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A Tier 3 Method for Enteric Methane in Dairy Cows Applied for Fecal N Digestibility in the Ammonia Inventory

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The current inventory of N emission from cow excreta relies on fecal N digestibility data in Dutch feeding tables, assuming additivity of dietary ingredients to obtain diet values (*CVB model*). Alternatively, fecal N digestibility can be estimated by a dynamic, mechanistic model of digestion in the gastrointestinal tract, currently used as Tier 3 for enteric methane prediction in the Netherlands (*Tier 3 model*). Estimates of *in situ* rumen degradation characteristics for starch, neutral detergent fiber (NDF) and crude protein used as an input for the *Tier 3 model* were based on Dutch feeding tables (the protein evaluation system). Both methods were evaluated on independent dataset on fecal N digestibility that was constructed from peer-reviewed papers on N balance data for dairy cows published since 1999 (54 trials, 242 treatment means). Results indicate that observed apparent fecal N digestibility (67.0 ± 6.77%) was systematically over-predicted in particular by the *CVB model* (73.8 ± 4.35%) compared to the *Tier 3 model* (69.8 ± 4.52%). For the dataset including only observations from Dutch trials the observed fecal N digestibility (70.4 ± 7.33%) was also systematically over-predicted by the *CVB model* (76.4 ± 5.27%) but not by the *Tier 3 model* (69.7 ± 5.81%). Mixed model analysis with study as random factor indicated the slope of the regression between observed and predicted fecal N digestibility to be smaller than 1, in particular for the *CVB model* (*CVB model* slope varied between 0.405 and 0.560 and *Tier 3 model* slope between 0.418 and 0.657). The over-prediction by the *CVB model* with 6–7%-units of digestibility will lead to an over-predicted ammoniacal N excretion (urinary N) in the ammonia inventory, and biased estimation of N mitigating potential of nutritional measures. The present study demonstrates the benefit of using the *Tier 3 model* to predict the average level of apparent fecal N digestibility compared to the *CVB model*. The general estimates of *in situ* rumen degradation characteristics for starch, NDF and crude protein used as input for the *Tier 3 model* seemed applicable for the Dutch trials but less so for the non-Dutch trials.

Keywords: models, Tier 3, dairy cows, nitrogen digestibility, nitrogen excretion

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INTRODUCTION

Ammonia emitted from dairy production systems is a major water and air pollutant, leading to eutrophication, acidification and fine particulate matter formation. These emissions are reported annually to the European Commission, according to the Göteborg and Kyoto protocols (e.g., Nielsen et al., 2018; Wakeling et al., 2018). Most inventory efforts adopt the concept of ammonia emission factors specific for an animal category or type of agricultural activity (Paulot et al., 2014). This implies a specific emission factor has to be allocated a priori to every management practice or abatement measure accounted for in the model. Actual modeling of the cause of variation in ammonia emission requires representation of details of the emission process itself. An ammonia emission model has been developed for inventory purpose in the Netherlands (Velthof et al., 2012) including the ammonia emissions from animal excreta. A crucial element in this model is the prediction of the urinary excretion rate of potentially volatile nitrogen, often referred to as total ammoniacal nitrogen (TAN). The proportion of nitrogen (N)-containing components in urine that is susceptible to almost instant volatilization varies considerably (Dijkstra et al., 2013, 2018), and mineralization of organic manure N (fecal N) also contributes to TAN (Vonk et al., 2016). For reasons of simplicity we refer to TAN as being the total amount of N excreted with urine, irrespective of the form of N present in urine and excluding a further input to TAN from mineralization of fecal excreted N.

In ammonia inventory methodology, accurate estimates of apparent fecal N digestibility are required to allow calculation of TAN excretion rate. This rate is calculated as the amount of N ingested that is apparently digested at the level of feces (by taking N intake times apparent fecal N digestibility of ingested feed), minus the amount of N retained in milk, body tissues, and offspring. Data on N intake and N retained by the cow can be retrieved from the activity data available in the inventory in the Netherlands (Velthof et al., 2012). Apparent fecal N digestibility is obtained or calculated from values for the dietary components given in Dutch tables of feed values for ruminant nutrition, also indicated as the CVB Feed Table (CVB, 2011; referred to from hereon as *CVB model*). These values have typically been determined in experiments with wethers rather than with cattle. However, since the introduction of the systems of evaluation of net energy for lactation (Schiemann et al., 1971; Van Es, 1978) differences in apparent fecal N digestibility between wethers and cattle have been documented. The Dutch evaluation system of net energy of lactation (VEM; Van Es, 1978) is part of the CVB Feed Table (CVB, 2011). These values are not directly applied with the purpose of estimating apparent fecal N digestibility in dairy cattle, but they are used in calculations of the energy value of feeds. For these reason some doubts could be raised on the accuracy of the current TAN excretion calculation for dairy cattle in the Netherlands. A preliminary evaluation (unpublished) of apparent fecal N digestibility predicted with the *CVB model* confirmed these doubts. Evaluation against a dataset of 69 dietary treatments from 13 trials indicated a large systematic over-prediction of apparent fecal N digestibility with 7.5 (±5.4) percent units of digestion, corresponding with 11.4% higher predicted than observed values for apparent fecal N digestibility (**Figure 1**). Prediction error appeared negatively related to the level of apparent fecal N digestibility and to the fraction of roughage in the diet, and positively related to DM intake ($R^2 =$ 0.26, 0.15, and 0.11, respectively).

Hence, it appeared that estimates of apparent fecal N digestibility with the CVB model might be biased. The aim of the present study was to evaluate the *CVB model*, as well as an alternative, more detailed candidate model, against an independent dataset of rather recent observations on apparent fecal N digestibility in dairy cows documented in peer-reviewed literature. As the alternative candidate model, a Tier 3 approach (from here on referred to as *Tier 3 model*) was chosen which is already in use to estimate enteric methane in dairy cattle (Bannink et al., 2011) in the greenhouse gas inventory in the Netherlands (Vonk et al., 2016).

MATERIALS AND METHODS

Data Collection Evaluation Databases

A literature search of the Scopus on-line database was conducted using the combination of words "dairy cattle OR dairy cows," "digestibility OR digestion." and "protein." The period covered was 2000–2016 and the search resulted in 1,207 articles. In order to be included in the dataset, studies had to provide information with respect to the ingredient composition of the diet, dry matter intake (DMI), and apparent fecal protein digestibility. Furthermore, as the CVB Feed Table (CVB, 2011) was used for recalculation of the diets, only those studies were selected in which the ingredients used were also present in the CVB Feed Table (CVB, 2011). Studies were removed from the database if grass silage was inoculated, cow body weight (BW) was lower than 550 kg and breeds other than Holstein Friesian were involved. Some digestion trials carried out by our own research group in the Netherlands were added to this database. This selection process resulted in an evaluation dataset containing a total of 54 studies containing 58 experiments and 242 treatment observations, including 9 Dutch studies containing 13 experiments and 62 treatment observations. A summary of cow and dietary characteristics for the selected studies is given in **Table 1** for the complete evaluation dataset, and in **Table 2** for the dataset of Dutch studies only. The 54 studies included in this analysis are listed in the footnotes of **Tables 1** and **2**.

