Final report

Effect of Bovaer®10 on performance, health, carcase characteristics and carbon footprint of Australian feedlot cattle

Project code: P.PSH.1375

Prepared by Robert Lawrence[A], Ian Loxton[B], Allan Lisle[C], Danielle Shirley[D] and John Doyle[A]
[A]Integrated Animal Production
[B]Yeppoon, Queensland
[C]University of Queensland
[D]NH Foods Australia Pty. Ltd.

Date published: 28-Feb-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

The Australian red meat industry has made a commitment to achieve a carbon neutral supply chain by 2030. A practical and immediate action is the use of methane mitigating feed additives. DSM Nutritional Products, Switzerland manufactures Bovaer®10 containing 100,000mg/kg of the active compound 3-nitrooxypropanol (3-NOP). Addition of 3-NOP has shown to effectively mitigate methane in cattle fed feedlot diets elsewhere, but limited work has been undertaken with this product in Australia.

The objectives of this project include:

(1) Determine the effect of Bovaer®10 on feedlot cattle performance, health, and carcass characteristics;
(2) Evaluate effect on carbon footprint of Australian feedlot cattle;
(3) Determine the cost-benefit of Bovaer®10 to the feedlot operation using three scenarios:
    (i) Assess cost of feeding Bovaer®10;
    (ii) Assess increase in carcass value ($/kg) required to offset cost of feeding Bovaer®10;
    (iii) Assess ability to monetise emissions reduction using an internationally recognised carbon trading scheme.

A commercial feedlot study was conducted between the 1st of June 2022 and 6th March 2023 at a feedlot in southern Queensland. A total of 7997 Black Angus steers (451.8 ± 10.4 kg SD) were allocated to project treatments and fed for an average of 151.5 days targeting a mid-fed export beef market. The study was designed as a randomised block with 16 blocks (replicates) of two treatments each, providing a total of 32 feedlot pens.

The treatments were:

- Control = standard feedlot diet with 0 mg 3-NOP/kg DM
- 3-NOP = standard feedlot diet with 100 mg 3-NOP/kg DM

Project cattle were fed the standard feedlot transition diets for the first 20 days on feed. The 3-NOP treatment cattle received the 3-NOP within a cereal based pellet included at 2% (As-Fed basis) within the standard feedlot finisher ration. The study was divided into two phases. Phase 1 (blocks 1 to 8), and Phase 2 (blocks 9 to 16).

Bovaer®10 had no effect on feedlot cattle performance to re-implant at an average 70 DOF (both Phase 1 & 2) or overall performance of cattle fed during Phase 2 of the study. Findings requiring further research include the 2.1% decrease in dry matter intake of cattle and a 4.2 kg decrease in hot carcase weight, in the latter half of the feeding period of Phase 1 of the project, which may be caused by wet weather conditions and/or high dry matter intakes (and 3-NOP intakes) observed. Furthermore, in Phase 2 during drier conditions during late/spring early summer there was no effect of Bovaer®10 on feedlot performance, carcass characteristics or animal health over the full feeding period.

This project demonstrated that Bovaer®10 lacked stability when applied through the supplemental pellet formulation used in this project. Bovaer®10 had active losses of 12.0% with pelleting and 7.3% during storage, and alternative supplementation mechanisms or stabilising technologies will need to be identified. Future research to understand energy pathways in the methane inhibited rumen such as hydrogen utilization will be important to develop mechanisms to improve cattle performance, given the absence of performance benefits in this study.

Using efficacy values from recently completed MLA research (Almeida et al. 2023) Bovaer®10 reduces feedlot Scope 1 and 2 carbon emissions by more than 50%, but when including Scope 3 (carbon associated with purchased cattle), reduction falls to 5%. If value is derived from carcass weight premiums breakeven prices of $0.017, $0.034, $0.069, $0.103 and $0.138/kg are required at 5, 10, 20, 30 c and 40 c/hd/d Bovaer®10 cost,

respectively assuming no performance loss at a 5-year average $7.40/kg carcass price ($/kg). If a 2.6 kg HCSW loss was factored, breakeven premiums increase to $0.061, $0.079, $0.113, $0.148 and $0.183/kg respectively.

Bovaer®10 as an intervention strategy is accepted by international carbon trading systems such as VERRA which offers feedlots the opportunity of monetising the reduction of methane. In our study breakeven carbon prices of $36, $59, $104, $148 and $193/t were required at Bovaer®10 cost of 5, 10, 20, 30 and 40 c/hd/d, respectively assuming no performance loss. If a 2.6 kg HSCW loss at a 5 year average hot-carcass weight of $7.40/kg is factored, breakeven carbon prices increase to $93, $116, $161, $206 and $251/t respectively.

Bovaer®10 is an effective tool for mitigating methane, but for broad industry adoption further research may occur in a number of areas. These include product stability under a range of supplemental forms, storage and weather conditions; understanding why performance was decreased during wet spring conditions and/or high feed intakes, further research in dose titration of Bovaer®10 and/or including an additional feed additive or probiotic that effectively captures ruminal hydrogen to improve cattle performance.

