Final report

Reducing emissions of backgrounded cattle - combining Bovaer®10 with supplementation to reduce methane and increase productivity

Project code: B.FLT.5015

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Date published: 01.02.2024

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

A suitable delivery mechanism is currently lacking to consistently supplement the anti-methanogenic compound Bovaer® to grazing cattle. This project aimed to develop a pellet attractive enough to ensure multiple feeding bouts throughout the day, with the aid of automated pellet delivery, on pasture. The stability of 3-NOP through pelleting and storage was assessed, in addition to various feed delivery protocols. Finally, a 150 head grazing trial was conducted to quantify methane emissions and cattle growth when supplemented with either a control pellet (Lucerne) to replicate grazing conditions, an energy pellet, or an energy pellet containing Bovaer®. Average daily gain (ADG) was similar in cattle that consumed no pellets, to those in the control group, indicating an accurate replication of a grazing only scenario. ADG was 18.6% and 6.6% greater (P=0.002) with Bovaer, compared to those supplemented with the Control or Energy pellets. Bovaer® inclusion reduced methane emissions by 15.9% as g CH_4/d, and 24.8% (g CH_4/kg ADG) when compared to Energy supplementation, and by 31.5% (g CH_4/kg ADG) compared to the Control. The increased growth rate with Bovaer has the potential to reduce the number of grazing days required to achieve a theoretical 200 kg weight gain by 28 or 54 d, compared to supplementation with Energy, or grazing only, respectively. Importantly, enteric methane emissions over a theoretical backgrounding period would be reduced by 351 kg CO_2 eq. compared to grazing cattle and 340.5 kg CO_2 eq. compared to the energy pellet group. When comparing to grazing only, Bovaer supplementation had the potential to achieve an additional $12.58/head profit in carbon credits.

Executive summary

As Greenhouse gas emissions from agriculture are becoming increasingly scrutinized, Australian red meat producers' need options to address enteric methane emissions in order to maintain their social licence to operate. For grass-finished beef, Wiedemann et al. (2016 a) reported mean greenhouse gas emissions ranged from 10.6 to 12.4 kg $CO2_e$/kg liveweight (excluding land use and direct land-use change emissions). Currently, there are multiple promising anti-methanogenic alternatives, but their utilisation in extensive or pasture-based production systems is limited due to the lack of delivery methods and the economic pressure that daily supplementation represents to Australian beef cattle producers.

Consequently, this project aimed to assess the use of a known anti-methanogenic supplement, Bovaer® in grazing cattle. Specifically, we aimed to:

1) Develop a stable pellet containing Bovaer® 10 to be fed in a pasture-based beef cattle backgrounding system.
2) Determine the optimal system to deliver Bovaer® 10 supplement in multiple bouts (up to 4 times per day) to cattle in a pasture based backgrounding system.
3) Determine the effect of Bovaer® 10 on methane emissions and performance of cattle during a defined backgrounding phase.
4) Measure methane emissions of cattle using the GreenFeed system (GEM).
5) Determine the value proposition for Bovaer® 10 in combination with energy supplementation in the backgrounding phase for achieving carbon neutrality or reducing carbon footprint, accessing carbon credits, productivity (rate of gain and cost per unit of gain), feeding profitability or marketing premiums/access.

This was achieved in three phases, where Phase 1 assessed the stability of 3-NOP under two different pellet manufacturing protocols (steam vs pressure), and its subsequent retention after one month of storage at different temperatures. Phase 2 optimised the use of the pellets under grazing conditions by assessing different pellet delivery protocols using a GreenFeed unit. Finally, Phase 3 assessed the supplementation of Energy and Bovaer pellets in 150 grazing heifers, in comparison to a Lucerne GEM attractant pellet (Control) for effects on methane emissions and cattle growth over 56 days.

Storage of pellets containing Bovaer® at 4°C is recommended, or use within two weeks of manufacture when stored at room temperature to minimise loss of active ingredient and maximise the dose received by cattle. Average daily gain was similar in cattle that consumed no pellets, to those in the control group, indicating an accurate replication of a grazing only scenario. Supplementation of Bovaer pellets on pasture reduced methane by 15.9% as g CH_4/d, and 24.8% as g CH_4/kg ADG, compared to Energy supplementation. Bovaer inclusion in Energy pellets did not alter methane (g/d) compared to the Control pellet, however when expressed as methane per kg ADG it was 31.5% lower. Average daily gain was 6.6% greater with Bovaer, compared to those supplemented with Energy pellets, and 18.6% greater than cattle just on pasture. If comparing energy supplementation with or without Bovaer, the addition of Bovaer has the potential to reduce the number of days cattle are backgrounded by 28 d or 54 d if compared to grazing only for a typical industry backgrounding program. The net benefit of a reduction in days of grazing would be a reduction of 351 kg CO_2 eq. compared to grazing cattle and 340.5 kg CO_2 eq. compared to the energy pellet group per animal. When comparing to grazing only, Bovaer supplementation had the potential to achieve an additional $12.58/head profit in carbon credits.

Future research should focus on the method of delivery to ensure a constant supply of Bovaer® to grazing cattle when the use of automated feeders is not a viable option, or distances are too far for cattle to feasibly visit several times per day. This work could also focus on pellet formulation with alternative minerals, treatment of Bovaer® or pellets to reduce loss of Bovaer during pelletisation and storage, or to extend the duration that Bovaer® is available in the rumen once consumed by the animal.

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1. Background

To enable sustainability in consumer sentiment towards beef, lamb and goat meat, whilst taking ownership of its contribution to the national carbon footprint, the red meat industry has outlined a target of becoming carbon neutral by 2030 (Wiedemann & Longworth 2021). Considerable attention has been placed on the Australian red meat sector as a result of this pledge, despite only producing 13.1% of national emissions (Australia 2017). For grass-finished beef, Wiedemann et al. (2016 a) reported mean greenhouse gas emissions ranged from 10.6 to 12.4 kg CO_2e/kg LW (excluding land use and direct land-use change emissions). There is the potential for large scale reduction in methane from the Australian herd by targeting the grazing phase of the production cycle. Not only would a reduction in methane (g/d) have a significant impact on national methane emissions (Wiedemann et al. 2016 a), but if an increase in livestock productivity could be realised in addition, a shortening of time required for growth would amplify these emissions reductions over the life of an animal.

DSM Nutritional Products have developed a novel feed additive (Bovaer-10®, 10% 3-Nitrooxypropanol) that has been shown to reduce enteric methane by an average of 30% when fed to cattle (Almeida et al., 2021), and up to 81% for feedlot finishing diets (Vyas et al., 2016; Alemu et al., 2021; Almeida et al., 2023). In a recent study by Alemu et al. (2021) the inclusion of Bovaer-10® into cattle feed at low (150 mg Bovaer®/kg DM), medium (175 mg Bovaer®/kg DM) or high levels (200 mg/kg DM), methane (g/d) decreased by 17.4, 28.8 and 28.1%, respectively and methane yield (g/kg DMI) by 17.2, 25.7 and 21.7%, respectively. This was without significant changes in dry matter intake, cattle performance or feed efficiency. The outcomes of these trials are similar to those presented in the most recent meta-analysis evaluating the anti-methanogenic properties and rumen fermentation impacts of Bovaer® (Yu et al. 2021).

Despite the promising results to reduce methane emissions, to-date there are no published studies evaluating Bovaer® in grazing cattle (Yu et al. 2021). This is likely due to the difficulties faced by all additives, in that constant and quantifiable intake of additives to grazing cattle is extremely difficult (Black et al. 2021). Alternatives have been proposed such as Bovaer® inclusion in a total mixed ration, lick blocks, or slow – release mechanisms, or Bovaer® pelleting for intensive production. Due to the mode of action (Duin et al., 2016) and short rumen retention time, it is hypothesised that multiple consumption bouts per day of the supplement would be necessary to maintain methane suppression in a grazing system. As such, this project aimed to evaluate the use of pellets voluntarily consumed throughout the day in the form of a high energy supplement on methane emission of grazing cattle.

The use of Bovaer® must also come with consideration for the inherent properties of 3-NOP, including heat sensitivity and stability when exposed to air. In a comprehensive evaluation of Bovaer® by Bampidis et al. (2021) concluded that 3-NOP retention after 12 and 18 months of storage in aluminium bags at ambient temperature was 95.5 and 97.3% of initial 3-NOP®, respectively. When this additive was included in a total mixed ration (TMR), 3-NOP retention decreased to 74% after three months of storage, compared to 83.3% after pelleting at 80°C and a further 3 months of storage (Bampidis et al. 2021). Therefore, this project also investigated the stability of 3-NOP in pellets under different pellet manufacturing processes, and across one month of storage under different temperature and humidity conductions.

2. Objectives

The objectives of this project were to:

1. Develop a stable pellet containing Bovaer® 10 to be fed in a pasture-based beef cattle backgrounding system.
2. Determine the optimal system to deliver Bovaer® 10 supplement in multiple bouts (up to 4 times per day) to cattle in a pasture-based backgrounding system.
3. Determine the effect of Bovaer® 10 on methane emissions and performance of cattle during a defined backgrounding phase.
4. Measure methane emissions of cattle in trial using appropriate field deployable equipment (Greenfeed system).
5. Determine the value proposition for Bovaer® 10 in combination with energy supplementation in the backgrounding phase for achieving carbon neutrality or reducing carbon footprint, accessing carbon credits, productivity (rate of gain and cost per unit of gain), feeding profitability or marketing premiums/access.

3. Methodology

3.1 Animal ethics approval

The animal trials (Phase 2 and phase 3) were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes with approval from The University of Queensland Animal Ethics Committee (SA2022/06/216).

3.2 Phase 1 – Pellet development

3.2.1 Pellet production and chemical composition

This phase of the project focused on formulating cattle feed pellets containing Bovaer and evaluating the physical and chemical properties of those pellet formulations. Energy pellets were designed for crossbred heifers (non-pregnant) with an initial weight of 250 kg. The pellets were formulated to provide 13.11 MJ/kg DM of metabolic energy, 7.41 MJ/kg of net energy for maintenance, and 5.80 MJ/kg of net energy for gain, allowing an average daily weight gain of 0.65 - 0.70 kg/d. A target of 2 kg (as fed) of pellet intake per animal per day was used to simulate common supplementary feeding or backgrounding for increased production. Physiologically regulated voluntary DMI was estimated using (Mertens 1987) equation which considered neutral detergent fibre (NDF) intake capacity, NDF content of pasture species (*Chloris gayana*) and expected initial body weight (iBW) of heifers. A total of 6.82 kg/d was estimated as daily dry matter intake (DMI), including 2 kg as pellets, and 4.82 kg of pasture consumption. Bovaer® was included at 0.51% of the total pellet ingredients, or 510 mg/kg. Assuming a total intake of 6.82 kg DM/head/day, as calculated above, the Bovaer pellets at 2 kg/hd/d would therefore provide the animals with a 3-NOP concentration of 150 mg/kg DMI (Table 1, referred to as Bovaer pellets from here onwards).

Table 1. Ingredients and chemical composition of the pellets produced using steam or pressure.

	% as fed				
Ingredient	Steam energy pellets	Pressure energy pellets batch 1	Pressure energy pellets batch 2	Pressure energy pellets batch 3	Control GEM attractant pellets
Barley	32.6	33.1	32.6	33.1	n/a
Wheat	25.6	25.6	25.6	25.6	n/a
Millrun	22.5	22.5	22.5	22.5	n/a
Canola meal	10	10	10	10	n/a
Bovaer®	0.51	0.51	0.51	0.51	n/a
Veg oil	2	2	2	2	n/a
Molasses	3	3	3	3	n/a
Aglime	1	1	1	1	n/a
Monodicalcium Phosphate	0.75	0.75	0.75	0.75	n/a
Magnesium Oxide	0.25	0.25	0.25	0.25	n/a
Urea	1.25	1.25	1.25	1.25	n/a
Vitamin 4 premix*	0.2	0.2	0.2	0.2	n/a
Mineral D premix	0.2	0.2	0.2	0.2	n/a
Chemical composition					
Crude protein, %DM	19.3	20.1	20.1	20.7	18.6
Acid detergent fibre, % DM	5.4	6.8	6.5	6	36.2
Neutral detergent fibre, % DM	15.4	17.3	15.7	15.1	43.2
Crude fat, % DM	4.68	2.67	2.79	2.51	1.4
Starch, % DM	35	32.3	35.1	37.7	2.2
NFC, % DM	51	49.4	49.7	51	30.3
Ash, % DM	5.46	6.14	6.58	5.85	9.5
DE, Mcal/kg	3.73	3.58	3.56	3.66	2.51
ME, Mcal/kg	3.33	3.17	3.15	3.24	2.09
NEm, Mcal/kg	2.08	1.98	1.97	2.03	1.05
NEg, Mcal/kg	1.41	1.33	1.32	1.37	0.50
Ca, %	1.08	1.05	1.04	1.01	0.89
P, %	0.67	0.74	0.69	0.7	0.23
K, %	0.7	0.79	0.7	0.73	2.56
Mg, %	0.32	0.44	0.41	0.43	0.32

Abbreviations: NFC, non-fibre carbohydrates; DE, digestible energy; ME, metabolic energy; Nem, net energy for maintenance; Neg, net energy for gain.

*Composition of Vitamin and Mineral premix is the proprietary information of Riverina Stockfeed.

All pellet ingredients were purchased in one batch to limit variation in the quality of ingredients. Chemical composition of pellets was performed at Dairy One laboratory (Ithaca, New York, United States of America), following the Association of Official Analytical Chemist (AOAC) procedures. Briefly, DM content was calculated at 105°C, following AOAC (2005, method 934.01). Ash was determined at 550°C for 8 h AOAC (2005, method 942.05). Acid detergent fibre (ADF) was calculated following AOAC (2005, method 973.18). Whilst, ash – free neutral detergent fibre (aNDF) was estimated using method described in Mertens (2002). Crude fat was calculated by extracting ether for lipid extraction AOAC (2006, method 2003.05). Nitrogen content was calculated using Dumas combustion with a carbon/nitrogen combustion analyser (Sweeney 1989). Crude protein content was estimated by multiplying nitrogen content with 6.25. Starch content was determined following (Hall 2009). Minerals was determined with the method described in AOAC (2000, method 985.01). Feed energy content and non-fibre carbohydrates (NFC) were calculated according to National Academies of Science, Engineering, and Medicine (NASEM 2001). The standard deviation and coefficient of variance was calculated to determine differences between commercial and non-commercial batches.

