

**FINAL PROJECT REPORT**

**Ruminant Nutrition Regimes To Reduce Methane And Nitrogen Emissions  
- A Meta-analysis of Current Databases**

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## EXECUTIVE SUMMARY

As a foundation for further research to develop new ruminant nutrition regimens for simultaneously reducing methane emissions and nitrogen excretion in growing and lactating ruminants, a meta-analysis of existing data was undertaken. Measurements of energy and/or nitrogen balance obtained using respiration calorimetry and digestion trials were accumulated into a database for meta-analysis of effects of key parameters on both methane and nitrogen excretion in growing and lactating beef cattle and lactating and non-lactating dairy cows. An existing database of individual measurements of energy and nitrogen balance from The University of Reading, which included measurements of methane and nitrogen excretion, was updated and expanded using more recent data from Reading and existing data from other laboratories as appropriate. Additional data were obtained from the USA, Wales, and the Netherlands, giving a total of 1819 individual measurements (1335 records of methane excretion). A multivariate analysis was conducted, with appropriate adjustments for variance associated with location and trial effects, to determine the most important dietary factors that influence methane and nitrogen excretion, based on both linear and nonlinear models.

Conclusions from the analyses conducted with the data available include:

- As observed in previous studies with smaller databases, total feed dry matter intake (DMI) has an overriding effect on the amount of methane produced by beef and dairy cattle, across a broad range of diet types and productive states. The prediction of methane production is improved only slightly on the basis of digestible energy, which is an indicator of the amount of digestible organic matter in the diet fed.
- In addition to DMI, the amount of starch fed relative to acid detergent fibre (ADF) had a significant effect on the amount of methane produced, but the effect of increasing amounts of starch relative to fibre was curvilinear and less pronounced at higher levels of starch feeding typical of rations fed to finishing beef cattle in the USA.

- There was no overall effect of the concentration of ether extract (largely fat) or crude protein in the diet fed on methane production. However, the amount of methane excreted per unit of energy consumed was slightly reduced as ether extract concentration of the diet increased. This effect was highly variable, reflecting the limitations of the ether extract analysis and the limited number of observations at higher levels of dietary ether extract.
  - Although in individual studies it is known that feeding fat decreases methane excretion for both beef and dairy animals, there are numerous other factors that influence the response within a population of animals and locations, which are represented by the database analyzed. This suggests that in practice, in a wider population of animals, the effect of individual diet components is minor relative to the dominant effect of total feed DMI.
- Nitrogen intake is the principal driver of nitrogen excretion for beef and dairy cattle, although the level of intake with respect to requirement modifies the response.
- As nitrogen intake relative to requirement increases, the proportion of total nitrogen excreted only increases marginally. However, the proportion of excreted nitrogen as urinary nitrogen increases significantly.
- Within this large population of measurements, nitrogen excretion was relatively unaffected by the balance between structural (fibre) and non structural (starch) carbohydrate.
- Lower producing dairy animals tend to lose more feed energy as methane per unit of milk produced. This is especially true at very low levels of milk yield, as observed in lactating beef cows.
  - A one size fits all estimate of the proportion of feed energy lost as methane is inappropriate.
  - As milk yield increases, methane energy relative to milk energy output decreases from 0.31 at 20 kg milk/d to 0.16 at 40 kg milk/d.

- As feed intake increases, methane energy production as a fraction of total gross energy intake decreases at a rate of 0.10% per kg dry matter intake increase.
- On average, lower producing animals also excrete more N to the environment per unit of N intake, but there is much variation in N excretion relative to milk yield.
- Provided information on an animal's DMI is available, a reliable estimate of methane emissions can be obtained for both beef and dairy animals.
- N excretion in urine and faeces can be estimated based on N intake alone. The same is true for milk N, although the error of prediction is increased.

## PRACTICAL RECOMMENDATIONS

- Directing dairy production systems towards those with high producing animals will reduce the amount of methane produced per unit of milk yield. Milk yields below 20 kg/d are associated with increasing amounts of methane excretion per kg milk yield, because DMI is the primary determinant of methane excretion.
  - Obviously, as feed efficiency (milk energy/kg DMI) improves the amount of methane excreted per kg milk energy yield will be reduced.
- Intensive beef production systems based around animals consuming high quantities of non-structural carbohydrates with associated high growth rates and hence an early age at slaughter, will produce less methane per unit of animal product than more extensive alternatives.
- Increasing dietary starch at the expense of more fibrous carbohydrates reduces the amount of methane produced per unit of feed dry matter consumed, but implications for rumen health and animal welfare must be carefully considered.
- By estimating nitrogen requirement and aiming to avoid excessive dietary N intake above that requirement, amounts of nitrogen excreted in urine as well as faeces can be reduced. Nitrogen requirement is determined primarily by body weight and growth rate and/or milk N output and efforts to prevent excessive feeding above requirement are likely to make economic sense given the expense associated with high protein feedstuffs.
- Assuming diet formulation is conducted with the aim of satisfying energy and nitrogen requirements for a given level of milk production and/or growth (without excessive overfeeding of nitrogen), diet formulation choices should prioritise starchy feeds or fat sources in the provision of energy supply. This will require careful attention to detail through routine forage analysis and nutritional guidance as needed to avoid potential problems of over-feeding energy supplements. In addition, these recommendations do not consider feed costs.
  - In practice this might mean:
    - Substitution of maize silage for grass silage.
    - Cereal grain (barley or wheat) inclusion instead of fibrous by-products.
    - Addition of oilseeds or rumen protected fats to supplement the diet and increase energy density thereby lowering the level of DMI required to meet energy requirements.

## **Background**

There have been a number of summarizations and reviews of existing experimental data from lactating dairy cows wherein the dietary and management factors that determine amounts of methane and nitrogen excreted have been investigated. A number of these reviews have been funded by DEFRA (or MAFF) and the intention of the project proposed is not to repeat the work that has been conducted previously, but to systematically integrate and extend current knowledge to hopefully develop a framework for practical application. In general, previous individual data summarizations have focused on distinct approaches for predicting methane or nitrogen output, as opposed to a unifying strategy for reducing their output per unit of product. More importantly, none of these reviews and summarizations has integrated the effects of dietary inputs and productive state on both methane AND nitrogen excretion simultaneously and there is a need to ascertain the most practical dietary regimens to reduce amounts of both pollutants relative to milk yield or growth.

## **Nitrogen excretion**

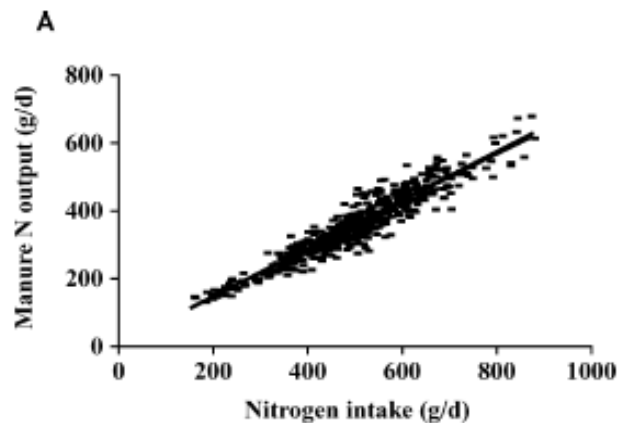
There is no question that the most important factor determining total nitrogen excretion as manure (faeces plus urine) in lactating dairy cows, and beef cattle, is total dietary nitrogen intake (James *et al.*, 1999; Castillo *et al.*, 2000; Kebreab *et al.*, 2002; Nennich *et al.*, 2005; Yan *et al.*, 2006). However, there is variation in the excretion measured at a given level of nitrogen intake (Figure 1), which may arise due to effects of experimental or animal variation, or dietary and other environmental variables affecting the supply of nitrogenous compounds relative to requirements. In this regard, the type of forage and concentrates included in the ration also has a small but important effect on total nitrogen excretion within individual experiments (Kebreab *et al.*, 2002). This implies that in spite of the overriding effect of total nitrogen intake, there is scope for reducing nitrogen excretion by reducing the amount fed, but also through other dietary management strategies. In addition, whilst the total amount of nitrogen excreted as manure increases linearly with increasing nitrogen intake, the proportion excreted in urine increases at an accelerating rate as nitrogen intake increases (Castillo *et al.*, 2001; Figure 2). This may to a large extent reflect increasing absorption of nitrogenous compounds in excess of

requirement, and an increasing proportion of N excretion as urea. This is a particular concern for ammonia emissions, as urinary urea is the primary source of nitrogen for ammonia generation in manure slurry (James *et al.*, 1999), and to a large extent reflects the relatively low efficiency of dietary nitrogen conversion to proteinaceous product in ruminants, which seldom exceeds 30% (Frank & Swensson, 2002). More precise nutritional management of growing and lactating ruminants will reduce the amount of manure nitrogen excreted, as well as the portion excreted as more volatile urinary nitrogen. In addition to a reduction in the amount of nitrogen fed (Castillo *et al.*, 2001), strategies suggested for reducing nitrogen excretion and ammonia losses from cattle include increasing the metabolizable energy concentration of the ration fed (Kebreab *et al.*, 2002), particularly through the use of maize based concentrates containing slowly degraded starch (Castillo *et al.*, 2000). All of these approaches will impact the amount and profile of absorbable amino acids provided for absorption from the small intestine, which are an important determinant of the overall efficiency of dietary nitrogen utilization (Noftsger & St-Pierre, 2003). In addition, there is currently considerable interest in identifying plant bioactive compounds, such as essential oils or tannins, which slow the degradation of protein in the rumen and thereby reduce ammonia absorption and urinary nitrogen excretion (e.g. Newbold *et al.*, 2004).

