Ministry for Primary Industries
Manatū Ahu Matua

Partitioning of animal excreta N into urine and dung and developing the N_2O inventory

MPI Technical Paper No: 2014/05

Prepared by AgResearch
June 2010

ISBN No: 978-0-478-42367-9 (online)
ISSN No: 2253-3923 (online)

March 2014

New Zealand Government

Growing and Protecting New Zealand

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(MAF POL 0910-11528, 09-03)

MAF

June 2010

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MAF

June 2010

Jiafa Luo, Frank Kelliher

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Abbreviations

DMI	Dry-matter intake in kg animal^{-1} day^{-1}
N_{intake}	N consumed in g animal^{-1} day^{-1}
N_{milk}	N contained in milk in g animal^{-1} day^{-1}
N_{urine}	N excreted as urine in g animal^{-1} day^{-1}
N_{dung}	N excreted as dung in g animal^{-1} day^{-1}
$\%N_{urine}$	Percent of total excretal N in the form of urine
$\%N_{dung}$	Percent of total excretal N in the form of dung
$N_{\%diet}$	Percentage weight of N in dry matter consumed

1. Abstract

The aim of this study was to refine the method of predicting the partitioning of N between urine and dung and to assess the impact on the national N_2O inventory.

A majority of the N ingested by ruminant livestock is excreted as either urine or dung. The form in which the N is excreted influences the extent of the environmental pollution caused. As the concentration of N in an animal's diet is increased, the amount of N excreted as urine increases sharply, while the amount of N in the dung remains relatively constant. The proportion of the total excretal N in the form of urine can be described as a linear function of the concentration of N in the diet. An equation developed from analysis of published data for dairy cattle did not differ significantly from an equation developed for dry stock (sheep and beef cattle). The combined data for dairy cattle, beef cattle and sheep can be described by the equation ($r^2 = 0.67$, $P < 0.01$):

% excretal N in form of urine ($\%N_{urine}$) *= 10.5(±1.1) × %N in the diet* ($N_{\%diet}$) *+ 34.4(±3.4)*

The percentage of excreted N in dung ($\%N_{dung}$)) is obtained by difference (subtracting $\%N_{urine}$ from 100%).

The agricultural soils N_2O emissions inventory has been developed by including the updated equation for partitioning total excretal N into urine and dung. Calculations were done for the years 1990 and 2007 using the current value of EF3 (1%) as well as EF3 for urine and dung equal to 1 and 0.25%, respectively, based on the recent recommendation. When urine and dung EF3 were 1 and 0.25%, respectively, total excretal N_2O emissions in 1990 and 2007 were 23.8 and 25.9 Gg N_2O yr^{-1} (7.4 and 8.0 Mt CO_2-eq yr^{-1}), respectively, i.e. an increase of 2.1 Gg N_2O yr^{-1} (about 0.6 Mt CO_2-eq yr^{-1}). These values were around 8 Gg N_2O yr^{-1} (2.5 Mt CO_2-eq yr^{-1}) less than those computed with both the urine and dung EF3 equal to 1%, the currently used value, while the increase of emissions between 1990 and 2007 was about 0.2 Mt CO_2-eq yr^{-1} less. Adopting the recently recommended values of EF3 for urine and dung would reduce total excretal N_2O emissions and the increase of them from 1990 to 2007, compared to the current inventory calculations.

Data on N partitioning into urine and dung for grazed sheep is very limited. In this review we can only include data for sheep from one published paper. Therefore, we recommend that trials be conducted for collection of more data about fate of dietary N in sheep fed predominantly on herbage diets. Accordingly, our developed equation for

predicting the percentage of excreted N in dung and urine can be improved and the national greenhouse gas inventory calculation can be more accurate.