Performance of the CVB model and the Tier 3 model was evaluated for the three different datasets: (1) the complete dataset including the diets containing rolled or cracked products, (2) the complete dataset excluding the diets containing rolled or cracked high moisture maize silage because for these products in particular representative data were lacking in the CVB Feed Table (CVB, 2011), and (3) a dataset containing the data from Dutch studies only.

Recalculation of Diets

Diets composition was recalculated using the CVB Feed Table (CVB, 2011) and, as far as available, analyzed nutrient composition of concentrates and roughages were used as inputs for the recalculation of diets. The unidentified fraction

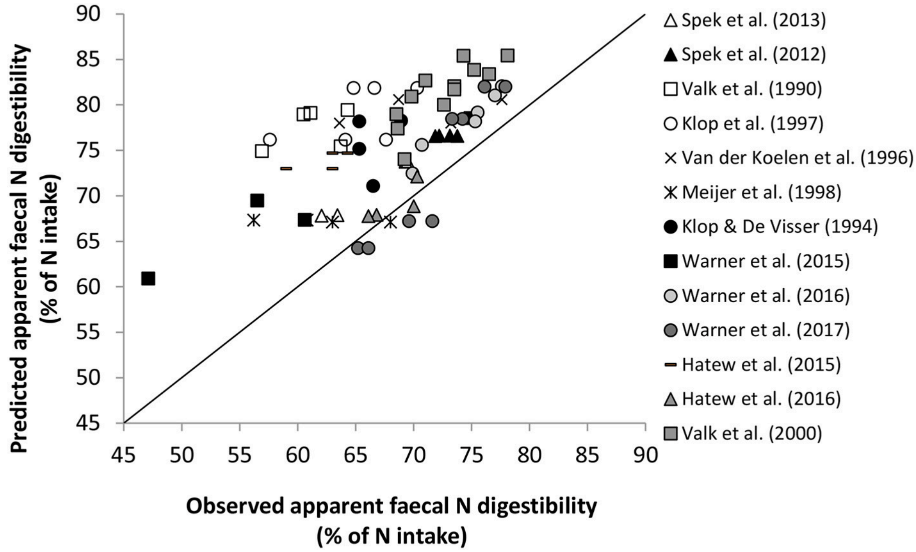

FIGURE 1 | A preliminary evaluation of prediction of apparent fecal N digestibility (% of N intake) by the *CVB model* for 13 Dutch trials with lactating cows on 69 dietary treatments. Observational data were retrieved from 10 studies published in journals (Valk et al., 1990, 2000; Spek et al., 2012, 2013; Hatew et al., 2015, 2016; Warner et al., 2015, 2016, 2017), and from 3 studies on the modulating effect of starch supplementation at various sites in the gastrointestinal tract on apparent N digestibility, reported in 4 internal documents (Klop and de Visser, 1994; Van der Koelen et al., 1996; Klop et al., 1997; Meijer et al., 1998). Dry matter (DM) intake ranged from 14.5 to 22.5 kg/d, crude protein content in dietary DM from 8.4 to 24.4%, roughage fraction in dietary DM from 0.45 to 0.91, and observed apparent fecal N digestibility from 47.1 to 78.1%.

of the dietary DM (in g/kg DM) was calculated as 1,000—crude protein (CP; excluding ammonia CP)—ammonia—crude fat—crude ash—neutral detergent fiber (NDF)—starch—sugar—fermentation products. This unidentified fraction was equally allocated to NDF and starch in cases when dietary starch content was higher than sugar content but equally allocated NDF and sugars in cases when sugar content was higher than the starch content. This was a pragmatic solution to allocate 100% of DM including the unidentified part which also contributes to fermentation, microbial growth, digestion and excretion. In a number of cases, rolled high moisture maize silage was used (involving North American studies) and for this product the values in the CVB Feed Table (CVB, 2011) for corn cob mix silage were adopted.

Calculation of Model Input Parameters

The required model input parameters for the *Tier 3 model* (and required input parameters related to CP for the *CVB model*) are summarized in **Table 3**. Ruminal *in situ* fermentation characteristics are required for starch, NDF and CP of the individual feed ingredients. These rumen fermentation characteristics for the individual feedstuffs include the washout fraction, the (non-washout) degradable fraction and the (non-washout) undegradable fraction of starch, NDF, and CP, as well as the respective ruminal *in situ* fractional degradation rates of the degradable fraction of starch, NDF, and CP. Values were adopted from those applied in the DVE/OEB2010 system (Van Duinkerken et al., 2011) as part of the CVB Feed Table (CVB, 2011). This feed evaluation system estimates requirements and supply of intestinal digestible protein in dairy cattle.

CVB Model

For all feedstuffs, the CVB Feed Table (CVB, 2011) contains estimates of the coefficient (%) of apparent fecal digestibility of CP (either as table values for concentrate ingredients, or as predictive equations for roughages; CVB, 2007). Digestibility data for the dietary components or ingredients were weighted according to their contribution to dietary DM.

Tier 3 Model

The *Tier 3 model* used in the present study to predict apparent fecal N digestibility, as an alternative to the *CVB model*, has been used in the greenhouse gas inventory in the Netherlands since 2005 to predict enteric methane emission in dairy cattle (Vonk et al., 2016). The *Tier 3 model* is a dynamic, mechanistic model describing the fermentative and digestive processes in the gastrointestinal tract of dairy cattle. The model is strongly based on the rumen and fermentation model of Dijkstra et al. (1992). This model was adapted by Mills et al. (2001) on post-ruminal digestion of nutrients and fermentation in the hindgut. Subsequently, it was adapted by Bannink et al. (2008) on the representation of the stoichiometry of production of volatile fatty acids from fermented substrate (soluble carbohydrates, starch, hemi-cellulose, cellulose and CP). Kebreab et al. (2004) used an extended version of this model, including prediction of nutrient utilization for milk yield according to Dijkstra et al. (1996), for the US greenhouse gas inventory purposes. The *Tier 3 model* represents fermentation and microbial metabolism processes in the rumen, including variation in microbial protein synthesis related to the type of carbohydrate and N-source available,

TABLE 1 | Summary statistics of cows and diets in the complete evaluation dataset[a].