### **Methane production**

As regards methane emission from ruminants, there have been a number of summarizations of data from measurements of energy balance of lactating dairy cows from laboratories in the US, UK and other parts of Europe (e.g. Moe & Tyrrell, 1979; Holter & Young, 1992; Kirchgeßner *et al.*, 1995; Wilkerson *et al.*, 1995; Yan *et al.*, 2000; Mills *et al.*, 2001; Mills *et al.*, 2003). Whilst the major determinant of total methane excretion is the amount of fermentable organic matter consumed, numerous other dietary factors have significant effects on methane excretion, including the amount and type of fibre, starch and sugars included in the diet. Based on the results of a dynamic model of methane excretion, Mills *et al.* (2001) suggested replacing soluble sugars with starch, and replacing grass silage with maize silage, as approaches that would reduce methane excretion from lactating dairy cows. In addition, it has long been known that feeding

supplemental fat reduces methane output (Czerkawski *et al.*, 1966; Andrew *et al.*, 1991), and other supplements are also known to reduce the total amount of methane produced relative to ME or milk yield (Johnson & Johnson, 1995). These previous reviews have provided a basis for predicting methane output, but each of the individual summarizations have in general focused on distinct approaches for predicting total methane output, depending on the available dietary composition data within the datasets analyzed. More importantly, none of these reviews and summarizations has integrated the effects of dietary inputs and productive state on both methane AND nitrogen excretion simultaneously and there is an urgent need to ascertain the most practical dietary regimens to reduce amounts of both methane and nitrogen excretion relative to milk yield, and their impact on costs of production. As regards the effect of dietary protein on methane excretion, the data are equivocal. Significant effects of dietary crude protein level have been reported for some analyses of available data (e.g. Holter & Young, 1995), but not all (e.g. Moe & Tyrrell, 1979), but the database of methane excretion in lactating dairy cows is largely based on rations containing protein in excess of requirements.



**Figure 1.** Relationship between nitrogen intake and total nitrogen excretion (faeces plus urine) in dairy cows during measurements of energy balance in Northern Ireland (Yan *et al.*, 2006).



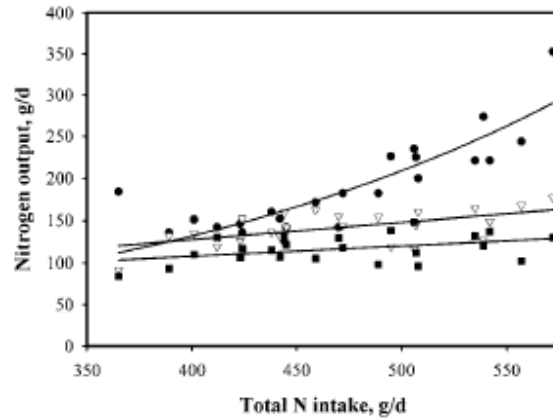


Figure 2. Relationship between total nitrogen intake and nitrogen excretion as faeces (▽), milk (■), and urine (●) in lactating dairy cows (Castillo *et al.*, 2001).

### Objectives

As a foundation for further research to develop new ruminant nutrition regimens for simultaneously reducing methane emissions and nitrogen excretion in lactating dairy cows and beef cattle, a meta-analysis of existing data will be undertaken. Measurements of energy and/or nitrogen balance accompanied by adequate dietary and animal descriptions will be accumulated into a database for meta-analysis of effects of key parameters, with practical relevance for diet management of growing and lactating ruminants in the UK, on both methane and nitrogen excretion. An existing database of individual measurements of energy balance from The University of Reading will be updated and expanded using data from other laboratories as appropriate. A multivariate analysis will be conducted, with appropriate adjustments for trial effects, to determine the most important dietary factors that influence methane and nitrogen excretion relative to growth rate and milk yield, based in part on response surface analysis. The meta-analysis will also consider non-linear biological relationships in the manner proposed by Mills *et al.* (2003). Unlike more traditional linear approaches, this will allow the development of a more comprehensive model of methane and nitrogen excretion that should be capable of broad practical application across a range of typical UK feeding regimes. A range of

non-linear equation forms will be selected and tested as part of the model construction process in an effort to minimise prediction error whilst maintaining biological validity through an appropriate mechanistic description. Including such mechanistic principles to the statistical modelling exercise should further our understanding of the biological nature for the relationships described.

### **Description of available data**

Measurements of energy and nitrogen balance accompanied by adequate dietary and animal descriptions were accumulated into a database for meta-analysis of effects of key parameters on both methane and nitrogen excretion. Our starting point was an existing database of 242 individual measurements of energy and nitrogen balance in dairy cattle from The University of Reading that was integrated for the Feed Into Milk project. Each measurement represents the simultaneous metabolism of energy and nitrogen, including methane and nitrogen excretion, measured in cow on a given diet or other treatment over the course of a 5 to 7 day collection period using open-circuit respiration calorimeters (e.g. Reynolds *et al.*, 2001). This was updated and expanded using data from Reading and other locations as follows:

1. University of Reading: an additional 94 more recent measurements of energy and nitrogen balance were included, as well as a further 187 measurements of nitrogen metabolism and excretion, obtained without simultaneous measurements of energy balance, giving a total of 523 observations of nitrogen excretion and 336 measurements of methane excretion.
2. A total of 368 measurements of energy and nitrogen balance from the USDA ARS Energy Metabolism Unit from the personal research of C. K. Reynolds were included. These were obtained primarily from growing beef cattle fed pelleted diets based on maize meal, soybean meal, and alfalfa meal in varying proportions. In addition, measurements from lactating beef cows (Reynolds and Tyrrell, 2000) and Holstein and Jersey cows (Tyrrell *et al.*, 1990) were included.
3. A total of 615 measurements of energy and nitrogen balance from research centres at Wageningen and Lelystad in the Netherland were included by kind permission of colleagues at Wageningen University (Dr. A. Bannink, Dr. Ad van Vuuren, Prof. Y. van

der Honing and Prof. S. Tamminga). These data include data from lactating cows fed fresh and frozen grass, and grass silage and were provided under condition that these data not be used for other projects and that they be included as authors on any publications arising.

4. A total of 312 measurements of nitrogen balance from the Institute of Grassland and Environmental Research, Aberystwyth were provided by Dr J. Moorby. These data were obtained from lactating and dry dairy cows fed grass-based diets primarily.

In total, the data set includes 1819 individual records of nitrogen excretion and 1335 records of methane excretion, along with varying amounts of supplementary information on diet formulation, diet composition, and the cows used. The availability of this supplemental information ranged from extensive (USDA) to very limited (IGER), which reflects the availability of resources for the measurements when the studies were conducted, or the resources needed for accessing the measurements from archives. In many cases funds were only available for essential measurements of diet composition when the studies were conducted, whilst for older data in many cases the data had not been retained (the Netherlands).

Additional databases exist, for example additional USDA data from Beltsville (Wilkerson *et al.*, 1995), data from the Ritzman Laboratory in New Hampshire (Holter *et al.*, 1992), data from Northern Ireland (Yan *et al.*, 2000 and 2006), and data from other locations in Europe with large animal calorimeters (e.g. Kirchgeßner *et al.*, 1995, Külling *et al.*, 2001) and around the world. Data from many of these other locations were also sought, but ultimately were not obtained due to issues of intellectual property and resource (labour) availability.

### **Data Analysis**

Data were integrated and corrected for variation due to location and experiment using Mixed Models procedures of SAS and linear regression models as described by St-Pierre (2001) and Mills *et al.* (2003). Covariance structures were selected based on fit criteria,

but in most cases an unstructured model was used for the data reported. In all cases there were significant effects of experiment and location.

For each relationship under investigation, the data were split onto two sets. Two thirds of the corrected data were used for model construction and the remaining third was allocated for model evaluation. Where a significant biologically meaningful relationship could be established between an independent variable and either methane or nitrogen excretion, a model was developed exclusively from the construction data set.

For many of the relationships investigated, a linear model proved to be the best description of the data. However, certain key relationships exhibited non-linear patterns and in these cases, suitable non-linear functions were selected based on their ability to describe the data. Where possible, parameters were ascribed biological meaning, thereby relating them to a variable present within the data set or they were fitted to the construction data set using the least squared means procedure in SigmaPlot (Systat Software Inc).

The various models of methane and nitrogen excretion were evaluated against their ability to predict the evaluation data set. A comparison of observed and predicted values was made initially using linear regression. The mean square prediction error (MSPE) was then used to demonstrate the overall error associated with the model as well as any bias that might have been evident. The MSPE is described as follows:

$$MSPE = \sum_{i=1}^n (O_i - P_i)^2 / n$$

Where  $i=1, 2, \dots, n$ ;  $n$  is the number of observations; and  $O_i$  and  $P_i$  are the observed and predicted values respectively. The square root of the MSPE is expressed in the same units as the observed values and a comparison of the root MSPE as a percentage of the observed mean provides an indication of the overall error of prediction.

# Ruminant nutrition regimes to reduce methane and nitrogen emissions

Meta-analysis and statistical modelling of methane and nitrogen excretion from cattle

## Objectives

- To update and expand our data base of measurements of methane excretion.
  - Additional energy balance records
  - Nitrogen balance data
- To conduct a meta-analysis of dietary factors that determine methane and nitrogen excretion.
- To define key relationships with statistical models that can be used to predict excretion based on dietary information.
- To produce recommendations on feeding strategies to limit methane and nitrogen excretion.

## Database structure

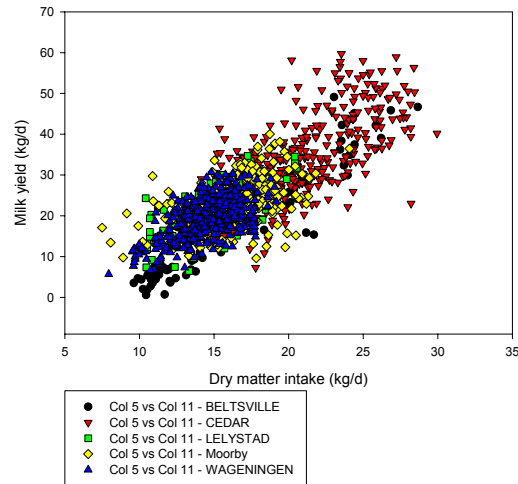
- Beltsville
  - 369 records
- Dutch
  - 615 records
- CEDAR
  - 523 records
- IGER
  - 312 records
- Total of 1819 individual records
  - *By comparison, previous modelling of methane emissions comprised 159 records (Mills et al. 2003)*

## Database structure

- Beltsville - USA
  - mostly growing beef cattle fed pelleted maize, soyabean meal and alfalfa
  - lactating/dry Jerseys vs. Holsteins
    - fed oil seeds
  - lactating beef cows
- Wageningen - Netherlands
  - fresh/frozen grass data = 83 observations
  - additional historical data = 532 observations
- CEDAR – UK
  - Lactating cows
- IGER
  - N balance data for grass & grass/concentrate diets