2. Introduction

In grazed pastures, a substantial amount of N is recycled through the direct deposition of animal excreta. Up to 25% of the total N consumed by grazing animals is retained in animal products (i.e. milk and meat) (van Vuuren and Meijs, 1987). The remainder is excreted in urine and dung. It has been shown that excreta patches, particularly urine patches, are important sources for N loss *via* ammonia volatilization (Jarvis *et al.*, 1989), nitrate leaching (Haynes and Williams, 1993), denitrification and N_2O emissions (Ryden, 1986). Research by Yamulki *et al.* (1998) in England suggests that the proportion of excreta-N loss as N_2O is lower for dung-N than urine-N. Research by the NzOnet team is also indicating differences in N_2O emission factor for dung relative to urine (Luo *et al.*, 2009a, b). If our future N_2O inventory calculations are to account for these differences, it will be important to have a reliable method for estimating the relative excretion of N in dung versus urine. The partitioning of N in dung and urine is dependent on the N content of the diet with relatively more N excreted in urine as the %N in diet increases. In a previous report to MAF, Ledgard *et al*. (2003) suggested Equation 1, derived from published data, as a way of allowing separate N_2O emission factors for urine and dung to be applied to existing models.

$$\%N_{urine} = 11.0(\pm 1.1) \times N_{\%diet} + 31.8(\pm 3.5) \qquad 1$$

where $\%N_{urine}$ (% of excretal N) is the percentage of excreted N in urine, and $N_{\%diet}$ is the N percentage in the diet consumed.

The aim of the present report is to examine alternative methods of predicting the partitioning of N between urine and dung in farmed ruminants and if appropriate to update this equation, taking into account additional data. Data were gathered from published N balance experiments for ruminants fed on pasture, as this is the most common feeding system used in New Zealand. However, feeding animals on pasture alone is not common in other countries and this restriction limited the available data. Data are included from experiments where dairy cattle were fed up to 1 kg of concentrate per day. No data, additional to that used by Ledgard *et al.* (2003), could be found for pasture-fed sheep or beef cattle.

There are difficulties involved in collecting the total dung and urine output from an animal while it roams pastures and the authors cited dealt with this in one of two ways. Some chose to house animals in metabolism stalls and provide pasture that had been cut up to 24 hours earlier (e.g. Peraud *et al.*, 1997). Others allowed animals to graze the pasture and estimated feed and faecal volumes with inert markers such as chromic oxide (Berry *et al.*, 2001) or alkanes (Peyraud *et al.*, 1997). The latter method also required amount of N excreted in urine (N_{urine}) to be approximated as the portion of N intake (N_{intake}) not accounted for elsewhere, often with the assumption that no N was retained as body tissue. Data from both types of experiments are included in this report.

Data from 5 additional studies were combined with the data used by Ledgard *et al.* (2003) and analysed to produce a revised equation to predict $\%N_{urine}$ as a function of $N_{\%diet}$.

3. Partitioning of N consumed by lactating dairy cows.

Table 1 shows the published N partitioning data gathered for dairy cattle, including data reported by Ledgard *et al*. (2003). On average only 22% of the nitrogen consumed was secreted as milk, which is comparable to the 25% that was observed when Castillo *et al.* (2000) analysed published data from dairy cows fed 91 different diets. On average only 3% of the N consumed was retained, as determined either by subtracting N in urine (N_{urine}), dung (N_{dung}) and milk (N_{milk}) from N_{intake} or by measuring changes in body weight. This low retention value supports the assumption of zero N retention made by several authors when calculating N_{urine}. However, N retention for individual treatments can range from 28% (Bargo *et al.,* 2002) to -17% (Berry *et al*, 2001). N balance studies are typically conducted after peak lactation or in early pregnancy when no changes in body weight are expected (Castillo *et al.,* 2000).

