



Assessing precision and accuracy of enteric methane flux measures using GreenFeed systems

G. Renand

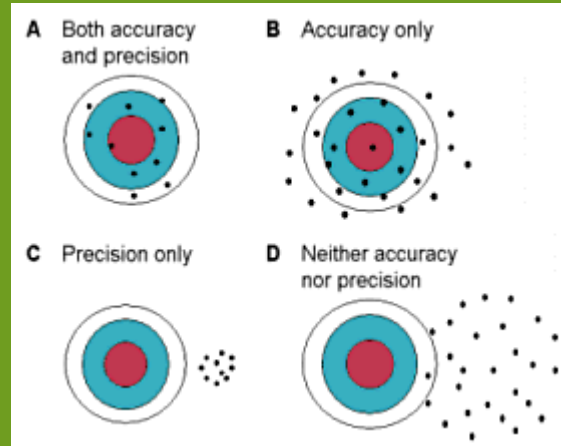
INRA, Animal Genetics Department,
Jouy en Josas, France



INTRODUCTION

- For selecting low emitting cattle there is a need to phenotype large reference populations where individual methane emission measures are associated with genomic markers.
- Among techniques for measuring enteric methane emission of a large number of animals, the GreenFeed system appears to be a potentially valuable tool for beef cattle.
- This technique can be implemented in performance testing stations where reference populations are currently evaluated for Feed Efficiency differences.

- For ranking animals on their methane emission, the measure should be precise and accurate



- The objective of the present work is to assess the precision and accuracy of GreenFeed measures

➤ **Animals and DMI recording:**

124 beef heifers (4 batches & 3 pens)

- ✓ **mean start age = 21 months**
- ✓ **3 to 4 weeks adaptation**
- ✓ **8 weeks measurements**
- ✓ **Wood shaving bedding**
- ✓ ***Ad libitum* fescue silage in trough equipped with Calan gate**
- ✓ **DMI controlled 3 times/week**

⇒ **24 DMI measures/heifer**



➤ Enteric methane emission recording:

One GreenFeed / pen (12 heifers)

✓ **Pellets** (alfalfa, beet pulp, cereal by-products)

✓ **5 drops** (30 g each) **at each visit**

✓ **45 seconds** between 2 drops

✓ **6 hours** minimum between 2 visits

⇒ **25,203 spot-measures**

⇒ **visit duration: 236 seconds**

⇒ **203 spot-measures / heifer**

⇒ **3.7 spot-measures / heifer / day**



1) Assessing precision of CH₄ emission measured with the GreenFeed system in comparison with the measure of DMI

➤ Estimation of the repeatability of DMI and CH₄ measures as influenced by the number of observations recorded during different test period length

Traits analyzed		CH ₄	CO ₂	CH ₄ /CO ₂	DMI	CH ₄ /DMI	CO ₂ /DMI	
Period length	Daily	N = 6,462						
	2-3 days					N = 2,825		
	1 week					N = 969		
	2 weeks					N = 486		
	4 weeks					N = 248		

➤ Estimation of variances across periods of measure

Model: $y_{ktij} = CG_k + \text{Period}_t + a_i + e_{ktij}$

Variances-Covariances of animal measures repeated across periods

$$V_i = \begin{array}{|c|c|c|c|} \hline \dots & \dots & \dots & \dots \\ \hline \dots & \sigma^2_t & \sigma_{t,t+1} & \dots \\ \hline \dots & \dots & \sigma^2_{t+1} & \dots \\ \hline \dots & \dots & \dots & \dots \\ \hline \end{array}$$

Constant Variances across periods

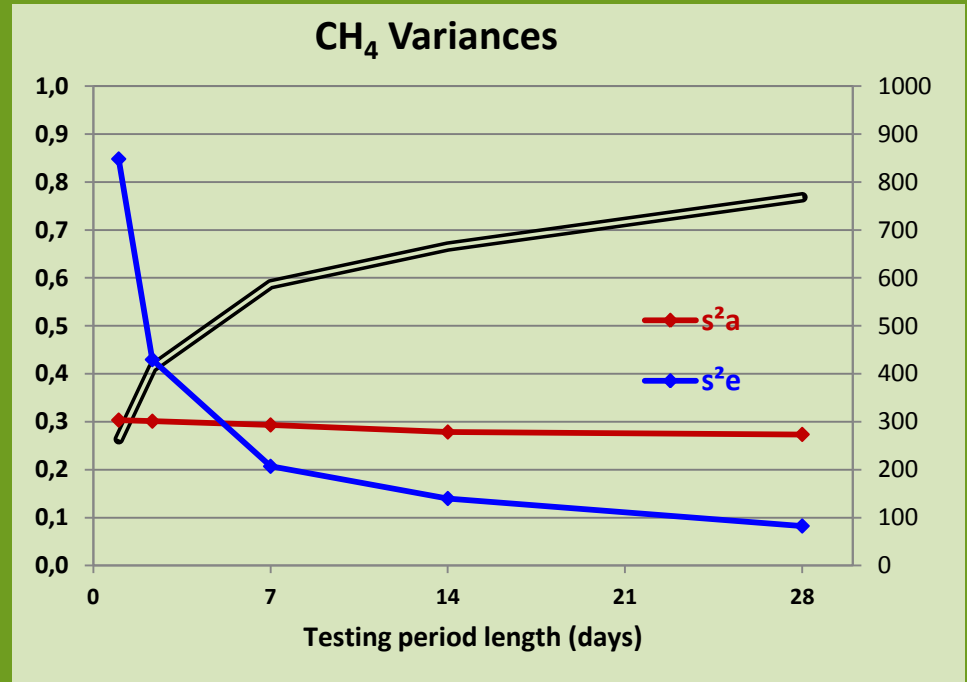
Proc Mixed (SAS) (type=cs)