For analysis of 3-NOP stability, batches of energy pellets incorporating Bovaer® were produced under commercial feed conditions (Steam), and at the Queensland Animal Science Precinct (QASP), at The University of Queensland, Gatton campus (Pressure). A total of 1 tonne was produced commercially with a maximum pelleting temperature of 67°C, steam pressure ~225 kPa, resulting in a 90.18% dry matter content (DM) after cooling. Three batches were produced at QASP using a feed mixer (Seko Samurai 5) and pressure based pelleting mill (flat pellet machine, model KL200). Pelleting temperatures for batch 1, 2 and 3 were 45°C, 47.3°C and 50.3°C, respectively, whilst the DM contents were 87.56%, 88.67% and 88.51%, respectively.

A pellet capable of attracting the control group to the GEM unit was required in Phase 3 to enable measurement of methane in these animals. A commercial Lucerne pellet (Control pellet; Lockyer Lucerne Pellets, Lockyer Lucerne, Gatton QLD) was selected to reflect nutrient availability of a pasture only diet as closely as possible. Chemical composition of the Control pellet is provided in Table 1.

To determine pellet durability, 500 g of pellets were sifted through a size 6 screen sifter to remove fines. Pellets were weighed and transferred into a tumbler can (~30 cm L, 30 cm W and 12.5 H), containing a metallic plate in the middle of the device (~23 cm large and ~5 cm width). Pellets were tumbled for 10 min at a speed of 50 RPM. Pellets were then sifted and weighed again to determine the pellet durability index (PDI) using the formula below. This methodology was described in The American Society of Agricultural and Biological Engineers (ASAE) standard S269.4 (ASAE 1991).

$$PDI = \frac{Final\ pellet\ weight\ (g)}{Initial\ pellet\ weight\ (g)}\ x\ 100$$

Pellet hardness was evaluated by randomly selecting 10 pellets, from a previous sub-sampled 100 g of pellets from each batch. A Hercules M hardness tester (Amandus Kahl GmbH & Co, Reinbek, Germany, Image 1) was used as per the manufacturer's instructions to determine the force required to break a pellet. For Kahl pellet hardness test, results are expressed in kilograms (Thomas & van der Poel 1996).

Image 1. Kahl harness tester, Hercules M

3.2.2 Accelerated 3-NOP stability sampling and test

The concentration of 3-NOP in the pellets was evaluated after pelleting, and after storage in four varying environmental conditions to determine the optimal conditions for energy pellet storage to limit 3-NOP disappearance (stability). A 20 kg bag from each batch was stored at 30°C (oven), 4°C (cold room), ambient temperature (ambient), or at 30°C with a relative humidity of 70% (incubator) to simulate average weather conditions of summer at Gatton, Queensland. A total of five samples per batch produced were collected weekly in HPDE containers and stored at -20°C until analysis. Pellet samples were collected over a month to replicate the expected storage time. The conditions in the incubator facilitated an accelerated stability test, replicating 4 months of storage. Analysis was conducted on two Pressure batches and one Steam batch to determine if the difference in pelleting processes significantly affected the stability of 3-NOP over time. The concentration of 3-NOP in Bovaer pellets (n=200 total, 5 replicate samples collected weekly from each of the 4 storage conditions, for each of the three pellets batches) was estimated using high performance high liquid chromatography coupled to spectrophotometric (UV) detection by Analytica Labs, NZ (Ezerskis 2020).

3.3 Phase 2 - Greenfeed intake optimisation trial

This phase of the project focused on animal behaviour and consisted of a small pilot study to optimise feed delivery and feeding visits with the GEM unit for capture of usable methane data (based on >3min visit, > 3 to ideally 4 visits per day), with the smallest amount of feed being dispensed each visit. Six Droughtmaster steers (412 ± 48.3 kg initial body weight) were grazed in a 3 ha paddock at QASP, UQ Gatton for 60 days. Animals had *ad libitum* access to mixed tropical pasture (predominately Rhodes grass) and water. A single GreenFeed Emission Monitoring (GEM) unit was available to evaluate visiting frequency and time of visits using different bait pellets. The GEM unit was located next to the water trough to promote steer utilisation. The GEM unit was freely accessible to all animals during the adaptation period to encourage access.

The trial comprised of 2 periods: 1) a 20 d period examining the use of the commercially available Control pellet, and 2) a 40 d period examining Energy pellets produced in Phase 1. In the first period, two feeding protocols were trialled. The first protocol provided a maximum of six visits per day, with each visit having a maximum 240 g of feed dispensed (8 feed drops, with each drop spaced 35 sec apart), such that the total daily intake was a maximum of 1.44 kg. In the second period, the target total daily intake was 2 kg, with a minimum of four visits per day. Four different GEM unit protocols were tested (Table 2), where the first two protocols increased pellet consumption up to ~2 kg intake, and the final two (protocol 3 and 4) were the experimental protocols.

Table 2. Alternative GreenFeed (GEM) protocols with energy pellets to maximise daily visit number, and usable methane data capture.

Protocol	Max. daily visits	Max. drops per visit	Pellets per drop (g)	Time between drops (sec)	Pellets per visit (g)	Total pellets per day (g)
1	6	6	30	35	180	1080
2	5	10	30	35	300	1500
3	5	13	30	35	390	1950
4	4	17	30	35	510	2040

Autocalibration of the GEM unit was conducted every 3 days by C-Lock. A $C0_2$ recovery test was conducted at the start of the experiment, monthly during the experiment and at the completion of the trial following C-Lock's protocol. Methane emissions were recorded by the GEM unit during this trial with the sole aim to determine if the settings were successful to enable capture of methane data, and therefore we are not reporting the methane emissions from these animals.

3.4 Phase 3 – Quantification of methane emissions during backgrounding

3.4.1 Animals and experimental design

A total of 150 crossbred heifers (initial weight of 298.0 ± 1.9 kg BW) were used in this study, conducted at Darbalara farm, The University of Queensland within an area of 25 ha. On arrival, heifers were housed together in a 3 ha paddock and had a minimum 14 d adaptation period to the GEM units, with the Control pellet as the feed offered. The study ran across two periods of 10 weeks, with 75 heifers used in each period. Each period comprised 2 weeks of dietary adaptation, and 8 weeks of experimental measurement. At the start of each period, heifers were randomly assigned to one of three treatments (n=25), blocked by bodyweight:

1) Grazing + Lucerne pellet @ 1 kg/hd/day (Control)
2) Grazing + Energy pellet @ 2kg/hd/day (Energy).
3) Grazing + Bovaer energy pellet @ 2kg/hd/day (Bovaer). Target dose of Bovaer® in pellets was 511 mg of 3-NOP/kg DM

The amount of pellets offered between treatment varied, with Control heifers provided with 1 kg/h/d to replicate grazing with minimal supplementation, compared to the Energy and Bovaer heifers, which were offered at 2 kg pellets/h/d. Heifers were vaccinated with 7 in 1 (Zoetis, New Jersey, USA), Piliguard Pinkeye vaccine (Cooper's, NSW, Australia), tick fever, bovine ephemeral fever (ULTRAVAC BEF VACCINE®, Zoetis, New Jersey, USA), and Baycox for clostridium (Bayer Ltd, Auckland, New Zealand), ear tagged and treated for internal and external parasites.

Heifers had *ad libitum* access to pastures, which comprised three main species (*Chloris Gayana, Megathyrsus maximus* and *Cenchrus clandestinus*), and water. Heifers, grouped by treatment, were rotated through six fertilised and irrigated paddocks (3.8 to 5.4 ha) every 12 d, such that each animal had access to the same pasture by the end of the trial. Pasture yield was calculated for each rotation using the visual yield score method (VYS) to monitor total biomass available in paddocks and estimate pasture intake. Briefly, the VYS method required standard calibrations where all pasture species were categorised into 5 different levels using a quadrat (50 cm x 50 cm). Pasture height was measured at each level, and a sample was collected and dried at 60°C in a force-air oven for 48 h to determine dry matter content (DM). Dry matter yield per hectare was estimated by using quadrat area and dry matter yield per quadrat. A linear regression model was developed for each pasture species to predict dry matter yield per hectare per centimetre. Approximately 100 quadrat samples were assessed at the beginning and end rotation of each rotation, and the difference in average dry matter yield between these sampling points were considered as average pasture intake (Haydock & Shaw 1975). A Rhodes grass (*Chloris Gayana)* hay bale was provided for each treatment group in the last rotation of each period to ensure adequate intakes were maintained.

Heifers were weighed every 12 d at the end of each pasture rotation. Average body weight gain was calculated as the difference between the initial and the final weight within sampling subperiod. Average daily gain (ADG) was calculated by dividing body weight gain by the number of days (i.e., 56 d). Individual methane (CH_4) emissions (g/d) were estimated using a GEM unit (Hristov et al. 2018). One GEM unit was allocated into each paddock (n=3), and randomly rotated through the treatment groups to avoid biases due to GEM unit. Animals had access to the GEM unit for 5 visits daily, with a minimum of 1 h between visits, and 30 g of pellets dropped at 30 sec intervals during each visit. Pellet intake was calculated by multiplying pellet drops per day and the amount of pellet per drop (g). Individual daily pellet intake was averaged by rotation within period. Calibration of the GEM unit was conducted automatically every 3 days by the manufacturer (C-Lock Inc, Rapid city, South Dakota, USA), while CO_2 recovery tests were performed every month.

Cattle were classified into users or non-users of the GEM unit based on selection criteria. Daily pellet intake for all animals was measured by the GEM units. Users were classified as those cattle with at least seven days where cattle visited the GEM unit ≥3 times, with each visit lasting ≥ 3 mins (Hammond et al. 2016), such that a minimum of 21 data collecting visits were achieved during the experimental period. Methane data was averaged per day and per rotation to estimate average daily methane emissions (g/d). Furthermore, methane emission data was averaged per period to calculate methane intensities by dividing methane emissions by ADG (CH_4 g/d per kg ADG), BW (CH_4 g/d per kg BW) and pellet intake (CH_4 g/d per kg pellet intake).

Rumen fluid was collected from 10 heifers per treatment classified as GEM unit users on d 46 of each period. Briefly, rumen fluid was collected via oesophageal tubing with the first sample discarded to avoid saliva contamination. Rumen fluid was filtered through four layers of cheesecloth and pH was immediately measured (Edge Benchtop HI2002, Hanna instruments, Melbourne, VIC, Australia). A total 1.5 mL of rumen fluid were transferred into 4 replicates, including 0.3 mL of metaphosphoric acid (25% w/v) for determination of volatile fatty acids (VFA). In addition, subsamples were transferred into 2 replicates tubes for ammonia (NH_3-N) determination (6 mL rumen fluid plus 2 mL 0.5 M H_2SO_4). Both VFA and NH_3-N samples were stored on iced and later stored into -20°C freezer for further analysis.

3.4.2 Laboratory analysis

Total and individual VFA samples were analysed using gas-liquid chromatograph system (Agilent 7820A, Santa Clara, California, USA) with a DB-FFAP column (30 m x 0.32 mm x 1.00 um), flame ionization detector (FID) at 250°C, air flow of 350 mL/min, hydrogen (H_2) fuel flow at 30 mL/min, nitrogen makeup flow at 30 mL/min split inlet heated at 225°C with PSI pressure of 9.526, helium flow at 33 mL/min. The septum purge flow was at 3 mL/min with a split ratio of 5:1 and split flow of 25 mL/min. VFA samples went through the system oven which the initial temperature was set at 195°C for 1 min. Later, the temperature was increased up to 195°C (5°C per minute) and samples were held for 3 min (Forwood et al. 2019). Rumen ammonia was analysed by colorimetric procedure using SEAL AQ400 discrete analyser (Seal Analytical Ltd, Southampton, United Kingdom). Briefly, rumen fluid samples (0.2 mL) were mixed with tartrate buffer (0.3 mL), salicylate-nitroprusside reagent (0.2 Ml) and bleach solution (0.1 mL). The analyser wavelength was set to 660 nm, and the calibration curve (0.1 – 4 mg/L-N) was performed for each sample analysed. Outcomes are expressed in absorbance unit, which later are converted into a mg/L – N value using a standard curve calculated from the results for the standards (Baethgen & Alley 1989).

3.4.3 Statistical analysis

Data were analysed as a randomized complete block design, using MIXED procedure of SAS (SAS Institute Inc. 2019 version 9.4) with individual heifer as experimental unit. Cattle growth performance, pellet intake, rumen fermentation and methane intensity parameters were analysed using a model considering treatment and period, as well as their interaction, as fixed effect and period and individual heifer interaction as a random effect. The model used to analyse methane emissions had the same fixed effect parameters, but rotation was considered a repetitive variable, and the interaction between individual heifer, treatment, and period as a subject. Covariance structure selection was performed using MIXED model of SAS program where unstructured structure covariance demonstrated the minimum values of Alaike's information criterion. Normality of residues were evaluated by Shapiro-Wilk test and homogeneity of variances was estimated with Levene test. Differences between treatments were considered significant at $P \leq 0.05$ and tendencies were declared when $P \leq 0.10$.

3.4.4 Cost benefit and emissions analysis

A number of scenarios were used to estimate the cost benefit of providing cattle on pasture with an energy pellet containing Bovaer:

1) The impact of supplementing grazing cattle with pellets (with and without Bovaer) on animal growth, compared to grazing only.

The equation used to calculate the gross margin was:

Gross margin ($/hd/d) =

$$\frac{\text{Sale income} - (\text{Purchase price} + \text{Health cost})}{\text{Number of days grazing}} + \text{Daily grazing cost} + \text{Daily supplementation cost}$$

2) The impact of supplementing grazing cattle with pellets (with and without Bovaer) on methane inhibition, compared to grazing only (Control pellet).
3) The ability to monetise use of a methane inhibitor in grazing cattle using Australian Carbon Credit Units as an example.