Figure 1 shows that N_{urine} increases sharply with increasing N_{Intake} while N_{dung} remains relatively constant. Lantinga *et al*. (1987) found that irrespective of the N level of feed intake, dairy cattle excreted an average of 132 g N cow^{-1} day^{-1} in the form of dung. Kebreab *et al.* (2001 and 2002) and Castillo (2000) observed that increasing N_{intake} caused a slight increase in N_{dung} and an exponential increase in N_{urine} (Table 2). Castillo (2000) suggested that this exponential relationship could also be described by two linear equations, one when N_{intake} < 400 g·cow^{-1} day^{-1} and one when N_{intake} > 400 g·cow^{-1} day^{-1}. As 75% of the data in Figure 1 are for N_{intake} > 400 g cow^{-1} day^{-1}, the relationship between N_{urine} and N_{intake} could therefore be expected to appear linear.

Table 1. Fate of dietary N in dairy cows fed predominantly on herbage diets (% of N intake).

Source	%N in diet	N intake ($g\ cow^{-1}\ day^{-1}$)	% of N intake			
			Milk	Urine	Dung	Retained
Carruthers *et al.* (1997)	3.0	354	16	60	27	-3
	3.8	563	21	52	21	6
Carruthers and Neil (1997)	2.8	409	24	46	28	2
	2.1	304	32	33	38	-2
Mackle *et al.* (1996)	3.7	485	20	48	25	8
	3.7	408	21	50	21	9
	3.5	507	20	48	22	10
	3.5	401	22	49	22	7
van der Meer (1982)	3.0	506	21	56	23	[b]
	4.4	647	16	66	17	[b]
Kemp (1979) from van der Meer (1982)	2.4	360	29	43	28	[b]
	3.0	444	24	53	23	[b]
	3.5	528	20	59	20	[b]
	4.1	612	17	65	18	[b]
van der Honing (1982) from van Vuuren and Meijs (1987)	3.7 [a]	521	17	54	24	5
	3.9 [a]	460	23	49	26	2
Berry *et al.* (2001)	1.9	328	31	50	35	-17
	2.2	385	22	39	42	-3
	3.2	550	16	49	36	-1
	2.4	415	24	56	29	-9
	1.8	309	26	46	31	-3
	2.9	508	16	62	27	-5
Astigarraga *et al.* (2002)	2.8	503	22	56	22	[b]
	2.4	423	26	48	26	[b]
Peyraud *et al.* (1997)	1.7	263	35	26	34	5
	2.4	367	25	45	25	5
Astigarraga *et al.* (1994)[c]	2.4	423	26	48	26	[b]
	2.8	503	22	56	22	[b]
Mulligan *et al.* (2004)	3.1 [a]	499	29	45	26	[b]
Mean:	**3.0**	**459**	**22**	**49**	**26**	**3**

[a] diet included a small amount of concentrate (<1 kg $cow^{-1}\ day^{-1}$)
[b] urine N estimated assuming zero N retention
[c] details of methods (Peyraud *et al.*, 1994) and certain data (Peyraud and Astigarraga, 1998) are given in separate publications

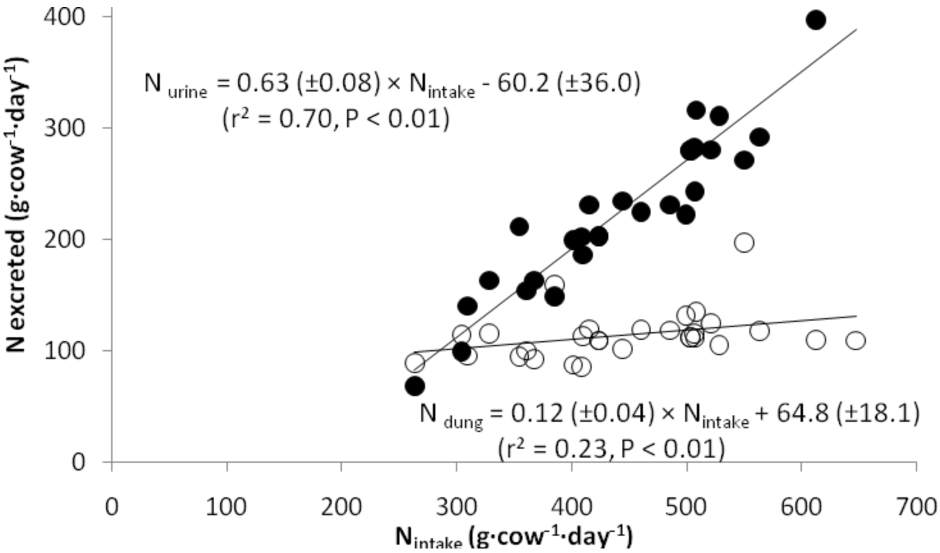

Figure 1. Relationship between N_{intake} and N_{urine} (●) or N_{dung} (○), data were derived from Table 1.

