Final report

Methane emissions of Australian feedlot cattle as influenced by 3-Nitrooxypropanol (Bovaer 10®)

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Abstract

Based on recent overseas literature, we hypothesized that methane (CH_4) reduction will be observed when feeding feedlot cattle increasing diet concentrations of 3-Nitrooxypropanol (3-NOP; Bovaer®, DSM Nutritional Products, Switzerland). In addition, the effect of 3-NOP on feed intake and ruminal fermentation profile was also investigated. This project also validated current methods for predicting methane production of feedlot cattle including the Moe and Tyrrell equations and the IPCC Tier 2 methodology.

The effect of increasing diet concentration of 3-NOP on rumen fermentation and methane emissions was evaluated with Angus steers fed typical Australian feedlot diets. Twenty Angus steers of initial liveweight (LW) of 356 ± 14.4 kg were allocated in a completely randomised block design. The experimental period was 112 days in which steers were housed in individual indoor pens, including the first 21 days of adaptation. Five different regimens were compared. Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112; and High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d 15 to 21, 125 mg/kg DM from d 22 to 112. For the 3-NOP titration regimens, the adaptation period followed a step-wise approach in terms of both increasing 3-NOP diet concentration and increasing grain level in the diet. The CH_4 emission, ruminal parameters and performance traits were measured. By using control steers, we assessed the accuracy of the equation used in the current National Inventory CH_4 calculations [Moe and Tyrrell (1979)].

This project demonstrated that all 3-NOP regimens significantly decreased methane of feedlot cattle from d 21 to 112 of the feeding period. The maximum CH_4 production abatement from 3-NOP was observed in day 28, averaging 99% reduction compared to control steers. For the overall feeding period, methane emissions (g/d) were 78% lower in the animals that followed the Medium B titration regimen than control animals. Further investigation is required in accelerated high-concentrate start-up phases to determine if greater methane inhibition is possible. Although this experiment was not designed to make conclusions on an effect of 3-NOP on feedlot performance (cattle were limit fed to 2.7 x maintenance; 4 steers per regimen) no negative effects of any 3-NOP regimen were detected on production parameters.

Using the data from control steers, is reported that the current National Inventory equation used to predict methane emissions from feedlot cattle appraisal in the Australian National Inventory, resulted in considerable overprediction of CH_4, by 95.5 g/day. Thus, it is proposed that the IPCC (2006) equation should yield more accurate CH_4 predictions. Additional research is recommended to further build a database of Australian feedlot cattle emissions on a range of diets.

Ultimately, the knowledge on the CH_4 suppression pattern of 3-NOP may facilitate sustainable pathways for the feedlot industry as well as enable Australian producers to benefit from carbon credit trading schemes.

Executive summary

Background

This project determined the effect of increasing diet concentration of 3-Nitrooxypropanol (3-NOP; Bovaer®, DSM) on methane (CH_4) emissions and rumen fermentation patterns of cattle fed typical Australian feedlot diets. It also evaluated the current baseline CH_4 prediction-equations for feedlot cattle in the Australian Government GHG accounting framework. Australian National Greenhouse Accounts uses the Moe & Tyrell (1979) methane calculation methodology for inventory purposes, whereas countries such as the United States utilise the IPCC Tier 2 methodology for estimating feedlot emissions. A comparison of the two methods is required.

Objectives

The project objectives were to:

- Determine the effect of 3-NOP titration regimen on rumen fermentation and methane emissions during starter- and finishing phases in beef cattle; and
- Validate current methods for predicting methane production of feedlot cattle, including the Moe and Tyrrell equations and the IPCC Tier 2 methodology.

Methodology

The effect of increasing diet concentrations of 3-NOP, and a placebo (control) was evaluated on rumen fermentation and methane emissions of Angus steers on feedlot diets. Twenty Angus steers of initial liveweight (LW) of 356 ± 14.4 kg and approximately 18 months of age were used in a completely randomised block design. The experimental period was 112 days in duration in which steers were housed in individual indoor pens, including the first 21 days of adaptation. Methane emission in respiration chambers for 24 hours (days 7, 14, 21, 49, 70, 91, and 112), ruminal parameters (the day before and after chambers) and performance traits (LW - the day before and after chambers; DMI – daily) were measured.

The adaptation period followed a step-wise approach in terms of both increasing 3-NOP dose concentration and increasing grain level in the diet. Five treatments were compared. Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112; and High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112 (Table 1). The CH_4 emission, ruminal parameters and performance traits were measured. By using control steers, we assessed the equation used in the current National Inventory CH_4 calculations [Moe and Tyrrell (1979)].

Results/key findings

- 3-NOP supplemented from 50 to 125 of mg 3-NOP/kg DM to Australian finishing diets containing 25 ppm of monensin and 7% fat (DM-basis) can reduce both production and yield of CH_4 by up to 99%.
- All 3-NOP titration regimens significantly reduced methane production and methane yield of feedlot cattle from d 21 to 112.
- The optimal methane suppression was obtained in the Medium B titration regimen, with reductions in methane yield (g/kg) and emissions (g/d) by 61 to 99 % in the finishing phase.

In the overall feeding period, methane emissions (g/d) was 78% lower in the Medium B titration regimen compared with control.

- Although this experiment was not designed to make conclusions on an effect of 3-NOP on feedlot performance (cattle were limit fed to 2.7 x maintenance; 4 steers per regimen) no negative effects of 3-NOP diet supplementation on the animal performance parameters measured were observed.
- The current equation used to predict methane emissions from feedlot cattle in the Australian National Inventory, from Moe and Tyrrell (1979), resulted in substantial overprediction of CH_4 by 95.5 g/day when tested against observed CH_4 emissions from the control cattle in this trial. An alternative equation, from IPCC (2006), predicted CH_4 production from control group cattle accurately and is more appropriate than Moe and Tyrrell (1979) for the diets tested, which are typical of the white-grain, high-fat diets of the Australian feedlot industry.

Benefits to industry

Supplemental 3-NOP in white-grain and high-fat finishing diets can allow Australian feedlots to produce low-methane-emission beef from the grain-finishing phase. It has also demonstrated that there is a low baseline level of CH_4 production from in feedlot diets tested during this project.

Ultimately, the knowledge on the CH_4 suppression pattern of 3-NOP may facilitate sustainable pathways for the feedlot industry as well as enabling Australian producers to benefit from carbon credit trading schemes.

Validation of the current and an alternative model for predicting CH_4 emissions of feedlot cattle demonstrated that the National Inventory currently overpredicts the contribution of feedlot finishing of cattle to Australia's Greenhouse Gas (GHG) emissions.

Future research and recommendations

Testing 3-NOP in larger-scale commercial-feedlot setting is recommended to investigate possible productivity co-benefits and practicalities of 3-NOP supplementation in feedlot diets.

It may be necessary to test the efficacy of 3-NOP in other diets used in the Australian industry as part of developing a system of carbon credits by verification organisations.

In this experiment, hydrogen emissions were not measured. We recommend that hydrogen emissions are examined in future trials to explain differences in rumen fermentation, as only small differences in ruminal fermentation parameters were found in the present study.

The method to predict methane emissions of lot-fed cattle in the National Inventory apparently is not suitable and further research should be undertaken to confirm if the National Inventory is over-predicting emissions from the feedlot sector, and if the methodology should be revised and alternative accredited models used.

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1. Background

As part of the red meat industry's CN30 Roadmap, MLA has identified ruminant greenhouse gas (GHG) emissions avoidance as one of four key work areas. MLA has identified 3-nitrooxypropanol (3-NOP, Bovaer®, DSM) as one of the most promising of the supplementary CH_4 inhibitors currently under development.

Although previous research was performed assessing the abatement potential of 3-NOP in a feedlot setting overseas (Romero-Perez et al., 2014 and 2015; Vyas et al., 2016, 2018a, and 2018b; Kim et al., 2019), this is the first study performed in Australia by using typical Australian feedlot diets containing white grains, rumensin and high levels of fat. Meta-analysis of previous peer-reviewed research during preliminary stages of this project reported that 3-NOP in feedlot backgrounding and finisher diets can suppress methane emissions in cattle by, on average, 29 and 27%, respectively (Figure 1). Limited research has been published in peer-reviewed journals on the effects of 3-NOP on methane emissions of finishing cattle in high fat and white-grain diets commonly used in the Australian feedlot industry.

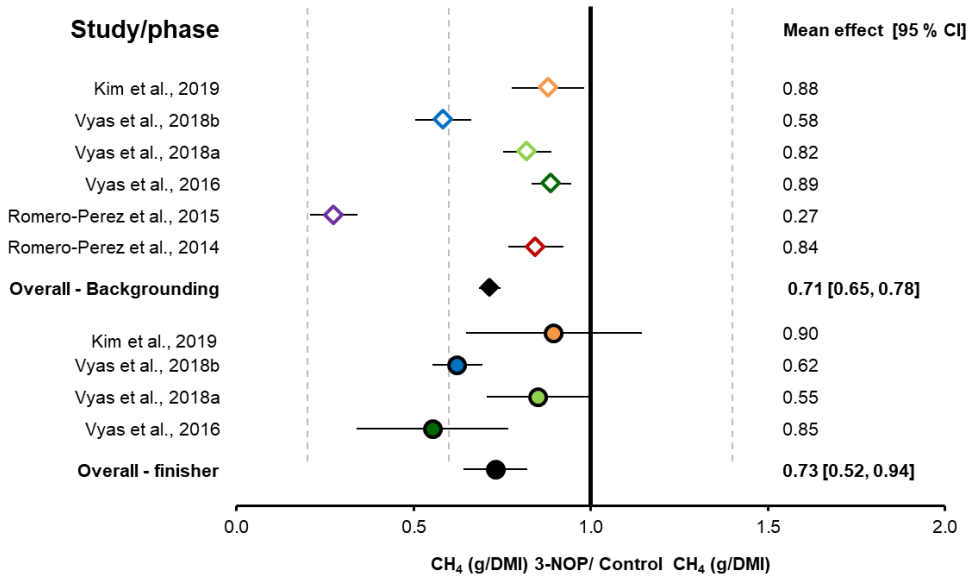


Figure 1. Forest plot with the standardized mean effect of the estimated ratio of methane yields for 3-NOP fed beef cattle vs control cattle emissions from previously published studies. Values below 1.0 indicate that 3-NOP yields reduction in methane emissions.

