

**Progress with genetic selection for low methane traits in dairy cows**

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**ABSTRACT:** Over the last five years a number of methods have been developed and adapted to measure and quantify methane emission from individual animals. Their approaches show similarities and yet also differences. In general, they focus on being noninvasive measuring in the cow's everyday environment. In order to make genetic selection for methane emission it will be necessary to make large scale measurements in commercial farms. International collaboration, comparison and validation are essential to make progress in this area. It will be necessary to have strategic collection and exchange of data. More work is needed on how to get small-scale phenotypes in to large-scale genomic selection.

**Keywords:** dairy cattle; methane; genetics; sustainability

### Introduction

Climate change is a growing international concern and it is well established that release of greenhouse gases (GHG) is a contributing factor (Gerber et al. (2010)). Of the various GHG produced by ruminants, enteric methane (CH<sub>4</sub>) is the most important contributor, with a global warming potential 25 times that of carbon dioxide (CO<sub>2</sub>). Today CH<sub>4</sub> emission as such does not have a direct economic value for the farmer but this may change if a tax is put on CH<sub>4</sub> emission or through the relation to feed efficiency since approximately 6% of gross energy in feed the cow eats is eructed as CH<sub>4</sub> (Johnson et al., (1993)). Therefore, a reduction in CH<sub>4</sub> emission will not just be beneficial for the environment; it will also mean more efficient animals for the farmer.

Our ability to reduce the carbon foot print of animal production through genetic strategies depends on the size of available genetic variation as well as accurate techniques needed for the measurement of phenotypes on a large scale. This means that starting selection for a new trait requires phenotypes measured in commercial farms. In order to get access to dairy cattle data from commercial farms it is important to develop methods that are non-invasive, require no training, and doesn't interfere with the normal dairy routines of commercial farms. The equipment should also be portable, low cost and preferably with limited maintenance and a high capacity.

With the recent successful incorporation of genomic information into breeding schemes the reliance on very large populations of phenotyped animals is somewhat relaxed. Nonetheless, a reference population of several thousand animals is still required to estimate the contribution of each genomic region to expression of the phenotype under investigation (Goddard and Hayes, (2009)). Therefore, a dataset of several thousand animals with similarly defined records for CH<sub>4</sub> emissions would be

sufficient for genetic evaluation of enteric CH<sub>4</sub> emissions. In this study we describe a number of methods to measure CH<sub>4</sub> from ruminants and the results in relation to potential to select for reduced CH<sub>4</sub> emission from dairy cattle.

### Materials and Methods

A number of approaches to measure CH<sub>4</sub> from the breath samples of the cow have been developed.

**Denmark.** In Denmark a method that measures gases during milking in milking robots has been used (Lassen et al., (2012)). A total of 2104 cows have been phenotyped in commercial farms and the focus is on using the ratio between CH<sub>4</sub> and carbon dioxide (CO<sub>2</sub>) concentrations in the breath as the phenotype to correct for the placement of the cow's head in relation to the measuring device. Another method, also measuring during robotic milking sessions is used at the Danish Cattle Research Centre. The method relies on infra-red detection with two commercially available instruments working in series (Guardian NG / Gascard; Edinburgh Instruments, UK) with data recording and logging every second. This equipment is the same as that used by Garnsworthy et al. (2012a). Readings are synchronized with robotic milking data for animal ID and links to other data. The research herd includes 120 Holsteins plus 60 Jerseys, all recorded for feed intake, production and live weight.

**The United Kingdom.** In the UK a method has been developed to measure CH<sub>4</sub> during milking in milking robots, which has been validated against measurements of CH<sub>4</sub> in respiration chambers (Garnsworthy et al. (2012a)). A total of 2659 cows have been phenotyped on commercial farms and the focus is on using CH<sub>4</sub> released by eructation as the phenotype to improve precision. A group of 215 cows was monitored continuously for 5 months to investigate within-cow variation (Garnsworthy et al. (2012b)).

In another UK study, the use of a proprietary laser CH<sub>4</sub> detector (LMD) in livestock is being investigated. This was first suggested by Chagunda et al. (2009) and its application has since been explored (Chagunda and Yan (2011); Ricci et al. (2012)). The LMD is a hand-held gas detector for remote measurement of column density for methane-containing gases. Methane measurements by the LMD are based on infrared absorption spectroscopy, using a semiconductor laser as a collimated excitation source and employing the second harmonic detection of wavelength modulation spectroscopy to establish a CH<sub>4</sub> concentration measurement (Iseki and Miyaji (2003)). Concentration of CH<sub>4</sub> is expressed in parts per million-metre (ppm-m), as the

amount itself relates to both the concentration and the depth of CH<sub>4</sub> respiratory plume.