Parameter	*N*	Mean	*SD*	Minimum	Maximum
COW CHARACTERISTICS/PERFORMANCE					
BW (kg)	234	625	41.7	557	784
DIM (d)	234	115	49.4	22	308
Milk (kg/d)	238	32	7.6	10	48
Fat (%)	238	3.8	0.48	2.7	5.2
Protein (%)	238	3.2	0.23	2.6	3.7
Lactose (%)	167	4.7	0.23	4.1	5.6
MUN (mg N/dL)	147	12	3.4	4	28
DMI (kg/d)	242	21	3.6	12	28
CALCULATED DIETARY PARAMETERS (G/KG DM)					
Roughage	242	591	165.5	283	100
CP	242	170	21.4	81	252
NDF	242	337	62.8	233	555
Ash	242	84	16.8	30	130
Sugar	242	47	44.2	0	264
Crude fat	242	36	13.0	17	80
Starch	242	206	94.9	3	393
Unidentified[b]	242	94	34.1	1	188
ANALYZED DIETARY PARAMETERS (G/KG DM)					
NDF	236	351	63.4	233	564
CP	236	168	21.9	82	251
Starch	156	208	96.8	2	412
NITROGEN BALANCE[c]					
N-intake (g N/d)	242	576	114.7	192	788
N-milk (g N/d)	242	154	38.5	0	227
N-feces (g N/d)	242	189	53.8	81	358
N-urine (g N/d)[d]	242	225	71.0	38	409

[a]*Data derived from Agle et al. (2010a,b), Akbari-Afjani et al. (2014), Arndt et al. (2015), Arriola et al. (2011), Bahrami-Yekdangi et al. (2014), Bahrami-Yekdangi et al. (2016), Beckman and Weiss (2005), Benchaar et al. (2013), Boerman et al. (2015), Brito and Broderick (2006), Brito et al. (2009), Broderick et al. (2000), Broderick et al. (2001), Broderick et al. (2002), Broderick and Radloff (2004), Broderick et al. (2009), Broderick and Reynal (2009), Colmenero and Broderick (2006), Dann et al. (2014), Doreau et al. (2014), Eun et al. (2014), Fanchone et al. (2013), Flis and Wattiaux (2005), Fredin et al. (2015), Hassanat et al. (2013), Hatew et al. (2015), Hatew et al. (2016), Hindrichsen et al. (2006), Khezri et al. (2009), Klevenhusen et al. (2011), Kowsar et al. (2008), Maesoomi et al. (2006), Mohammadzadeh et al. (2014), Mosavi et al. (2012), Olijhoek et al. (2016), Petit (2002), Peyrat et al. (2016), Poorkasegaran and Yansari (2014), Rafiee-Yarandi et al. (2016), Ruppert et al. (2003), Sinclair et al. (2015), Spek et al. (2013), Stojanovic et al. (2014), Tas et al. (2005), Valk et al. (2000), Warner et al. (2013a), Warner et al. (2013b), Warner et al. (2015), Warner et al. (2016), Weiss et al. (2009), Weiss et al. (2011), Yang and Beauchemin (2006), Yang and Beauchemin (2007).*

[b]*The unidentified fraction (g) in 1 kg of dietary DM is calculated as 1,000—CP (excluding ammonia CP)—ammonia—crude fat—crude ash—NDF—starch—sugar—fermentation products.*

[c]*N balance results includes observations for all dairy cows, including lactating as well as non-lactating cows.*

[d]*For 155 treatments (36 experiments) urine N was not observed but estimated as apparent fecal N digested minus N in milk.*

TABLE 2 | Summary statistics of cows and diets in the evaluation dataset, containing Dutch experiments only[a].

Parameter	*N*	Mean	*SD*	Minimum	Maximum
COW CHARACTERISTICS/PERFORMANCE					
BW (kg)	62	600	26.9	567	653
DIM (d)	62	132	49.2	53	222
Milk (kg/d)	62	26	4.4	17	36
Fat (%)	62	4.3	0.35	3.8	5.2
Protein (%)	62	3.4	0.16	2.9	3.7
Lactose (%)	34	4.6	0.10	4.4	4.9
MUN (mg N/dL)	28	11	4.2	4	19
DMI (kg/d)	62	18	2.2	13	22
CALCULATED DIETARY PARAMETERS (G/KG DM)					
Roughage	62	775	99.2	600	905
CP	62	173	32.4	81	252
NDF	62	400	47.4	320	505
Crude ash	62	92	21.2	56	130
Sugar	62	95	58.8	9	264
Crude fat	62	40	11.5	23	60
Starch	62	100	92.0	3	325
Unidentified[b]	62	87	18.4	48	118
ANALYZED DIETARY PARAMETERS (G/KG DM)					
NDF	56	410	41.3	325	501
CP	56	174	34.6	82	251
Starch	40	106	99.6	7	326
NITROGEN BALANCE					
N-intake (g N/d)	62	505	107.0	192	723
N-milk (g N/d)	62	136	23.0	84	195
N-feces (g N/d)	62	146	40.2	93	281
N-urine (g N/d)[c]	62	218	90.6	38	409

[a]*Data derived from Hatew et al. (2015), Hatew et al. (2016), Spek et al. (2013), Tas et al. (2005), Valk et al. (2000), Warner et al. (2013a), Warner et al. (2013b), Warner et al. (2015) and Warner et al. (2016).*

[b]*The unidentified fraction (g) in 1 kg of dietary DM is calculated as 1,000—CP (excluding ammonia CP)—ammonia—crude fat—crude ash—NDF—sugar—starch—fermentation products.*

[c]*For 46 treatments (10 experiments) urine N was not observed but estimated as apparent fecal N digested minus N in milk.*

retention time of substrate, acidity of rumen contents, intraruminal microbial N recycling, recycling of N to the rumen via saliva and through the rumen wall. The model distinguishes bacteria and protozoal metabolism and predicts variation in rumen (and large intestinal) metabolism instead of adopting fixed values (reviewed by Bannink et al., 2016). Representation of such aspects is relevant to prediction of variation in outflow of microbial and non-microbial protein from the rumen and its subsequent digestion in the intestines.

For the present study, rather limited adaptations were made to the model. These adaptations did not affect predicted enteric methane emission (Vonk et al., 2016), but they were required for accurate prediction of apparent fecal N digestibility. A representation of endogenous protein in the small intestine was included, similar to that in the DVE/OEB 2010 system (Van Duinkerken et al., 2011). The following equation was used to calculate the production rate of endogenous protein (CP_{End}; g/d) from the flow of undigested feed (Van Duinkerken et al., 2011): $CP_{End} = 50 \times Feed\ ((1\text{-}DC_{OM}/100) + Fr_{Ash}/1000 \times 0.5)$, with $Feed$ as DM intake (kg/d), DC_{OM} as fecal digestibility of organic matter (% of organic matter intake), Fr_{Ash} as the fraction of crude ash in feed (g/kg DM), and assuming 50%

fecal digestibility of crude ash. It was assumed that 60% of the ileal outflow of endogenous protein and microbial crude protein to the large intestine is potentially degradable in the large intestine.

