Final Report of METHAGENE Working Group 1

September 2015

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1) Summary of tasks and objectives of WG1

The Scientific program defined for Working Group 1 (Methane-determining factors) is stipulated to compile:

a) an inventory and discuss weighting of methane-determining factors,
b) standardized definitions for CH_4 measurements, and
c) combined and integrated data into novel genetic models.

The following further tasks have been identified for WG1:

- To establish a network of nutritionists, physiologists and geneticists, animal breeders, and microbiologists working in the field of methane production from ruminants
- To establish a beneficial interaction between researchers working on environmental and genetic factors determining methane production
- To provide the most specific and sensitive means of assessing methane production of animal origin and to recommend the use of standardized protocols and units
- To exchange experience and knowledge, protocols, experimental design and data analysis with other WGs especially with Early Stage Researchers in an international forum
- To communicate what environmental factors should be considered in calibrating techniques, validating methane indicators and integrating into genetic selection strategies
- To collaborate with the other WGs of this COST Action
- To exploit data already available to be integrated into novel genetic models

2) Overview of collected CH_4-determining factors

For this overview more than 30 published studies were provided by the involved countries. The majority of the studies used a meta-analysis approach. The trials were setup to study effects of diet, rumen microbiota, host genetics, physiological stage, environment or a combination on methane production. Most studies examined diet effects (13), one was on rumen microbiota, another one on the effects between the animal and the microbiota, seven on host genetics, one on the interaction between host genetics and physiological stage, four on the physiological stage, and two on environment. Almost all trials concerned cattle (20), but there were also 1 goat, 2 sheep and 1 cattle plus small ruminants trials included. In the studies, methane data are given in various units where 1 L = 0.716 g = 55 kJ.

2a) Diet and rumen microbiota

There are different CH_4 determining factors in diet trials. Dry matter intake (DMI) is the most determining factor and is easy to be analyzed. A regression line can sometimes be fitted more precisely when different feed components are analyzed to the level of the main nutrients. The study of Ellis et al. (J. Dairy Sci. 2007;90:3456) used 83 beef and 89 dairy data sets. In their CH_4 emissions prediction models they considered DMI, metabolizable energy (ME) intake, neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), lignin (ADL), and forage proportion. For the beef database, the equation CH_4 (MJ/d) = 2.94 + 0.059 × ME (MJ/d) + 1.44 × ADF (kg/d) - 4.16 × ADL (kg/d) resulted in the lowest root mean square prediction error (RMSPE). For the dairy database, the equation CH_4 (MJ/d) = 8.56 + 0.14 × forage (%) resulted in the lowest RMSPE value. An equation based on DMI also performed well for the dairy database: CH_4 (MJ/d) = 3.23 + 0.81 × DMI. When the dairy and beef databases were combined, the equation CH_4 (MJ/d) = 3.27 + 0.74 × DMI resulted in the lowest RMSPE

Jentsch et al. (Archives of Animal Nutrition, 2007;61:10) used data from cattle of both sexes, fed 337 rations. They made a regression to predict total CH_4 emission on the basis of DMI: $CH_4(kJ)=8427.5+164.18\times DMI(kg)$ and on the basis of nutrient composition: $CH_4(kJ)=1.3\times dCP(g)-0.31\times dCF(g)+1.31\times dstarch(g)+1.1\times dsugar(g)+2.4\times dNFR(g)+1835$, where dCP=digestible crude protein and dCF= digestible crude fat. They stated that a major component of the measured CH_4 emission cannot be explained by DMI but is rather due to differences in dietary nutrient composition. The amount of digestible nutrients consumed especially of the carbohydrate fraction (starch, sugar, N-free residuals) is reliable to estimate CH_4 release with high precision. Furthermore, diets rich in fat reduced CH_4 formation in the rumen. These regression equations are applicable to various types of production systems.

Kirchgessner et al. (Agribiol.Res. 1991;44:2) evaluated 153 two-day gaseous emissions of lactating cows to quantify the release of methane from dairy cattle. All animals received nutrients according to their requirements. In this experiment DMI was also the most important determining factor, but there were different regression lines for maize silage and dried grass as the main roughage component: $CH_4(g)=93+16.8\times DMI(kg)$ and $CH_4(g)=81+14.0\times DMI(kg)$, respectively. Methane release was particularly dependent on the intake of crude fiber (CF) and ether extract (EE): $CH_4(g)=63+80xCF (kg)+11xNFE (kg)+19xCP(kg)-195xEE (kg)$.