⇒ estimates of σ^2_a and σ^2_e

$$\text{rep} = \sigma^2_a / (\sigma^2_a + \sigma^2_e)$$

$$V_i =$$

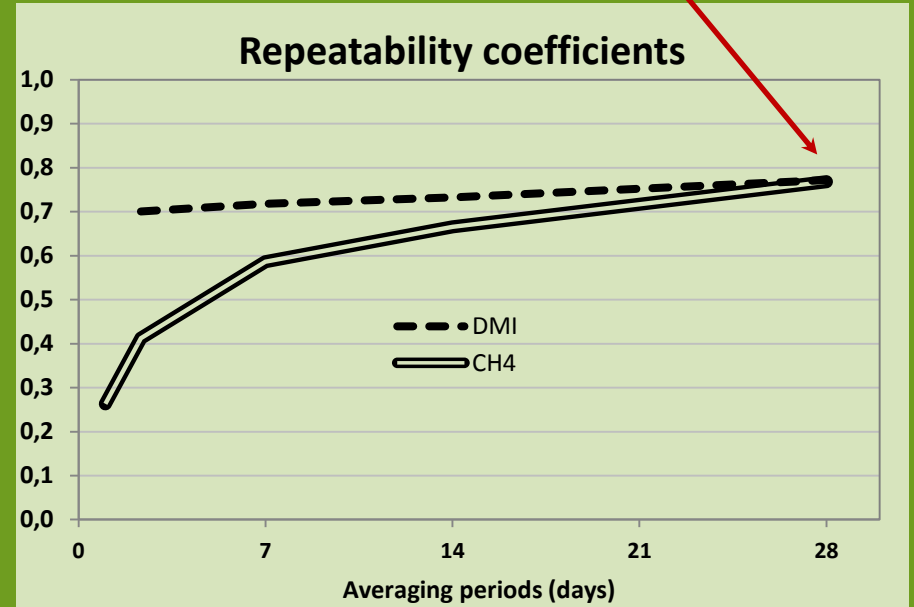
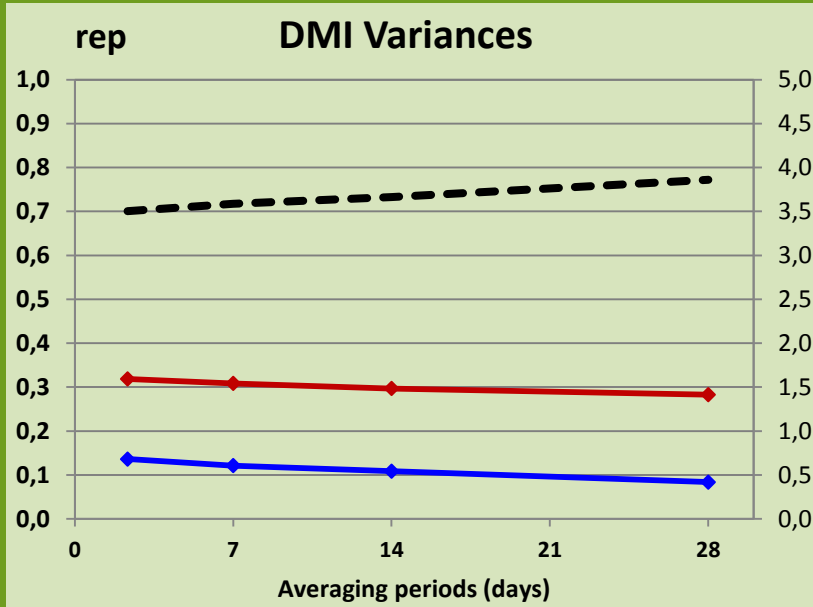
...
...	$\sigma^2_a + \sigma^2_e$	σ^2_a	...
...	...	$\sigma^2_a + \sigma^2_e$...
...



Constant Variances across periods

Comparison with DMI repeatability

Rep = 0.77
with 4 weeks
≈ 100 spot-measures



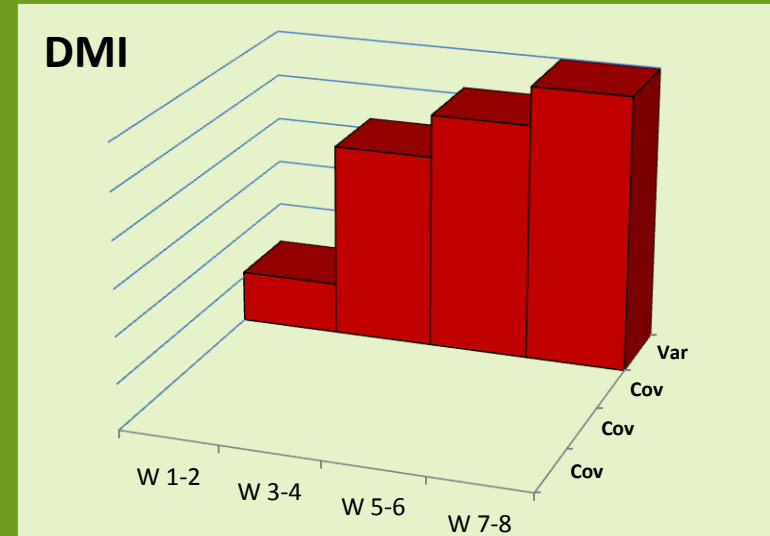
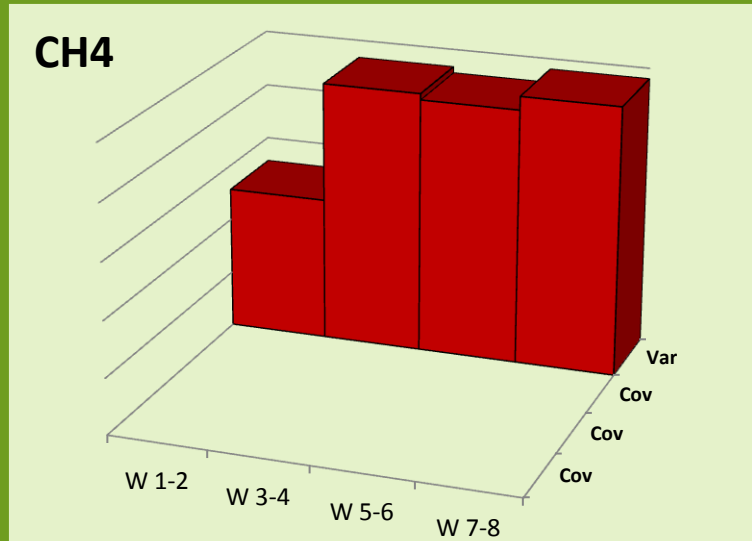
Unstructured Variances-Covariances with period duration = 2 weeks

Proc Mixed (SAS) (type=un)

⇒ estimates of Variances across periods

$V_i =$

...
...	σ^2_t	$\sigma_{t,t+1}$...
...	...	σ^2_{t+1}	...
...