Executive summary

Background

The Australian red meat industry has made a commitment to achieve a carbon neutral supply chain by 2030 (CN30). A key component of CN30 is reducing methane production from ruminant livestock. A practical and immediate option is the use of methane mitigating feed additives. DSM Nutritional Products, Switzerland (DSM) manufactures a feed additive called Bovear®10 which contains 100,000mg/kg of the active compound 3-nitrooxypropanol (3-NOP). Addition of 3-NOP has shown to reduce methane production and yield by more than 80% in feedlot diets (Vyas *et al*., 2016; Almeida et al. 2023). However, limited data has been generated to assess the effect of 3-NOP on feedlot cattle performance, with research to date mostly in small-pen environments. Assessment of Bovaer®10 within a large-scale commercial feedlot in Australia enables evaluation of the practicalities of using this feed additive and implications on cattle performance, health, and carcass characteristics.

As part of the long-term sustainability of red meat production there is interest within the Australian feedlot industry for determining its carbon footprint as well as identifying methods of monetising the reduction of methane. Generating a return from methane reduction either through increased product value or selling carbon within a certified trading system are possible drivers that will be explored in this project.

Objectives The objectives of this project include:

(1) Determine the effect of Bovaer®10 on feedlot cattle performance, health, and carcass characteristics;
(2) Evaluate effect on carbon footprint of Australian feedlot cattle;
(3) Determine the cost-benefit of Bovaer®10 to the feedlot operation using three scenarios:
    (i) Assess cost of feeding Bovaer®10;
    (ii) Assess increase in carcass value ($/kg) required to offset cost of feeding Bovaer®10;
    (iii) Assess ability to monetise emissions reduction using an internationally recognised carbon trading scheme.

Methodology

A commercial feedlot study was conducted between the 1st of June 2022 and 6th March 2023 at a feedlot in southern Queensland. A total of 7997 Black Angus steers (451.8 ± 10.4 kg SD) were allocated to project treatments and fed for an average of 151.5 days targeting a mid-fed export beef market. The study was designed as a randomised block with 16 blocks (replicates) of two treatments each, providing a total of 32 feedlot comparable pens.

The treatments were:

- Control = standard feedlot diet with 0 mg 3-NOP/kg DM
- 3-NOP = standard feedlot diet with 100 mg 3-NOP/kg DM

To coordinate with available feedlot cattle supply and an abattoir Christmas shutdown period, the project was divided into 2 experimental time-based phases (from the 16 blocks; numbers 1 to 8 as Phase 1, with feedlot placement from 1st June 2022 to 19th July 2022; numbers 9-16 as Phase 2, with feedlot placement from 17th August 2022 to 7th October 2022). For each block, cattle were individually randomised to experimental treatments alternately (2 consecutive cattle at a time to each treatment pen) at feedlot induction.

Project cattle were fed the standard feedlot transition rations to 20 DOF, after which the 3-NOP treatment received Bovaer®10 within a cereal based pellet provided at 2% as-fed inclusion. The control treatment continued being fed the standard feedlot finisher ration. The project ration protocol was followed for every project block from DOF 20 until the blocks' exit from the feedlot.

Feedlot exit occurred at an average of 151.5 DOF for each project block. At exit, steers were transported in multi combination truck-livestock trailers to a collaborating abattoir, a journey on average ~ 4 hours. Each project block was slaughtered within 12 hours of arrival at the abattoir. Some exceptions occurred - from project Phase 1, Block 1 was slaughtered on 2nd-3rd November, 2022 and Block 8 on December 20th, 2022. From project Phase 2, Block 9 was slaughtered on 19th January, 2023 and Block 16 on 7th March, 2023, that date being the end of the project's experimental phase.

The measurements recorded and nutritional analyses conducted during the project included hourly weather data measurements collected from a feedlot automated weather station, daily observations of treatment cattle health and welfare, full (non-fasted) live weights at induction, re-implantation at 70 DOF and final tag check (average 140 DOF), 11.5 days prior to exit (average 151.5 DOF); assays of 3-NOP concentration within the pellets, nutritional analysis of the finisher ration, daily feed intake (as-fed and dry matter) and calculated feed conversion ratios (as-fed, dry matter).

The measurements and assessments recorded at slaughter of the project steers included hot standard carcass weight (HSCW, kg), dressing percentage, fat depth (subcutaneous rib fat, P8), dentition and bruising.

Measurements and assessments recorded in the carcass chiller following 18-21 hours post slaughter chilling at the 12th/13th rib quartering point on either the left or right-hand carcass side included ossification, eye muscle area (cm^2), meat colour, intermuscular fat colour, AUS-MEAT marbling and MSA chiller assessments (MSA marbling score, rib fat depth, MSA index).

