



## Effect of fat supplementation and stage of lactation on methane production in dairy cows



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### ABSTRACT

The aim was to determine the effect of fat supplementation on methane (CH<sub>4</sub>) production and to study if the effect persists over time as lactation progresses. Rumen microbial protein synthesis and thereby milk yield may be reduced when fermentable organic matter (OM) is replaced by nonfermentable fat. Therefore the effect of concurrent supplying hydroxy-methionine-analog-isobutyrate (HMBi) to overcome this potential reduction in supply of metabolisable protein was also studied. Twelve multiparous lactating Danish Holstein cows were used in a randomised block design experiment with four treatments: Control (CON), CON supplemented with whole cracked rapeseed (WCR), CON supplemented with vegetable rumen protected fat (Lipmix 40/60, Lipitec, NLM Vantage Aps, Ringe, Denmark) (RPF), and RPF supplemented with HMBi (MetaSmart, Adisseo, France) (RPFA). All diets contained maize silage (230 g/kg dry matter (DM)), grass clover silage (230 g/kg DM), and concentrate (540 g/kg DM). Dietary crude fat concentration was 27 g/kg DM in CON and approximately 56 g/kg DM in the three other diets. All cows were offered 3 kg per day of the same standard concentrate during milking in addition to the different mixed rations fed ad libitum. Production of CH<sub>4</sub> was measured for four d in open-circuit respiration chambers in four periods during lactation: at 48, 125, 164, and 212 d in milk (DIM), and feed intake and milk yield were recorded at the same time. DIM did not affect DM intake (DMI). Increased DIM decreased milk yield, both measured as kg milk and kg energy corrected milk (ECM). Fat supplementation increased milk yield compared to CON, however ECM yield was not affected. Milk protein concentration decreased with fat supplementation. Methane production increased with DIM when measured in L/d, L/kg DMI and L/kg ECM. However, when CH<sub>4</sub> production was expressed as L/kg ECM there was a tendency for interaction between DIM and treatment. Methane production also increased with DIM when expressed as % of gross energy (GE) intake. The present study demonstrates that CH<sub>4</sub> production increases with DIM, especially when expressed as L/kg ECM. The reduction in CH<sub>4</sub> production when fat is added to the diet persists throughout the lactation. Supplementation with HMBi tended to decrease CH<sub>4</sub> production further.

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**Abbreviations:** AMS, automatic milking system; aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; CH<sub>4</sub>, methane; CO<sub>2</sub>, carbon dioxide; CON, control; DIM, days in milk; DM, dry matter; DMI, dry matter intake; ECM, energy corrected milk; FA, fatty acid; GE, gross energy; HMBi, hydroxy-methionine-analog-isobutyrate; N, nitrogen; NE<sub>l</sub>, net energy lactation; OM, organic matter; RPF, control supplemented with vegetable rumen protected fat; RPFA, RPF supplemented with HMBi; SFU, Scandinavian feed unit; WCR, control supplemented with whole cracked rapeseed.

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## 1. Introduction

Methane is produced in ruminants as a by-product of OM fermentation in the rumen and hindgut; this CH<sub>4</sub> is an energy loss to the animal and will vary with feed composition and intake. Under extreme circumstances 2–12% of GE intake is converted into CH<sub>4</sub> (Johnson and Johnson, 1995), although values between 3 and 7% are more realistic in intensive dairy production (Martin et al., 2008). Supplementation of fatty acids (FA) to the feed decreases CH<sub>4</sub> production on a short term (Martin et al., 2008; Brask et al., 2013b), and is one of the most promising dietary strategies to reduce enteric CH<sub>4</sub> production (Hristov et al., 2013). However, the persistency of this reduction has been questioned (Woodward et al., 2006; Hristov et al., 2013). Fat supplementation reduces the fermentable substrate and can lower OM and fibre degradability (Knapp et al., 2014). As a consequence, the amount of hydrogen produced during fermentation is reduced, resulting in decreased CH<sub>4</sub> production. Besides, supplementation with fat may reduce the activity of ruminal methanogens and protozoal numbers (Hristov et al., 2013). An increased degree of FA unsaturation will increase the negative effect on fibrolytic bacteria and methanogens (Giger-Reverdin et al., 2003). Supplementation with fat increases energy density in the diet and often increases milk yield (Larsen et al., 2012). However, the response in milk yield to supplementary fat is complex and not easy to predict (Garnsworthy, 1997). The supplementation of fat often replaces easily digestible carbohydrates and thereby reduces fermentation and changes fermentation pattern in the rumen. Reduced fibre digestibility is often followed by reduced DMI and milk production and therefore it is important to take the CH<sub>4</sub> reduction per kg of DMI or per kg of product into account. Substituting fermentable OM with nonfermentable fat will reduce microbial fermentation and microbial protein synthesis and thereby microbial protein supply for milk production (Canale et al., 1990). Supplementation of potential limiting amino acids in the form of esterified hydroxylated analogue of methionine (HMBi) would be a strategy to compensate this potentially lower metabolisable protein supply, when fat is added to the diet. The hydroxylated analogue HMBi has an acceptable bioavailability (Graulet et al., 2005) and may increase milk yield (St-Pierre and Sylvester, 2005). Supplementation of fat right after calving, where the cow mobilise on its own fat storage, may have a negative effect on milk yield (Weisbjerg et al., 2013), and therefore supplementation of fat should be postponed to later in lactation.

