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**Citation:** Foskolos A, Moorby JM (2018) Evaluating lifetime nitrogen use efficiency of dairy cattle: A modelling approach. PLoS ONE 13(8): e0201638. https://doi.org/10.1371/journal. pone.0201638

Editor: Juan J Loor, University of Illinois, UNITED STATES

Received: May 31, 2017

Accepted: July 19, 2018

Published: August 2, 2018

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**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** Financial support was provided by the Welsh Government and Higher Education Funding Council for Wales through the Sêr Cymru National Research Network for Low Carbon, Energy and Environment. Project: Consequential Life Cycle Assessment of Environmental and Economic Effects of Dairy and Beef Consolidation and Intensification Pathways - "Cleaner Cows." Financial support from the UK Department for **RESEARCH ARTICLE** 

# Evaluating lifetime nitrogen use efficiency of dairy cattle: A modelling approach

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# Abstract

The increased nitrogen (N) use efficiency in cattle farming is proposed as a key action to improve N management and reduce the environmental impact of cattle farming systems. Most attention has been given to lactating cow nutrition, excluding the elements of fertility, disease, and the non-lactating animals within the herd. Therefore, the aim of the current study was to develop a herd-level simulation model incorporating these elements to assess dairy farm N use efficiency. We developed a cattle N use efficiency (CNE) model with six primary compartments: (i) heifer growth, (ii) heifer removal, (iii) pregnancy, (iv) cow removal, (v) disease and fertility, and (vi) milk production. The CNE model calculates N loss or gain for each compartment, and then calculates the lifetime N loss or gain taking into account the replacement rate (rep) and/or the corresponding number of lactations in a herd (Lact = 1/rep). Finally, three N use efficiencies were estimated: (i) RepINE: replacement cattle N use efficiency, (ii) LactNE: lifetime N use efficiency for lactation, and (iii) LNE: lifetime N use efficiency. The sensitivity of the model to variation in farm- and animal-related input values was evaluated using Monte Carlo simulation. Values for a model dairy farm were used based on published data reflecting typical dairy farming practices in the United Kingdom. To assist reporting net values of main N outputs, a dairy herd of 100 lactating cows was modelled. Productive N outputs (1000s of kg) over the course of an animal's lifetime, partitioned into milk and meat, were dominated by milk production (89% of total N output). We estimated a mean RepINE of 23.7%, affected most by the last stage of heifer growth. The Monte Carlo sensitivity analysis suggested that variation in time to first calving (T<sub>1stCal</sub>) might cause larger changes on RepINE than variation in feed. The sensitivity analysis revealed a strong positive correlation between dietary oriented milk N use efficiency (MNE) and LactNE and LNE (r = 0.99 and 0.97 for LactNE and LNE, respectively). However, our study highlighted two other model variables that affected LNE. Variation in calving interval (CI; r = -0.15) and T<sub>1stCal</sub> (r = -0.15) may cause measurable reductions of overall LNE. The first is an indicator of lactating cattle fertility, and the second an indicator of replacement cattle growth and fertility efficiency. In conclusion, with the current study we provided a dairy cattle herd model that is sensitive in elements of diet, fertility and health. Lifetime N use efficiency of dairy cattle is dominated by MNE, but we detected specific nondiet related variables that affect RepINE, LactNE and LNE.

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Environment, Food and Rural Affairs is also gratefully acknowledged. The Institute of Biological, Environmental & Rural Sciences gratefully acknowledges funding from the Biotechnology and Biological Sciences Research Council.

**Competing interests:** The authors have declared that no competing interests exist.

# Introduction

Increasing public awareness about environmental issues and the environmental impacts of dairy production challenge the dairy industry to perform in a more environmentally responsible way [1, 2]. Nitrogen (N) is an essential component of human food production, determining the productivity of crops and animals in fertilizers and feeds [3]. However, its extensive use has led to the so-called N cascade phenomenon, which refers to the circulation of anthropogenic N in ecosystems causing multiple effects on atmospheric, freshwater and marine environments [4–6]. European data suggest that agriculture is the main contributor to this phenomenon accounting for approximately 78% of total N entering the ecosystem [7] and an increase in the efficiency of N use in crop and animal production is proposed as a key action to improve N management [8].

Nitrogen use efficiency can be defined at the levels of the animal, the farm, and the entire agricultural supply chain. In milk production, milk N use efficiency (MNE), defined as the amount of milk N produced relative to N intake at an individual dairy cow level, is commonly used [9–11]. However, this approach focuses almost exclusively on the lactating cow and her nutrition, and generally excludes elements of fertility and disease as well as growing and nonlactating animals within the herd. Animal disease and fertility status affect milk production and it is possible to quantify these effects [12-15]. Moreover, growing animals can represent a significant proportion of animals in the production system [16]. Calves are a required consequence of milk production from cows, and contribute to herd replacements and to meat production. Therefore, besides the economic cost of heifer rearing, there is an impact on overall herd N use efficiency. Gross N use efficiency of milk-fed calves between 39 and 50%, and of growing heifers between 20 and 28% were reported [17]. Various studies have evaluated N use efficiency either at a cow level [9, 18] or at a farm level [15, 19, 20]. As a result, relative N use efficiency at a system level has been proposed [21]. However, none of the previous studies have incorporated elements of performance, fertility, and diseases of lactating and replacement cattle into a single model. Therefore, the aim of the current study was to develop a herd-level simulation model incorporating these elements to assess dairy cattle lifetime N use efficiency.

# Materials and methods

# Model description

The dairy cattle N use efficiency (CNE) model was developed as a spreadsheet using Microsoft Excel and consists of six primary compartments: (i) heifer growth, (ii) heifer removal, (iii) pregnancy, (iv) cow removal, (v) disease and fertility, and (vi) milk production. Other potential farm parameters, such as the use and fate of N in manures, fertilisers, and male and surplus heifer calves sold off farm, were not considered. The definitions and abbreviations of model entities are presented in Table 1 and a schematic description of the CNE model is given in Fig 1.

**Heifer growth.** For the heifer growth compartment, the equations of the 2001 Dairy NRC model were used [22] to calculate N loss or gain. Four stages of growth were considered: (i) from birth to weaning, (ii) from weaning to  $BW_{100}$ , (iii) from  $BW_{100}$  to first service, and (iv) from first service to first calving. For the stages i and ii, N requirements (in the 2001 Dairy NRC model denoted as CPreq; currently expressed in N using the N<sub>6.25</sub> factor) at birth, weaning and  $BW_{100}$  were calculated assuming daily BW gain of 400, 800 and 800 g, respectively, for calves fed milk replacer and starter diets, or weaned calves fed solid diets [22]. Cumulative amounts of N required for each stage (N<sub>FeedReq\_w</sub>, N<sub>FeedReq\_BW100</sub>) were calculated as the area under the interpolated line assuming N requirement was a linear function of time (T<sub>W</sub>, T<sub>100</sub>;



Entity	Unit	Level	Definition
Heifer growth a	nd removal		
BW <sub>100</sub>	kg	animal	Heifer at 100 kg of body weight
BW <sub>1stCal</sub>	kg	animal	Body weight at first calving
BW <sub>1stSer</sub>	kg	animal	Body weight at first service
BWB	kg	animal	Body weight at birth
BW <sub>c</sub>	kg	animal	Body weight of heifer at 6 months of age
BWn	kg	animal	Body weight of neonatal calf
CPreq	kg	animal	Crude protein requirements for heifer growth
m <sub>c</sub>	N/A <sup>a</sup>	herd	Calf mortality rate
m <sub>h1stCal</sub>	N/A	herd	Heifer mortality rate from first service to first calving
m <sub>h1stSer</sub>	N/A	herd	Heifer mortality rate from six months of age to first service
m <sub>n</sub>	N/A	herd	Neonatal mortality rate
m <sub>p</sub>	N/A	herd	Perinatal mortality rate
MP:CP <sub>1stCal</sub>	N/A	animal	Metabolizable protein to crude protein ratio at first calving
MP:CP <sub>1stSer</sub>	N/A	animal	Metabolizable protein to crude protein ratio at first service
MPGrowth	kg	animal	Metabolizable protein requirements for growth
N <sub>6.25</sub>			Protein to nitrogen conversion constant (N = protein/6.25)
N <sub>FeedRea</sub>	kg	animal	Total feed nitrogen required for heifer's growth
N <sub>FeedReg</sub> BW100	kg	animal	Feed nitrogen required for calf growth from weaning to reach 100 kg of body weight
N <sub>FeedReg</sub> c	kg	animal	Feed nitrogen required for calf growth
N <sub>FeedReg</sub> h1stCal	kg	animal	Feed nitrogen required for heifer growth from first service to first calving
N <sub>FeedReg_blstSer</sub>	kg	animal	Feed nitrogen required for heifer growth from 100 kg of body weight to first service
N <sub>FeedReg</sub> n	kg	animal	Feed nitrogen required for neonatal growth
N <sub>FeedReq</sub> w	kg	animal	Feed nitrogen required for calf weaning
N <sub>G1stCal</sub>	kg	animal	Nitrogen gained from heifers removed between first service and first calving
N <sub>G1stSer</sub>	kg	animal	Nitrogen gained from heifers removed between six months of age and first service
N <sub>Ghm</sub>	kg	animal	Nitrogen gained from culled replacement heifers
N <sub>Hbody</sub>	kg	animal	Nitrogen retained in heifer's body
NLer	kg	animal	Nitrogen lost for heifer growth
N <sub>Lhm</sub>	kg	animal	Nitrogen lost due to heifer mortality
N <sub>Lm</sub>	kg	animal	Total nitrogen losses due to heifer mortality
N <sub>Lm1stCal</sub>	kg	animal	Nitrogen lost due to heifer mortality to first calving
N <sub>Lm1stSer</sub>	kg	animal	Nitrogen lost due to heifer mortality to first service
N <sub>Lmc</sub>	kg	animal	Nitrogen lost due to calf mortality
N <sub>Lmn</sub>	kg	animal	Nitrogen lost due to neonatal mortality
N <sub>Lmp</sub>	kg	animal	Nitrogen lost due to perinatal mortality
P <sub>%B</sub>	%	animal	Cattle body protein content
\$ <sub>h1stCal</sub>	N/A	herd	Sold rate (proportion of animals sold for meat out of total animals removed) of heifers between first service and first calving
\$ <sub>h1stSer</sub>	N/A	herd	Sold rate of heifers between six months of age and first service
T <sub>100</sub>	day	animal	Age when body weight reaches 100 kg
T <sub>1stCal</sub>	day	animal	Age at first calving
T <sub>1stSer</sub>	day	animal	Age first service
T <sub>c</sub>	day	animal	Heifer at 6 months of age
T <sub>n</sub>	day	animal	Age of neonatal calf
Tw	day	animal	Age at weaning
Pregnancy	1		
N <sub>Calf</sub>	kg	animal	Nitrogen in calf's body
	0	1	