# Database structure

This plot shows the range of dry matter intake and milk yield for lactating animals



# Database summary

Diet composition summary (all nutrients stated as g/kg DM, energy as MJ/kg DM)									
	EE	N	GE	ASH	NDF	OM	WSC	ADF	STARCH
<b>Beltsville</b>	27	26	18.6	69	322	917		175	408
<b>CEDAR</b>	23	27	20.6	71	361	872	98	189	229
<b>IGER</b>		27							
<b>Dutch</b>	41	28	18.4			910			

	DMI, kg/d	Milk Yield, kg/d	Milk fat, g/kg	Milk protein, g/kg	Milk lactose, g/kg
<b>Beltsville</b>	8.15	17.96	43	36	
<b>CEDAR</b>	19.07	32.06	40	32	47
<b>IGER</b>	15.35	18.59		31	
<b>Dutch</b>	14.81	20.13	40	30	
<b>All data</b>					
<b>Max</b>	28.70	59.7	67	48	53
<b>Min</b>	2.10	0.57	20	21	40

# Modelling approach 1

- An initial investigation was conducted to determine most useful relationships
  - Significant / reliable effects
  - Practical
    - Based on easily available diet characteristics
  - Logical
    - Biologically meaningful relationships showing cause and effect

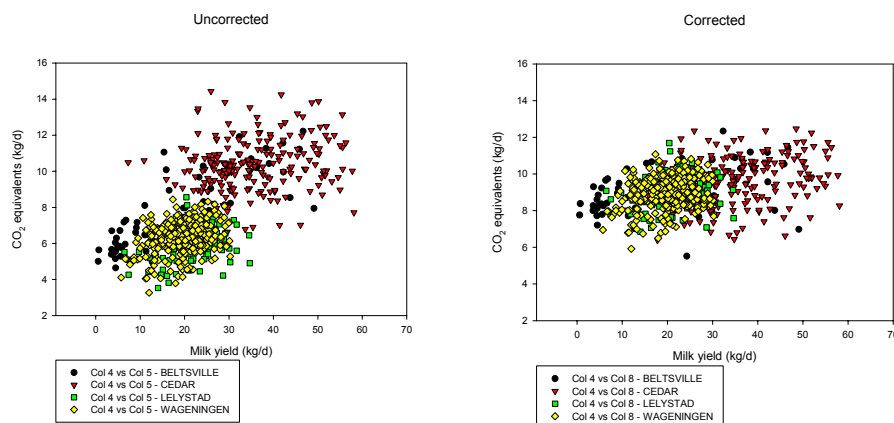


## Modelling approach 2

- The data were adjusted for trial effects for each of the most promising relationships
  - SAS mixed model procedure
    - Location & experiment were highly significant in most cases
  - Data were subsequently split into two thirds and one third for construction and evaluation respectively
  - Adjusted data used to build the models

### Example of correction for location and experiment

Methane excretion as CO<sub>2</sub> equivalents versus milk yield



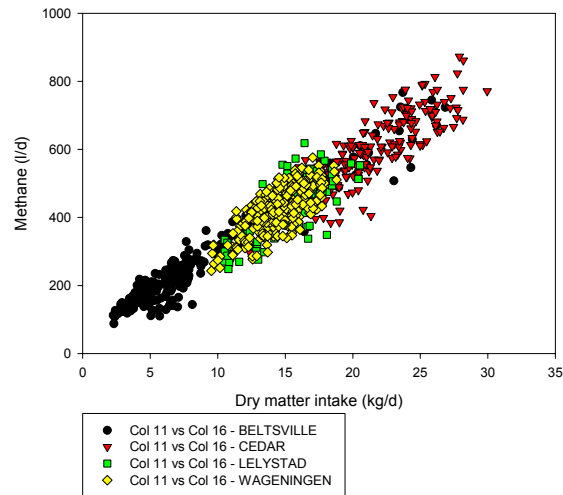
## Modelling approach 3

- Models developed using construction data set
- Suitable linear and non-linear models were chosen
  - Biologically meaningful parameters
- Models programmed and fitted using SigmaPlot
- Models predicted values for evaluation data set
  - Plots of observed vs predicted
    - Compared with line of unity
    - Linear regression through predicted data
    - Mean square prediction error

# Methane

Models to predict methane emissions

## DMI vs Methane production (litres)

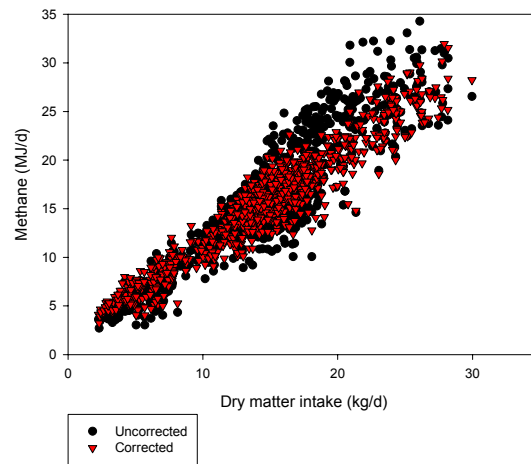


## DMI vs Methane production (litres)

- This plot shows the relationship between dry matter intake (DMI) and methane production.
- The database includes values for methane production which range from 100-800 litres/d, which in terms of energy equates to approximately 2-30 MJ/d.
- Some of the methane data was only provided as an amount of energy, rather a volume, with no correction for standard temperature and pressure. Therefore, throughout the rest of this report we discuss methane production in terms of energy as MJ/d rather than a volume.

# DMI vs Methane

Uncorrected and corrected methane data

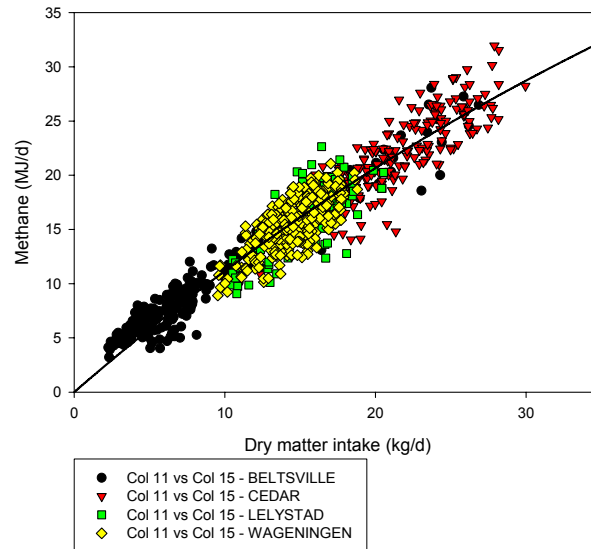


# DMI vs Methane

- This plot shows the relationship between DMI and methane production.
- There is a particularly large spread of data at the higher levels of DMI for the uncorrected data.
- Following correction for the effects of location and experiment in SAS, the spread of the data is reduced.

# DMI vs Methane

Non-linear Mitscherlich model



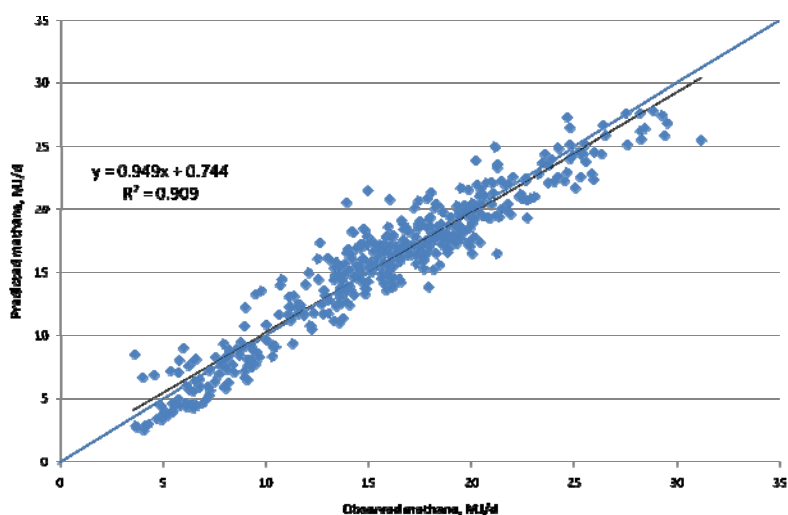
# DMI vs Methane

Non-linear Mitscherlich model

- A Mitscherlich model proved to be the best fit for these data and this is shown on the adjacent graph. As intake increases there is a small decrease in the relative rate of methane emission leading to the curvilinear shape.
- The model shows good agreement with the independent data set (see next slide). No overall bias was detected.

## DMI vs Methane

Observed vs predicted methane emissions (Mitscherlich model)



## DMI vs Methane

- Previous research has shown that the relationship between DMI and methane emissions can be improved by accounting for the ratio of dietary starch to acid detergent fibre (ADF), an estimate of cellulose and lignin.
- As starch levels increase at the expense of more fibrous carbohydrates, rumen fermentation is shifted along glucogenic pathways that are less conducive to producing methane.
- Therefore, in the second model the slope of the curve relating DMI to methane production was related to starch:ADF ratio.

# DMI vs Methane

Mitscherlich model modified for starch:ADF ratio

- Mitscherlich (monomolecular) model

$$y = a - (a + b)e^{-cx}$$

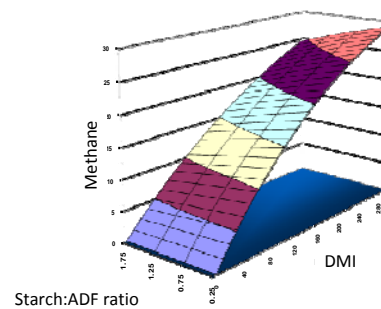
- where

$a$  = maximum methane

$b$  = minimum methane

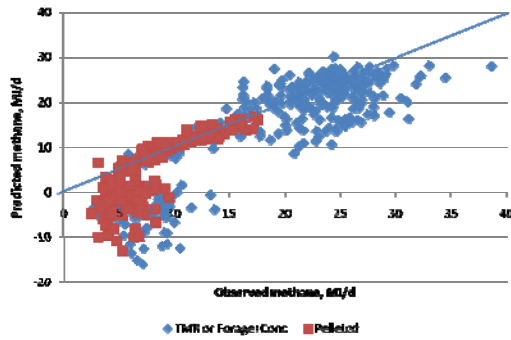
$c$  = Starch:ADF ratio

$x$  = DM intake





# Modelling methane emissions



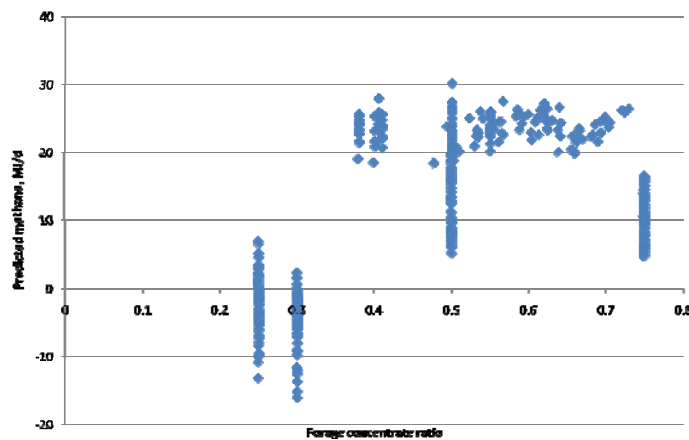
Using the existing non linear model yielded negative predictions of methane emission for some animals.

