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

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Abstract

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

Aims. The aim of the present study was to develop and evaluate a dynamic mechanistic research-oriented model that explicitly represents N partition into faeces, urine and milk in dairy goats fed total mixed rations.

Methods. Data from five N-balance dairy-goat 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 were proposed.

Key results. The data for faecal 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 the predicted N excretion in faeces (15.6 g/day), urine (15.4 g/day) and milk N output (11.7 g/day) being very close to the observed values, namely, 15.31 g N/day in faeces, 18.78 g N/day in urine and 12.24 g N/day in milk. Independent datasets with 12 studies were used to evaluate the model. The model tended to under-predict faecal N outflow at a lower N intake level and urinary N outflow at a higher N intake level, with the lowest mean bias for milk N outflow.

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

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

Additional keywords: allocation, efficiency, protein, regulation.

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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 1006 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 among 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 maximisation 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 (AFRC 1993; NRC 2001; 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 0.64 for milk, as suggested by the INRA (1989). This can be problematic for practical ration formulation because predicted performance losses and gains at varying levels of N intake could be biased, and the extent of such bias will entail financial expense from costly protein sources to guarantee performance levels or lower output of valuable milk protein from underfeeding N. It is documented that this efficiency varies in lactating cows according to diet and animal's potential (Kebreab *et al.* 2002; INRA 2018) and, recently, INRA (2018) proposed an approximation where protein incorporation into milk depends non-linearly on dietary supply of truly digestible protein (g/kg DM) about a

pivot value of 0.66 in goats. This model describes empirically metabolisable protein inputs and outputs and has the potential to be readily applied to diet formulation in the field so as to optimise N use. However, explicitly representing other physiological processes that largely affect N economy and productivity in goats, such as recycling, body growth and the overall dynamics of N allocation to these functions in relation to milk N incorporation throughout lactation, provides for a longer-term research framework that can support even more flexible decision systems. The aim of the present study was to develop and evaluate a dynamic mechanistic research-oriented model that explicitly represents N partition into faeces, urine and milk in dairy goats fed total mixed rations.

Materials and methods

The experimental procedures were approved by the Committee on Animal Use and Care at the Universitat Politècnica de Valencia in Spain. Animals were cared for by trained personnel and managed in accordance with the Spanish guidelines for experimental 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; European Union 2017).

Data origin

Data from five N-balance experiments (López *et al*. 2014; Criscioni and Fernández 2016; Ibáñez *et al*. 2016; unpubl. data from two experiments) conducted at the Universitat Politècnica de Valencia were used to develop the model. 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 by-products. The trial of López *et al*. (2014) studied the effect of replacing corn grain with citrus pulp (trial A), Ibáñez *et al*. (2016) replaced barley grain with fibrous by-products (trial B), one unpublished study replaced mixed cereals with beet pulp (Trial C), Criscioni and Fernández (2016) replaced oats with rice bran (trial D), and the other unpublished study replaced barley with orange pulp (trial E). 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 was 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 faecal, urinary and milk N were recorded. In addition, feed concentration of DM, crude protein (CP), neutral detergent fibre, starch, ash and metabolisable energy were recorded.

In developing the conceptual model, a reference state was defined as a goat weighing 43 kg, producing 2.0 kg of milk/day, consuming 1.8 kg DM/day. Mixed diets ranged from 13% to 17% in CP, 1.5% to 46% in starch and 23% to 59% in neutral detergent fibre concentration on a DM basis. Intake was *ad libitum*, with diets being offered at 110% of consumption on the preceding few days. Half the daily ration was offered at 0800 hours and half at 1600 hours respectively. Goats had free access to water. A summary of the data used in the model development is given in Table 1.

Table 1. Summary of the data used in the construction of the model
CPd, crude protein of diet; DMI, DM intake; ME, metabolisable energy; N, nitrogen; NDF, neutral detergent fibre; NI, nitrogen intake

Variable	Lactating dairy goat fed mixed diet (n = 104)			
	Mean	Min.	Max.	s.d.
Intake				
DMI (g/day)	1854	1079	2287	272.8
NI (g/day)	48	23	60	11.0
N excretions (g/day)				
Faecal	15	2	10	3.2
Urinary	19	8	34	7.1
Body	2	–4.0	7	2.4
Diet composition				
Forage to concentrate ratio	40 : 60			
DM percentage	88.6	87.5	90.2	0.87
CPd (% DM)	16.3	13.2	16.5	2.17
N (% DM)	2.6	2.1	2.6	0.35
NDF (% DM)	39.8	22.8	59.0	9.66
Ash (% DM)	8.1	6.9	10.7	0.99
Starch (% DM)	18.4	1.5	41.6	13.02
ME (MJ/kg DM)	11	9	13	0.69
Milk yield and composition				
Yield (g/day)	2003	1043	2977	434.0
N (g/day)	12.4	6.9	16.2	2.27
Goat characteristics				
Bodyweight (kg)	43	35	59	4.2

When estimating models using data arising from multiple different studies, it is important to know whether 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). Figure 1 illustrates the relationship between N intake and faecal, 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 a similar slope for urinary and faecal 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, so as 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

