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## Association of Nitrate and Linseed Oil Effectively Reduces Methane Emission in Ruminants

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Using linseed oil or nitrate in diets are long-term viable strategies for mitigating enteric methane (CH<sub>4</sub>) emission from ruminants (Hristov *et al* 2013). These additives individually reduce methanogenesis by affecting the rumen hydrogen (H<sub>2</sub>) pool: linseed oil reduces H<sub>2</sub> production through its toxic effect against rumen protozoa (Doreau and Ferlay 1995) and nitrate acts as a H<sub>2</sub> sink through its reduction into nitrite then ammonia (Van Zijderveld *et al* 2010). As the mechanisms of action of these additives in the rumen are different, we hypothesized that their combination would lead to lower CH<sub>4</sub> emissions than when they are fed individually.

Four multiparous dry Holstein cows fitted with rumen cannulas were randomly assigned to 4 dietary treatments in a 4 x 4 Latin square design. Each experimental period lasted 5 weeks with measures performed in the last 2 weeks (wk1 and wk2). Diets were on a dry matter (DM) basis: 1) control diet (CON, 50% natural grassland hay and 50% concentrate), 2) control diet with 4% linseed oil (LIN), 3) control diet with 3% calcium nitrate (NIT), and 4) control diet with 4% linseed oil and 3% calcium nitrate (LIN+NIT). Diets were offered twice daily and formulated to have similar amounts of crude protein (12.2%), starch (25.5%) and neutral detergent fiber (NDF, 39.5%). Feed availability was restricted to 90% of voluntary intake (average 9.2 kg DM/day). Total tract digestibility of organic matter (OM) and NDF was determined from total feces collection for 6 days in wk1. Daily CH<sub>4</sub> emissions were determined in kinetics using open chambers for 4 days in wk2. Methane was expressed as a function of gross energy (GE) intake, DM intake and digested OM. Data were analyzed using the MIXED procedure of SAS (SAS, 2009) with a model including period and diet as fixed effects, and cow as random effect.

**Table 1. Digestibility and CH<sub>4</sub> emission of dry cows fed linseed oil and/or nitrate**

	Diet				s.e.	P Value
	CON	LIN	NIT	LIN+NIT		
CH <sub>4</sub> (% of GE intake)	7.2 <sup>a</sup>	5.6 <sup>b</sup>	5.8 <sup>b</sup>	4.8 <sup>c</sup>	0.20	<0.001
CH <sub>4</sub> (g/kg DM intake)	25.0 <sup>a</sup>	20.7 <sup>b</sup>	19.4 <sup>b</sup>	17.0 <sup>c</sup>	0.70	<0.001
CH <sub>4</sub> (g/kg digested OM)	46.2 <sup>a</sup>	37.6 <sup>b</sup>	35.1 <sup>bc</sup>	31.4 <sup>c</sup>	1.31	<0.001
OM digestibility (%)	66.6	66.9	67.2	66.4	1.19	0.891
NDF digestibility (%)	42.6	42.2	43.2	38.1	2.67	0.109

<sup>a,b</sup> Within a row, means without a common superscript differ (P<0.05).

Linseed oil and nitrate fed individually decreased CH<sub>4</sub> emissions by 17 and 22%, respectively (Table 1). These results are in accordance with previous data (Van Zijderveld *et al* 2010; Grainger and Beauchemin 2011). The association of these two additives induced the lowest CH<sub>4</sub> emissions (-32%), which can be explained by their different mechanisms of action throughout the day. Indeed, daily pattern of CH<sub>4</sub> emissions showed that LIN decreased emissions throughout the day, NIT strongly reduced production during the 3 hours postfeeding and LIN+NIT combined the two effects (data not shown). For the first time, we demonstrated the partial additivity of nitrate and linseed oil on methanogenesis in ruminants, without altering diet digestibility.

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