Therefore the data were filtered to identify the cause of this under prediction

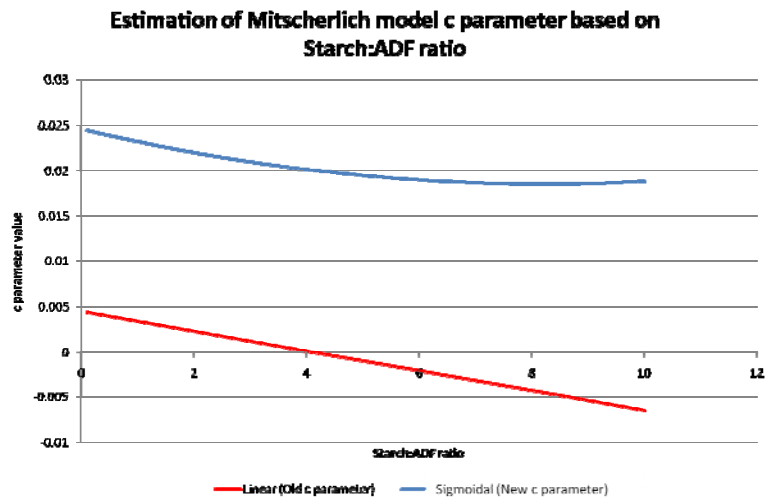
A plot of forage concentrate ratio against predicted methane shows that the negative values are entirely associated with very high concentrate diets.

# Modelling methane emissions

- This slide shows the old non linear model's negative methane estimates for diets comprising very high concentrate fractions (>70% concentrate).



# Modelling methane emissions

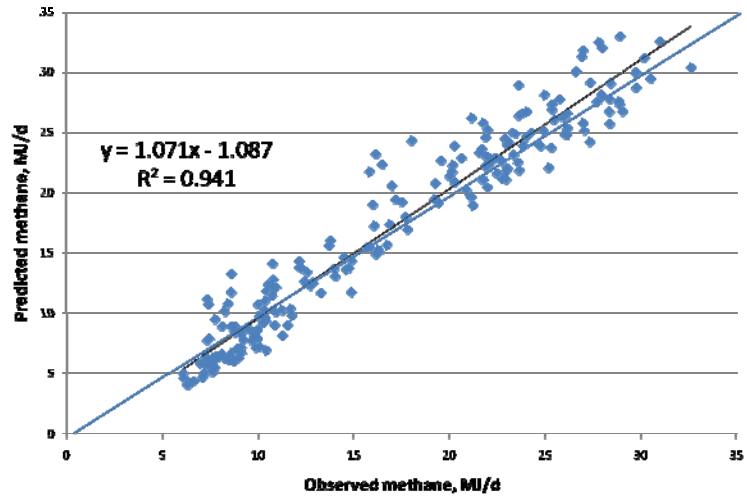


# Modelling methane emissions

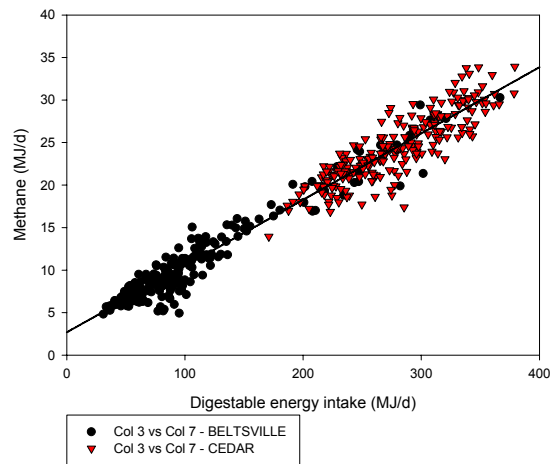
- The existing calculation of the slope in the Mitscherlich model was based on a linear function.
- $c = -0.0011x(\text{Starch}/\text{ADF}) + 0.0045$
- However, for very high concentrate diets with high levels of starch this function produced negative values.
- A new sigmoidal function was devised that better described the diminishing returns nature of the relationship.
- This new relationship was then integrated into the Mitscherlich model of DMI vs methane production.
- The revised model gave predictions that were highly correlated with observations.

# DMI vs Methane modified for starch:ADF ratio

Observed vs predicted methane emissions



## Digestible energy vs Methane energy

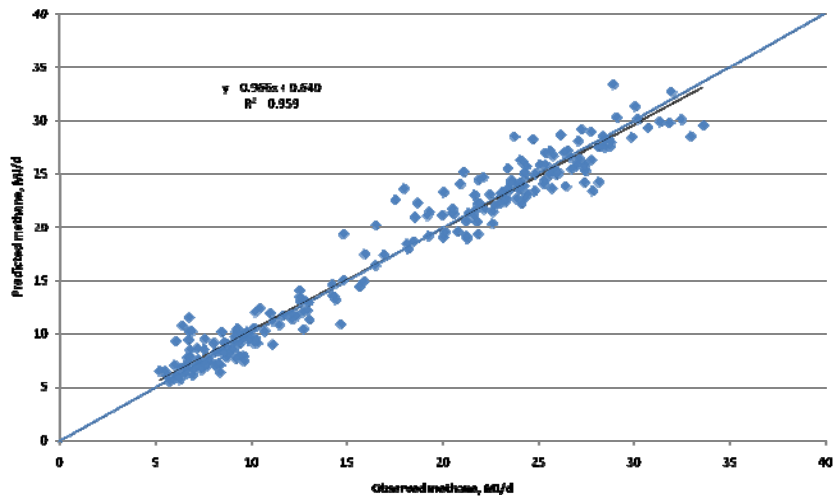


## Digestible energy vs methane energy

- As digestible energy intake (DEI) increases, methane output rises in a linear manner.
- The linear nature of this relationship is in contrast to the non linear model relating DMI to methane. This may reflect a depression in digestibility at the highest levels of DMI.

## DEI vs methane

Observed vs predicted methane emissions

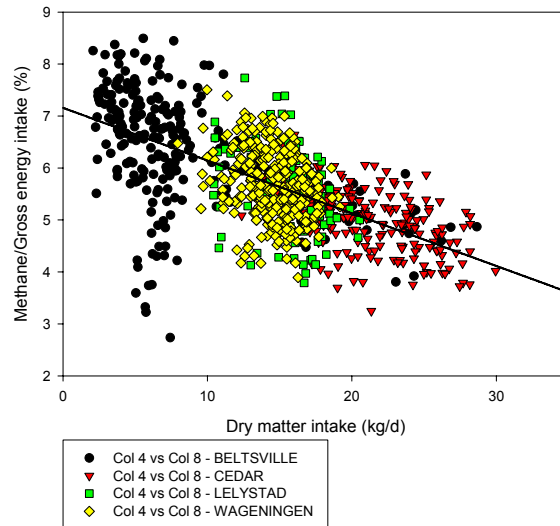


## DEI vs Methane

Observed vs predicted methane emissions

- The relationship between DEI and methane emission is highly significant with the highest correlation coefficient of all the models.
- DEI accounts for faecal energy loss directly leading to the marginal improvement when compared with models based on DMI.

## DMI vs Methane as % GE intake



## DMI vs Methane as % GE intake

- This plot shows that as intake increases the proportion of feed energy lost as methane declines.
- The line represents a linear model with a correlation coefficient of 0.56 and an overall prediction error of 23%.
- The mean amount of gross energy intake lost as methane is commonly quoted as 8%, but this data shows this values is approximately 5% for modern dairy cows

## Predicting methane emissions

Model Description		
Model	Description	Parameters
DMI vs CH <sub>4</sub>	CH <sub>4</sub> (MJ/d) = $a-(a+b)e^{-cx}$	$a=74.43, b=0, c= 0.0163, x=DMI$
DMI <sub>Starch:ADF</sub> vs CH <sub>4</sub>	CH <sub>4</sub> (MJ/d) = $a-(a+b)e^{-cx}$	$a=74.43, b=0, x=DMI$ $c=0.0187+0.0059/(1+\exp(\text{Starch:ADF}-3.1003))/0.6127$
DMI vs CH <sub>4</sub> % GEI	CH <sub>4</sub> (%GEI) = $m DMI + c$	$m = -0.101, c = 7.16$
DEI vs CH <sub>4</sub>	CH <sub>4</sub> (MJ/d) = $m DEI + c$	$m = 0.0779, c = 2.6861$

Model Evaluation				
Model	Observed mean	Predicted mean	r <sup>2</sup>	Root MSPE (% of observed mean)
DMI vs CH <sub>4</sub>	15.65	15.60	0.91	11.02%
DMI <sub>Starch:ADF</sub> vs CH <sub>4</sub>	16.73	16.83	0.94	12.48%
DMI vs CH <sub>4</sub> % GEI	5.70%	6.55%	0.56	23.5%
DEI vs CH <sub>4</sub>	17.22	17.28	0.96	9.65%

## Predicting methane emissions

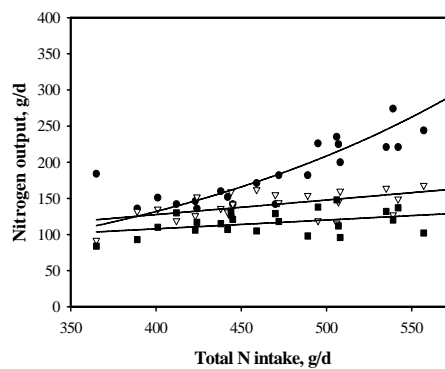
- The four models described in the above tables all show a low error of prediction against the evaluation data set.
- As well as overall error, end users need to consider practicality and likely variation between any diets being tested.
- The straightforward relationship between DMI and methane is highly practical (probably more so than DEI) given the information available on farm.
- Accounting for variation in the starch:ADF ratio may only show a limited reduction in prediction error against the evaluation data set, but it is likely to prove more robust across a wider range of diets from other data sources than the other models listed.