**Finland.** In Finland, the CH<sub>4</sub> output of first-lactation cows from the MTT Agrifood Research experimental dairy farm has been continuously monitored using a F10 multi-gas analyzer (GASERA Ltd. Turku, Finland) that is based on Photoacoustic Infrared Spectroscopy technique (Negussie et al. (2013)). This method is used for the measurement of individual cow CH<sub>4</sub>, CO<sub>2</sub> and acetone outputs from the breath sample of cows via sampling tubes of the analyzer that are fitted to separate individual feeding kiosks. Simultaneously, along with the measurements of the gases, daily feed intake, production and functional traits are also recorded. From the measured gasses, for each cow CH<sub>4</sub>:CO<sub>2</sub> was calculated and based on ME intake, daily CH<sub>4</sub> outputs are estimated as shown in (Madsen et al. (2010)). Currently the database contains entire first-lactation records of more than 90 cows with both daily feed intake, production as well as daily CH<sub>4</sub> outputs (Table 1).

**The Netherlands.** The same equipment and measurement strategy as in Denmark has been used in the Netherlands. Breath air was sampled and analyzed directly for CH<sub>4</sub> and CO<sub>2</sub> using a portable Fourier Transformed Infrared (FTIR) gas analyzer with 2 air inlets (GASMET 4030; Gasmot Technologies Oy, Helsinki, Finland). One instrument air inlet was placed in front of the cow's head in the milking robot, and one air inlet was placed at 2.5 meters height near the milking robot to measure background concentrations. Measurements were alternatively taken from one of the 2 air inlets; each measurement involved continuous sampling for 1 minute. Cows visited the milking robot between 1 and 4 times per day, and between 3 to 8 measurements of 1 minute were recorded per visit. To date, 400 genotyped cows have been phenotyped. Enteric CH<sub>4</sub> emissions measured from the breath during milking have been recorded for at least 6 weeks, and 100 of them have been continuously recorded for a full year.

## Results and Discussion

Overall means and standard deviations for the different types of measuring units and approaches are presented in Table 1.

In the Danish data a repeatability of 0.35 between measurements of the ratio between CH<sub>4</sub> and CO<sub>2</sub> has been found. Also a heritability of 0.21±0.06 of the same phenotype has been reported using data from milking robots (Lassen and Løvendahl (2013)). A heritability of that magnitude opens up for selection based on direct measurements

In the UK data the repeatability of CH<sub>4</sub> measurements online during milking ranged from 0.55 in cows measured at an interval of 5 months to 0.89 in cows measured at an interval of 1 week (Garnsworthy et al. (2012b)). Methane emission rate recorded during milking on farm showed a linear relationship ( $R^2 = 0.79$ ) with daily CH<sub>4</sub> output by the same cows when housed subsequently in respiration chambers (Garnsworthy et al. (2012a)).

**Table 1. Country, number of records, phenotype used, phenotypic means and standard deviations, repeatability and heritability in different European studies of methane (CH<sub>4</sub>) emission from dairy cattle**

CO	#	PH	MEAN	SD	t	h <sup>2</sup>
DK	2104	CH <sub>4</sub> /CO <sub>2</sub>	0.089	0.03	0.35	0.21
FIN	90	CH <sub>4</sub> g/day	330	58	0.27-	0.65
NL	400	CH <sub>4</sub> ppm	386	99		
UK	2659	CH <sub>4</sub> g/d	418	349	0.55-	0.89
UK	180	CH <sub>4</sub> ppm	396	183	0.48	

CO = country  
 # Number of animals with registrations  
 PH = phenotype registered  
 MEAN =phenotypic mean  
 SD = Phenotypic standard deviation  
 t = Repeatability  
 h<sup>2</sup> = Heritability  
 DK = Denmark  
 FIN = Finland  
 UK = United Kingdom  
 NL = The Netherlands

Using the LMD To date 180 cows from the long-running genetic x management systems study based at Scotland's Rural College (SRUC) Dairy Research Centre, Crichton Royal Farm, UK have been phenotyped. After adjusting for plume effect, in dairy cows the mean breath CH<sub>4</sub> has been found to be 395.8 sd 182.7 ppm while that for sheep was 68.9 sd 32.6 ppm (Chagunda et al. (2013)). In dairy cows, repeatability of the measurements 0.48 has been found (Chatepa (2012)).

In the Finnish data, CH<sub>4</sub> outputs of first-lactation cows were monitored for the entire lactation. Across lactation analysis of this data showed that the repeatability of CH<sub>4</sub> output (gram/day) ranged from 0.27 to 0.65 for different stages of lactation (Negussie et al. (2014)). The result indicates that there is potential between-animal variation for the trait which could possibly be used for developing genetic tools to lower CH<sub>4</sub> output from ruminant production systems.