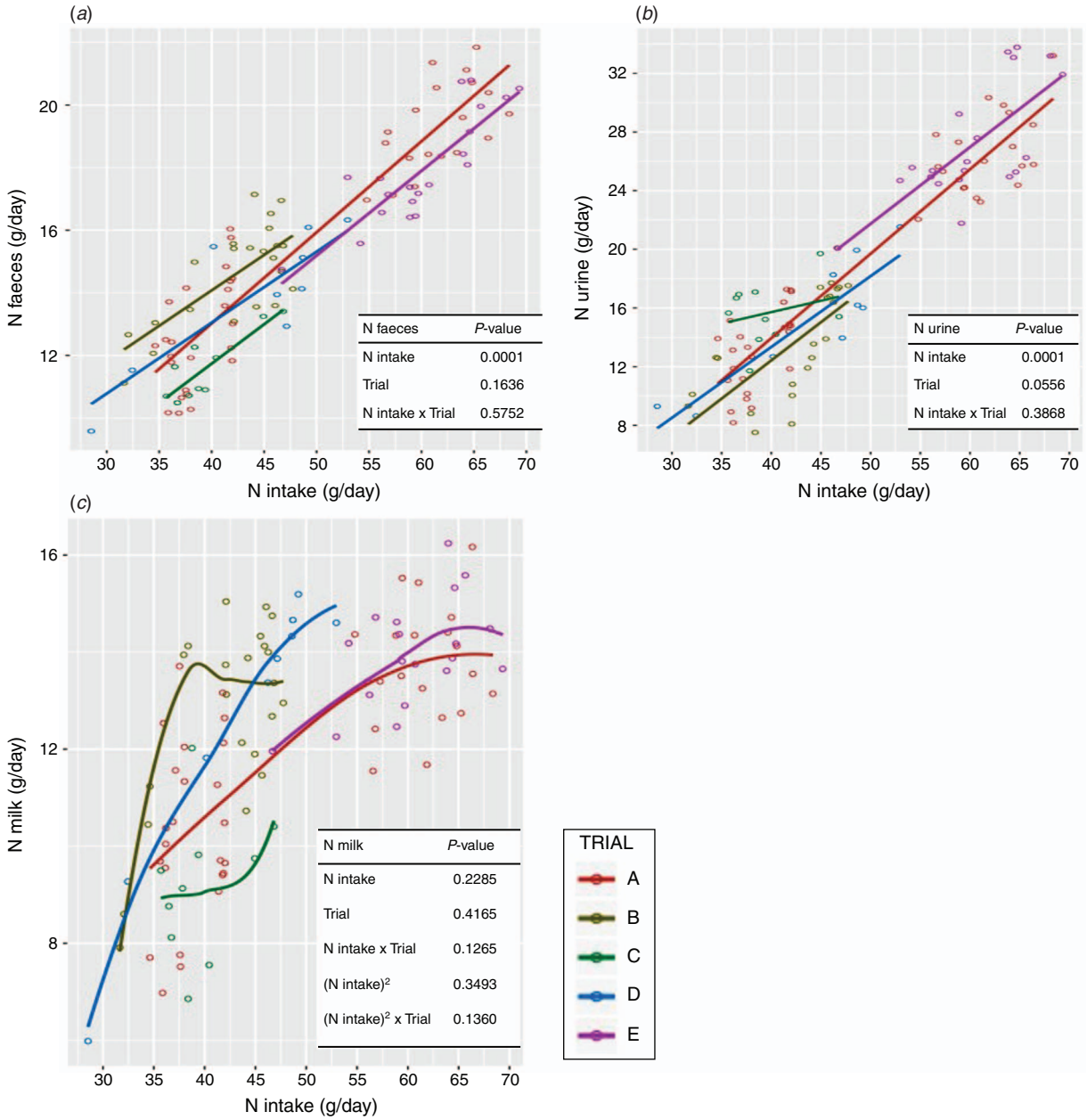

Fig. 1. Graphical representation of the dataset for (*a*) nitrogen (N) faeces, (*b*) N urine and (*c*) N milk. Data points from the same experiment are connected by solid lines.

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 days 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. 2, 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 three versions of the same process model, differing only in the type of kinetics (i.e. mass action vs

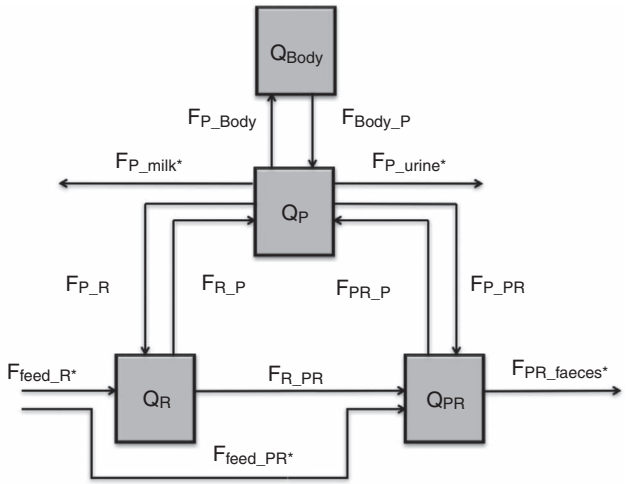

Fig. 2. Schematic representation of model of nitrogen (N) excretion in dairy goats. Boxes indicate N pools, and arrows indicate fluxes. Symbols used are defined in Tables 1 and 2. Asterisks indicate experimental values.

Table 2. Pools, fluxes and symbols used in the model

Label	Description
	Nitrogen pools (g)
Q_R	Reticulo-rumen pool (microbial nitrogen (N) plus ammonia-N)
Q_{PR}	Rest of the gut pool (post-rumen compartments)
Q_P	Plasma pool (total N; including urea-N and ammonia-N)
Q_{Body}	Body pool (total N retained in the body)
	Nitrogen fluxes (g/day)
F_{feed_R}	Degradable-N intake to Q_R
F_{feed_PR}	Undegraded-N intake to Q_{PR}
F_{R_PR}	Microbial protein N passage from Q_R to Q_{PR}
F_{R_P}	Ammonia-N flux from Q_R to Q_P through rumen walls
F_{PR_faeces}	Total faecal N excretion
F_{PR_P}	Duodenal absorption of N flux from microbial protein and undegraded protein through gut wall
F_{P_R}	Plasma urea-N flux from Q_P to Q_R through blood and saliva
F_{P_PR}	Plasma urea-N flux from Q_P to Q_{PR} through blood
F_{P_milk}	N secreted in milk
F_{P_Body}	N retention
F_{Body_P}	N mobilisation
	Fractional rates (per day)
k_{R_PR}	Fractional rate of F_{R_PR}
k_{R_P}	Fractional rate of F_{R_P}
k_{PR_faeces}	Fractional rate of F_{PR_faeces}
k_{PR_P}	Fractional rate of F_{PR_P}
k_{P_R}	Fractional rate of F_{P_R}
k_{P_PR}	Fractional rate of F_{P_PR}
k_{P_urine}	Fractional rate of F_{P_urine}
k_{P_milk}	Fractional rate of F_{P_milk}
k_{P_Body}	Fractional rate of F_{P_Body}
	Reference constants (g/day)
P_f	Metabolic faecal N
P_u	Endogenous urinary N
P_{milk}	Intercept for linear milk excretion
V_{max}	Maximum milk N rate
K_m	Affinity constant in Michaelis–Menten equation
	Inputs
DMI (g/day)	Input value of DM intake
CPd (%)	Diet input value of crude protein
RUPd (%)	Diet input value of rumen-undegraded protein
RDPd (%)	Diet input value of rumen-degraded protein

saturation kinetics), to assess which would fit the data better. Hence, Model 1 assumed mass action flow in faeces, urine and milk with no intercept ($F = k \times Q$; k being the fractional rate constant). Model 2 was a mass action type flux in faeces, urine and milk and allowed for intercepts ($F = k \times Q + P_i$, where k and P_i are the fractional rate constant and intercept for N excretion respectively). Model 3 assumed mass action in faeces 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 1/2 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 parameterisation of the dynamic model in ACSLX (k_{PR_faeces}, k_{P_urine}, k_{P_milk}, P_f, P_u, P_{milk}, V_{max} and K_m), linear and non-linear regressions were performed first by minimisation of least-squares by 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 faecal N (MFN; i.e. intercept value in regression of faecal 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