Statistical analysis of the project data following compilation and verification, was conducted, being:

- For live animal performance and carcass data, the 32-pen means were analysed using a mixed model ANOVA, with means weighted by the number of observations used. Project data were split into two phases with Blocks 1 to 8 as Phase 1 and Blocks 9 to 16 as Phase 2. The mixed model estimated fixed effects of Phase, Treatment and Phase x Treatment. Random effects were estimated for 'Blocks within Phase';
- For the intervals corresponding to liveweight measurement, data for daily feed intake and feed conversion ratios for both 'As fed' and 'DM' basis were analysed using a similar linear mixed model to carcass and performance data. That is, with fixed effects for project phase, treatment, and their interaction, with block within phase as a random effect;
- Data for the supplement pellet and 3-NOP intake and 3-NOP concentration was summarised across the 16 blocks;
- Animal health data were analysed as a generalized linear model assuming a binomial distribution with the default logit link.

An assessment of the effect of 3-NOP utilisation on the carbon footprint of Australian feedlot cattle was conducted by a 3rd party.

Three scenarios were used to estimate the cost benefit of Bovaer®10 to feedlot operations, these being:

(i) Assess the cost of feeding Bovaer®10;
(ii) Assess the increase in carcass value ($/kg) required to offset the cost of feeding Bovaer®10;
(iii) Assess the ability to monetize emissions reduction using an internationally recognised carbon trading scheme.

Results/key findings

- The study provided further evidence that 3-NOP had no effect on the animal health of the project animals.
- The project highlighted responses to the feeding of Bovaer®10 at a recommended formulated rate of 100 mg 3-NOP/kg DM differed within the two project phases of the experimental period;
- The two phases while only separated by a 4-week interval provided data on the responses to Bovaer®10 in a southern Queensland commercial feedlot during a winter-spring-early summer period which provided cool and wet conditions known as Phase 1 ; and during a late spring-full summer period which provided hot and dry conditions known as Phase 2.
- A statistically significant treatment x phase interaction was reported for dry matter intake ($P < 0.01$) between reimplant (average 70 DOF) and final tag check (average 140 DOF), which translated to a trend for a treatment x phase interaction for overall HSCW ($P = 0.068$). A treatment within phase analysis was conducted to examine the interactions.
- The treatment within phase data analysis outlined that:
  - There was no effect of Bovaer®10 on animal performance to DOF 70 HGP re-implant across both Phase 1 & Phase 2.
  - In Phase 1, the inclusion of Bovaer®10 resulting in an estimated mean consumption of 82.22 mg 3-NOP/kg DM for the overall feeding period, did not affect overall dry matter intake yet decreased ADG by 1.9% (P<0.0001), decreased carcass weight by 4.2 kg ($P < 0.01$), reduced subcutaneous fat at the P8 site (P<0.05) (but did not affect subcutaneous rib fat) and reduced MSA index (P<0.01). There was however, a 2.1% decrease (P<0.01) in dry matter intake of cattle in the latter half of the feeding period of this phase (days 70 to 140). This may have contributed to the lower ADG reported;
  - In Phase 2, the inclusion of Bovaer®10 resulting in an estimated mean consumption of 89.91 mg 3-NOP/kg DM for the overall feeding period had no effect on dry matter intake, animal performance their carcass characteristics, value, and quality.
- Further research is required to understand if this decrease in carcass weight in Phase 1 was the result of Bovaer®10 inclusion rate, wet weather and/or the high magnitude of dry matter feed intake observed.
- Including Bovaer®10 in its current composition within a cereal based pellet does not avoid degradation and loss of the active ingredient, 3-nitrooxypropanol;
- This project demonstrated that Bovaer®10 lacked stability in the supplemental pellet utilized in its delivery for this project. Bovaer®10 had active losses of 12.0% with pelleting and 7.3% during storage, and alternative supplementation mechanisms will need to be identified. Future research to understand energy pathways in the methane inhibited rumen such as hydrogen utilization will be important to develop mechanisms to improve cattle performance, given the absence of performance benefits in this study.
- Using efficacy values from recently completed MLA research study (Almeida et al. 2023) Bovaer®10 reduces feedlot Scope 1 and 2 carbon emissions by more than 50%, but when including Scope 3 (carbon associated with purchased cattle), reduction falls to 5%;
- If value is derived from carcass weight premiums breakeven prices of $0.017, $0.034, $0.069, $0.103, and $0.138/kg are required at 5, 10, 20, 30 c and 40 c/hd/d Bovaer®10 cost, respectively assuming no

performance loss at a 5-year average $7.40 carcass price ($/kg). If a 2.6 kg HCSW loss was factored, breakeven premiums increase to $0.061, $0.079. $0.113, $0.148 and $0.183/kg respectively.
- Bovaer®10 as an intervention strategy is accepted by international carbon trading systems such as VERRA which offers feedlots the opportunity of monetising the reduction of methane. In our study breakeven carbon prices of $36, $59, $194, $148 and $193/t were required at Bovaer®10 cost of 5, 10, 20, 30 and 40 c/hd/d, respectively assuming no performance loss. If a 2.6 kg HSCW loss at a 5 year average hot-carcass weight of $7.40/kg is factored, breakeven carbon prices increase to $93, $116, $161, $206 and $251/t respectively.