4. Results

4.1 Phase 1 - Pellet chemical composition and quality

Chemical composition of the pellets was consistent across all batches (Table 3). Crude fat presented the highest variation when pellets produced using steam were compared with pressure, but low variation was observed among the three pressure batches. Pellet durability was similar between batches. Though, pellets produced with steam had the highest durability with 98.40%, followed by pressure batch 2 with 95.80%, pressure batch 1 with 94.80% and pressure batch 3 with 93.60%. All batches had a higher than the expected pellet durability index under commercial conditions, at 90% (Winowiski 2014). Steam pellets were twice as resistant to breakage (17 kg), compared to pressure pellets (average 8.3±0.8 kg) as measured using the hardness test.

4.1.1 3-NOP stability test

The retention of 3-NOP through pelleting was different between steam pellets, and pressure pellets, where pressure batch 2 which had the greatest retention (Figure 1). Samples of steam pellets, and two pressure batches (batch 2 and 3) were analysed for 3-NOP stability, due to the capacity of the lab (200 samples, 5 replicates collected weekly for one month, from each of the 4 storage conditions, for each batch). Pellets produced with pressure showed greater 3-NOP stability, especially pressure batch 2 which retained 97.26 ± 0.34% of 3-NOP post-pelleting. Whereas samples from pressure batch 3 showed a great variability with an average 3-NOP disappearance of 29.6%. The steam pellets had the greatest 3-NOP disappearance during pelleting, averaging 37.2%.

Table 3. Differences in chemical composition between energy pellet batches

Parameter	Pressure pellets			Steam pellets		
	Mean	SD	CV	Mean	SD	CV
Dry matter, %	95.23	0.32	0.34%	95.8	0.06	0.06%
Crude protein, %	20.3	0.35	1.71%	19.3	0.57	2.87%
Acid detergent Fibre, %	6.43	0.4	6.28%	5.4	0.61	9.93%
Neutral detergent fibre, %	16.03	1.14	7.09%	15.4	0.98	6.18%
Ether extract, %	2.66	0.14	5.29%	4.68	1.02	32.19%
Starch, %	35.03	2.7	7.71%	35	2.21	6.30%
Non-fibrous carbohydrates, %	50.03	0.85	1.70%	51	0.85	1.68%
Ash, %	6.19	0.37	5.94%	5.46	0.47	7.87%
Ca, %	1.03	0.02	2.01%	1.08	0.03	2.76%
P, %	0.71	0.03	3.73%	0.67	0.03	4.21%
K, %	0.74	0.05	6.19%	0.7	0.04	5.81%
Mg, %	0.43	0.02	3.58%	0.32	0.05	13.69%

Abbreviations: SD, standard error; and CV, coefficient of variation.

Stability analysis across four weeks of storage indicated the highest retention of 3-NOP under cold room conditions (Figure 2) for both processing types (steam vs pressure). Specifically, the concentration of 3-NOP in pressure batch 2 pellets declined 1.23% after one month of cold room storage, 9.93% at ambient temperature, 10.63% in an incubator, and 11.21% in an oven, over the same time period. The 3-NOP concentration in steam pellets decreased 7.35% when stored in a cold room, 21.67% at ambient temperature, 30.79% in an incubator, and 49.33% in an oven, respectively, when stored for four weeks.

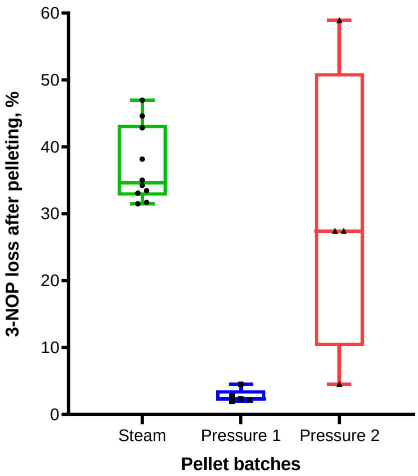


Figure 1. Differences in 3-NOP concentration (%) between energy pellet from formulated values, to after pelleting

Interestingly, pressure batch 3 presented an inverse relationship, where cold room and ambient conditions showed the greatest 3-NOP loss, while the greatest 3-NOP retention was under oven conditions. The large variation between each replicate and the unexpected concentrations observed resulted in the exclusion of this batch from our analysis and interpretation.

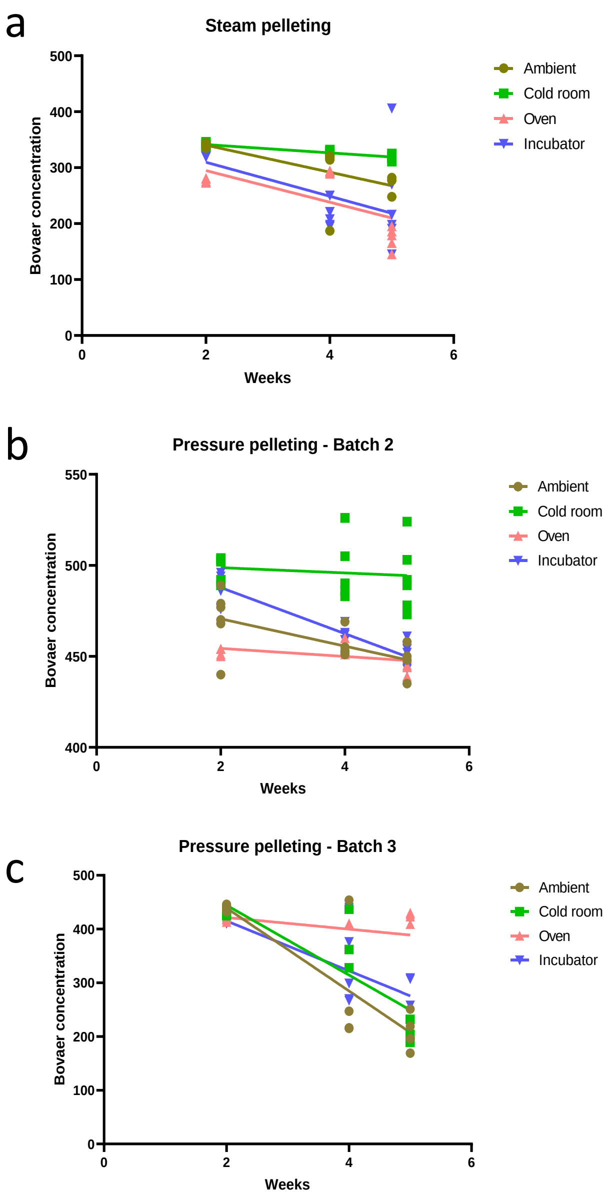


Figure 2. Concentration of 3-NOP (mg/kg as fed) in a) steam pellets and pressure pellets [batches 2 (b) and 3 (c)], and, when stored at ambient temperature in a shaded shed, cold room (4°C), incubator (30°C at 70% humidity), or Oven (30°C).

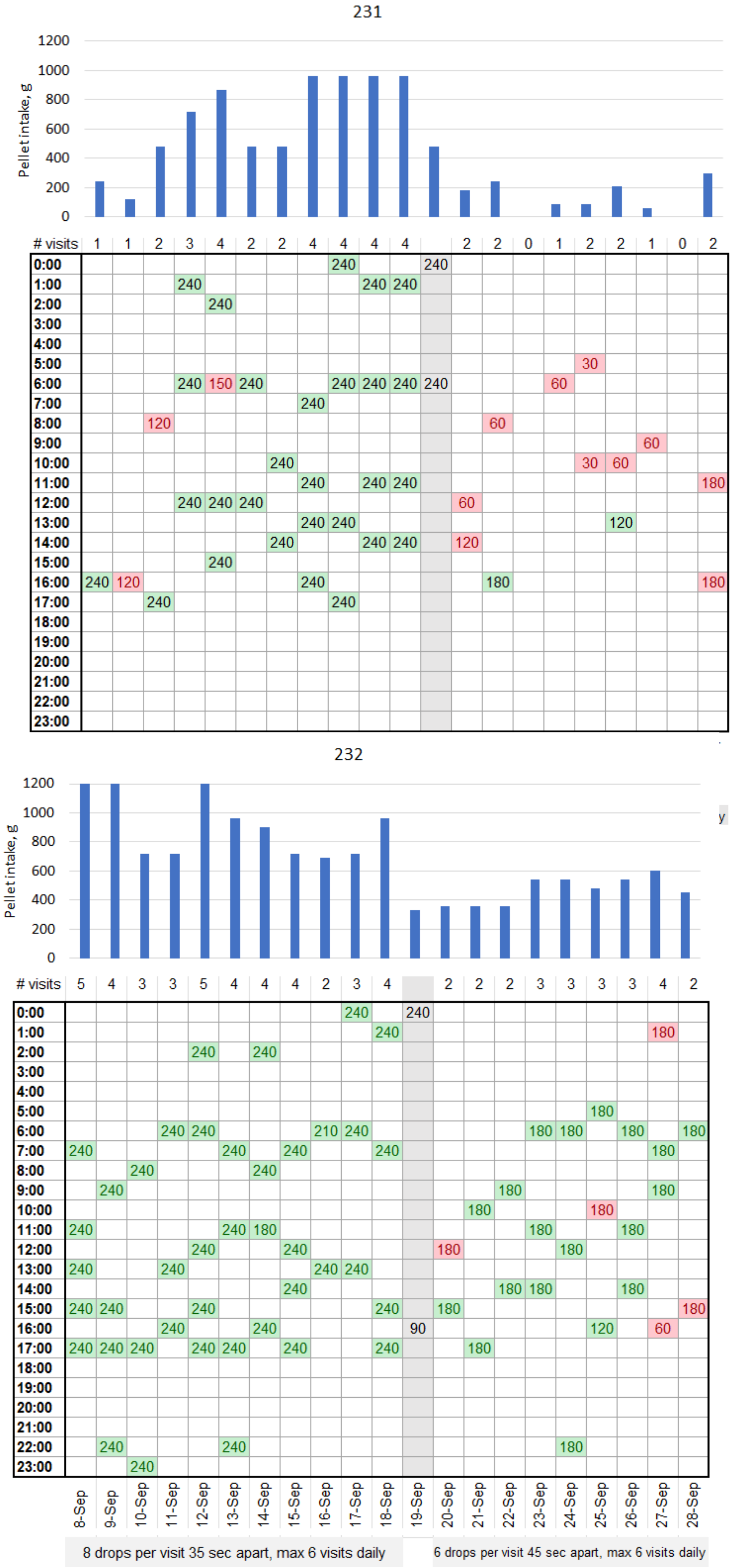
4.2 Phase 2 - Greenfeed intake optimisation trial

As expected, altering the GEM protocol and pellet type altered the behaviour of the steers, such that, the number of steer visits, time spent at the device and pellet intake changed. Offering GEM attractant pellets up to a maximum 6 visits per day, with 8 drops each visit spaced 35 sec apart, resulted in steers visiting the GEM unit on average 2.6 (range 1.9 to 3.7) times per day (Figure 3). Five of the six steers visited the GEM unit on a consistent basis, averaging 3.36 visits per day. Steer 254 had a much lower visit occurrence at 1.9 visits per day. Providing 240 g was sufficient to keep animals at the machine long enough to obtain reliable methane data from a visit. Overall, this suggests that 3 – 4 visits per day with 240 g each visit will be suitable to obtain reliable data, capturing varying timepoints throughout the day. The maximum intake with these settings would be 960 g, if 4 visits are achieved.

Using the second protocol (6 visits and 6 drops/visit, with drops spaced at 45 seconds apart), there were a clear reduction in the number of visits, now ranging between 2.0 – 3.1 per day. This is below the required number to obtain reliable and repeatable methane measurements from a GEM unit ($n \geq 3$). Additionally, the number of visits that did not result in good methane data collection were increased, as steers appeared to not like waiting the longer time period for more feed to drop, and left the machine. Steer 254 was again an anomaly, increasing the number of visits with these settings, and visited the machine more consistently throughout the day, than with the previous quicker drop settings. This indicates there will be individual preferences between animals, however the majority of steers preferred the faster drop protocol, and as such this protocol was used for the control group in Phase 3.

The second period of measurement examined the use of the energy supplement pellet (Figure 4). The steers showed a very strong interest in these pellets, with almost all offered feed being consumed, regardless of the settings. We achieved consistent visits throughout the day – with all but one animal (254) visiting the maximum number of times allowed each day (regardless of the number allowed). An interesting trend appeared, where most visits occurred between 5 am and 5 pm, with clear patterns of visit times emerging for some animals throughout the day. Similarly, almost all visits provided reliable methane data, indicating that the visits were long enough to ensure the animals remained at the feeder.

Figure 3. Total pellet intake and GEM unit visits by individual animals in Phase 2 with the control commercial lucerne pellet. The bar graph represents total daily intake of pellets, in grams. The boxes below indicate the amount consumed in each visit during the day, in grams, the time of that visit and if the visit duration was successful to obtain good methane data (green = successful, red = unsuccessful). The total number of visits per day is reported along the upper x-axis, and the GEM unit settings on that particular date is represented on the lower x-axis.