Table 3. Initial and final parameter estimation and standard deviation of optimised model parameters, other parameters and pools

Abbreviations are defined in Table 2. s.d., standard deviation; CV, variation coefficient

Parameter	Initial value	Final value	s.d.	CV	Reference
	Model 1; mass action				
k_{PR_faeces}	0.380	0.139	0.0036	3	
k_{P_urine}	0.528	0.990	0.1751	18	
k_{P_milk}	0.344	0.010	0.0021	21	
	Model 2; linear regression				
k_{PR_faeces}	0.265	0.107	0.0050	5	
P_f	2.516	11.579	0.2020	2	
k_{P_urine}	0.805	0.233	0.0413	18	
P_u	–7.752	-4.032	0.7542	19	
k_{P_milk}	5.688	12.248	0.5351	4	
P_{milk}	0.199	0.097	0.0191	20	
	Model 3; linear regression and Michaelis–Menten equation				
k_{PR_faeces}	0.265	0.208	0.0046	2	
P_f	2.516	7.138	0.2012	3	
k_{P_urine}	0.805	0.215	0.0217	10	
P_u	–7.752	–2.679	0.3158	12	
V_{max}	26.588	16.726	1.1271	7	
K_m	37.530	36.642	4.1709	11	
	Other parameters				
k_{R_PR}	0.65				Malecky *et al.* (2009)
k_{R_P}	0.15				Domingue *et al.* (1991)
k_{PR_P}	0.68				AFRC (1993)
k_{P_PR}	0.047				Harmeyer and Martens (1980)
k_{P_PR}	0.001				Harmeyer and Martens (1980)
k_{P_Body}	0.056				Observed
	Pools (g)				
Q_R	53				Malecky *et al.* (2009)
Q_PR	40				Brun-Bellut *et al.* (1991)
Q_P	36				Observed
Q_Body	1238				AFRC (1997)

k_{P_PR}) were obtained from the literature and not estimated (Table 3).

Schematic representation of the model is shown in Fig. 2. Description of pools and the associated differential equations describing the 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 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/day. 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. These authors recorded variables related to rumen fermentation, duodenal nutrient flow and milk yield, and determined k_{R_PR} to be 0.65/day. They also estimated a rumen pool size, including diet and recycled N, to be ~53 g. Pool-size change over time and fluxes are defined below.

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

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

Inflows:

$$F_{feed\_R} = NI \times ((100 - RUPd)/100),$$
$$F_{PR} = k_{PR} \times Q_P$$

Outflows:

$$F_{R\_PR} = k_{R\_PR} \times Q_R,$$

$$F_{R\_P} = k_{R\_P} \times Q_R,$$

where NI is N intake (NI = (DMI × CPd/100))/6.25). DMI is daily DM intake and CPd is the diet CP.

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 40 g, based on the study of N flows through rumen, duodenum ileum and rectum by Brun-Bellut *et al*. (1991) with lactating Saanen goats having a bodyweight of 48 kg, 1541 g DM intake/day 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 faecal N excretion (F_{PR_faeces}). The rate constant k_{PR_P} (0.68/day) was calculated from the estimated apparent total-tract CP 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 CP; and the proportion of microbial CP present that is microbial true protein was assumed to be 75% and with digestibility of 85% (NRC 2007). The flux from post-rumen to faeces was the experimentally observed average N excreted (15 g/day), and the estimated rate constant k_{PR_faeces} was 0.375/day in Model 1; whereas, the rate constant and intercept in Models 2 and 3 were the same at: k_{PR_faeces} = 0.265/day and P_f = 2.52 g N/day. Pool-size change over time and fluxes are defined below.

Change over time in post-rumen N pool (g N/day):

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

Inflows:

$$F_{feed\_PR} = NI \times (RUP/100),$$
$$F_{R\_PR} = k_{R\_PR} \times Q_R$$

Outflows:

$$F_{PR\_faeces} = k_{PR\_faeces} \times Q_{PR}(\text{Model 1}),$$

$$F_{PR\_faeces} = k_{PR\_faeces} \times Q_{PR} + P_f(\text{Models 2, 3}),$$

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

where P_f is the intercept of the regression line, representing the MFN.

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

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

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

Plasma pool, Q_P (g N)

The plasma pool includes the total peptide-N, urea-N and ammonia-N and an amount of 36 g was obtained from

blood-sample analyses and plasma-volume measures (Trials C and D; Criscioni and Fernández 2016; Ibáñez *et al*. 2016). This pool has three inflows; one comes from rumen ammonia-N absorption through the rumen wall (F_{R_P}), another one from microbial protein absorbed from the small intestine (F_{PR_P}) and the last one from body protein catabolism (F_{Body_P}). The fluxes F_{R_P} and F_{PR_P} were defined previously. The muscle N anabolic and catabolic fluxes were assumed equal for mid- and late-lactation goats ($F_{Body\_P} = -F_{P\_Body}$). There are five outflows from the plasma pool. Two of them are plasma N flux to rumen (F_{P_R}) and post-rumen (F_{P_PR}), and the other three are urinary N excretion (F_{P_urine}), N excreted in milk (F_{P_milk}) and N retention in body 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), who considered plasma urea-N entering the rumen with saliva to be 1.68 g/day ($k_{P\_R} = 0.047$) and plasma urea-N entering the gut to be 0.03 g/day ($k_{P\_PR} = 0.001$). The observed average N outflows in urine and milk from our dataset were 19 g/day and 12.4 g/day respectively. Initial parameter values (i.e. to be used to initialise the likelihood-based parameter estimation in the dynamic model) describing such fluxes were obtained from preliminary linear and non-linear regression, as indicated previously. The following 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 $k_{P\_urine} = 0.528$ and $k_{P\_milk} = 0.344$. For (2), the initial estimates for the rate constant and intercept for urine N excretion were $k_{P\_urine} = 0.805$/day and $P_u = -7.75$ g N/day, and, for milk N excretion, they were $k_{P\_milk} = 0.199$/day and $P_{milk} = 5.69$ g N/day. Finally, for (3), the maximal daily N excretion (V_{max}) was 26.59 g/day 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/day), so the k_{P_Body} was 0.056/day. 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/day):

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

Inflows:

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

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

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

Outflows:

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

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

$$F_{P\_urine} = k_{P\_urine} \times Q_P \text{ (Model 1)}$$

$$F_{P\_urine} = k_{P\_urine} \times Q_P + P_u \text{ (Models 2, 3)}$$

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

$$F_{P\_milk} = k_{P\_milk} \times Q_P \text{ (Model 1)}$$

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

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

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), with iQ_P being 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 mobilisation 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. Brown and Taylor (1986) reported the mean composition of a heterogeneous group of 15 French Alpine, Nubian and Toggenburg females ranging in liveweight from 38 to 70 kg, and from 2 to 5 years of age, including both lactating and pregnant animals. Mean data for this group were 7.9 kg of protein, which, converted to percentage of body CP in Murciano–Granadina goats, was 18%. Thus, the body N pool with an average bodyweight of 43 kg was 1238 g N. Pool-size change over time and fluxes are defined below.

