



## Effect of dietary starch concentration and fish oil supplementation on milk yield and composition, diet digestibility, and methane emissions in lactating dairy cows

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### ABSTRACT

The aim of this study was to evaluate the effects of diets with different starch concentrations and fish oil (FO) supplementation on lactation performance, in vivo total-tract nutrient digestibility, N balance, and methane (CH<sub>4</sub>) emissions in lactating dairy cows. The experiment was conducted as a 4 × 4 Latin square design with a 2 × 2 factorial arrangement: 2 concentrations of dietary starch [low vs. high: 23.7 and 27.7% on a dry matter (DM) basis; neutral detergent fiber/starch ratios: 1.47 and 1.12], the presence or absence of FO supplement (0.80% on a DM basis), and their interaction were evaluated. Four Italian Friesian cows were fed 1 of the following 4 diets in 4 consecutive 26-d periods: (1) low starch (LS), (2) low starch plus FO (LSO), (3) high starch (HS), and (4) high starch plus FO (HSO). The diets contained the same amount of forages (corn silage, alfalfa and meadow hays). The starch concentration was balanced using different proportions of corn meal and soybean hulls. The cows were housed in metabolic stalls inside open-circuit respiration chambers to allow measurement of CH<sub>4</sub> emission and the collection of separate urine and feces. No differences among treatments were observed for DM intake. We observed a trend for FO to increase milk yield: 29.2 and 27.5 kg/d, on average, for diets with and without FO, respectively. Milk fat was affected by the interaction between dietary starch and FO: milk fat decreased only in the HSO diet. Energy-corrected milk (ECM) was affected by the interaction between starch and FO, with a positive effect of FO on the LS diet. Fish oil supplementation decreased the n-6:n-3 ratio of milk polyunsaturated fatty acids. High-starch diets negatively influenced all digestibility parameters measured except starch, whereas FO improved neutral detergent fiber digestibility (41.9 vs. 46.1% for diets without and with FO, respectively, and ether extract

digestibility (53.7 vs. 67.1% for diets without and with FO, respectively). We observed a trend for lower CH<sub>4</sub> emission (g/d) and intensity (g/kg of milk) with the high-starch diets compared with the low-starch diets: 396 versus 415 g/d on average, respectively, and 14.1 versus 14.9 g/kg of milk, respectively. Methane intensity per kilogram of ECM was affected by the interaction between starch and FO, with a positive effect of FO for the LS diet: 14.5 versus 13.3 g of CH<sub>4</sub>/kg of ECM for LS and LSO diets, respectively.

**Key words:** methane, starch, fish oil, digestibility, dairy cow

### INTRODUCTION

Decreasing the potential of global warming by reducing emissions of greenhouse gases is a social and environmental priority. Methane (CH<sub>4</sub>) is a potent greenhouse gas that is produced in the rumen by highly specialized bacteria, and a recent review (Hristov et al., 2013) reports wide variability for CH<sub>4</sub> yield: 16 to 26 g/kg of DMI. The variability in CH<sub>4</sub> yield depends on several factors, and the chemical composition of TMR fed to cattle strongly affects emissions. For example, it is well known that increasing the concentrate proportion of the diet (especially increasing starch concentration) generally decreases CH<sub>4</sub> emissions. Using a modeling approach, Benchaar et al. (2001) showed that CH<sub>4</sub> yield was reduced when beet pulp (fibrous concentrate) was replaced by barley (starchy concentrate), although a recent study (Hassanat et al., 2013) suggests that a critical dietary concentration of starch is required to alter ruminal methanogenesis. Usually, corn meal is used in dairy cow rations as the starchy ingredient; however, high usage of corn meal is not desirable for 2 primary reasons: a higher risk of rumen acidosis and the economic cost. Cereal prices are predicted to increase in the next years as a consequence of the increased demand from developing countries and the growing market for bio-fuels (Godfray et al., 2010). Consequently, major use of by-products to partly replace corn meal in TMR is a

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strategy to provide cost-effective and environmentally sustainable feed to dairy cattle. Among by-products, soybean hulls, despite their high NDF concentration, are characterized by a high energy value, and they can thus represent an important energy source for dairy cattle. Ipharraguerre et al. (2002b) showed that soybean hulls can replace corn to supply up to 30% DM of TMR for mid-lactating cows. The replacement of corn meal by soybean hulls decreases dietary starch concentration and increases the NDF concentration. As a consequence, higher daily CH<sub>4</sub> emission would be expected; however, the high NDF digestibility of soybean hulls could improve animal performance and lower CH<sub>4</sub> yield (g/kg of DMI) or intensity (g/kg of milk). To the best of our knowledge, no in vivo studies have been conducted to evaluate the effect on rumen methanogenesis of partial replacement of corn meal by soybean hulls. Furthermore, as reported by Martin et al. (2010), only a few studies have been conducted to evaluate the effects of the nature of concentrate on methanogenesis.