231

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5:00																	30				
6:00				240	150	240			240	240	240	240				60					
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232

	8-Sep	9-Sep	10-Sep	11-Sep	12-Sep	13-Sep	14-Sep	15-Sep	16-Sep	17-Sep	18-Sep	19-Sep	20-Sep	21-Sep	22-Sep	23-Sep	24-Sep	25-Sep	26-Sep	27-Sep	28-Sep
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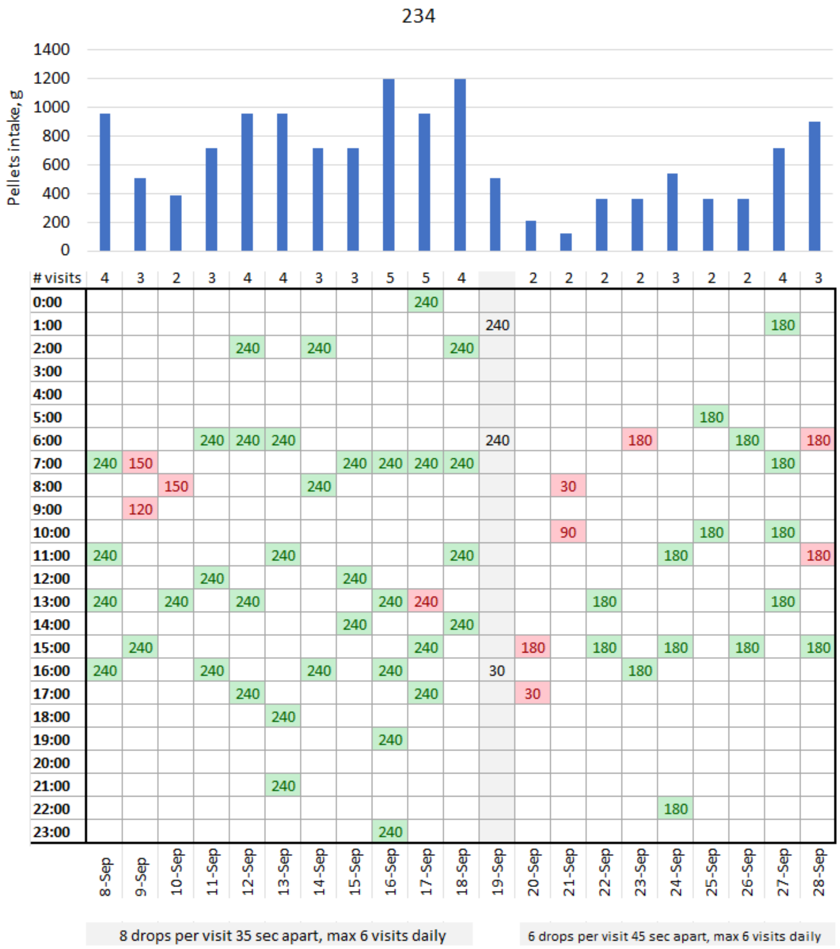
22:00		240				240											180				
23:00			240																		

Figure 3 (continued). Total pellet intake and GEM unit visits by individual animals in Phase 2 with the control commercial lucerne pellet. The bar graph represents total daily intake of pellets, in grams. The boxes below indicate the amount consumed in each visit during the day, in grams, the time of that visit and if the visit duration was successful to obtain good methane data (green = successful, red = unsuccessful). The total number of visits per day is reported along the upper x-axis, and the GEM unit settings on that particular date is represented on the lower x-axis.



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7:00	240	150						240	240	240	240									180	
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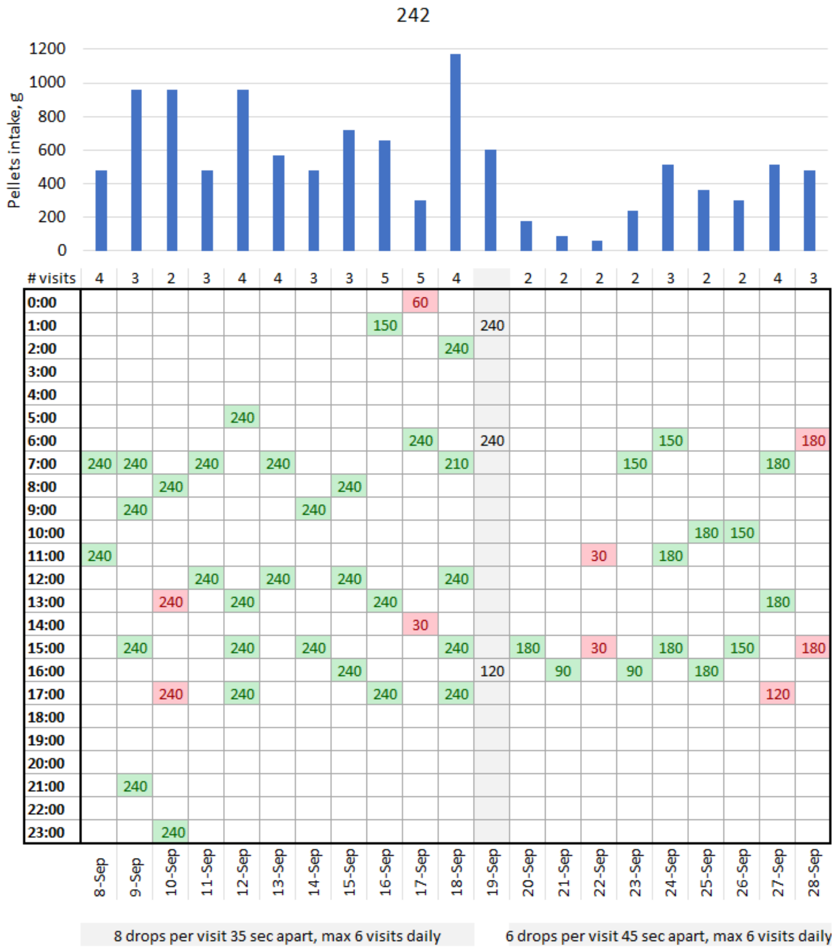

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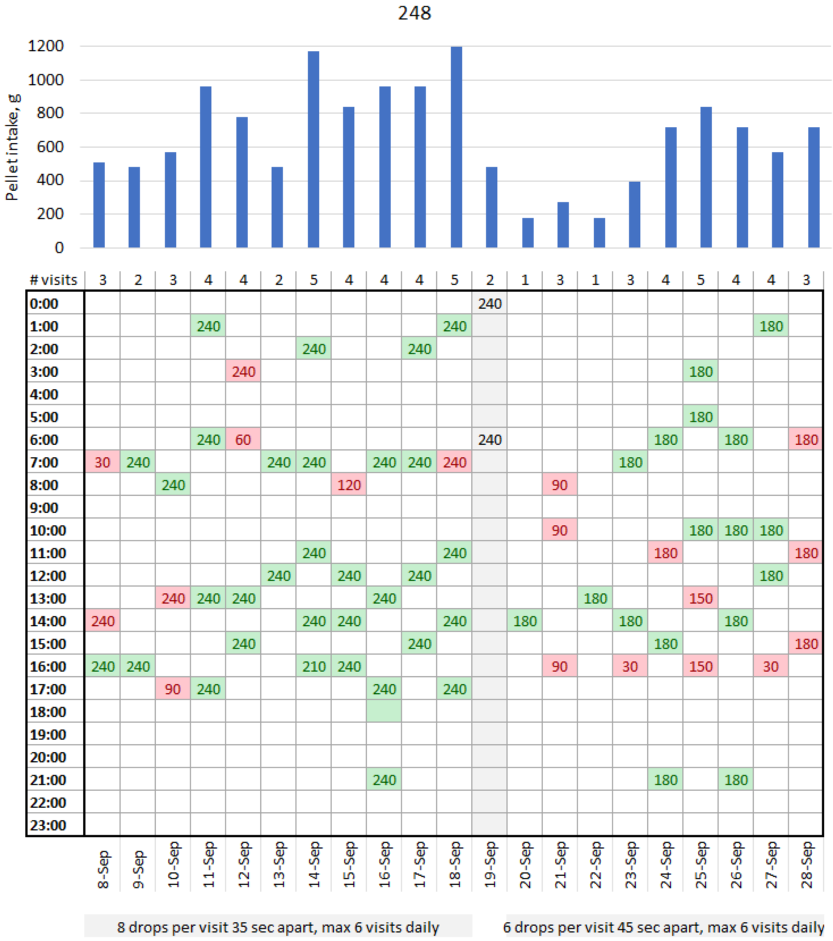
Figure 3 (continued). Total pellet intake and GEM unit visits by individual animals in Phase 2 with the control commercial lucerne pellet. The bar graph represents total daily intake of pellets, in grams. The boxes below indicate the amount consumed in each visit during the day, in grams, the time of that visit and if the visit duration was successful to obtain good methane data (green = successful, red = unsuccessful). The total number of visits per day is reported along the upper x-axis, and the GEM unit settings on that particular date is represented on the lower x-axis.



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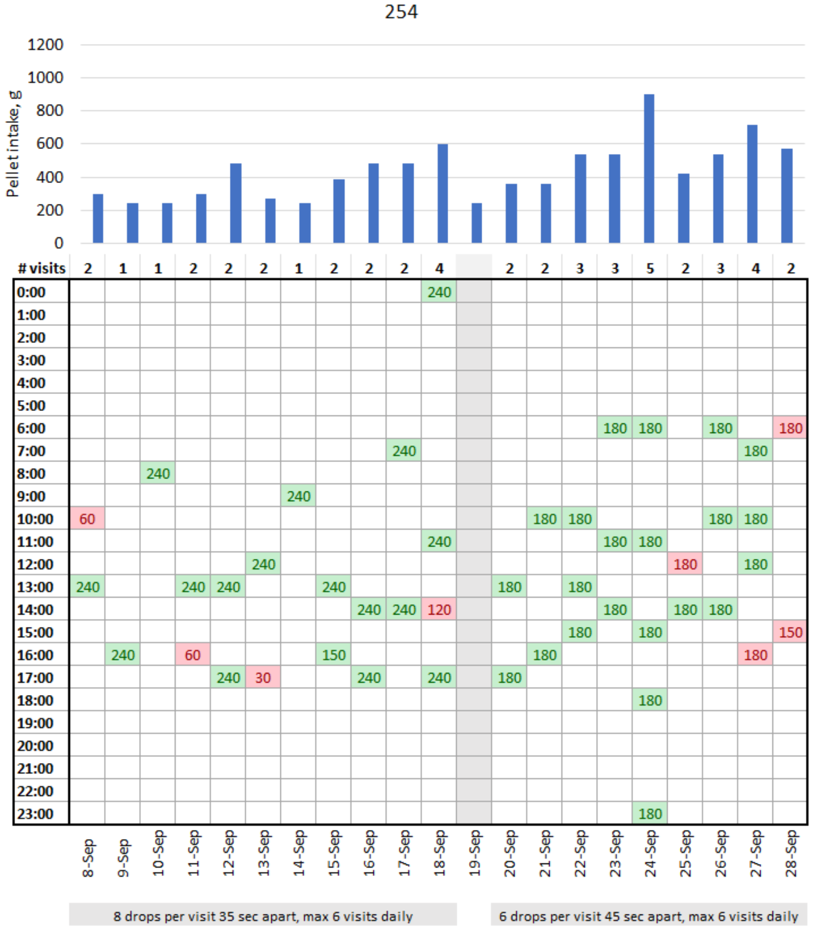

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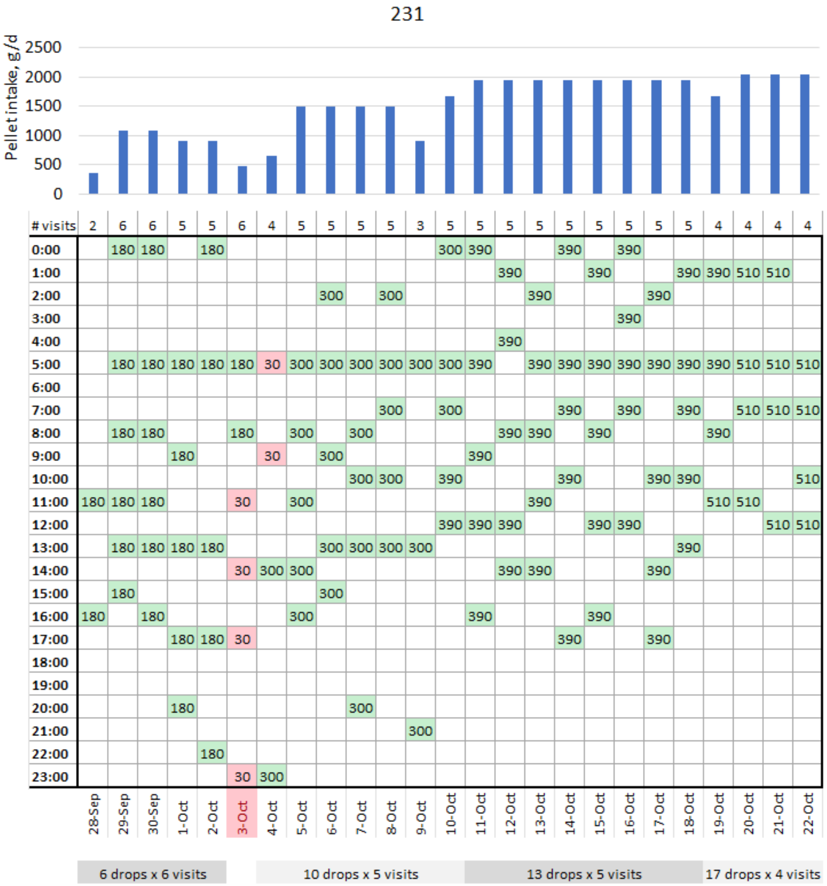
Figure 4. Total pellet intake and GEM unit visits by individual animals in Phase 2 with the Energy pellet. The bar graph represents total daily intake of pellets, in grams. The boxes below indicate the amount consumed, in grams, in each visit during the day, the time of that visit, and if the visit duration was successful to obtain good methane data (green = successful, red = unsuccessful). The total number of visits per day is reported along the upper x-axis, and the GEM unit settings on that particular date are represented on the lower x-axis.