Estermann et al. (J. Anim. Sci. 2002;80: 4:1124) found that methane linearly increased with NDF intake ($CH_4(L)=59.4\times NDF[kg]+ 64.6$) for cows together with their calves independent of the breed.

Hindrichsen et al. (Environment Monitor Assessm, 2005;107:329) investigated dietary carbohydrate effects on methane emission from cows and their slurry. Twelve dairy cows (6 per diet) consumed a diet with a forage-to-concentrate ratio of 1:1 (DM basis), designed to cover the cows' requirements. The enteric CH_4 could be predicted with the equation: $CH_4(g/d)=84+47\times cellulose(kg/d)+32\times starch(kg/d)+62\times sugars (kg/d)$.

Total CH_4 emission could be explained by $CH_4(g/d)=123+84\times cellulose(kg/d)-30\times hemicelluloses(kg/d)+58\times starch(kg/d)+73\times sugars(kg/d)-95\times ADL(kg/d)$.

Other factors that influence the methane production or methane conversion rate (Ym) are the concentrate:forage ratio and the passage rate. The higher the percentage concentrate the lower Ym (Zeitz et al., 2012, J. Integr. Environ. Sci. 9:199).When giving different proportions of highly digestible grass and concentrate, the positive effect on the emissions is less clear (Patel et al., Acta Agric. Scand., Section A, 2011;61:128). A 29% decrease in methane production (L/d) of steers was related to a 63% increase in fractional passage rate of fiber from the rumen and a 43%

increase in ruminal fluid dilution rate (Okine et al., J. Anim. Sci. 1989;67:3388). This is consistent with reports of a 30% decline in methane production with 54 and 68% increases in ruminal passage rate constants of fluid and particulate matter in cold-adapted sheep at a constant intake (Kennedy and Milligan, 1978).

Additives can sometimes have a methane reducing effect. Best researched are fats/oils and tannins. In both groups of compounds there are specific differences with some representatives being particularly effective, but both groups also seem to have an underlying dose-response effect where higher dosages are mitigating methane more (Beauchemin et al., 2008, Aust. J. Exp. Agric. 48, 21-27, for lipids; Jayanegara et al., 2012, J. Anim. Physiol. Anim. Nutr. 96, 365-375, for tannins; Zmora et al., 2012 Acta Agr. Scand., Section A–Animal Science, 2012, 62.1: 46-52, for raw material as a source of plant bioactive components). Research with dairy cattle showed that the addition of condensed tannins (2 g/kg dietary DM) caused mitigation of methanogenesis mainly resulting from a reduction in protozoal numbers without a negative effect on the digestibility of organic matter and VFA production (Cieslak et al., 2012, Anim. Feed Sci. Tech. 176, 102–106). Some studies also suggested that the basic components of plants or plant extracts fed to animals as natural feed additives could interact with plant bioactive components (phytochemicals) or that the phytochemicals became physically less available for microbiota, resulting in a decreased antimethanogenic activity of the raw material *v.* the extract (Cieslak et al., 2014, J. Agric. Sci. 152, 981–993). Moreover, it was generally concluded that saponins mitigate methanogenesis mainly by reducing the number of protozoa whereas condensed tannins act both by reducing the number of protozoa and by a direct toxic effect on methanogens (Cieslak et al., 2013, Animal 7.s2: 253-265).

Khiaosa-ard and Zebeli (J. Anim Sci. 2014;91:1819) did a meta-analysis on the effects of essential oils and their bioactive compounds (EOBC). Despite diverse types of EOBC, doses used so far showed the potential to mitigate methane and increase the acetate:propionate ratio. These changes may favour beef production rather than dairy production. Nevertheless, high doses of EOBC might not necessarily facilitate rumen fermentation or promote animal performance and feed efficiency. Diet composition may be a determining factor in the use of EOBC in ruminants.

Nitrate addition to maize silage-based dairy cow diets for 89 d persistently decreased in 20 lactating HF cows the enteric methane emissions by 16% without negatively affecting diet digestibility and milk production. The energetic benefit from the decreased methane production did not appear to benefit the animal, as milk production and energy balance were not affected (van Zijderveld et al., 2011; J. Dairy Sci. 94:4028).