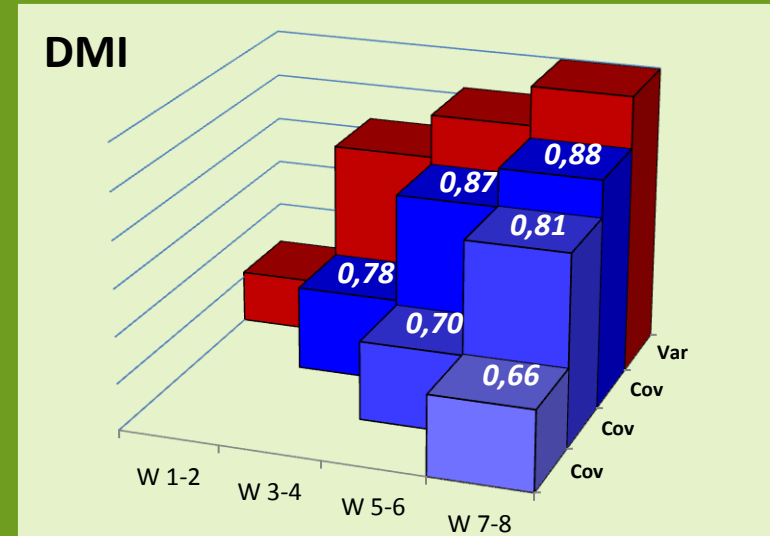
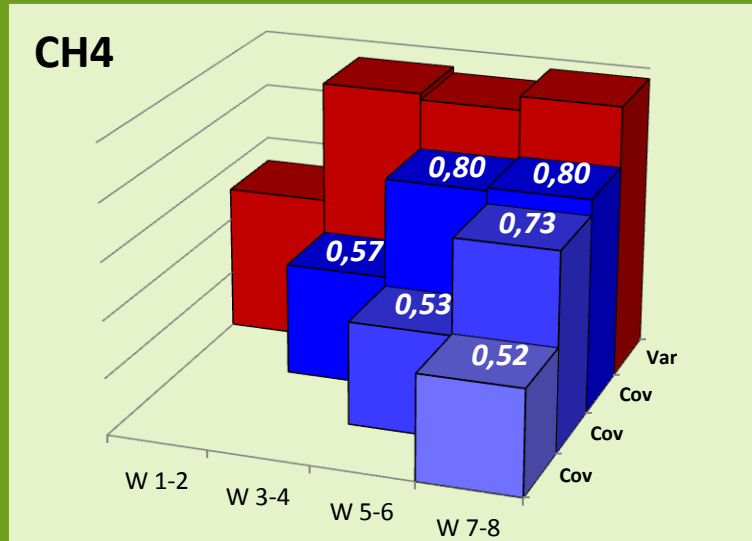
Unstructured Variances-Covariances with period duration = 2 weeks

Proc Mixed (SAS) (type=un)

⇒ estimates of Variances-Covariances across periods

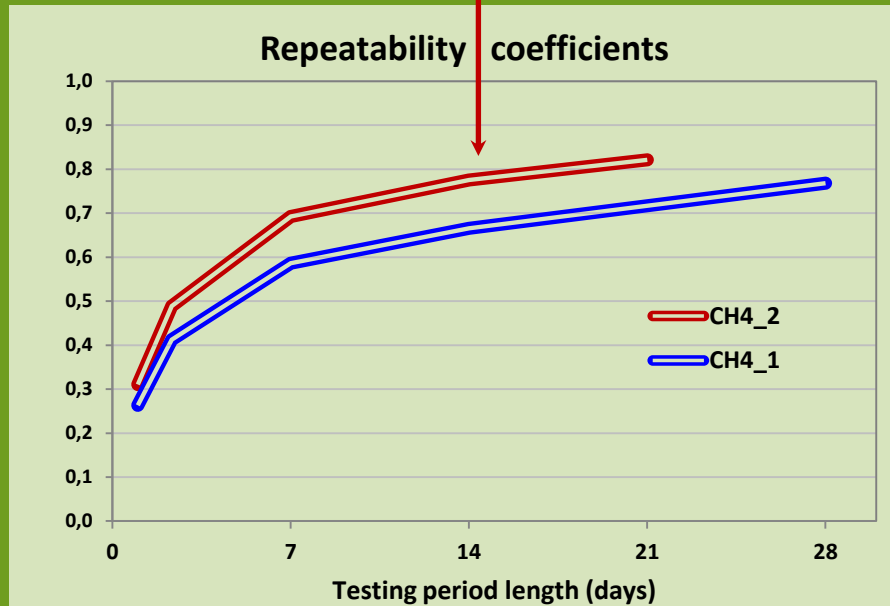
$$V_i =$$

...
...	σ^2_t	$\sigma_{t,t+1}$...
...	...	σ^2_{t+1}	...
...



Comparison of repeatability coefficients according to the lengths of the adaptation and of the testing period duration

Rep = 0.77
with 2 weeks
≈ 50 spot-measures



adaptation = 40 days
adaptation = 26 days

1) Conclusion on precision

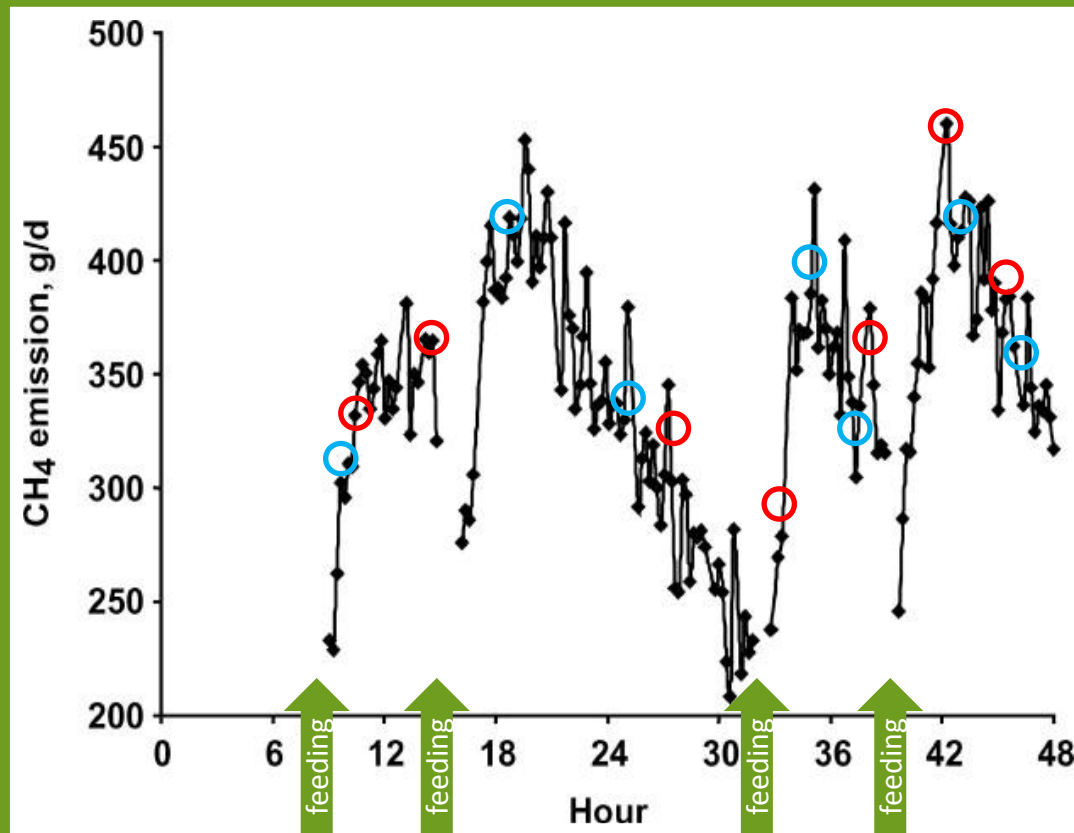
- In studies where the animals are fully adapted to their diet before the test, **≈ 50 spot-measures** could be sufficient for limiting the variance of spot sampling and for providing precise individual estimates of enteric methane emission.
- In performance testing stations where 3-4 weeks of adaptation may not be long enough, it would be wise to record **≈ 100 spot-measures** for providing precise individual estimates
- As compared with recommendations for testing differences of DMI of growing animals, it would be wise therefore to fix the same length of GreenFeed testing length as for recommended for feed intake, i.e. 5 weeks (35 days)