Figure 2 show the relationship between $\%N_{urine}$ and $N_{\%diet}$, which can be described by the equation ($r^2 = 0.54$, $P < 0.01$):

$$\%N_{urine} = 8.65(\pm 1.53) \times N_{\%diet} + 39.7(\pm 4.7) \qquad 2$$

Hutanen *et al.* (2008) examined a very large set of data and suggested a model in which $\%N_{urine}$ linearly related to the percentage of crude protein in the diet (Table 2). By converting % crude protein to %N by the standard factor of 6.25, this equation can be put into a similar format to Equation 2:

$$\%N_{urine} = 15.5 \times N_{\%diet} + 15.5 \qquad 3$$

The slope and intercept of Equation 3 appear quite different to Equation 2, but animals in the trials studied by Hutanen *et al.* (2008) were fed silage-based diets, and both N_{urine} and N_{dung} were calculated, rather than measured, based on various feed parameters.

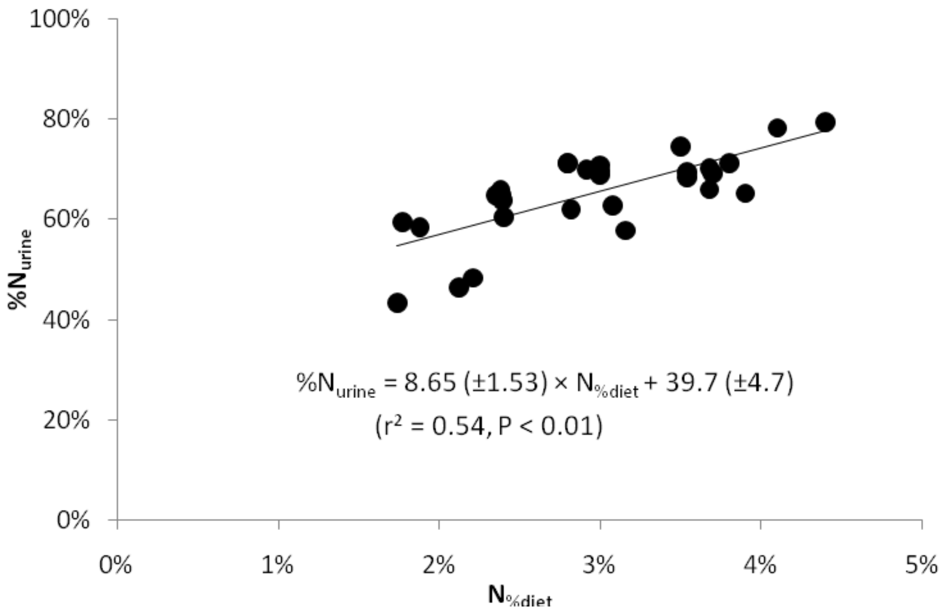

Figure 2. Relationship between $N_{\%diet}$ concentration and $\%N_{urine}$ for dairy cattle, data derived from Table 1.

Table 2. Equations predicting N_{urine}, N_{dung} and $\%N_{urine}$.

