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Use of 3-nitrooxypropanol as feed additive for mitigating enteric methane emissions from ruminants: a meta-analysis

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ABSTRACT

This study aimed to perform a meta-analysis on the effect of 3-nitrooxypropanol (3-NOP) on enteric methane (CH_4) emissions from ruminants. A total of 12 *in vivo* studies from 10 articles were integrated into a database. Ruminant species included were dairy cows, beef cattle and sheep. Concentration of 3-NOP in diets varied from 0 to 280 mg/kg dry matter intake (DMI). Parameters included were CH_4 emissions, rumen fermentation, microbial population, nutrient digestibility and animal performance. Meta-analysis of data was performed by using mixed model methodology in which different studies were treated as random effects whereas 3-NOP addition levels in diets of ruminants were treated as fixed effects. Results showed that increasing level of 3-NOP addition in diets of ruminants decreased enteric CH_4 emissions per unit of body weight, CH_4/DMI, CH_4/milk produced, CH_4/digested organic matter or CH_4/gross energy intake ($p < .05$). Production of H_2 was higher with increasing level of 3-NOP addition ($p < .001$). Addition of 3-NOP decreased total VFA concentration ($p < .01$), and decreased and increased proportions of C_2 and C_3, respectively ($p < .001$). Addition of 3-NOP decreased archaea population ($p < .01$) but it did not change total bacteria and protozoa populations. The substance had minor effect on digestibility of nutrients. Production performance of dairy cows and beef cattle was limitedly influenced by the addition of 3-NOP in the diets, and it had no negative effect on DMI of ruminants. It is concluded that 3-NOP is an effective feed additive to mitigate enteric CH_4 emissions without compromising productive performance of ruminants.

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Introduction

Methane (CH_4) has been considered as a main greenhouse gas (GHG) after carbon dioxide (CO_2) that causes global warming phenomenon. Although it is the second in term of quantity, the capacity of CH_4 to retain heat or so-called global warming potential is 28 times greater than that of CO_2 at a time horizon of 100 years (Tian et al. 2016). Livestock particularly ruminants contribute to the accumulation of atmospheric CH_4, and contribution of enteric fermentation accounts for 17% of global methane sources (Knapp et al. 2014). Methane in the rumen of ruminants is produced by methanogenic archaea that utilise CO_2 and H_2 as their primary substrates to generate CH_4 (Morgavi et al. 2010), although other substrates may also be used to a lesser extent. Methane emitted by ruminants does not only contribute to global warming but it also represents considerable energy loss and thus cannot be used by the animals for production purposes. Therefore, mitigating enteric methane emissions from ruminants is favourable from the point of environmental conservation and animal production.

Various approaches have been attempted to mitigate enteric methane emissions, including the use of feed additives. Scientists have developed certain compounds that directly target methanogenesis in the rumen such as bromoethane sulphonate (BES) and bromopropane sulphonate (BPS). These compounds are specific inhibitors of methyl-coenzyme M reductase (MCR), an enzyme that is involved in methane formation of methanogenic archaea at the terminal step. However, BES has been considered as a toxic substance (Grawert et al. 2014), making its use as a feed additive is not authorised. In recent years, another molecule that specifically inhibit MCR has been developed, that is, 3-nitrooxypropanol (3-NOP; Duval and

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Kindermann 2012). Apparently the earliest work reported the use of nitrocompounds for reducing ruminal CH_4 emissions was Anderson and Rasmussen (1998). Since then various nitrocompounds have been investigated such as 2-nitropropanol, nitroethane, nitroethanol, 2-nitro-1-propanol, 3-nitro-1-propionic acid, dimethyl-2-nitroglutarate and 2-nitro-methyl-propionate (Anderson et al. 2003, 2010). With regard to 3-NOP, the compound has been experimentally tested to some ruminant species, that is, dairy cows, beef cattle and sheep at various levels, and generally the molecule is able to lower enteric CH_4 emissions. However, to date, there is no study attempted to quantitatively summarise the effect of 3-NOP in mitigating enteric methane emissions.