Apparent Fecal N Digestibility in Inventory Methodology

Activity data for the diet and performance of the average dairy cow in the Netherlands allowed to generate model inputs for the *Tier 3 model* to predict apparent fecal N digestibility (% of N intake). The activity data include statistics on the number of dairy cows in the Netherlands, delivered and recorded amounts and composition of tank milk in two identified regions (i.e., the North and West, and the East and South). The data include for each region an estimate of DM intake by cows based on milk yield and milk composition, components in the cow ration and the feeding of these components. The specific task to collect the statistics for these data is allocated to the WUM working group

TABLE 3 | Model inputs required for prediction of apparent fecal N digestibility with the Dutch Tier 3 model (and some for the CVB model[a]).

Model inputs	Comments	Abbreviation used in model[b]
Dry matter intake (kg/d)		Feed
Roughage proportion (%)		RP
Dietary content (g/kg)		
Sugar		Frwr
Rumen washout starch		Frsr
Rumen degradable, non-washout starch		Frsf
NDF		Fndf
Rumen degradable NDF		Frff
Rumen washout N		Fsln
Rumen undegradable N		Frpi
NH_3 – N		Fram [a]
Total N		Fn [a]
Crude fat		Frli
Crude ash		Frash
Acetic acid	Total fermentation products in roughage × 0.3	Frac
Propionic acid	Total fermentation products in roughage × 0.05	Frpr
Butyric acid	Total fermentation products in roughage × 0.05	Frbu
Lactic acid	Total fermentation products in roughage × 0.6	Frla
Average kd-starch (/24 h)[c]	Calculated as the ∑ (individual ingredients degradable fraction of starch, NDF, or N × fractional degradation rate (/d) of these fractions)/∑ (individual ingredients degradable fraction of starch, NDF, or N)	ksfdi
Average kd-NDF (/24 h)		kffdi
Average kd-N (/24 h)		kpddi

[a] *Parameter inputs required with predictive equations for roughages in the CVB model.*
[b] *Model inputs according to Dijkstra et al. (1992) and the Dutch Tier 3 for prediction of enteric methane in cows. See Bannink et al. (2011) for further explanation.*
[c] *kd, fractional rate of degradation during in situ incubation in the rumen under standardized conditions.*

TABLE 4 | Summary of observed and predicted apparent fecal N digestibility (as % of N intake) for the complete dataset, the complete dataset excluding rolled or cracked high moisture maize silage, and the Dutch dataset.

	Complete dataset			Complete dataset, excluding cracked, or rolled products			Dutch dataset		
	Observed	Predicted		Observed	Predicted		Observed	Predicted	
		Tier 3	CVB		Tier 3	CVB		Tier 3	CVB
Number (n)	242	242	242	197	197	197	62	62	62
Mean	67.0	69.8	73.8	68.3	69.9	74.2	70.4	69.7	76.4
SD	6.77	4.52	4.35	6.44	4.89	4.49	7.33	5.81	5.27
Minimum	46.2	46.5	59.1	46.2	46.5	59.1	47.1	46.5	59.1
Maximum	80.6	79.5	86.3	80.6	79.5	86.2	80.6	79.5	86.3

who interpret statistics of the aforementioned data according to a standardized methodology (CBS, 2010). A yearly estimate is delivered of the proportion of each component in dietary DM (grass herbage, grass silage, maize silage, standard concentrates, protein-rich concentrates, and wet by-products), DM intake, and milk yield and composition. Statistics (across season and across postal code reflecting soil and farm type) on the chemical composition, digestibility and feeding value of roughages are obtained from a commercial laboratory (https://www.eurofins.com/agro) that analyses the majority of silage samples offered for analysis by Dutch dairy farmers as almost all Dutch dairy farmers offer samples their silos for analysis. Furthermore, estimates are made for which part of prepared silages are fed within the year of preparation or are fed in subsequent years. The approach was consistent with that followed in the inventory of enteric methane in dairy cattle in the Netherlands from 1990 till 2016 (Vonk et al., 2016). Required model inputs have been described by Bannink et al. (2011) and are listed in **Table 3**. Values achieved with the *CVB model* have been drawn from the inventory reports in the Netherlands and were available from 1990 till 2014.

Statistical Analysis

Data were analyzed based on the three different datasets; (1) the complete dataset of observations without any exclusions, (2) the complete dataset excluding diets containing rolled or cracked products, and (3) the dataset containing observations from Dutch studies only. A separate analysis was carried out for the complete excluding rolled or cracked products because no similar products have been listed in the Dutch Feed Table (CVB, 2011) and assumptions on inputs were hence expected to be rather inaccurate. A separate analysis was carried out for the Dutch dataset because the values adopted in the DVE/OEB 2010 system (Van Duinkerken et al., 2011) on rumen degradation characteristics of feedstuffs and diet components are thought to be most reliable for trials performed in the Netherlands. These values have typically been established in *in situ* degradation studies conducted under Dutch feeding conditions. These values are likely less applicable to the same type of feedstuffs or dietary components (especially for roughages) tested in other countries due to differences in climate, harvest management, varieties of maize and grass, and post-harvest treatment and conservation.

Data were analyzed using the PROC MIXED procedure of SAS (version 9.3). Model predicted apparent fecal N digestibility (%) based on the *Tier 3 model* and the *CVB model* were tested against observed apparent fecal N digestibility. Study effect was included as a random effect in the model. Model predictions of apparent fecal N digestibility were evaluated using two methods as described in Ellis et al. (2010). The square root (RMSPE) of the mean square prediction error (MSPE) was calculated and expressed as percentage of the observed mean. The RMSPE was decomposed into error due to overall bias (ECT), error due to deviation of the regression slope from unity (ER), and error due to the disturbance (random error) (ED) (Bibby and Toutenburg, 1977).

Furthermore, concordance correlation coefficient analysis (CCC) was performed (Lin, 1989) where CCC is calculated as:

$$\mathrm{CCC} = \mathrm{R} \times \mathrm{Cb}, \tag{1}$$

where Cb is a bias correction factor and is a measure of accuracy, and the R variable (the Pearson correlation coefficient) gives a measure of precision. A higher CCC value indicates a better prediction of observed values. The Cb is calculated from SD_O and SD_P as the standard deviation of observed and predicted values, respectively, and M_O and M_P as the mean of observed and predicted values respectively, where υ provides a measure of scale shift (i.e., the change in standard deviation between predicted and observed values), and μ provides a measure of location shift (i.e., under-prediction