2) Assessing accuracy of CH₄ emission measured with the GreenFeed system

Methane emission highly fluctuates in relation with the feeding pattern.

Spot -measure sampling with the GreenFeed system

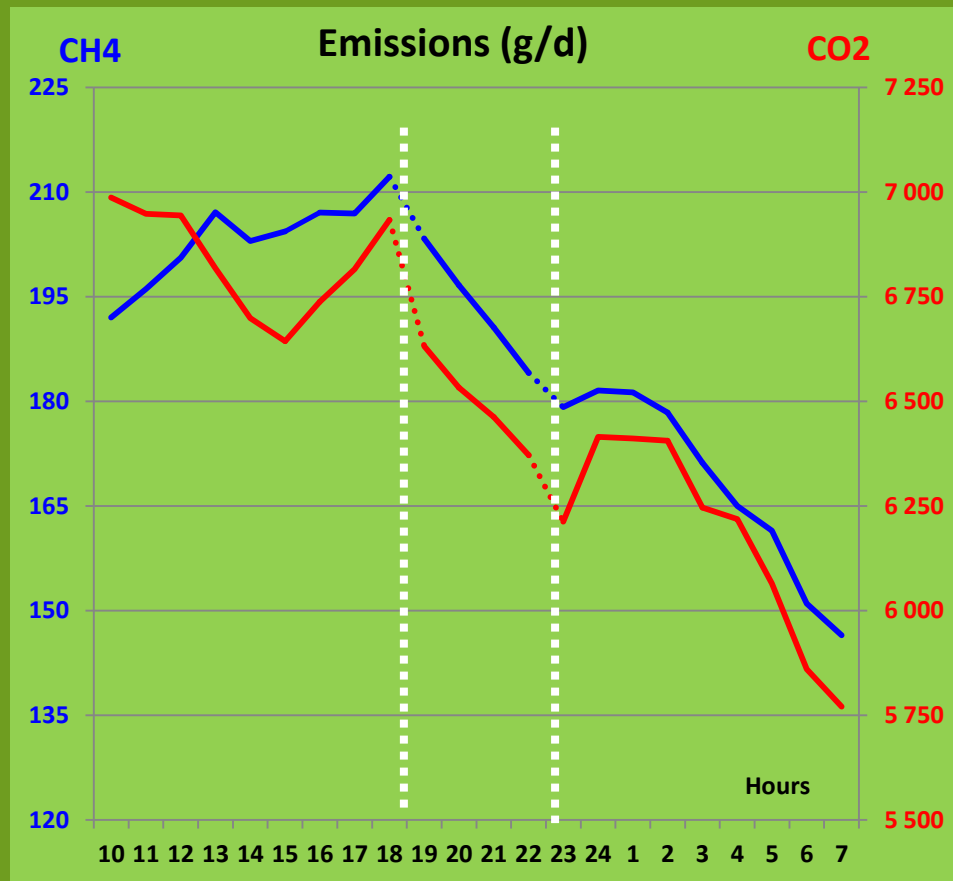
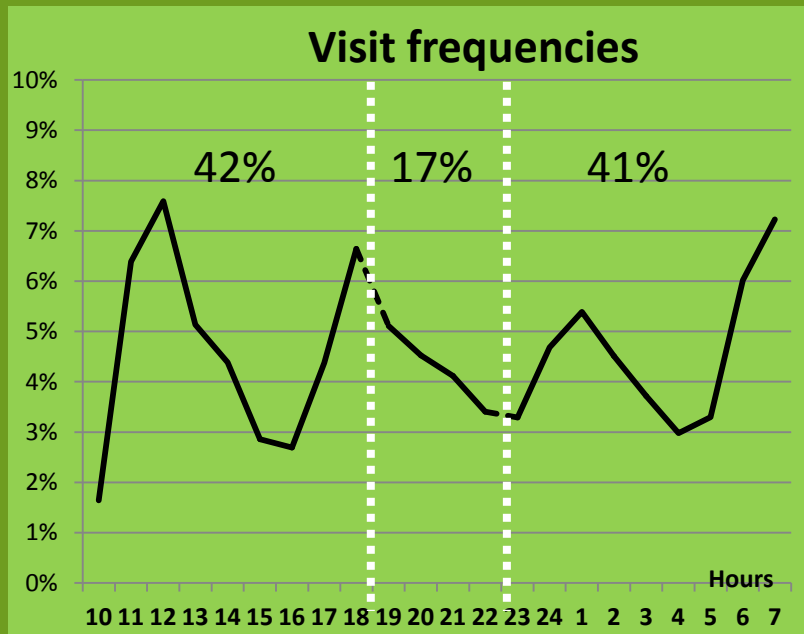
↳ Does the visit pattern influences the animal estimates ?