Each study used in Figure 1 showed reduced enteric CH_4 emissions when using 3-NOP. Moreover, studies have demonstrated the safety (no residues or animal health issues) in supplementing 3-NOP to ruminant animals (Thiel et al., 2019a and 2019b). Previously, CH_4 abatement with dietary 3-NOP in ruminants was associated with a decrease in dry matter intake (DMI) (Romero-Perez et al., 2014; Vyas et al., 2016 and 2018b), and in some instances resulted in increased feed efficiency (Vyas et al., 2016 and 2018b). Considering the studies reviewed to this date, the in-feed doses of 3-NOP used for feedlot cattle ranged from 0 to 280 mg 3-NOP/kg DM and responses have been dose-dependent

(Figure 2). Moreover, 3-NOP is suppressing CH_4 in both high-forage and high-grain feedlot diets (Figure 2).

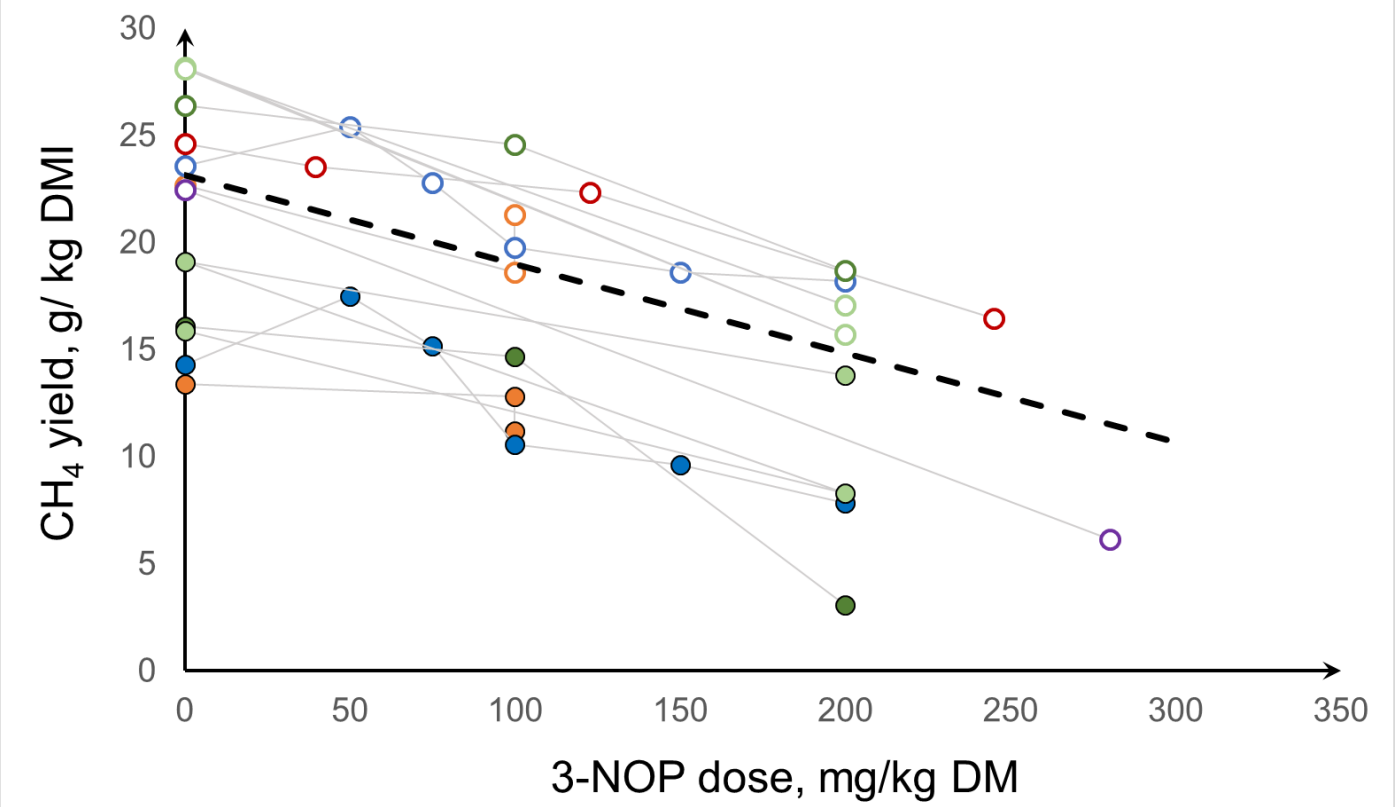


Figure 2. Linear relationship between 3-NOP dose (mg/kg DM) and CH_4 yield (g/kg DMI). Open circles represent studies that tested background feedlot diets, whereas closed circles show studies with finishing diets. CH_4 yield (g/kg DMI) = 23.2 ± 1.72 - 0.0416 ± 0.00997 × 3-NOP dose (mg/kg DM); P <0.01; RMSE = 5.15 g CH_4 /kg DMI. This analysis was performed using the MIXED procedure of SAS (version 9.4, SAS/STAT, SAS Institute Inc., Cary, NC), considering study as a random effect. Furthermore, to account for variations in precision across studies, the inverse of the squared standard error of the mean (SEM) (Wang & Bushman, 1999) of CH_4 yield was used as a factor in the WEIGHT statement of the model (St-Pierre, 2001).

Regulatory approval to improve environmental sustainability of cattle feeding is currently underway in the European Union, Canada and the United States. The 3-NOP is not yet approved for ruminants in Australia by the APVMA (Australian Pesticides and Veterinary Medicines Authority), and evidence of dose-response relationships of 3-NOP with methane emissions, and animal health and productivity may be necessary for such a registration. This project provides evidence of the dose-response relationship of 3-NOP with methane emissions and rumen fermentation patterns.

This project also evaluates the current baseline CH_4 prediction equations for feedlot cattle in the Australian Government GHG accounting framework. Australian National Greenhouse Accounts National Inventory of GHG emissions uses the Moe & Tyrrell (1979) equation, which was derived from studies with dairy cattle and consider ingested hemicellulose, cellulose and soluble residue (kg/day) to estimate CH_4 production. Previous MLA research from CH_4 measures in Australian

feedlots has suggested that feedlot emissions are lower than predicted by the National Inventory method. An alternative predictive equation is the Intergovernmental Panel on Climate Change (IPCC, 2006) Tier 2 methodology. In this method, a CH_4 emission factor (Y_m, % of GEI – Gross Energy Intake) is applied to specific feedlot diet categories. Previous modelling exercises have shown that uncertainty of the IPCC Tier 2 emission-factors at a national level can be high across diverse production systems (Monni et al., 2007; Karimi-Zindashty et al. 2012), as the Y_m value directly influences the estimated CH_4 inventory. However, if the Y_m is correctly estimated for the relevant production system with accurate activity data, the prediction may be useful. Currently, the United States uses the Y_m methodology for estimation of emissions of feedlot cattle. This project evaluates both the Moe & Tyrrell (1979) and the IPCC (2006) equations for predicting CH_4 emissions from cattle fed typical Australian feedlot diets.

Results of this project will contribute to determining which 3-NOP doses should be used in future performance studies before making final recommendations on 3-NOP use for the feedlot cattle industry. It will also provide evidence of the most appropriate predictor of baseline methane emissions in feedlot cattle to be used in the Australian Government GHG accounting framework.

2. Objectives

(1) Determine the effect of 3-NOP diet concentration on rumen fermentation and methane emissions during starter and finishing phases in beef cattle.
(2) Validate current methods for predicting methane production of feedlot cattle including the Moe and Tyrrell equations and the IPCC Tier 2 methodology

3. Methodology

3.1 Effect of 3-NOP on rumen fermentation and methane emissions

3.1.1 Animals, diets and experimental design

This experiment evaluated the effect of increasing 3-NOP diet concentration on rumen fermentation characteristics and methane emissions of Angus steers on typical Australian feedlot diets. All procedures were approved by the University of New England Animal Ethics Committee (Authority number 20-061). The experiment was conducted at the University of New England Centre for Animal Research and Teaching (CART), Armidale, NSW, Australia. Twenty Angus steers (15 – 18 months of age) of initial liveweight (LW) of 356 ± 14.4 kg were selected from a single source. After transport to the research facility, the steers were inducted with visual identification (eartag), vaccinated (Ultravac® 7-in-1, Zoetis, Melbourne Australia and Bovilis MH+IBR, Coopers Animal Health, Macquarie Park, NSW, Australia) and received a pour-on anthelmintic (Cydectin plus Fluke: Virbac, Milperra NSW, Australia). Throughout the experiment, the steers were housed in individual indoor pens. During an initial 7-day acclimation period before the trial, the steers were fed a 100% forage diet, *ad libitum*, to permit acclimation to the housing. Initial LW was recorded (Gallagher W310 (Gallagher, Hamilton, NZ) and used to split the steers into two blocks (light and heavy LW) of 10 steers each. Throughout the experiment, LW was recorded on days 6, 13, 20, 27, 48, 69, 90 and 111, 4 hours after feeding and on days 8, 15, 22, 29, 50, 71, 92, and 113 before feeding. Prior to each weighing procedure, the scale was calibrated with a known weight of 300 to 400 kg. The second block of steers commenced the experiment one day after the first block, so that all procedures were staggered by one day for the second block. The purpose of this staggered design was to account for

the limited number of respiration chambers, which allowed for only 10 animals per day. Animals in both blocks completed a 112-day experimental feeding period.

Within each block, the steers were randomly allocated to one of 5 treatments, being a placebo (control) and four 3-NOP titration regimens, as Bovaer 10®, a blend of 3-NOP included at a minimum of 10% on a carrier of silicate and dried propylene glycol (DSM Nutritional Products AG, Basel, Switzerland). Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112; and High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112.

A number of treatment premixes were mixed via a ribbon mixer into a dry supplement containing vitamins, minerals and monensin (25 ppm) (DSM, Wagga Wagga, Australia) at the University of New England. Treatment premixes supplied either 0, 50, 75, 100 or 125 mg/kg 3-NOP and were balanced across treatments to the same DM inclusion level by a silicate and dried propylene glycol carrier. During the adaptation phase of the experiment, the inclusion rate of 3-NOP was titrated up to the finisher inclusion rate, to permit rumen adaptation of the 3-NOP, as per Table 1.