The objective of the present study therefore was to perform a meta-analysis from published experiments on the effect of 3-NOP on methane emissions from ruminants. Other related parameters such as rumen fermentation, nutrient digestibility, blood metabolites, production performance of ruminants and product characteristics were also evaluated in order to comprehensively assessed the effect of 3-NOP on ruminants.

Materials and methods

Database development

A database was developed from studies that reported the use of 3-NOP to mitigate enteric methane emissions originated from ruminants. Criteria for an article to be included in the database were: (1) experiments were performed directly to ruminants (*in vivo*), not *in vitro*, (2) methane emissions were directly measured, not estimated by any predictive equations and (3) articles were published in English. A total of 12 studies from 10 articles were integrated into the database (Haisan et al. 2014, 2017; Martinez-Fernandez et al. 2014; Reynolds et al. 2014; Romero-Perez et al. 2014, 2015; Hristov et al. 2015; Lopes et al. 2016; Vyas et al. 2016a, 2016b). Number of articles reported 3-NOP were not many since it is considered as a novel compound for mitigating enteric methane emissions. Ruminant species included were dairy cows, beef cattle and sheep. Breeds for dairy cows, beef cattle and sheep were Friesian Holstein, Angus and Segurena, respectively. Diets provided for ruminants were total mixed ration (TMR) with either high forage or high concentrate diets. Concentration of 3-NOP in diets varied from 0 to 280 mg/kg dry matter intake (DMI). Methane emissions were measured either by employing sulphur hexafluoride (SF_6) tracer technique, Green Feed system or respiratory chamber. Parameters included were enteric methane emissions, rumen fermentation and microbial population, nutrient digestibility, blood metabolites, production performance of animals and milk composition.

Data analysis

Prior to statistical meta-analysis, data were transformed into similar units of measurements in order to enable direct analysis within a particular parameter. Values in some parameters were transformed as proportions to body weight of the animals to account for the difference among ruminant species, especially between small and large ruminants. Rumen microbial population data were transformed into their logarithmic values to allow linear relationship with the dependent variable. Meta-analysis of data was performed by using mixed model methodology according to St-Pierre (2001), in which different studies were treated as random effects whereas 3-NOP addition levels in diets of ruminants were treated as fixed effects. Model statistics used were p value, root mean square error (RMSE) and coefficient of determination (R^2). The statistical analysis was performed in SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA) by using mixed procedure (PROC MIXED).

Results

Increasing level of 3-NOP addition in diets of ruminants decreased enteric CH_4 emissions per unit of BW, CH_4/DMI, CH_4/milk produced or CH_4/DOM ($p < .05$; Table 1). Amount of energy loss as CH_4, proportionally to GEI, was also reduced by addition of 3-NOP ($p < .001$; Figure 1(a,b)). Production of H_2 was higher with increasing level of 3-NOP addition ($p < .001$). The substance had minor effect on digestibility of nutrients (DM, OM, CP, NDF and ADF). Addition of 3-NOP increased rumen pH ($p < .01$) but decreased total VFA concentration ($p < .01$; Table 2). With regard to VFA composition, proportions of C_2 and C_3 were decreased and increased ($p < .001$), respectively, due to 3-NOP addition. The 3-NOP also altered C_4 and C_5 proportions ($p < .01$). A decrease of rumen NH_3 concentration was observed with increasing level of 3-NOP addition ($p < .01$). Addition of 3-NOP decreased archaea population ($p < .01$) but it did not change total bacteria and protozoa populations.

Blood metabolites such as glucose, insulin, NEFA, BHBA and BUN were not affected due to 3-NOP addition (data not shown). Production performance of dairy cows (milk production, FCM, ECM, efficiency of milk production) and beef cattle (ADG) was limitedly

Table 1. Effects of 3-nitrooxypropanol (3-NOP) addition (in g/kg DMI) on enteric methane (CH_4) emissions and nutrient digestibility of ruminants.