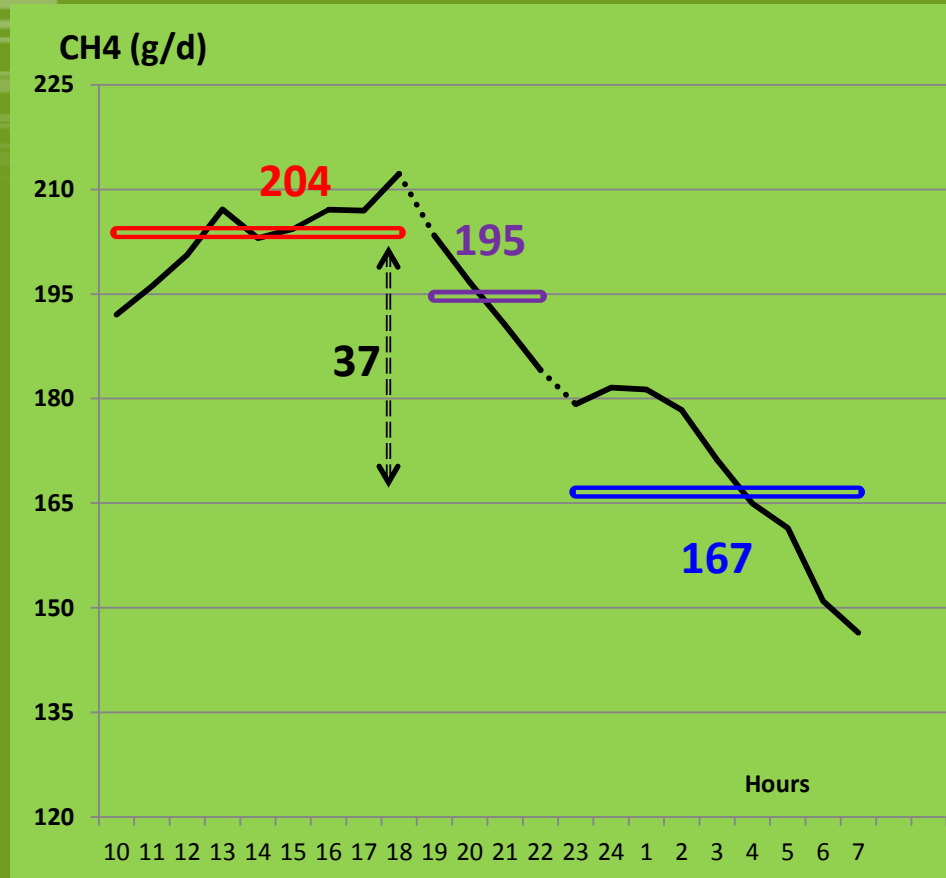
Grainger et al., 2007

014

Circadian GHG emissions



Differences between Day and Night visit emissions



Simulation of populations with different Visit Patterns

for quantifying the bias generated by different Visit Patterns

1) Extraction of the data from 70 heifers with 14 days and exactly 4 visits/day

=> 56 visits/heifer

=> “Initial” CH₄ measure = average of 56 spot-measures

regularly spread over day (n=23), evening (n=10) and night (n=23)

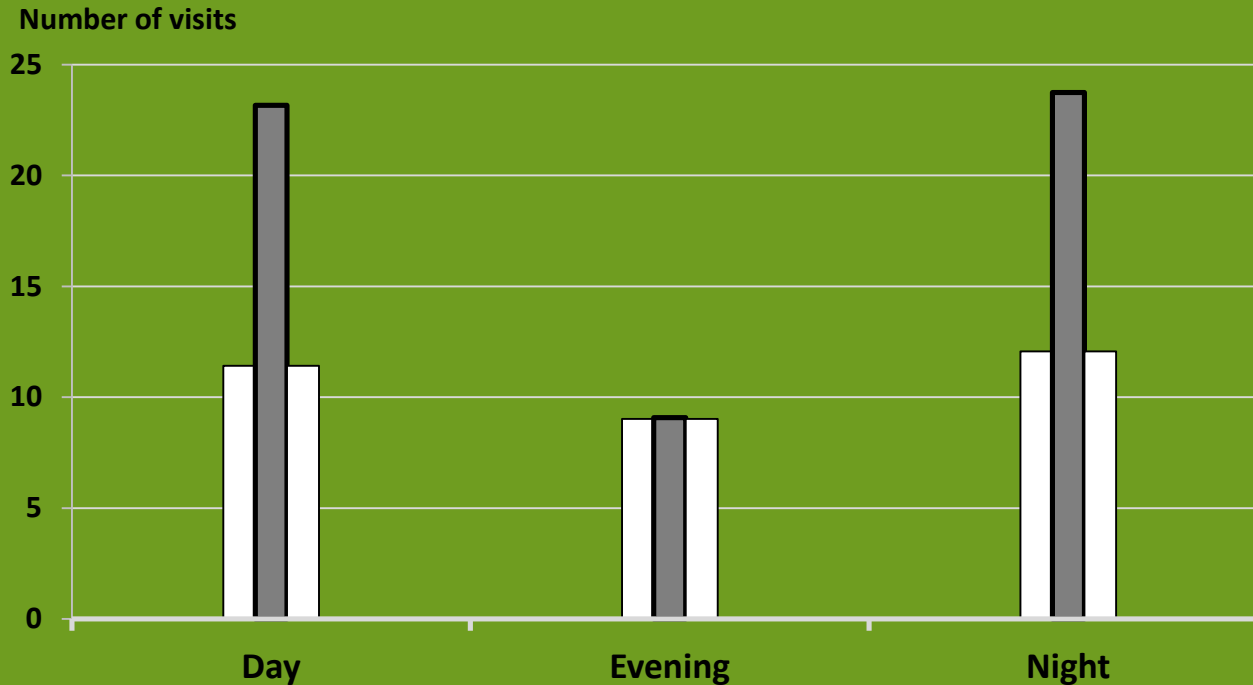
2) Discarding at random 1/2 data from day or night visits