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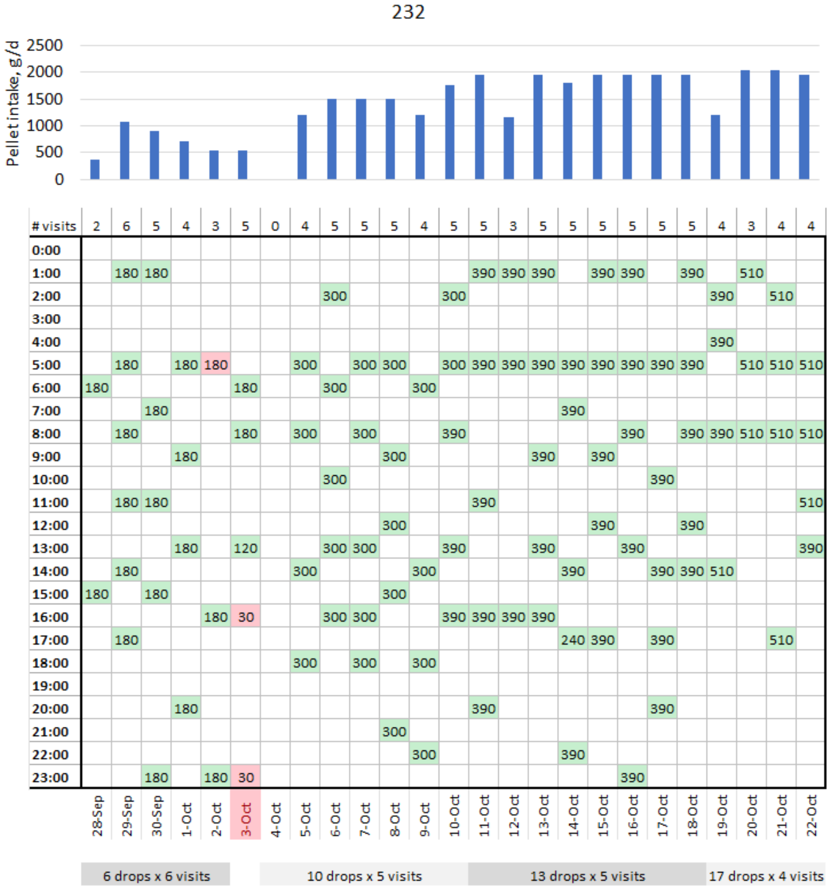

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# visits	2	6	5	4	3	5	0	4	5	5	5	4	5	5	3	5	5	5	5	5	5	4	3	4	4
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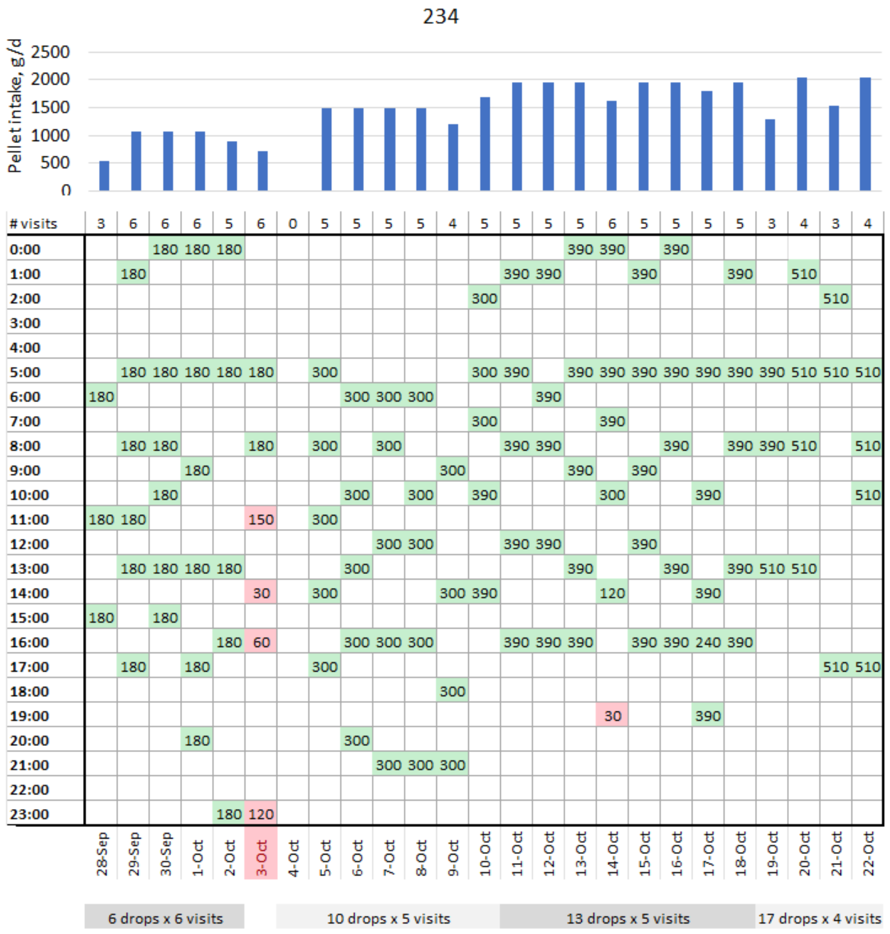
Figure 4 (continued). Total pellet intake and GEM unit visits by individual animals in Phase 2 with the Energy pellet. The bar graph represents total daily intake of pellets, in grams. The boxes below indicate the amount consumed, in grams, in each visit during the day, the time of that visit, and if the visit duration was successful to obtain good methane data (green = successful, red = unsuccessful). The total number of visits per day is reported along the upper x-axis, and the GEM unit settings on that particular date are represented on the lower x-axis.



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7:00													300				390								
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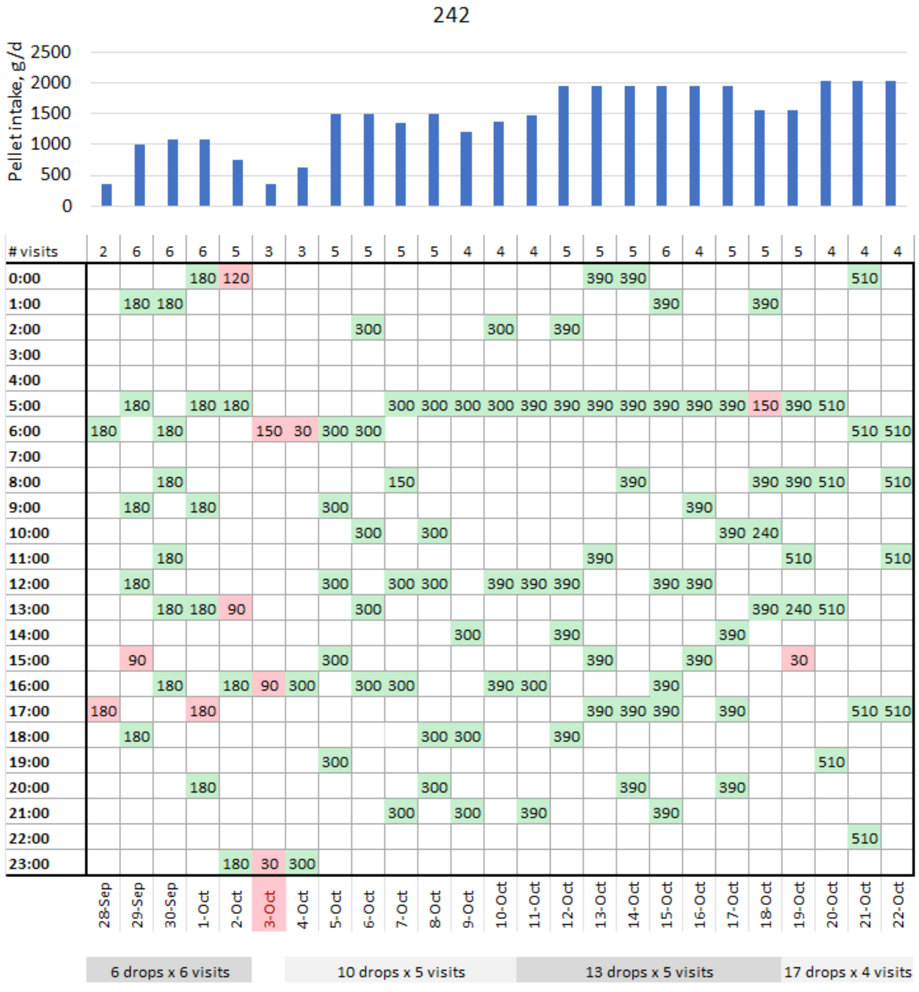
	28-Sep	29-Sep	30-Sep	1-Oct	2-Oct	3-Oct	4-Oct	5-Oct	6-Oct	7-Oct	8-Oct	9-Oct	10-Oct	11-Oct	12-Oct	13-Oct	14-Oct	15-Oct	16-Oct	17-Oct	18-Oct	19-Oct	20-Oct	21-Oct	22-Oct



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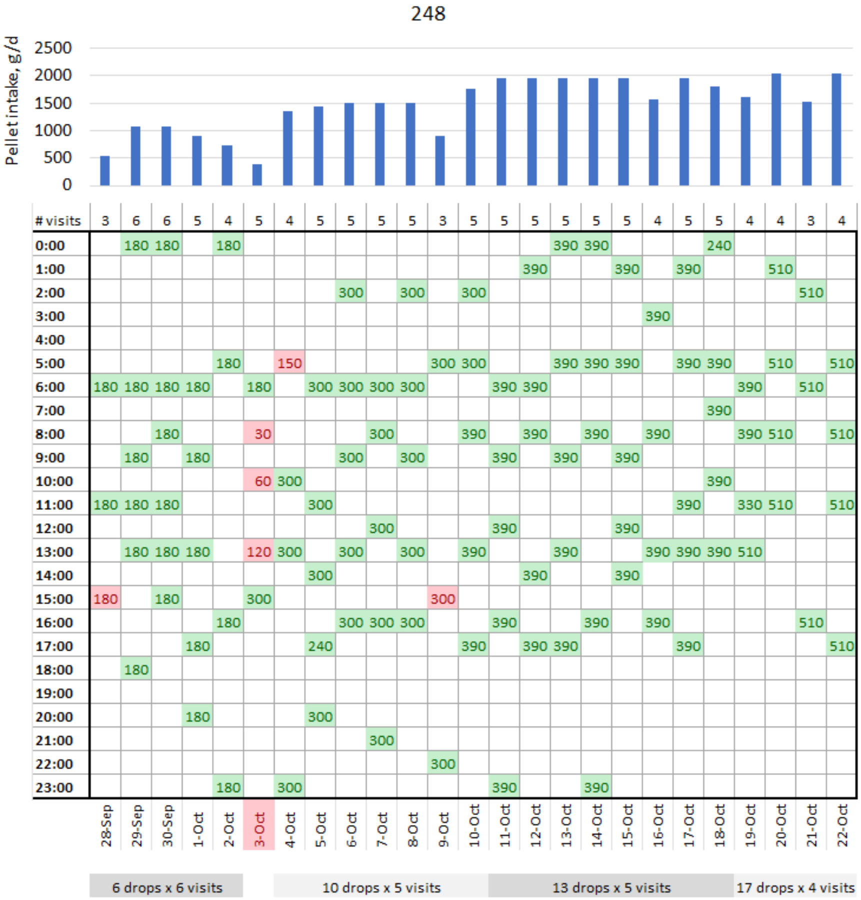
23:00					180	🟥30	300																		
	28-Sep	29-Sep	30-Sep	1-Oct	2-Oct	3-Oct	4-Oct	5-Oct	6-Oct	7-Oct	8-Oct	9-Oct	10-Oct	11-Oct	12-Oct	13-Oct	14-Oct	15-Oct	16-Oct	17-Oct	18-Oct	19-Oct	20-Oct	21-Oct	22-Oct

Figure 4 (Continued). Total pellet intake and GEM unit visits by individual animals in Phase 2 with the Energy pellet. The bar graph represents total daily intake of pellets, in grams. The boxes below indicate the amount consumed, in grams, in each visit during the day, the time of that visit, and if the visit duration was successful to obtain good methane data (green = successful, red = unsuccessful). The total number of visits per day is reported along the upper x-axis, and the GEM unit settings on that particular date are represented on the lower x-axis.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# visits	3	6	6	5	4	5	4	5	5	5	5	3	5	5	5	5	5	5	4	5	5	4	4	3	4
0:00		180	180		180											390	390				240				
1:00															390			390		390			510		
2:00									300		300		300											510	
3:00																			390						
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5:00					180		150					300	300			390	390	390		390	390		510		510
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9:00		180		180					300		300			390		390		390							
10:00						60	300														390				
11:00	180	180	180					300												390		330	510		510
12:00										300				390				390							
13:00		180	180	180		120	300		300		300		390			390			390	390	390	510			
14:00								300							390			390							
15:00	180		180			300						300													
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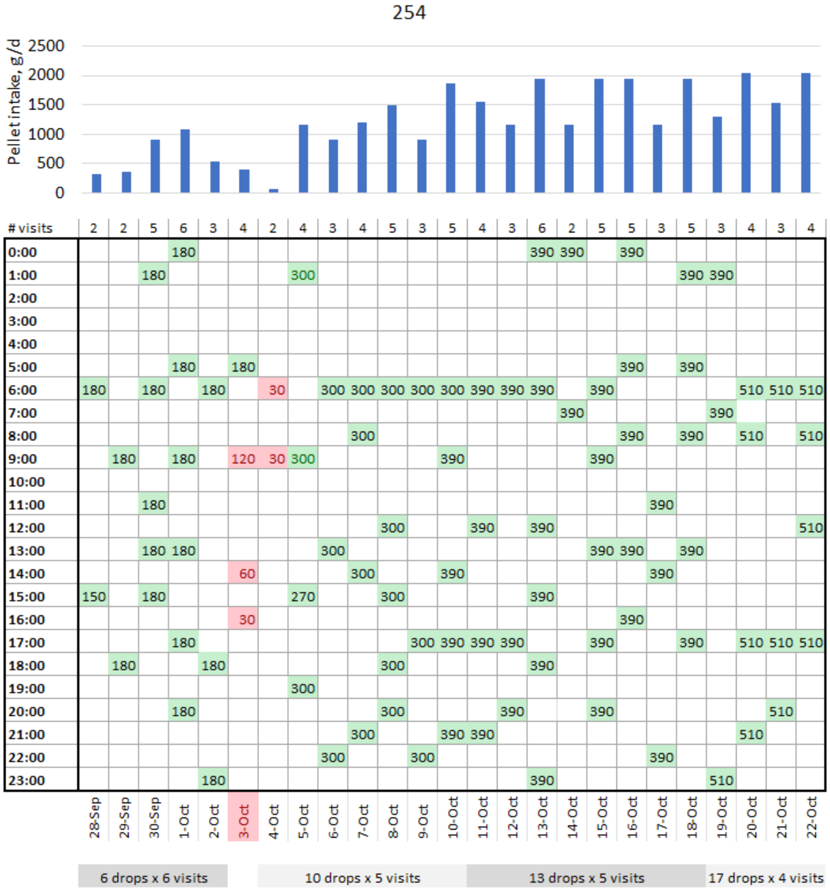
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	28-Sep	29-Sep	30-Sep	1-Oct	2-Oct	3-Oct	4-Oct	5-Oct	6-Oct	7-Oct	8-Oct	9-Oct	10-Oct	11-Oct	12-Oct	13-Oct	14-Oct	15-Oct	16-Oct	17-Oct	18-Oct	19-Oct	20-Oct	21-Oct	22-Oct
# visits	2	2	5	6	3	4	2	4	3	4	5	3	5	4	3	6	2	5	5	3	5	3	4	3	4
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6:00	180		180		180		30		300	300	300	300	300	390	390	390		390					510	510	510
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4.3 Phase 3 - Backgrounding trial

4.3.1 Pasture composition and Pellet formulation

At the beginning of period 1, available pasture biomass was estimated at 31,379 kg across the entire 21 ha where 8,814 kg was available for consumption by heifers in Energy treatment, 12,568 kg for those in Control treatment, and 9,997 kg for grazing heifers supplemented with Bovaer pellets. At the completion of period 1 where all paddocks were utilised by all treatments groups, the pasture biomass remaining was 16,347 kg, indicating that 40% of total biomass was consumed. In Period 2, the initial pasture biomass for all paddocks was 18,844 kg, of which 6,280 kg was available to Energy supplemented heifers, 6,000 kg to Bovaer supplemented heifers, and 6,565 kg to Control heifers. At the end of this final period, entire pasture biomass was 12,588 kg, such that heifers consumed 33.2% of available biomass.