#### Table 1. Definition of entities used in dairy cow nitrogen use efficiency (CNE) model.

(Continued)

### Table 1. (Continued)

Entity	Unit	Level	Definition				
N <sub>Lpreg</sub>	kg	animal	Nitrogen lost during pregnancy				
N <sub>PregReq</sub>	kg	animal	Feed nitrogen required for pregnancy				
Cow remo	Cow removal						
BWM	kg	animal	Mature body weight				
N <sub>Gsl</sub>	kg	animal	Vitrogen gain due to culled cows				
N <sub>Lcul</sub>	kg	animal	Nitrogen losses due to cattle culling				
s <sub>c</sub>	N/A	herd	Sold rate of dairy cows				
Disease an	d fertility						
CI	day	animal	Calving interval				
M <sub>LCI</sub>	Kg	animal	Milk lost due to extended calving interval				
M <sub>Lcm_m</sub>	Kg/lactation	animal	Milk lost due to mild milk clinical mastitis				
M <sub>Lcm_s</sub>	Kg/lactation	animal	Milk lost due to severe milk clinical mastitis				
M <sub>LD</sub>	Kg/lactation	animal	Cumulative milk lost due to diseases				
M <sub>Ldl</sub>	Kg/lactation	animal	Milk lost due to digital lameness				
M <sub>Lil</sub>	Kg/lactation	animal	Milk lost due to interdigital lameness				
M <sub>Lmf_m</sub>	Kg/lactation	animal	Milk lost due to mild milk fever				
M <sub>Lmf_s</sub>	Kg/lactation	animal	Milk lost due to severe milk fever				
M <sub>Lop</sub>	kg	animal	Milk lost due to disease and fertility problems				
M <sub>Lpm</sub>	Kg/lactation	animal	Milk lost due to perinatal calf mortality				
M <sub>Lrp</sub>	Kg/lactation	animal	Milk lost due to retained placenta				
$M_{Lsu}$	Kg/lactation	animal	Milk lost due to sole ulcer				
M <sub>Lvd</sub>	Kg/lactation	animal	Milk lost due to vulval discharge				
N <sub>Lop</sub>	kg	animal	Opportunity N losses				
r <sub>pm</sub>	%	animal	Risk factor for perinatal calf mortality				
r <sub>cm_m</sub>	%	animal	Risk factor for mild milk clinical mastitis				
r <sub>cm_s</sub>	%	animal	Risk factor for severe milk clinical mastitis				
r <sub>dl</sub>	%	animal	Risk factor for digital lameness				
r <sub>il</sub>	%	animal	Risk factor for interdigital lameness				
r <sub>mf_m</sub>	%	animal	Risk factor for mild milk fever				
r <sub>mf_s</sub>	%	animal	Risk factor for severe milk fever				
r <sub>rp</sub>	%	animal	Risk factor for retained placenta				
r <sub>su</sub>	%	animal	Risk factor for sole ulcer				
r <sub>vd</sub>	%	animal	Risk factor for vulval discharge				
Milk Prod	uction						
MNE	g/g	herd	Milk nitrogen use efficiency				
MY	kg	animal	Annual milk yield				
N <sub>6.38</sub>			Milk protein to nitrogen conversion constant (N = milk protein/6.38)				
N <sub>Lmilk</sub>	Kg/lactation	animal	Nitrogen lost due to milk production				
N <sub>Omilk</sub>	Kg/lactation	animal	Cumulative milk nitrogen output				
P%	%	herd	Milk protein content				
Herd Level							
Lact		herd	Lactations (1 / cattle replacement rate)				
n		herd	Lactating cattle in herd				
N <sub>L1st</sub>	kg	herd	Nitrogen lost from birth to first calving				
N <sub>Llact</sub>	kg	herd	Nitrogen losses in lactation for lifetime				
N <sub>LmilkLT</sub>	kg	herd	Nitrogen lost for milk in lifetime				
N <sub>LopLT</sub>	kg	herd	Opportunity nitrogen losses in lifetime				

(Continued)

#### Table 1. (Continued)

Entity	Unit	Level	Definition			
N <sub>LpregLT</sub>	kg	herd	itrogen lost for pregnancy in lifetime			
N <sub>Lrepl</sub>	kg	herd	itrogen lost for replacement cattle			
N <sub>OmeatLT</sub>	kg	herd	Nitrogen output in meat for lifetime			
N <sub>OmilkLT</sub>	kg	herd	Nitrogen output in milk for lifetime			
N <sub>Prod</sub>	kg	herd	oduced nitrogen			
N <sub>ReplBW</sub>	kg	herd	trogen retained in replacement cattle body			
rep	N/A	herd	attle replacement rate			
Efficiency						
LactNE	%	herd	Lactation nitrogen use efficiency			
LNE	%	herd	Lifetime nitrogen use efficiency			
ReplNE	%	herd	Replacement nitrogen use efficiency			

<sup>a</sup> N/A: not applied. This refers to proportions that have the same units in both parts of the ratio (e.g. cow/cow)

https://doi.org/10.1371/journal.pone.0201638.t001

measured in days). Similarly, for the following two stages (iii and iv) MPGrowth (denoted as such by the NRC model) was calculated at  $BW_{100}$ , first service, and first calving based on corresponding live weights ( $BW_{100}$ ,  $BW_{1stSer}$  and  $BW_{1stCal}$ ) and assuming net energy for growth from diets of 5.61, 9.63 and 12.98 MJ/d, respectively, to allow shrunk body weight gains higher



**Fig 1. Schematic description of cattle nitrogen (N) use efficiency model (CNE) for dairy cattle.** Where,  $N_{Hbody}$ : N retained in heifer's body;  $N_{Lgr}$ : N lost for heifer growth;  $N_{Lhm}$ : N lost due to heifer mortality;  $N_{Ghm}$ : N gained from culled heifers;  $N_{Lpreg}$ : N lost during pregnancy;  $N_{Lcul}$ : N losses due to cattle culling;  $N_{Gsl}$ : N gain due to sold cattle;  $N_{Lop}$ : opportunity N losses;  $N_{Omilk}$ : cumulative milk N output;  $N_{Lmilk}$ : N lost due to milk production;  $N_{L1st}$ : N lost from birth to first calving;  $N_{Lrepl}$ : N lost for replacement cattle;  $N_{Llact}$ : N losses in lactation for lifetime;  $N_{Prod}$ : Produced N; ReplNE: replacement N use efficiency; LactNE: lactation N use efficiency; LNE: lifetime N use efficiency.

https://doi.org/10.1371/journal.pone.0201638.g001

than 0.6 kg/d. Cumulative MPGrowth was calculated as the area under the interpolated line assuming MPGrowth requirement was a linear function of time ( $T_{1stSer}$  and  $T_{1stCal}$ ; measured in days). To convert from metabolizable protein (MP) requirements to crude protein (CP) inputs, MP:CP constants were used (MP:CP<sub>1stSer</sub>, and MP:CP<sub>1stCal</sub>, respectively). Metabolizable protein in ruminants comprises undegraded feed protein and microbial protein that leaves the rumen. Both undegraded feed and microbial protein are feedstuff- and animal-specific and vary depending on the animal's diet and stage of growth. Mechanistic models, such as the 2001 Dairy NRC and the Cornell net carbohydrate and protein system (CNCPS) [18, 23] may be used to estimate CP intake and corresponding MP supply of a given diet and stage of growth; then, the ratio between these two will be the MP:CP constants. After converting to CP, N<sub>FeedReq\_h1stSer</sub> and N<sub>FeedReq\_h1stCal</sub> were calculated using N<sub>6.25</sub>. Finally, overall N<sub>FeedReq</sub> was estimated as the sum of all stages:

$$N_{\text{FeedReq}} = N_{\text{FeedReq}_w}, + N_{\text{FeedReq}_BW100} + N_{\text{FeedReq}_h1stSer} + N_{\text{FeedReq}_h1stCal}$$
(1)