In the Dutch data, mean CH<sub>4</sub> concentration was 386 ppm, with a range of individual cow means between 196 and 724 ppm, and a standard deviation of 99 ppm. Mean CO<sub>2</sub> concentration was 6380 ppm, with a range between 4280 and 14,500 ppm, and a standard deviation of 1900 ppm. Daily means of the background concentrations were 35 ppm for CH<sub>4</sub> and 591 ppm for CO<sub>2</sub>. The high concentrations measured in the milking robot indicate that the sampled air included a high portion of exhaled air of the milked cow.

Direct measurements might not be the only opportunity to select for lower CH<sub>4</sub> emissions from dairy cattle. Studies have shown promising results in using milk spectral data to predict CH<sub>4</sub> emission (Dehareng et al. (2012)) or feed intake data (de Haas et al. (2011)). The dairy cattle sector is very experienced in handling milk analysis and the farmers are used to having milk samples recorded on farm so if the use of milk spectral data can be

used it would be a much more efficient and powerful way to select for reduced CH<sub>4</sub> emission from dairy cattle. In order to justify selection for less emitting dairy cattle an economic value for the trait has to be estimated. This could come from a tax on CH<sub>4</sub> emission or from establishing the genetic relation to feed intake and/or feed efficiency.

In all data that is presented in this study cows were feed ad libitum, and no restrictions were made that could affect the repeatability. Had restrictions been made daily variation could be relatively controlled and that would affect repeatability. Feeding ad libitum is more and more the feeding regime in dairy cattle production in Europe.

A number of international initiatives have been established to help the process of validation, exchange of data and experiences and to make consensus on which phenotypes should be reported for CH<sub>4</sub> production from dairy cattle. This is mainly important to be able to combine CH<sub>4</sub> measurements performed with different equipment and protocols. Even though a number of countries have started to record individual CH<sub>4</sub> data, none of them has enough records available to get accurate breeding values for this trait to be used in their national breeding program. The only way to use selective breeding as a mitigation strategy that is permanent and cumulative, is to create an international database with individual measurements. Therefore, a work group has been initiated under ICAR, and the ASGGN network has also been running and worked on a white paper for CH<sub>4</sub> phenotypes, and in December 2013 the EU-COST-Action METHAGENE was established to work on aspects of selection for reduced CH<sub>4</sub> emission from dairy cattle.

### Conclusion

A number of approaches are currently being tested and evaluated to measure CH<sub>4</sub> from dairy cattle. In general they show promising results concerning repeatability between measurements. An upcoming task is to compare these methods with each other in order to make international collaboration and to be able to merge data from different devices and environments.

### Literature Cited

- Chagunda, M. G. G., D. Ross, and D. J. Roberts. (2009) *Comp. Electr. Agric.* 68:157-160.
- Chagunda, M. G. G. & Yan, T. (2011) *Anim. Feed Sci. Techn.* 165, 8-14.
- Chagunda, M.G.G., Ross, D., Rooke, J., et al. (2013) *Acta Agr. Scand., Section A – Anim. Sci.* 63 (2): 68-75.
- Chatepa, L.E.C. (2012) *Mphil Thesis, University of Glasgow.*
- Dehareng F., Delfosse, C., Frodimont, E. et al. (2012) *Anim.* 10:1694-1701.
- De Haas Y, Windig JJ, Calus MPL, et al. (2011) *J Dairy Sci* 94:6122-34.
- Garnsworthy, P. C., J. Craigon, J. H. Hernandez-Medrano et al. 2012 a. *J. Dairy Sci.* 95:3166–3180.
- Garnsworthy, P. C., J. Craigon, J. H. Hernandez-Medrano et al. 2012 b. *J. Dairy Sci.* 95:3181–3189.
- Gerber, P., Key, N., Portet, F. et al (2010) *Anim.* 4:393-406.
- Goddard M. E. and B. Hayes 2009 *Nat Rev Gen* 10:381-391.
- Iseki, T. & Miyaji, M. (2003). *Proceedings, 22nd World Gas Congress, June 1-5, Tokyo, Japan.*
- Johnson DE, TM Hill, GM Ward, et al (1993) *Global Env. Change*, 13:219-229 .
- Lassen, J., Løvendahl, P., J. Madsen (2012) *J. Dairy Sci.* 95:890-898.
- Lassen, J. and Løvendahl, P. 2013. *Adv in Anim Biosci* 4:280.
- Negussie, E., Mäntysaari, P., Mäntysaari, E, et al. 2013. *Adv in Anim Biosci* 4:463.
- Negussie, E., Mäntysaari, P., Mäntysaari, E, et al. 2014. *Proceedings of the 10th WCGALP, Canada.*
- Ricci, P., Chagunda, M. G. G., Duthie, et al. (2012). *Proceedings of the 2nd Joint Conference of the New Zealand and Australian Societies of Animal Production, July 2-5, Lincoln University, Lincoln.*