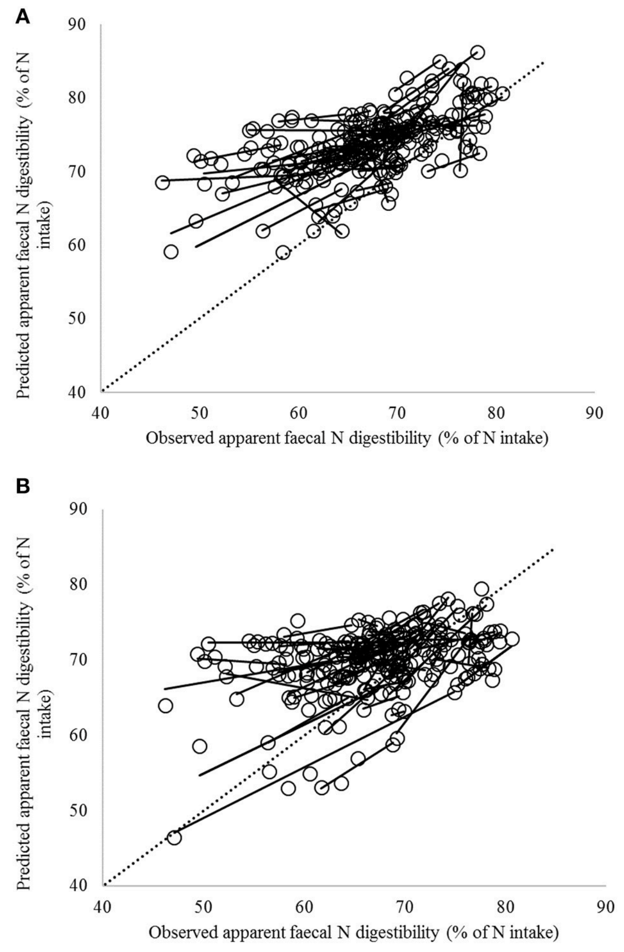

FIGURE 2 | Comparison of observed and predicted apparent fecal N digestibility values according to **(A)** the *CVB model* (the current Dutch feed evaluation system of net energy of lactation; Van Es, 1978) and **(B)** the enteric methane emission *Tier 3 model* (Bannink et al., 2011) for the complete dataset with observations for lactating cows from 58 trials including 242 dietary treatments. Solid lines indicate regression of predicted against observed values within trial; the dashed line indicates the line of unity.

with a positive value and over-prediction with a negative value):

$$\upsilon = SD_O / SD_P,$$
$$\mu = [M_O - M_P] / [SD_O \times SD_P]^{1/2},$$
$$Cb = 2/[\upsilon + 1/\upsilon + \mu^2].$$

RESULTS

Model Evaluation Results

Table 4 shows summarizing statistics of observed and predicted values of apparent fecal N digestibility (as % of N intake) for the three datasets. For the complete dataset, observed variation in apparent fecal N digestibility (referring to the SD values reported in **Table 4**) was 50 and 56% greater than predicted by the *Tier 3 model* and *CVB model*, respectively. For the complete dataset excluding rolled or cracked products it was 32 and 43% greater, and for the Dutch dataset 26 and 39% greater, respectively. The *CVB model* over-predicted apparent fecal N digestibility by 6.8% units of digestibility for the complete dataset, by 6.0% units for the complete dataset excluding rolled and cracked products, and by 6.0% units of digestibility for the Dutch dataset (**Table 4**). The *Tier 3 model* over-predicted by 2.8 and 1.7% units and under-predicted by 0.7% units for these datasets, respectively. Results have been represented graphically in **Figure 2** for the complete dataset and **Figure 3** for the Dutch dataset.

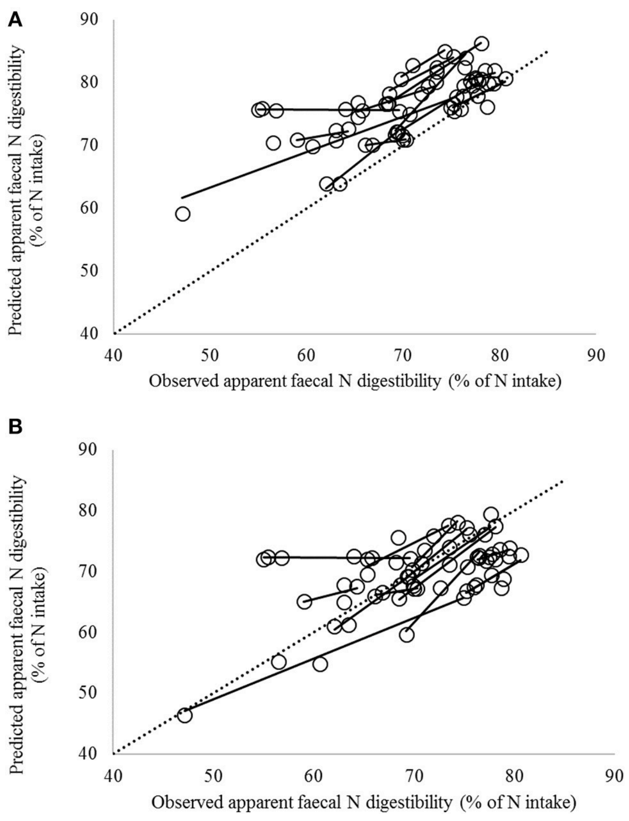

FIGURE 3 | Comparison of observed and predicted apparent fecal N digestibility values according to **(A)** the *CVB model* (the current Dutch feed evaluation system of net energy of lactation; Van Es, 1978) and **(B)** the *Tier 3 model* currently used for enteric methane emission (Bannink et al., 2011) for the dataset with observations for lactating cows in 13 Dutch trials including 62 dietary treatments. Solid lines indicate regression of predicted against observed values within trial; the dashed line indicates the line of unity.

More qualifying statistics on model prediction performance are given in **Table 5**. The relationship between observed and predicted apparent fecal N digestibility, taking into account the study effect, indicated a better prediction by the *Tier 3 model.* Slope estimates (between 0.418 and 0.657) were greater and intercept estimates smaller (between 23.4 and 41.6) with the *Tier 3 model* compared to slope estimates obtained for the *CVB model* (slope estimates between 0.405 and 0.560; intercept estimates between 37.2 and 46.4; **Table 5**).

Consistent results were obtained with RMSPE and CCC analysis of overall performance for these three datasets. A consistently smaller RMSPE was established with the *Tier 3 model* compared to the *CVB model* for the complete dataset, the complete dataset excluding rolled or cracked products and the Dutch dataset (a 1.5, 1.4, and 16% units of digestibility smaller prediction error with the *Tier 3 model*, respectively, based on RMSPE and observed means in **Table 5**). Predictive performance in terms of RMSPE value was highest with the Dutch dataset and lowest with the complete dataset. The RMSPE for the *Tier 3* model was almost totally attributed to error due to disturbance (ED) with % of RMSPE attributed to bias (ECT) and regression (ER) not exceeding 16% (**Table 5**). In contrast, with the *CVB model* most error was due to the bias (more than 58%; ECT) and the contribution of disturbance error (DE) to total error was about half that of the *Tier 3 model*.