Source	Animal	Equation
Ledgard *et al.* (2003)	Dairy cattle	$\%N_{urine} = 10.1 \times N_{\%diet} + 34.2$
	Dry stock	$\%N_{urine} = 11.9 \times N_{\%diet} + 29.9$
	Combined	$\%N_{urine} = 11.0 \times N_{\%diet} + 31.8$
Kebreab *et al.* (2001)	Dairy cattle	$N_{urine} = 0.003 \times N_{intake}^{1.8}$
		$N_{dung} = 0.16 \times N_{intake} + 76.7$
Castillo *et al.* (2000)	Dairy cattle	$N_{urine} = 30.4 \times e^{0.0086 \times N_{intake}}$
		$N_{dung} = 0.21 \times N_{intake} + 52.3$
Hutanen *et al.* (2008)	Dairy cattle	$\%N_{urine} = 2.48 \times crude\ protein_{\%diet} + 15.5$
		$N_{urine} = 0.844 \times N_{intake} - 13 \times DMI + 27$
Patra (2009)	Sheep	$N_{urine} = 0.20 \times N_{\%diet} - 0.022$
Jonker *et al.* (1998)	Dairy cattle	$N_{urine} = 12.54 \times milk\ urea\ nitrogen\ (mg \cdot dl^{-1})$

4. Partitioning of N consumed by sheep and cattle

Ledgard *et al.* (2003) assembled the data in Table 2 from the few published N balance studies involving sheep and non-lactating cattle. The retention of N by cattle ranged between 4 and 44% of that ingested. The higher values reported by Betteridge *et al.* (1986) are probably unrealistically high and an artefact of the method used to measure N retention, as acknowledged by the authors themselves. Ledgard *et al.* (2003) found that the equation for $\%N_{urine}$ as a function of $N_{\%diet}$ (Table 2, Figure 3) did not differ significantly from their equation for dairy cows and therefore chose to combine data from dairy cows and dry stock. No additional data could be found for dry stock for the present report.

When data from dairy cattle and dry stock were combined, the relationship between $N_{\%diet}$ and $\%N_{urine}$ was ($r^2 = 0.67$, $P < 0.01$):

$$\%N_{urine} = 10.5(\pm 1.1) \times N_{\%diet} + 34.4(\pm 3.4) \qquad 4$$

Table 2. Fate of dietary N in dairy cows fed predominantly on herbage diets (% of N intake), reproduced from Ledgard *et al.* (2003).

Source	%N in diet	N intake (g animal^{-1} day^{-1})	% of N intake: Urine	Dung	Retained
Cattle					
Henzell and Ross (1973)	1.3	39 [a]	38	51	10
	3.5	105 [a]	73	23	4
Jarvis *et al.* (1989)	2.7	140	46	31	23
	2.6	160	43	28	29
	3.9	200	73	23	4
	2.3	110	41	42	17
	2.2	120	51	36	13
	3.2	200	57	24	19
Betteridge *et al.* (1986)	2.7	263	51	24	25
	3.4	231	40	16	44
	3.0	242	46	21	33
Mean	**2.8**	**165**	**51**	**29**	**20**
Sheep					
Henzell and Ross (1973)	1.4	38 [a]	39	47	14
	3.5	94 [a]	71	23	6