Table 4. Proportion of each pasture species in 6 experimental paddocks across the two 56 d periods

	Period 1						Period 2					
Pasture species	E1	E2	E3	W1	W2	W3	E1	E2	E3	W1	W2	W3
Couch	77.8	35.9	48.7	68.9	49.6	54.8	69.8	42.5	62.6	75.4	69.7	57.9
Green panic	17.1	51.6	32.2	14.5	5.6	15.1	19.6	45	21.7	12.2	2.5	15.7
Rhodes grass	3.5	11.8	18.2	16	44.8	27.5	2.1	7.9	9.9	12.2	27.5	22.1
Weed	1.6	0.6	0.9	0.2	0	2.4	8.5	4.6	5.7	0.2	0.3	4.4
Other grass	0	0	0	0.4	0	0.2	0	0	0	0	0	0

Abbreviations: E1-E3, east paddocks; W1-W3, west paddocks

Species composition of each paddock is presented in Table 4. Chemical composition of pastures in the paddocks showed similar dry matter content (%DM) between pastures species and periods (Table 5). In period 2, crude protein (CP) content was 40.2% greater in Green Panic, 12.2% greater in Couch grass, and 19.5% greater in Rhodes grass. Similarly, fat content was ≥15% greater in all three grasses in Period 2.

Table 5. Chemical composition of pasture available throughout the two grazing periods.

Parameter	Period 1				Period 2			
	Rhodes grass	Cough grass	Green panic	Rhodes grass hay	Rhodes grass	Cough grass	Green panic	Rhodes grass hay
DM, %	40.7	42.9	34.8	81.4	33.1	36.6	27.1	81.8
CP, %DM	6.2	8.6	7.0	10.6	7.7	9.8	11.7	10.0
ADF, %DM	39.9	37.5	39.0	44.5	42.3	36.2	39.1	48.5
NDF, %DM	70.2	69.1	64.9	68.7	71.8	66.3	65.4	69.8
EE, %DM	1.77	1.26	2.0	1.75	2.26	1.96	2.35	1.16
Ash, %DM	7.29	6.53	8.58	11.65	6.92	7.3	10.1	8.68
NFC, %DM	14.5	14.5	17.5	7.3	11.4	14.6	10.5	10.4

Abbreviations: DM, Dry Matter; CP, Crude Protein; ADF, Acid Detergent Fibre; NDF, Neutral Detergent Fibre; EE, Ether extract; NFC, Non-Fibrous Carbohydrates.

Ingredients and chemical composition of pellets offered are presented in Table 6. As Phase 1 indicated a 40% loss of 3-NOP during the pelleting process, to ensure the target concentration of 150 mg/kg DMI was provided to the grazing heifers, the concentration of 3-NOP was increased by 40% in the formulation. Quantification of 3-NOP showed a 7.53% greater concentration than that formulated (511 mg/kg DM), immediately after pelleting (Table 7). Energy pellets, and Bovaer pellets were stored at 4^{o}C in a cold room for the duration of the trial to eliminate any external influence on 3-NOP concentration (mg/kg DMI) as per Phase 1 results. Concentration of 3-NOP in pellets reduced 6.57% over the duration of period 1, and 1.58% in period 2.

Table 6. Ingredients and chemical composition of pellets available to the three treatment groups.

Ingredients (%)	Control Pellets	Bovaer Pellets	Energy Pellets
Barley hammered	N/A	25.60	25.60
Wheat hammered	N/A	32.31	33.16
Millrun	N/A	22.59	22.59
Canola meal	N/A	10.00	10.00
Bovaer	N/A	0.85	N/A
Recycle vegetable oil	N/A	2.00	2.00
Molasses	N/A	3.00	3.00
Aglime*	N/A	1.00	1.00
Mono dicalcium phosphate	N/A	0.75	0.75
Magnesium oxide	N/A	0.25	0.25
Urea	N/A	1.25	1.25
Vitamin mix	N/A	0.20	0.20
Mineral premix	N/A	0.20	0.20
Chemical composition			
DM, %	90.9	95.7	92.3
CP, %DM	15.7	19.7	19.1
ADF, %DM	37.5	7.2	7.3
NDF, %DM	46.6	19.6	19.5
Ether Extract, %DM	1.72	4.37	5.41
Ash, %DM	7.76	6.3	6.38
NFC	28.2	50	49.7

Abbreviations: DM, dry matter; CP, crude protein; ADF, Acid detergent acid; NDF, Neutral detergent acid; EE, ether extract; NFC, non-fibre carbohydrates; * main component is limestone.

Table 7. Concentration of 3-NOP (mg/kg DMI) in pellets immediately after pellets were made, and at the end of period 1 and period 2.

Pellets samples	3-NOP	SEM	CV
Pelleting	549.5	4.839	1.76%
Period 1	513.4	3.932	1.71%
Period 2	540.8	6.583	2.72%

Abbreviations: SEM, standard mean error; CV, coefficient of variation.

4.1.2 Greenfeed usage, productivity and methane emissions

Twenty-two heifers (44.0%) in the Control group were classified as users of the GEM unit, comprising nine in period 1 and 13 in period 2. Of the cattle offered the Energy supplement, 27 heifers (54.0%) were classified as GEM users, 14 in period 1 and 13 in period 2. Lastly, a total of 33 heifers (66.0%) offered the Bovaer supplement were classified as users of the GEM unit, which included 14 heifers in Period 1 and 19 heifers in period 2. Heifers that were classified as non-users, i.e. did not generate reliable CH_4 data or never visited the GEM unit, were kept in the treatment group for the duration of the trial to quantify productivity data for grazing only heifers.

A direct comparison of heifers using or not using the GEM units to obtain pellets within the same paddock showed the consumption of Bovaer pellets increased final body weight and ADG by 3.2% (P<0.047) and 16.1% (P<0.009), respectively, compared to heifers consuming pasture alone. Similarly, heifers with Energy supplementation had 4.9% greater (P<0.004) final BW, but ADG was similar to heifers not consuming any pellets within the same paddock (P=0.167). Heifers consuming Control pellets had similar final body weights (P=0.304), and ADG (P=0.238), compared to heifers not consuming any pellets within the same paddock, indicating that this group successfully replicated performance of a grazing only cohort.

Table 8. Productivity parameters of grazing heifers not using the GreenFeeds within each treatment paddock.

Parameters	Control	Energy	Bovaer	SEM	P-value
Body weight, kg	331.1	334.4	337.4	3.37	0.250
Body weight change, kg	41.5	39.3	43.5	2.105	0.153
Average daily gain, kg	0.74	0.7	0.78	0.032	0.153

Abbreviations: SEM, standard mean error.

For heifers classified as non-users, no differences were observed in final BW, body weight gain or ADG across the treatment groups (P > 0.15; Table 8). There was no interaction of period x treatment (P>0.09) for initial or final body weight (kg), body weight gain or average daily gain for heifers using the GEM units (Table 9). Heifers supplemented with Bovaer had the greatest (P = 0.009) pellet intake (on a DM basis) in both periods, with no differences between periods. Similarly, no changes in pellet intake between period were observed with Energy supplementation. Control heifers in period 1 had the lowest pellet intake and greatest difference between periods with 14.0% lower consumption during period 2. When comparing the effect of treatment, average DMI of pellets was greater (P < 0.001) for Bovaer heifers, compared to Energy heifers. The consumption of the Control pellet was restricted to 1 kg maximum per day, with an actual intake of 950 g (Table 9).

Table 9. Effect of Energy, Bovaer and Control pellet supplementation on productivity and pellet intake of grazing heifers

	Treatment				P-value		
Parameters	Control	Energy	Bovaer	SEM	TRT	Period	TRT*Period
Final body weight, kg	340.8b	347.3ab	352.6a	3.157	0.042	0.004	0.898
Body weight gain, kg	42.0b	46.5b	52.7a	2.364	0.002	0.599	0.101
Average daily gain, kg	0.75b	0.83b	0.94a	0.042	0.003	0.600	0.101
Pellet intake, kg DM	0.95c	1.76b	1.91a	0.021	<0.0001	0.03	0.009

Abbreviations: SEM, standard mean error.

Heifers supplemented with Bovaer grew 10.7 kg, and 6.2 kg more (P = 0.003), than Control, or Energy supplemented heifers, respectively, across the entire trial duration. Weight gain of Control and Energy supplemented heifers was similar, averaging 44.25 kg (Table 9). Similarly, Bovaer heifers had 20.2% and 11.7% greater (P=0.002) average daily gain (ADG), compared to Control or Energy, whilst ADG was similar between Energy and Control heifers, averaging 0.79 kg/d. Additionally, there was an effect of period on productivity, where heifers in period 2 had 4.2% lower initial weight (P<0.01), 4.2% higher pellet DMI (P=0.02), and a 3.1% lower final body weight (P=0.004), than heifers in period 1.

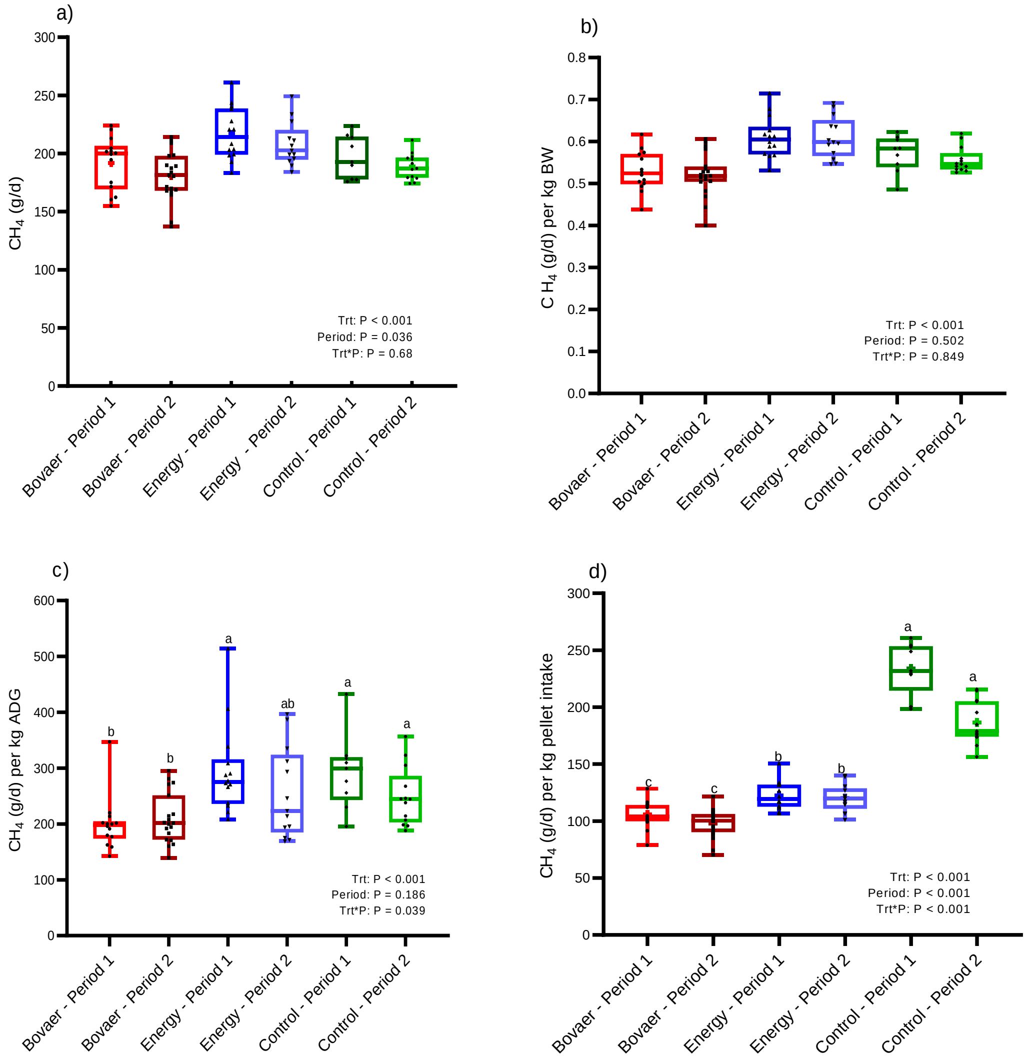

Figure 5 Methane emissions from heifers expressed as a) Grams of methane per day; b) grams methane per kilogram of body weight, c) grams of methane per kilogram of average daily gain (ADG), and d) Methane (g) per kilogram of pellet dry matter intake.