# Nitrogen (N)

## Models to predict excretion

## Previous research

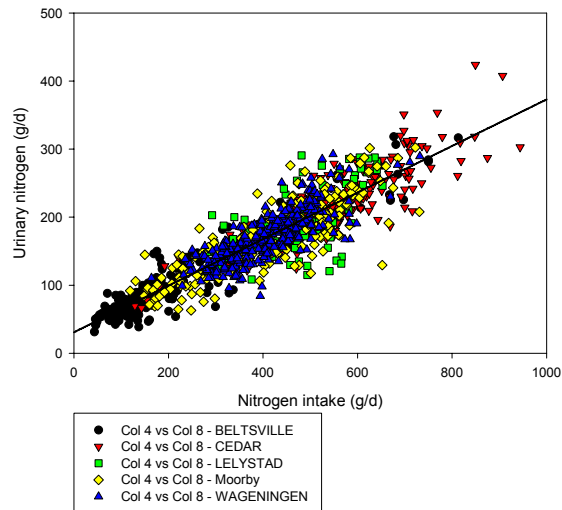
Relationship between total N intake (g/d) and N output as urine (●), faeces (▽), and milk (■).



Source: Castillo AR, Kebreab E, Beaver DE, Barbi JH, Sutton JD, Kirby HC and France J. (2001). *J. Anim. Sci.*, 79: 247-253.



## N intake vs Urine N

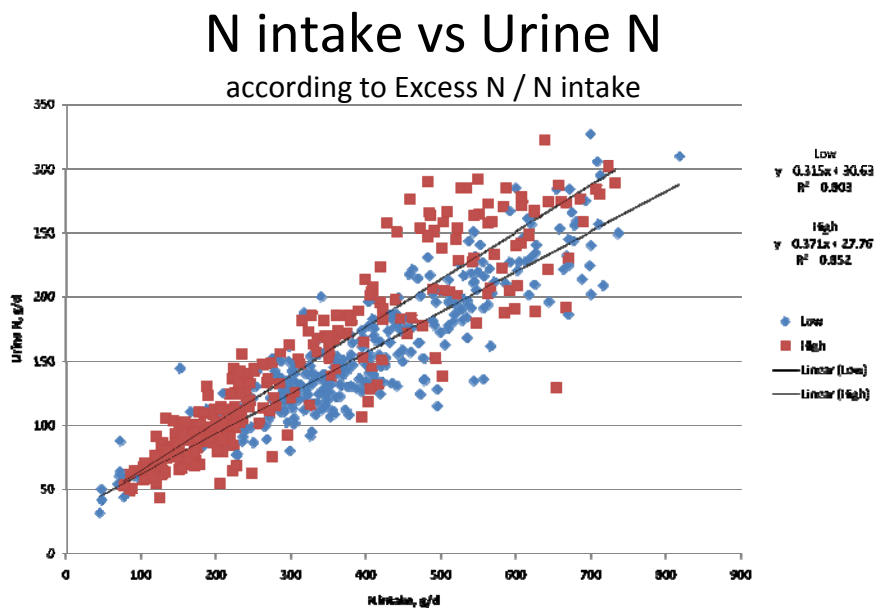


## N intake vs Urine N

- Linear relationship between N intake and urine N observed that does not conform to the 'broken stick' model of Castillo et al. (2001).
- However, there is some variation that appears to be related to level of over or under feeding of N relative to requirement.

## N intake vs Urine N

- The data were grouped according to 'Excess N' which was defined as the level of N intake relative to each animal's calculated requirement, as described previously by Moe et al. (1972).
- Data for animals at the extreme ends of this spectrum (underfed or overfed) was plotted to determine if there was an impact on urinary N excretion.
- Underfed are grouped as 'Low'
- Overfed are grouped as 'High'



## N intake vs Urine N

- Two models

- Model 1

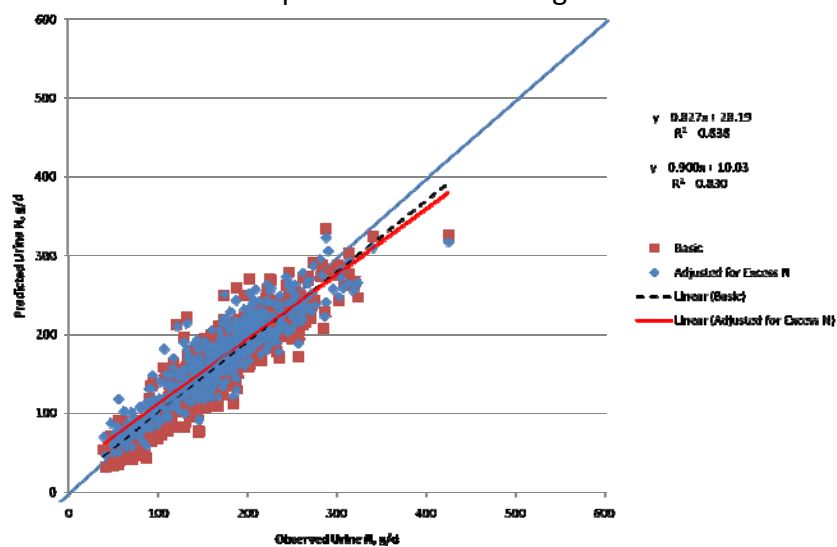
- Linear relationship between N intake and urine N
      - »  $\text{Urine N (g/d)} = 0.366 * \text{N intake} + 14.52$

- Model 2

- Linear relationship between N intake and urine N, adjusted for calculated level of over or under feeding of N in relation to requirement (Excess N)
      - $\text{Urine N (g/d)} = m * \text{N intake} + 32$ 
        - » Where  $m = 0.315 * (\text{Excess N} / \text{N intake}) + 0.106$

## N intake vs Urine N

Observed vs predicted urine nitrogen excretion

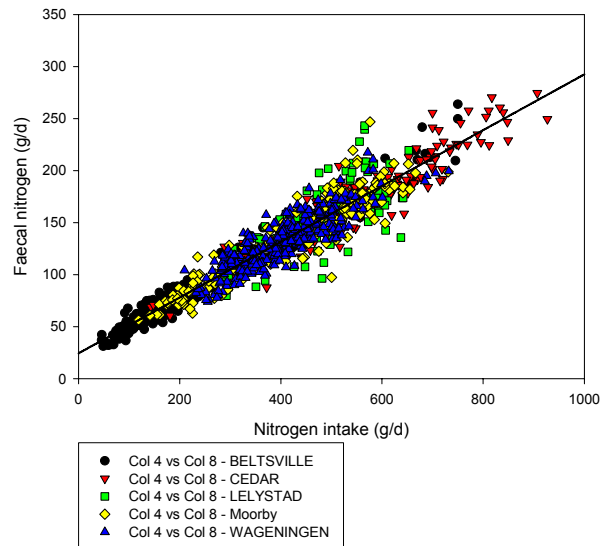




## N intake vs Urine N

- Adjusting for the level of feeding in relation to requirement marginally improves the predictive of urine N excretion.
- However, the principal determinant of urine N excretion is N intake .

## N intake vs Faecal N

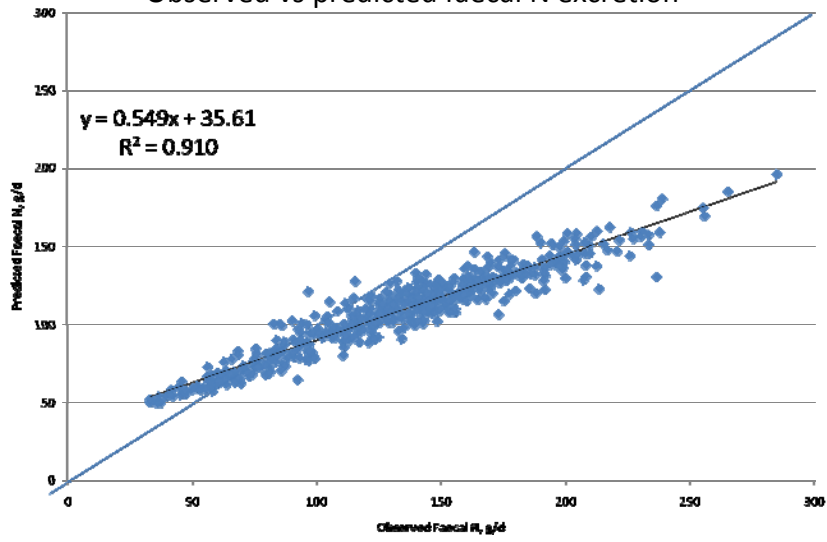


## N intake vs Faecal N

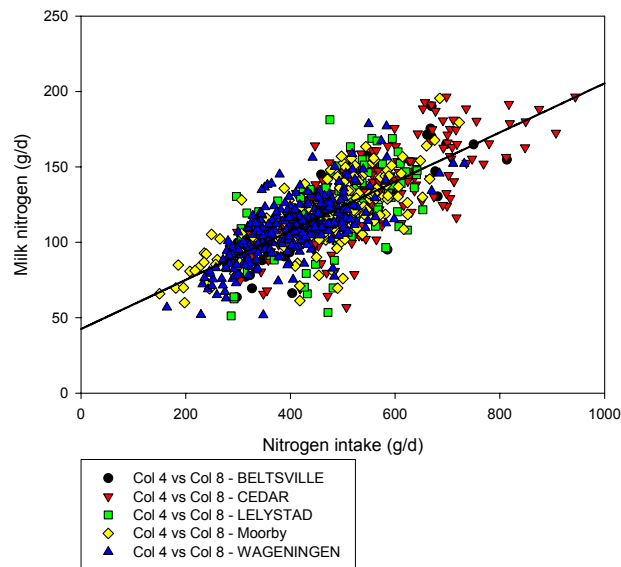
- Faecal N is also highly correlated with total N intake. However when this model is used to predict independent data there is a bias associated with the linear relationship as shown on the subsequent slide. This implies that the relationship is actually multi-factorial with N intake alone being an inadequate predictor of faecal N over a wide data range. It is also likely that relatively few data points at very high levels of N intake predominantly from one source (CEDAR) has contributed to this bias.

