J. Dairy Sci. 96:4643–4646
http://dx.doi.org/10.3168/jds.2013-6556

Short communication: Measurements of methane emissions from feed samples in filter bags or dispersed in the medium in an in vitro gas production system

M. Ramin,*[1] S. J. Krizsan,* F. Jančík,† and P. Huhtanen*
*Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, S-901 83 Umeå, Sweden
†Institute of Animal Science, Prague-Uhříněves, Přátelství 815, 104 00 Prague, Czech Republic

ABSTRACT

The objective of this study was to compare methane (CH_4) emissions from different feeds when incubated within filter bags for in vitro analysis or directly dispersed in the medium in an automated gas in vitro system. Four different concentrates and 4 forages were used in this study. Two lactating Swedish Red cows were used for the collection of rumen fluid. Feed samples were milled to pass a 1.0-mm screen. Aliquots (0.5 g) of samples were weighed directly in the bottles or within the F57 filter bags that were placed in the bottles. Gas samples were taken during 24 and 48 h of incubation, and CH_4 concentration was determined. The data were analyzed using a general linear model. Feeds differed significantly in CH_4 emission both at 24 and at 48 h of incubation. The interaction between feed and method on methane emission in vitro was significant, indicating that the ranking of feeds was not consistent between the methods. Generally, greater amounts of CH_4 were emitted from samples directly dispersed in the medium compared with those incubated within the filter bags, which could be a result of lower microbial activity within the filter bags. The ratio of CH_4 to total gas was greater when the feeds were incubated within bags compared with samples directly dispersed in the medium. Incubating samples in filter bags during 48 h of incubation cannot be recommended for determination of CH_4 emission of feeds in vitro.
Key words: filter bag, gas production, in vitro, methane emission

Short Communication

Methane (CH_4) is the greenhouse gas that has recently been subject to most attention with regard to the environmental impact of different ruminant livestock production systems (Cederberg et al., 2013). Dietary and animal factors considerably influence methane emissions from ruminants (Ramin and Huhtanen, 2013). Methane is produced as a result of microbial breakdown of carbohydrates in the rumen and it represents an energy loss of 0.02 to 0.12 of gross energy intake to the animal (Johnson and Johnson, 1995). Recently, in vitro methods have been applied to evaluate the effects of diet composition and feed additives on CH_4 emissions (Becker and van Wikselaar, 2011; Opsi et al., 2012; Sakthivel et al., 2012). The feed samples incubated in vitro can be enclosed in filter bags for fiber and in vitro studies (F57 bags, Ankom Technology Corp., Macedon, NY) or directly dispersed in the in vitro medium. Incubating feeds in filter bags makes simultaneous determination of in vitro digestibility easier by applying fewer transfers of sample residues after incubation, but also seems to be less accurate compared with in vivo degradability (Krizsan et al., 2013). However, bags can restrict microbial digestion, particles can be lost from the bags during incubation, and the bag can act as a physical hindrance for removal of end products from the degradation (Nozière and Michalet-Doreau, 2000). We hypothesized that CH_4 emission in vitro could be lower if samples are incubated within filter bags compared with when they are directly dispersed in the medium. The objective of this study was to compare CH_4 emission from different feeds measured using an in vitro method and incubated within filter bags or directly dispersed in the medium.

The study was conducted with the permission of the Swedish Ethical Committee on Animal Research. The details of the feed samples used in the current study and their chemical composition are reported by Krizsan et al. (2013). Eight feed samples were used: 4 concentrates and 4 forages. The 4 concentrates were barley, oat, canola meal, and dried sugar beet pulp; CP on a DM basis was 12.1, 10.8, 34.6, and 10.1%, respectively; and NDF concentration on a DM basis was 24.9, 35.5, 30.4, and 31.7%, respectively. The 4 forages were grass silage 1 (DM of 29.9%) and grass silage 2 (DM of 31.4%) of a mixed sward timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.) harvested as a

Received January 8, 2013.
Accepted March 7, 2013.
[1]Corresponding author: mohammad.ramin@slu.se

first cut (grass silage 1) and second cut (grass silage 2), respectively, a grass hay, and an alfalfa sample; CP on a DM basis was 15.0, 13.7, 10.2, and 19.1%, respectively; and NDF concentration on DM basis was 56.6, 60.9, 61.3, and 47.4%, respectively. Samples were dried at 60°C for 48 h and ground through a 1.0-mm screen using a cutting mill (Retsch SM 2000; Retsch GmbH, Haan, Germany) before the in vitro incubations.