Methane emissions (g/d) from Bovaer heifers were 15.9% lower (P < 0.001), compared to Energy heifers, but were similar to Control heifers. Energy supplemented heifers emitted 9.0% more methane that Control heifers (Figure 5; Table 10). A period effect was observed on methane emissions (g/d), where heifers in period 2 emitted 5.8% less than those in period 1 (P = 0.03). Similarly, Bovaer heifers had 24.9% and 31.6% lower (P < 0.001) methane emission per kilogram of ADG, compared to Energy and Control heifers, respectively, which were similar. Additionally, Bovaer supplemented heifers emitted 17.3% and 7.7% less ($P < 0.001$) methane per kilogram of BW, compared to Energy and Control heifers, respectively. Bovaer heifers had 19.3% and 106.6% lower methane per kilogram of pellet DMI, compared to Energy and Control heifers, respectively ($P < 0.001$). Similarly, heifers in period 2 emitted 12.6% less methane per kilogram of pellet intake than their counterparts in period 1 ($P < 0.001$).

Table 10. Methane emissions of grazing heifers supplemented with Bovaer, Energy, or Control pellets.

Parameters	Treatment group			SEM	P-value
	Control	Energy	Bovaer		
CH_4, g/d	192.97b	211.93a	182.87b	4.256	<0.0001
CH_4, g/kg pellet DM intake	210.22a	121.40b	101.75c	3.227	<0.0001
CH_4, g/kg ADG	262.22a	248.83a	199.18b	9.915	<0.0001
CH_4, g/kg body weight	0.56b	0.61a	0.52c	0.01	<0.0001

Abbreviations: CH_4, methane; ADG, average daily gain; SEM, standard error mean.

4.1.3 Rumen volatile fatty acids

All rumen fermentation parameters were influenced by the interaction treatment x period, except ruminal ammonia concentration (Table 11). While period effect was observed on Total VFAs and butyrate, as a percentage of total VFAs, where heifers in period 2 had 11.2% more total VFAs and 5.8% butyrate in the rumen fluid ($P \leq 0.05$). Heifers supplemented with Energy pellets in period 1 had the highest acetate proportion, compared with Bovaer by 6.2% and Control heifers by 11.4% ($P < 0.001$). In period 2, all heifers had similar acetate concentrations. Heifers supplemented with Control pellets in period 1 had the highest percentage of propionate, followed by Bovaer in period 1 ($P < 0.001$), while all others were similar. This resulted in the same trend for the A:P ratio ($P < 0001$). Branched chain fatty acids were greatest in Bovaer and Control supplemented heifers in period 1, while all others were similar.

Comparisons across treatments revealed Total VFA (av. 69.3 ± 3.2 mM) and butyrate (av. 10.3 ± 0.71 % of total VFAs) were similar ($P \geq 0.20$; Appendix 1). Control heifers had the lowest percentage of acetate, and highest propionate, compared to all other treatments, resulting in the lowest A:P ratio ($P<0.001$; Table 11). Bovaer heifers had 3.3% less acetate, 6.2% more propionate, and consequently decreased the A:P ratio by 9.9%, compared to Energy supplemented heifers ($P<0.001$). Ammonia concentration (mg/L of N) was similar between Bovaer and Energy, but greater ($P<0.001$) in Control heifers. Branched chain VFA were similar in Bovaer and control, and greater than Energy supplemented heifers. Conversely, Valerate (as a % of total VFAs) was greatest in Control, followed by Bovaer, then Energy supplemented heifers.

Table 11. Rumen fermentation parameters in grazing heifers supplemented with either Bovaer, Energy, or Control pellets for 56d.

Parameters	Control		Energy		Bovaer		SEM	P-value		
	P1	P2	P1	P2	P1	P2		TRT	P	T*P
Total VFA, mM	70.29ab	66.31bc	68.26ab	77.87a	56.92c	75.84ab	5.223	0.19	0.01	0.03
Acetate, %TVFA	63.79d	70.30b	71.99a	69.70b	68.04c	69.16bc	0.93	<0.001	0.005	<0.001
Propionate, %TVFA	19.91a	16.57c	15.84c	16.36c	18.01b	16.34c	0.606	<0.001	<0.001	<0.001
Butyrate, %TVFA	10.58ab	10.12b	9.24c	11.04a	10.13bc	10.63ab	0.426	<0.001	0.005	<0.001
BCVFA, %TVFA	2.54a	2.16b	1.98bc	1.91bc	2.48a	1.75c	0.124	<0.001	<0.001	<0.001
Valerate, %TVFA	1.15a	0.78cd	0.77cd	0.72d	0.92b	0.81c	0.024	<0.001	<0.001	<0.001
A:P	3.27c	4.25a	4.56a	4.29a	3.81b	4.26a	0.011	<0.001	0.001	<0.001
NH_3-N, mg/L of N	49.76bc	46.34c	67.44a	58.39ab	62.92a	59.76ab	4.125	<0.001	0.08	0.65

Abbreviations: VFA, volatile fatty acids; BCVFA, branched chain fatty acids; A:P, acetic to propionic ration; NH_3-N, rumen ammonia; SEM, standard error mean.

4.1.4 Cost benefit and emissions analysis

A cost benefit and emissions analysis aimed to assess the following scenarios:

1) The impact of supplementing grazing cattle with Energy supplementation (with and without Bovaer) on animal growth, compared to grazing only.
2) The impact of supplementing grazing cattle with Energy supplementation (with and without Bovaer) on methane emissions, compared to grazing only (Control pellet).
3) The ability to monetise use of a methane inhibitor in grazing cattle using Australian Carbon Credit Units as an example.

A list of assumptions used in these analyses are presented in Table 12.

Table 12. Assumptions made for cost benefit analysis based on typical industry backgrounding program

Assumption	Metric	Value
Target weight gain during backgrounding period	kg liveweight	200
Backgrounding starting liveweight	kg liveweight	200
Backgrounding finishing liveweight	kg liveweight	400
Cost of grazing animals	$/head/day	1
Health, morbidity and mortality	$/head (one off fixed cost)	25

The number of days feeding required in the backgrounding period was calculated by dividing the targeted body weight gain of 200 kg with the ADG observed in each treatment group. Heifers in the grazing only group (Control) reached the target weight gain of 200 kg in 267 d. Whereas, Energy supplementation shortened the backgrounding period by 26 d, and Bovaer supplementation shortened it by 54 d, compared to the grazing only (Table 13).

Table 13. Cost of supplementing grazing cattle with Energy pellets (with and without Bovaer) to increase productivity

Parameter	Scenario		
	Grazing only	Grazing plus energy pellets	Grazing plus energy pellets with Bovaer
Average daily gain (ADG), kg	0.75	0.83	0.94
Marginal increase in ADG, kg	N/A	0.08	0.19
Days to gain 200 kg	267	241	213
Pellet costs, $/kg	0	0.5	0.5 + Bovaer
Pellet intake, kg	0	2	2

A sensitivity analysis revealed the potential gross margin ($/head/d) for animals gaining 0.83 kg liveweight daily through Energy supplementation, at different liveweight values and supplement costs (Table 14). Using the assumed parameters, Energy supplementation of grazing cattle is an economically viable option if the value of liveweight was ≥ $3/kg liveweight and supplementation costs were ≤ $500/tonne, with a maximum return of $2.76/hd/d.

Table 14. Sensitivity analysis for grazing cattle supplemented with Energy pellets to achieve 200 kg weight gain at various liveweight values and supplement costs. Outcome represents the gross margin ($/head/d) of supplementing Energy pellets.

Liveweight value ($/kg Liveweight)	**Supplement cost ($/tonne)**				
	300	**400**	**500**	**600**	**700**
2	-0.15	-0.35	-0.55	-0.75	-0.95
3	0.58	0.38	0.18	-0.02	-0.22
4	1.30	1.10	0.90	0.70	0.50
5	2.03	1.83	1.63	1.43	1.23
6	2.76	2.56	2.36	2.16	1.96

The total cost of Bovaer® inclusion at 511.5 mg 3-NOP kg/DM was $0.17/kg. Therefore, the cost of supplementing 2 kg of pellet, equates to $0.34, per head per day. This was factored into an additional sensitivity analysis (Table 15), which shows a reduction in days grazing with the increased ADG from Bovaer supplementation can increase the gross margin up to $0.23/hd/d greater than with Energy supplementation alone.

Table 15. Sensitivity analysis for grazing cattle supplemented with Bovaer to achieve 200 kg weight gain at various liveweight values and supplement costs. Outcome represents the gross margin ($/head/d) of supplementing Bovaer pellets.

Liveweight value ($/kg Liveweight)	**Supplement cost ($/tonne)**				
	300	**400**	**500**	**600**	**700**
2	-0.30	-0.50	-0.70	-0.90	-1.10
3	0.52	0.32	0.12	-0.08	-0.28
4	1.35	1.15	0.95	0.75	0.55
5	2.17	1.97	1.77	1.57	1.37
6	2.99	2.79	2.59	2.39	2.19

Table 16. Impact of supplementing grazing cattle with Energy pellets (with and without Bovaer) on methane emissions during the grazing period, compared to grazing only (Control pellets)

Parameter	Scenario		
	Grazing plus control pellets	Grazing plus energy pellets	Grazing plus energy pellets with Bovaer
Methane emissions (kg CH_4/200 kg BW gain)	51.46	51.07	38.91
CO_2eq, kg/head	1,440.80	1,429.90	1,089.40

The next stage of the assessment was to model the ability to monetise reductions in emissions attributable to the use of Bovaer as a methane mitigating supplement using Australian carbon credits units (ACCUs) as an example. It is also currently possible to generate ACCUs under the herd productivity method, which would be possible for supplementation of grazing cattle. A price of $38.5 per tonne of CO_2e for each ACCU was assumed, as published by the clean energy regulator of Australian government for first quarter 2023 (CER 2023). The inclusion of Bovaer would result in a reduction of 351 kg CO_2eq/head, compared to animals that were grazing only, across the projected backgrounding period. This equates to a carbon credit worth $12.58/head. Although supplementing grazing animals with Energy pellets alone generated greater daily methane emissions than grazing animals, cattle fed Energy pellets achieved 200 kg of gain 26 days faster, resulting in a 0.8% reduction in total methane emissions and CO_2e for the backgrounding period compared to those grazing alone. This equated to a carbon credit worth $0.39 per head more when feeding Energy pellets, compared with animals that were grazing only.

5 Discussion

This project involved a series of three studies to develop and evaluate an energy-based supplement containing Bovaer, suitable for backgrounding cattle. In what was a world first for this application, this study showed the inclusion of Bovaer in the pellet significantly decreased enteric methane emissions and increased productivity (liveweight gain) of grazing cattle. When the results of this project were applied to a typical backgrounding period in cattle, it was shown that provision of an energy-based pellet containing Bovaer to grazing cattle resulted in 351 kg CO_2 eq less emissions than grazing alone, with 54 less grazing days. In addition to the direct emissions mitigation and productivity increases observed, the provision of an energy-based supplement to growing cattle had additional ancillary benefits. This included additional pasture that could either be conserved or utilised by the business depending on the environmental conditions, market conditions and business drivers which are unique to each individual operation.

Currently the best available strategies for supplementation of anti-methanogenic additives to grazing animals are driven by the physical and chemical properties of the products and their active ingredients. These include, but are not limited to pelleting, mixing with feed ingredients or vitamin and mineral pre-mixes, addition into water troughs, or lick blocks (Van Wesemael et al, 2019). Bovaer-10® (Bovaer) is a powdered formulation consisting of 10% 3-Nitrooxypropanol in a silica carrier. Once consumed by the animal in feed, 3-NOP has been shown to have an immediate effect on methanogenesis, however this effect may only last up to 12 h (Gruninger et al., 2022). Accordingly, reliable and significant reductions in enteric methane emissions have been demonstrated with animals provided regular feed rations such as dairy cattle and lot fed cattle (Yu et al., 2021). The short half-life of 3-NOP in the rumen creates a challenge for achieving significant emissions reductions when supplementing grazing animals with products containing Bovaer. The use of automated feeders which can allocate set amounts to animals throughout the day were hypothesised as being able to overcome this challenge. As such, this project consisted of three phases to determine the effect of pelleting conditions (steam vs. pressure pelletisation) on pellet quality, and 3-NOP stability (Phase 1), to optimise cattle utilisation of an automated feeder (GEM unit) on pasture (Phase 2), and to utilise this information to assess the effect of frequent energy supplementation in the form of pellets on the productivity and methane emissions of grazing cattle prior to feedlot entry, with and without Bovaer® inclusion (Phase 3). The hypothesis was that Bovaer inclusion in Energy pellets would reduce methane emissions from grazing beef cattle while increasing cattle growth, compared to other treatment groups.

The initial experiment (Phase 1) evaluated the production of energy-based pellets as a delivery mechanism for Bovaer. The pellets formulated in the current study, were balanced for nutritional requirements of growing heifers, with considerations for inclusion limitations of pellet ingredients, and ingredient cost at the time of manufacture. The intention was to develop a high-quality pellet easily replicable by producers, at the lowest cost. A viable pellet formulation was developed as part of this project (Table 1), where pellets produced using steam were twice as resistant to breakage and had a greater durability, compared to those made using pressure.

There were, however, challenges with the development of the pellets containing Bovaer. Despite best efforts a loss of 30% of 3-NOP occurred through pellet production. The chemical properties of Bovaer are such that pelleting temperature cannot exceed 85°C. This was able to be achieved at a commercial feed mill. In addition, the mineral premix used in this study contained iron(II) which has been known to react with 3-NOP causing significant losses throughout the pelleting process (DSM, unpublished data). It is recommended that iron be included in the form iron(III) when mixed with 3-NOP to avoid excessive losses. As this was not known at the time of pelleting, the concentration of 3-NOP was increased (average) by 40% to achieve our targeted 3-NOP concentration in Phase 3. Additionally, to produce the required 10 tonnes of pellets for Phase 3, pelleting was undertaken by a commercial feed manufacturer. Based on the outcomes of Phase 1, this ensured pellets were of a high quality (hardness and durability).

