

**Table S1.** In vivo experimental treatments resulting in 60% or more decrease in enteric methane ( $\text{CH}_4$ ) production.

Reference	Animal, diet	Inhibitor/algae (g/kg diet DM <sup>1</sup> )	Treatment period (d)	Inhibition relative to Control treatment (% decrease in $\text{CH}_4$ animal <sup>-1</sup> d <sup>-1</sup> )	Comments
Trei et al. (1971) experiment 1	Lambs, mixed	2, 2, 2-trichloroacetamide (0.10)	7	94 <sup>2</sup>	Decreased rumen acetate to propionate concentration ratio
Trei et al. (1971) experiment 2	Lambs, mixed	Hemiacetal of chloral and starch (2.0)	90	67 <sup>2</sup>	Additive tended to improve G: F
Johnson (1972)	Lambs, mixed	Hemiacetal of chloral and starch (2.2)	9	49 to 82	Restricted DMI. Inhibition was greater at high intake level
Johnson et al. (1972)	Steers, mixed	BCM (0.50)	28	~65 <sup>2,3</sup>	ADG unaffected
Johnson (1974)	Lambs, high concentrate	Hemiacetal of chloral and starch (1.1)	3	64	N retention improved
Sawyer et al. (1974)	Sheep	BCM (0.1 – 0.3)	26	83 to 86	Improved DM and N digestibility
Davies et al. (1982)	Calves, mixed	ICI 13409 (0.20)	196	63 <sup>2</sup>	Improved ADG and G: F
Mathers and Miller (1982)	Ewes, mixed	Chloral hydrate (intraruminal, ~ 1-4)	10	96	Digestibility unaffected. Decreased rumen acetate to propionate concentration ratio
McCrabb et al. (1997)	Steers, roughage	BCM (0.26)	28	$\text{CH}_4$ not detected	No difference in final body mass
Tomkins and Hunter (2004)	Steers, high concentrate	BCM (0.15-0.20)	28	88-91	No effects on animal performance

Knight et al. (2011)	Cows, roughage	Chloroform (0.27)	42	~38 to 97 <sup>6</sup>	Extent of inhibition of methanogenesis decreased as the experiment progressed
Mitsumori et al. (2012)	Goats, mixed	BCM (2.6)	22	91	No effects on digestibility. Decreased rumen acetate to propionate concentration ratio
Haisan et al. (2014)	Cows, mixed	3-NOP (0.13)	28	60	No change in DMI and milk production and composition. Greater body mass recovery with 3-NOP
Romero-Perez et al. (2015)	Heifers, mixed	3-NOP (0.28)	112	59	No effect on ADG <sup>4</sup> . Decreased rumen acetate to propionate concentration ratio
Li et al. (2016)	Wethers, mixed	<i>Asparagopsis taxiformis</i> (2.7)	72	81	No effects on ADG. Decreased rumen acetate to propionate concentration ratio. Ruminal mucosa alterations identified
Martinez-Fernandez et al. (2016)	Steers, mixed	Chloroform (1.50)	10	65	Decreased rumen acetate to propionate concentration ratio
Vyas et al. (2016), finishing diet	Steers, high concentrate	3-NOP (0.2)	105	84	DMI and ADG tended to decrease
McGinn et al. (2019)	Cattle, high concentrate	3-NOP (0.125)	123	~70	Experiment conducted in a commercial feedlot
Roque et al. (2019)	Cows, mixed	<i>Asparagopsis armata</i> (10)	21	67	DMI decreased. Milk production and protein decreased with highest dose
Kinley et al. (2020)	Steers, high concentrate	<i>Asparagopsis taxiformis</i> (1.8)	90	98	Increased ADG
Roque et al. (2021)	Steers, high concentrate	<i>Asparagopsis taxiformis</i> (4.7)	63	82	No effects on ADG. Tendency to decreased DMI and improved F:

					G. No effects on carcass or meat quality
Alemu et al. (2021)	Steers, high concentrate	3-NOP (0.108)	112	77	Decreased DMI
Cristobal-Carballo et al. (2021)	Calves, milk replacer, concentrate, partial mixed ration, pasture	Chloroform (0.050) plus 9, 10-anthraquinone (0.50)	84	90 <sup>3</sup>	No effects on DMI and ADG
Garcia et al. (2022)	Cows, mixed	3-NOP (0.10)	15	60	Decrease in CH <sub>4</sub> despite more methanogenic diet in 3-NOP period

<sup>1</sup>Abbreviations: 3-NOP = 3-nitrooxypropanol; ADG = average daily body mass gain; BCM = bromochloromethane; CH<sub>4</sub> = methane; DM = dry matter; DMI = dry matter intake; G: F = body mass gain per kilogram of dry matter intake; ICI 13409 = 2,4-bis(trichloromethyl)-benzo [1, 3]dioxin-6-carboxylic acid .

<sup>2</sup>Methane concentration, rather than production, was measured, by rumenotomy (Treij et al., 1971; Treij et al., 1972), rumen headspace sampled through the cannula (Johnson et al., 1972), or air expelled in hood (Davies et al., 1982).

<sup>3</sup>Daily average, estimated from graph.

<sup>4</sup>A. Romero-Perez, pers. comm.

## References

- Alemu, A.W., Shreck, A.L., Booker, C.W., McGinn, S.M., Pekrul, L.K.D., Kindermann, M. *et al.* (2021). Use of 3-nitrooxypropanol in a commercial feedlot to decrease enteric methane emissions from cattle fed a corn-based finishing diet. *J. Anim. Sci.* 99. doi: 10.1093/jas/skaa394
- Cristobal-Carballo, O., McCoard, S.A., Cookson, A.L., Ganesh, S., Lowe, K., Laven, R.A. *et al.* (2021). Effect of Methane Inhibitors on Ruminal Microbiota During Early Life and Its Relationship With Ruminal Metabolism and Growth in Calves. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.710914
- Davies, A., Nwaonu, H.N., Stanier, G., and Boyle, F.T. (1982). Properties of a novel series of inhibitors of rumen methanogenesis; in vitro and in vivo experiments including growth trials on 2,4-bis (trichloromethyl)-benzo [1, 3]dioxin-6-carboxylic acid. *Brit. J. Nutr.* 47: 565-576. doi: 10.1079/bjn19820068
- Garcia, F., Muñoz, C., Martínez-Ferrer, J., Urrutia, N.L., Martínez, E.D., Saldivia, M. *et al.* (2022). 3-Nitrooxypropanol substantially decreased enteric methane emissions of dairy cows fed true protein- or urea-containing diets. *Heliyon* 8: e09738. doi: <https://doi.org/10.1016/j.heliyon.2022.e09738>
- Haisan, J., Sun, Y., Guan, L.L., Beauchemin, K.A., Iwaasa, A., Duval, S. *et al.* (2014). The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation. *J. Dairy Sci.* 97: 3110-3119. doi: 10.3168/jds.2013-7834
- Johnson, D.E. (1972). Effects of a hemiacetal of chloral and starch on methane production and energy balance of sheep fed a pelleted diet. *J. Anim. Sci.* 35: 1064-1068. doi: not available
- Johnson, D.E. (1974). Adaptational responses in nitrogen and energy balance of lambs fed a methane inhibitor. *J. Anim. Sci.* 38: 154-157. doi: not available
- Johnson, E.D., Wood, A.S., Stone, J.B., and Moran Jr, E.T. (1972). Some effects of methane inhibition in ruminants (steers). *Can. J. Anim. Sci.* 52: 703-712. doi: 10.4141/cjas72-083
- Kinley, R.D., Martinez-Fernandez, G., Matthews, M.K., de Nys, R., Magnusson, M., and Tomkins, N.W. (2020). Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. *J. Clean. Prod.* 259: 120836. doi: 10.1016/j.jclepro.2020.120836
- Knight, T., Ronimum, R.S., Dey, D., Toottill, C., Naylor, G., Evans, P. *et al.* (2011). Chloroform decreases rumen methanogenesis and methanogen populations without altering rumen function in cattle. *Anim. Feed Sci. Technol.* 166-167: 101-112. doi: 10.1016/j.anifeedsci.2011.04.059
- Li, X., Norman, H.C., Kinley, R.D., Laurence, M., Wilmot, M., Bender, H. *et al.* (2016). *Asparagopsis taxiformis* decreases enteric methane production from sheep. *Anim. Prod. Sci.* 58: 681-688. doi: [doi.org/10.1071/AN15883](https://doi.org/10.1071/AN15883)
- Martinez-Fernandez, G., Denman, S.E., Yang, C., Cheung, J., Mitsumori, M., and McSweeney, C.S. (2016). Methane inhibition alters the microbial community, hydrogen flow, and fermentation response in the rumen of cattle. *Front. Microbiol.* 7: 1122. doi: 10.3389/fmicb.2016.01122
- Mathers, J.C., and Miller, E.L. (1982). Some effects of chloral hydrate on rumen fermentation and digestion in sheep. *J. Agric. Sci. (Camb.)* 99: 215-224. doi: 10.1017/S0021859600055234