Response parameter	Unit	*n*	Intercept	SE intercept	Slope	SE slope	*p* value	RMSE	R^2
Methane									
CH_4/BW	g/kg BW	39	0.486	0.04	−0.94	0.19	<.001	0.083	0.420
CH_4/DMI	g/kg DMI	39	20.2	1.25	−38.7	6.3	<.001	2.73	0.590
CH_4/milk	g/kg milk	12	14.0	1.90	−29.5	11.9	.042	1.80	0.459
CH_4/DOM	g/kg DOM	10	30.6	1.32	−54.6	13.3	.006	2.99	0.679
H_2	g/kg DMI	23	0	0	1.26	0.190	<.001	64.9	0.681
Digestibility									
DMD	%	12	69.8	3.99	6.3	6.4	.360	1.45	0.125
OMD	%	10	69.7	3.57	5.4	6.8	.455	1.44	0.100
CPD	%	10	64.7	2.05	8.8	9.5	.391	2.03	0.144
NDFD	%	10	48.8	8.25	14.0	11.6	.281	2.47	0.191
ADFD	%	7	55.8	11.7	−1.5	11.1	.900	2.11	0.003

ADFD: acid detergent fibre digestibility; BW: body weight; CPD: crude protein digestibility; DMD: dry matter digestibility; DMI: dry matter intake; DOM: digested organic matter; H_2: hydrogen; *n*: number of data; NDFD: neutral detergent fibre digestibility; OMD: organic matter digestibility; R^2: coefficient of determination; RMSE: root mean square error; SE: standard error.