Often the storage of feed additives, or their bioactive component, is not considered when evaluating the efficacy of a product for methane reduction. The current study showed that storage conditions of pellets containing 3-NOP are crucial for the retention of the 3-NOP, and therefore the dose consumed by cattle (Bampidis et al. 2021). Our results suggest the optimal storage conditions are at 4°C. As most producers lack facilities to guarantee this temperature, and on the scale required to store feed, the most realistic alternative to store Bovaer pellets on-farm would be ambient conditions, which resulted in a potential 3-NOP disappearance of 21.7% monthly. More frequent pelleting batches (every 2 weeks) could be produced to limit storage time, and therefore ensure a dose closest to the target dose. This increases transport costs and must be considered in the overall cost of supplementation.

Methods to accurately and reliably quantify enteric methane emissions of grazing cattle is currently restricted to research settings with limitations on the number of animals that can be measured at any one time. This project used GEM units as a method of not only measuring methane, but as a method for pellet delivery, and concurrently, quantified supplement intake for GEM user animals. As the GEM unit takes spot samples of methane, numerous visits (>15 total data captures per animal) spaced throughout the day are necessary to capture diurnal variation in methane emissions (McGinn et al. 2021). This is vital with anti-methanogenic products such as 3-NOP which have been shown to have a short retention time in the rumen, and as such if methane emissions are captured 12 h after consumption of pellets no effect on methane would be observed, severely underestimating the effect. To combat this, Phase 2 of this study determined the optimal GEM unit settings (drops per visit, drop rate, visits allowed per day and time between visits) to ensure that methane emissions of cattle on pasture would be accurately measured. With 6 visits daily and 8 pellet drops allowed each visit, the amount of time animals spent at the feeder increased, achieving better data capture (visits >3 mins on more occasions). Previous studies have demonstrated that the average number of voluntary visits is 3.2 per head per day, much lower than that achieved here (av. 6.1 visits per head per day (Della Rosa et al. 2021). In the current trial, palatability of grain-based pellets facilitated an average visitation rate of 80.0% of the total targeted visits. In comparison with data obtained feeding GEM attractant pellets, Energy pellets enhanced pellet intake and reliable CH_4 data capture. These protocols were applied in Phase 3 to ensure consistent consumption of Bovaer in the backgrounding trial to maximise its capacity to inhibit methane emissions (Duin et al. 2016).

Phase 3 of this study successfully quantified methane emissions and productivity of grazing cattle supplemented with Bovaer® for the first time. As with any grazing system, seasonality and subsequent pasture quality are important considerations in determining methane emissions. Therefore, this trial covered two pasture growing seasons, period 1 in Summer, and period 2 in Autumn. The pasture was irrigated and fertilised for most of period 1 and the beginning of period 2 to limit deterioration of pasture quality from grazing pressure, pasture dieback and weed competition, and to guarantee enough pasture biomass for the experimental period. The differences in pasture quality among periods should be interpreted with caution as pasture samples were collected at the beginning and middle of each period, potentially limiting the ability to quantify pasture quality deterioration towards the end of the period. However, it is known that increases in structural carbohydrates and lignin content of forage reduces ruminal passage rate and organic matter digestibility (OMD), resulting in low pasture intake and consequently lower methane emissions. In the current trial, the lower methane emissions in period 2 may also be explained by the lighter initial heifer body weight. Interestingly, heifers had higher pellet intakes in period 2, irrespective of treatment, increasing their capacity to grow, without increasing methane emissions (Beauchemin et al. 2009; Knapp et al. 2014).

To entice grazing animals to use the GEM unit, they had to be provided with a pellet attractant. A commercial lucerne pellet was selected as our control, with intake limited to 1 kg/d. Weight gain and final body weights of animals using the GEM unit to obtain the Control pellet and those not consuming any pellets were similar, indicating the control pellets did not alter productivity and successfully replicated a grazing only scenario. However, is it possible that this is also due to the improved nature of the pasture being consumed with regular fertiliser application and irrigation providing a high-quality feed base, as the ADG observed was greater than the 0.5 kg per day estimated at the start of the trial, and was similar to that of heifers supplemented with Energy pellets. This indicates that there may be the potential for greater weight gains with energy supplementation in cattle grazing lower quality pastures.

Supplementation with 3-NOP at 150 mg/kg DMI reduced methane emissions from heifers by 15.9%, expressed as grams per day, compared with Energy supplementation alone, and methane intensity (g methane/kg ADG) compared to both Energy (24.8%) and Control (31.5%) heifers, confirming the anti-methanogenic property of this additive on pasture. The effectiveness of 3-NOP is a result of its capacity to bind with the MCR enzyme, responsible for the formation of methane in the last methanogenesis step, avoiding the attachment of an intermediate compound which carries methyl group that later is transformed into methane, and at the same time inactivating MCR enzyme (Duin et al. 2016). In addition, 3-NOP can be reduced by rumen microbiota into different compounds including nitrates which have been demonstrated to directly compete with rumen methanogens for hydrogen (van Lingen et al. 2021). Previous studies have shown 3-NOP supplementation in forage based total-mixed ration can reduce methane emissions (g/d) by 40.7% (Alemu et al. 2023), while trials evaluating this additive in grain-based diets have shown reductions between 25.7 to 87.6% (Vyas et al. 2016; Vyas et al. 2018b; Alemu et al. 2021; Araújo et al. 2023). The capacity of Bovaer to inhibit methane emissions in the current trial are on the lower end of values observed in the literature, but this is the first study to examine its effect in a pasture-based system. A greater proportion of structural carbohydrates and lignin in the pasture diet, as well as lower diet digestibility would increase the concentration of rumen fermentation subproducts, such as CO_2 and H_2 which are used by methanogens to produce methane (Shibata & Terada 2010).

The lack of difference in methane yield (g/d) between the Control and Bovaer heifers was surprising. However, this likely reflects the difference in body weight between the two groups (Gonzalez et al. 2014), evidenced as a difference in methane when expressed as grams methane per kilogram of body weight, and gram methane per kg ADG, indicating that the Bovaer supplemented animals emitted the equivalent methane to an animal that was 12 kg smaller. Consequently, Bovaer supplementation on pasture not only reduced energy lost as methane, but was able to redirect that energy to growth (Hristov et al. 2015). This outcome is not always realised with Bovaer supplementation, with variable results on average daily gain reported (Vyas et al. 2018a; Alemu et al. 2021). It is hypothesised that these cattle were able to utilise the additional energy for growth on a pasture-based diet as cattle are not growing at their maximum potential on these diets, unlike studies in feedlots, which have not observed consistent increases in weight gain, as production efficiency is already being maximised with high grain diets. The mechanism by which this occurred however, is not clear, as Bovaer pellets reduced propionate, compared to control pellets as more starch was available for rumen microbiota to generate this VFA. Propionate synthesis is generated via two different metabolic pathways. In the acrylate pathway, starch and lactate are degraded by starch-fermenting bacterial communities, such as *Megasphaera elsdenii* and *Selonomonas ruminantium* to generate propionate (Pereira et al. 2022). This pathway is not upregulated in high forage diets (Wang et al. 2020), indicating that for the current trial, the low proportion of pellets in the heifers diets (29.3% of total DMI), may have limited propionate production via the acrylate pathway in both groups consuming grain-based pellets. In contrast, the Control pellet consumption may have increased hemicellulose and lignocellulosic content available in the rumen, which are then degraded into succinic acid by rumen bacterial communities such as *Fibrobacter succinogenes*, and immediately reduced into propionate, through the succinate pathway (Neumann et al. 2018). In this sense, more propionate was generated in the current trial by Control pellets as they comprised more hemicellulose polysaccharides which upregulated the succinic pathway. The high acetate, and lack of differences in butyrate were not expected due to previous evidence that a high concentration of H_2 downregulates enzymes involved in fibre degradation by rumen fibrolytic bacterial communities (Greening et al. 2019). While the presence of 3-NOP in the rumen upregulates enzymes participating in butyrate synthesis (Pitta et al. 2022). Although the mechanism is not clear, the productivity benefits observed with Bovaer supplementation in the current trial have the potential to be of great significance to the Australian grazing industry. For example, in the context of backgrounding growing cattle in preparation for lot fed finishing, the grazing time to achieve a 200 kg weight gain would be shortened by 54 d, when compared to grazing only (control), or by 28 d in comparison to Energy supplementation alone.

When combining the reduction in methane emissions and increased productivity observed in grazing heifers, the potential for adoption of Bovaer supplementation in grazing cattle is clear. However, careful consideration must be given to supplement cost. A cost benefit analysis indicated the reduction in the number of days required to achieve a 200 kg weight gain with Energy supplementation alone presented a profitable scenario when cattle liveweight prices were at least $3/kg and the cost of supplementation was kept below $500/tonne. The inclusion of Bovaer at $0.34 per head per day altered the scenario, such that cattle prices needed to be at least $4/kg liveweight with cost of supplementation at or below $700/tonne to obtain a higher gross margin ($/hd/d) over Energy supplementation alone. These profits, however, do not account for additional costs to deliver pellets to animals and manufacture to maintain stable 3-NOP levels in pellets, as our pellets were stored in a cold room for the duration of the trial. Additionally, the mechanism of delivering 3-NOP consistently throughout the day is difficult to achieve in extensive systems as not all producers have access to automated feeders that can limit intake, and as such, this is an avenue for further research.

The outcomes observed in the current trial may also provide ancillary benefits. Dependant on the time of year and season, environmental outlook, market conditions and business objectives producers may be able to:

- Retain more pasture biomass and rest areas introducing environmental and biodiversity benefits, while also reducing the operations carbon account.
- Utilise the additional feed either by increasing the number of cattle per hectare, or introducing an additional group of cattle as the first group were able to be turned off sooner.
- Make hay or silage with any surplus feed, if applicable.
- Be more drought resilient as cattle can be fed to a marketable endpoint, and sold sooner, especially when seasonal outlook is not favourable.

5. Conclusion

The project demonstrated for the first-time improvements in productivity and reductions in emissions from the use of 3-NOP (as Bovaer®) in supplements for grazing cattle. Delivery of pellets was successfully achieved using GreenFeed units in up to 6 visits per day to ensure consistent supply of 3-NOP to the rumen, and to quantify methane throughout the day. The results confirm the anti-methanogenic potential in grazing cattle, with reductions in methane yield observed (15.9% reduction in g CH_4/d, and 24.8% lower as g CH_4/kg ADG), in addition to a 6.6% greater average daily gain of cattle supplemented with Bovaer pellets, versus those supplemented with the Energy pellets. This increased ADG would result in cattle reaching a predicted weight gain of 200 kg for feedlot entry 28 d faster than those consuming Energy pellets, and 54 d faster than grazing cattle offsetting the cost of adding Bovaer® to the pellets and in fact offering a potential gain of up to $0.23/head/d above that of energy supplementation alone. If this is combined with carbon credits from reduced methane emissions, the potential for additional profit increases to $61.52/hd over the backgrounding period.

5.1 Key findings

- Optimal storage conditions for pellets containing Bovaer® is 4°C. As most producers lack facilities that guarantee this temperature, and on the scale required to store feed, therefore the most realistic solution to store Bovaer pellets on-farm would be at ambient temperature with storage for a maximum of 2 weeks.
- Delivery of pellets was successfully achieved using GreenFeed units in up to 6 visits per day to ensure consistent supply of 3-NOP to the rumen, and to quantify methane throughout the day.
- Potential of 3-NOP as Bovaer® to reduce methane emissions in grazing cattle was confirmed, with reductions in methane yield observed (15.9% reduction in g CH_4/d, and 24.8% lower as g CH_4/kg ADG)
- Average daily gain of cattle supplemented with Bovaer pellets was 6.6% greater, compared to those supplemented with the Energy pellets. This increased ADG would result in cattle reaching a predicted weight gain of 200 kg for feedlot entry 28 d faster than those consuming Energy pellets.
- Provision of Bovaer would reduce total enteric methane emissions over a 200 kg liveweight increase backgrounding period by 351 kg CO_2 eq. compared to grazing cattle and 340.5 kg CO_2 eq. compared to an energy supplement alone, per head.

5.2 Benefits to industry

- First evidence of Bovaer reducing methane emissions whilst increasing cattle growth in a backgrounding system. These outputs provide the baseline of future research confirming Bovaer capacity in grazing systems, but more importantly, developing alternative mechanisms to deliver Bovaer daily with/without feed supplementation or additional farm infrastructure and cattle management.
- Bovaer supplementation may decrease days and costs of feeding in the backgrounding phase, allowing animals to commence the feedlot phase earlier.
- The energy pellet formulation that was developed contained limited feedstuff which minimized pellet price and guaranteed ADG of 0.85 kg/h/d. This formulation can be easily replicated by producers at low costs.
- Allow Australian meat industry to access or expand participation in markets focusing on carbon neutral or environmentally friendly meat products.

5.2 Future research and recommendations

- Investigation of pelleting ingredients, coatings and storage on the long term stability of Bovaer
- Explore its interaction with minerals in pre-mix supplements to reduce losses during pelleting
- Investigate the use of alternative feeding mechanisms, auto feeders, grain supplementation, lick blocks to deliver Bovaer to grazing cattle throughout the day.

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8. Appendix

Appendix 1. Rumen fermentation parameters of grazing heifers supplemented with GEM attractant, Energy or Bovaer pellets.

Rumen fermentation parameters	Treatment pellets			SEM	P value		
	Control	Energy	Bovaer		TRT	P	TRT*P
Total VFA, mM	68.3	73.07	66.38	3.2	0.2	0.02	0.03
Acetate, %TVFA	67.04c	70.85a	68.59b	0.529	<0.001	0.01	<0.001
Propionate, %TVFA	18.24a	16.10c	17.17b	0.357	<0.001	<0.001	<0.001
Butyrate, %TVFA	10.35	10.14	10.37	0.259	0.71	0.04	0.006
BCVFA, %TVFA	2.35a	1.94b	2.11a	0.077	<0.001	<0.001	0.005
Valerate, %TVFA	0.96a	0.75c	0.86b	0.029	<0.001	<0.001	<0.001
A:P	3.76c	4.43a	4.03b	0.107	<0.001	0.002	<0.001
NH_3-N, mg/L of N	48.05b	62.91a	61.34a	2.69	<0.001	0.09	0.66

Abbreviations: VFA, volatile fatty acids, BCVFA, branched chain fatty acids, A:P, acetic to propionic ration, NH_3-N, rumen ammonia; SEM, standard error mean.