- McCrabb, G.J., Berger, K.T., T., M., May, C., and Hunter, R.A. (1997). Inhibiting methane production in Brahman cattle by dietary supplementation with a novel compound and the effects on growth. *Aust. J. Agr. Res.* 48: 323-329. doi: 10.1071/AR96119
- McGinn, S.M., Flesch, T.K., Beauchemin, K.A., Shreck, A., and Kindermann, M. (2019). Micrometeorological Methods for Measuring Methane Emission Reduction at Beef Cattle Feedlots: Evaluation of 3-Nitrooxypropanol Feed Additive. *J. Environ. Qual.* 48: 1454-1461. doi: <https://doi.org/10.2134/jeq2018.11.0412>
- Mitsumori, M., Shinkai, T., Takenaka, A., Enishi, O., Higuchi, K., Kobayashi, Y. et al. (2012). Responses in digestion, rumen fermentation and microbial populations to inhibition of methane formation by a halogenated methane analogue. *Brit. J. Nutr.* 108: 482-491. doi: 10.1017/S0007114511005794
- Romero-Perez, A., Okine, E.K., McGinn, S.M., Guan, L.L., Oba, M., Duval, S.M. et al. (2015). Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. *J. Anim. Sci.* 93: 1780-1791. doi: 10.2527/jas.2014-8726
- Roque, B.M., Salwen, J.K., Kinley, R., and Kebreab, E. (2019). Inclusion of *Asparagopsis armata* in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. *J. Clean. Prod.* 234: 132-138. doi: 10.1016/j.jclepro.2019.06.193
- Roque, B.M., Venegas, M., Kinley, R.D., de Nys, R., Duarte, T.L., Yang, X. et al. (2021). Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. *Plos One* 16: e0247820. doi: 10.1371/journal.pone.0247820
- Sawyer, M.S., Hoover, W.H., and Sniffen, C.J. (1974). Effect of a ruminal methane inhibitor on growth and energy metabolism in the ovine. *J. Anim. Sci.* 38: 908-914. doi: not available
- Tomkins, N.W., and Hunter, R.A. (2004). "Methane reduction in beef cattle using a novel antimethanogen," in. Proceedings of the Australian Society of Animal Production 25th Biennial Conference : the new realities, ed. R. Stockdale, J. Heard and M. Jenkin (University of Melbourne, Parkville, Vic.: CSIRO Publishing 329.
- Trei, J.E., Parish, R.C., and Singh, Y.K. (1971). Effect of methane inhibitors on rumen metabolism and feedlot performance of sheep. *J. Dairy Sci.* 54: 536-540. doi: not available
- Trei, J.E., Scott, G.C., and Parish, R.C. (1972). Influence of methane inhibition on energetic efficiency of lambs. *J. Anim. Sci.* 34: 510-515. doi: not available
- Vyas, D., McGinn, S.M., Duval, S.M., Kindermann, M., and Beauchemin, K.A. (2016). Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle. *J. Anim. Sci.* 94: 2024-2034. doi: 10.2527/jas.2015-0268