Total N retained in a heifer's body was calculated assuming 16% P<sub>%B</sub> [24, 25] and N<sub>6.25</sub>:

$$N_{Hbody} = BW_{1stCal} x(P_{\% B}/100) / N_{6.25}$$
(2)

Finally, N losses for growth were calculated with the following equation:

$$N_{Lgr} = N_{FeedReq} - N_{Hbody}$$
(3)

Heifer removal. Losses or gains of N due to calf and heifer removal from the herd were calculated for five lifetime stages. Causes of removal included mortality (death) and culling (voluntary removal due to either productive issues related to health problems, infertility, or any other negative reason, or sale as healthy animals). Animal removals at five different stages were considered: (i) perinatal: stillbirths and mortality within the first 24 h of birth of male and female calves; (ii) neonatal: the number of female calves that died or were euthanized between 24 h and 28 d of age; (iii) calf: the number of female calves that died or were euthanized between 1 and 6 months of age; (iv) heifers to first service: the number of heifers that died or were culled between 6 months old and the commencement of breeding (defined as the time of first insemination, first contact with a bull, or first embryo transfer); and (v) heifers to first calving: the number of heifers that died or were culled between the first breeding service and first calving. This last stage included those animals that failed to conceive. Losses of N to reach an animal a specific growth stage were calculated based on specific mortality rates ( $m_p, m_o, m_{h1stSer}$ ) and *m*<sub>h1stCal</sub>) and the N<sub>FeedReq</sub> for each stage (N<sub>FeedReq\_n</sub>, N<sub>FeedReq\_c</sub>, N<sub>FeedReq\_h1stSer</sub> and N<sub>FeedReg h1stCal</sub>) with the exception of N<sub>Lmp</sub>. These were calculated by the heifer growth submodel using specified BW (BWn, BWc, BWhlstSer and BWhlstCal) and times (Tn, Tc, ThlstSer and Th1stCal) of dead or culled heifers in each stage. For the perinatal stage it was assumed that calves were not fed and  $N_{Lmp}$  was therefore based on  $m_p$  and  $BW_B$ .

To differentiate between system N losses and gains from heifers removed from the herd of the stages "heifers to first service" and "heifers to first calving" sold constants were used to represent the proportion of animals sold at market ( $s_{h1stSer}$  and  $s_{h1stCab}$  proportion of heifers sold

PLOS

for meat out of total heifers removed). The equations were:

$$\mathbf{N}_{\mathrm{Lmp}} = \mathbf{BW}_{\mathrm{B}} \times m_{p} \times (\mathbf{P}_{\mathrm{MB}}/100) / \mathbf{N}_{6.25} \tag{4}$$

$$N_{Lmn} = m_n \times N_{FeedReq_n} \tag{5}$$

$$N_{Lmc} = m_c \times N_{FeedReq_c} \tag{6}$$

$$N_{Lm1stSer} = m_{h1stSer} \times N_{FeedReq\_h1stSer} \times (1 - s_{h1stSer})$$
(7)

$$N_{Lm1stCal} = m_{h1stCal} \times N_{FeedReq-h1stCal} \times (1 - s_{h1stCal})$$
(8)

Finally, overall N losses from the compartment due to replacement heifer mortality were calculated with the following equation:

$$N_{Lhm} = N_{Lmp} + N_{Lmn} + N_{Lmc} + N_{Lm1stSer} + N_{Lm1stCal}$$
(9)

Within the heifer removal compartment, N gained from removed heifers that were sold for meat was calculated with the following equations:

$$N_{G1stSer} = BW_{h1stSer} \times s_{h1stSer} \times (P_{\%B}/100)/N_{6.25}$$
(10)

$$N_{G1stCal} = BW_{h1stCal} \times s_{h1stCal} \times (P_{\%B}/100)/N_{6.25}$$
(11)

$$N_{Ghm} = N_{G1stSer} + N_{G1stCal}$$
(12)

**Pregnancy.** A third compartment was used to calculate  $N_{Lpreg}$  based on  $N_{PreReq}$  for days 190 to 279 of pregnancy using equations of the 2001 Dairy NRC model [22]. Nitrogen required for pregnancy was calculated for days 190 and 279 of pregnancy and the corresponding cumulative  $N_{PreReq}$  was calculated as the area under the interpolated line. The N retained in the developing calf ( $N_{Calf}$ ) was calculated from BW<sub>B</sub> using  $N_{6.25}$  and 16%  $P_{\%B}$ . Then,  $N_{Lpreg}$  was calculated with the following equation:

$$N_{Lpreg} = N_{PreReq} - N_{Calf}$$
(13)

**Cow removal.** To account for N lost through death or gained when sold for meat by removing cows from the herd ( $N_{Lcul}$  or  $N_{Gsb}$ , respectively), the breed related BW<sub>M</sub> was considered to be the final weight. To differentiate between cows that were removed without any use of their carcass and those that were sold for meat a constant was used ( $s_c$ ; proportion of cows sold for meat out of total animals removed). Then,  $N_{Lcul}$  and  $N_{Gsl}$  were calculated with the following equations:

$$N_{Lcul} = BW_{M} \times (1 - s_{c}) \times (P_{\%B}/100) / N_{6.25}$$
(14)

$$\mathbf{N}_{\rm Gsl} = \mathbf{B}\mathbf{W}_{\rm M} \times \mathbf{s}_{\rm c} \times (\mathbf{P}_{\rm HB}/100) / \mathbf{N}_{6.25} \tag{15}$$

**Disease and fertility.** Another set of equations was used to estimate opportunity costs related to health issues and were expressed in terms of a loss of MY. In the current study, opportunity costs reflect milk losses caused by diseases, disorders or sub-optimal fertility

Average

8.0

4.0

7.1

0.9

14.0

17.1

1.9

6.6

6.1

3.4

9.0

5.0

9.8

1.2

31.0

45.0

5.0

14.8

13.7

7.6

Table 2. Health management index used to calculate opportunity losses due to health issues (adapted from [26]).						
		Minimum	Maximum			
Perinatal calf mortality	117	5.0				
Retained placenta	415	2.0				
Milk fever—mild	215	1.8				
Milk fever—severe	540	0.2				
Vulval discharge	325	9.0				

<sup>a</sup> Tables 4.10–4.26 in the original study [26] assuming a dairy cow with average annual milk production of 7,000 kg

350

1050

505

160

506

<sup>b</sup> Adapted from Appendix 5.1 in the original study [26], including prevalence of average milk fever (89 and 11% for mild and severe cases, respectively; Table 4.18, correcting for fatal cases that are included in mortality rates in the current study), clinical mastitis (90 and 10% for mild and severe cases, respectively; Table 4.22) and lameness (41, 38 and 21% for digital, interdigital and sole ulcer, respectively; Table 4.28)

https://doi.org/10.1371/journal.pone.0201638.t002

Clinical mastitis-mild

Digital lameness

Sole ulcer

Interdigital lameness

Clinical mastitis-severe

compared with full productivity from healthy, fertile cows. An early attempt to incorporate opportunity costs through milk yield reductions was described by Kossaibati and Esslemont [14]. The following issues and disorders were considered: perinatal calf mortality, retained placenta, milk fever-mild, milk fever-severe, vulval discharge, clinical mastitis-mild, clinical mastitis-severe, digital lameness, interdigital lameness and sole ulcer. Milk yield reductions for each issue and disorder are presented in Table 2 and are based on those reported by Kossaibati and Esslemont [14] and refined by Esslemont and Kossaibati [26]. The overall milk lost per cow due to disease (M<sub>LD</sub>) and fertility issues was calculated, based on a risk factor (r<sub>cm m</sub>, r<sub>cm\_s</sub>, r<sub>dl</sub>, r<sub>il</sub>, r<sub>mf\_m</sub>, r<sub>mf\_s</sub>, r<sub>pm</sub>, r<sub>rp</sub>, r<sub>su</sub>, and r<sub>vd</sub>) and milk yield losses (M<sub>Lcm\_m</sub>, M<sub>Lcm\_s</sub>, M<sub>Ldl</sub>, M<sub>Lil</sub>, M<sub>Lmf\_m</sub>, M<sub>Lmf\_s</sub>, M<sub>Lpm</sub>, M<sub>Lrp</sub>, M<sub>Lsu</sub>, and M<sub>Lvd</sub>) for each disease. For example, for retained placenta, a r<sub>rp</sub> of 3.9% and estimated M<sub>Lrp</sub> per cow each year of 415 kg were used (Table 2); thus, the overall opportunity loss of milk per cow and lactation was  $16.2 \text{ kg} (415 \times 3.9 / 100)$ . In addition, milk losses due to extended CI (M<sub>LCI</sub>) were calculated as a loss of 0.2% of MY daily for each day above 365 [26]. Then, M<sub>Lop</sub>, the sum of opportunity costs, and a specified  $P_{\%}$  were used to estimate  $N_{Lop}$  at a cow level per lactation using the  $N_{6.38}$  conversion factor for milk:

15.3

1.7

3.7

3.4

1.9

$$M_{Lop} = M_{LCI} + M_{LD}$$
(16)

$$N_{Lop} = M_{Lop} \times (P_{\%}/100) / N_{6.38}.$$
 (17)

Milk production. The last compartment was used to calculate  $N_{Omilk}$  from MY,  $P_{\%}$  and  $N_{6.38}$ . Thus, the overall  $N_{Lmilk}$  was calculated with the following equations:

$$N_{Omilk} = MY \times (P_{\%}/100) / N_{6.38}$$
(18)

$$N_{Lmilk} = N_{Omilk} \times (1/MNE - 1)$$
<sup>(19)</sup>

where MNE represents the value for a given diet fed to the healthy cows of the herd. The major determinants of MNE is diet composition (in particular its protein concentration and

fermentable energy density) and feed intake relative to productivity [9]. The former varies considerably between farms depending on feed resource availability, and for this reason we chose not to include direct feed variation in our analysis for lactating cattle, and to treat MNE as the principal input for it.