Benefits to industry

With the Australian red meat industry seeking a carbon neutral supply chain by 2030, feed additives which mitigate methane provide an efficient tool to meeting this objective. Research has shown that Bovaer®10 effectively mitigates methane of cattle on feedlot diets by more than 80%.

This project has generated a large-scale commercial dataset, to inform Australian lot feeders and consulting nutritionists on the effect of Bovaer®10 on feed additive stability, performance, health, carcass characteristics and carbon footprint of Australian feedlot cattle.

Future research and recommendations

The findings from this project identifies the following further research and recommendations:

- The carrier matrix for Bovaer® 10 and its delivery mechanism requires further research to avoid activity loss associated with commercial feedlot ration manufacture, delivery, and tenure in the feedbunk; The inherent volatility of 3-NOP increases its loss under pelleting and storage as demonstrated in this research project. Further research into the manufacture and presentation of Bovaer®10 to enable inclusion into existing feed additive delivery systems such as micro dosing equipment where it can be supplemented fresh daily. Incorporation of Bovaer® into stable liquid forms should also be explored.
- Reassess Bovaer®10 inclusion levels to minimise reduction in feed intake, animal performance and carcass weight observed during the latter half of Phase 1 in this research project. Further performance trials should occur at reduced dose levels of 50 to 75 mg/kg DM, as initial MLA research (Almeida et al. 2023) reports excellent efficacy of methane yield suppression at these levels (65.5 to 80.1%). The initial respiratory calorimeter studies of Almeida et al (2023) were generally too small to identify 3-NOP dose effects on animal production.
- Conduct further studies on the utilisation of Bovaer®10 in feedlot diets during different seasonal periods, market categories and levels of feed intake. Further research on effect of 3-NOP on palatability through diet preference tests with feed exposed to simulated rainfall is required. If wet weather is proven to influence diet palatability this could simply be managed by withdrawing 3-NOP during rain events.
- Identify existing or novel feed additives and probiotics which capture rumen hydrogen but are not contraindicated to Bovaer®10 and its efficacy or compromising of animal performance.

Table of contents

1. Background

The Australian red meat industry has made a commitment to achieve a carbon neutral supply chain by 2030 (CN30). This commitment supports the long-term sustainability of red meat production in Australia by reducing carbon emissions and promoting product acceptability within current and developing markets, both domestic and internationally.

A key component of CN30 is reducing methane production from ruminant livestock. Several methods of methane mitigation have been explored, however one of the more practical and immediate options is the use of feed additives. These additives have a range of mode of actions including blocking methane production, inhibiting methanogen bacteria, or reducing the availability of hydrogen for methane production.

DSM Nutritional Products, Switzerland (DSM) manufactures a feed additive called Bovear®10 which contains 100,000mg/kg of 3-nitrooxypropanol (3-NOP). This compound functions by blocking the methanogen enzyme, methyl co-enzyme M reductase, required for the final step of methane production (Duin *et al*., 2016). Much of the initial work with 3-NOP has investigated level of methane mitigation with different feeding regimes (pasture, feedlot) and 3-NOP feeding levels (100 to 200mg/kg DM) (Vyas *et al*., 2016). Addition of 3-NOP has shown to reduce methane production consistently by more than 80% in feedlot diets (Vyas *et al*., 2016). A recently funded MLA study (Almeida et al. 2023) reported decreases in finisher period methane yield of 65.5 to 87.6% for steers supplemented with 3-NOP and fed a tempered barley diet with canola oil.

Commercial feedlot projects using 3-NOP have been undertaken in the USA, Canada and Brazil (Alemu *et al*., 2021a & 2021b; Vyas *et al*., 2016 & 2018; Araujo et al. 2023, Pedrini et al. 2023). These projects have shown inclusion of 3-NOP at 100 to 200mg/kg DM effectively reduce methane production. Effects of 3-NOP on feedlot cattle performance however differ between studies and dietary concentrate level. A number of backgrounding studies on barley silage-based diets have reported improved feed conversion (2.5 to 7.6 %) with no change in average daily gain (Vyas et al. 2016; Vyas et al. 2018; Alemu et al. 2021b). Effects in finishing phases however vary between studies.

Vyas et al. (2016) reported 3-NOP fed at 100 mg/kg DM tended to reduce dry matter feed intake and daily gain in the finishing phase for cattle fed dry-rolled barley-based diets as described by Table 1 below. A follow-up study by Vyas et al. (2018), however reported a 5% decrease in DMI and 3% improvement in feed efficiency for steers fed 125 mg/kg DM 3-NOP. Alemu et al. (2021a) measured variable responses of increasing doses of 3-NOP on dry matter intake of corn-based diets in a small pilot trial. Araujo et al. (2023) evaluated feedlot performance of Nellore cattle fed a ground corn-citrus pulp diet supplemented with either 100 or 150 mg/kg DM of 3-NOP over a 96-d period in Brazil. A trend for reduced final body weight (P = 0.063) was reported although this did not translate to significant differences in hot carcass weight in this small pen study (9 replicates per treatment; 4 to 5 bulls per pen). Finally, in a small pilot study with individual intake measured, Pedrini et al. 2023 reported improved ADG (1.73 vs. 1.63 and 1.60 kg/d) and carcass weight efficiency for feedlot bulls fed 75, 0 (control) and 100 mg/kg DM 3-NOP, respectively.

