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## Short communication: Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type

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### ABSTRACT

3-Nitrooxypropanol (NOP) is a promising methane (CH<sub>4</sub>) inhibitor. Recent studies have shown major reductions in CH<sub>4</sub> emissions from beef and dairy cattle when using NOP but with large variation in response. The objective of this study was to quantitatively evaluate the factors that explain heterogeneity in response to NOP using meta-analytical approaches. Data from 11 experiments and 38 treatment means were used. Factors considered were cattle type (dairy or beef), measurement technique (GreenFeed technique, C-Lock Inc., Rapid City, SD; sulfur hexafluoride tracer technique; and respiration chamber technique), dry matter (DM) intake, body weight, NOP dose, roughage proportion, dietary crude protein content, and dietary neutral detergent fiber (NDF) content. The mean difference (MD) in CH<sub>4</sub> production (g/d) and CH<sub>4</sub> yield (g/kg of DM intake) was calculated by subtracting the mean of CH<sub>4</sub> emission for the control group from that of the NOP-supplemented group. Forest plots of standardized MD indicated variable effect sizes of NOP across studies. Compared with beef cattle, dairy cattle had a much larger feed intake ( $22.3 \pm 4.13$  vs.  $7.3 \pm 0.97$  kg of DM/d; mean  $\pm$  standard deviation) and CH<sub>4</sub> production ( $351 \pm 94.1$  vs.  $124 \pm 44.8$  g/d). Therefore, in further analyses across dairy and beef cattle studies, MD was expressed as a proportion (%) of observed control mean. The final mixed-effect model for relative MD in CH<sub>4</sub> production included cattle type, NOP dose, and NDF content. When adjusted for NOP dose and NDF content, the CH<sub>4</sub>-mitigating effect of NOP was less in beef cattle ( $-22.2 \pm 3.33\%$ ) than in dairy cattle ( $-39.0 \pm 5.40\%$ ). An increase of 10 mg/kg of DM in NOP dose from its mean (123 mg/kg of DM) enhanced the NOP effect on CH<sub>4</sub> production decline by  $2.56 \pm 0.550\%$ .

However, a greater dietary NDF content impaired the NOP effect on CH<sub>4</sub> production by  $1.64 \pm 0.330\%$  per 10 g/kg DM increase in NDF content from its mean (331 g of NDF/kg of DM). The factors included in the final mixed-effect model for CH<sub>4</sub> yield were  $-17.1 \pm 4.23\%$  (beef cattle) and  $-38.8 \pm 5.49\%$  (dairy cattle),  $-2.48 \pm 0.734\%$  per 10 mg/kg DM increase in NOP dose from its mean, and  $1.52 \pm 0.406\%$  per 10 g/kg DM increase in NDF content from its mean. In conclusion, the present meta-analysis indicates that a greater NOP dose enhances the NOP effect on CH<sub>4</sub> emission, whereas an increased dietary fiber content decreases its effect. 3-Nitrooxypropanol has stronger antimethanogenic effects in dairy cattle than in beef cattle.

**Key words:** 3-nitrooxypropanol, cattle, methane

### Short Communication

Enteric methane (CH<sub>4</sub>) production is among the main targets of greenhouse gas mitigation practices for the dairy and beef production sector. Several CH<sub>4</sub>-mitigation strategies have been proposed, including improving genetic potential, reproductive efficiency, and health of animals; increasing animal productivity; improving forage quality; and using feed additives (Hristov et al., 2013a,b). Recently, a compound called 3-nitrooxypropanol (NOP) has been reported to substantially decrease CH<sub>4</sub> emissions from ruminants (Duin et al., 2016). The molecular shape of NOP is similar to that of methyl-coenzyme M, and NOP specifically targets methyl-coenzyme M reductase (MCR), which catalyzes the last step in the CH<sub>4</sub>-forming pathway of rumen archaea (Duin et al., 2016). Several studies have investigated the effects of NOP on CH<sub>4</sub> emission in cattle, but the results have not been fully consistent. Large variation in response to addition of NOP was reported; namely, between a decrease of 84.3% (Vyas et al., 2016) and an increase of 7.1% (Vyas et al., 2018) in CH<sub>4</sub> production compared with the control diet. In a recent meta-analysis, Jayanegara et al. (2018)

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showed that increasing levels of NOP addition in diets of ruminants decreased enteric CH<sub>4</sub> emissions. In the present meta-analysis, we hypothesize that (in addition to NOP dose), DMI, nutrient composition of the diet, BW, and type of animal might explain the variability in NOP effect. The objective of this study was to quantitatively evaluate the factors that explain heterogeneity in response to NOP using meta-analytical approaches.