**Herd level calculations.** Once N loss of gain were calculated for each compartment, the lifetime losses were estimated taking into account the specified replacement rate (*rep*) and/or the corresponding average number of lactations for each cow in a herd (Lact = 1/rep). These model flows were then expressed at a herd level (where *n* is the specified number of lactating cows in the herd) and were calculated with the following equations:

$$N_{L1st} = (N_{Lgr} + N_{Lhm} + N_{Lpreg}) \times n$$
<sup>(20)</sup>

$$N_{Lrepl} = N_{L1st} + N_{Lcul} \times n \tag{21}$$

$$N_{\text{ReplBW}} = N_{\text{Hbody}} \times n \tag{22}$$

$$N_{\rm LmilkLT} = N_{\rm Lmilk} \times Lact \times n \tag{23}$$

$$N_{LopLT} = N_{Lop} \times Lact \times n \tag{24}$$

$$N_{LpregLT} = N_{Lpreg} \times (Lact - 1) \times n$$
(25)

$$N_{Llact} = N_{LmikLT} + N_{LopLT} + N_{LpregLT}$$
(26)

$$N_{\text{OmilkLT}} = N_{\text{Omilk}} \times \text{Lact} \times n \tag{27}$$

$$N_{OmeatLT} = (N_{Gsl} + N_{Ghm}) \times n$$
(28)

$$N_{Prod} = N_{OmilkLT} + N_{OmeatLT}$$
<sup>(29)</sup>

**Efficiencies of N utilization.** As the last step of the CNE model, N use efficiencies were calculated as follows:

$$RepINE = N_{RepIBW} / (N_{RepIBW} + N_{LrepI})$$
(30)

$$LactNE = N_{OmikLT} / (N_{OmikLT} + N_{Llact})$$
(31)

$$LNE = N_{Prod} / (N_{Prod} + N_{Lrepl} + N_{Llact})$$
(32)

#### Model sensitivity analysis

The sensitivity of the model to variation in farm- and animal-related input values was evaluated with a Monte Carlo simulation using @Risk version 7.1 (Palisade, West Drayton, UK). Values for a modelled dairy farm were used based on published data related to dairy farming practices in the United Kingdom [27–33]. To assist reporting net values of main N outputs, an example herd of a fixed size of 100 lactating dairy cows, plus the heifers needed to replace these

Variable	No	rmal	Non-normal <sup>a</sup>			Ref
	Mean	SD	Max	Likely	Min	
Annual milk yield (MY), kg			7870	7096	6449	AHDB <sup>b</sup>
BW <sup>c</sup> at first calving (BW <sub>1stCal</sub> ), kg	544	25				[33]
BW at first service (BW <sub>1stSer</sub> ), kg	368	29				[30]
BW mature (BW <sub>M</sub> ), kg	748	75				[18]
BW at birth (BW <sub>B</sub> ), kg	43.4	4.9				[33]
Calf mortality rate (m <sub>c</sub> )	0.034	0.036				[29]
Calving interval (CI), d			600	385	365	[27]
Cattle replacement rate ( <i>rep</i> )			0.287	0.238	0.175	[31, 32]
Heifer mortality rate to first calving (m <sub>h1stCal</sub> )	0.037	0.05				[29]
Heifer mortality rate to first service (m <sub>h1stSer</sub> )	0.032	0.046				[29]
Milk nitrogen use efficiency (MNE)	0.277	0.036				[9]
Milk protein content (P%),%	3.21	0.17				[9]
Neonatal mortality rate (m <sub>n</sub> )	0.032	0.040				[29]
Perinatal mortality rate (m <sub>p</sub> )	0.081	0.036				[29]
Sold rate of dairy cattle (s <sub>c</sub> )	0.93	0.01				[32]
Sold rate of heifers to first calving $(s_{h1stCal})$	0.95	0.09				[29]
Sold rate of heifers to first service (sh1stSer)	0.19	0.02				[29]
Age at weaning (T <sub>w</sub> ), d	42	4.2				[29]
Age to first calving $(T_{1stCal})$ , m			50.9	26.4	21.2	[28]
Age to first service (T <sub>1stSer</sub> ), d			963	473	357	[28]

#### Table 3. Distribution characteristics of inputs used in Monte Carlo sensitivity analysis.

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<sup>a</sup> The triangular distribution was used for age to first calving and age to first service, and the program evaluation and review technique (PERT) for annual milk yield, calving interval, and cattle replacement rate.

<sup>b</sup> From Agriculture and Horticulture Development Board (AHDB) using average annual production. Then, SD reflects annual variation and not cow-herd variation; http://dairy.ahdb.org.uk/market-information/farming-data/milk-yield/average-milk-yield

<sup>c</sup> BW: body weight

https://doi.org/10.1371/journal.pone.0201638.t003

cows, was modelled. Male and surplus female calves were assumed to be sold at birth to be reared elsewhere. Productive N output in milk and cull-cow and heifer meat (in 1000s of kg) was calculated for the whole herd over the average animal's lifetime and each output was expressed as a percentage of the total. Probability density functions were fitted to farm and animal input values. Table 3 describes tested variables, their type of distribution and their selected values. All variables and their range were evaluated for their biological correctness and correlation. For example, the onset of puberty is determined by BW as heifers start to cycle at approximately 43% of mature BW[33]. We used a mature BW of 748 kg that requires BW at first service of about 321 kg. In our dataset the minimum BW at first service is 320 kg. Moreover, we chose to use a MNE value (0.277) reported by Huhtanen and Hristov [9] for the North European dataset that reflected diets similar to those used in the UK within a similar MNE range [34–37].

Most variables were described with a normal distribution except those for which limited or apparently extreme data were available (e.g. annual milk production, where the Agriculture and Horticulture Development Board (AHDB) annual data were used, representing the country's annual variation rather than cow-herd variation) or when the variable is not distributed normally (e.g.  $T_{1stCal}$  [38]). In this case, we used either the triangular or the Program Evaluation and Review Technique (PERT) distribution. Both distributions require 3 estimates: (i) the most likely result, (ii) the minimum expected result, and (iii) the maximum expected result.

With the triangular distribution values around the most likely result are more likely to occur. The PERT distribution is similar to a  $\beta$  or triangular distribution and is useful to describe variation in a situation where limited data exists [39]. The distribution of selected inputs is presented in Fig 2. We used contemporary peer-reviewed data to build our dataset where the range of T<sub>1stCal</sub> was up to 50 months [28]. However, a recently published study analysing the cost of heifer growth in the UK reported a narrower range than the one we used in our analysis, where T<sub>1stCal</sub> ranged from 21.3 to 32.4 with a mean of 26.1 months [40]. Therefore, we



**Fig 2. Frequency distributions of major inputs used in Monte Carlo sensitivity analysis.** (A) Normal distribution of milk nitrogen use efficiency (MNE), (B) program evaluation and review technique (PERT) distribution of calving interval (CI), (C) triangular distribution of time to first calving (T<sub>1stCal</sub>) based on Brickell et al. [28], (D) triangular distribution of T<sub>1stCal</sub>, based on Boulton et al.[40].

https://doi.org/10.1371/journal.pone.0201638.g002

performed the same sensitivity analysis but using the values of the later study for  $T_{1stSer}$  (509, 365, and 700 days of age for most likely, minimum expected and maximum expected result, respectively) and  $T_{1stCal}$  [40]. In both cases, triangular distributions were considered in our analysis for  $T_{1stSer}$  and  $T_{1stCal}$  as suggested by published data [38].