Change over time in N body pool (g N/day):

$$dQ_{Body}/dt = F_{P\_Body} - F_{Body\_P}$$

$$\text{Inflow} : F_{P\_Body} = k_{P\_Body} \times Q_P$$

$$\text{Outflow} : F_{Body\_P} = -F_{P\_Body}$$

The body N pool size was expressed by the integral equation

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

representing the quantity of N accumulated from initial time (t_0) to final time (t), with iQ_{Body} being the initial pool size.

Model development: parameter estimation and adequacy assessment

Conceptual model structure was defined from biological definitions of N utilisation by lactating animals (NRC 2001; Kebreab *et al.* 2002) and the parameter estimation was performed by minimising the negative log-likelihood function (LLF) by using an adaptive non-linear least-square optimisation algorithm (Generalised NL2SOL, Dennis *et al.* 1981) available in ACSLX (Aegis Technologies Group). An LLF-based goodness-of-fit method, Bayesian information criterion (BIC), was used to compare Models 1, 2 and 3. A smaller BIC indicates a better fit to the data. In general, BIC penalises models with more

parameters; thus, larger models with the same LLF values have a larger BIC.

Subsequently, to characterise model inadequacy (i.e. bias) in the range of our observations, the observed values of faecal, urinary and milk N were compared with model predictions and the discrepancy was calculated as the root mean-square prediction error (RMSPE). The RMSPE was then decomposed into error due to the overall bias of prediction (i.e. mean bias), error due to deviation of the regression slope from unity (i.e. slope bias), and error due to the disturbance or random variation (Bibby and Toutenburg 1977). The model adequacy of the best-fitting model was further assessed outside the range of our observations by fitting a regression line between observed and predicted values and considering the intercept and slope deviations from 0 and 1 (i.e. unity line) respectively. This exercise extrapolates to zero and beyond the maximum observed values, and, thus, quantifies the applicability domain for the model under consideration.

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

Sensitivity analysis

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

Model evaluation against external data

The final chosen model was compared against a set of external data to assess its predictive ability. Twelve studies were used to evaluate the predictive ability of the model (see Table 4 for details). These studies contained a total 42 different treatments with varying levels of protein (from 10% to 20%), combined different breeds (Granadina, Murciano–Granadina, Saanen and Alpine), milk production levels and stages of lactation. NI was estimated from the reported diet composition and table values for each ingredient (FEDNA 2010). The description of the database used to independently challenge the model is shown in Table 4. The metric implemented to compare the model prediction against the independent experimental observations, for the outflows of N in urine, faeces and milk, was the RMSPE as described previously.

Results and discussion

Figure 2 shows the five datasets for N faeces (*a*), N urine (*b*) and N milk (*c*) outflows. Data points from the same experiment share the same colour and were connected by solid lines. In obtaining initial parameter estimates for the subsequent parameterisation of the dynamic model, the data for faecal and urine N responses were best fit by a straight line, whereas data for milk N responses were best fit by curvilinear

Table 4. Summary of the data used for model evaluation
CPd, crude protein of diet; DMI, DM intake; ME, metabolisable energy; N, nitrogen; NDF, neutral detergent fibre; NI, N intake

Variable (g/day)	Mean	Min.	Max.	s.d.
Aguilera et al. (1990)				
Treatments: 6				
Breed: Granadina				
Stage of lactation: early–mid–late lactation				
NDF (%)	40	–	–	–
CPd (%)	14	12	16	1.5
Bodyweight (kg)	38.6	34.1	41.4	3.32
Milk yield (kg/day)	1.01	0.38	1.51	0.467
DMI	1218	989	1616	251.0
N intake	27.9	22.0	38.1	6.14
N faeces	9.5	7.9	13.0	2.04
N urine	8.8	5.6	13.0	2.82
N milk	6.4	5.0	8.5	1.37
N body	3.2	2.4	3.7	0.50
Schmidely et al. (1999)				
Treatments: 4				
Breed: Saanen, Alpine				
Stage of lactation: mid-lactation				
NDF (%)	28.6	14.6	42.6	16.17
CPd (%)	14.2	13.9	14.7	0.34
Bodyweight (kg)	66.0	63.0	69.0	3.00
Milk yield (kg/day)	3.0	2.8	3.2	0.19
DMI	2200	2000	2400	230.9
N intake	49.8	44.4	54.2	4.93
N faeces	15.2	14.2	15.9	0.70
N urine	16.8	15.0	19.0	1.98
N milk	11.8	9.8	13.0	1.53
N body	6.1	5.1	6.9	0.98
Bava et al. (2001)				
Treatments: 4				
Breed: Saanen				
Stage of lactation: early–mid–late lactation				
NDF (%)	34	33	37	1.9
CPd (%)	16	15	20	2.6
Bodyweight (kg)	57.4	52.0	61.1	3.85
Milk yield (kg/day)	3.13	1.89	4.37	1.18
DMI	2312	1975	2627	358.9
N intake	60.2	48.1	65.9	8.21
N faeces	19.4	14.0	21.8	3.69
N urine	24.5	16.0	29.0	5.81
N milk	13.2	12.0	14.0	1.00
N body	2.8	0.9	6.1	2.41
Rapetti et al. (2005)				
Treatments: 3				
Breed: Saanen				
Stage of lactation: mid-lactation				
NDF (%)	32	30	34	2.04
CPd (%)	18	17	18	0.9
Bodyweight (kg)	55.0	46.0	64.0	9.00
Milk yield (kg/day)	3.30	3.01	3.68	0.347
DMI	2170	2054	2354	161.4
N intake	61.9	56.4	69.5	6.81
N faeces	19.5	18.4	20.4	1.02
N urine	24.0	23.0	26.0	1.73
N milk	13.3	13.0	14.0	0.58
N body	5.1	–0.1	9.9	4.97