In line with RMSPE results, the results from CCC analysis indicate that predictive performance of both the *Tier 3 model* and the *CVB model* improved in the order of the complete dataset, the complete dataset excluding rolled or cracked products and the Dutch dataset. The CCC value increased, the Pearson correlation coefficient R changed in the direction of 1, as well the bias correction factor Cb and the υ parameter indicating scale shift, whereas the μ parameter indicating location shift became smaller (results for both models in **Table 5**). Simultaneously, the CCC value (a high value indicating better prediction) increased by 0.25 for the *Tier 3 model* and by 0.15 for the *CVB model.* With the complete dataset, performance by the *CVB model* was similar comparable to that of the *Tier 3 model* with a CCC value of 0.32 (**Table 5**). This changed into a slightly better performance by the *Tier 3 model* for the complete dataset excluding rolled or cracked products (0.04 higher CCC value), and a better performance with the Dutch dataset (0.09 higher CCC value). For all three datasets tested, precision (R; **Table 5**) was higher for the *CVB model*, whereas accuracy (Cb; **Table 5**) was higher for the *Tier 3 model*. Higher accuracy (Cb) of the *Tier 3 model* remained with differences in Cb value of 0.29, 0.33 and 0.31 for the complete dataset, the complete dataset excluding rolled or cracked products and the Dutch dataset, respectively. Precision remained lower for the *Tier 3 model* but the difference in *R*-value with the *CVB model* declined from 0.22 to 0.19 to 0.14, respectively. The standard deviation of predicted values was smaller than that of observed values leading to υ values (scale

TABLE 5 | Relationship between observed apparent fecal N digestibility (%) and predicted apparent fecal N digestibility (%) with the *Tier 3 model* and the *CVB model* (values and calculations based on studies with wethers fed on maintenance level) for the complete dataset, the complete dataset excluding rolled or cracked high moisture maize silage, and the Dutch dataset.

	Intercept Value ± SE[a]	Slope Value ± SE[a]	RMSPE[b]	ECT[c]	ER[d]	ED[e]	CCC[f]	Cb[g]	υ[h]	μ[i]	R[j]	Observed mean[k]
COMPLETE DATASET (*N* = 242)												
Tier 3	41.6 ± 2.81*	0.418 ± 0.0410*	10.6 *(7.1)*	15.6	7.3	77.2	0.317	0.825	1.499	−0.507	0.384	*67.0*
CVB	46.4 ± 2.47*	0.405 ± 0.0361*	12.9 *(8.6)*	61.0	0.1	38.9	0.323	0.535	1.555	−1.241	0.604	
COMPLETE DATASET WITHOUT ROLLED OR CRACKED PRODUCTS (*N* = 197)												
Tier 3	36.1 ± 3.08*	0.494 ± 0.0442*	9.2 *(6.3)*	6.5	10.2	83.3	0.414	0.927	1.316	−0.287	0.447	*68.3*
CVB	43.0 ± 2.69*	0.455 ± 0.0388*	11.3 *(7.7)*	58.2	0.3	41.5	0.379	0.600	1.434	−1.096	0.632	
DUTCH DATASET (*N* = 62)												
Tier 3	23.4 ± 5.14*	0.657 ± 0.0716*	8.7 *(6.2)*	1.3	6.4	92.4	0.563	0.968	1.261	0.110	0.581	*70.4*
CVB	37.2 ± 4.47*	0.560 ± 0.0625*	11.1 *(7.8)*	58.4	0.0	41.6	0.473	0.658	1.390	−0.964	0.718	

[a]*P-value indicates significance of the estimate being different from zero at level of $P < 0.001$, indicated by *.*
[b]*RMSPE as root of mean square prediction error (MSPE) expressed as a percentage of the observed mean, and in parentheses as % units of apparent fecal N digestibility.*
[c]*Error due to bias, as a percent of total MSPE.*
[d]*Error due to regression, as a percent of total MSPE.*
[e]*Error due to disturbance, as a percent of total MSPE.*
[f]*Concordance correlation coefficient.*
[g]*Bias correction factor.*
[h]*Scale shift.*
[i]*Location shift.*
[j]*Pearson correlation coefficient.*
[k]*Observed mean of apparent fecal N digestibility (% of N intake).*

shift) higher than 1, more so for the CVB model than for the *Tier 3 model* however (**Table 5**). Both the *CVB model* and the *Tier 3 model* over-predicted apparent fecal N digestibility as indicated by the negative μ values (**Table 5**). Absolute values of μ were 2.4, 3.8, and 8.8 times as large for the *CVB model* compared to the *Tier 3 model* with the complete dataset, the complete dataset excluding rolled or cracked products, and the Dutch dataset, respectively. There was essentially (only) a small under-prediction of apparent fecal N digestibility with the *Tier 3 model* (**Tables 4**, **5**).

Prediction of Apparent Fecal N Digestibility in Inventory

Figure 4 demonstrates the consequences on predicted apparent fecal N digestibility in the ammonia inventory methodology with the *CVB model* or the alternative the *Tier 3 model* (Vonk et al., 2016). The average of predictions by the *CVB model* for the period of 1990 till 2014 was 5.9% units of digestibility higher than the average of predictions by the *Tier 3 model* from 1990 till 2016. The annual predictions by the *CVB model* were 5.6 (±0.93) % units of digestibility higher compared to the Tier 3 model. The results further demonstrate a continuous decline in predicted N digestibility since 1990 following the trend in the activity data of a declining dietary N content (data not shown here; Vonk et al., 2016). The predicted decline in the apparent fecal N digestibility from 1990 till 2010 was about 6.5% units of digestibility. Since 2010 the decline leveled off (despite some remaining variation predicted by the *Tier 3 model*) together with activity data indicating a rather constant dietary N content (data not shown here; Vonk et al., 2016).

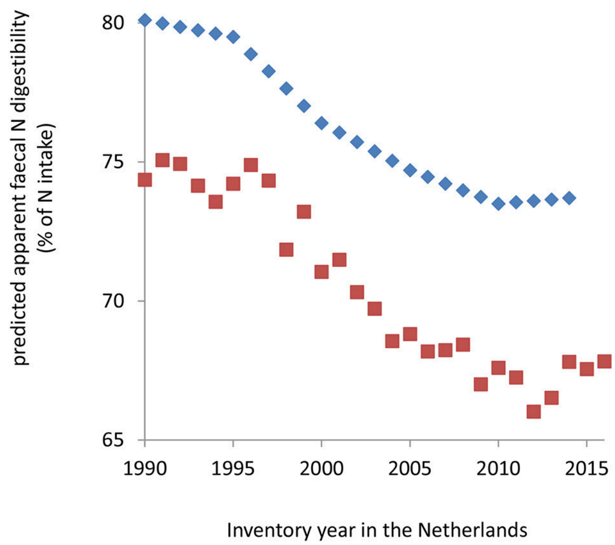

FIGURE 4 | Apparent fecal N digestibility (%) in dairy cows predicted with the *CVB model* (diamonds; reported values available till 2014), or predicted with the *Tier 3 model* (squares; activity data for calculations available till 2016), according to available activity data in the ammonia and greenhouse gas emissions inventory in the Netherlands (Velthof et al., 2012; Vonk et al., 2016).