# N intake vs Faecal N

Observed vs predicted faecal N excretion



## N intake vs Milk N



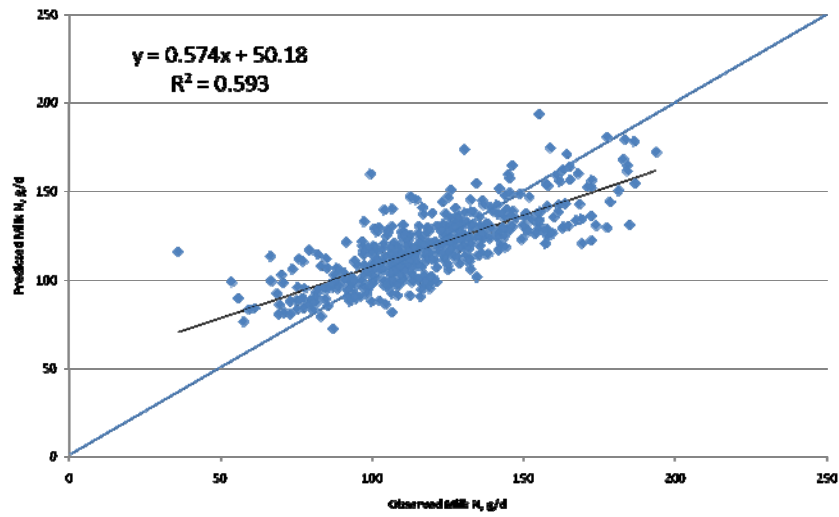
## N intake vs Milk N

- As N intake increases for lactating animals, the level of milk N output per day increases in a linear fashion.
- This is in agreement with previous modelling studies.
- Variation tends to be greater than for urinary or faecal nitrogen.
- As for faecal N, there is a bias associated with this linear model as depicted on the following slide. Although it seems likely that this bias is introduced due to the relatively small number of data points at either extreme of this relationship.
- Milk N is a highly complex variable with many different dietary, genetic, physiological and environmental, factors determining its output.



# N intake vs Milk N

Observed vs predicted milk N



## Predicting nitrogen excretion

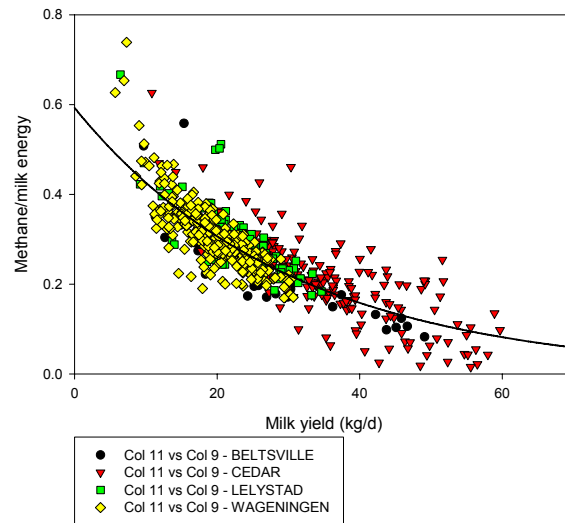
Model Description		
Model	Description	Parameters
N intake vs Urine N	Urine N (g/d) = $m(N \text{ intake}) + c$	$m = 0.366, c = 14.52$
N intake <sub>ExcessN</sub> vs Urine N	Urine N (g/d) = $m(N \text{ intake}) + c$	$m = 0.315*(\text{Excess N} / N \text{ intake}) + 0.106, c = 32$
N intake vs Faecal N	Faecal N (g/d) = $m(N \text{ intake}) + c$	$m = 0.268, c = 24.34$
N intake vs Milk N	Milk N (g/d) = $m(N \text{ intake}) + c$	$m = 0.163, c = 42.47$

## Predicting nitrogen excretion

Model Evaluation				
Model	Observed mean	Predicted mean	r <sup>2</sup>	Root MSPE (% of observed mean)
N intake vs Urine N	170	163	0.83	15.5
N intake <sub>ExcessN</sub> vs Urine N	170	169	0.84	14.4
N intake vs Faecal N	131	108	0.91	25.0
N intake vs Milk N	119	119	0.59	14.37

## Other relationships

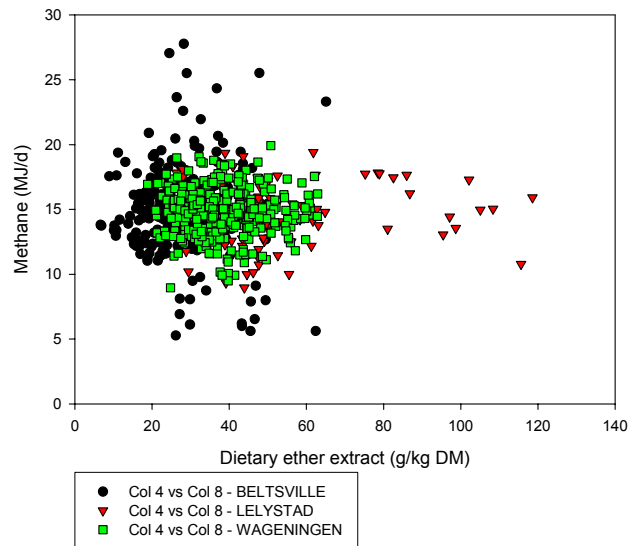
## Methane energy as a proportion of milk energy



## Methane energy as a proportion of milk energy

- As milk yield increases there is a decline in the excretion of methane energy relative to milk energy.
- This indicates that higher yielding cows will be producing less methane per unit of milk produced.

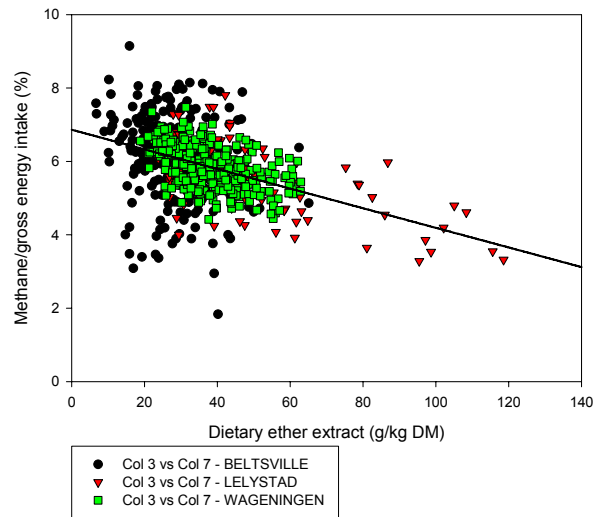
## Dietary fat vs Methane



## Dietary fat vs Methane

- There does not appear to be a reliable relationship between dietary fat and methane emission across the whole dataset.
- Dietary fat is characterised poorly by traditional analytical techniques (ether extract) and this fails to show the complex behaviours of different fat sources, where it is known that for a given diet supplemental fat can reduce methane excretion per kg DMI (e.g. Andrew et al., 1991).

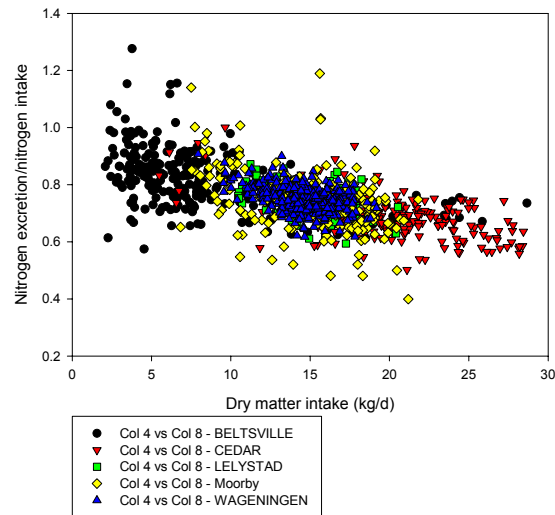
## Dietary fat vs Methane



## Dietary fat vs Methane

- When dietary fat is correlated with methane energy as a proportion of gross energy intake, there appears to be a slight negative trend. However, due to the limited data range and the reliance on a few extreme data points to direct the plot only a low degree of confidence can be placed in the observed pattern.

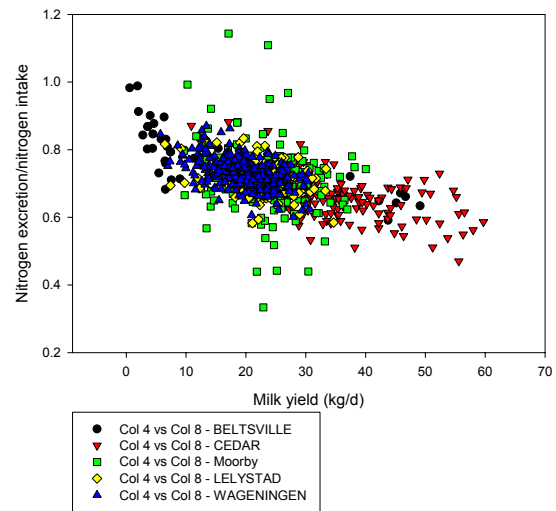
## DMI vs Excreted N/N intake



## DMI vs Excreted N/N intake

- As intake increases there is a tendency for the proportion of N excreted to decline. This represents an increasing proportion of dietary N being directed towards milk production as shown on the following slide.

# Milk Yield vs Excreted N/N intake





## Conclusions

- Higher producing animals tend to lose less feed energy as methane per unit of milk produced.
  - A one size fits all estimate of feed energy lost as methane is inappropriate.
  - On average, higher producing animals also excrete less N to the environment per unit N intake, but there is much variation in N excretion relative to milk yield.
- Nitrogen intake is the principal driver of N excretion although the level of N intake with respect to requirement modifies the response.
- As N intake increases above requirement, the excess N is partitioned largely towards urine N excretion with relatively modest increases in faecal and milk N.
- Nitrogen excretion remains relatively unaffected by the balance between structural and non structural carbohydrate.