In addition, dietary inputs for growing animals necessary to run the simulation were obtained using the CNCPS assuming animals were grazing perennial ryegrass with varying CP concentrations. Three CP concentrations of ryegrass were considered (10, 16 and 20% on a dry matter basis) and the chemical composition necessary to run a simulation with the CNCPS was obtained from the literature [36, 37, 41-44] and the CNCPS feed library. Simulations were run for each CP concentration and animal type (heifers at first service and heifers at first calving) using animal inputs (body weight and age) reported in Table 3 for each stage, and feed dry matter intake as predicted by the CNCPS (9.4 and 12.6 kg of dry matter intake daily for first service and first calving heifers, respectively). Then metabolizable protein supply was calculated by the CNCPS and the ratio of metabolizable protein supply to crude protein intake (MP:CP) was estimated. Due to feeding similarities for both stages, MP:CP<sub>1stSer</sub> and MP: CP<sub>1stCal</sub> were similar. Therefore, a merged factor was used (MP:CP<sub>heifer</sub>) and variation in MP: CP<sub>heifer</sub> was described with a PERT distribution using the following values reflecting feed variation: (i) minimum expected result = 0.505, obtained by feeding ryegrass with a CP concentration of 20% on a dry matter basis, (ii) most likely result = 0.605, obtained by feeding ryegrass with a CP concentration of 16% on a dry matter basis, and (iii) maximum expected result = 0.850, obtained by feeding ryegrass with a CP concentration of 10% on a dry matter basis. Frequency distributions for model outputs were generated using a Monte Carlo simulation with 10,000 iterations to describe the range of possible outcomes for each output and the relative likelihood of occurrence.

# Results

Productive N outputs over the course of an animal's lifetime were partitioned into milk  $(N_{OmilkLT})$  and meat  $(N_{OmeatLT})$ , and they were dominated by milk production. Indeed,  $N_{OmilkLT}$  represented on 89% of total N output, and the remainder 11% was partitioned in  $N_{OmeatLT}$  (Fig 3). As presented in Table 4 for the modelled farm of 100 lactating dairy cows, a net production between 12,700 and 18,400 kg of  $N_{OmilkLT}$  was estimated, with the range being most significantly affected by variation in cattle replacement rate, milk protein concentration and milk yield. Similarly, a total production between 1,420 and 2.280 kg of  $N_{OmeatLT}$  was calculated, with the range being most significantly affected by variation in cow sold rate, mature body weight, and heifer mortality to first calving.

However, this overall production was achieved with substantial N losses. Nitrogen lost during lifetime milk production at a herd level were on average 41,000 kg but may reach 57,600 kg for a 100-cow dairy (Table 4), mainly affected by milk N use efficiency, replacement rate and production characteristics, such as milk protein concentration and milk yield. Nitrogen losses incurred by replacing dairy cows within the herd represented a lower portion of losses (mean = 4,760 ± 1,350 kg of N<sub>Lrepl</sub>) than those during lifetime milk production and were strongly affected by the last time point of heifer growth (T<sub>1stCal</sub> and its related mortality rate) rather than feed variation, as assessed by variation in MP:CP<sub>heifer</sub>. Even though the overall contribution of N<sub>LopLT</sub> was relatively small compared with N losses during lactation, it was estimated to be between 530 and 1,170 kg, mainly being affected positively by variation in calving interval and disease index, and negatively by variation in cattle replacement rate. Further, a much lower proportion of N losses were partitioned in pregnancy (mean = 850 kg of N<sub>LpregLT</sub>),



Fig 3. Frequency distributions of major lifetime nitrogen outputs and losses at a herd level for lifetime expressed as % of total N losses in lifetime. (A) Nitrogen output in milk ( $N_{OmilkLT}$ ), (B) Nitrogen output in meat ( $N_{OmeatLT}$ ), (C) Nitrogen lost for milk production ( $N_{LmilkLT}$ ), (D) Nitrogen lost for replacement cattle ( $N_{Lrepl}$ ), (E) Opportunity nitrogen losses ( $N_{LopLT}$ ), and (F) Nitrogen lost for pregnancy ( $N_{Lrepl}$ ).

https://doi.org/10.1371/journal.pone.0201638.g003



Item <sup>a,b</sup>	Mean	SD	5%	95%	Effect on output mean		r
					Form	То	
N <sub>OmilkLT</sub>	15.4	1.7	12.7	18.4			
rep					13.2	18.0	-0.81
P%					13.9	16.9	0.45
МҮ					14.3	16.3	0.32
N <sub>OmeatLT</sub>	1.83	0.26	1.42	2.28			
s <sub>c</sub>					1.51	2.14	0.67
BW <sub>M</sub>					1.52	2.14	0.66
m <sub>h1stCal</sub>					1.72	1.93	0.22
N <sub>LmilkLT</sub>	41.0	9.0	28.6	57.6			
MNE					29.8	56.7	-0.84
rep					35.3	47.8	-0.42
P%					37.3	45.0	0.23
МҮ					38.2	43.5	0.17
N <sub>Lrepl</sub> ,	4.76	1.35	2.86	7.25			
T <sub>1stCal</sub>					3.08	6.96	0.88
MP:CP <sub>heifer</sub>					3.91	5.53	-0.36
m <sub>h1stCal</sub>					4.34	5.21	0.19
s <sub>c</sub>					4.43	5.03	-0.14
m <sub>h1stSer</sub>					4.55	4.95	0.10
N <sub>LopLT</sub>	2.24	1.17	0.378	4.50			
CI					0.81	4.61	0.97
Rep					1.92	2.59	-0.18
Health index					2.06	2.59	0.12
N <sub>LpregLT</sub>	0.85	0.20	0.53	1.17			
rep					0.61	1.07	-0.66
BW <sub>M</sub>					0.65	1.05	0.58
BW <sub>B</sub>					0.79	0.91	-0.17

Table 4. Factors affecting nitrogen (N) gains and losses (values in 1000s of kg in lifetime at a herd level), corresponding effects on output means, and their correlation coefficients (r).

<sup>a</sup>  $N_{OmilkLT}$ : nitrogen output in milk for lifetime,  $N_{OmeatLT}$ : nitrogen output in meat for lifetime,  $N_{LmilkLT}$ : nitrogen lost for milk in lifetime,  $N_{Lrepl}$ : nitrogen lost for replacement cattle,  $N_{LopLT}$ : opportunity nitrogen losses,  $N_{LpregLT}$ : nitrogen lost for pregnancy in lifetime, *rep*: cattle replacement rate,  $P_{\%}$ : milk protein content, MY: annual milk yield,  $s_c$ : sold rate of dairy cows,  $BW_M$ : mature body weight,  $m_{h1stCal}$ : heifer mortality rate from first service to first calving, MNE: milk nitrogen use efficiency,  $T_{1stCal}$ : age at first calving, MP:CP<sub>heifer</sub>: metabolizable protein to crude protein ratio for heifer diet,  $m_{h1stSer}$ : heifer mortality rate from six months to first service, CI: calving interval,  $BW_B$ : body weight at birth

 $^{\rm b}$  Factors that affect a variable were listed when  $r \geq \pm \ 0.1$ 

https://doi.org/10.1371/journal.pone.0201638.t004

and these were positively affected by variation in mature body weight and negatively by variation in replacement rate and body weight at birth.

Replacement heifers form an important part of the dairy herd in terms of animal numbers and overall cost. With the CNE model we estimated a mean ReplNE of 23.7%, which was most substantially affected by the last stage of heifer growth. Variation in time to first calving may cause larger changes on ReplNE than variation in feed as assessed by the MC:CP<sub>heifer</sub> (Fig 4A). Both weight and heifer mortality to first calving were correlated with ReplNE: positively with BW<sub>1stCal</sub> (r = 0.19) and negatively with m<sub>1stCal</sub> (r = -0.17). The sensitivity analysis showed a very strong positive correlation between LactNE and MNE (r = 0.99; Fig 4B). Variation in MNE was found to cause changes in LactNE mean from 20.5 to 31.8%. However, a small negative relationship of opportunity losses was found with extended calving interval (r = -0.14). Within the structure of the CNE model, the combination of the efficiency of N use by replacement animals (i.e. ReplNE) and the efficiency of use of N for lactation (i.e. LactNE) is expressed in LNE. We estimated a mean of 26.3% for LNE (Fig 4C), and it was dominated by MNE as indicated by r = 0.97. However, our study highlighted two other model variables that affected LNE. Variation in CI (r = -0.15) and T<sub>1stCal</sub> (r = -0.15) may cause measurable



Fig 4. Frequency distributions and tornado diagrams showing the change in means outputs and correlation coefficients of overall lifetime use efficiencies. (A) Replacement nitrogen use efficiency (RepINE). (B) Lactation nitrogen use efficiency (LactNE). (C) Lifetime nitrogen use efficiency (LNE). Where, MNE: milk nitrogen use efficiency, CI: calving interval,  $T_{1stCal}$ : age at first calving, MP:CP<sub>heifer</sub>: metabolizable protein to crude protein ratio of feed fed to heifers after 100 kg of body weight,  $m_{1stCal}$ : heifer mortality rate from first service to first calving, BW<sub>1stCal</sub>: body weight at first calving,  $s_c$ : sold rate for dairy cows,  $m_{1stSer}$ : heifer mortality rate from six months to first service.

https://doi.org/10.1371/journal.pone.0201638.g004

reductions of overall LNE. A recent study [40] published  $T_{1stCal}$  values that were less variable than those of previous studies used to define the frequency distributions for initial Monte Carlo simulations. Therefore, we performed an additional sensitivity analysis using these new values. In this case, reduced variation in  $T_{1stCal}$  indicated weaker effects in mean LNE provoking mean changes from 26.5 to 27.1% (r = -0.04), but remained the principal variable responsible for changes in mean ReplNE (from 24.3 to 32.5%; r = -0.59). In addition, the mean values of both ReplNE and LNE increased to 28.1 ± 3.9% and 26.8 ± 3.1%, respectively.