=> New possibly biased CH₄ measures = average of 33 spot-measures

influenced by different visit pattern, with different day/night balance

Simulation of populations with different Visit Patterns

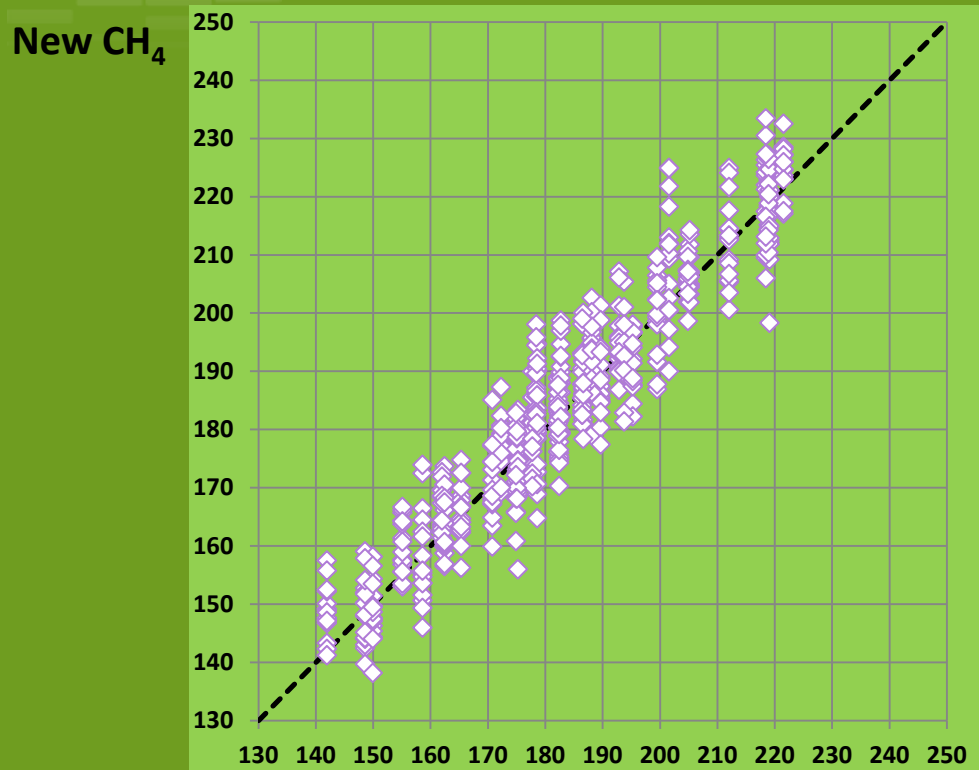
⇒ Discarding at random ½ data from day and night visits ⇒ 33 visits/heifer



Simulation of populations with different Visit Patterns

⇒ Discarding at random 1/2 data from day and night visits (8 times for each heifer)

⇒ 560 heifers
with 33 visits/heifer



Correlation = 0,95

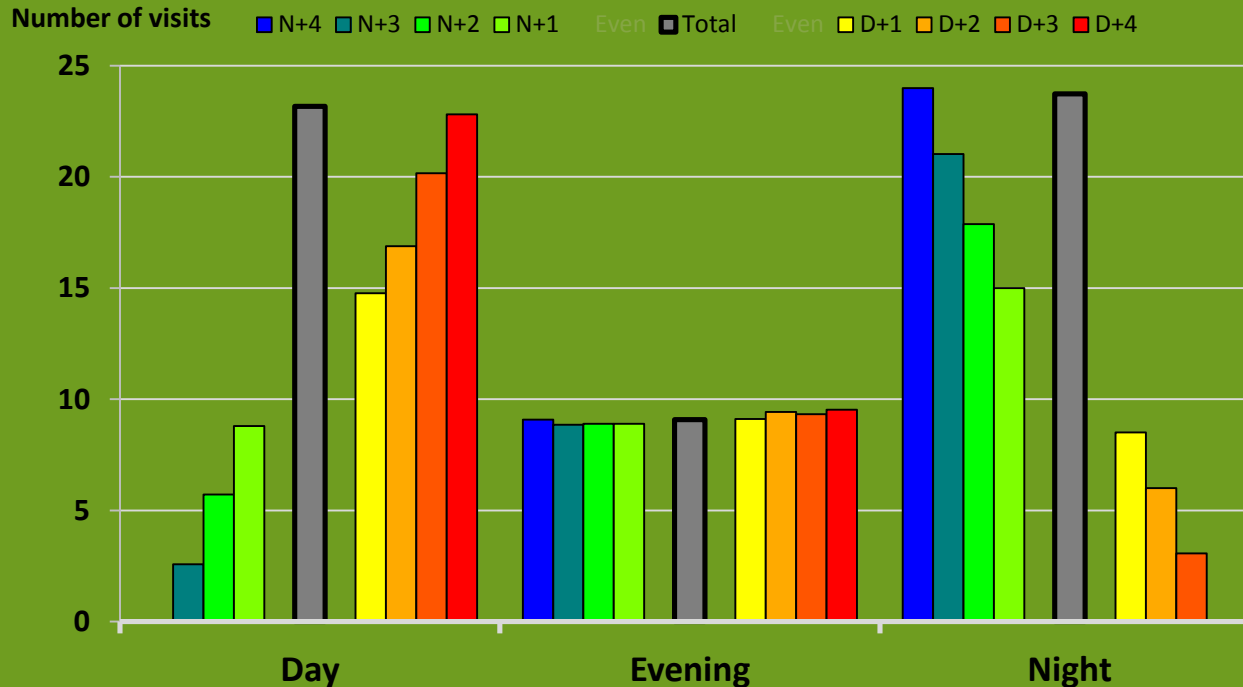
difference (New-Initial)
s.d. = 6.6

Initial CH₄

Simulation of populations with different Visit Patterns

⇒ Unbalanced discarding ½ data from day and night visits (*15 times for each heifer*)

⇒ 1,050 heifers with 8 different Visit Patterns and 33 visits/heifer



Simulation of populations with different Visit Patterns

⇒ Unbalanced discarding ½ data from day and night visits (*15 times for each heifer*)

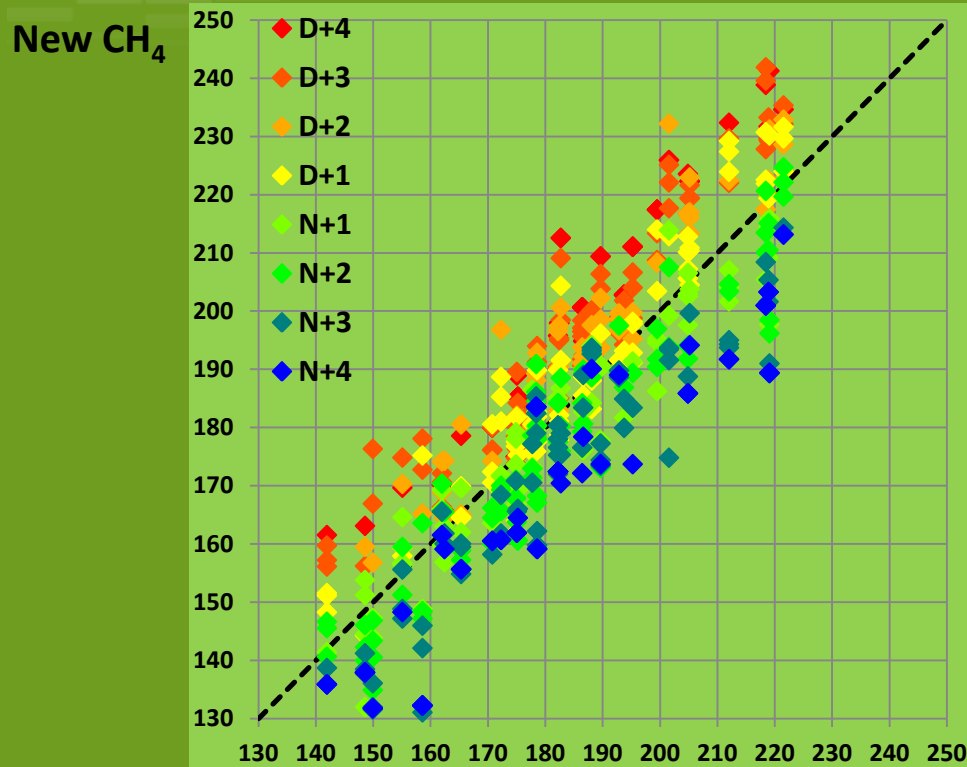
15 times for each heifer ⇒ 1,050 heifers with 8 different Visit Patterns and 33 visits/heifer