[a] data converted from kg of N hectare^{-1} year^{-1}

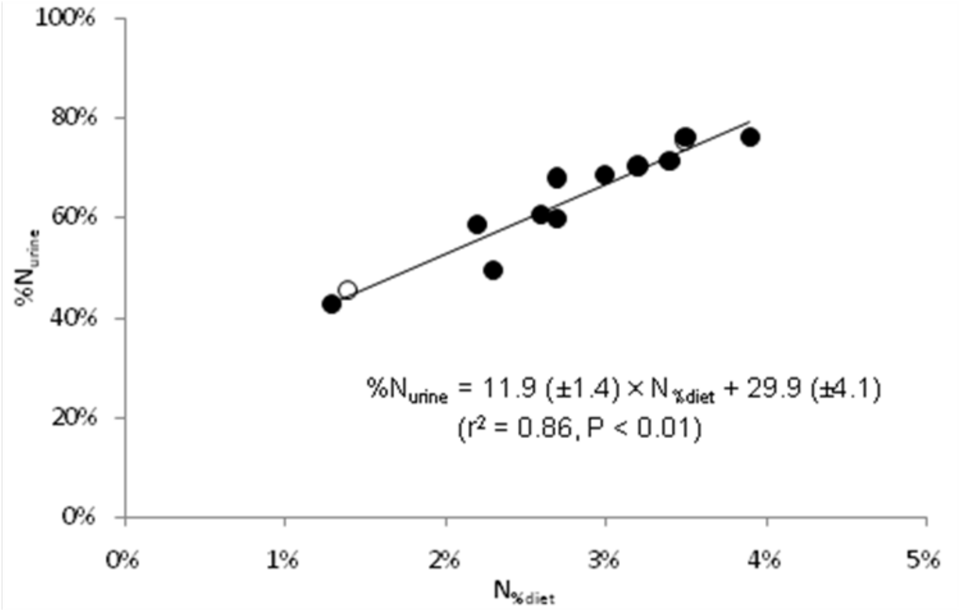

Figure 3. Relationship between N%diet and the %Nurine for sheep and beef cattle (hollow points represent data from sheep), reproduced from Ledgard *et al.* (2003).

5. Other models predicting N_{urine} and $\%N_{urine}$

A number of different models have been used for predicting N_{urine} and $\%N_{urine}$ and these are outlined:

Jonker *et al.* (1998) proposed that N_{urine} is proportional to milk urea nitrogen for lactating dairy cows, as both are proportional to the concentration of urea in the blood:

$$N_{urine} = 12.54 \times milk\ urea\ nitrogen\ (mg \cdot dl^{-1}) \qquad 5$$

Body weight and milk production were also shown to influence this relationship. This approach is not appropriate for the present report as it is not applicable to non-lactating animals and milk urea nitrogen is not commonly tested in New Zealand.

Kebreab *et al.* (2002) proposed a dynamic model for N partitioning based on modelling the quantity of N present in an animal as digesta, microbes, amino acids and ammonia/urea. Differential equations were used to describe movement of N between these N pools and its conversion to faeces, urine, milk and body tissue. In this model urine N was produced from the ammonia/urea N pool, which was fed by the digesta N pool, representing the breakdown of unwanted amino acids by microbes, and the amino acid N pool, representing amino acid catabolism by the animal. This model was shown to accurately predict N partitioning for a single animal.

A major source of urine N is excess N from the rumen. The protein requirement of the rumen microbes is determined by the amount of energy available and excess amino acids are broken down to urea which is excreted as urine (Kebreab *et al*., 2002). This is seen in the model of Hutanen *et al.* (2008) which shows a linear relationship between $\%N_{urine}$ and percent crude protein in the diet (Table 2). Interestingly, this model was improved slightly when milk urea nitrogen was included as a second variable. As indicated earlier, milk urea nitrogen is not commonly tested in New Zealand.

6. Developing the agricultural soils N_2O emissions inventory

This section develops the agricultural soils N_2O emissions inventory by including the updated equation for partitioning total excretal N into urine and dung. Calculations were done for the years 1990 and 2007 using the current value of EF3 (1%) as well as EF3 for urine and dung equal to 1 and 0.25%, respectively, based on the recommendation of Luo *et al.* (2009a, b).

Through a literature review, an additional 15 means from N balance studies on dairy cattle were identified and added to the data examined by Ledgard *et al.* (2003). However, no new data for sheep or beef cattle could be found. A linear relationship with $N\%_{diet}$ was the most appropriate way to predict $\%N_{urine}$. When data for dairy cattle and dry stock were combined, the relationship between $N\%_{diet}$ and $\%N_{urine}$ was:

% excretal N in form of urine ($\%N_{urine}$) = 10.5(±1.1) × %N in the diet ($N_{\%diet}$) + 34.4(±3.4)

This new equation should be used in place of the previous one, developed by Ledgard *et al.* (2003), when calculating the partitioning of excretal N into urine and dung for New Zealand's agricultural soils national N_2O emissions inventory. The $\%N_{dung}$ can be calculated by subtracting $\%N_{urine}$ from 100%. The new equation yields similar results to the one that is currently used in the inventory (Table 4).