DISCUSSION

In cattle, utilization of dietary N is relatively inefficient with some 50–85% of consumed N excreted in feces and urine (Moore et al., 2014). The amount of N excreted is related to several factors, with dietary protein content and its apparent digestibility being major determinants. Decreasing dietary protein content is among the most effective strategies to reduce ammonia emissions from dairy manure (Agle et al., 2010b). In a meta-analysis, Bougouin et al. (2016) identified DM intake, milk production, and dietary protein content being key explanatory variables in predicting ammonia emission from dairy housing. The estimation of urine N, or TAN, excretion requires knowledge of dietary N consumption, apparent fecal N digestibility and the amount of N retained in animal products. Activity data in the inventory methodology in the Netherlands already deliver insight into N consumption and N retained by cows in milk, growth and offspring. However, these data do not indicate apparent digestibility of dietary N which hence needs to be predicted.

Comparison of *Tier 3 Model* and *CVB Model*

The average of predicted and observed values for apparent fecal N digestibility were closer for the *Tier 3 model* compared to the *CVB model* with the difference becoming <1% unit of digestibility (**Table 4**) for the Dutch dataset. Also the better correspondence between predicted and observed N digestibility values when accounting for study effect (**Table 5**) indicates an improved applicability of the *Tier 3 model* on account of representation of fermentative and digestive mechanisms. The statistical results consistently indicate a better prediction performance by the *Tier 3 model* although it may remain hard to distinguish the better capture by the model of the within trial treatment differences in **Figures 2**, **3**. This was also demonstrated by a much smaller RMSPE value and more than 77% of error attributed to disturbance (ED) instead of bias (ECT) and regression (ER) (**Table 5**). Furthermore, CCC analysis indicated that the *CVB model* was less capable than the *Tier 3 model* to predict apparent fecal N digestibility for the Dutch dataset in particular. With a lower Cb value the *CVB model* appeared always less accurate (**Table 5**), although demonstrating a higher *R*-value indicating a better correlation between predicted and observed values (measure for precision).

The complete dataset was restricted, first by exclusion of rolled or cracked products because estimates of *in situ* degradation characteristics were highly uncertain and not available in the Dutch Feed Table (CVB, 2011), and second by selecting the Dutch dataset with studies conducted in the Netherlands only. Accuracy of the *Tier 3 model* was high for the Dutch dataset with a Cb value of 0.97 (**Table 5**), and the majority of observations well predicted. Three observations of exceptionally small values of apparent fecal N digestibility by Warner et al. (2013b) could not be reproduced accurately by the *Tier 3 model* (observations below 60% and prediction above 70%; **Figure 3**). These observation were obtained for three out of six maize silage treatments used in that particular experiment, with a CP content in dietary DM of 18% and maize silage a third of dietary DM with all 6 treatments. Due to the fact that the *Tier 3 model* received the same *in situ* degradation characteristics from the Dutch Feed Table (CVB, 2011) as an input for these six maize silages, it is no surprise that the model could not separate out the two groups of observations.

Both the *CVB model* and the *Tier 3 model* predicted less variation than observed which is clearly demonstrated by υ values >1, in particular for the *CVB model*, and more so for the complete dataset than for the Dutch dataset (**Table 5**). Ellis et al. (2010) compared the RMSPE and CCC statistics in an evaluation study of enteric methane prediction equations that are adopted in farm systems modeling. They demonstrated and discussed that when models are unable to describe adequate amounts of the observed variation, CCC analysis is likely the preferred evaluation tool to be used. When mainly focussing on the results of CCC statistics in the present study, the conclusion remain however that the *Tier 3 model* outperforms the *CVB model* based on the results obtained for the Dutch dataset to which the model inputs derived from Dutch Feed Table (CVB, 2011) will comply most.

The results depicted in **Figures 2**, **3** show a large positive bias in predicted apparent fecal N digestibility for the *CVB model*. This is demonstrated by the stronger negative value of the μ parameter from CCC analysis which indicates a stronger over-prediction by the *CVB model*. Over-prediction is clearly far less with the *Tier 3 model* and almost absent with the Dutch dataset (**Table 5**). The main reason for the over-prediction with the *CVB model* is likely that it bases its prediction on digestion data retrieved from wethers instead of dairy cattle. The latter are reported to have a lower apparent fecal N digestibility due to a different contribution of endogenous and microbial N sources to fecal N (Schiemann et al., 1971; Van Es, 1978). Results from Soto-Navarro et al. (2014) suggest that also digestibility data for steers might not be representative for dairy cattle. Apparent fecal N digestibility was reported to be equal or higher than in sheep (2.6, 8.6, and 51.5% units of digestibility for alfalfa, high-quality grass hay and low-quality grass hay, respectively). Therefore, any empirical database to be applied to dairy cows should best be obtained from observations on dairy cows under representative nutritional conditions. Furthermore, the relatively small bias (small ECT values and high Cb values; **Table 5**) with the *Tier 3 model* for all three datasets, suggests that the *Tier 3 model* performance in predicting the average level of apparent fecal N digestibility is satisfactory. Accurate prediction of such an average level is of particular importance for the *Tier 3 model* to be used for the national inventory purposes, as these are based on calculations with averaged and consolidated data at the regional or national level. It is noted that the consistent bias obtained with the *CVB model* (high ECT values and low Cb values; **Table 5**) could be removed by applying a fixed correction factor based on the present findings. It remains to be demonstrated that such a correction factor holds when evaluating the *CVB model* against another dataset which is independent from the results obtained in the present study. Moreover, the results from mixed model analysis (**Table 5**), in which bias for each study is accounted for by including a random effect of study, show that the *CVB model* suffers more from the regression slope differing from the optimal value of 1 than the *Tier 3* model. This holds in particular again for the Dutch dataset to which the model inputs used comply most (**Table 5**).

Predictive Performance of the *Tier 3 Model*

Standard dietary characteristics were obtained from the Dutch Feed Table (CVB, 2011) and served to calculate model inputs for the *Tier 3 model*. However, these are likely inaccurate for the wide range of roughage types and feed qualities encountered under non-Dutch conditions. This probably contributed to the poorer prediction of apparent fecal N digestibility for the non-Dutch trials, whereas statistical analysis the Dutch dataset was more satisfactory. Even for these Dutch trials, however, the dietary ingredients and roughages must have differed strongly from the standard *in situ* degradation characteristics that are listed in the Dutch Feed Table (CVB, 2011; Van Duinkerken et al., 2011). Allowing for variation in these *in situ* degradation characteristics and adopting more realistic estimates reflecting the treatments reported would probably have increased the capacity of the model to capture observed variation in apparent fecal N digestibility. For example, the model cannot be expected to accurately predict the consequence of variation in differences in roughage quality when standardized *in situ* degradation characteristics of roughages are used as an input. In the present study we used such standardized input from the Dutch Feed Table (CVB, 2011).