# Discussion

Despite the European dairy herd's main role as a producer of milk, is also a very important source of beef meat, with approximately 50% of produced beef estimated to come from culled dairy cows and 15% from male dairy calf systems [45]. In 2014, the EU-28 countries produced 151.7 million tonnes of liquid bovine milk with an average protein concentration of 3.37%, resulting into 0.805 million tonnes of milk N being produced [46]. For the same period, the overall bovine carcass production was 7.59 million tonnes and assuming an average carcass protein concentration of 16.5%, the overall N output in bovine meat production was 0.200 million tonnes [46]. Considering approximately 50% of this was from culled dairy cows [45], we can estimate meat N output at 11% of total N output from dairy cattle. This matches very well the calculations in the current study of meat N output being 10.7% of total N produced by a dairy farm, for an annual MY range from 6,449 to 7,870 kg/cow (Table 3).

With this modelling exercise and by describing the UK dairy sector, we estimated significant lifetime N losses at a herd level of up to 57,600 kg of N for a 100-cow dairy herd. This may happen in a high-yielding herd with high milk protein content, low replacement rate but which produce milk protein using dietary nitrogen with a low rate of efficiency. For example, a 100-cow herd with 7000 kg /cow milk produced per year with 3.5% milk protein content, a replacement rate of 0.25 (4 lactations) and a milk nitrogen use efficiency of 0.22, will excrete into the environment 54,460 kg of N in lifetime. If we calculate the daily N excretion of the lactating cow for this herd (total excretion / lactations / n / 305 days of milk production) we will estimate a daily N excretion of 446 g / cow, which is within the normal range of N excretion reported in the literature [9, 18, 34]. Moreover, it should be noted that in the current analysis replacement rate is negatively correlated with lifetime N excretion. However, this is mainly related to the lifetime calculation of excretion and does not indicate a recommendation for higher replacement rate.

The majority of lifetime N losses were accounted for by losses in the milk production process. Besides the importance of milk production of the dairy herd in terms of total outputs, this is also because the efficiency of converting feed N into milk N is relatively poor, with a large proportion of feed N being excreted in faeces and urine (on average 72% of N intake) [11]. Variation in MNE was the principal cause of changes in  $N_{LmilkLT}$  and this was reflected in LactNE and consequently in overall LNE, which was highly correlated with MNE. In the current study, we considered baseline MNE to be the efficiency of N utilization for milk protein production for healthy lactating animals within the herd. Several studies have reported MNE, ranging from 14.0 to 45.3% [9–11] and reaching a theoretical maximum of about 45% for a 600 kg dairy cow producing daily 25 kg of milk [47]. The major determinant of MNE is nutrition [9], and in our sensitivity analysis reflects different feeding scenarios and production levels in the UK. Use of the model at a farm level will require knowledge of that farm's baseline MNE as an input. However, the model could be linked to nutritional models (e.g. CNCPS) that could provide MNE predictions based on different feeding scenarios. Besides the dominant effects of MNE on LNE, we detected the effects that variation in specific non-diet related variables have on changes in LactNE and LNE means. In particular, variation in CI may cause measurable changes in LactNE and LNE. To our knowledge, this study is the first to have assessed the implications of factors such as CI with the effects of opportunity costs of disease and infertility leading to N losses during lactation. Traditionally, CI has been used as an indicator of herd fertility [48, 49] and a short CI of 10 to 12 months (300–365 days) has been recommended for maximizing herd profitability [50, 51]. However, several studies have questioned this approach, suggesting that a longer CI, even of up to 24 months, may be beneficial either as a practice to avoid high replacement rates caused by infertility in seasonalcalving-based systems [52, 53], or for high-producing dairy cows that are dried off with more than 25 kg/d of milk production [54].

An extended lactation length, and therefore an extension of CI, may increase yields per lactation but will depress annualized MY (expressed on a 305-day basis) by delaying the following lactation [26]. This demonstrates the opportunity costs due to extended CI in the current study. We calculated annual MY (305-day) reductions for each extra day of CI above 365 days, based on the best alternative which is calving in 365-day cycle, considering a 12 months CI to be the standard management decision within our dataset [27]. Therefore, milk opportunity cost reflects the theoretical additional amount of milk that would have been produced if cows had been in a following lactation assuming a typical lactation curve, which increases rapidly from calving to a peak at about 6 weeks of lactation and then decreases gradually as lactation progresses [55].

Reduced annualized MY (= MY × 12 / CI) up to 10.5% for cows with 24 months CI compared with those having a CI of 12 months was reported [53]. In a following study, cows with extended CI (24 months) produced 7.1% less milk in two years compared with the 12 months CI group [56]; in terms of annualized MY, cows with extended CI produced 22% less milk. Similarly, pasture-based cows with 24 months CI produced on average 21% less milk during the second year (13 to 24 m) compared with the first year (1 to 12 m) [52], suggesting an opportunity cost of 21% for the extended CI. These findings are in accordance with our results, where opportunity costs of milk production due to CI averaged 10.5% of annual milk yield (results not shown) for extended CI from 366 to 600 days (Table 3). Thus, these losses resulted in a negative correlation with both LactNE and LNE.

A negative correlation with both LNE and RepINE was found for T<sub>1stCa</sub>, which is an indicator of replacement cattle growth and fertility efficiency. This suggested that the efficiency by which replacement heifers are grown affects overall LNE. Replacement heifers represent a major economic expense for dairy operations, being the second largest input after feed costs, and accounting for 15 to 20% of total milk production costs [57]. Several studies in the USA and Europe suggested that T1stCal is the primary variable to define net cost for replacement cattle [58, 59]. Within the structure of our model,  $T_{1stCal}$  affected total feed N requirements for heifer growth (in the model: N<sub>FeedReq</sub>) and consequently N losses for growth for the period between first service and first calving. A mean  $N_{\text{FeedReg}}$  of 42.9 ± 5.6 kg of N per heifer for a BW<sub>1stCal</sub> of 544 kg (Table 3) was estimated, suggesting a feed N utilization efficiency of 25.2%, which is within the range reported in the literature [17]. Feed N requirements for the heifer between first service and first calving was 44% of N<sub>FeedReq</sub>. Similarly, for the period between BW100 and first service, feed N requirements were 49% of NFeedReq, but variation in T1stSer did not cause significant changes in either LNE or RepINE. This is because we calculated N<sub>FeedReg</sub> as a cumulative growth function of MP requirements in time for three stages of heifer growth after weaning (BW100, first service and first calving). Using this approach, MP requirements for a heifer at first calving is 28% higher than those for a heifer at first service due to BW differences. For this reason, variation in T<sub>1stCal</sub> led to larger changes in N<sub>FeedReg</sub> (from 36.1 to 50.3

kg of N per heifer) than variation in  $T_{1stSer}$  (from 42.0 to 43.8 kg of N per heifer), and consequently to significant changes in ReplNE (Fig 4).

This variation in  $T_{1stCal}$  does not reflect growth variation only but incorporates variation in fertility of the replacement heifers as well. Once heifers have reached an adequate  $BW_{1stSer}$  they are ready for breeding, but they rarely conceive immediately. A mean of 3 services before conception, with a range between 1 and 9, was reported for the UK [29]. The delay in conception increases the time to first calving, extending the time between  $T_{1stSer}$  to  $T_{1stCal}$  beyond 280 days. Therefore, an overall improvement of  $T_{1stCal}$  can be achieved from both better heifer growth rates and improved conception rates. Interestingly, variation in  $T_{1stCal}$  caused larger changes in ReplNE than feed variation as included by MP:CP<sub>heifer</sub>. In our analysis we avoided the inclusion of a wide variety of feeding strategies for heifers and we focused on grazing systems as the principal strategy in the UK. Links of the CNE model to feed models, such as the CNCPS, could be used to incorporate and analyse this variation in future work. Variation in heifer mortality was negatively correlated to ReplNE than variation in mortality rates from first service to first calving caused larger changes to ReplNE than variation in mortality rates from first service to higher N losses due to mortality.

In any sensitivity analysis, the results depend on the range of values used as inputs. Different studies describing the UK dairy sector have reported different range for  $T_{1stSer}$  and  $T_{1stCal}$ [28, 40] We used contemporary peer-reviewed data to build our dataset with a range of  $T_{1stCal}$ up to 50 months [28]. This may be considered extreme for modern dairy farming, even though this high value may include extensive dairy systems that do exist in the UK. Even though the most likely value, according to both studies, was similar (approximately 26 months for  $T_{1stCal}$ ) the maximum likely value was very different. Boulton et al. [40] reported a maximum  $T_{1stCal}$  of 32.4 months, which is similar to that reported for Holstein heifers in the USA in 2004 [38]. We cannot confirm if this narrower range from earlier data reflects an improvement of UK dairy farming practices or the description of different population sample. In any case, these findings suggest that better management strategies that reduce the range of  $T_{1stCal}$  may help reduce N losses during heifer growth due to extended  $T_{1stCal}$ . If all heifers calved for the first time by about 32 months, LNE would be practically insensitive to replacement heifer variables.