Table 4. Feed N content by type of animal in the agricultural soils N_2O emissions inventory. Also shown are percentages of excretal N in the form of urine based on the currently used relation with feed N content and values based on an updated relation that has included data from 15 additional studies.

Animal	Feed N content	Current % excretal N in the form of urine	Updated% excretal N in the form of urine
	%	%	%
Dairy cattle	3.7	73.6	73.2
Beef cattle	3.0	66.1	65.9
Sheep	3.0	66.1	65.9
Deer	3.0	66.1	65.9

For the inventory calculations, we extracted mean (total) N excretion rate per animal, population and total N excretion by animal populations for 1990 and 2007 from the 2007 agricultural N_2O emissions inventory report (common reporting format tables) (Table 5). The total N_{ex} data were combined with the updated equation to partition it into N excretion as urine and dung. These were then multiplied by the different values of EF3 with conversions to compute the N_2O emissions from urine and dung by animal type as well as total excretal N_2O emissions for the two years (Tables 6 and 7).

Table 5. Mean (total) N excretion rate per animal, population and total N excretion by animal populations in 1990 and 2007 taken from the 2007 agricultural N_2O emissions inventory report (common reporting format tables).

Animal	Total N_{ex} kg N head^{-1} year^{-1}	Population on 30 June	Total N_{ex} Gg N year^{-1}
1990			
Dairy cattle	103.9	3,448,200	358
Beef cattle	65.5	4,593,160	301
Sheep	12.6	57,852,190	730
Deer	24.9	976,290	24
All animals			***1,413***
2007			
Dairy cattle	113.6	5,260,850	598
Beef cattle	74.0	4,393,620	625
Sheep	15.0	38,460,480	576
Deer	29.7	1,396,020	41
All animals			***1,840***

Table 6. Urine and dung N_2O emissions by animal populations in 1990 and 2007 when urine EF3 = dung EF3 = 1.0%.

Animal	N_2O emissions from urine Gg N_2O year^{-1}	N_2O emissions from dung Gg N_2O year^{-1}	N_2O emissions from urine and dung Gg N_2O year^{-1}
1990			
Dairy cattle	5.8	2.1	7.9
Beef cattle	4.4	2.3	6.7
Sheep	10.6	5.5	16.1
Deer	0.3	0.2	0.5
All animals	***21.1***	***10.1***	***31.2***
All animals (Mt CO_2-eq yr^{-1})	6.5	3.2	9.7
2007			
Dairy cattle	9.6	3.5	13.1
Beef cattle	4.7	2.4	7.1
Sheep	8.3	4.3	12.6
Deer	0.6	0.3	0.9
All animals	***23.2***	***10.5***	***33.7***
All animals (Mt CO_2-eq yr^{-1})	7.2	3.3	10.5

Table 7. Urine and dung N_2O emissions by animal populations in 1990 and 2007 when urine EF3 was 1% and dung EF3 was 0.25%.

Animal	N_2O emissions from urine Gg N_2O year^{-1}	N_2O emissions from dung Gg N_2O year^{-1}	N_2O emissions from urine and dung Gg N_2O year^{-1}
1990			
Dairy cattle	5.8	0.5	6.3
Beef cattle	4.4	0.6	5.0
Sheep	10.6	1.4	12.0
Deer	0.4	0.05	0.45
All animals	***21.2***	***2.6***	***23.8***
All animals (Mt CO_2-eq yr^{-1})	6.6	0.8	7.4
2007			
Dairy cattle	9.6	0.9	10.5
Beef cattle	4.7	0.6	5.3
Sheep	8.3	1.1	9.4
Deer	0.6	0.1	0.7
All animals	***23.2***	***2.7***	***25.9***
All animals (Mt CO_2-eq yr^{-1})	7.2	0.8	8.0

When EF3 was 1%, total excretal N_2O emissions were 31.2 and 33.7 Gg yr^{-1} in 1990 and 2007, respectively, i.e. an increase of 2.5 Gg yr^{-1}. For sheep and dairy cattle, the corresponding values were 16.1 (52% of that for all animals), 12.6 (37%) and -3.5 Gg yr^{-1} and 7.9, 13.1 and 5.2 Gg yr^{-1}. Over the 17 years, total excretal N_2O emissions increased by 8%.

