculated from the experimental diet according to Sniffen *et al.* (1992); this technique assumes that the neutral detergent insoluble protein represents the primary RUP fraction in feedstuffs (15% across studies). The degradable N (RDPd) of the diet was calculated by difference from rumen undegraded protein (RUPd): RDPd = (100 – RUPd). The outflows are the ammonia N flux from rumen to plasma through the rumen wall (F_{R_P}) and the microbial N passing from rumen to small intestine (F_{R_PR}). Both fluxes were represented as mass action and the fraction of rumen ammonia going to plasma (i.e. k_{R_P}) was assumed from F_{R_P} according to Domingue *et al.* (1991); whereas, the fraction of microbial N passing to lower intestine (i.e. k_{R_PR}) was taken from estimations made by Malecky *et al.* (2009). Domingue *et al.* (1991) measured N

metabolism and water flows along the digestive tract in red deer, goats (castrate Angora) and sheep fed a chaffed lucerne hay diet *ad libitum*; under these conditions, the k_{R_P} obtained was of 0.15/d. Malecky *et al.* (2009) fitted a rumen cannula and T-type cannula into the duodenum of lactating Alpine and Saanen goats and fed them total mixed diets. They recorded variables related to rumen fermentation, duodenal nutrient flow and milk yield, and determined k_{R_PR} to be 0.65/d. These authors also estimated a rumen pool size, including diet and recycled N, to be about 53g. Pool size change over time and fluxes are defined below:

Change over time in the rumen N pool size (g N/d)

$dQ_R/dt = F_{feed\_R} + F_{P\_R} – F_{R\_PR} – F_{R\_P}$

Inflows:

$F_{feed\_R} = Ni \cdot ((100 – RUPd)/100)$

$F_{PR} = k_{PR} \cdot Q_P$

Outflows:

$F_{R\_PR} = k_{R\_PR} \cdot Q_R$

$F_{R\_P} = k_{R\_P} \cdot Q_R$

Where Ni is nitrogen intake [$Ni = (DMI \cdot CPd/100))/6.25$]. DMI is daily dry matter intake and CPd is the diet crude protein.

The rumen N pool size was expressed by the integral equation:

$$Q_R = \int_{to}^{t} \frac{dQ_R}{dt} + iQ_R$$

Representing the quantity of N accumulated from initial time (t_0) and final time (t), and iQ_R being the initial pool size.

Post-rumen pool, Q_{PR} (g N). The post-rumen pool includes all small intestine and the lower digestive tract. The initial amount of N in the post-rumen pool was set at 40g, based on the study of N flows through rumen, duodenum ileum and rectum by Brun-Bellut *et al.* (1991) with lactating Saanen goats weighing 48 kg body weight, 1541 g DM intake/d and fed with concentrate-hay mixtures. This pool has three inflows and two outflows. The inflows are microbial protein N (F_{R_PR}), undegraded protein N intake (F_{feed_PR}) and plasma urea N entry from plasma to post-ruminal and lower digestive tract through blood (F_{P_PR}). The amount of non-degradable dietary protein (i.e. RUP) N varies according to the chemical composition of the diet, but an average value of 15% was calculated for the diets given to the goats in the experiments, as mentioned above (Sniffen *et al.* 1992). The two outflows are the duodenal absorption of N flux from small intestine to blood through the intestinal epithelium (F_{PR_P}) and the total fecal N excretion (F_{PR_feces}). The rate constant k_{PR_P} (0.68/d) was calculated from the estimated apparent total tract crude protein digestibility (69%) for RUP and the RDP according to the assumptions of AFRC (1997) and NRC (2007); 85% of the RDP was assumed to be converted to microbial crude protein; and the proportion of microbial crude protein present that is microbial true protein was assumed to be 75% and with digestibility of 85% (NRC, 2007). The flux from post rumen to feces was the experimentally observed average N excreted (15 g/d), and the estimated rate constant k_{PR_feces} was 0.375/d in model 1; whereas, the rate constant and intercept in models 2 and 3 were the same at: k_{PR_feces} = 0.265/d and Pf = 2.52 g N/d. Pool size change over time and fluxes are defined below:

Change over time in Post-rumen N pool (gN/d)

$$dQ_{PR}/dt = F_{feed\_PR} + F_{R\_PR} - F_{PR\_feces} - F_{PR\_P}$$

Inflows:

232 $F_{feed\_PR} = Ni \cdot (RUP/100)$

233 $F_{R\_PR} = k_{R\_PR} \cdot Q_R$

234 Outflows:

235 $F_{PR\_feces} = k_{PR\_feces} \cdot Q_{PR}$ Model 1

236 $F_{PR\_feces} = k_{PR\_feces} \cdot Q_{PR} + P_f$ Model 2 and 3

237 $F_{PR\_P} = k_{PR\_P} \cdot Q_{PR}$

238 Where P_f is the intercept of the regression line, representing the MFN.

239 The post-rumen N pool size is expressed by the integral equation:

240 $$Q_{PR} = \int_{to}^{t} \frac{dQ_{PR}}{dt} + iQ_{PR}$$

241 Representing the quantity of N accumulated post-ruminally from initial time (t_0) to final time
242 (t), being iQ_{PR} the initial pool size.

243

244 *Plasma pool, Q_P (g N).* The plasma pool includes the total peptide N, urea N and ammonia
245 N and an amount of 36 g was obtained from blood sample analyses and plasma volume
246 measures (unpublished data and, Criscioni and Fernández (2016) and Ibáñez *et al.* (2016)).
247 This pool has three inflows; one comes from rumen ammonia N absorption through the
248 rumen wall (F_{R_P}), another one from microbial protein absorbed from the small intestine
249 (F_{PR_P}) and the last one from body protein catabolism (F_{Body_P}). The fluxes F_{R_P} and F_{PR_P}
250 were defined previously. The muscle N anabolic and catabolic fluxes were assumed equal
251 for mid and late lactation goats ($F_{Body\_P} = - F_{P\_Body}$). There are five outflows from the plasma
252 pool. Two of them are plasma N flux to rumen (F_{P_R}) and post rumen (F_{P_PR}), and the other
253 three are urinary N excretion (F_{P_urine}), N excreted in milk (F_{P_milk}) and N retention in body
254 tissue protein (F_{P_Body}). The plasma N secretion flux into rumen (F_{P_R}) and post rumen (F_{P_PR})

was obtained from Harmeyer and Martens (1980), which considered that the plasma urea nitrogen entering the rumen with saliva is 1.68 g/d (k_{P_R} = 0.047) and plasma urea N entering the gut was 0.03 g/d (kP_PR = 0.001). The observed average N outflows in urine and milk from our dataset were 19 g/d and 12.4 g/d, respectively. Initial parameter values (i.e. to be used to initialize the likelihood-based parameter estimation in the dynamic model) describing such fluxes were obtained from preliminary linear and non-linear regression as indicated previously. Three equation types were evaluated: 1) linear relationship between N intake and, urine and milk N outflow without an intercept; 2) linear relationship between N intake and, urine and milk N outflow with an intercept; and 3) same description for urine N outflow as in 2) and a saturating relationship between N intake and milk N outflow. For 1) the initial estimates for the rate constants were kP_urine = 0.528 and kP_milk = 0.344. For 2) the initial estimates for the rate constant and intercept for urine N excretion were k_{P_urine} = 0.805/d and P_u = -7.75 g N/d, and for milk N excretion were k_{P_milk} = 0.199/d and P_{milk} = 5.69 g N/d. Finally, for 3) the maximal daily N excretion (V_{max}) was 26.59 g/d and 50% of such excretion (i.e. the affinity constant) occurred at a N intake of 37.53 g.