## Conclusions

- Prediction:
  - Provided information on an animal's DMI is available, a reliable estimate of methane emission can be given for both beef and dairy animals.
  - Nitrogen excretion in urine and faeces can be estimated based on N intake alone. The same is true for milk N although the error of prediction is increased.

## Practical recommendations

- Direct production systems towards those with high producing animals.
  - Aim for high growth rates or milk yields and estimate energy & protein requirements using the most appropriate available models (e.g. FiM, NRC)
  - Milk yields below 20 kg/d are associated with increasing amounts of methane excretion per kg milk yield, because DMI is the primary determinant of methane excretion.
- Increase dietary starch at the expense of more fibrous carbohydrates (whilst being mindful of implications for rumen health).
  - Aim to increase diet energy density using starch, fats and oils
- Estimate N requirement and aim to avoid excessive dietary N intake above the requirement based on estimates of metabolizable protein supply .
  - Nitrogen requirement is determined primarily by body weight and milk N output.
- As feed efficiency (meat or milk per kg DMI) improves, the amount of methane excreted per kg milk or meat will be reduced.

## Practical recommendations

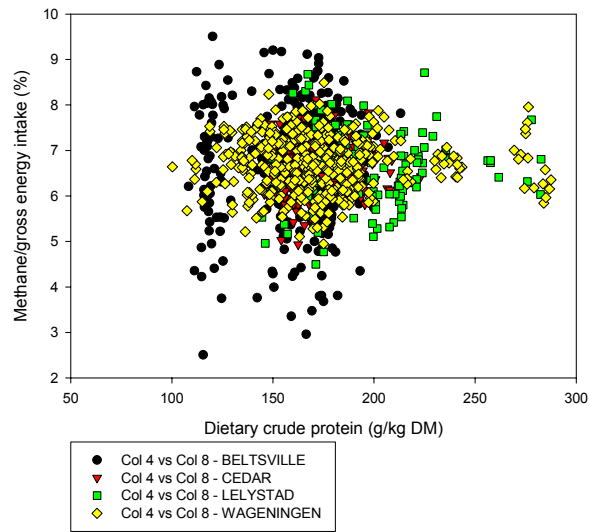
- When formulating diets for dairy or beef cattle:
  - Prioritise:
    - Maize silage
    - Cereal grains
    - Starch rich root crops
    - Oils (protected fats, crushed oilseeds)
  - Where possible limit:
    - Grass silage (particularly late maturity)
    - Hays and straws
    - Fibrous concentrates

# Appendix

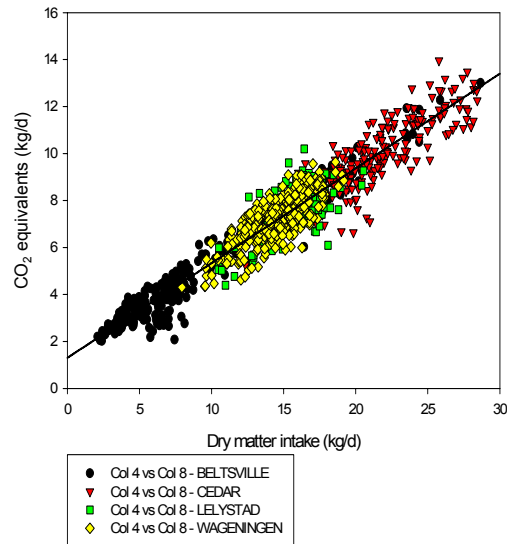
## Methane

Other relationships

# Diet crude protein vs Methane



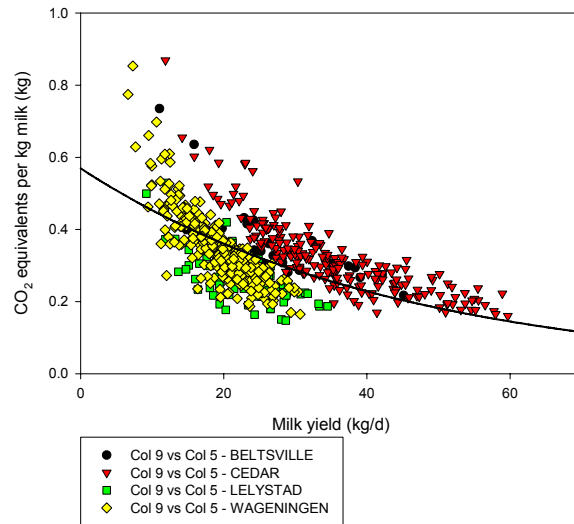
## DMI vs CO<sub>2</sub> equivalent emissions



## DMI vs CO<sub>2</sub> equivalent emissions

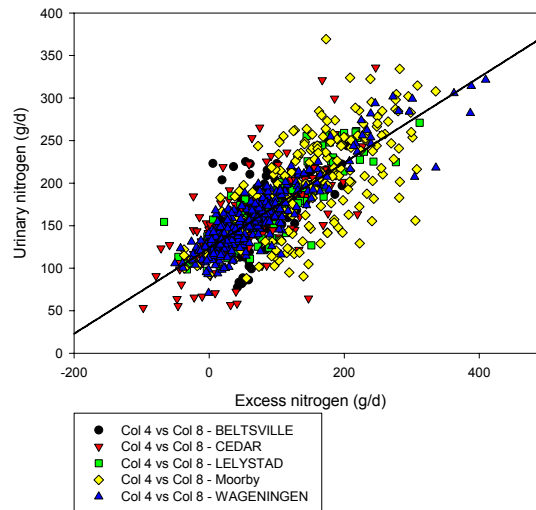
- For purposes of comparison with other environmental emissions, methane emissions can be expressed as equivalent to kilograms of CO<sub>2</sub> emitted per day. The range is between 2 and 15 kg/d depending on intake.
- Above figures based on a global warming potential (GWP) for methane set at 23 with CO<sub>2</sub> set at 1.

# Milk yield vs CO<sub>2</sub> equivalent emissions



Nitrogen  
Other relationships

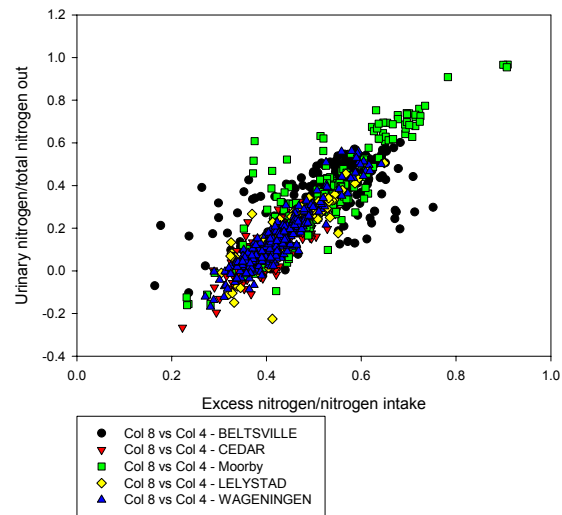
## Excess N vs Urine N



## Excess N vs Urine N

- Excess N is defined as the surplus of N intake relative to the calculated requirement. Used on its own this does not provide as good a predictor of N excretion as total N intake, although the general trend is clear.

## Excess N/N intake vs Urine N

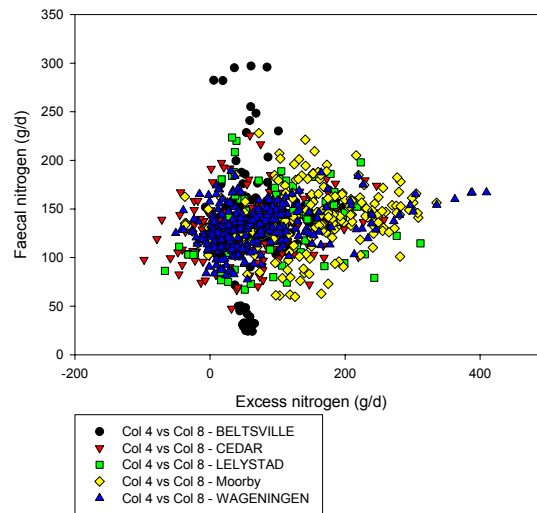


## Excess N/N intake vs Urine N

- As N is increasingly overfed in relation to requirement the proportion of N directed towards urine increases in a linear fashion.



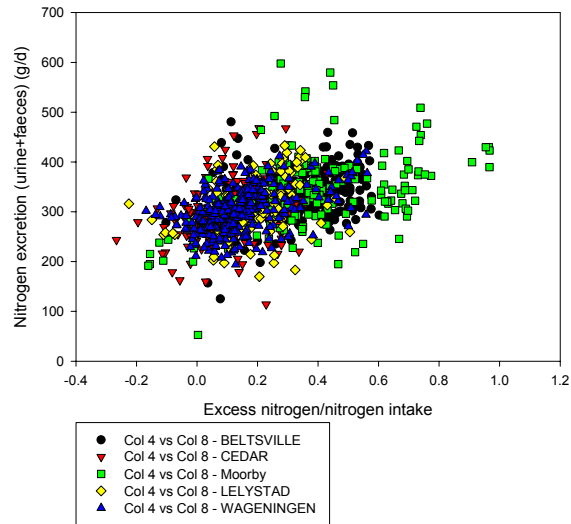
## Excess N vs Faecal N



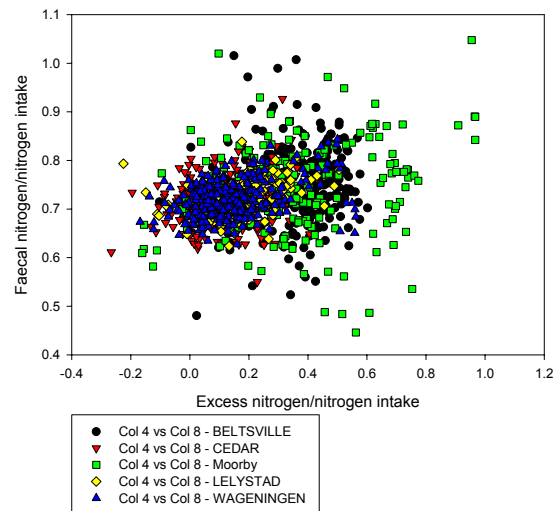
## Excess N vs Faecal N

- Faecal N excretion can not be predicted accurately from the N intake relative to requirement.