(*continued next page*)

Table 4. (*continued*)

Variable (g/day)	Mean	Min.	Max.	s.d.
Sari et al. (2009)				
Treatments: 4				
Breed: Saanen				
Stage of lactation: early lactation				
NDF (%)	36	–	–	–
CPd (%)	16	16	17	0.2
Bodyweight (kg)	41.0	39.5	42.5	1.50
Milk yield (kg/day)	1.88	1.75	1.95	0.0866
DMI	2160	1985	2250	119.2
N intake	56.9	53.1	59.0	2.63
N faeces	15.3	14.7	16.1	0.57
N urine	22.3	20.0	24.0	1.71
N milk	13.1	13.0	13.3	0.13
N body	6.2	4.4	7.2	1.28
Molina-Alcaide et al. (2010)				
Treatments: 3				
Breed: Murciano–Granadina				
Stage of lactation: mid-lactation				
NDF (%)	55	47	62	7.49
CPd (%)	17	16	17	0.6
Bodyweight (kg)	38.9	38.3	39.5	0.60
Milk yield (kg/day)	1.09	0.97	1.26	0.149
DMI	1358	1295	1405	56.6
N intake	36.1	34.3	37.9	1.77
N faeces	10.7	9.9	11.9	1.04
N urine	13.5	12.8	14.2	0.71
N milk	9.3	8.5	10.0	0.76
N body	2.5	1.7	3.8	1.13
Romero-Huelva et al. (2012)				
Treatments: 4				
Breed: Murciano–Granadina				
Stage of lactation: mid-lactation				
NDF (%)	60	59	60	0.619
CPd (%)	20	19	20	0.3
Bodyweight (kg)	39.5	38.6	40.3	0.96
Milk yield (kg/day)	1.00	0.94	1.04	0.042
DMI	1572	1548	1631	39.5
N intake	49.1	48.1	50.3	1.01
N faeces	11.5	11.2	11.8	0.30
N urine	21.8	20.3	23.4	1.35
N milk	12.3	12.0	12.6	0.25
N body	3.5	3.1	4.2	0.50
Santos et al. (2014)				
Treatments: 4				
Breed: Alpine				
Stage of lactation: mid-lactation				
NDF (%)	44	42	45	1.24
CPd (%)	10	10	11	0.3
Bodyweight (kg)	42.0	35.9	48.1	6.10
Milk yield (kg/day)	1.70	1.30	2.10	0.4
DMI	1955	1845	2024	83.2
N intake	32.1	30.4	34.0	1.48
N faeces	15.1	13.3	17.5	2.06
N urine	10.5	10.0	11.1	0.61
N milk	8.4	8.0	9.0	0.51
N body	–2.0	–3.2	–0.1	1.34

Table 4. (*continued*)

Variable (g/day)	Mean	Min.	Max.	s.d.
Dos Santos et al. (2016)				
Treatments: 4				
Breed: Saanen				
Stage of lactation: early lactation				
NDF (%)	31	25	33	1.96
CPd (%)	15	10	19	3.9
Bodyweight (kg)	42.7	41.3	44.1	1.43
Milk yield (kg/day)	2.00	1.78	2.22	0.22
DMI	1815	1569	1970	175.0
N intake	41.7	29.1	50.4	9.58
N faeces	10.1	7.7	11.1	1.58
N urine	13.3	8.0	18.0	4.99
N milk	9.8	7.5	12.0	2.08
N body	8.6	2.7	12.0	4.05
Criscioni et al. (2016)				
Treatments: 2				
Breed: Murciano–Granadina				
Stage of lactation: late lactation				
NDF (%)	34	32	35	2.47
CPd (%)	16	16	16	0.4
Bodyweight (k)g	45.8	45.6	45.9	0.21
Milk yield (kg/day)	1.71	1.66	1.76	0.076
DMI	1700	1600	1800	141.4
N intake	46.6	42.1	51.1	6.38
N faeces	12.3	10.5	14.1	2.53
N urine	12.8	10.5	15.0	3.16
N milk	11.4	10.5	12.3	1.28
N body	10.1	9.7	10.5	0.59
Fernández et al. (2018)				
Treatments: 2				
Breed: Murciano–Granadina				
Stage of lactation: late lactation				
NDF (%)	26	21	31	7.07
CPd (%)	17	16	17	0.7
Bodyweight (kg)	44.1	41.5	46.7	3.68
Milk yield (kg/day)	1.75	1.70	1.80	0.071
DMI	1600	1500	1700	141.4
N intake	43.7	40.0	47.4	5.23
N faeces	13.0	10.9	15.0	2.93
N urine	13.8	12.7	15.0	1.64
N milk	9.9	9.7	10.1	0.35
N body	7.0	6.8	7.2	0.32
Fernández et al. (2019)				
Treatments: 2				
Breed: Murciano–Granadina				
Stage of lactation: mid-lactation				
NDF (%)	29	26	32	3.75
CPd (%)	16	15	17	1.8
Bodyweight (kg)	43.3	42.5	44.1	1.31
Milk yield (kg/day)	1.29	1.25	1.33	0.040
DMI	1525	1360	1690	233.4
N intake	40.6	34.1	47.1	9.15
N faeces	12.5	9.0	16.1	5.02
N urine	11.2	8.5	13.9	3.79
N milk	9.3	7.7	11.0	2.36
N body	7.6	6.1	9.0	2.03

saturating curve (Fig. 2*a*, *b*). Visually, the efficiciency of conversion between N intake and milk N, across all trials, appears non-constant across studies, in agreement with previous observations that N partition towards milk marginally decreases with an increasing N intake (Doepel *et al*. 2004; Dijkstra *et al*. 2013). No significant ($P > 0.05$) effect of the study was observed during this preliminary analysis. In addition, the interaction between study and the linear and quadratic components of the function was not different from zero, suggesting consistency of the milk N-excretion response across trials.

During parameterisation of the dynamic model, the negative LLF was –722.31 for Model 1 and –711.96 for Models 2 and 3. Also, BIC was lower in Models 2 and 3 than in Model 1 (1451.79 vs 1458.55 respectively; Table 5). On the basis of the BIC, Models 2 and 3 fitted the data better than did Model 1, but Models 2 and 3 seemed to fit the data equally well, hence suggesting that the flux of milk N output can be described well both by a mass action or a Michaelis–Menten function. However, parameter estimates were more precise when the saturating function was assumed (Table 3). The fractional rate k_{P_urine} had a variation coefficient (CV) of ~18% in Models 1 and 2, but it was reduced to 10% in Model 3. The fractional rate k_{P_milk} had a CV of 21% in Model 1, but was reduced to 4% in Model 2. However, the intercept for milk N output at zero N intake (P_{milk}) was high at 20%. In comparison, the k_m and V_{max} parameters of saturating representation in Model 3 had a rather low CV at 7% and 11% respectively.

