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Evaluation of a gas in vitro system for predicting methane production in vivo

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ABSTRACT

Methane production from ruminant livestock varies with the diet as a result of factors such as dry matter intake, diet composition, and digestibility. To estimate the effect of dietary composition and feed additives, CH_4 production can be measured in vitro as a first step because large numbers of samples can be incubated and analyzed at the same time. This study evaluated a recently developed in vitro method for prediction of in vivo CH_4 production by examining the relationship between predicted and observed CH_4 production values. A total of 49 different diets (observations), used in previous 13 in vivo studies, were selected to include diets varying in nutrient composition. Methane production was measured in all in vivo studies by respiration chambers or the GreenFeed system (C-Lock Inc., Rapid City, SD). Overall, the in vitro system predicted CH_4 production well ($R^2 = 0.96$), but the values obtained were slightly underestimated compared with observed in vivo values (mean 399 L/d compared with 418 L/d: root mean square prediction error = 51.6 L/d or 12.3% of observed mean). Further analysis of the effect on residuals showed no significant relationship between CH_4 production and most factors known to affect CH_4 production such as dry matter intake, digestibility, and dietary concentrations of fat and starch. However, some factors included in the model were not well predicted by the system, with residuals negatively related to neutral detergent fiber concentration and positively related to concentrate proportion. The in vitro system can thus be useful for screening diets and evaluation of feed additives as a first step that can be best interpreted when feeding cows at maintenance level.

Key words: in vitro, in vivo, predicting methane production

INTRODUCTION

Methane is one of the major greenhouse gases. Dairy cows contribute CH_4 to the atmosphere due to microbial fermentation of feed in the rumen and hindgut. The production of CH_4 by ruminants also causes energy losses for the animal, corresponding to 2 to 12% of gross energy (**GE**) intake (Johnson and Johnson, 1995). The total amount of CH_4 released is dependent on several factors, such as DMI, type of feed, feed quality, and OM digestibility (Johnson and Johnson, 1995; Ramin and Huhtanen, 2013). Different strategies have been evaluated with the aim of reducing enteric CH_4 production and interesting possibilities are offered by feed supplements [e.g., dietary fat, ionophores, plant compounds, and enzymes (Beauchemin et al., 2009; Hook et al., 2010; Knapp et al., 2014)]. To evaluate the effect of dietary composition and feed additives, reliable measurement of CH_4 production is essential. Some common in vivo measurement techniques are available, all of which have advantages and disadvantages in terms of, for example, accuracy and cost. The most consistent is the respiration chamber technique, where the concentrations of CH_4 and CO_2 in air flux (L/min) are measured (Johnson and Johnson, 1995; Yan et al., 2010), but this technique is very costly and is not suitable for measurements on many animals at the same time. Another technique that can be used for on-farm measurements is the sulfur hexafluoride (SF_6) tracer technique (Johnson et al., 1994), which is based on the ratio of SF_6 to CH_4. Results obtained using this technique show higher variation than chamber values (Hammond et al., 2009). Yet another technique that can be used to estimate total daily CH_4 emissions of individuals is based on spot sampling over several days of breath in feed troughs in automatic milking systems or in concentrate feeders, such as the GreenFeed system (C-Lock Inc., Rapid City, SD) and the spot sampling method used by Madsen et al. (2010). These techniques may give higher variation than chamber techniques, but this can be compensated for by a large number of animals for measurements.