Table 1. Effect of 3-NOP at 100 and 200mg/kg DM on dry matter intake, daily gain, feed conversion and ruminal methane and hydrogen production of cattle fed a dry rolled barley finisher diet (Vyas *et al*., 2016).

	3-NOP mg/kg DM				
	Control	**100**	**200**	**SE**	**P-value**
DM intake, kg/head/day	10.9	10.2	9.9	0.28	0.06
Average daily gain, kg/head	1.55	1.48	1.39	0.05	0.07
Feed conversion (F:G)	7.0	6.9	7.1	0.05	0.85
Methane emissions, g/day	116[a]	102[a]	18[b]	10.70	<0.01
Hydrogen emissions, g/day	0.02[a]	2.8[b]	12.4[c]	1.90	<0.01

Different subscripts in the same row represent significant difference P<0.05

Normal rumen metabolism by microbial communities generate dissolved hydrogen (H_2) as a by-product of glycolysis (Ungerfeld, 2022). This hydrogen is typically utilised by methanogens to produce methane gas to maintain rumen homestatasis. A consequence of reducing methanogen activity with inhibitors is an increase in both dissolved H_2 and ruminal hydrogen emissions as described in Table 1. The rumen possess alternative hydrogen pathways which could, be facilitated with the use of other feed additives (Ungerfeld et al., 2022). Hydrogen re-directed into volatile fatty acid production could provide a pathway of recovering energy loss associated with methane production (Johnson & Johnson, 1995). This pathway could be facilitated using probiotics (Jeyanathan et al., 2014). Other pathways include use of organic acids (Beauchemin and McGinn, 2006) to promote hydrogen use into intermediatery fermentation end-products (Ungerfeld, 2015) such as formate, sucinate and lactate. Facilitating rumen reduction reactions by the addition of nitrate (Lee et al., 2015), direct uptake of hydrogen with the addition of acetogens which utilise hydrogen to produce acetate Nollet et al, 1997; Le Van et al. 1998; Lopez et al. 1999; Karekar and Ahring, 2023) or use of novel hydrogen accepting compounds such as phenolic substances (Huang et al., 2022). These types of feed additives facilitate efficient rumen fermentation by reducing accumulation of ruminal hydrogen and may avoid depressing animal production when effective methane mitigation products like 3-NOP are used. Further large scale feedlot performance trials are required with 3-NOP to understand is such strategies are required to optimise performance outcomes with 3-NOP or if the additive can be fed without them.

One of the challenges of ensuring the efficacy of 3-NOP is the method of addition to ensure consistency of supply within total mixed rations. Research has shown that 3-NOP is rapidly degraded in the rumen to an inactive form (1,3-propandiol) within 15 hours (Figure 1.) and eliminated from the rumen completely within 24 hours (Duin *et al*., 2016). Methanogens can rapidly recover their ability for methanogenesis by ATP driven reduction of the methyl co-enzyme M reductase enzyme (Duin *et al*., 2016).

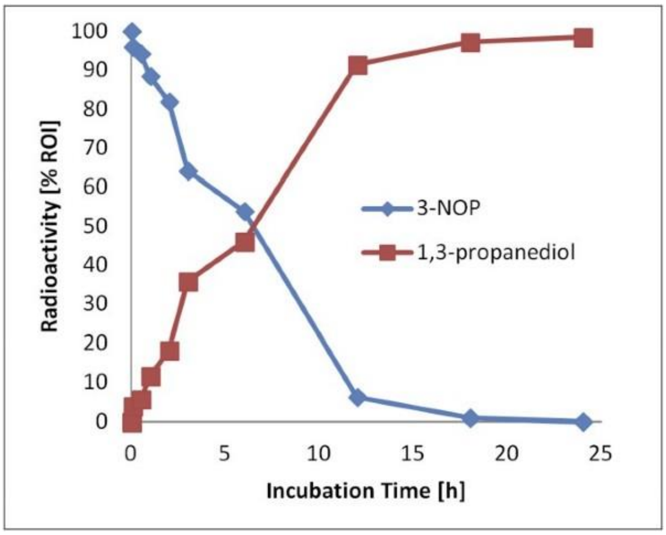


Figure 1. Conversion rate of 3-NOP to 1,3-propandiol (Duin *et al*., 2016)