Adding salts of nitrate or sulphate to the diet of sheep reduced enteric methane production. Moreover, the effects of both products on methane production were additive. Provided that these substances can be fed in a safe way, they are powerful agents to reduce methane production from sheep (van Zijderveld et al., 2010, J. Dairy Sci. 3:5856).

Abecia et al. (J. Dairy Sci. 2012;95:2027) fed complexed bromochloromethane to lactating dairy goats. The goats showed a reduction in methane emissions that was associated with an increase in milk yield, probably due to favourable propionic rumen fermentation, but fat, protein, casein, and lactose concentrations in milk were unaffected by treatment. Bromochloromethane did not affect either the abundance of rumen bacteria and protozoa or that of total methanogenic archaea. Moreover, in this study, a minor change in milk fatty acid profile was observed, which suggests that the important decrease in methane production was not related to alterations in ruminal biohydrogenation pathways.

Schönhusen el al. (Arch. Anim. Nutr. 2003;57:279) demonstrated that the methanogenesis in the rumen of calves is associated with the development of the ruminal protozoa population.

The absence of protozoa in the rumen reduced CH_4 production and the digestibility of carbohydrates. Thereby, the concentration of metabolizable energy in the diet did not rise in comparison to the presence of protozoa. In the absence of protozoa the hydrogen recovery in the rumen is not balanced, thus, the utilization of other routes of metabolic hydrogen utilization or production of free hydrogen should be considered.

In grass-based dairy cow production, controlling the herbage mass of the grass grazed by the cows is an important management tool, as herbage mass is related to grass quality. High herbage mass grass generally has lower quality than low herbage mass grass. The effect of grass herbage mass on the enteric CH_4 emissions of dairy cows in mid lactation (summer) was measured by Wims et al. (J. Dairy Sci. 2010;93:4976). Enteric CH_4 emissions were measured using the SF_6 technique, which was conducted for 5 days, on two occasions. Cows grazing low HM swards produced less CH_4 per cow, per kg milk, per kg milk solids and per kg grass DMI, through intake of higher quality grass. Cows grazing the low HM swards lost a lower proportion of their gross energy intake as CH_4, demonstrating the benefit of grazing low HM swards to improve the GHG efficiency of milk production from pasture. This study found that implementing good grazing management reduced gross energy intake loss as CH_4 by 14%.

Enriquez Hidalgo et al (J. Dairy Sci. 2014;97:1400) carried out an experiment to investigate the effect of white clover inclusion in grass swards (GWc) compared with grass-only (GO) swards on herbage and dairy cow productivity, and enteric CH_4 emissions. In September (late lactation) individual cow CH_4 emissions were estimated using the SF_6 technique (5 consecutive days). Annual clover proportion in the GWc swards was 20%. Similar sward and animal performance was observed during the CH_4 estimation period, but GWc swards had 7.4% less NDF than GO swards. Cows had similar daily and per-unit-of-output CH_4 emissions but cows grazing GWc swards had 11.9% lower CH_4 emissions per unit of feed intake than cows grazing GO swards due to the numerically lower CH_4 per cow per day and a tendency for the GWc cows to have greater DMI than the GO cows. Although GWc cows emitted less CH_4 per unit of feed intake than GO cows, GWc cows had a tendency to consume more, and so no difference was observed in daily or per-unit-of-output CH_4 emissions.

O'Neill et al. (J. Dairy Sci. 2011;94:1941) compared the enteric CH_4 emissions and milk production of spring-calving Holstein-Friesian cows offered either a grazed perennial ryegrass diet or a total mixed ration (TMR) diet for 10 wk in early lactation (spring). The TMR was composed of 36% maize silage, 41% concentrate, 17% grass silage, 3.5% molasses and 2.5% straw (DM basis). Enteric CH_4 emissions were measured using the SF_6 technique for two 5-day periods. The grass diet produced less enteric CH_4 per cow, per unit of DMI, and per unit of fat+protein yield than the TMR diet did. The TMR diet used gave rise to higher milk yield and fat+protein yield due to higher DMI, but this increase was not large enough to offset the substantially greater quantity of enteric CH_4 produced.