The anabolic flow F_{P_Body} was the N retained in body (2 g/d), so the k_{P_Body} was 0.056/d. The catabolic flow (F_{Body_P}) is of equal magnitude by definition under the assumption of zero growth. Pool size change over time and fluxes are defined below:

Change over time in Plasma pool (g N/d)

$$dQ_P/dt = F_{R\_P} + F_{PR\_P} + F_{Body\_P} - F_{P\_R} - F_{P\_PR} - F_{P\_urine} - F_{P\_milk} - F_{P\_Body}$$

Inflows:

$$F_{R\_P} = k_{R\_P} \cdot Q_R$$

$$F_{PR\_P} = k_{PR\_P} \cdot Q_{PR}$$

$$F_{Body\_P} = - F_{P\_Body}$$

Outflows:

$F_{P\_R} = k_{P\_R} \cdot Q_P$

$F_{P\_PR} = k_{P\_PR} \cdot Q_P$

$F_{P\_urine} = k_{P\_urine} \cdot Q_P$ Model 1

$F_{P\_urine} = k_{P\_urine} \cdot Q_P + P_u$ Model 2 and 3

$F_{P\_Body} = k_{P\_Body} \cdot Q_P$

$F_{P\_milk} = k_{P\_milk} \cdot Q_P$ Model 1

$F_{P\_milk} = k_{P\_milk} \cdot Q_P + P_{milk}$ Model 2

$F_{P\_milk} = V_{max} / (1 + (K_m/Q_P))$ Model 3

Where P_u is the regression line intercept, representing EUN. In the Michaelis-Menten equation V_{max} was the maximum milk yield and K_m the affinity constant.

The plasma N pool size was expressed by the integral equation:

$$Q_P = \int_{to}^{t} \frac{dQ_{RP}}{dt} + iQ_P$$

Representing the quantity of N accumulated from initial time (t_0) to final time (t), being iQ_P the initial pool size.

Body pool, Q_{Body} (g N). The body pool includes one inflow and one outflow. The inflow is the N flow from plasma to body (F_{P_Body}) and the other is the N mobilization from body reserves to plasma (F_{Body_P}). According to AFRC (1997), only one reference by Brown and Taylor (1986) was found relating to the body composition of adult females. They reported the mean composition of a heterogeneous group of 15 French Alpine, Nubian and Toggenburg females ranging in live weight from 38 to 70 kg, and from 2 to 5 years of age, including both lactating and pregnant animals. Mean data for this group was 7.9 kg of protein,

302 which converted to percentage of body CP in Murciano-Granadina goats was 18%. Thus, the
303 Body N pool with an average BW of 43 kg was 1238 g N. Pool size change over time and
304 fluxes are defined below:
305
306 *Change over time in N Body pool (g N/d)*
307 $dQ_{Body}/dt = F_{P\_Body} - F_{Body\_P}$
308 Inflow: $F_{P\_Body} = k_{P\_Body} \cdot Q_P$
309 Outflow: $F_{Body\_P} = - F_{P\_Body}$
310 The body N pool size was expressed by the integral equation:

311 $$Q_{Body} = \int_{to}^{t} \frac{dQ_{Body}}{dt} + iQ_{Body}$$

312 Representing the quantity of N accumulated from initial time (t_0) to final time (t), being iQ_{Body}
313 the initial pool size.
314 *Model development: parameter estimation and adequacy assessment*
315 Conceptual model structure was defined from biological definitions of N utilization by
316 lactating animals (NRC, 2001; Kebreab et al., 2002) and the parameter estimation was
317 performed by minimizing the negative log likelihood function (LLF) using an adaptive
318 nonlinear least square optimization algorithm (Generalized NL2SOL, Dennis et al., 1981)
319 available in ACSLX (Aegis Technologies Group). A LLF based goodness of fit method,
320 Bayesian Information Criterion (BIC), was used to compare models 1, 2 and 3. A smaller
321 BIC indicates a better fit to the data. In general, BIC penalizes models with more parameters,
322 thus larger models with same LLF values have a larger BIC .
323 Subsequently, to characterize model inadequacy (i.e. bias) in the range of our
324 observations, the observed values of fecal, urinary and milk N were compared with model