Table 1. Protocol for titrated adaptation of 3-NOP to finisher diet inclusion rates (mg 3-NOP per kg DM in diet offered).

Experiment days	Treatment				
	Control	Low	Medium A	Medium B	High
Adaptation period					
1-7	0	50	50	50	50
8-14	0	50	75	75	75
15-21	0	50	75	100	100
Finisher period					
22-112	0	50	75	100	125

DM - dry matter; 3-NOP – 3-nitrooxypropanol

The diet and feeding regimen were based on commercial best-practice in the Australian grain-fed cattle industry. The initial 21 days of the experiment was a 'adaptation period', when the steers were adapted to the finisher 3-NOP inclusion rates, and adapted from 100% forage in the pre-experimental acclimation period, to 80% tempered barley (DM-basis, Table 2). During the adaptation period, the cattle were 'stepped up' in DM offered each day by using increasing multiples of maintenance energy requirement. During the finisher period (days 22 – 112), the steers were fed at a fixed rate of 2.7 x of the maintenance energy requirement (**NASEM, 2016**). Steers were fed once daily, at 0800h (block 1) or 0900h (block 2). Feed offered and refusals were recorded daily, and sampled for DM and proximate analysis. All steers had *ad libitum* access to clean, fresh water.

DM of feed offered and refusals were measured daily. Grab samples (500 g) were collected from each mixer load of the main diet and bulked weekly and analysed for content of crude protein (AOAC Method 2001.11), acid detergent fibre (ADF, AAFCO Method 008.08), neutral detergent fibre (NDF, NFTA Method 2.2.2.5), organic matter (ISO 5984:2002(E)), ether extract (AFIA Method 1.14R), starch (AOAC Method 996.11), water soluble carbohydrates (AFIA Method 1.11A), digestible dry and organic matter (AFIA Method 1.7R) (NSW DPI Laboratory Services - Wagga Wagga Chemistry Services Laboratory, PMB Pine Gully Road, Wagga Wagga NSW 2650 (Table 2)). Metabolisable energy (ME) content was calculated according to the equations for grains and concentrates of AFIA Method 2.2R (MAFF, 1990). Please refer to Appendix 1 for wet chemistry results.

Approximately 100 g of the starter, intermediate I, intermediate II and finisher were sent to DSM Switzerland in HDPE bottles for 3-NOP quantification. The 3-NOP concentration was 64.0 ± 2.98% of the target dose, across all 3-NOP diets.

Table 2. Formulated composition of feedlot total mixed rations feed to Angus steers during the 112-d feeding period.

	Diet (feeding days)[1]			
Item	Starter (0-7)	Intermediate I (8-14)	Intermediate II (15-21)	Finisher (22-112)
Ingredient, % DM				
Tempered Barley	35.27	49.89	64.48	80.75
Cereal hay	23.97	16.93	9.85	-
Oaten Chaff	12.16	8.59	5.00	3.11
Whole cottonseed	12.17	11.25	10.31	9.35
Mill run	10.45	7.09	3.64	-
Molasses	3.44	2.88	2.38	1.79
Canola Oil[2]	-	0.81	1.75	2.39
Treatment Premix[3]	0.04	0.06	0.09	0.11
Dry Supplement[4]	2.50	2.50	2.50	2.50
Formulated Chemical Composition (DM-basis)[5]				
Dry Matter (DM)	100	100	100	100
Organic Matter, % DM	81.6	85.6	89.8	94.2
Ash, % DM	5.14	4.50	3.86	3.17
Crude Protein, % DM	13.6	13.8	13.9	14.0
Fat, % DM	4.66	5.44	6.34	6.98
TDN, % DM	71.5	74.0	76.5	79.4
NDF, % DM	37.4	32.7	27.9	23.5
Starch, % DM	24.7	32.2	39.6	47.9
ME, Mcal/kg DM	2.58	2.74	2.90	3.05
NE_m, Mcal/kg DM	1.68	1.81	1.95	2.07
NE_g, Mcal/kg DM	1.07	1.19	1.31	1.42
Ca, % DM	0.69	0.70	0.65	0.63
P, % DM	0.35	0.34	0.32	0.31

[1]Diets were formulated using the Concept 5 software. [2] Please refer to Appendix 1 & 2 for the analyzed diet and canola oil composition. [3]Treatment premixes supplied either 0, 50, 75, 100 or 125 mg/kg 3-NOP, balanced across treatments to the same DM inclusion level by a silicate and dried propylene glycol carrier. [3] Supplied

per kilogram of supplement (DM basis) 6.0 mg cobalt (MICROGRAN Co 5% BMP), 400 mg copper (Copper Sulphate Pentahydrate, 20mg iodine (Calcium iodate anhydrous 63%), 800 mg manganese (Manganese Sulphate 31%), 4mg selenium (MICROGRAN Se 4.5% BMP), 2.4 g zinc (Zinc Sulphate Monohydrate 35%), 2.0% magnesium (Magnesium Oxide 54%), 22.0% calcium (calcium carbonate), 2.0% sulfur (Calcium Sulphate), 10% salt (feed grade salt), 11% urea, 1 g monensin (Rumensin 200), 88 KIU vitamin A (ROVIMIX A 1000), 11 KIU vitamin D (ROVIMIX D3-500) and 1.1 KIU vitamin E (ROVIMIX E-50 Adsorbate)[5]. TDN = total digestible nutrients; NDF = neutral detergent fibre; ME = metabolisable energy; NE_m = Net for maintenance; NE_g = Net energy for gain; all diets contained 25 ppm of Monensin.

3.1.2 Measurements of methane production

The CH_4 production was estimated by confining each steer in an individual open circuit respiration chamber (Hegarty et al., 2012) for 24 hours on days 7 (last day of starter diet), 14 (last day of the intermediate I diet), and 21 (last day of the intermediate II diet) of the adaptation period and on days 28, 49, 70, 91 and 112 of the finisher period. The period of confinement commenced at the usual time of feeding. A Servomex analyser was used to quantify production of CH_4, O_2, and CO_2. The CH_4-production data were corrected for recovery of a known quantity of pure CH_4, which was introduced via a mass flow controller (Smart Trak 2 Series 100, Sierra Instruments, Monterey, CA, USA) and measured by the Servomex analyser before and after each CH_4 measurement-period (Hegarty et al., 2012). Mean CH_4 recovery was 92.8% ± 1.36%.

Besides total methane production (g of CH_4/d) and methane yield (g of CH_4/kg DMI) by day, a weighted CH_4 is also reported. The weighted CH_4 (g/d) was calculated multiplying the methane yield by daily dry matter intake of starter, intermediate I, intermediate II and finisher periods, weighting for the days that each steer received those diets.

3.1.3 Sampling for VFA concentrations, rumen ammonia and protozoal enumeration

Post-feeding rumen-fluid samples (~70 mL) were collected oro-gastrically from each animal 4 hours after feed was offered on days 6 (Starter diet), 13 (- Intermediate diet I), 20 (Intermediate diet II), 27 (Finisher diet), 48 (Finisher diet), 69 (Finisher diet), 90 (Finisher diet), and 111 (Finisher diet). Pre-feeding rumen samples were collected from each steer 1 hour before feeding on days 9 (Starter diet), 15 (First Intermediate diet), 23 (Second Intermediate diet), 29 (Finisher diet), 50 (Finisher diet), 71 (Finisher diet), 92 (Finisher diet), and 113 (Finisher diet). The rumen fluid was tested for pH (EcoScan Portable pH/ORP meter with TPS pH Sensor) and redox potential (Mettler Toldeo SevenEasy S20 pH meter with TPS Intermediate Junction Redox Sensor) immediately after sampling, and then subsampled for measurement of volatile fatty acid (VFA) profiles, rumen ammonia, and rumen protozoa enumeration.

Volatile fatty acids were determined by gas chromatography (Nolan et al., 2010). Rumen ammonia-N was determined by Skalar methodology, based on the modified Berthelot reaction (de Raphelis et al., 2016). For protozoa enumeration, 4 mL of rumen fluid was suspended in 16 mL of an isotonic formaldehyde-saline of 4%, then subsampled and stained with brilliant green (Nguyen and Hegarty, 2016) before microscopic enumeration of ciliate protozoa on a Fuchs – Rosenthal optical counting chamber (0.0625 mm^2, 0.2 mm depth) by using a technique adapted from Dehority (1984).

3.1.4 Statistical analysis of animal performance, methane emission and rumen function parameters

To achieve the first objective of determining the effect of 3-NOP diet concentration on rumen fermentation and methane emissions during the 112-d feedlot period (starter + finishing phases) in

beef cattle, the data from all 20 steers were analysed as a completely randomized block design including block as a random effect. Diet changes during adaptation coincided with the 3-NOP dose titration changes; therefore, we included the 3-NOP regimen (Control, Low, Medium A, Medium B, High, see Table 1) as a fixed effect and analysed data obtained within each timepoint. The PROC MIXED procedure of SAS (version 9.4, SAS Systems Inc., Cary, NC, USA) was used to perform all the statistical analyses. Statistical significance was declared at $P < 0.05$, in which case a pairwise comparison was performed using Tukey's adjustment.

3.2 Validation of current methods for predicting methane of feedlot cattle

3.2.1 Sampling and analysis of nutrient composition of feeds

Grab samples (~500 g) were collected at mixing of each batch of the diet. The samples were sealed and frozen at -20 °C before later analysis. Gross energy was determined by using an adiabatic calorimetric bomb (Parr Instrument Co., Moline, Il). Fat content (AOAC, 1990, method 930.15), protein content by N analysis with Dumas combustion by using Leco FP-528LC (Etheridge et al., 1998) were also analysed. The NDF was determined with amylase and without sodium sulphate (Van Soest et al., 1991), and ADF and ADL was determined (Goering and Van Soest, 1970) in an ANKOM 2000 Fibre Analyser (ANKOM Technology, Macedon NY, USA). Hemicellulose was calculated as the difference between NDF and ADF, cellulose was calculated as the difference between ADF and ADL, soluble residue was the sum of crude fat, crude protein and soluble carbohydrates.