When urine and dung EF3 were 1 and 0.25%, respectively, the corresponding total excretal N_2O emissions were 23.8 and 25.9 Gg yr^{-1} i.e. an increase of 2.1 Gg yr^{-1}. These emissions were around 8 Gg N_2O yr^{-1} (2.5 Mt CO_2-eq yr^{-1}) less than those computed with both urine and dung EF3 equal to 1% and the increase of emissions from 1990 to 2007 was about 0.2 Mt CO_2-eq yr^{-1} less. For sheep and dairy cattle, the corresponding values were 12.0, 9.4 and -2.6 Gg N_2O yr^{-1} and 6.3, 10.5 and 4.2 Gg N_2O yr^{-1}.

In summary, when urine and dung EF3 were 1 and 0.25%, respectively, as recently recommended, total excretal N_2O emissions in 1990 and 2007 were 23.8 and 25.9 Gg N_2O yr^{-1} (7,4 and 8,0 Mt CO_2-eq $year^{-1}$), respectively, i.e. an increase of 2.1 Gg N_2O $year^{-1}$ (0.6 Mt CO_2-eq $year^{-1}$). These values were around 8 Gg N_2O yr^{-1} (2.5 Mt CO_2-eq yr^{-1}) less than those computed with EF3 equal to 1%, the currently used value, and the increase of emissions was 0.2 Mt CO_2-eq yr^{-1} less. Adopting the recently recommended values of EF3 for urine and dung would reduce total excretal N_2O emissions and the increase of them from 1990 to 2007, compared to the current inventory calculations.

7. Conclusions

Through a literature review, an additional 15 means from N balance studies on dairy cattle were identified and added to the data examined by Ledgard *et al.* (2003), but no new data for sheep or beef cattle could be found. A linear relationship with $N\%_{diet}$ was the most appropriate way to predict $\%N_{urine}$. When data for dairy cattle and dry stock were combined, the relationship between $N\%_{diet}$ and $\%N_{urine}$ was:

% excretal N in form of urine $(\%N_{urine}) = 10.5(\pm 1.1) \times \%N$ *in the diet* $(N_{\%diet}) + 34.4(\pm 3.4)$

The New Zealand agricultural soils N_2O emissions inventory has been developed by including the updated equation for partitioning total excretal N into urine and dung. When urine and dung EF3 were 1 and 0.25%, respectively, as recently recommended (Luo et *al.* 2009b), total excretal N_2O emissions in 1990 and 2007 were 23.8 and 25.9 Gg N_2O yr^{-1} (7.4 and 8.0 Mt CO_2-eq $year^{-1}$), respectively, i.e. an increase of 2.1 N_2O Gg yr^{-1} (0.6 Mt CO_2-eq $year^{-1}$). These values were around 8 Gg N_2O yr^{-1} (2.5 Mt CO_2-eq $year^{-1}$) less than those computed with EF3 equal to 1%, the currently used value, and the increase of emissions was 0.2 Mt CO_2-eq yr^{-1} less. Adopting the recently recommended values of EF3 for urine and dung would reduce total excretal N_2O emissions and the increase of them from 1990 to 2007, compared to the current inventory calculations.

There could be merit in conducting further experiments to explore the partitioning of N into N in urine and dung, particularly for grazed sheep. We have only been able to include one single study of sheep into the regression equation for use in calculation of the GHG inventory. Therefore, more data for sheep is required that can be used to develop regression parameters with small uncertainties.

8. Acknowledgement

The authors would like to acknowledge the assistance with statistical matters provided by Dr John Waller.

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