The aim of this work was to describe whole-lifetime N use efficiency, which, by definition, limits the applicability of the model. For example, cattle replacement rate was found to be negatively correlated with lifetime N excretion but had no effect on LNE; a higher replacement rate reduces lifetime N excretion because fewer lactations are included. However, there was no effect on LNE because it is a ratio, and both numerator and denominator variables are assessed for the same number of lactations. Similarly, herd size was considered to be unchanged in the current study, and even though herd size is a dynamic variable [60, 61] that might be beneficially included for production and policy matters, its effect remains to be incorporated in future work.

# Conclusion

With the current study, we developed a dairy cattle herd model that is sensitive to elements of performance, fertility and health. Lifetime N use efficiency of dairy cattle was shown to be dominated by MNE, the short-term efficiency of use of feed N for milk production. However, we have demonstrated important effects of both the replacement cattle growth period and the opportunity costs of disease and fertility on N use efficiency. The considerable economic cost of the replacement cattle part of the dairy herd is well established. Here we demonstrated that replacement cattle have a considerable impact in terms of farm N losses. Further, we detected

specific non-diet related variables that affect the efficiency of use of N in the growth of replacement cattle (ReplNE) and during lactation (LactNE), and therefore overall lifetime N use efficiency (LNE) of dairy cattle.

# Acknowledgments

Financial support was provided by the Welsh Government and Higher Education Funding Council for Wales through the Sêr Cymru National Research Network for Low Carbon, Energy and Environment. Project: Consequential Life Cycle Assessment of Environmental and Economic Effects of Dairy and Beef Consolidation and Intensification Pathways —"Cleaner Cows." Financial support from the UK Department for Environment, Food and Rural Affairs is also gratefully acknowledged. The Institute of Biological, Environmental, and Rural Sciences gratefully acknowledges funding from the Biotechnology and Biological Sciences Research Council.

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#### References

- 1. Tamminga S. A review on environmental impacts of nutritional strategies in ruminants. J Anim Sci. 1996; 74(12):3112–24. PMID: 8994925
- 2. Capper JL, Cady RA, Bauman DE. The environmental impact of dairy production: 1944 compared with 2007. J Anim Sci. 2009; 87(6):2160–7. https://doi.org/10.2527/jas.2009-1781 PMID: 19286817
- Jensen LS, Schjoerring JK, van der Hoek KW, Poulsen HD, Zevenbergen JF, Palliere C, et al. Benefits of nitrogen for food, fibre and industrial production. In: Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, et al., editors. The European Nitrogen Assessment. New York: Cambridge University Press; 2011. p. 32–61.
- Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, et al. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science. 2008; 320(5878):889–92. https://doi.org/10.1126/science.1136674 PMID: 18487183
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, et al. Nitrogen cycles: past, present, and future. Biogeochemistry. 2004; 70(2):153–226. <u>https://doi.org/10.1007/s10533-004-0370-0</u>

- Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, et al. The nitrogen cascade. BioScience. 2003; 53(4):341–56. https://doi.org/10.1641/0006-3568(2003)053[0341:tnc]2.0.co;2
- Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, et al. Assessing our nitrogen inheritance. In: Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, et al., editors. The European Nitrogen Assessment. New York: Cambridge University Press; 2011. p. 1–6.
- Sutton MA, Billen G, Bleeker A, Bouwman AF, Bull K, Erisman JW, et al. Summary for policy makers. In: Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, et al., editors. The European Nitrogen Assessment. New York: Cambridge University Press; 2011. p. xxiv–xxxiv.
- Huhtanen P, Hristov AN. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. J Dairy Sci. 2009; 92(7):3222–32. <u>https://doi.org/10.3168/jds.2008-1352</u> PMID: 19528599
- Lund P, Søegaard K, Weisbjerg MR. Effect of strategies regarding concentrate supplementation and day-time grazing on N utilization at both field and dairy cow level. Livest Sci. 2008; 114(1):93–107. http://dx.doi.org/10.1016/j.livsci.2007.04.014.
- Calsamiglia S, Ferret A, Reynolds CK, Kristensen NB, van Vuuren AM. Strategies for optimizing nitrogen use by ruminants. Animal. 2010; 4(7):1184–96. <u>https://doi.org/10.1017/S1751731110000911</u> PMID: 22444616.
- Bruijnis MRN, Hogeveen H, Stassen EN. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. J Dairy Sci. 2010; 93(6):2419–32. http://dx.doi.org/ 10.3168/jds.2009-2721. PMID: 20494150
- Enting H, Kooij D, Dijkhuizen AA, Huirne RBM, Noordhuizen-Stassen EN. Economic losses due to clinical lameness in dairy cattle. Livest Prod Sci. 1997; 49(3):259–67. http://dx.doi.org/10.1016/S0301-6226 (97)00051-1.
- Kossaibati MA, Esslemont RJ. The costs of production diseases in dairy herds in England. Vet J. 1997; 154(1):41–51. http://dx.doi.org/10.1016/S1090-0233(05)80007-3. PMID: 9265852
- Garnsworthy PC. The environmental impact of fertility in dairy cows: a modelling approach to predict methane and ammonia emissions. Anim Feed Sci Tech. 2004; 112(1–4):211–23. <u>http://dx.doi.org/10.1016/j.anifeedsci.2003.10.011</u>.
- Mohd Nor N, Steeneveld W, Mourits MCM, Hogeveen H. The optimal number of heifer calves to be reared as dairy replacements. J Dairy Sci. 2015; 98(2):861–71. <u>http://dx.doi.org/10.3168/jds.2014-8329</u>. PMID: 25497803
- Zanton GI, Heinrichs AJ. Analysis of nitrogen utilization and excretion in growing dairy cattle. J Dairy Sci. 2008; 91(4):1519–33. http://dx.doi.org/10.3168/jds.2007-0624. PMID: 18349245
- Van Amburgh ME, Collao-Saenz EA, Higgs RJ, Ross DA, Recktenwald EB, Raffrenato E, et al. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J Dairy Sci. 2015; 98(9):6361–80. http://dx.doi.org/10.3168/jds.2015-9378. PMID: 26142847
- Beukes PC, Palliser CC, Macdonald KA, Lancaster JAS, Levy G, Thorrold BS, et al. Evaluation of a whole-farm model for pasture-based dairy systems. J Dairy Sci. 2008; 91(6):2353–60. http://dx.doi.org/ 10.3168/jds.2007-0728. PMID: 18487657
- Powell JM, Gourley CJP, Rotz CA, Weaver DM. Nitrogen use efficiency: A potential performance indicator and policy tool for dairy farms. Environmental Science & Policy. 2010; 13(3):217–28. http://dx.doi. org/10.1016/j.envsci.2010.03.007.
- Godinot O, Leterme P, Vertès F, Faverdin P, Carof M. Relative nitrogen efficiency, a new indicator to assess crop livestock farming systems. Agron Sust Dev. 2015; 35(2):857–68. https://doi.org/10.1007/ s13593-015-0281-6
- 22. NRC. Nutrient requirements of dairy cattle. Washington, D.C.: Natl. Acad. Press; 2001. 381 p.
- Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE, et al. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim Feed Sci Tech. 2004; 112(1–4):29–78. https://doi.org/10.1016/j.anifeedsci.2003.10.006
- Hammond AC, Waldo DR, Rumsey TS. Prediction of body composition in Holstein steers using urea space. J Dairy Sci. 1990; 73(11):3141–5. https://doi.org/10.3168/jds.S0022-0302(90)79003-0 PMID: 2273144
- Tomlinson DL, James RE, Bethard GL, McGilliard ML. Influence of undegradability of protein in the diet on intake, daily gain, feed efficiency, and body composition of Holstein Heifers. J Dairy Sci. 1997; 80(5): 943–8. https://doi.org/10.3168/jds.S0022-0302(97)76018-1 PMID: 9178135
- 26. Esslemont R, Kossaibati M. The costs of poor fertility and disease in UK dairy herds: trends in DAISY herds over 10 seasons. Walton, UK: Intervet; 2002.
- 27. Royal MD, Pryce JE, Woolliams JA, Flint APF. The genetic relationship between commencement of luteal activity and calving interval, body condition score, production, and linear type traits in Holstein-

Friesian dairy cattle. J Dairy Sci. 2002; 85(11):3071–80. http://dx.doi.org/10.3168/jds.S0022-0302(02) 74394-4. PMID: 12487474

