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In vitro gas and methane production of silages from whole-plant corn harvested at 4 different stages of maturity and a comparison with in vivo methane production

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ABSTRACT

The current study investigated the relationship between in vitro and in vivo CH_4 production by cows fed corn silage (CS)-based rations. In vivo CH_4 production was measured in climate respiration chambers using 8 rumen-cannulated Holstein-Friesian cows. In vitro CH_4 production was measured using rumen fluid from the 8 cows that were fully adapted to their respective experimental rations. The animals were grouped in 2 blocks, and randomly assigned to 1 of the 4 total mixed rations (TMR) that consisted of 75% experimental CS, 20% concentrate, and 5% wheat straw [dry matter (DM) basis]. The experimental CS were prepared from whole-plant corn that was harvested at either a very early (25% DM), early (28% DM), medium (32% DM), or late (40% DM) stage of maturity. The 4 experimental TMR and the corresponding CS served as substrate in 2 separate in vitro runs (each run representing 1 block of 4 animals) using rumen fluid from cows fed the TMR in question. No relationship was found between in vivo CH_4 production and in vitro CH_4 production measured at various time points between 2 and 48 h. None of the in vitro gas production (GP) and CH_4 production parameters was influenced by an interaction between substrate and origin of rumen fluid. In vitro measured 48-h GP was not affected by the maturity of whole-plant corn, irrespective whether CS alone or as part of TMR was incubated in adapted rumen inoculum. Incubation of the experimental TMR did not affect the kinetics parameters associated with gas or CH_4 production, but when CS alone was incubated the asymptote of GP of the soluble fraction was slightly decreased with increasing maturity of CS at harvest. In vitro CH_4 production expressed as a percent of total gas was not affected by the maturity of whole-plant corn at harvest. Several in vitro parameters were significantly affected (GP) or tended to be affected (CH_4) by diet fed to donor cows. It was concluded that the current in vitro technique is not suitable to predict in vivo CH_4 production from CS-based rations.
Key words: methane, corn silage, maturity, in vitro, in vivo

INTRODUCTION

Whole-plant corn silage (**CS**) is commonly used in rations of dairy cows in many parts of the world. It has a high content of starch and generally good ensiling characteristics (Khan et al., 2015). The nutritional value of such CS largely depends on the content and degradability of the starch. The starch content, as well as the vitreousness of corn kernels, increases with maturity, and the fractional rate of ruminal starch degradation of corn decreases with maturity (Philippeau and Michalet-Doreau, 1997). The stage of maturity of the corn plant at harvest, therefore, has a significant effect on the nutritive value of CS, feed intake, milk yield (Johnson et al., 1999; Cammell et al., 2000; Warner et al., 2013) and CH_4 production (Hatew et al., 2016). Enteric CH_4 is a potent greenhouse gas (Moss et al., 2000) and constitutes a loss of dietary energy to the animal (Johnson and Johnson, 1995).

Assessment of in vitro gas production (**GP**) is largely used to evaluate the nutritive value of ruminant feeds by incubating substrate in buffered rumen fluid (Cone et al., 1996; Getachew et al., 1998; Dijkstra et al., 2005). This in vitro approach can also be used to evaluate different feeding strategies for their potential to mitigate CH_4 production (Pellikaan et al., 2011; Holtshausen et al., 2012; Hatew et al., 2015). Currently, only a limited number of studies are available reporting in vivo CH_4 production of cattle upon changes in maturity of whole-plant corn at harvest (Cammell et al., 2000; Mc Geough

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et al., 2010; Hatew et al., 2016), and a dearth of direct in vitro-in vivo comparisons exist with respect to CH_4 production (Yáñez-Ruiz et al., 2016). Such comparisons are essential to evaluate the robustness of in vitro techniques to represent and simulate rumen fermentation including in vivo CH_4 production. Furthermore, it offers the possibility to predict in vivo CH_4 production, which is of practical interest to mitigate enteric CH_4 production by dairy cows.