Literature searches of the Web of Science (Thomson Reuters Science, New York, NY), CAB Direct (CAB International, Wallingford, UK), and Scopus (Elsevier, Amsterdam, the Netherlands) online databases were conducted using keywords “NOP” (including all variants, such as “nitrooxypropanol”) + “cattle” + “methane” (or “CH<sub>4</sub>”). The search resulted in 12 articles related to effect of NOP on methane emissions. For inclusion in the database, the studies were required to include a control treatment group that did not receive NOP, to be conducted in vivo using cattle, and to include measured CH<sub>4</sub> production. Two articles were rejected because they reported in vitro experiments only. Another study (a short communication) was rejected because it repeated data from another paper included in our analysis. Data from 9 articles (11 experiments) met the selection criteria, and 38 treatment means were used for dairy cattle (Haisan et al., 2014, 2017; Reynolds et al., 2014; Hristov et al., 2015; Lopes et al., 2016) and beef cattle (Romero-Perez et al., 2014, 2015; Vyas et al., 2016, 2018). 3-Nitrooxypropanol was delivered twice daily directly into the rumen (Reynolds et al., 2014), top-dressed on a TMR that was offered once daily (Romero-Perez et al., 2014; NOP consumed by animals within 10 min of presentation), or was mixed in a TMR that was offered once daily (all others; continuous NOP dose). Methane emissions were estimated using the respiration chamber technique (7 studies; 1 involving dairy cattle and 6 involving beef cattle), the sulfur hexafluoride (SF<sub>6</sub>) tracer technique (2 studies, both involving dairy cattle), or the GreenFeed technique (2 studies, both involving dairy cattle; C-Lock Inc., Rapid City, SD). Usually, CH<sub>4</sub> production was reported in grams per day and CH<sub>4</sub> yield in grams per kilogram of DMI consumed. If reported in liters rather than grams, the values were converted assuming a molar weight of 16.0 g and volume of 22.4 L, respectively. A summary of the database is presented in Table 1.

Effect size estimates and corresponding sampling variances were obtained using the “metaphor” (version 2.0–0) and “robumeta” (version 2.0) packages in R (version 3.1.1, R Foundation for Statistical Computing, Vienna, Austria). The mean difference (MD) of CH<sub>4</sub> production or CH<sub>4</sub> yield was calculated as NOP treatment mean minus control treatment mean. Individual studies were weighted by their corresponding sample variation

**Table 1.** Descriptive statistics of feed intake, dietary characteristics, and methane emission<sup>1</sup>

Item	Dairy cattle					Beef cattle				
	Mean	Median	SD	Minimum	Maximum	Mean	Median	SD	Minimum	Maximum
DMI (kg/d)	22.3	19.5	4.13	18.3	28.0	7.3	7.3	0.97	5.5	8.7
Roughage proportion (% of diet DM)	55	60	7.7	38	61	42	60	28.3	8	70
NDF (g/kg of DM)	319	309	52.2	265	398	337	370	78.6	192	417
CP (g/kg of DM)	178	182	15.3	161	196	132	133	11.2	113	145
BW (kg)	632	664	44.0	573	673	422	376	89.9	319	637
NOP <sup>2</sup> dose (mg/kg of DM)	81	68	41.2	27	135	144	125	82.3	50	345
CH <sub>4</sub> production (g/d)	351	368	94.1	132	487	124	126	44.8	18	207
MD <sup>3</sup> CH <sub>4</sub> production (g/d)	-126	-147	64.7	-240	-27	-37	-28	32.6	-98	10
Relative MD CH <sub>4</sub> production (% of control)	-29.6	-30.8	16.89	-64.5	-6.4	-25.2	-17.5	23.80	-84.3	7.1
CH <sub>4</sub> yield (g/kg of DMI)	16.1	16.3	4.61	7.2	22.4	17.7	18.4	6.27	3.1	26.4
MD CH <sub>4</sub> yield (g/kg of DMI)	-5.2	-5.0	2.94	-10.6	-1.0	-4.0	-3.8	4.54	-13.3	3.2
Relative MD CH <sub>4</sub> yield (% of control)	-28.1	-29.1	16.41	-59.6	-4.8	-20.1	-18.6	24.86	-80.8	22.4

<sup>1</sup>Statistics of all diets (including control) except for mean difference (MD) characteristics, where statistics relate to 3-nitrooxypropanol treatment mean compared with control treatment mean.

<sup>2</sup>3-Nitrooxypropanol.

<sup>3</sup>MD (mean difference) is NOP treatment mean – control group mean.

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