N+4	N+3	N+2	N+1	D+1	D+2	D+3	D+4
-0.72	-0.56	-0.37	-0.19	0.17	0.34	0.53	0.72

$$\text{Balance Day/Night} = (N_{\text{Day}} - N_{\text{Night}}) / N_{\text{Tot}}$$

Unbalanced discarding 1/2 data from day and night visits

⇒ 1,050 heifers with 8 different Visit Patterns



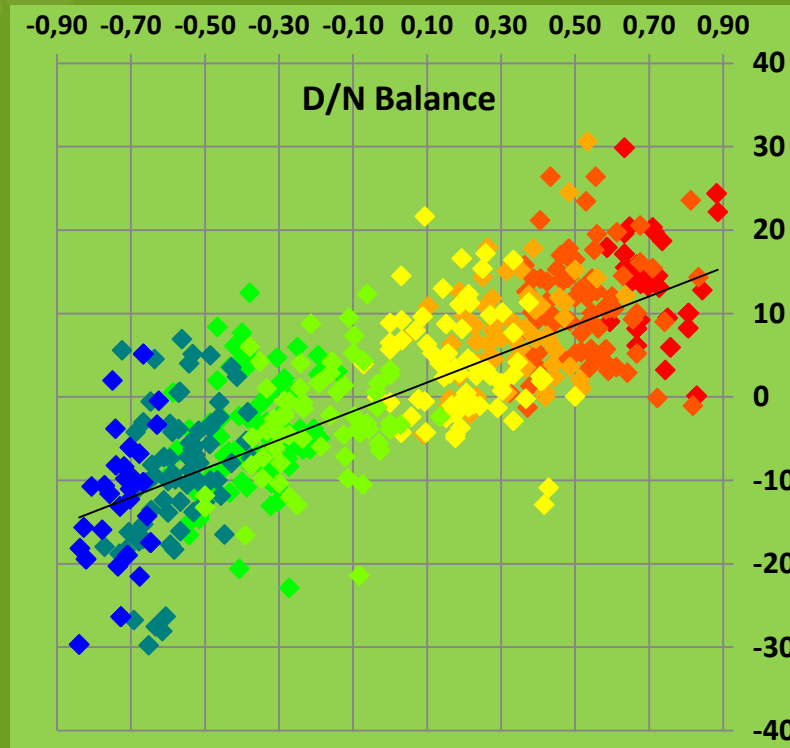
Correlation = 0,88

difference (New-Initial)
s.d. = 11.3

Initial CH₄

Incidence of discarding process on the measure of CH₄

Incidence of the Day/Night balance on the bias in measuring CH₄



Correlation = 0,78

**Bias (New-Initial)
s.d. = 11.3**

Simulation of populations with different Visit Patterns

⇒ 1,050 heifers with 8 different Visit Patterns

⇒ Incidence of Day/Night balance on daily CH₄ emission estimates

	N+4	N+3	N+2	N+1	D+1	D+2	D+3	D+4
Balance	-0.72	-0.56	-0.37	-0.19	0.17	0.34	0.53	0.72
Bias g/d	-12.0	-9.2	-4.9	-2.4	4.3	7.4	10.9	14.0



First conclusion: in a situation where

- ✓ **there is one main meal in the morning**
- ✓ **some animals have very different visit patterns**
 - **day visitors are over-estimated**
 - **night visitors are under-estimated**

there is a need to adjust for hour effects for avoiding possible bias



Adjusting CH₄ measures for hour effects

in a linear model including simultaneously the animal and hour effects

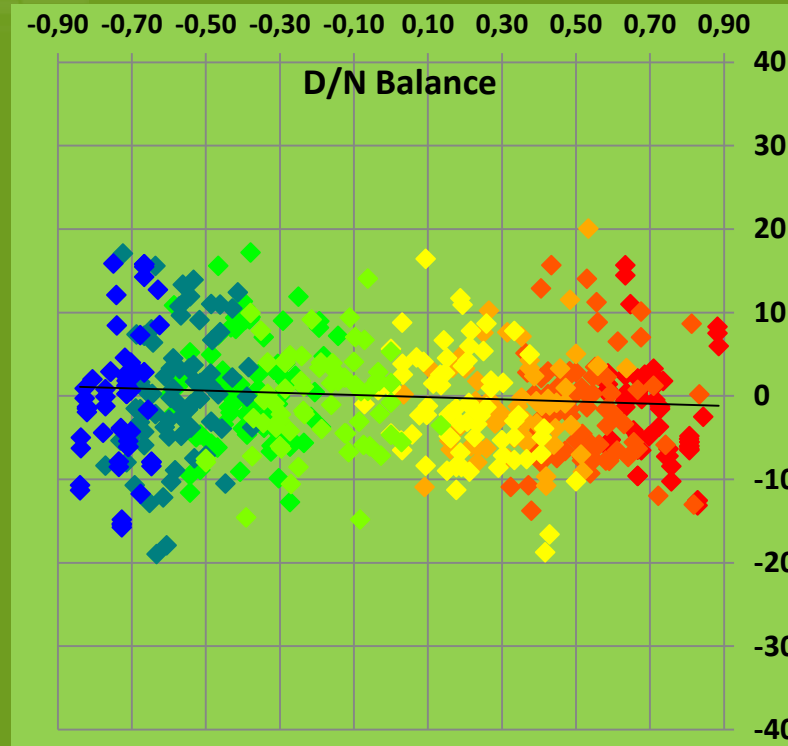
$$\text{CH}_4 = \mu + \text{Pen} + \text{Day} + \text{Animal} + \text{Hour} + \text{residual}$$

