



## Increasing linseed supply in dairy cow diets based on hay or corn silage: Effect on enteric methane emission, rumen microbial fermentation, and digestion

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### ABSTRACT

We investigated the effects of increasing extruded linseed supply in diets based on hay (H; experiment 1) or corn silage (CS; experiment 2) on enteric methane ( $\text{CH}_4$ ) emission, rumen microbial and fermentation parameters, and rumen and total-tract digestibility. In each experiment, 4 lactating Holstein cows fitted with cannulas at the rumen and proximal duodenum were used in a  $4 \times 4$  Latin square design (28-d periods). Cows were fed ad libitum a diet [50:50 and 60:40 forage:concentrate on a dry matter (DM) basis for experiments 1 and 2, respectively] without supplementation (H0, CS0) or supplemented with extruded linseed at 5% (H5, CS5), 10% (H10, CS10), and 15% (H15, CS15) of dietary DM (i.e., 1.8, 3.6 and 5.4% total fatty acids added, respectively). All measurements were carried out during the last 8 d of each period. Linseed supply linearly decreased daily  $\text{CH}_4$  emission in cows fed H diets (from 486 to 289 g/d for H0 to H15, on average) and CS diets (from 354 to 207 g/d for CS0 to CS15, on average). The average decrease in  $\text{CH}_4$  per kilogram of DM intake was, respectively,  $-7$ ,  $-15$ , and  $-38\%$  for H5, H10, H15 compared with the H0 diet, and  $-4$ ,  $-8$ , and  $-34\%$  for CS5, CS10, and CS15 compared with the CS0 diet. The same dose-response effect was observed on  $\text{CH}_4$  emission in percent of gross energy intake, per kilogram of nutrient digested, and per kilogram of 4% fat- and 3.3% protein-corrected milk (FPCM) in both experiments. Changes in the composition of rumen volatile fatty acids in response to increasing linseed supply resulted in a moderate or marked linear decrease in acetate:propionate ratio for H or CS diets, respectively. The depressive effect of linseed on total protozoa concentration was linear for H diets ( $-15$  to  $-40\%$ , on average, for H5 to H15 compared with H0)

and quadratic for CS diets ( $-17$  to  $-83\%$ , on average, for CS5 to CS15 compared with CS0). Concentration of methanogens was similar among H or CS diets. The energetic benefits from the decreased  $\text{CH}_4$  emission with linseed supply in diets based on hay or corn silage did not improve digestibility or milk yield. Milk efficiency (kg of FPCM/kg of DM intake) was improved with linseed supply up to H10 in H diets and was unchanged in CS diets. Lower  $\text{CH}_4$  enteric emission from dairy cows fed linseed helps limit the environmental footprint of ruminant livestock.

**Key words:** dairy cow, digestion, hay or corn silage, linseed, methane

### INTRODUCTION

Methane ( $\text{CH}_4$ ) released by ruminants is the main greenhouse gas at the farm level (Veyssset et al., 2010) and constitutes an energetic loss for the animal ranging from 2 to 12% of its gross energy (GE) intake (Johnson and Johnson, 1995). Decreasing enteric  $\text{CH}_4$  emission from ruminants without altering animal production is desirable both as a strategy to reduce global greenhouse gas emissions, and thus the negative environmental impact of livestock, and as a means of improving feed conversion efficiency.

Several reviews have reported that dietary fatty acid (FA) supply (Grainger and Beauchemin, 2011; Hristov et al., 2013), particularly FA from linseed (Martin et al., 2010), is one of the most efficient dietary strategies to mitigate enteric  $\text{CH}_4$  emission from dairy cows. In addition, linseed supply to dairy cow diets helps improve the nutritional value of milk through a slight increase in linolenic acid (Doreau et al., 2011). The extent of the linseed  $\text{CH}_4$ -mitigating effect varies depending on many factors, including FA availability in the rumen and the dose included in the diet (Martin et al., 2010). Low supply of linseed oil ( $<2\%$  added FA) did not modulate  $\text{CH}_4$  emission in dairy cows (Livingstone et al., 2015), whereas higher supply (3.7–5.7% added FA) of differ-

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ent linseed forms (crude, crushed, or extruded whole grains, or oil) resulted in a lower CH<sub>4</sub> yield (g/kg of DM intake) but had deleterious effects on OM and fiber digestibility and, for the highest FA level, impaired intake and dairy cow performance (Martin et al., 2008; Beauchemin et al., 2009b). In addition, the extent of the effect of linseed supply on CH<sub>4</sub> emission may also depend on diet composition, especially the nature of forage and the percentage of concentrate (Martin et al., 2010). Only Chung et al. (2011) compared the effect of linseed with different forage-based diets fed to dry cows, and found a decrease in CH<sub>4</sub> emission with barley silage, but not with hay. To our knowledge, the dose-response effect of dietary linseed FA on CH<sub>4</sub> emission has not yet been studied. In addition, only a few *in vivo* studies have considered, in the same experiment, the effects of linseed FA supply on both CH<sub>4</sub> emission and the rumen fermentation and microbial processes involved.