Hence, assumptions on *in situ* degradation characteristics probably have been too generic to capture the variation in apparent fecal N digestibility that was observed in the various N balance trials selected from literature. Differences and inaccuracies in experimental set-ups and measuring techniques have contributed to this variation. In many studies N excreted with urine was calculated by difference method (**Tables 1**, **2**), whereas in the others a full N balance was determined (including measurement of N excreted with urine). This difference between in studies in quantifying urine N excretion will have contributed strongly to the variation not captured by both models. Nevertheless, the most likely explanation of the lowest prediction capacity for the most complete dataset (**Figure 2**) remains the too narrow range or the bias in values retrieved from Dutch Feed Table (CVB, 2011), not being representative for the range of conditions met in international trials. Improving prediction performance of the *Tier 3 model* for non-Dutch conditions would require such model inputs to be derived from local, non-Dutch conditions as well. Such an approach was followed in studies that aimed to predict enteric methane emission in dairy cattle in various regions in the US (Kebreab et al., 2008) and digestibility (including apparent fecal N digestibility) of various diet types and production conditions (Hanigan et al., 2013). In these studies, similar dynamic, mechanistic models were used, requiring inputs similar to those of the *Tier 3 model* used the present study.

N Digestion Models in Ammonia Inventory

There is an urgent need to account for the effect of the ammonia mitigation measures taken in livestock operations. Both farm accounting tools and Life Cycle Analysis methodology would benefit from a more accurate and more case-specific quantification of sources of emissions (Cederberg et al., 2013), such as the amount of volatile N excreted as a source of ammonia. Both accuracy and precision is needed to identify the level and size of trade-offs between various sources and types of emissions. The highly volatile urine N as a source of ammonia is the ingested amount of digestible N by cattle which is not retained in animal product. This becomes apparent in various literature surveys (e.g., Kebreab et al., 2002). In a companion study, Dijkstra et al. (2018) explored how various dietary measures to mitigate N excretion affect the composition and characteristics of C and N containing fractions in urine and feces. The quantitative terms used to characterize manure correspond with the fermentation and digestibility concepts applied in ruminant feed evaluation. Despite the large impact of dietary N mitigation measures on the proportion of urine N in total N excreted, and on the C:N ratio of manure, inventory methodology seldom represents the variation in these proportions to calculate ammonia emissions (EEA, 2016; Nemecek and Ledgard, 2016). However, under various production conditions the proportion of urine N as well as the volume and frequency of urine excretion may impact immediate ammonia and nitrous oxide emissions from urine N (Ledgard et al., 2015; Selbie et al., 2015). Also with regard to ammonia emission from stored manure, complex mechanisms are responsible for variation in emission rates which includes the amount of urine N and the volume of urine excreted in housing (Sommer et al., 2006).

Despite the complexity of the mechanisms underlying the variation in these emissions, rather constant emission factors are often applied in inventory which in principle lack a relationship with nutritional measures and details on excreta composition, N excretion rate and excreted volumes. All models represent mass flows on a dairy farm. The more detailed ammonia emission models such as the dairy farming systems model developed by Rotz et al. (2014) represent details on the effect of type and fate of excreted N and of excreta volumes on ammonia emission. Excreted urine and fecal N are calculated by functions of animal size, feed intake and protein intake, and milk production, but not protein digestibility characteristics. More recently, Chai et al. (2016) added such detail in a model used for an ammonia inventory on Ontario dairy farms in four ecoregions. The Canadian ammonia emission inventory and survey model was refined by introducing a representation of the effect of dietary mitigation measures. They derived a linear equation to estimate the fraction of urine N, or TAN, in total N excretion from dietary CP content. The range of dietary CP content used (123, 153, and 164 g CP/kg DM with TAN proportion in manure N of 0.42, 0.50, and 0.56, respectively) covers the lower half of the range in the database used in the present study (**Tables 1**, **2**). The relationship between dietary CP content and TAN excretion may be considered intrinsically non-linear however. This non-linear effect on TAN proportion may be covered by the approach of Velthof et al. (2012), who use a method adopting estimates of apparent fecal N digestibility retrieved from the Dutch Feed Table (CVB, 2011), evaluated in the present study as the *CVB model*. This method attributes all digested N not retained in animal products to TAN and, therefore, with further increase of dietary CP content, the estimated TAN proportion in total excreted N increases non-linearly. Notwithstanding the fact that current methodologies may capture the non-linear increase of proportion of TAN with total N excretion, it is of importance that variation in apparent fecal N digestibility on proportion of TAN is captured as well. The present study focussed on an independent evaluation and improvement of the *CVB model* as the method use by Velthof et al. (2012). The *Tier 3 model* was evaluated as well, as an alternative candidate model which takes details on fermentative and digestive aspects into account. Based on the promising findings in the present study, and the fact that this model is already in use in the greenhouse gas inventory for estimating enteric methane in dairy cattle, the *Tier 3 model* has replaced the *CVB model* in the ammonia inventory in the Netherlands since 2015 (**Figure 4**; Vonk et al., 2016). The studies of Dijkstra et al. (2013, 2018) demonstrate that further detailing of the composition of urine and feces (and manure) is possible if needed for the purpose of a more detailed inventory.

CONCLUSIONS

Upon using *the CVB model* to predict apparent fecal N digestibility in dairy cows in the ammonia emissions inventory in the Netherlands, a large systematic bias of 6–7% units of digestibility occurs. This bias can almost entirely be prevented by the use of the *Tier 3 model* which is extant methodology to estimate enteric methane in dairy cattle in the greenhouse inventory in the Netherlands. The more mechanistic representation of fermentation and digestion in the gastro-intestinal tract of dairy cows allows a more accurate and acceptable precision of predicted apparent fecal N digestibility under Dutch feeding conditions. Model performance was less satisfactory on the complete dataset, likely because of less valid standardized inputs to the model (in particular ruminal *in situ* degradation characteristics) when derived from distinct world regions. Satisfactory prediction of the overall average apparent fecal N digestibility demonstrates applicability of the *Tier 3 model* for the calculation of TAN excretion in the ammonia emissions inventory.

AUTHOR CONTRIBUTIONS

AB, LŠ, and JD developed the concepts. AB developed the equations. WS and AB performed the simulations and analyzed the data. AB and WS wrote the original draft of the manuscript and LŠ and JD contributed to discussion and revision of this manuscript.

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