There are only few studies concerning long term effects of feeding strategies for reduction of CH<sub>4</sub> production, and the ruminal microflora seems to adapt to different CH<sub>4</sub> inhibitors (Johnson and Johnson, 1995). Woodward et al. (2006) suggest that this will also be the case for fat supplementation, whereas others point out that the effect of fat will persist for up to 16 weeks (Holter et al., 1992; Grainger et al., 2010; Moate et al., 2011). As the effect of fat supplementation on CH<sub>4</sub> mainly is due to a reduction in OM fermented in the rumen, the CH<sub>4</sub> mitigation effect of fat supplementation is hypothesised to be persistent.

The aim of this present research was to study the long term effect of fat supplementation in two different forms on CH<sub>4</sub> production in dairy cows. Further, the effect of additional supplementation of HMBi to the RPF diet was studied. The hypothesis was that the reducing effect of fat supplementation on CH<sub>4</sub> production is persistent. As fat supplementation reduces microbial protein synthesis and milk protein, it was hypothesised that additional supplementation of HMBi would increase milk production, and thereby reduce the CH<sub>4</sub> production per kg milk.

## 2. Materials and methods

### 2.1. Cows, experimental design, facilities and feeding

The experiment complied with the guidelines set out by the Danish Ministry of Justice with respect to animal experimentation and care of animals used for scientific purposes.

Twelve multiparous Danish Holstein cows were selected from a group of 107 Holstein cows being a part of a larger experiment focusing on different feeding strategies and their effect throughout lactation on feed intake, milk production, mammary nutrient supply, and energy and nitrogen (N) efficiency, reported in Alstrup et al. (2015). In this larger production experiment cows were kept in a loose-housing system and milked in an automatic milking system (AMS) (DeLaval AB, Tumba, Sweden), equipped with a device for concentrate feeding, removal and weighing of concentrate refusals at the end of each cow visit. The four different diets were as follows: Control (CON), CON supplemented with whole cracked rapeseed (WCR), CON supplemented with vegetable rumen protected fat (Lipmix 40/60, Lipitec, NLM Vantinge Aps, Ringe, Denmark) (RPF), and RPF supplemented with HMBi (MetaSmart, Adisseo, France) (RPFA). The RPF consisted of calcium soaps of palm FA (400 g/kg) and hydrogenated palm FA (600 g/kg). All diets consisted of maize silage (230 g/kg DM), grass clover silage (230 g/kg DM), concentrate in the mixed ration (430 g/kg DM), and concentrate in the AMS (110 g/kg DM) (Tables 1 and 2). The rations were fed ad libitum as mixed rations and 3 kg of concentrate was offered daily in the AMS.

At four time points in the lactation the selected cows were moved from the loose housing system to the tie stall, where the methane chambers are located. Cows entered the chambers at ( $\pm$ SD),  $48 \pm 5$  DIM (DIM48),  $125 \pm 5$  DIM (DIM125),  $164 \pm 11$  DIM (DIM164) and  $212 \pm 11$  DIM (DIM212), after an adaptation period in the tie stall (7 d at DIM48 and 3 d at DIM125, DIM164 and DIM212) before entering the chambers, in order to insure that animals were adapted to being tie stalled and manually milked. Eleven cows were of second parity and one cow was of third parity. At the beginning of experiment (DIM48) cows averaged ( $\pm$ SD)  $600 \pm 76$  kg bodyweight,  $38.9 \pm 3.8$  kg ECM, and  $20.4 \pm 2.3$  kg DMI. In the chamber period cows were fed and milked twice daily at 0600 h and 1700 h. Three kilograms of AMS concentrate was fed separately, with 1.5 kg per milking, to simulate the conditions in the loose housing/AMS system.

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