O'Neill et al. (J. Dairy Sci. 2012;95:6582) compared the enteric CH_4 emissions and milk production of spring-calving Holstein-Friesian dairy cows during mid to late lactation offered one of the following diets for 8 wk: (1) low grass allowance (LGA) (13.9 kg grass DM/cow/d) + partial mixed ration (PMR) (4.1 kg PMR DM/cow/d), (2) high grass allowance (HGA) (19.3 kg DM/cow/d), or (3) LGA (14.4 kg grass DM/cow/d). The PMR offered was composed of 450 g maize silage/kg DM, 450 g concentrate blend/kg DM, and 100 g barley straw/kg DM. Daily enteric CH_4 emissions were determined using the SF_6 technique, for 5 consecutive days, on two occasions. Enteric CH_4 emissions per cow were greater on the PMR-supplemented diet than on the grass-only diets. When PMR was offered, DMI and milk production per cow also increased. The partial supplementation of a grazing diet with PMR did not confer any reduction in enteric

CH_4 emissions per unit of DMI, milk yield, solids-corrected milk yield or fat+protein yield. The PMR treatment effects were attributed solely to the increased DMI, rather than to any particular nutritional characteristic of the PMR.

2b) Host genetics, physiology and environment

Garnsworthy et al. (J. Dairy Sci. 2011;95:3166) examined the animal variation, breed, feed intake, digestibility and rumen microbes as determining factors, during the evaluation of their own developed on-farm methane emissions technique (MERm). With this technique they quantify methane emissions from individual cows during milking. For 82 cows, methane emission rate during milking increased with daily milk yield ($r = 0.71$), but varied between individuals with the same milk yield and fed the same diet. For 42 cows, the methane emission rate during milking was greater on a feeding regimen designed to produce high methane emissions.

During on-farm measurements with 215 cows Garnsworthy et al. (J. Dairy Sci. 2011;95:3181) found that between-cow variation in MERm, was greater than within-cow variation, but ranking of cows for methane emissions is consistent across time. Variation was related to body weight, milk yield, parity, and week of lactation/days in milk. Estimation of daily methane emissions from MERm data, produced ranges from 278 to 456 g of CH_4/d and were commensurate with values predicted from ME requirements for observed body weight and milk yield. The monitored variation might offer opportunities for genetic selection.

Mills et al. (J. Anim Sci. 2001;79:1584) demonstrated that the mechanistic modelling approach is reliable for the prediction of methanogenesis in the lactating dairy cow. The ability of the model to simulate methanogenesis for a wide range of dietary inputs allows its application as a tool for determining dietary strategies to reduce environmental impact of dairy systems and to maximize feed energy utilization. This investigation has shown the potential for dietary intervention as a means of substantially reducing methane emissions without adverse effects on dietary energy supply.

Lassen et al. (J. Dairy Sci. 2012;95:890) recorded repeatedly individual methane and CO_2 production on 50 Holsteins and 43 Jerseys dairy cows during milking in an automatic milking system, with the aim of estimating individual cow differences in CH_4 production. The cows were from mixed parities and at all stages of lactation. The repeatability of the CH_4-to-CO_2 ratio was 0.39 for Holsteins and 0.34 for Jerseys. Both concentrate intake and total mixed ration intake were positively related to CH_4 production, whereas milk production level was not correlated with CH_4 production. The results suggest that the CH_4-to-CO_2 ratio measured using the non-invasive method is an asset of the individual cow and may be useful in both management and genetic evaluations.

Renand et al. (2013; Thouly, 64th EAAP Meeting, Nantes, France) saw time of day, day and animal variance in methane production by young beef bulls with the same pellet diet. Dehareng et al. (Animal 2012;6:1694) ascertained the effect of milk yield and diet on methane production.

Kandel et al. (J. Dairy Sci. 2013;95:388) found that estimated heritability for CH_4 g/day and CH_4 g/kg of FPCM were lower than common production traits but would still be useful in breeding programs. While selection for cows emitting lower amounts of MIR predicted CH_4 (g/d) would have little effect on milk production traits, selection on MIR predicted CH_4 (g/kg of FPCM) would decrease FPCM, fat and protein yields. These genetic parameters of CH_4 indicator traits might be an entry point for selection that accounts mitigation of CH_4 from dairy farming.