Across the three models, the errors of prediction in the range of our observations were ~21% for faecal, 19% for milk and 37% for urine N flows respectively (Table 6). The mean and slope bias were zero for all fluxes in Models 2 and 3, but not for the flux from faeces and urine in Model 1, which presented an error of 3.28% in faeces and 0.68% in urine. Model adequacy was, therefore, better for Models 2 and 3 than for Model 1.

Table 5. Model prediction errors and decomposition associate with prediction of the outputs

Variable abbreviations are defined in Table 2. RMSPE, root mean-square prediction error as a percentage of observed mean

Variable	RMSPE (%)	Mean bias (%)	Slope bias (%)	Random bias (%)
		Model 1		
F_{PR_faeces}	21.08	3.28	0	96.72
F_{P_urine}	37.70	0.68	0	99.32
F_{P_milk}	19.47	0	0	99.99
		Model 2		
F_{PR_faeces}	20.73	0	0	100
F_{P_urine}	37.57	0	0	99.99
F_{P_milk}	19.47	0	0	100
		Model 3		
F_{PR_faeces}	20.73	0	0	100
F_{P_urine}	37.57	0	0	100
F_{P_milk}	19.47	0	0	100

Thus, the goodness-of-fit measures suggested Model 1 to provide inferior fit to data but it did not clearly discriminate between Models 2 and 3. However, Model 3 had more precise parameter estimates. Furthermore, because experimentally we have consistently observed that the average milk N output progressively decreases as N intake increases (Fig. 2*c*), we decided to retain the Michaelis–Menten representation depicted by Model 3 as a more biologically meaningful description of N partition. In summary, in the range of our observations, Model 3 predicted N excretion in faeces (15.6 g/day) and urine (15.4 g/day) and milk N output (11.7 g/day), whereas the observed values were 15.31 g N/day in faeces, 18.78 g N/day in urine and 12.24 g N/day in milk, as shown in Table 1.

Gauging the domain of applicability of the chosen Model 3, Fig. 3 displays observed versus predicted values and the corresponding unity regression equation (i.e. observed = predicted). The model presented the least bias for the faecal N data in the range of 14–20 g/day, but below and above this range, it underestimated and overestimated. Also, it had a nearly unbiased fit to urinary N data from 10 to 25 g/day; however, above 25 g/day, the model tended to underestimate urinary N output. For milk N, the model bias was minimal in the range of 9–14 g/day, whereas above 14 g/day, it overestimated milk N output. The residual standard error for faecal, urinary and milk N showed that the model was off by 1.38, 2.68 and 1.63 g/day. Figure 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 faecal, urine and milk N flows, within and outside the range of observed data. For the ranges between 14 and 20 g/day, 10 and 25 g/day and 9 and 14 g/day for faecal, 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 faeces and milk, indicating that the model overpredicted flows as the predicted flow increased. The slope was negative for urinary N, indicating that the model underpredicted flows as the predicted flow increased. Therefore, extrapolating outside the above ranges will yield increasingly biased predictions.

Sensitivity analysis of faecal N, urinary N excretions and milk N to the model parameters was performed (Table 7). The F_{PR_faeces} was sensitive to the digestibility coefficient and F_{P_urine} was sensitive to both digestibility coefficient and urinary loss-rate constant. This implied 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

Table 6. Goodness-of-fit models

LLF, likelihood function; *k*, numbers of parameters; *n*, number of observations; AIC, Akaike information criterion; BIC, Bayesian information criterion

Model	LLF	*k*	*n*	BIC	AIC
1	–722.31	3	104	1458.55	14.75
2	–711.96	6	104	1451.79	15.67
3	–711.96	6	104	1451.79	15.67

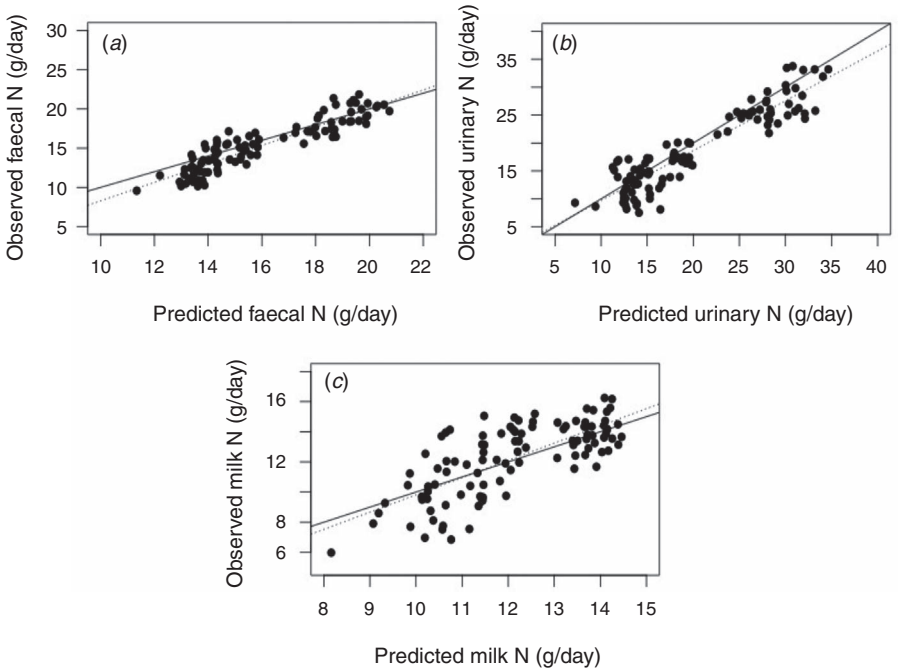

Fig. 3. Observed versus predicted plot of (*a*) faecal, (*b*) urine and (*c*) milk output in Model 3. The regression equations were as follow: faeces, $Y = -3.19 + 1.18X$ (s.e. = 0.84 and 0.05 for the intercept and slope respectively; residual standard error = 1.32; $R^2 = 0.83$); urine, $Y = -0.49 + 0.95X$ (s.e. = 0.81 and 0.04 for the intercept and slope respectively; residual standard error = 2.67; $R^2 = 0.86$); milk, $Y = -1.62 + 1.14X$ (s.e. = 1.27 and 0.01 for the intercept and slope respectively; residual standard error = 1.63; $R^2 = 0.54$).