325 predictions and the discrepancy was calculated as the root mean squared prediction error
326 (RMSPE). The RMSPE was then decomposed into error due to the overall bias of prediction
327 (i.e. mean bias), error due to deviation of the regression slope from unity (i.e. slope bias), and
328 error due to the disturbance or random variation (Bibby and Toutenburg, 1977). The model
329 adequacy of the best fitting model was further assessed outside the range of our observations
330 by fitting a regression line between observed and predicted values and considering the
331 intercept and slope deviations from 0 and 1 (i.e. unity line), respectively. This exercise
332 extrapolates to zero and beyond the maximum observed values, and thus quantifies the
333 applicability domain for the model under consideration.

334 Afterwards, residual plots verifying the assumptions that errors are normally and
335 identically distributed about zero with constant variance were elaborated. Since residuals are
336 not correlated with predictions, the slope of the regression of residuals on predictions must
337 be zero if the model is unbiased.

338 *Sensitivity analysis*

339 Once one of the three models was selected based on goodness of fit and adequacy, a global
340 sensitivity analysis (Saltelli *et al.* 1999) was performed to assess the sensitivity of N excretion
341 and transfer into milk to the model inputs and the parameters. This exercise provides insight
342 of the most critical aspects of the system to guide future research and model improvement.

343 *Model evaluation against external data*

344 The final chosen model was compared against a set of external data to assess its predictive
345 ability. Twelve studies were used to evaluate the predictive ability of the model (see Table 7
346 for details). These studies contained a total 42 different treatments with varying levels of
347 protein (from 10 to 20%), combined different breeds (Granadina, Murciano-Granadina,

348 Saanen and Alpine), milk production levels and stages of lactation. Nitrogen intake was
349 estimated from the reported diet composition and table values for each ingredient (FEDNA
350 2010). The description of the database used to independently challenge the model are shown
351 in Table 7. The metric implemented to compare the model prediction against the independent
352 experimental observations, for the outflows of N in urine, feces and milk was the RMSPE as
353 described previously.

354 **Results and discussion**

355 Fig. 2 shows the 5 data sets for N feces (*a*), N urine (*b*) and N milk (*c*) outflows. Data points
356 from the same experiment shared the same colour and were connected by solid lines. In
357 obtaining initial parameter estimates for the subsequent parameterization of the dynamic
358 model, the data for fecal and urine N responses were best fit by a straight line; whereas, data
359 for milk N responses were best fit by curvilinear saturating curve (Figs. 2*a* and 2*b*). Visually,
360 the efficiciency of conversion between N intake and milk N, across all trials, appears non-
361 constant across studies, in agreement with previous observations that N partition towards
362 milk marginally decreased with increasing N intake (Doepel *et al.* 2004; Dijkstra *et al.* 2013).
363 No significant ($P > 0.05$) effect of the studies were observed during this preliminary analysis.
364 In addition, the interaction between between study and the linear and quadratic components
365 of the function was not different from zero, suggesting consistency of the milk N excretion
366 response across trials.

367 During parameterization of the dynamic model, the negative LLF was -722.31 for
368 model 1 and -711.96 for models 2 and 3. Also, BIC was lower in model 2 and 3 than in 1
369 (1451.79 vs. 1458.55, respectively) (Table 4). Based on the BIC, models 2 and 3 fit the data
370 better than 1, but 2 and 3 seem to fit the data equally well, hence suggesting the flux of milk

371 N output can be described well both by a mass action or a Michaelis-Menten function.
372 However, parameter estimates were more precise when the saturating function was assumed
373 (Table 3); The fractional rate k_{P_urine} had a variation coefficient (CV) around 18% in models
374 1 and 2, but it was reduced to 10% in model 3. The fractional rate k_{P_milk} had a CV of 21% in
375 model 1, which lowered in model 2 to 4%. The intercept for milk N output at zero N intake
376 (P_{milk}) was high at 20%, however. In comparison, the k_m and V_{max} parameters of saturating
377 representation in model 3 had rather low CV at 7 and 11%, respectively.