Previous research has used different methods of adding 3-NOP to total mixed rations including direct addition in its concentrate form (Vyas *et al*., 2016) or via liquid carriers including a molasses/water blend prepared daily (Martinez-Fernandez *et al*., 2018) and water via micromachines (Alemu et al. 2021a). Most feedlots in Australia currently utilise molasses-based suspension supplements, however no published research has occurred on stability of 3-NOP for this supplementation method over typical periods of retention in feedlot storage tanks (weeks to months). Given these factors and the currently marketed concentration of 100,000mg/kg, 3-NOP is most practically incorporated into total mixed rations in Australia via mineral concentrate (Almeida et al. 2023) or pelleted with a cereal carrier. A pellet is easily handled in most commercial feedlot operations where large quantities of feed (20 tonnes) are batched for delivery. Effects of pelleting at 80°C on 3-NOP recovery have been previously investigated by DSM (Bampidis et al., 2021) The recovery of 3-NOP after pelleting was 89%. After pelleting, the samples were stored for 3 months at 25° C, and 50% relative humidity and the recovery was 83.3%.

As part of the long-term sustainability of red meat production there is interest within the Australian feedlot industry for determining its carbon footprint as well as identifying methods of monetising the reduction of methane. Generating a return from methane reduction either through increased product value or selling carbon within a certified trading system will be investigated in this project.

The results from this project will assess the manufacture, storage, and use of a pelleted Bovaer®10 supplement to supply 100mg 3-NOP/kg DM of feed to feedlot cattle. The project will assess cattle performance, health and carcass characteristics of cattle fed Bovaer®10 in a commercial Australian feedlot. Closeout data will be used to determine; 1. profit/loss associated with Bovaer®10 use and 2. additional carcass value required to breakeven as well as determine value generated from selling the carbon offset by use of Bovaer®10. Finally, a qualified consultant will conduct a full carbon footprint of the project's experimental treatments.

2. Objectives

(1) Determine the effect of Bovaer®10 on feedlot cattle performance, health, and carcass characteristics.
(2) Evaluate effect on carbon footprint of Australian feedlot cattle.
(3) Determine the cost-benefit of Bovaer®10 to the feedlot operation using three scenarios:
   (i) Assess cost of feeding Bovaer®10
   (ii) Assess increase in carcass value ($/kg) required to offset cost of feeding Bovaer®10
   (iii) Assess ability to monetise emissions reduction using an internationally recognised carbon trading scheme.

3. Methodology

3.1 Animal ethics approval

This project was approved (AE000901) by the University of Queensland Production and Companion Animals Ethics Committee.

3.2 Methodology

3.2.1 Study design and treatments

A commercial feedlot study was conducted between the 1st of June 2022 and 6th March 2023 at a feedlot in southern Queensland. A total of 7997 Black Angus steers (451.8 ± 10.4 kg SD) were allocated to the project treatments, were on feed for average 151.5 days and were targeted at a mid-fed export beef market. The study was a randomised block design with 16 blocks of two treatments.

Two dietary treatments were used in a replicated study of 16 pairs of pens, 1 pen for each treatment for a total of 32 pens. A pair of pens constituted 1 replicate and was referred to as a Block. Thus, there were 16 blocks in the study numbered 1 to 16. Due to an abattoir shutdown period during the 2022 Christmas—2023 New Year period, the project was separated into two experimental phases. Blocks 1 to 8 as Phase 1 and Blocks 9-16 as Phase 2. The dietary treatment under study was 3-nitrooxyproponal (Bovaer®10; DSM Nutritional Products, Switzerland)(3-NOP).

The treatments were:

- Control: standard feedlot diet with 0 mg 3-NOP/kg DM
- 3-NOP: standard feedlot diet with 100 mg 3-NOP/kg DM

For all measurements the pen was the experimental unit of interest, and all analyses were planned around the use of pen means or pen aggregate figures.

3.2.2 Study period time sequence terminology

A total of 16 induction/allocation sessions (one session per Block) in two periods were conducted to allocate the steers to treatments between 1st June 2022 and 7th October 2022. Resulting from the two periods of project steer induction/allocation, the first period of 1st June 2022 until 19th July 2022 for Blocks 1-8 was referred to as 'Phase 1' and the second period of 17th August 2022 until 7th October 2022 referred to as 'Phase 2'.

The study period for each block commenced on the date of completion of induction/allocation and the receipt of their first ration and was referred to as day 0 or Days on Feed (DOF 0). The study period for each block completed on average 151.5 ± 0.89 SD days on feed (DOF), referred to as exit DOF. This convention was followed for all 16 project blocks. The project timeline is outlined in a schema shown in Appendix 10.1.

Measurement sessions of individual liveweight were conducted at the time of each individual steer HGP reimplantation session at an average 69.8 ± 0.58 SD DOF and at each final quality assurance tag check session (to scan electronic identification RFID tags, check for veterinary chemical withholding periods) at an average 139.8 ± 0.58 SD DOF , with final liveweight measured approximately 12 days prior to exit.