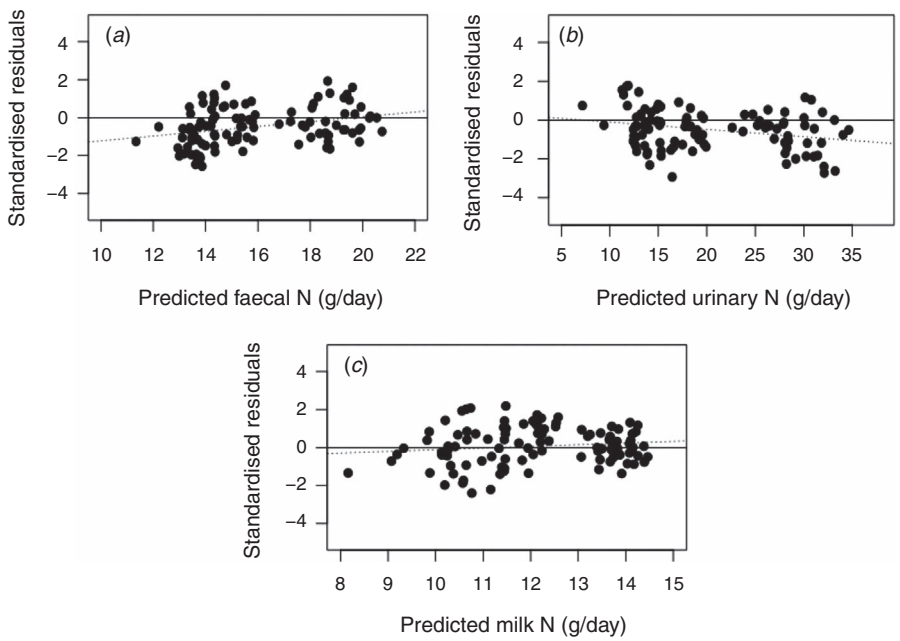

Fig. 4. Residual plot of (*a*) faecal, (*b*) urine and (*c*) milk output in Model 3.

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/day (Harmeyer and Martens 1980) via mass action with a more flexible, possibly non-linear, representation accounting for

Table 7. Global sensitivity analysis to model parameters
Abbreviations are defined in Table 2

Parameter	F_{PR_faeces}	F_{P_urine}	F_{P_milk}
k_{PR_faeces}	0.7954	0.4128	0.0464
P_f	0.1627	0.0844	0.0138
k_{P_urine}	0.0135	0.1981	0.2016
P_u	0.0038	0.0858	0.1360
V_{max}	0.0007	0.1308	0.4327
K_m	0.0002	0.0290	0.0853

Table 8. Evaluation of Model 3 with original values obtained by literature
N, nitrogen; RMSPE, root mean-square prediction error as a percentage of observed mean

Variable	Observed	Predicted	RMSPE (%)	Mean bias (%)	Slope bias (%)	Random bias (%)
N in faeces	13.56	14.95	19.12	23.73	0.25	76.01
N in urine	16.13	18.91	19.87	54.55	19.27	26.18
N in milk	10.55	10.65	7.85	1.35	21.35	77.29

carbohydrate profile, supply and fermentation, microbial growth and the resulting ammonia–urea exchanges (Reynolds and Kristensen 2008). Similarly, the assumption of zero growth currently included in the model is likely to be equivocal, and generating data on body N accretion by goats during first and second lactation and throughout the full lactation would provide a better description of N allocation and recycling into urea towards the rumen. However, 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 characterise N-use efficiency (Hanigan *et al*. 2008).

In the following, we compare our basal faecal and urinary N-loss parameter estimates with values reported historically in the experimental literature. The N in the faeces 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 faecal N 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 grams of faecal N on grams of 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 g N/kg $W^{0.75}$. Published values for goats are few and Sahlu *et al*. (2004, included in NRC 2007), reported a mean value for MFN of 4.27 g N/kg DMI. The value estimated for our model was 3.85 g N/kg DMI, being similar to the NRC (2007) estimates.

With respect to urinary N excretion, it has traditionally been divided into two components, namely, 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 goats of an average of 43-kg bodyweight, the EUN was 2.245, 1.671 and 2.788 g N/day respectively, which is similar to the intercept value obtained in our model, i.e. 2.679 g N/day.

Following is a test of the predictive ability of the model against an independent dataset, and results are reported in Table 8. Aguilera *et al*. (1990) found 9, 8 and 6 g/day of N in faeces, urine and milk respectively, in Granadina goats in mid-lactation fed alfalfa hay and barley diets (CP 14% and 16%). The simulated values from our chosen model (Model 3) were 11, 9 and 7 g/day, which resulted 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, being similar to those in our studies. Some diets replaced part of the cereal in the grain mix with nutrients blocks than incorporated by-products 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 faecal, urinary and milk N outflows were 11, 18 and 6 g/day, whereas values predicted by the model were 15, 18 and 11 g/day, resulting 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 the same level of CP, i.e. 10%), the values simulated were close to the observed values when the source of protein was soybean meal; observed faecal, urinary and milk N outflows were 13, 6 and 7 g/day, whereas predicted values were 12, 8 and 9 g/day, resulting in an error of 8%, 20%, 27% respectively. The study of Bava *et al*. (2001) was conducted with lactating Saanen goats at early, mid- and late lactation, and the goats were fed with silage and non-forage diets. For this trial, the average error was 13%, 22% and 26% for faecal, urinary and milk N respectively. Dos Santos *et al*. (2016) fed Saanen lactating goats with pelleted diets, increasing the CP of the diet from 10% to 19% (by substitution of alfalfa hay with soybean meal). When goats were fed 10% CP, the observed faecal, urinary and milk N outflows were 11, 4 and 8 g/day, whereas our predicted values were 12, 6 and 9 g/day, resulting in an error of 6%, 36%, 16% respectively. The prediction was worse when goats were fed 19% CP, with observed faecal, urinary and milk N outflows at 8, 7 and 9 g/day, whereas predicted values were 15, 16 and 12 g/day, resulting in an error of 49%, 58% and 26%.