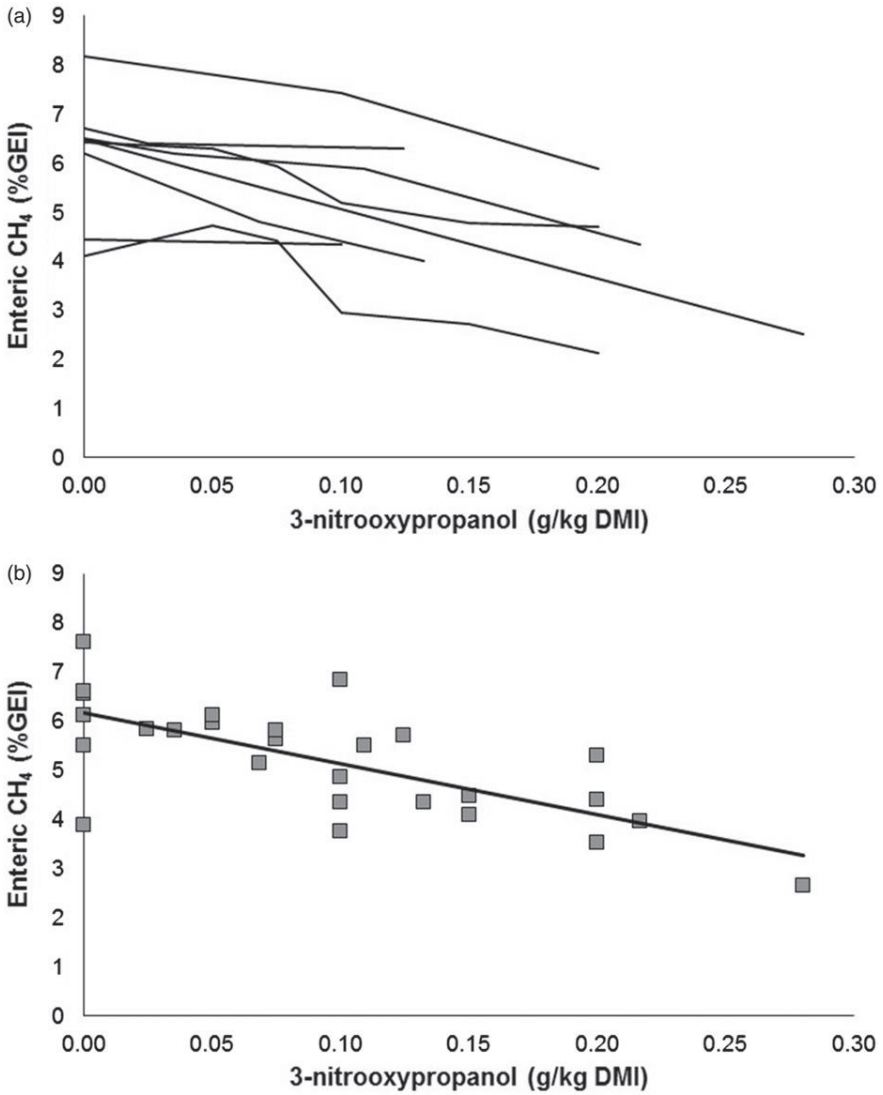

Figure 1. Effects of 3-nitrooxypropanol (3-NOP) addition (in g/kg dry matter intake, DMI) on enteric methane (CH_4) emissions as percentage of gross energy intake (GEI) of ruminants, before (a) and after (b) meta-analysis. CH_4 (%GEI) = 6.16–10.3 × 3-NOP ($n = 29$; $p < .001$; RMSE = 0.90; $R^2 = 0.491$).

Table 2. Effects of 3-nitrooxypropanol (3-NOP) addition (in g/kg DMI) on rumen fermentation and microbial population of ruminants.

Response parameter	Unit	*n*	Intercept	SE intercept	Slope	SE slope	*p* value	RMSE	R^2
pH average		14	6.40	0.046	0.56	0.13	.002	0.038	0.694
Total VFA	mmol/L	18	113	8.30	−35.0	10.7	.008	3.41	0.524
C_2	%	18	62.3	1.90	−31.0	4.1	<.001	1.31	0.855
C_3	%	18	19.6	1.24	18.0	3.8	<.001	1.22	0.703
C_4	%	18	12.8	0.53	9.3	2.3	.002	0.740	0.625
isoC_4	%	18	1.13	0.178	0.12	0.08	.180	0.026	0.171
C_5	%	18	1.81	0.116	2.1	0.22	<.001	0.070	0.899
isoC_5	%	18	1.68	0.213	1.6	0.75	.063	0.241	0.285
C_6	%	8	0.788	0.137	1.3	0.92	.244	0.148	0.301
C_2/C_3		18	3.34	0.293	−4.2	0.66	<.001	0.211	0.800
NH_3	mg/dL	16	8.50	1.56	−7.4	1.7	.002	0.505	0.691
Bacteria	log cell/mL	15	10.9	0.18	0.36	0.35	.328	0.106	0.124
Protozoa	log cell/mL	13	5.35	0.087	0.07	0.33	.850	0.100	0.001
Archaea	log cell/mL	15	8.42	0.301	−1.3	0.3	.002	0.091	0.720

C_2: acetate; C_3: propionate; C_4: butyrate; C_5: valerate; C_6: caproate; *n*: number of data; NH_3: ammonia; R^2: coefficient of determination; RMSE: root mean square error; SE: standard error; VFA: volatile fatty acid.

Table 3. Effects of 3-nitrooxypropanol (3-NOP) addition (in g/kg DMI) on production performance of ruminants.

Response parameter	Unit	*n*	Intercept	SE intercept	Slope	SE slope	*p* value	RMSE	R^2
DMI	g/kg BW	39	27.7	2.76	−1.15	3.2	.722	1.37	0.003
Dairy cow									
Milk production	kg/d	14	33.8	3.34	−9.9	5.6	.116	0.986	0.279
FCM	kg/d	14	32.6	3.62	−1.8	6.3	.789	1.12	0.009
ECM	kg/d	11	35.0	3.82	−4.2	5.5	.480	0.83	0.083
Efficiency	kg FCM/kg DMI	14	1.45	0.089	0.04	0.19	.840	0.034	0.004
Milk fat	%	14	3.78	0.172	1.5	0.57	.034	0.100	0.467
Milk protein	%	14	3.22	0.088	0.46	0.22	.067	0.038	0.371
Milk lactose	%	14	4.60	0.090	0.15	0.11	.212	0.019	0.189
Beef cattle									
ADG	kg/d	5	1.43	0.088	−0.65	0.8	.477	0.134	0.180
G:F	g/g	5	0.143	0.00085	0.05	0.008	.007	0.001	0.938