Two Swedish Red cows were used as donor animals of rumen fluid 2 h after the morning feeding. The cows were fed a diet consisting of 60% grass silage and 40% concentrate on a DM basis. The rumen fluid was filtered through 2 layers of cheesecloth into preheated thermos flasks previously flushed with CO_2 and further mixed with a buffer-mineral solution according to Menke and Steingass (1988). The in vitro procedure was performed as described by Krizsan et al. (2013). The ratio of rumen fluid to buffer was 1:2 and peptone (Merck KGaA, Darmstadt, Germany) was added to the buffer to supply the protein source for rumen microorganisms. Samples of 0.5 g were weighed directly in 250-mL serum bottles (Schott, Mainz, Germany) or into F57 filter bags (for fiber and in vitro studies; Ankom Technology Corp., Macedon, NY) that were sealed and placed in the serum bottles. Samples were incubated in 60 mL of buffered rumen fluid and the bottles were then placed in a water bath at 39°C and gently and continuously agitated for 72 h. All samples were incubated in 2 consecutive runs, including duplicate samples of blanks and standard hay; that is, each treatment was represented in 2 experimental units totally. To avoid any over-pressure in the bottles, the gases were released from the system at a preset pressure by the opening of an electric gas valve (approximately 1 mL of gas was released at each opening).

Measurement of CH_4 emission was conducted as described by Ramin and Huhtanen (2012). In brief, gas samples were drawn using a gas-tight syringe (1 mL; Hamilton, Bonaduz, Switzerland) from each bottle at 24 and 48 h of incubation. Methane concentration was determined by injecting 0.2 mL of gas into a Varian Star 3400 CX gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) using thermal conductivity detection. Separation was achieved using a 1.8-m × 3.0-mm stainless steel HayeSep T 80/100 mesh column (Varian Analytical Instruments, Walnut Creek, CA) with argon as the carrier gas at a flow rate of 32 mL/min and an isothermal oven temperature of 32°C. The injector and detector temperatures were set to 110°C and 135°C, respectively. The peaks were identified and quantified by comparison with a standard gas mixture of CO_2 (90 mol %) and CH_4 (10 mol %) prepared by AGA Gas AB (Sundbyberg, Sweden). Other details and calculations of methane emission are given by Ramin and Huhtanen (2012). Methane emission at each time point (24 or 48 h) was calculated as 265 × CH_4 concentration + total gas production × CH_4 concentration × 0.55, where 265 is the total headspace volume in mL and 0.55 is the constant (the ratio of CH_4 emission in the outflow gas from the in vitro system). Total gas production was measured with an automated system and the readings were done every 12 min and corrected to normal air pressure (101.3 kPa) according to Cone et al. (1996). Mean blank gas production within run was subtracted from the sample gas production.

Methane emission was reported in milliliters per gram of DM, milliliters per gram of truly digested OM (**TDOM**; determined at 72 h of incubation), and as a proportion of the total gas recorded in the automated gas in vitro system.

The data were analyzed using the GLM procedure (release 9.2; SAS Institute Inc., Cary, NC) by applying the following model:

$$Y_{ijk} = \mu + F_i + M_j + (FM)_{ij} + R_k + e_{ijk},$$

where Y_{ijk} = dependent variable, μ = overall mean, F_i = feed i, M_j = method j, represented either by samples incubated directly dispersed in the medium or within filter bags in the gas in vitro system, $(FM)_{ij}$ = interaction between feed i and method j, R_k = run k, and $e_{ijk} \sim N\left(0, \sigma_e^2\right)$ is the random residual error. Least squares means are reported, and mean separation was done by least significant difference to test differences between treatments.

Methane emissions for all feeds at 24 and 48 h of incubation, incubated within filter bags or directly dispersed in the medium in the gas in vitro system, are presented in Table 1. Methane emission in milliliters per gram of DM was different between feeds at both 24 and 48 h of incubation ($P < 0.01$; Table 1). A significant feed × method interaction ($P < 0.01$; Table 1) showed that CH_4 emission was lower for barley, oat, and sugar beet pulp when incubated within filter bags compared with directly dispersed in the medium in the gas in vitro bottles ($P \leq 0.02$). This was true at both time points except at 48 h of incubation for sugar beet pulp ($P = 0.91$). When CH_4 emission was expressed in milliliters per gram of TDOM, CH_4 emission was different between feeds at both 24 and 48 h of incubation ($P < 0.01$; Table 1). A significant feed × method interaction ($P < 0.01$; Table 1) showed that CH_4 emission was higher for the grass silage and grass hay samples when incubated within filter bags compared with directly dispersed in the bottles at both 24 and 48 h of incubation ($P < 0.01$). We observed a weak relationship between CH_4 emission (mL/g of DM) from feeds