where CH₄ are the flux measures at each visit

=> the animal emission rates are the animal LSMeans estimates

Adjusting CH₄ measures for hour effects in a GLM model

=> Lack of relationship between Day/Night balance and hour adjusted CH₄ measure

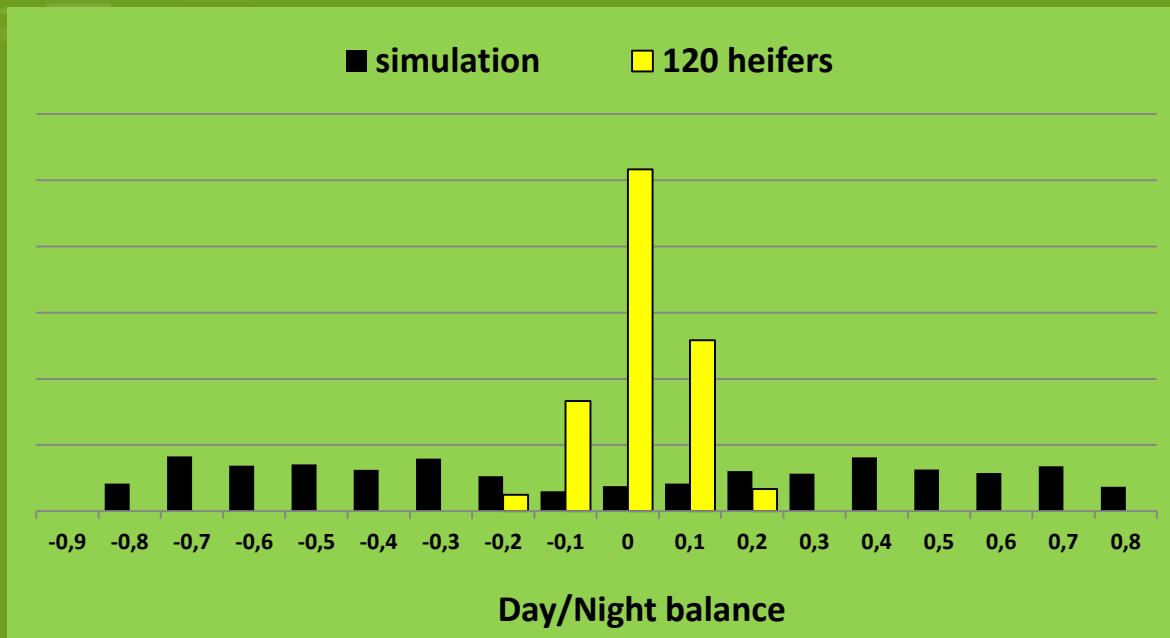


Correlation = -0,04

difference (LSMeans-Initial)
s.d. = 6.7

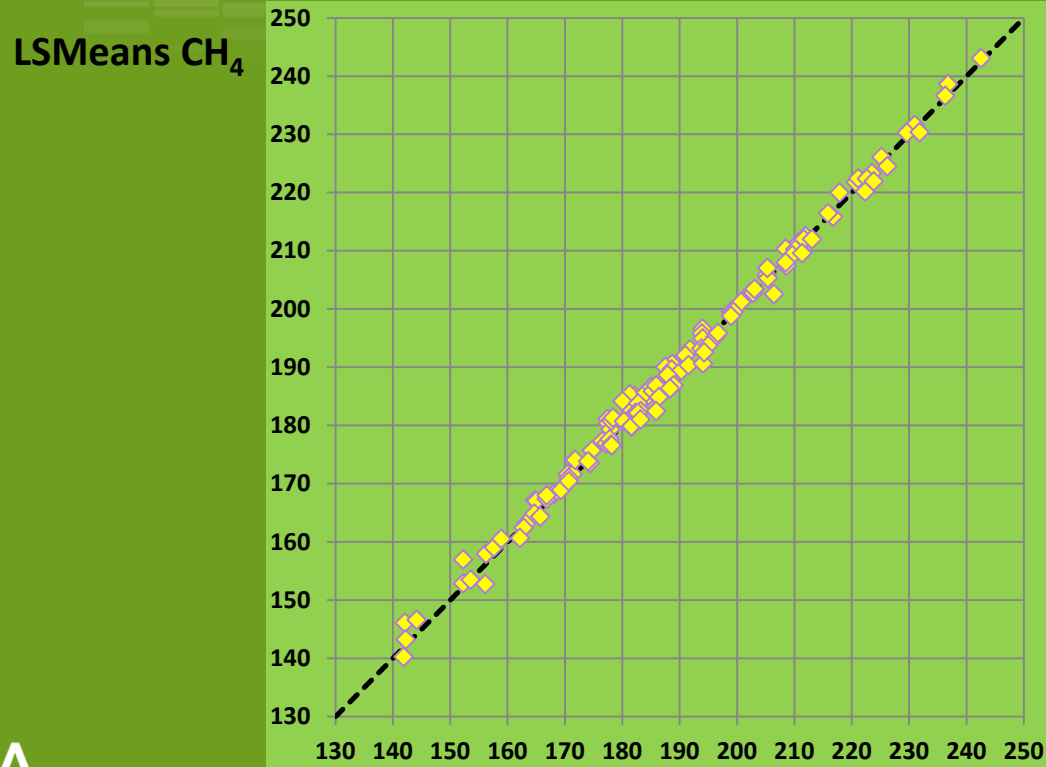
120 heifers with 8 weeks recording (200 visits/heifer in average)

Observed Day/Night balance



120 heifers with 8 weeks recording (200 visits/heifer in average)

Adjusting for hour effects



Correlation = 0,998

difference (LSM-Mean)
s.d. = 1.6

Mean CH₄

2) Conclusion on accuracy

➤ In a situation where

- ✓ there is one main meal in the morning
- ✓ some animals have very different visit patterns

adjusting for hour effects with LSMeans in a GLM will suppress the bias.

➤ In a situation where

- ✓ there are several meals
- ✓ there are limited differences in the visiting patterns
- ✓ there are enough visit measures

averaging all visit measures provides accurate enough estimates

Conclusions

- 1) **GreenFeed system provides precise measure of CH₄ emission rates as long as 50 spot-measures are averaged.**
- 2) **The possible bias generated by marked differences in visiting pattern will be suppressed when taking into account the hour of visit when averaging spot-measures of each animal.**

Final Conclusions

In my experiment I'll be confident in the relationship I'll estimate between
Feed Efficiency and **Methane Emission Rate**



of the beef heifers.