3.2.2 Statistical analysis of methane prediction equations

For validating current methods for predicting methane production of feedlot cattle energy intake, methane emissions and energy losses were measured from the four Control steers during each of the eight periods of methane measurement in the respiration chambers. The observed energy and fibre-fraction intakes from the diet, and methane emissions were compared with the predicted methane emissions from Moe & Tyrrell (1979) and IPCC (2006).

$$\boldsymbol{CH_4} = (Y_m \times \boldsymbol{GEI})/0.05565$$ IPCC (2006)

CH_4 = Methane production in g/day;

Y_m = Emission factor: adopted 0.063 for starter, intermediate I, and intermediate II diets; and 0.03 for finisher diet;

GEI = Gross energy intake in MJ/day.

$$\boldsymbol{CH_4} = [3.406 + 0.510 \times \boldsymbol{S} + 1.736 \times \boldsymbol{HC} + 2.648 \times \boldsymbol{CEL}]/0.05522$$ Moe & Tyrrell (1979)

CH_4 = Methane production in g/day used by the National Inventory;

S= Soluble residue (calculated by subtracting crude protein and ether extract from the neutral-detergent solubles) in kg/day;

HC = Hemicellulose in kg/day;

CEL = Cellulose in kg/day.

For each equation, the residual (observed – predicted) values were regressed on the predicted values centred on their mean (St-Pierre, 2003) by using the PROC MIXED of SAS 9.4 (SAS Systems Inc., Cary, NC, USA), to ensure that intercept and slope estimates remain independent. The slopes and intercepts of each equation were estimated by using the ESTIMATE statement of the MIXED

procedure in SAS. The intercepts of the regression equations represent the mean biases, whereas the slopes of such regression equations are the linear biases. A t-test on the estimate of the intercept and slope determined the statistical significance of mean and linear biases. The root mean squared error (RMSE) of this evaluation indicates the variations within the evaluation database.

4. Results and Discussion

4.1 Animal Productivity

There was no negative effect on animal performance of any 3-NOP titration regimen on live weight (LW), average daily gain (ADG), dry matter intake (DMI) and gain to feed (G:F) (Table 3), during the adaptation (day 0 to 21) or the overall period (day 0 to 112).

Previous studies have shown DMI (Romero-Perez et al., 2014; Vyas et al. 2016) and ADG reduction and increased G:F response in feedlot cattle fed 3-NOP (Romero-Perez et al., 2014; Vyas et al. 2016). None of those performance responses were observed in the current study, as it was not powered to address differences in those traits. We propose that a commercial feedlot type of study with a larger number of animals should be designed and performed to address the influence of 3-NOP on cattle performance, as previous research has demonstrated a 3-5% difference in those traits.

Table 3. Responses of live weight (LW), average daily gain (ADG), dry matter intake (DMI), and gain to feed ratio (G:F) in Angus steers fed different 3-NOP titration regimens during the overall feeding period (day 0 to 112).

	3-NOP Regimen						
Item	Control	Low	Medium A	Medium B	High	SE	*P-Value*
Initial LW, kg	354	356	356	355	359	8.04	0.82
d 21 LW, kg	385	398	394	392	392	8.14	0.64
Final LW, kg	558	561	560	563	567	9.60	0.96
ADG, kg							
d 0 to 21	1.45	2.02	1.82	1.77	1.55	0.247	0.52
d 0 to 112	1.82	1.83	1.83	1.86	1.85	0.092	0.99
DMI, kg/d							
d 0 to 21	7.30	7.65	7.70	7.75	7.39	0.148	0.19
d 0 to 112	9.76	9.79	9.83	9.84	9.63	0.131	0.79
G:F							
d 0 to 21	0.189	0.253	0.229	0.218	0.199	0.0306	0.63
d 0 to 112	0.184	0.186	0.185	0.187	0.191	0.00825	0.96

[1]3-NOP Regimens were Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112 ; High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112

[2]Standard error of the mean [3]When P-value for 3-NOP regimen was significant (≤ 0.05) pairwise comparison was performed using Tukey's test, in that case means within a row showing different superscripts depict significant effects of 3-NOP regimen.

4.2 Methane Emission

The CH_4 production, yield and emissions as a percentage of gross energy intake (GEI) were influenced by 3-NOP regimen on days 21, 49, 70, 91 and 112 ($P \leq 0.02$; Table 4). The maximum CH_4 production abatement from 3-NOP was observed in day 28, averaging 99% reduction compared to control steers. The weighted CH_4 (calculated from methane yield and daily DMI of starter, intermediate I, intermediate II and finisher periods, g/d) was greater in control steers compared to steers in medium and high 3-NOP regimen (Table 4). The observed percentage reduction relative to control in weighted CH_4 was 58%, 73%, 79% and 76% in the low, medium A, medium B and high 3-NOP regimen, respectively (Table 4 and Figure 3).

Table 4. Methane (CH_4) emissions of Angus steers fed 3-NOP titration regimens during the eight measurement times during the overall feeding period (day 0 to 112).

	3-NOP Regimen[1]						
Item[4]	Control	Low	Medium A	Medium B	High	SE[2]	*P-value*[3]
DMI, kg/d							
d 7	6.74	6.74	5.90	6.95	6.35	0.389	0.37
d 14	8.41	8.68	8.80	8.64	8.33	0.161	0.28
d 21	8.53	9.13	9.09	8.95	8.98	0.250	0.48
d 28	10.0	10.2	10.1	10.1	10.0	0.144	0.71
d 49	8.91	8.86	8.69	8.63	8.83	0.133	0.47
d 70	10.1	9.45	9.95	9.67	9.84	0.282	0.51
d 91	11.5	10.9	11.6	11.4	10.7	0.340	0.24
d 112	10.6	10.8	10.6	10.6	8.90	0.544	0.15
CH_4, g/d							
d 7	90.6	54.6	58.5	51.6	81.7	16.4	0.38
d 14	89.8	43.5	46.4	33.9	44.9	15.7	0.17
d 21	57.2[a]	4.80[b]	1.07[b]	1.16[b]	2.32[b]	6.35	<0.01
d 28	34.7	0.09	0.34	0.00	0.30	9.30	0.07
d 49	49.9[a]	1.37[b]	5.59[b]	1.97[b]	1.86[b]	6.35	<0.01
d 70	53.3[a]	9.95[b]	6.85[b]	1.68[b]	1.39[b]	4.60	<0.01
d 91	57.2[a]	50.9[a]	10.3[b]	5.36[b]	9.18[b]	9.83	<0.01
d 112	63.8[a]	37.0[ab]	25.3[ab]	25.1[ab]	11.7[b]	9.89	0.02
Overall weighted CH_4, g/d[5]	59.1[a]	24.9[ab]	16.0[b]	12.1[b]	14.0[b]	9.46	<0.01
CH_4, g/kg DMI							
d 7	13.5	8.07	9.77	7.48	13.4	2.51	0.31
d 14	10.7	5.01	5.28	3.98	5.38	1.86	0.15
d 21	6.71[a]	0.526[b]	0.117[b]	0.132[b]	0.258[b]	0.682	<0.01
d 28	3.48	0.00864	0.0342	0	0.03	0.936	0.07
d 49	5.57[a]	0.159[b]	0.641[b]	0.233[b]	0.212[b]	0.696	<0.01
d 70	5.34[a]	1.07[b]	0.709[b]	0.178[b]	0.137[b]	0.509	<0.01
d 91	4.99[a]	4.68[a]	0.887[b]	0.467[b]	0.917[b]	1.01	<0.01
d 112	6.00	3.47	2.41	2.36	1.55	0.958	0.05

CH_4, % GEI

d 7	8.85	5.11	5.23	4.58	10.1	1.96	0.22
d 14	6.42	3.07	3.27	3.27	2.44	3.35	0.19
d 21	3.94 [a]	0.323 [b]	0.0723 [b]	0.0811 [b]	0.162 [b]	0.401	<0.01
d 28	2.09	0.00520	0.0205	0.0180	0.00	0.562	0.07
d 49	2.95 [a]	0.0830 [b]	0.326 [b]	0.119 [b]	0.111 [b]	0.367	<0.01
d 70	2.97 [a]	0.585 [b]	0.409 [b]	0.0978 [b]	0.0730 [b]	0.281	<0.01
d 91	3.03 [a]	2.70 [a]	0.533 [b]	0.280 [b]	0.489 [b]	0.531	<0.01
d 112	3.35 [a]	2.05 [ab]	1.40 [ab]	1.36 [ab]	0.731 [b]	0.531	0.03

[1]3-NOP Regimen were Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112 ; High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112

[2]Standard error of the mean [3]When P-value for 3-NOP Regimen was significant (≤ 0.05) pairwise comparison was performed using Tukey's test, in that case means within a row showing different superscripts depict significant effects of 3-NOP Regimen. [4]Methane measured in chambers on days 7 (last day of starter diet), 14 (last day of the intermediate I diet), and 21 (last day of the intermediate II diet) of the adaptation period and finisher diet on days 28, 49, 70, 91 and 112 [5]Weighted average feeding period methane calculated from methane yield and daily dry matter intake of starter, intermediate I, intermediate II and finisher periods.

The CH_4 reduction in the present study was greater than previous studies involving 3-NOP (Romero-Perez et al., 2014 and 2015; Vyas et al., 2016, 2018a, and 2018b; Kim et al., 2019). The remarkably greater CH_4 reduction observed in the present study might be due to synergistic effects of polyunsaturated fatty acids and monensin in the diet as these two dietary strategies to tackle CH_4 in the rumen have different modes of action compared with 3-NOP. A previous meta-analysis indicated that for each 1% oil added to the diet, methane yield was reduced by 5.6% (Beauchemin et al., 2007). Monensin is an ionophore that may anchor to the cell membranes of organisms and translocate protons (H+) and metal ions through the membrane leading to eventual selective death of microbes in the rumen (i.e., Gram positive, H+-, ammonia-, and lactate-producing organisms, Russell and Strobel, 1989, Chow et al., 1994). This shift in the microbial population results in lower CH_4 and higher propionate production in the rumen. Noteworthy, all diets had the same amount of oil and monensin, therefore a synergistic effect is hypothesised.