Kandel et al. (19th National Symposium on Applied Biological Sciences 2014, p12) estimated the genetic correlations between CH_4 intensity and milk production traits on Holstein cows from correlations of estimated breeding values. Genetic correlation between CH_4 intensity and milk yield (MY) was -0.67 and with milk protein yield (PY) was -0.46.

Vanrobays et al. (2013; 64th Annual meeting of the European Federation of Animal Science, p498) showed that milk production and CH_4 emissions of dairy cows seemed to be influenced by the temperature humidity index. Vanrobays et al. (2013; 64th Annual meeting of the European Federation of Animal Science, p344) found that the herd-test-day effects on milk production and on MIR CH_4 emissions varied through herds and seasons. However, Vanlierde et al. (J. Dairy Sci. 2015;98:5740) showed that the prediction of methane emission of dairy cows from milk MIR data is quite robust across breeds, diets and countries.

3) Overview of methane measurements in Europe

The most common equipment used in Europe to record individual methane emissions of ruminants are:

- Respiration chamber
- SF_6
- GreenFeed
- Laser
- Sniffer methods

The current 'gold standard' for measuring methane emissions is respiration chambers, but these are expensive and impractical for large-scale data collection. The sulphur hexafluoride (SF_6) technique can be used for field-scale data collection, but requires insertion of rumen boluses, daily animal handling and laboratory measurement of gases (McGinn et al., 2006; J. Environ Qual. 35:1686). The GreenFeed system (C-Lock Inc, Rapid City, USA; http://www.c-lockinc.com) also uses a tracer (propane) to calculate volumetric flux of air (L/min) to measure CH_4 and CO_2 during feeding in cattle visiting a "baiting" station. The laser methane detector system (LMD) is a non-invasive and non-contact technique which entails directing a laser beam at the methane point source, in this case a cow's nostrils, to perform highly sensitive infrared absorption measurements (Iseki and Miyaji, 2003; Chagunda et al., 2013; Animal 2:394). This remote way of enteric methane detection is useful because it enables measurements to be taken without disturbing the animals from exhibiting their normal behaviour (Chagunda et al., 2013; Animal 2:394). In recent years researchers, including those involved in METHAGENE, have developed many innovative non-invasive techniques, either by infrared (Garnsworthy et al., 2012; J Dairy Sci. 95:3166; Lassen et al., 2012; J. Dairy Sci. 95:890) or photo acoustic (Negussie et al., 2012) gas analysers, or by using a laser methane detector (Chagunda et al., 2013; Animal 2:394).

An inventory among the members of the METHAGENE consortium was held, and representatives of 14 countries have indicated that they have been recording individual methane measurements of ruminants: Austria, Belgium, Switzerland, Germany, Denmark, France, Finland, Netherlands, Poland, Spain, Sweden, UK, Ireland and Italy. Most countries have nationally funded projects to perform the measurements, but many animals were recorded specifically within the FP7 project *RuminOmics*.

According to the inventory, there are currently 10,690 individual animals recorded for their methane emission, using one of the methodologies mentioned above. Based on aims of approved projects ~6600 animals will be recorded in the near future. Most records are taken in research farms, but a few studies also take records on commercial farms (UK, DK, NL, IT). The measurements are mainly performed on Holstein cows, but in some studies Jerseys, Red breeds, Finish Ayrshire, Brown Swiss and Simmentals were also recorded. Some studies have been performed on beef cattle, in Spain the measurements are done on goats, and in France one study was done on sheep. The length of measurement differed between studies. Some studies measured 4 days per animal, and other studies measured up to 400 days per animal.

Individual feed intake records were taken on ~5,500 animals out of the 10,690, which is approximately 50%. Live weight was recorded on ~3,600 animal (approximately 33%), and milk production was known for almost all dairy cows.

The animals were not genotyped in all studies, neither were rumen microbiota samples always taken. The genotyping is under progress for most studies, and it is expected that at least half of the phenotyped animals will also have genotypes. Rumen microbiota samples were collected from approximately 1500 animals, but more studies are underway. It is therefore expected that this number will increase.