The exit and slaughter of the treatment pens of steers commenced when the first of the project blocks reached their days on feed target and the blocks were slaughtered as per the two induction phases described above. Block 1 exited the feedlot on 1st November 2022 and was slaughtered at a collaborating abattoir on 2nd November 2022. Block 16, the final block of project steers was slaughtered on 7th March 2023.

3.2.3 Animals and feedlot description

3.2.3.1 Animals

Seven thousand, nine hundred and ninety-seven (7997) phenotypically Black Angus steers aged 18-24 months of age were purchased by the feedlot as part of their routine purchase protocol from properties throughout Victoria and NSW, Australia. Purchases occurred just prior to the first project induction/allocation session on June 1st, 2022 and continued until early October 2022. Where numbers of suitable phenotypically Black Angus steers were not available for purchase, the shortage of numbers for an induction/allocation session were made up from suitable steers from backgrounding in paddocks surrounding the feedlot.

3.2.3.2 Feedlot description

The project pens were in the 'new side' of the feedlot. The selected pens were not located in any end row nor were they end pens of any row. This was to ensure that none of the project steers suffered end pen nor end row effects. The project pens have a north south alignment. All pens had the same surface area of shade available. At an initial stocking rate of 250 steers/pen, there was on average 2.72 m^2/steer of available shade. The surface of the pens was a crushed granite base with a manure top layer. The layout of pens in the new side, the actual location of the project treatment home pens and the 'dispatch pens' are shown in the graphics of Appendix 10.2. Also shown in the same appendix are the linear measurements of the concrete feedbunks and concrete water troughs.

The treatment home pens selected for the project were those most similar available in respect to:
- Pen surface area;
- Feed bunk length;
- Water trough length;
- Shade area;

The dispatch pens were used for approximately the final 10 to 12 days of the project feeding period for each block. Cattle were moved to dispatch pens at 135.0 + 1.06 SD DOF, then weighed at 139.8 + 0.58 SD DOF. The control treatment pen and the adjacent 3-NOP treatment pen steers were walked as separate mobs to a pair of adjacent dispatch pens. Thus, matched pairs of pens from each block were moved from their respective home pens to a matched pair of dispatch pens in the same row.

3.2.4 Induction of cattle to the feedlot

Steers for feedlot induction and project treatment allocation were walked from receival pens or from backgrounding paddocks to the feedlot induction processing shed. Steers held in receival pens had access to oaten hay and water. Steers from backgrounding paddocks were receiving the R1 Starter ration. Steers from the backgrounding paddocks had been previously inducted to the feedlot, however each of these steers were processed through the induction/allocation sessions as though they were new receivals and received the same processing treatments. Upon arrival at the processing shed, all steers were worked through the yards by feedlot personnel to the handling race at which point individual steers entered the Veterinary Crush where the following procedures occurred:

- Measurement and recording of liveweight as per feedlots' protocol;
- Given an individual visual identification eartag and lot identification eartag;
- For Blocks 1-4, 6-9 and 16 each steer was treated, vaccinated and HGP implanted with:
  - 8 in 1 Clostridial vaccine;
  - Pasteurella Multocida vaccine;

  - Rhinoguard Herpesvirus Freeze dried vaccine;
  - Bovilis MH Mannheimia haemolytica vaccine;
  - HGP Implant - Revalor S (28mg oestradiol 17β, 140mg of trenbolone acetate);
  - Flukazole C Unselenised anthelmintic vaccine;
  - Paramectin RV pour on for treatment immature internal and adult external parasites.
- For Block 5, each steer was treated, vaccinated and HGP implanted with the same veterinary medicine compounds as Blocks 1-4, 6-9 and 16, except:
  - 5 in 1 Clostridial vaccine was administered instead of 8 in 1 vaccine.
- For Blocks 10-15, each steer was treated, vaccinated and HGP implanted with the same veterinary medicine compounds as Blocks 1-4, 6-9 and 16, with the addition of:
  - Coopers Easy Dose for control of nuisance flies and lice.

There were two exceptions for the above treatment/vaccination treatments above for Blocks 14 and 15, being:

- At block 14 allocation, 16 steers from backgrounding in the 3-NOP treatment received Coopers Easy Dose treatment, while the rest of the group did not. Similarly, 18 steers from Backgrounding in the control treatment received Coopers Easy Dose treatment, while the rest of the group did not;
- At block 15 allocation, 152 steers from Backgrounding in the 3-NOP treatment did not receive Coopers Easy Dose treatment item and 1 steer did. All steers from Backgrounding in the control treatment did receive Coopers Easy Dose treatment.

3.2.5 Live animal phase measurement schedule of events

Detailed schedules of project events are included in numerous appendices of Appendix 10. Specific event procedures are outlined in the following subsections.