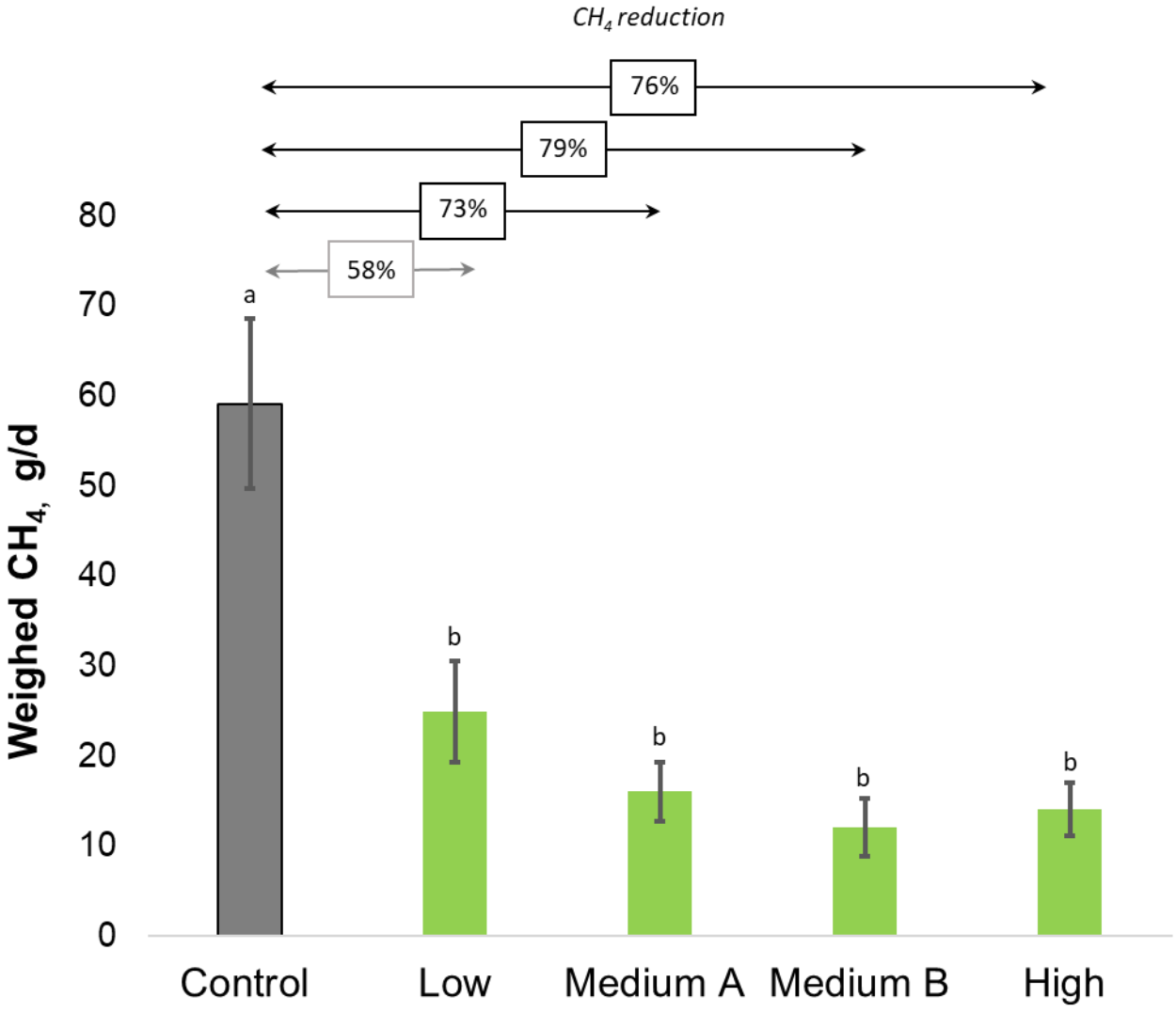


Figure 3. The weighted CH_4 (calculated from methane yield and daily dry matter intake of starter, intermediate I, intermediate II and finisher periods, g/d) and methane reduction of animals that followed each titration regimen relative to control during the overall feeding period (day 0 to 112). Means with different letters depict significant effects of 3-NOP regimen.

4.3 Rumen function

The effect of 3-NOP regimen on VFA concentration and profile pre-feeding and after four hours of feeding was mainly around days 20 and 29, when the lowest CH_4 production was recorded (Tables 5 and 6). Previous research has indicated that feeding of 3-NOP to feedlot cattle does not cause changes in total VFA production but will shift the molar percentage of acetate, propionate and butyrate, increasing propionate and butyrate at the expense of decreased acetate production, as propionate production may act as a hydrogen sink in the rumen (Romero-Perez et al., 2015). In day 29, we observed greater A:P ratios in the pre-feeding rumen fluid of control steers than in steers fed medium A and B and high 3-NOP regimens. Day 29 coincides with the first week that the steers were receiving the finisher diet.

No effect on pre- or post-feeding ammonium-N was observed as a result of 3-NOP administration (Tables 5 and 6), which is in agreement with previous research by Romero-Perez et al. (2015).

Table 5. Pre-feeding ammonium, volatile fatty acid (VFA) amounts and profile in the rumen fluid of Angus steers fed 3-NOP titration regimens during the eight measurement times during the overall feeding period (day 0 to 112).

	3-NOP Regimen[1]						
Item[4]	Control	Low	Medium A	Medium B	High	SE[2]	*P-value*[3]
Ammonium-N, ug/mL							
d 8	78.5	98.9	96.9	67.6	97.8	19.6	0.69
d 15	62.4	87.9	76.8	82.7	79.0	21.6	0.88
d 22	65.2	71.6	54.0	57.6	69.6	11.3	0.69
d 29	71.8	67.9	41.9	51.5	53.9	17.2	0.68
d 50	92.3	146	88.3	87.9	90.9	16.3	0.11
d 71	69.7	82.1	92.6	81.4	89.0	20.0	0.79
d 92	100	101	100	106	97.9	21.5	0.99
d 113	131	173	132	142	159	29.5	0.82
Total VFA, mmol/L							
d 8	38.4	38.6	42.9	33.0	44.8	6.64	0.61
d 15	41.7	30.8	42.2	37.9	34.4	9.50	0.87
d 22	27.7	20.4	17.4	18.8	17.2	6.96	0.82
d 29	51.7[a]	15.5[b]	26.9[ab]	35.6 [ab]	36.4 [ab]	8.68	0.04
d 50	23.8	28.3	28.0	26.9	26.9	2.62	0.76
d 71	29.0	25.3	26.1	28.8	31.6	7.08	0.91
d 92	39.2	42.2	28.3	41.0	37.0	6.52	0.60
d 113	29.8	37.3	41.1	30.0	42.4	6.67	0.40
Acetate, %							
d 8	59.2	57.0	58.2	59.6	62.5	1.59	0.08
d 15	60.3	56.8	57.8	59.4	58.8	1.91	0.67
d 22	59.0	54.9	57.6	56.3	58.9	1.34	0.24
d 29	61.3	59.6	57.4	52.3	57.7	2.63	0.16
d 50	56.7	56.8	58.9	55.3	58.7	1.60	0.49
d 71	58.0	56.7	58.8	60.1	60.3	2.32	0.83
d 92	53.4	55.2	56.9	56.2	56.1	4.01	0.96
d 113	54.4	51.7	53.2	55.0	50.5	2.75	0.77
Propionate, %							

d 8	26.9	27.5	25.6	24.6	20.7	2.88	0.37
d 15	22.4	26.5	25.2	22.1	24.3	2.03	0.50
d 22	20.8	21.8	26.0	21.2	21.2	2.08	0.27
d 29	17.2 [b]	19.2 [ab]	25.6 [ab]	26.9 [a]	25.7 [a]	2.14	<0.01
d 50	23.3	23.7	22.5	21.4	21.8	2.00	0.91
d 71	20.0	22.5	19.3	18.4	18.6	1.76	0.43
d 92	31.1	27.7	25.8	23.5	25.7	4.70	0.63
d 113	28.4	29.6	32.1	24.8	31.0	4.78	0.77
Iso-butyrate, %							
d 7	1.67	1.77	2.18	1.78	1.60	0.261	0.57
d 14	1.41	1.99	1.84	1.85	1.28	0.183	0.05
d 21	1.75	1.97	1.96	2.28	2.07	0.237	0.28
d 28	1.38	1.29	1.23	1.55	1.27	0.333	0.96
d 49	1.90	1.74	1.54	1.99	1.76	0.163	0.34
d 70	1.56	1.86	1.85	1.63	1.71	0.187	0.29
d 91	1.46	1.30	1.48	1.67	1.89	0.354	0.80
d 112	1.27 [b]	1.03 [b]	0.94 [b]	2.17 [a]	1.29 [b]	0.163	<0.01
Butyrate, %							
d 8	8.06	8.14	7.75	8.36	10.9	1.05	0.28
d 15	10.8	9.07	8.92	10.2	10.7	1.21	0.64
d 22	11.4 [ab]	14.2 [a]	8.08 [b]	12.2 [ab]	10.4 [ab]	1.21	0.02
d 29	11.9	11.4	7.81	10.2	7.21	2.23	0.49
d 50	10.3	9.70	9.08	12.5	9.68	1.33	0.45
d 71	13.2	10.8	12.0	12.1	11.6	2.02	0.91
d 92	7.31	8.40	8.28	10.4	8.41	1.42	0.18
d 113	8.81	7.93	7.20	9.66	9.69	1.61	0.68
Iso-valerate, %							
d 7	2.90	3.54	4.09	3.19	3.24	0.814	0.84
d 14	3.32	3.15	3.16	4.51	2.18	0.619	0.09
d 21	3.02	2.04	2.52	2.45	2.49	0.418	0.48
d 28	1.44	2.40	1.37	1.68	1.50	0.317	0.20
d 49	2.05	1.96	1.73	2.25	2.01	0.175	0.34
d 70	1.77	2.21	2.05	1.82	1.93	0.242	0.41
d 91	1.81	1.57	1.85	1.90	2.26	0.432	0.82
d 112	1.53	3.11	1.02	2.38	1.92	1.14	0.64
Valerate, %							
d 8	1.24	1.84	1.61	2.12	1.12	0.401	0.39
d 15	1.62	2.25	2.40	1.60	2.26	0.532	0.59
d 22	2.77	3.72	3.12	4.15	3.59	0.450	0.08
d 29	4.09	4.08	5.08	5.44	5.04	0.366	0.06
d 50	4.04	4.14	4.33	4.25	4.26	0.262	0.94
d 71	3.56	4.02	3.68	3.65	3.33	0.416	0.78
d 92	4.00	4.07	4.37	4.30	4.16	0.529	0.96
d 113	4.42	5.08	4.22	4.03	4.23	0.432	0.39
Caproate, %							
d 8	0.0569	0.175	0.173	0.383	0.0475	0.181	0.68
d 15	0.142	0.485	0.613	0.216	0.545	0.280	0.59
d 22	1.28	1.71	0.642	1.47	1.37	0.379	0.15
d 29	2.58	2.03	1.52	1.95	1.59	0.346	0.26
d 50	1.74	1.91	1.85	2.39	1.82	0.309	0.60
d 71	1.92	2.08	2.29	2.14	2.34	0.476	0.91

d 92	0.952	1.76	1.41	2.14	1.62	0.509	0.43
d 113	1.17	1.53	1.31	1.25	1.49	0.418	0.94
Acetate:Propionate							
d 8	2.36	2.07	2.44	2.43	3.06	0.302	0.13
d 15	2.83	2.15	2.36	2.72	2.44	0.287	0.42
d 22	3.10	2.59	2.26	2.70	2.79	0.388	0.52
d 29	3.57 [a]	3.14 [a]	2.26 [b]	2.08 [b]	2.30 [b]	0.250	<0.01
d 50	2.46	2.52	2.67	2.73	2.70	0.280	0.95
d 71	2.90	2.67	3.09	3.29	3.28	0.321	0.56
d 92	1.88	2.47	3.40	3.07	2.58	0.774	0.78
d 113	1.96	2.43	1.67	2.18	1.89	0.653	0.88