4) Training School on Methane Physiology & Modelling for Geneticists in Dummerstorf

The training school on 'Methane Physiology & Modelling for Geneticists' in Dummerstorf can be understood as the counterpart of 'Methane Data Handling Analysis for Nutritionists and Physiologists', which was held in Poznan in September 2015. The training school in Dummerstorf was held from September 29th to October 1st 2014 and was hosted and organised by the Leibniz Institute of Farm Animal Biology, Dummerstorf, Germany. Among 29 participants, 22 trainees and 7 lecturers came from 10 different countries (B, D, DK, FIN, I, NL, PL, SL, UK and TR). The 4 main talks were given by Paul Boettcher (Animal Production Officer, FAO); Diego Morgavi (INRA Centre Theix, St Genes Champanelle, F), Jan Dijkstra (Wageningen University, NL) and Björn Kuhla (FBN Dummerstorf, D). Besides these, 3 tutorial and case study reports were provided by Frank Lehmann (Sensors Europe, D), Michael Derno and Cornelia Metges (both FBN Dummerstorf, D). Scientific topics addressed in the training school were 1) Global climate change and involvement of ruminants, 2) Rumen microbiology, hydrogen, methanogens, metagenomics, 3) Modelling methane production from ruminants, 4) Nutrition and physiology affecting methane production, and 5) Technical aspects of methane measurement using GreenFeed and Respiration Chambers.
Trainees were asked to answer 7 questions in an evaluation form resulting in an overall score:
17 Excellent 5 Good O Average O Below average O Bad

However, trainees also made the following suggestions for the next Training School:

1) Different measurement techniques:
   Pros and cons
   How do they work
   What data comes out of it (unit, frequency)
   Analysing real data
2) Animal variation:
   Host genetics
   Genetic parameters (common unit!)
   Breeding goals (economic/environmental values)

5) Major Outcome of Joint Work group meeting in Granada, 2014

The workshop was organised by EEZ-CSIC in Granada (Spain) on 5-7 November 2015. The objective of the workshop was to gather scientists from different disciplines to generate stimulating discussions on the different factors that contribute to between-animal variation in methane production in ruminants. We also aimed to address the challenge of combining measurements using different techniques and protocols for potential breeding programmes.

The program was designed to stimulate interaction among participants by combining introductory talks to the main topics and small group sessions for discussions. As part of Working Group 1, three main sessions were held: i) 'Diet associated effects that contribute to among-animal variation in methane production', ii) 'Rumen microbiome associated effects that contribute to variation' and iii) 'Host-genetic associated effects that contribute to variation'.

Introductory talks were given for each by Prof. Michael Kreuzer (ETZH, Switzerland), Prof. Jamie Newbold (Aberystywth University, UK) and Dr. Donagh Berry (Teagasc, Ireland), respectively. The talks were followed by discussion in small groups, which were arranged in a way to ensure that all expertise from participants were evenly distributed. Each session ended with a general discussion. We also had the opportunity to have a presentation on the Australian Research Program 'Pangenome' presented by Dr. Philip Vercoe (University of Western Australia) and on the 'New Zealand breeding program for low emissions' presented by Suzanne Rowe (AgResearch Ltd) to find common areas of interest and complementarity with METHAGENE activities and other European projects.

A total of 53 researchers from 17 countries participated in the workshop and the content of the presentations is available in the COST Action web site (www.methagene.eu). The main outcomes from the discussion groups can be outlined as follows:

1. Many different components of the diet are associated with variations in CH_4 emissions (nutrient composition, secondary compounds, fat content and intake level). Although they are widely recognized, they are often not fully considered when CH_4 emissions measurements are undertaken.
2. Both archaea and the balance of the microbial population are important predictors of methane emissions and the microbial population in the rumen is effected by the host genetics but also by early life.
3. Genetics is improving environmental footprint and can directly reduce CH_4 emissions. The heritability of the trait does not provide that much information. Firstly we need to know the genetic variation in CH_4 emissions independent of performance.

6) Summary and Conclusion

Based on the scientific program defined in (1), Working Group 1 successfully worked out a comprehensive inventory on methane-determining factors as listed in (2). One of the major contributors to methane production from ruminants is the level of dry matter intake and feed composition, despite further factors such as host genetics and early life accounting for it as well.
Care must be taken in standardizing definitions for CH_4 measurements, as the different techniques listed in (3) provide methane data in different units or frequencies. Next to the techniques available, the unit of how methane is expressed depends to a large extent on the specific research question, making a standardization across research disciplines hard to realize. However, there is still work going on in terms of “comparison and calibration of measurements” which is headed by Working Group 2. This work needs to be finished before methane data can be combined and integrated into novel genetic models.