ADG: average daily gain; DMI: dry matter intake; ECM: energy corrected milk; FCM: fat corrected milk; G:F: gain to feed ratio; *n*: number of data; R^2: coefficient of determination; RMSE: root mean square error; SE: standard error.

influenced by the addition of 3-NOP in the diets except for G:F (Table 3); the G:F ratio was improved by the addition of 3-NOP ($p < .01$). Further, it had no negative effect on DMI of ruminants. The 3-NOP increased milk fat concentration ($p < .05$) and tended to elevate milk protein concentration ($.05 < p < .1$). Concentration of lactose, MUN and milk FA profiles were not influenced by addition of 3-NOP (data not shown).

Discussion

Effects of 3-nitrooxypropanol on CH_4 emissions, rumen fermentation and nutrient digestibility

The present meta-analysis study reveals that 3-NOP is an effective feed additive to mitigate enteric methane emissions from ruminants. The effect of methane decrease is linear within the range of 3-NOP between 0 and 280 mg/kg DMI. Use of 3-NOP at 100 mg/kg DMI, for instance, would decrease CH_4/BW, CH_4/DMI, CH_4/milk produced, CH_4/DOM and CH_4/GEI by 19.3%, 19.2%, 21.1%, 17.8% and 16.7% from control (diet unsupplemented with 3-NOP), respectively. When the 3-NOP used is 200 mg/kg DMI, it would theoretically decrease CH_4 emissions by double, that is, 33.4–42.1%. Mechanism by which 3-NOP reduce CH_4 emissions has been elucidated by Duin et al. (2016). The 3-NOP is structurally analogue to methyl-coenzyme M, a cofactor in the terminal step of methanogenesis that transfers a methyl group to MCR. The MCR itself contains nickel bound in a tetrapyrrole derivative known as cofactor F_{430} (Thauer et al. 2008). In order to be in the active form, the nickel-containing cofactor should be in the Ni(I) oxidation state. The 3-NOP acts by directly targeting MCR and oxidising its active site Ni(I) in which the MCR inactivation by the molecule is accompanied by formation of nitrate, nitrite and 1,3-propanediol (Duin et al. 2016). Hristov et al. (2015) suggested that 3-NOP should be continuously delivered to ruminants by mixing it into daily ration in order to obtain consistent CH_4-mitigating effect effectively; if the compound is fed as a pulse-dose, reduction of CH_4 emissions would be transient.

An elevation of H_2 concentration due to 3-NOP addition is expected since methanogenesis is a main sink of H_2 in the rumen. The H_2 itself is an important

metabolic intermediate in the rumen and serves as a substrate for methanogenic archaea to generate energy. Therefore, inhibition of methanogenesis would theoretically reduce utilisation of H_2 and thus increase its concentration. Elevation of H_2 further leads to down-regulation of H_2-generating pathways and up-regulation of H_2-consuming pathways (Janssen 2010). Simultaneous decrease of acetate (that produce H_2) and increase of propionate (that consume H_2) proportions found in this meta-analysis study is apparently related to the mechanisms of H_2 regulation in the rumen. Additionally, formation of propionate is considered to be the second most important H_2 sink in the rumen after methanogenesis (McAllister and Newbold 2008). Such accumulation of H_2 is typical when using feed additives that act as methanogenesis inhibitors (Soliva et al. 2011; O'Brien et al. 2014).