[1]3-NOP regimen were Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112 ; High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112

[2]Standard error of the mean [3]When P-value for 3-NOP regimen was significant (≤ 0.05) pairwise comparison was performed using Tukey's test, in that case means within a row showing different superscripts depict significant effects of 3-NOP regimen.

Table 6. Post-feeding ammonium, volatile fatty acid (VFA) amounts and profile in the rumen fluid of Angus steers fed 3-NOP titration regimens during the eight measurement times during the overall feeding period (day 0 to 112).

	3-NOP Regimen[1]						
Item	Control	Low	Medium A	Medium B	High	SE[2]	*P-value*[3]
Ammonium-N, ug/mL							
d 6	37.2	39.3	53.2	72.5	70.6	23.6	0.71
d 13	22.6	24.6	10.0	30.5	28.5	12.7	0.81
d 20	2.28	6.25	20.4	35.0	41.9	19.4	0.55
d 27	26.5	15.3	20.3	8.25	24.1	7.43	0.45
d 48	55.1	39.8	34.8	32.9	26.7	18.8	0.77
d 69	17.9	76.5	39.7	48.0	79.0	23.9	0.40
d 90	43.2	35.4	40.8	27.4	38.8	15.3	0.95
d 111	66.8[ab]	35.3[b]	81.6[a]	54.7[ab]	82.6[ab]	14.6	0.02
Total VFA, mmol/L							
d 6	70.1	82.8	69.4	63.3	65.2	10.6	0.64
d 13	96.2	63.9	59.3	57.5	67.6	10.6	0.08
d 20	70.5	67.3	62.2	55.2	86.6	10.3	0.32
d 27	62.0	107	89.0	67.5	69.2	18.6	0.33
d 48	76.0	71.1	67.2	58.8	76.6	14.6	0.69
d 69	67.5	71.9	106	63.5	69.3	24.9	0.65
d 90	73.0	61.9	80.7	67.0	77.5	11.7	0.79
d 111	85.2	101	79.1	82.9	106	9.06	0.14
Acetate, %							
d 6	59.3	55.0	58.5	59.2	55.4	2.47	0.58
d 13	58.0	55.8	57.0	54.7	53.7	2.78	0.81
d 20	57.8	59.7	56.2	58.9	60.0	1.77	0.58
d 27	63.4	64.9	60.1	57.0	65.9	3.46	0.38
d 48	57.7	59.3	53.8	60.0	58.5	2.50	0.48
d 69	52.9	56.6	60.7	64.2	64.7	3.77	0.12
d 90	52.7	57.6	57.5	59.7	57.4	5.83	0.79
d 111	49.3	47.0	50.1	47.2	47.4	2.32	0.82

Propionate, %							
d 6	29.2	27.5	25.2	28.8	26.8	3.77	0.94
d 13	24.5	23.4	27.2	26.0	24.6	3.27	0.93
d 20	24.5	18.9	24.4	16.7	17.6	3.94	0.22
d 27	17.2	15.0	22.2	23.3	19.5	3.22	0.12
d 48	27.2	26.2	30.8	21.2	22.9	3.07	0.26
d 69	28.2	28.5	17.2	22.1	18.7	3.27	0.05
d 90	31.3	26.1	24.3	23.7	27.9	5.72	0.70
d 111	29.8	32.1	28.9	26.8	31.8	3.19	0.48
Iso-butyrate, %							
d 6	0.537	0.620	0.710	0.463	0.812	0.145	0.49
d 13	0.633	0.679	0.642	0.650	0.741	0.0715	0.82
d 20	0.548	0.630	0.507	0.682	0.799	0.118	0.47
d 27	0.485	0.426	0.407	0.486	0.341	0.162	0.97
d 48	0.293	0.291	0.439	0.457	0.484	0.0659	0.15
d 69	0.318	0.294	0.480	0.260	0.316	0.137	0.82
d 90	0.529	0.464	0.551	0.469	0.548	0.0780	0.87
d 111	0.535	0.467	0.499	0.811	0.428	0.126	0.04
Butyrate, %							
d 6	12.7	12.9	9.65	13.3	17.5	2.68	0.41
d 13	14.3	14.6	11.3	15.0	19.1	3.16	0.41
d 20	10.0	12.0	11.4	12.4	12.0	1.52	0.72
d 27	9.32	11.1	8.57	9.96	7.40	2.45	0.83
d 48	7.15	7.87	7.69	10.0	9.50	1.02	0.26
d 69	10.3	7.13	11.0	6.50	8.19	1.91	0.22
d 90	8.58	8.35	9.18	8.45	7.37	1.41	0.75
d 111	12.8	10.7	10.9	14.5	11.3	1.71	0.43
Iso-valerate, %							
d 7	1.32	3.10	2.86	2.79	2.65	1.13	0.81
d 14	1.53	1.17	1.27	1.77	1.46	0.549	0.93
d 21	1.16	0.583	0.575	0.666	0.749	0.309	0.66
d 28	0.506	0.383	0.449	0.506	0.360	0.145	0.92
d 49	0.339	0.297	0.440	0.508	0.438	0.0674	0.24
d 70	0.354	0.327	0.528	0.320	0.347	0.180	0.86
d 91	0.566	0.548	0.533	0.456	0.549	0.101	0.94
d 112	0.547	1.10	0.478	0.707	0.716	0.408	0.80
Valerate, %							
d 6	1.39	2.05	2.37	1.75	1.42	0.461	0.53
d 13	2.20	4.54	3.33	1.63	1.01	0.927	0.21
d 20	4.22[b]	5.20[ab]	4.71[b]	6.73[a]	5.62[ab]	0.545	<0.01
d 27	3.98	4.55	5.38	4.80	3.46	0.852	0.57
d 48	4.16	3.21	3.67	3.31	3.85	0.343	0.32
d 69	4.51	3.77	9.96	3.19	3.05	0.518	0.18
d 90	4.27	3.40	4.30	3.66	3.00	0.873	0.63
d 111	4.31	5.95	4.67	5.70	4.71	0.627	0.25
Caproate, %							
d 6	0.115	0.550	0.423	0.269	0.0663	0.273	0.70
d 13	0.437	1.55	0.864	1.13	0.280	0.608	0.59
d 20	1.65	3.04	2.13	3.96	3.23	0.575	0.09
d 27	5.07	3.72	2.84	4.00	3.07	1.28	0.65
d 48	3.11	2.85	3.10	4.48	4.29	0.477	0.08

d 69	3.27	3.63	6.12	3.43	4.76	0.852	0.14
d 90	2.07	3.61	3.66	3.54	3.24	0.773	0.52
d 111	2.75	2.67	4.46	4.22	3.63	0.590	0.17
Acetate:Propionate							
d 6	2.04	2.25	2.53	2.39	2.14	0.442	0.94
d 13	2.45	2.47	2.20	2.72	2.18	0.510	0.94
d 20	2.63	3.28	2.38	3.82	3.69	0.508	0.25
d 27	5.76	4.94	2.74	2.60	4.59	0.866	0.09
d 48	2.16	2.39	1.83	3.28	2.71	0.495	0.34
d 69	1.89	2.25	3.76	3.12	3.63	0.598	0.11
d 90	1.77	3.50	3.63	4.25	2.97	1.69	0.77
d 111	1.92	1.59	1.79	1.77	1.50	0.312	0.79

[1]3-NOP regimen were Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112 ; High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112.
[2]Standard error of the mean [3]When P-value for 3-NOP regimen was significant (≤ 0.05) pairwise comparison was performed using Tukey's test, in that case means within a row showing different superscripts depict significant effects of 3-NOP regimen.

There was not important differences on ruminal pH redox potential or rumen protozoa population pre-or post-feeding of feedlot steers in control or 3-NOP titration regimens from 0 to 112 days (Tables 7 and 8). Romero-Perez et al. (2015) previously reported an increase in rumen protozoa in feedlot cattle fed 3-NOP. This does not agree with results reported herein, as we observed absence of rumen protozoa after day 22.

Table 7. Pre-feeding protozoa enumeration, pH and redox potential in the rumen fluid of Angus steers fed 3-NOP titration regimens during the eight measurement times during the overall feeding period (day 0 to 112).