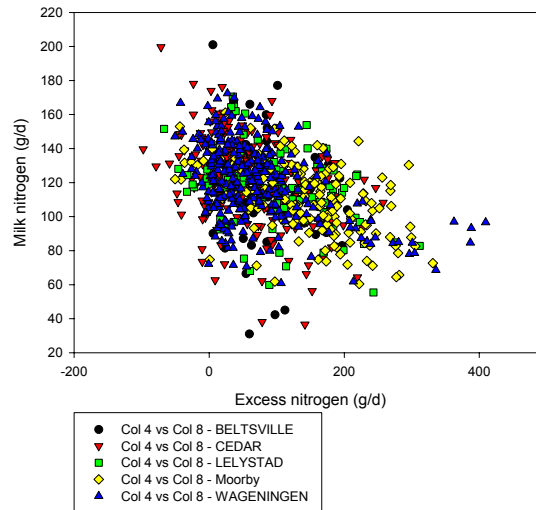
## Excess N vs Excreted N



## Excess N vs Faecal N/N intake



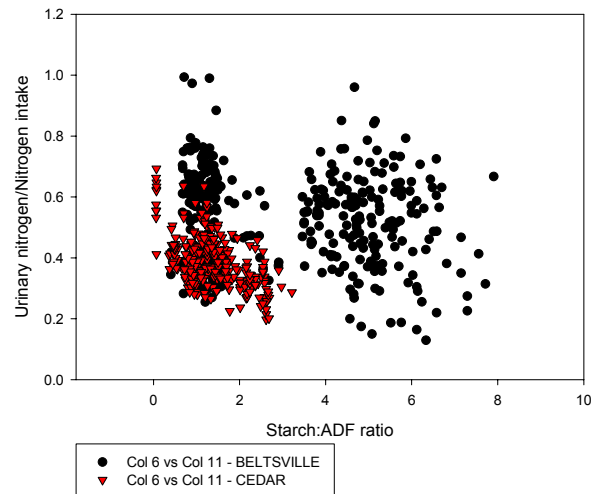
## Excess N vs Milk N



## Excess N vs Milk N

- The intake of N relative to requirement appears to be a poor predictor of milk nitrogen output.

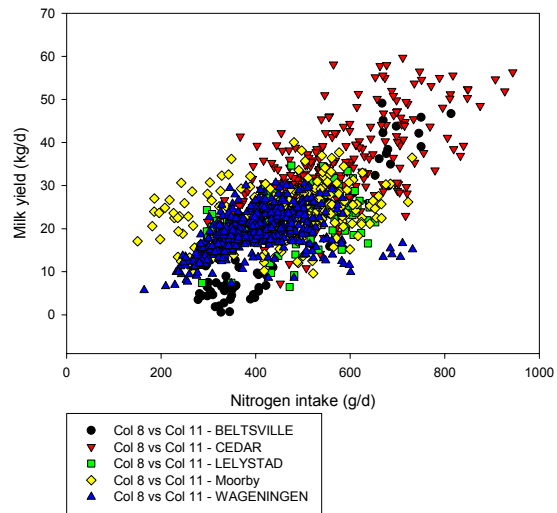
## Starch:ADF ratio vs Urine N



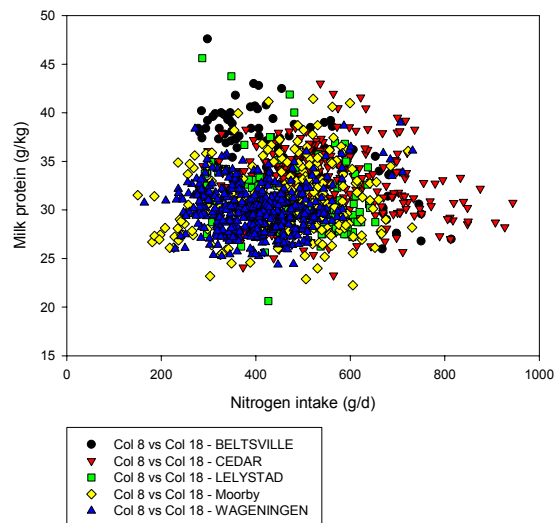
## Starch:ADF ratio vs Urine N

- The data suggest that starch:ADF ratio is unrelated to the proportion of ingested N excreted in urine across all the diets fed. As for fat effects on methane, the response is complex and determined by a number of dietary and physiological factors (Reynolds et al., 2001; Reynolds, 2006).

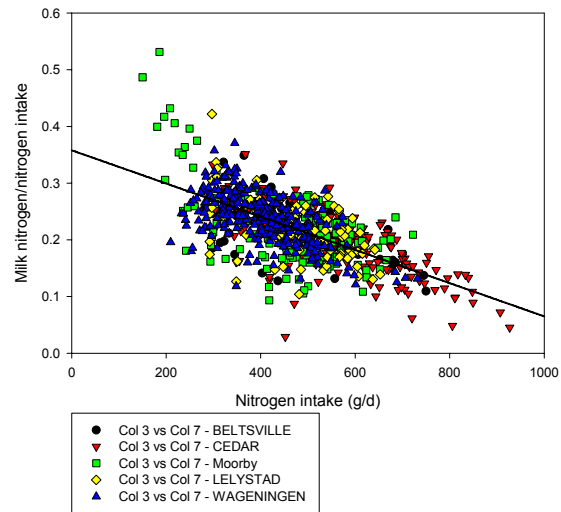
## N intake vs Milk yield



## N intake vs Milk protein



# N intake vs Milk N/N intake



## References:

Andrew, S. M., H. F. Tyrrell, C. K. Reynolds, and R. A. Erdman. 1991. Net energy value for lactation of a dietary fat, calcium salts of long-chain fatty acids, for cows fed silage-based diets. *J. Dairy Science* 74:2588-2600.

Castillo, A. R., E. Kebreab, D. E. Beever, and J. France. 2000. A review of the efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *J. Anim. Feed Sci.* 9:1-32.

Castillo, A. R., E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby, and J. France. 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.* 79:247-253.

Frank, B., and C. Swensson. 2002. Relationship between content of crude protein in rations for dairy cows and milk yield, concentration of urea in milk and ammonia emissions. *J. Dairy Sci.* 85:1829–1838.

Holter, J. B., and A. J. Young. 1992. Methane prediction in dry and lactating Holstein cows. *J. Dairy Sci.* 75:2165.

James, T., D. Meyer, E. Esparza, E. J. DePeters, and H. Perez-Monti. 1999. Effects of dietary nitrogen manipulation on ammonia volatilization from manure from Holstein heifers. *J. Dairy Sci.* 82:2430–2439.

Kebreab, E., J. France, J. A. N. Mills, R. Allison, and J. Dijkstra. 2002. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. *J. Anim. Sci.* 80:248-259.

Kirchessner, M., W. Windisch, and H. L. Muller. 1995. Nutritional factors for the quantification of methane production. In, W.v. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke (ed.), *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Proceedings of the 8th International Symposium on Ruminant Physiology.* Ferdinand Enke Verlag, Stuttgart, Germany, 333-348

Külling, D. R., F. Dohme, H. Menzi, F. Sutter, P. Lischer, and M. Kreuzer. 2002. Methane emissions of differentially fed dairy cows and corresponding methane and nitrogen emissions from their manure during storage. *Environmental Monitoring and Assessment* 79:129-150.

Nennich, T. D., J. H. Harrison, L. M. Van Wieringen, D. Meyer, A. J. Heinrichs, W. P. Weiss, N. R. St-Pierre, R. L. Kincaid, D. Davidson, and E. Block. 2005. Prediction of manure and nutrient excretion of dairy cattle. *J. Dairy Sci.* 88:3721-3733.

Mills, J. A. N., J. Dijkstra, A. Bannink, S. B. Cammell, E. Kebreab, and J. France. 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation and application. *J. Anim. Sci.* 79:1584-1597.

Mills J.A.N., Kebreab E., Yates C.M., Crompton L.A., Cammell S.B., Dhanoa M.S., Agnew R.E., France J. 2003. Alternative approaches to predicting methane emissions from dairy cows *J. Anim. Sci.* 81: 3141-3150.



Moe, P. W. and H. F. Tyrrell. 1979. Methane production in dairy cows. *J. Dairy Sci.* 62:1583-1586.

Moe, P. W., H. F. Tyrrell, and W. P. Flatt. 1972. Net energy value of feeds for lactation. *J. Dairy Sci.* 55:945-958.

Newbold, C. J., F. M. McIntosh, P. Williams, R. Losa, and R. J. Wallace. 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. *Anim. Feed Sci. Technol.* 114:105-112.

Noftsker, S., and N. R. St-Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. *J. Dairy Sci.* 86:958-969.

St-Pierre, N. R. 2001. Integrating quantitative findings from multiple studies using mixed model methodology. *J. Dairy Sci.* 84:741-755.

Reynolds, C. K. and H. F. Tyrrell. 2000. Energy metabolism in lactating beef heifers. *J. Anim. Sci.* 78:2696-2705.

Reynolds, C. K., S. B. Cammell, D. J. Humphries, D. E. Beever, J. D. Sutton, and J. R. Newbold. Effects of post-rumen starch infusion on milk production and energy metabolism in dairy cows. *J. Dairy Sci.* 84:2250-2259.

Reynolds, C. K. 2006. Production and metabolic effects of site of starch digestion in lactating dairy cattle. *Anim. Feed Sci. Technol.* 130:78-94.

Tyrrell, H. F., C. K. Reynolds, and H. D. Baxter. 1990. Energy metabolism of Jersey and Holstein cows fed total mixed diets with or without whole cottonseed. *J. Dairy Sci.* 73 (supplement 1):192.

Wilkerson, V. A., D. P. Casper, and D. R. Mertens. 1995 The prediction of methane production of Holstein cows by several equations. *J. Dairy Sci.* 78:2402-2414.

Yan, T., R. E. Agnew, F. J. Gordon, and M. G. Porter. 2000. Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. *Livestock Prod. Sci.* 64:253-263.

Yan, T., J. P. Frost, R. E. Agnew, R. C. Binnie, and C. S. Mayne. 2006. Relationships among manure nitrogen output and dietary and animal factors in lactating dairy cows. *J. Dairy Sci.* 89:3981-3991.