378 Across the 3 models, the errors of prediction in the range of our observations were
379 about 21% for fecal, 19% for milk, and 37% for urine N flows, respectively (Table 5). The
380 mean and slope bias were zero for all fluxes in models 2 and 3 but not for the flux from feces
381 and urine in model 1; which presented an error of 3.28% in feces and 0.68% in urine. Model
382 adequacy was therefore better for models 2 and 3 compared to 1.

383 Thus, the goodness of fit measures suggested model 1 to provide inferior fit to data
384 but it did not clearly discriminate among models 2 and 3. Yet, model 3 had more precise
385 parameter estimates. Furthermore, because experimentally we have consistently observed
386 that the average milk N output progressively decreases as N intake increases (Fig. 2*c*), we
387 decided to retain the Michaelis-Menten representation depicted by model 3 as a more
388 biologically meaningful description of N partition. In summary, in the range of our
389 observations, model 3 predicted N excretion in feces (15.6 g/d), urine (15.4 g/d) and milk N
390 output (11.7 g/d); whereas, the observed values were 15.31 gN/d in feces, 18.78 gN/d in urine
391 and 12.24 gN/d in milk as shown in Table 1.

392 Gauging the domain of applicability of the chosen model 3, Fig. 3 displays observed
393 versus predicted values and the corresponding unity regression equation (i.e. Observed =
394 Predicted). The model presents the least bias for the fecal N data in the range of 14 and 20

g/d, but below and above this range it under and overestimates. Also, it has a nearly unbiased fit to urinary N data from 10 to 25 g/d; however, above 25 g/d the model tended to under estimate urinary N output. For milk N, the model bias is minimal in the range of 9 and 14 g/d; whereas, above 14 it overestimates milk N output. The residual standard error for fecal, urinary and milk N shows the model is off by 1.38, 2.68 and 1.63 g/d. Fig. 3 provides intercept and slope estimates with their standard errors for the interested reader.

Analyses of residuals for model 3 are shown in Fig. 4. Results are consistent with the biases illustrated in Fig. 3 for fecal, urine and milk N flows, within and outside the range of observed data. For the ranges between 14 and 20 g/d, 10 to 25 g/d and 9 to 14 g/d for fecal, urinary and milk N flow, residuals appear to be randomly distributed about zero. Slopes of regression lines for residuals versus predicted were positive for N in feces and milk, indicating that the model overpredicted flows as predicted flow increased. The slope was negative for urinary N, indicating that the model underpredicted flows as predicted flow increased. Therefore, extrapolating outside the above ranges will yield increasingly biased predictions.

Sensitivity analysis of fecal N, urinary N excretions and milk N to the model parameters was carried out (Table 6). The F_{PR_feces} were sensitive to the digestibility coefficient and F_{P_urine} was sensitive to both digestibility coefficient and urinary loss rate constant. This implies that: 1) Good understanding of N digestibility is critical to predict supply and post-absorptive responses; therefore, validating any currently proven equations from large or small ruminants to these types of diets to predict digestible N flows to small intestine should be a relatively straightforward and fruitful exercise. Moreover, understanding, at least empirically, the control underlying the urinary loss rate constant could explain some of the residual error of prediction (~21%). That would entail replacing the presently assumed constant urea N recycling at 1.68 g/d (Harmeyer and Martens, 1980) via

mass action with a more flexible, possibly non-linear, representation accounting for carbohydrate profile, supply and fermentation, microbial growth and the resulting NH3-urea exchanges (Reynolds and Kristensen, 2008). Similarly, the assumption of zero growth currently included in the model is likely equivocal and generating data on body N accretion by goats during 1st and 2nd lactation and throughout the full lactation would provide a better description of N allocation and recycling into urea towards the rumen. On the other hand, F_{P_milk} was highly sensitive to the V_{max} parameter, which represents the maximum potential of milk protein synthesis by the goat's mammary gland. This suggests that experimental work considering the modulatory effect of lactation stage or genetic merit on the N partitioning in response to intake, will provide important quantitative information to better characterize N use efficiency (Hanigan *et al.* 2008).