	3-NOP Regimen[1]						
Item[4]	Control	Low	Medium A	Medium B	High	SE[2]	*P-value*[3]
pH							
d 8	7.25	7.69	7.31	7.96	7.28	0.226	0.18
d 15	7.43	7.60	7.33	7.65	7.85	0.184	0.25
d 22	7.76	7.85	7.78	7.92	8.00	0.203	0.87
d 29	7.28[b]	8.29[a]	7.52[b]	7.26[b]	7.54[b]	0.157	<0.01
d 50	8.98	8.60	8.48	8.62	8.73	0.125	0.39
d 71	8.15	8.09	8.38	7.87	7.97	0.250	0.61
d 92	7.76	7.54	8.12	7.79	7.80	0.210	0.40
d 113	7.99	7.93	8.08	8.11	7.86	0.176	0.77
Redox potential							
d 8	-104	-87.3	-119	-69.3	-84.5	17.8	0.43
d 15	-79.3	-50.8	-30.5	-6.7	-29.8	31.6	0.42
d 22	-35.8	-46.8	-44.5	-65.8	-41.5	22.3	0.86
d 29	-119	-153	-152	-171	-147	59.2	0.98
d 50	-54.0	-65.3	-55.0	-44.5	-59.0	46.4	0.77
d 71	-177[b]	-210[ab]	-214[ab]	-213[ab]	-314[a]	33.1	0.02
d 92	-220	-234	-175	-233	-217	23.3	0.32
d 113	-287	-292	-223	-266	-270	26.3	0.41

Total protozoa (×10^3/mL)							
d 8	1.02	1.58	0.757	0.669	1.79	0.485	0.40
d 15	2.01	2.24	0.690	0.127	1.39	0.976	0.53
d 22	0.0170	0.00990	0.0115	0.00833	0.0210	0.00979	0.88
d 29	-	-	-	-	-	-	-
d 50	-	-	-	-	-	-	-
d 71	-	-	-	-	-	-	-
d 92	-	-	-	-	-	-	-
d 113	-	-	-	-	-	-	-
Large holotrich (×10^3/mL)							
d 8	0.0341	0.0761	0.0318	0.0324	0.126	0.178	0.98
d 15	0.0241	0.00625	0.00313	1.69E-06	0.0137	0.0777	0.99
d 22	-	-	-	-	-	-	-
d 29	-	-	-	-	-	-	-
d 50	-	-	-	-	-	-	-
d 71	-	-	-	-	-	-	-
d 92	-	-	-	-	-	-	-
d 113	-	-	-	-	-	-	-
Small holotrich (×10^3/mL)							
d 8	0.00469	0.0115	0.00868	0.00156	0.0307	0.00916	0.25
d 15	0.0145	0.00424	0.00204	0.000794	0.00154	0.0326	0.99
d 22	-	-	-	-	-	-	-
d 29	-	-	-	-	-	-	-
d 50	-	-	-	-	-	-	-
d 71	-	-	-	-	-	-	-
d 92	-	-	-	-	-	-	-
d 113	-	-	-	-	-	-	-
Entodiniomorphs (×10^3/mL)							
d 8	0.991	1.49	0.727	0.640	1.63	0.481	0.50
d 15	1.96	2.23	0.684	0.127	1.37	0.966	0.54
d 22	-	-	-	-	-	-	-
d 29	-	-	-	-	-	-	-
d 50	-	-	-	-	-	-	-
d 71	-	-	-	-	-	-	-
d 92	-	-	-	-	-	-	-
d 113	-	-	-	-	-	-	-

[1]3-NOP regimens were Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112 ; High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112

[2]Standard error of the mean [3]When P-value for 3-NOP regimen was significant (≤ 0.05) pairwise comparison was performed by using Tukey's test, in that case means within a row showing different superscripts depict significant effects of 3-NOP regimen.

Table 8. Post-feeding protozoa enumeration, pH and redox potential in the rumen fluid of Angus steers fed 3-NOP titration regimens during the eight measurement times during the overall feeding period (day 0 to 112).

	3-NOP Regimen[1]						
Item[4]	Control	Low	Medium A	Medium B	High	SE[2]	*P-value*[3]
pH							
d 6	6.45	6.82	6.72	6.63	6.92	0.235	0.67
d 13	6.63	6.92	6.79	6.92	7.25	0.430	0.86
d 20	6.60	6.62	7.03	7.20	6.29	0.324	0.33
d 27	6.15	6.28	6.54	6.08	6.50	0.393	0.89
d 48	6.47	6.98	7.05	7.56	6.88	0.433	0.54
d 69	6.63	6.25	6.09	6.69	6.38	0.509	0.71
d 90	6.69	7.00	6.58	6.69	7.19	0.452	0.61
d 111	6.95	6.93	6.68	7.15	6.58	0.290	0.20
Redox potential							
d 6	-133	-154	-173	-88.5	-173	55.8	0.34
d 13	-126	-94	-102	-75.5	-136	35.6	0.75
d 20	-77.8	-47.0	-34.5	-67.0	-90.5	30.9	0.57
d 27	-145	-163	-172	-178	-196	30.2	0.77
d 48	-144	-119	-171	-135	-162	34.4	0.61
d 69	-131	-224	-181	-129	-128	51.7	0.29
d 90	-243	-194	-215	-199	-201	26.6	0.57
d 111	-178	-179	-194	-179	-167	34.2	0.99
Total protozoa (×10^3/mL)							
d 6	2.35	1.28	1.14	1.80	3.10	0.880	0.32
d 13	1.67	0.645	0.836	0.379	2.40	1.36	0.42
d 20	-	-	-	-	-	-	-
d 27	-	-	-	-	-	-	-
d 48	-	-	-	-	-	-	-
d 69	-	-	-	-	-	-	-
d 90	-	-	-	-	-	-	-
d 111	-	-	-	-	-	-	-
Large holotrich							
d 6	0.0872	0.0947	0.135	0.0904	0.258	0.254	0.96
d 13	0.0162	0.00296	0.0192	0.0029	0.0295	0.0859	0.99
d 20	-	-	-	-	-	-	-
d 27	-	-	-	-	-	-	-
d 48	-	-	-	-	-	-	-
d 69	-	-	-	-	-	-	-
d 90	-	-	-	-	-	-	-
d 111	-	-	-	-	-	-	-
Small holotrich							
d 6	0.0102	0.00535	0.0141	0.00844	0.0394	0.0595	0.99
d 13	0.0186	0.00434	1.81E-06	0.00278	0.0210	0.0725	0.99
d 20	-	-	-	-	-	-	-
d 27	-	-	-	-	-	-	-
d 48	-	-	-	-	-	-	-
d 69	-	-	-	-	-	-	-
d 90	-	-	-	-	-	-	-

d 111	-	-	-	-	-	-	-
Entodiniomorphs							
d 6	2.31	1.24	1.01	1.76	2.82	0.844	0.37
d 13	1.64	0.641	0.812	0.374	2.35	1.34	0.43
d 20	-	-	-	-	-	-	-
d 27	-	-	-	-	-	-	-
d 48	-	-	-	-	-	-	-
d 69	-	-	-	-	-	-	-
d 90	-	-	-	-	-	-	-
d 111	-	-	-	-	-	-	-

[1]3-NOP regimens were Control = no added 3-NOP; Low = 50 mg/kg DM 3-NOP from d 0 to 112; Medium A = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 112 ; Medium B = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg from d 15 to 112 ; High = 50 mg/kg DM from d 0 to 7, 75 mg/kg DM from d 8 to 14, 100 mg/kg DM d15 to 21, 125 mg/kg DM from d22 to 112

[2]Standard error of the mean [3]When P-value for 3-NOP regimen was significant (≤ 0.05) pairwise comparison was performed by using Tukey's test, in that case means within a row showing different superscripts depict significant effects of 3-NOP regimen.

4.4 Validation of current methods for predicting methane of feedlot cattle

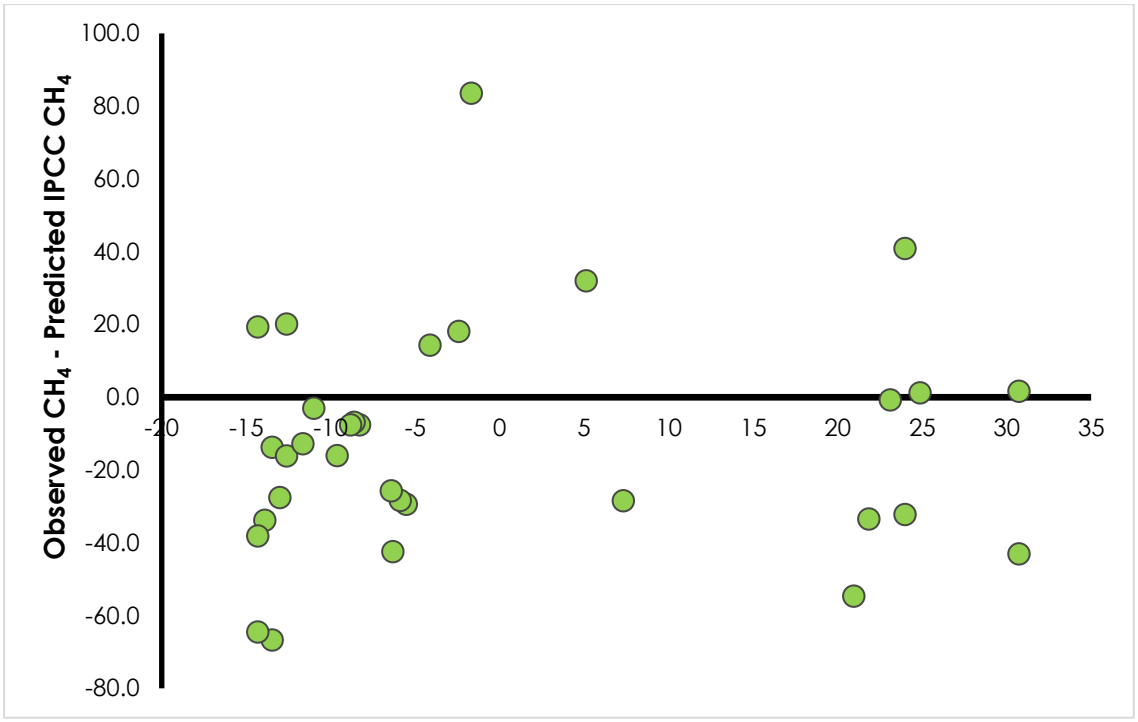
Descriptive statistics of the feed proximate analyses and methane emissions used in this model evaluation are presented in Table. By using results from both the adaptation and finisher periods, a broad range of GEI could be evaluated resulting in a robust analysis.

Table 8. Summary of descriptive statistics of CH_4 production of steers fed control diets (n=32) used in this model evaluation (day 0 to 112).