- Brickell JS, Bourne N, McGowan MM, Wathes DC. Effect of growth and development during the rearing period on the subsequent fertility of nulliparous Holstein-Friesian heifers. Theriogenology. 2009; 72(3): 408–16. http://dx.doi.org/10.1016/j.theriogenology.2009.03.015. PMID: 19481791
- Brickell JS, McGowan MM, Pfeiffer DU, Wathes DC. Mortality in Holstein-Friesian calves and replacement heifers, in relation to body weight and IGF-I concentration, on 19 farms in England. Animal. 2009; 3(08):1175–82. https://doi.org/10.1017/S175173110900456X PMID: 22444847
- Brickell JS, McGowan MM, Wathes DC. Effect of management factors and blood metabolites during the rearing period on growth in dairy heifers on UK farms. Dom Anim Endocrinol. 2009; 36(2):67–81. <a href="http://dx.doi.org/10.1016/j.domaniend.2008.10.005">http://dx.doi.org/10.1016/j.domaniend.2008.10.005</a>.
- Brickell JS, Wathes DC. A descriptive study of the survival of Holstein-Friesian heifers through to third calving on English dairy farms. J Dairy Sci. 2011; 94(4):1831–8. http://dx.doi.org/10.3168/jds.2010-3710. PMID: 21426972
- Esslemont RJ, Kossaibati MA. Culling in 50 dairy herds in England. Vet Rec. 1997; 140(2):36–9. <a href="https://doi.org/10.1136/vr.140.2.36">https://doi.org/10.1136/vr.140.2.36</a> PMID: 9123795
- Coffey MP, Hickey J, Brotherstone S. Genetic aspects of growth of Holstein-Friesian dairy cows from birth to maturity. J Dairy Sci. 2006; 89(1):322–9. http://dx.doi.org/10.3168/jds.S0022-0302(06)72097-5. PMID: 16357296
- Moorby JM, Ellis NM, Davies DR. Assessment of dietary ratios of red clover and corn silages on milk production and milk quality in dairy cows. J Dairy Sci. 2016; 99(10):7982–92. <u>http://dx.doi.org/10.3168/</u> jds.2016-11150. PMID: 27474976
- Moorby JM, Lee MRF, Davies DR, Kim EJ, Nute GR, Ellis NM, et al. Assessment of dietary ratios of red clover and grass silages on milk production and milk quality in dairy cows. J Dairy Sci. 2009; 92(3): 1148–60. http://dx.doi.org/10.3168/jds.2008-1771. PMID: 19233807
- Moorby JM, Evans RT, Scollan ND, MacRae JC, Theodorou MK. Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.). Evaluation in dairy cows in early lactation. Grass Forage Sci. 2006; 61(1):52–9. https://doi.org/10.1111/j.1365-2494.2006.00507.x
- Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, et al. Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.): milk production from late-lactation dairy cows. Grass Forage Sci. 2001; 56(4):383–94. https://doi.org/10.1046/j.1365-2494. 2001.00288.x
- Hare E, Norman HD, Wright JR. Trends in calving ages and calving intervals for dairy cattle breeds in the United States. J Dairy Sci. 2006; 89(1):365–70. http://dx.doi.org/10.3168/jds.S0022-0302(06) 72102-6. PMID: 16357301
- **39.** Johnson D. The triangular distribution as a proxy for the beta distribution in risk analysis. J R Stat Soc Series D (The Statistician). 1997; 46(3):387–98. https://doi.org/10.1111/1467-9884.00091
- 40. Boulton AC, Rushton J, Wathes DC. An empirical analysis of the cost of rearing dairy heifers from birth to first calving and the time taken to repay these costs. Animal. 2017; 11(8):1372–80. Epub 02/08. https://doi.org/10.1017/S1751731117000064 PMID: 28173887
- Lee MRF, Brooks AE, Moorby JM, Humphreys MO, Theodorou MK, Macrae JC, et al. In vitro investigation into the nutritive value of Lolium perenne bred for an elevated concentration of water-soluble carbohydrate and the added effect of sample processing: freeze-dried and ground vs. frozen and thawed. Anim Res. 2002; 51(4):269–77. https://doi.org/10.1051/animres:2002023.
- Lee MRF, Harris LJ, Moorby JM, Humphreys MO, Theodorou MK, MacRae JC, et al. Rumen metabolism and nitrogen flow to the small intestine in steers offered Lolium perenne containing different levels of water-soluble carbohydrate. Anim Sci. 2002; 74(3):587–96. https://doi.org/10.1017/ S1357729800052747
- Lee MRF, Jones EL, Moorby JM, Humphreys MO, Theodorou MK, Scollan ND. Production responses from lambs grazed on Lolium perenne selected for an elevated water-soluble carbohydrate concentration. Anim Res. 2001; 50(6):441–9. https://doi.org/10.1051/animres:2001106.
- 44. Evans JG, Fraser MD, Owen I, Davies DA. An evaluation of two perennial ryegrass cultivars (AberDart and Fennema) for sheep production in the uplands. J Agri Sci. 2010; 149(2):235–48. https://doi.org/10. 1017/S0021859610001048
- Nguyen TLT, Hermansen JE, Mogensen L. Environmental consequences of different beef production systems in the EU. J Clean Prod. 2010; 18(8):756–66. http://dx.doi.org/10.1016/j.jclepro.2009.12.023.
- EUROSTAT. Agriculture European Commission; 2016 [cited 2016]. http://ec.europa.eu/eurostat/web/ agriculture.

- 47. Van Vuuren AM, Meijs JAC. Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows. In: Van Der Meer HG, Unwin RJ, Van Dijk TA, Ennik GC, editors. Animal Manure on Grassland and Fodder Crops Fertilizer or Waste? Proceedings of an International Symposium of the European Grassland Federation, Wageningen, The Netherlands, 31 August–3 September 1987. Dordrecht: Springer Netherlands; 1987. p. 17–25.
- Plaizier JCB, Lissemore KD, Kelton D, King GJ. Evaluation of overall reproductive performance of dairy herds. J Dairy Sci. 1998; 81(7):1848–54. http://dx.doi.org/10.3168/jds.S0022-0302(98)75755-8. PMID: 9710751
- 49. Esslemont R. Measuring dairy herd fertility. Vet Rec. 1992; 131(10):209–12. PMID: 1441105
- Holmann FJ, Shumway CR, Blake RW, Schwart RB, Sudweeks EM. Economic value of days open for Holstein cows of alternative milk yields with varying calving intervals. J Dairy Sci. 1984; 67(3):636–43. https://doi.org/10.3168/jds.S0022-0302(84)81349-1
- Schmidt GH. Effect of length of calving intervals on income over feed and variable costs. J Dairy Sci. 1989; 72(6):1605–11. https://doi.org/10.3168/jds.S0022-0302(89)79272-9
- Butler ST, Shalloo L, Murphy JJ. Extended lactations in a seasonal-calving pastoral system of production to modulate the effects of reproductive failure. J Dairy Sci. 2010; 93(3):1283–95. http://dx.doi.org/ 10.3168/jds.2009-2407. PMID: 20172248
- Auldist MJ, O'Brien G, Cole D, Macmillan KL, Grainger C. Effects of varying lactation length on milk production capacity of cows in pasture-based dairying systems. J Dairy Sci. 2007; 90(7):3234–41. <u>http://</u> dx.doi.org/10.3168/jds.2006-683. PMID: 17582106
- Arbel R, Bigun Y, Ezra E, Sturman H, Hojman D. The effect of extended calving intervals in high lactating cows on milk production and profitability. J Dairy Sci. 2001; 84(3):600–8. http://dx.doi.org/10.3168/ jds.S0022-0302(01)74513-4. PMID: 11286412
- 55. Lopez S, France J, Odongo NE, McBride RA, Kebreab E, AlZahal O, et al. On the analysis of Canadian Holstein dairy cow lactation curves using standard growth functions. J Dairy Sci. 2015; 98(4):2701–12. https://doi.org/10.3168/jds.2014-8132 PMID: 25648814
- Grainger C, Auldist MJ, O'Brien G, Macmillan KL, Culley C. Effect of type of diet and energy intake on milk production of Holstein-Friesian cows with extended lactations. J Dairy Sci. 2009; 92(4):1479–92. http://dx.doi.org/10.3168/jds.2008-1530. PMID: 19307629
- Heinrichs AJ. Raising dairy replacements to meet the needs of the 21st century. J Dairy Sci. 1993; 76(10):3179–87. http://dx.doi.org/10.3168/jds.S0022-0302(93)77656-0. PMID: 8227639
- Tozer PR, Heinrichs AJ. What affects the costs of raising replacement dairy heifers: A multiplecomponent analysis. J Dairy Sci. 2001; 84(8):1836–44. http://dx.doi.org/10.3168/jds.S0022-0302(01) 74623-1. PMID: 11518308
- 59. Mohd Nor N, Steeneveld W, Mourits MCM, Hogeveen H. Estimating the costs of rearing young dairy cattle in the Netherlands using a simulation model that accounts for uncertainty related to diseases. Prev Vet Med. 2012; 106(3–4):214–24. http://dx.doi.org/10.1016/j.prevetmed.2012.03.004. PMID: 22487166
- Styles D, Gonzalez-Mejia A, Moorby J, Foskolos A, Gibbons J. Climate mitigation by dairy intensification depends on intensive use of spared grassland. Global Change Biology. 2018; 24(2):681–93. https://doi.org/10.1111/gcb.13868 PMID: 28940511
- Gonzalez-Mejia A, Styles D, Wilson P, Gibbons J. Metrics and methods for characterizing dairy farm intensification using farm survey data. PloS one. 2018; 13(5):e0195286. https://doi.org/10.1371/journal. pone.0195286 PMID: 29742166