It is interesting to note that although H_2 is accumulated by the addition of 3-NOP, it does not hamper nutrient digestibility in the digestive tract of ruminants. The increase of H_2 partial pressure is generally known to cause negative feedback on fermentation process in the rumen, especially fermentation that involves cofactors such as NADH, NADPH and reduced ferridoxins (Leng 2014). A plausible explanation is that there might be a shift from H_2 to formate when adding methanogenesis inhibitor like 3-NOP. Unfortunately formate concentration was not reported in the literatures used for meta-analysis and thus could not be integrated in the database. Through this mechanism H_2 partial pressure remains low or within the range that allows cofactor regeneration and, hence, fermentation to continue (Leng 2014). Further, formate may diffuse to rumen liquid phase to form HCO_3^- and H_2 (Leng 2014), and formation of the former product may increase the buffering capacity of rumen fluid. This is confirmed by the increase of average pH in the rumen with addition of 3-NOP.

Since methanogenesis is a pathway to generate energy for methanogenic archaea, addition of 3-NOP apparently causes energy deprivation on the microbes thus reduces their population as confirmed in the present meta-analysis study. The 3-NOP has minor effect on bacteria and protozoa populations, and this confirms the specificity of the compound to target MCR that present only in archaea methanogen, not in other rumen microbes. Although there is a symbiotic relationship between protozoa and methanogens in which protozoa become the hosts of some methanogen population through inter-species H_2 transfer (Morgavi et al. 2010), apparently such symbiosis is not affected in the presence of 3-NOP. In agreement with the present finding, a study from Karnati et al. (2008) reported that BES, a specific methanogenesis inhibitor other than 3-NOP, did not affect population and generic distribution of protozoa such as *Entodinium*, *Diplodiniinae*, *Epidinium*, *Isotricha* and *Dasytricha*.

Effects of 3-nitrooxypropanol on animal performance and product quality

It is apparent that 3-NOP does not compromise feed intake, productive performance and product quality of ruminants. Improvement of G:F observed in beef cattle may suggest a more efficient of energy utilisation by the animals through reduction of energy loss via CH_4 emissions, although it has to be interpreted cautiously due to the small number of data available. Such more efficient energy utilisation may lead to performance improvement when GE intake and nutrient digestibility are not reduced due to 3-NOP addition (Vyas et al. 2016b). Despite DMI and nutrient digestibility are not negatively affected by 3-NOP, G:F improvement is relatively modest, taking into consideration that energy saving through CH_4 mitigation is obvious. It seems that a decrease in total VFA concentration by addition of 3-NOP may explain the effect since VFA can be converted to energy and may contribute up to 70% of energy requirement of ruminants (Bergman 1990). Additionally, the accumulation of H_2 due to 3-NOP addition may also explain the effect since its production is energetically inefficient, as H_2 possesses a high energy yield, that is, 122 kJ/g (Sharma and Ghoshal 2015). A more efficient use of energy by adding 3-NOP is not reflected from production performance (milk production and milk efficiency) of dairy cows. Despite such fact, Haisan et al. (2014) and Hristov et al. (2015) recorded a greater body weight gain of lactating dairy cows (the parameter was not integrated in the current meta-analysis) fed with 3-NOP in comparison to control diets. The increase of body weight gain of dairy cows in both experiments was observed during mid-lactation in which milk production is not an energetic priority during that lactation phase. Therefore, such condition is considered as a better energy balance by 3-NOP addition. Kirkland and Gordon (2001) observed that with the advancement of lactation stage, partition of energy intake toward milk energy decreases but the partition toward body tissue energy increases. An increase of milk fat concentration of lactating dairy cows administered with 3-NOP is apparently related to an increase of energy availability as a result of CH_4 reduction.

Conclusions

The 3-NOP is apparently an effective feed additive to mitigate enteric methane emissions from ruminants.

Methane decrease by addition of 3-NOP is accompanied by an elevation of H_2 concentration and simultaneous decrease of acetate and increase of propionate proportions. Methanogenic archaea population is reduced by 3-NOP without affecting bacteria and protozoa populations. Further, 3-NOP does not hamper nutrient digestibility in the digestive tract of ruminants, and it does not compromise feed intake, productive performance and product quality of ruminants. Despite the promising effects of 3-NOP, further studies are required to assess carry-over of the compound into animal products and food safety concern when the products are consumed by human.

Disclosure statement

The authors declare that there is no conflict of interest.

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