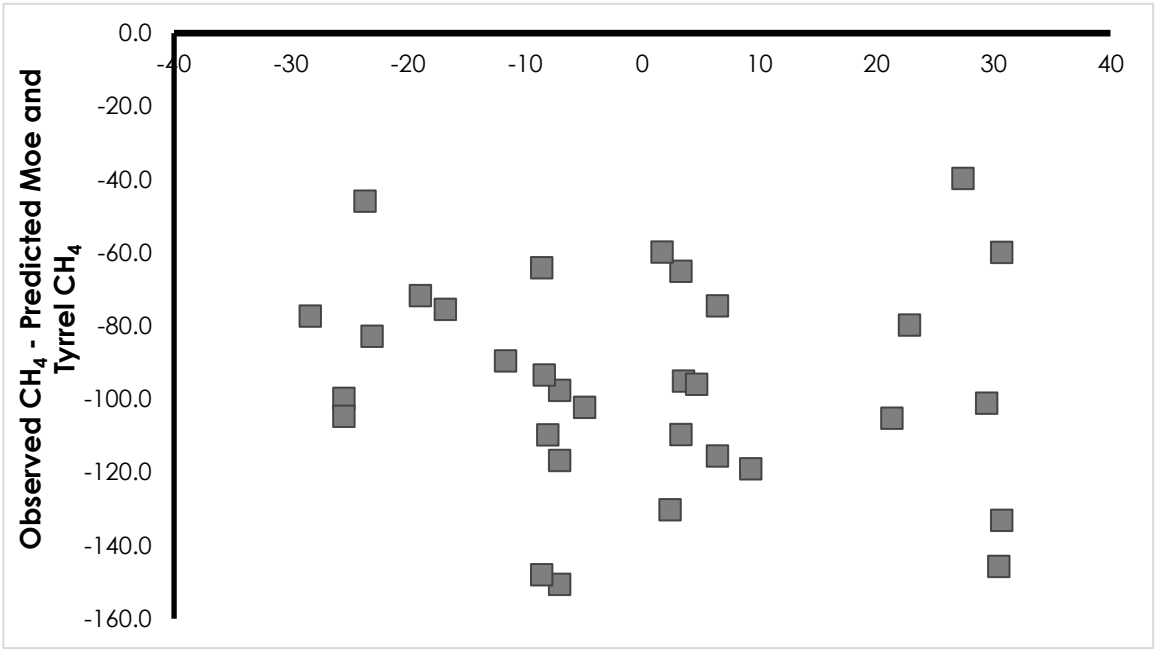
Trait	**Units**	**Median**	**SD**[1]	**Minimum**	**Maximum**
DMI	kg/d	9.11	1.53	6.24	11.9
CH_4 production	g/day	55.6	31.9	0	145
CH_4 production	MJ/day	3068	1762	0	8030
Gross energy intake	MJ/day	92400	15784	52443	113312
CH_4 production	%GEI	3.56	2.91	0	14.7
Soluble residues	kg/d	0.572	0.582	0.409	2.06
Hemicellulose	kg/d	1.87	0.223	1.32	2.18
Cellulose	kg/d	0.559	0.168	0.389	0.977

[1]Standard deviation

The results of the evaluation are presented in Figure 4. Both models displayed limited linear bias, and were consistent in their predictions of CH_4. IPCC (2006). In this regard, the estimated linear bias by IPCC (2006) and Moe and Tyrrell (1979) was -0.323 ± 0.351 (P = 0.37) and -0.187 ± 0.287 (P = 0.52), respectively. The IPCC (2006) CH_4 prediction displayed a negligible mean bias of -1.43 ± 5.41 g CH_4/day (P = 0.79). Whereas Moe and Tyrrell (1979) prediction had substantial mean bias, overpredicting CH_4 by 95.5 ± 5.09 gCH_4/day (P <0.01). These results show a potential of 'reducing' CH_4 output in feedlot animals in Australia by using the more suitable equation to estimate it (IPCC, 2006), which is strongly recommended after the evaluation presented herein.



(a)



(b)

Figure 4. Regression of residuals (observed – predicted CH_4) on predicted CH_4 production, modelled using the equations of IPCC (2006, a) and Moe and Tyrrell (1979, b). Estimated by IPCC (circle; Y = -1.43 ± 5.41 – 0.322 ± 0.351 × X ; P = 0.37; RMSE = 30.6) or Moe and Tyrrell (square; Y = -95.5 ± 5.09 – 0.187 ± 0.287 × X ; P = 0.52; RMSE = 28.8) equations) centred on their mean value from control group feedlot steers over 8 periods.

5. Conclusion

5.1 Key findings

- 3-NOP added to typical Australian feedlot diets containing 25 ppm of monensin and 7 % fat (DM-basis) reduced both production and yield of CH_4 by up to 99%.
- Although this experiment was not designed to make conclusions on an effect of 3-NOP on feedlot performance (cattle were limit fed to 2.7 x maintenance; 4 steers per titration regimen), no detrimental effect of 3-NOP administration was detected on the production parameters measured.
- The IPCC (2006) equation to predict CH_4 production is appropriate for the diets tested, which are typical of the white-grain, high-fat diets of the Australian feedlot industry. An alternative model tested, Moe and Tyrrell (1979) resulted in substantial overprediction of CH_4. Further investigation is required as the National Greenhouse Inventory utilises this Moe & Tyrrell equation.

5.2 Benefits to industry

This project has demonstrated that daily supplemental feeding of Bovaer® (3-NOP) may allow Australian feedlots to produce low emission beef during the grain-finishing phase. During the overall feeding period methane emissions (g/d) were 78% lower in the optimal 3-NOP regimen (medium B) compared with control animals. Early indications suggest that this high percentage methane reduction comes without cost to production, although further research is required to demonstrate any productivity costs or benefits to mitigating CH_4 emissions with 3-NOP at a commercial scale. Further modelling of benefits and costs of implementing 3-NOP supplementation as a carbon neutrality strategy will be required to demonstrate its advantages relative to other strategies, such as off-sets. While this project has demonstrated almost complete mitigation of CH_4 emissions in cattle fed a high-fat grain diet containing monensin, for benefits to flow from this research into effective implementation and adoption of 3-NOP as a CH_4 mitigator in the wider red meat industry, further research is required to support commercialisation.

6. Future research and recommendations

The research team recommends that a performance trial using a larger number of animals be implemented to test the effect of Bovaer®, on a typical Australian feedlot diet in order to evaluate the liveweight gain, intake and feed efficiency in feedlot cattle.

It may be necessary to test the efficacy of 3-NOP in other diets commonly used in the Australian industry as part of development of carbon credit systems by verification organisations. The effect of 3-NOP in diets varying in fat concentration will be interesting to research in the future. It is likely that the high fat content in combination with the high starch/ low NDF and inclusion of rumensin of the present finisher diet is one of the causes of the low CH_4 emissions from the control diet. Results from grain-based diets tested overseas with lower fat contents have not been able to achieve the same extent of CH_4 mitigation as reported here. In addition, testing of the effect of 3-NOP on methane emissions from higher forage diets utilised in Wagyu and growing animal production is recommended.

In this experiment hydrogen emissions were not measured. We recommend future research to examine hydrogen emissions to explain differences in rumen fermentation as only small differences in rumen fermentation parameters were found in the present study.

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8. Appendix

8.1 Analysed chemical composition of feedlot total mixed rations feed to Angus steers during the 112-d feeding period.

	Diet (feeding days)[1]			
Item	Starter (0-7)	Intermediate I (8-14)	Intermediate II (15-21)	Finisher (22-112)
Analysed Chemical Composition (DM-basis)[1]				
Neutral Detergent Fibre, %	40.5	33.7	31.3	29.0
Acid Detergent Fibre, %	22.5	17.7	15.0	11.8
Crude Protein, %	13.9	13.8	14.4	15.1
DM digestibility, %	69.0	73.3	77.3	82.0
Metabolisable Energy, MJ/kg DM	11.5	12.2	12.9	13.8
Crude Fat, %	4.35	4.70	5.58	6.66
Water Soluble Carbohydrates, %	12.2	10.9	7.73	4.44
Starch Total, %	20.0	28.3	35.7	42.3
Gross Energy, MJ/kg DM	18.6	18.7	18.8	19.3
DOMD, %	68.0	72.7	76.3	81.0
Inorganic Ash, %	6.00	6.00	4.75	3.80
Organic Matter, %	94.0	94.0	95.25	96.2
Moisture, %	12.9	14.4	15.6	16.4

[1]Diets were formulated by using the Concept 5 software. [2] 3-NOP was added to the experimental diets such that the placebo (Propylene glycol adsorbed on silicic acid, precipitated and dried) + Bovaer 10 (minimum 10% 3-NOP) added up to 0.15% of the diet. [2] DM= dry matter; DOMD = dry organic matter digestibility; analysed for content of crude protein (AOAC Method 2001.11), acid detergent fibre (ADF, AAFCO Method 008.08), neutral detergent fibre (NDF, NFTA Method 2.2.2.5), organic matter (ISO 5984:2002(E)), ether extract (AFIA Method 1.14R), starch (AOAC Method 996.11), water soluble carbohydrates (AFIA Method 1.11A), digestible dry and organic matter (AFIA Method 1.7R) (NSW DPI Laboratory Services - Wagga Wagga Chemistry Services Laboratory, PMB Pine Gully Road, Wagga Wagga NSW 2650 (Table 2)). Metabolisable energy (ME) content was calculated according to the equations for grains and concentrates of AFIA Method 2.2R (MAFF, 1990).

8.2 Fatty acid profile of the canola oil added to the feedlot total mixed rations feed to Angus steers during the 112-d feeding period.

Fatty acid	%
Myristic acid C14:0	0.07
Palmitic acid C16:0	4.4
Palmitoleic acid C16:1	0.3
Heptadecanoic acidC17:0	0.1
Heptadecenoic acidC17:1	0.1
Stearic acid C18:0	2.1
Oleic acid C18:1	62.3
Linoleic acid C18:2	18.4
Linolenic acid C18:3	10.1
Arachidic acid C20:0	0.6
Eicosenoic acid C20:1	1.0
Behenic acid C22:0	0.3
Erucic acid C22:1	<0.1
Lignoceric acid C24:0	0.1
Tetracosenoic acidC24:1	0.1
Total Fatty Acids	100

NSW DPI Laboratory Services - Wagga Wagga Chemistry Services Laboratory, PMB Pine Gully Road, Wagga Wagga NSW 2650. Edible Oil Fatty Acids Profile: 2-1702.