Another strategy that can reduce CH<sub>4</sub> emission is fat supplementation, and feeding fat can also modify the milk FA profile. In practice, polyunsaturated fats are fed to dairy cows to manipulate milk FA profiles, increasing the concentrations of PUFA and CLA, which have potential beneficial effects on human health (Mele, 2009). Generally, vegetable oils (e.g., soybean, canola, linseed) are used as fat supplementation, whereas the use of alternative oils rich in n-3 PUFA, such as fish oil (FO), is not very common. Fish oil is characterized by a high concentration of long-chain unsaturated fatty acids, which have been shown to decrease methanogenesis (Fievez et al., 2003). This CH<sub>4</sub>-suppressing effect may relate to the degree of unsaturation of these FA as they undergo biohydrogenation in the rumen, their reactivity in the rumen, and their effects on specific rumen microorganisms (e.g., cellulolytic bacteria and protozoa). Although interesting, to the best of our knowledge, the existing experimental data on the effects of specific long-chain PUFA of FO on CH<sub>4</sub> emission are scarce. The few studies involved are primarily based on in vitro procedures (e.g., Fievez et al., 2003; Patra and Yu, 2013) or on in vivo studies conducted at pasture (Woodward et al., 2006). Particularly, Woodward et al. (2006) showed a positive effect of FO on reducing CH<sub>4</sub> emissions in a short-term study, whereas no reduction was observed for a longer-term study. Hence, there is a need for further in vivo research to evaluate the effects of FO on methanogenesis and animal productive performance.

The aim of the present study was to evaluate the effects of diets with different starch concentrations (using soybean hulls in partial replacement for corn meal)

supplemented or not with FO on productive performances, milk FA profile, digestibility, and methanogenesis of dairy cows.

## MATERIALS AND METHODS

### *Animals, Experimental Design, and Diets*

The experiment was conducted at the Research Center of the Department of Agricultural and Environmental Sciences, University of Milan, Italy. Trial animals were handled as outlined by the guidelines of the Italian law on animal welfare for experimental animals (Italian Ministry of Health, 1992) and of the University of Milan Ethics Committee for animal use and care. Four lactating secondiparous Italian Friesian cows with mean ( $\pm$ SD) BW of 617 kg ( $\pm$ 18), 177 DIM ( $\pm$ 46), and producing an average of 30.3 kg of milk/d ( $\pm$ 3.43) at the start of the trial were used. The experiment was conducted as a 4  $\times$  4 Latin square design balanced for carryover effect with a 2  $\times$  2 factorial arrangement: treatments were arranged to evaluate the main effects of 2 dietary starch concentrations (low vs. high), the presence or absence of FO supplement, and their interaction. The 4 dietary treatments were as follows: (1) low-starch diet (**LS**), (2) low-starch diet supplemented with FO (**LSO**), (3) high-starch diet (**HS**), and (4) high-starch diet supplemented with FO (**HSO**). The FO supplement (Danish Fish Oil HF, MagriOtello SRL, San Cesario sul Panaro, MO, Italy) was included in the LSO and HSO diets to provide a theoretical concentration of 0.80% on a DM basis. To balance for the different starch concentrations, corn meal and pelleted soybean hulls were included in the experimental diets in different proportions. In the 2 experimental diets supplemented with FO (0.80% DM), the same amount of corn meal was replaced by the fat supplement. The diets (Tables 1 and 2) were formulated using the CNCPS model (version 6.1; Cornell University, Ithaca, NY) to meet the protein and energy requirements of lactating cows weighing 625 kg and producing 32.0 kg of milk/d containing 4.60% fat and 3.49% CP, that represents the average milk yield at 100 DIM of the experimental cows. Due to the higher ME concentration (Mcal/kg of DM) of corn meal (3.3) compared with soybean hulls (2.8), the ME concentration of the HS diets was slightly higher than that of the LS diets.

Each cow was fed the 4 diets in 4 consecutive experimental periods of 26 d, including 21 d of adaptation and 5 d of sample collection and data registration. During the entire experiment, the cows had free access to water and were fed ad libitum twice daily (0730 and 1830 h). Orts were recorded once daily, and the feeding rate was adjusted to yield Orts on the basis of at

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