The use of rumen fluid from donor cows is inherent to the in vitro GP technique. It is generally accepted that rumen fluid of donor cows has to be adapted to the substrate that is subjected to in vitro GP and CH_4 measurements. To our knowledge, however, a lack of studies have addressed the issue of adaptation of rumen inoculum with CS-based rations. It was, therefore, considered opportune to address the issue of adaptation of rumen inoculum to substrate in the current study as well.

The objective of this research was to investigate the relationship between in vitro and in vivo CH_4 production of silages from whole-plant corn harvested at 4 different stages of maturity. We hypothesized that in vitro CH_4 production is related to the in vivo CH_4 production when identical dietary material is used both in vivo and in vitro and those dietary materials are inoculated with rumen fluid obtained from donor animals adapted to those dietary materials.

MATERIALS AND METHODS

Donor Animals and Substrates

The in vitro experiment was conducted in parallel with the in vivo trial of Hatew et al. (2016), where 28 lactating Holstein-Friesian cows were used for in vivo CH_4 measurements using climate-controlled respiration chambers. Eight of the 28 cows in the latter study (Hatew et al., 2016) had a permanent rumen cannula, and these 8 animals served as donors of rumen fluid for the current in vitro incubations. Housing, animals, feeding regimens, and all procedures related to the in vivo trial are described in detail by Hatew et al. (2016). Briefly, cows were allocated to 7 blocks of 4 cows each, based on parity, DIM, fat- and protein-corrected milk at the start of the trial, and presence of a rumen cannula. Within blocks, cows were randomly assigned to 1 of the 4 TMR that consisted of 75% experimental CS, 20% concentrate, and 5% wheat straw (DM basis). The experimental CS were harvested in 2013 at 4 different stages of maturity; that is, very early (September 20, 25% DM; **CS25**), early (September 28, 28% DM; **CS28**), medium (October 9, 32% DM; **CS32**), or late (October 31, 40% DM; **CS40**). Five days before the start of the adaptation period, cows received a high-CS diet containing a nonexperimental corn silage. The 4 experimental TMR and the corresponding CS (Table 1) served as substrate in 2 separate in vitro runs (each run with 1 block of 4 animals) using rumen fluid from cows fed the TMR in question. The in vitro incubations were run simultaneously with the in vivo CH_4 measurements, and the timespan between the 2 in vitro runs was 1 wk.

Rumen fluid was collected on the last day of each 12-d experimental period, thereby assuming that the cows were adapted to their respective experimental rations. The experimental CS (n = 4) were used as sole substrates and incubated separately with each of the rumen fluid inocula types (n = 4). Furthermore, the CS-based TMR (n = 4) were used as substrate and incubated with rumen fluid from cows adapted to the

Table 1. Chemical composition of corn silages differing in maturity at harvest and of TMR; data are adopted from Hatew et al. (2016)

	Corn silage (CS)[1]				TMR[2]			
Parameter	CS25	CS28	CS32	CS40	TMRCS25	TMRCS28	TMRCS32	TMRCS40
Growing days[3]	128	136	147	169	NA[4]	NA	NA	NA
DM content (g/kg)	283	292	318	396	437	444	463	522
Chemical composition (g/kg of DM)								
Ash	39	37	37	35	56	55	55	53
CP	83	83	80	79	145	145	142	142
NDF	407	394	359	349	369	359	332	325
ADF	242	233	207	195	219	212	193	183
ADL	11	11	9	10	13	12	11	12
Crude fat	26	27	25	24	26	26	25	24
Starch	275	305	356	385	243	266	304	326

[1]Whole-plant corn was harvested at targeted DM contents of 25, 28, 32, or 40% for CS25, CS28, CS32, and CS40, respectively.

[2]Total mixed rations had corn silage:wheat straw:concentrate ratio of 75:5:20 (DM basis). TMRCS25, TMRCS28, TMRCS32, and TMRCS40 contained either CS25, CS28, CS32, or CS40, respectively.

[3]Number of days from planting until harvesting of the whole plant for ensiling.

[4]NA = not applicable.