Following, we compare our basal fecal and urinary N loss parameter estimates with values reported historically in the experimental literature. The N in the feces of animals given N-free diets is represented by MFN. All the MFN would be endogenous if the animal ate a N free diet, but this state is experimentally difficult to achieve with ruminants. A long period elapses before fecal nitrogen excretion falls to a baseline because recycling of N to the rumen and large intestine continues to provide some N for microbial activity (AFRC, 1997). The most common method of estimation is by extrapolating to zero (i.e. the intercept) from the regression of g fecal N on g N intake. The results generally obtained have indicated that MFN is in the order of 5 g/kg DMI, which is equivalent to 0.35 gN/kg $W^{0.75}$. Published values for goats are relatively few and Sahlu *et al.* (2004), included in NRC (2007), reported a mean value for MFN of 4.27 gN/kg DMI. The value estimated for our model is 3.85 gN/kg DMI, similar to the NRC (2007) estimates.

With respect to urinary N excretion, it has traditionally been divided into two components; a relatively constant component termed EUN and an exogenous component arising from the protein turnover. EUN is assumed to be the minimum urinary N excretion of an animal maintained for an extended period on a diet that contains little or no protein, but is adequate in energy and other nutrients. It can be estimated either by regressing urinary N on N supply. Brody (1945) found that EUN for a very wide range of animal species was related to basal metabolic rate, and the general value was 0.141 g EUN/kg $W^{0.734}$. Applying Brody's equation, AFRC (1997) and Sahlu *et al.* (2004) to our 43 kg average W goat, the EUN was 2.245, 1.671 and 2.788 g N/d, respectively, which is similar to the intercept value obtained in our model; 2.679 g N/d.

Following is a test of the model's predictive ability against an independent dataset and results are reported in Table 8. Aguilera *et al.* (1990) with Granadina goats in mid lactation fed alfalfa hay and barley diets (CP 14% and 16%) found values of N in feces, urine and milk of 9, 8 and 6 g/d respectively. The simulated values from our chosen model (#3) were 11, 9 and 7 g/d which results in an error of 18, 11 and 14%, respectively. The studies of Molina-Alcaide *et al.* (2010) and Romero-Huelva *et al.* (2012) were conducted with Murciano-Granadina goat as well. The diets were mixed diets with alfalfa hay as forage, similar to our studies. Some diets replaced part of the cereal in the grain mix with nutrients blocks than incorporated byproducts from agriculture (tomato, cucumber and olive cake waste) and the level of CP was 15% on average. Goats were in mid lactation and under these conditions observed fecal, urinary and milk N outflows were 11, 18 and 6 g/d; whereas, predicted values by model were 15, 18 and 11 g/d which results in an error of 27, 0, 45%, respectively. In the study of Santos *et al.* (2014) with Alpine lactating goats consuming mixed diets containing different protein sources (and same level of CP; 10%), the values

466 simulated were close to the observed values when the source of protein was soybean meal;
467 observed fecal, urinary and milk N outflows were 13, 6 and 7 g/d; whereas, predicted values
468 were 12, 8 and 9 g/d which results in an error of 8, 20, 27%, respectively. The study of Bava
469 *et al.* (2001) was conducted with lactating Saanen goats at early, mid and late lactation, which
470 were fed with silage and non-forage diets. For this trial the average error was 13, 22 and 26%
471 for fecal, urinary and milk N, respectively. Dos Santos *et al.* (2016) with Saanen lactating
472 goats as well, fed goats with pelleted diets increasing the CP of the diet from 10% to 19%
473 (by substitution of alfalfa hay with soybean meal). When goats were fed 10% of CP the
474 observed fecal, urinary and milk N outflows were 11, 4 and 8 g/d; whereas, our predicted
475 values were 12, 6 and 9 g/d which results in an error of 6, 36, 16%, respectively. The
476 prediction was worse when goats were fed 19% of CP with observed fecal, urinary and milk
477 N outflows at 8, 7 and 9 g/d; whereas, predicted values were 15, 16 and 12 g/d which results
478 in an error of 49, 58, 26%, respectively.