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However, in vivo studies are expensive and to reduce costs and effects on animals, various in vitro systems have been developed. Continuous culture experiments as described by Czerkawski and Breckenridge (1977) and batch culture experiments as reported by van Nevel and Demeyer (1981) are commonly used for evaluating the effects of diets and additives on enteric CH_4 production. Recently, Ramin and Huhtanen (2012) developed an in vitro method for prediction of CH_4 production in the rumen of cows using the kinetic parameters from an automated in vitro gas production (**GP**) system in a 2-compartment rumen model. This approach takes rumen dynamics (digestion kinetics) into account and may have advantages compared with single time point batch culture systems. However, in vitro techniques are applied to predict treatment effects in vivo, and it is therefore important that these techniques are reliable and well validated. In the review of Yáñez-Ruiz et al. (2016) where the designs, implementation, and interpretation of in vitro batch systems were reviewed, it was proposed that studies with direct comparisons between in vitro and in vivo systems would allow better interpretation of data and treatments suitable for evaluating the in vitro GP system. The aim of this study was to investigate the potential of the in vitro GP system for ranking different type of diets according to predicted CH_4 production, compared with in vivo CH_4 production values.

MATERIALS AND METHODS

Studies and Treatments

The diets for which in vitro GP and CH_4 production were predicted were selected from 13 different in vivo studies and consisted of 49 diets in total (Appendix). The majority of these diets were formulated based on a larger data set previously used to develop prediction equations for methane production (Ramin and Huhtanen, 2013). They were selected to include different dietary composition: feeding levels, proportion of concentrate, carbohydrate composition of concentrates, protein and fat supplementation, forage type, and maturity of forage (Table 1). Measurement of CH_4 production in the original in vivo studies was mainly performed in respiration chambers, with the exception of the study by Gidlund et al. (2015) where the GreenFeed system was used (C-Lock Inc.). In 4 cases, the original ingredients used in the in vivo studies (21 diets) were also used for the in vitro system. The in vitro diets for the remaining 9 of the 13 in vivo studies (28 diets) were formulated to be as similar as possible to the original feeds used in vivo in terms of ingredient composition and concentrations of ME, CP, and NDF. Digestibility of silage samples was determined either in vivo in sheep or in vitro. Ingredients for those 9 studies were provided by the Swedish University of Agricultural Sciences Research Centers in Umeå and Uppsala (Sweden), LUKE National Resources Institute (Finland), and the feed companies Raisio Ltd. (Raisio, Finland) and Teknosan (Vänersborg, Sweden).

Animals, Experimental Design, and Laboratory Procedures

The study was performed at the Swedish University of Agricultural Sciences in Umeå, Sweden. All handling of animals was approved by the Umeå Ethics Committee for Animal Research, Sweden. Three dairy cows of the Swedish Red breed in late lactation, fed a TMR (grass silage/concentrate ratio 600/400 g/kg on a DM basis) ad libitum, were used as donors of ru-

Table 1. Description of diets and type of animal used in in vivo studies for which original[1] or constructed diets were analyzed in vitro

Diet	Reference	Forage	Animal type[2]
1–4	Ferris et al., 1999	Grass silage and different proportions of concentrate	1
5–7	Keady and Mayne, 1998	Grass silage and different energy sources for concentrate	1
8–11	Beever et al., 1988	Grass silage cut at different date and barley substitution	3
12–14	Kirkpatrick et al., 1997	Different types of grass silage and different levels of concentrate	2
16–17	Gordon et al., 1995	Grass silage and different types of concentrate	1
18–20	Jentsch et al., 1972	Hay, different levels of added canola oil	1
21–23	Moss et al., 1995	Grass silage and different levels of barley	4
24–26	Moss and Givens, 2002	Grass silage and supplementation with soybean meal	4
27–28	Tyrrell et al., 1992	Direct-cut alfalfa or orchard grass ensiled	3
29–32	Brask et al., 2013a	Corn and grass silage and different physical forms of canola	1
33–38	Brask et al., 2013b	Early/late grass silage or corn silage, with/without canola oil supplement	1
39–45	Gidlund et al., 2015	Grass silage and soybean meal or canola meal	1
46–49	Hellwing et al., 2013	Grass silage and different types of treated wheat	1

[1]Original diets were also used in vitro for diets 29 to 49.

[2]1 = dairy cows; 2 = beef cattle; 3 = growing cattle; 4 = sheep.