Across the models, the predicted faecal and urinary N excretion with acceptable RMSPE was between 19% and 20%, and milk N excretion was ~8%. Unexplained random error made up the largest portion of the predicted flows for faecal and milk N, namely ~76–77%. Mean and slope bias in

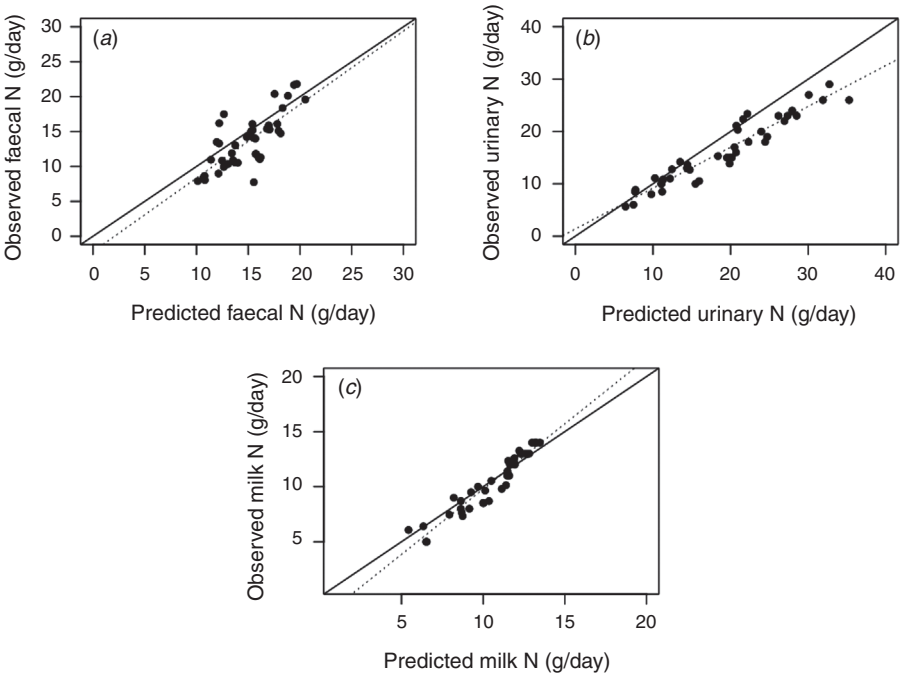

Fig. 5. Observed versus predicted plot of (*a*) faecal, (*b*) urine and (*c*) milk output for external evaluation. The regression equations were as follow: faeces, $Y = -2.19 + 1.05X$ (s.e. = 2.23 and 0.14 for the intercept and slope respectively; residual standard error = 2.56; $R^2 = 0.56$); urine, $Y = -1.37 + 0.78X$ (s.e. = 0.82 and 0.04 for the intercept and slope respectively; residual standard error = 1.97; $R^2 = 0.90$); milk, $Y = -2.08 + 1.19X$ (s.e. = 0.61 and 0.06 for the intercept and slope respectively; residual standard error = 0.75; $R^2 = 0.92$).

predicted faecal N output were ~24% and 0% respectively, whereas for predicted milk N output, they were 1% and 21% respectively (Fig. 5*a*, *c*).

The majority of the error in urine N flow predictions (19.87%) is due to mean bias (55%) and slope bias (19%; Fig. 5*b*), both of which sum up to ~74% (Table 8). Mostly, the issue is one of overpredicting N loss in urine (i.e. the goats urinated less N than the model predicted; Figs 5*b*, 6*b*), especially in the studies that use rations with high concentrations of CP, such as those from Rapetti *et al*. (2005,18%), Criscioni *et al*. (2016, 16%) and Schmidely *et al*. (1999, 16%), which resulted in urine N excretion levels beyond 20–25 g/day. Nonetheless, acceptable predictions were observed when dietary CP ranged between 10% and 15%, with N urine excretion between 7–15 g/day; it is important to recall that the model was parameterised and shown to be fairly adequate in the range of 10–25 g/day of urinary N output. However, while extrapolating the model perhaps explains some portion of the prediction bias, other factors may also partially explain such systematic error in urine-N flow predictions. These include (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.

However, overall, the largest errors observed against the independent dataset for faecal, urine and milk N predictions are in the magnitude of 1–3 g/day, with respect to mean fluxes of ~15, 20 and 10 g/day, which suggests that the model structure reflects well the biology of N use by goats.

So as 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, DMI, carbohydrate source and concentration, and production potential, and their interactions with N supply on its partition.

It, thus, appears that the model satisfactorily characterises N excretion and milk N secretion in lactating goats fed mixed diets that supply dietary N in the range of 30–70 g/day. Extrapolating beyond this level of N intake, our estimations of N excretion are inflated because we are likely to be 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

Of the various models evaluated here, the best one presented here simulated the effect of N intake on N excretion in faeces, 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 ~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

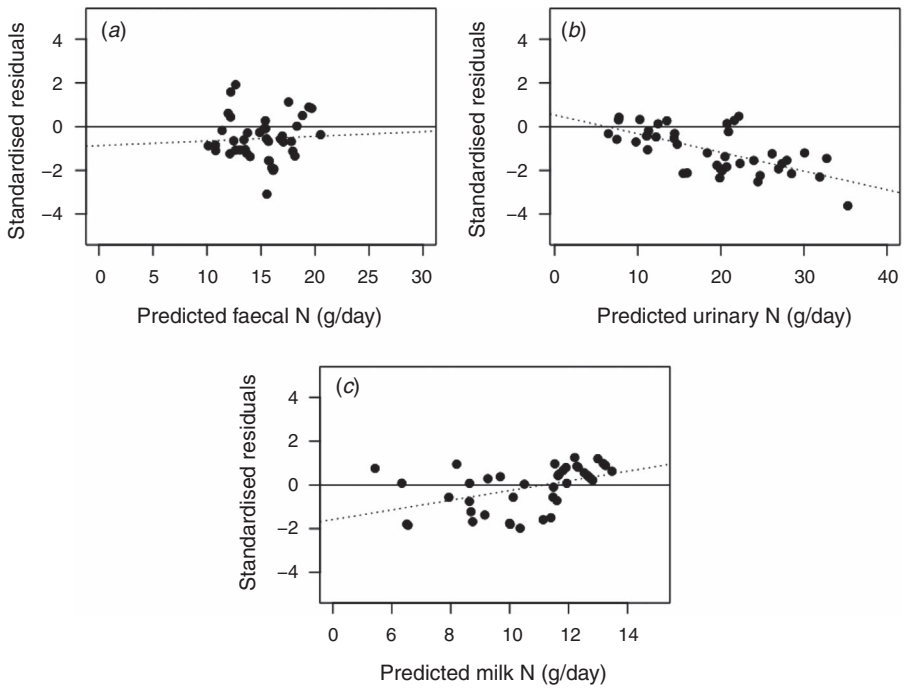

Fig. 6. Residual plot of (*a*) faecal, (*b*) urine and (*c*) milk output for external evaluation.

accretion during simultaneous growth and lactation, N recycling under different dietary-N and carbohydrate regimes, and N allocation towards milk at different 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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