479 Across models, the model predicted fecal and urinary N excretion with acceptable
480 RMSPE between 19 and 20 %, and milk N with about 8%. Unexplained random error made
481 up the largest portion for feces and milk N predicted flows, around 76-77%. Mean and slope
482 bias in predicted fecal N output were about 24 and 0%, respectively; whereas for predicted
483 milk N output they were 1 and 21%, respectively (Fig. 5*a* and 5*c*).

484 Of the error in urine N flow predictions (19.87%), the majority is due to mean bias
485 (55%) and slope bias (19%) (Fig. 5*b*), both of which sum up to about 74% (Table 8). Mostly,
486 the issue is one of overpredicting N loss in urine (i.e. the goats urinated less N than the model
487 predicted) (Fig. 5*b* and 6*b*), especially in the studies that used rations with high CP levels
488 such as those from Rapetti *et al.* (2005) (18%), Criscioni *et al.* (2016) (16%) and Schmidely
489 *et al.* (1999) (16%), which resulted in urine N excretion levels beyond 20-25 g/d.

Nonetheless, acceptable predictions were observed when dietary CP ranged between 10 and 15% with N urine excretion between 7-15 g/d; and it is important to recall that the model was parameterized and shown to be fairly adequate in the range of 10 to 25 g/d of urinary N output. However, while extrapolating the model perhaps explain some portion of the prediction bias, other factors may also partially explain such systematic error in N urine flow predictions: 1) non-linear mechanisms other than simple mass action underlying urine N loss, specifically, N recycling as related to ruminal fermentation and microbial growth efficiency with varying carbohydrate types and supply, and 2) changes in body N accretion depending on maturity and stage of lactation of experimental goats.

Overall, however, the largest errors observed against the independent data set for fecal, urine and milk N predictions are in the magnitude of 1-3 g/day with respect to mean fluxes of about 15, 20 and 10 g/day, which suggests the model structure reflects well the biology of N use by goats.

In order to further our quantitative understanding of N metabolic usage by goats, it is critical to experimentally evaluate the main effects of factors such as lactation stage, dry matter intake, carbohydrate sources and concentration, and production potential, and their interactions with N supply on its partition.

It thus appears that the model satisfactorily characterizes N excretion and milk N secretion in lactating goats fed mixed diets supplying dietary N in the range of 30 to 70 g/d. Extrapolating beyond this level of N intake our estimations of N excretion are inflated because we are likely failing to account for some physiological N retaining process.

This model is only a basis for a mechanistic approach that needs to be updated as more information on biological processes in goats becomes available.

Conclusions

From various models evaluated here, the best one presented here simulated the effect of N intake on N excretion in feces, urine and milk, and included a Michaelis-Menten representation of N use for milk suggesting a system that responds decreasingly at higher protein supplies. This model presented about 20% prediction error against independent data, mostly systematic, in its description of urinary N losses indicating the need to understand and account for N retaining processes other than milk output. Sensitivity analysis encourages work on body N accretion during simultaneous growth and lactation, N recycling under different dietary N and carbohydrate regimes, and N allocation towards milk at different of lactational stages for goats with different genetic potential. This model provides a framework to embed future research hypothesis in view of the experimental work needed to better describe and learn to manage N under different diets and lactation stages for dairy goats.

Conflicts of interest

The authors declare no conflicts of interest.

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