To address these questions, we performed 2 experiments in dairy cows, the first with a hay-based diet and the second with a corn silage-based diet. These diets are representative of winter diets used frequently in Europe for dairy cows. Diets were supplemented with extruded linseed at 3 levels of FA supply (1.8, 3.6, and 5.4% of DM). The highest FA supply used here is not currently recommended at the farm level, but the wide variation in FA level was chosen to establish the dose-response relationship regarding enteric CH<sub>4</sub> emission and associated digestive processes such as rumen fermentation and microbial parameters and rumen and total-tract digestibility of diets. Data on milk production and composition have been published previously (Ferlay et al., 2013).

## MATERIALS AND METHODS

Two experiments were performed during 2 successive years at the animal experimental facilities of the Herbivores Research Unit at Institut National de la Recherche Agronomique (INRA, Saint-Genès-Champagne, France). Procedures on animals were in accordance with the guidelines for animal research of the French Ministry of Agriculture and all other applicable national and European guidelines and regulations for experimentation with animals (see [http://www2.vet-lyon.fr/ens/expa/acc\\_regl.html](http://www2.vet-lyon.fr/ens/expa/acc_regl.html) for details).

### Animals, Experimental Design, and Diets

The main description of the experiments are given below. Additional details are found in Ferlay et al. (2013). In each experiment, 4 lactating multiparous Holstein

cows fitted with rumen and proximal duodenum cannulas were used after lactation peak (experiment 1:  $117 \pm 26$  DIM; experiment 2:  $96 \pm 29$  DIM) in  $4 \times 4$  Latin square designs. Each experimental period lasted 28 d with the first 5 d as a transition period between treatments and the last 8 d in wk 4 (d 1 to 8) as a measurement period. Cows were housed in a tiestall barn during the whole experiment.

Linseeds were supplied as extruded linseed mixture (70% linseed, 30% wheat bran, Valorex, Combourtillé, France) at 0, 7, 14, or 21% of inclusion in the diet, which represented 0, 5, 10, or 15% of linseed in dietary DM. This mixture was included in the diets substituting for expeller linseed meal, wheat bran, and corn grain, and it provided a supply level of 1.8, 3.6, or 5.4% FA for 5, 10, and 15% of linseed, respectively. In experiment 1, the diet was composed of 50% hay and 50% concentrates on a DM basis; the experimental diets were called **H0**, **H5**, **H10**, and **H15**, according to the level of inclusion of linseeds. In experiment 2, the diet was composed of 60% forages (55.5% corn silage plus 4.5% hay) and 40% concentrates on a DM basis; experimental diets were called **CS0**, **CS5**, **CS10**, and **CS15** according to the level of inclusion of linseeds. Diets were given *ad libitum*. In both experiments, concentrates were offered at 60 and 40% of the daily amount at 0900 and 1630 h, respectively. In experiment 1, hay was offered in equal amounts at 0900, 1330, and 1630 h. In experiment 2, forages was offered in equal amounts at 0900 and 1630 h. The forage:concentrate ratio was maintained as close as possible to the target by adjusting the amount of feed offered daily. Ingredient and chemical composition of experimental diets as consumed are presented in Table 1. The diets were formulated to cover 105% of the INRA energy and protein requirements for maintenance and lactation of dairy cows (INRA, 2007).

### Measurements

**Intake, Milk Production, and BW.** Intake and milk production were recorded daily by weighing throughout the experiment. Feeds (offered and refused) and milk were sampled in wk 4 until biochemical analyses as described in Ferlay et al. (2013). Animals were weighed at the beginning and the end of the experiment.

**Total-Tract and Rumen Digestibility.** Total-tract digestibility was measured by total feces collection from d2 to d7 of each experimental period. Duodenal flow was determined using ytterbium chloride (YbCl<sub>3</sub>) as described by Fanchone et al. (2013). Briefly, a YbCl<sub>3</sub> solution was infused continuously into the rumen (1.2 g of Yb daily) via the rumen cannula using a peristaltic

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