3.2.5.1 Allocation of cattle to treatment pens

A total of 16 induction/allocation sessions (one session per block) were conducted to allocate the steers to treatments between 1st June 2022 and 7th October 2022. To avoid treatment pens of steers completing their 150-day feeding period during the scheduled 2022 Christmas-2023 New Year abattoir closure; induction/allocation sessions ceased on 19th July 2022 and recommenced on 16th August 2022 and were completed by 7th October 2022. Resulting from the two periods of project steer induction/allocation sessions, the first period of 1st June 2022 until 19th July 2022 for Blocks 1-8 was referred to as 'Phase 1', and the second period of 16th August 2022 until 7th October 2022 referred to as 'Phase 2'. The schedule of induction/allocation session dates is shown in Appendix 10.3.

Around 700 suitable steers arrived at the feedlot on a weekly basis. During periods of the project when there was a shortage of suitable new steers, then steers originating from onsite backgrounding paddocks were used to make up the required stock number for a project induction/allocation session. Specifically, steers from backgrounding were required for the induction/allocation sessions of Blocks 2 and 3, and 9 to 16 inclusive.

The liveweight of newly arrived steers (unadjusted for shrink) were recorded individually during the induction/allocation session in the induction shed. Individual steer RFID numbers were scanned by a RFID scanner and recorded. Steers originating from onsite backgrounding paddocks were similarly weighed individually during the induction/allocation session and adjusted to an shrunk weight using a 5% correction.

The weekly induction/allocation session procedure involved: Up to 700 suitable steers processed as per the feedlot routine for the mid-fed 100% Black Angus genotype cattle class. During processing, 500 suitable steers were identified and allocated to one of the two treatments and put into their treatment pens. The selection of each steer for a block was a fully randomised procedure i.e., the steers suitability determined on its liveweight and other criteria such as phenotype, temperament, or any fault as it moved through the race, scales box and into the Veterinary crush/chute. For e.g., the first 3 steers in the race could be randomly allocated to, Project Group 1 (or I1) or Project Group 2 (or I2) or Group 3 Outliers (Out of Spec., or I3). There was one 'block' allocation per week that was repeated weekly for 16 weeks.

Groups 1 and 2 of 250 steers each were randomly allocated to one each of the two treatment diets as shown in the table of Appendix 10.3. A comprehensive description of the induction and allocation of steers to the two-project treatments is given in Appendix 10.3.

Each randomly allocated treatment group was designated a feedlot 'Lot' code number referred to as a project lot with:

- Control treatments 22 DTnn (nn being a sequential numerical number, commencing with 23 for Block 1 through to 30 for Block 8 – Phase 1 and 34 for Block 9 through to 41 for Block 16 – Phase 2);
- 3-NOP treatments 22 CTnn (nn being a sequential numerical number, commencing with 23 for Block 1 through to 30 for Block 8 – Phase 1 and 34 for Block 9 through to 41 for Block 16 – Phase 2).

Each Lot was randomly allocated to a project pen.

Immediately following the completion of the project steer Induction processing, each Group 1(I1) and 2(I2) of 250 steers each were randomly allocated to a set of 2 adjacent feedlot pens – Group 1(I1) in one pen; Group 2(I2) in a separate but adjacent pen in the same pen row. The allocation to pens is shown in the table of Appendix 10.4.

The 32 project lots inducted and allocated from 7997 steers, had an average induction/allocation liveweight of 451.8 kg ± 10.40 kg SD, over the range of 427.7 to 467.9 kg. The average induction/allocation liveweight for each project Lot is shown in Appendix 10.5.

3.2.5.2 Commencement of study period for each project block

The study period for each block commenced on the date of completion of induction/allocation and the receipt of their first ration and was referred to as day 0 or Days on Feed (DOF 0). The study period for each block completed after average 151.5 ± 0.89 SD DOF, also referred to as exit DOF. This convention was followed for all 16 project blocks. The actual dates of the project induction/allocation sessions including liveweight measurement is shown in Appendix 10.6. The project timeline is also outlined in a schema shown in Appendix 10.1.

3.2.5.3 Revaccination sessions

At approximately DOF 20, prior to introduction of each block's finisher ration, each block was yarded on its scheduled date for a revaccination session.
The revaccination treatments administered to each steer by Block were:

- For Block 1 and Lot 22 CT24 (3-NOP) from Block 2:
    - Bovilis MH Mannheimia haemolytica +IBR vaccine;
    - Micotil for bovine respiratory disease (BRD);
    - Pasteurella Multocida vaccine.
- For Lot 22 DT24 (Control) from Block 2 and Block's 3-5 and 7:
    - Bovilis MH Mannheimia haemolytica +IBR vaccine;
    - Pasteurella Multocida vaccine.
- For Block 6:
    - Bovilis MH Mannheimia haemolytica +IBR vaccine;

  - 8 in 1 Clostridial vaccine (block had received 5 in 1 Clostridial vaccine, instead of 8 in 1 Clostridial vaccine at Induction/allocation);
  - Pasteurella Multocida vaccine.
- For Block 8-16:
  - Bovilis MH Mannheimia haemolytica +IBR vaccine;
  - 8 in 1 Clostridial vaccine;
  - Pasteurella Multocida vaccine.

3.2.5.4 Reimplant weight

At 69.8 ± 0.58 SD DOF individual steers within each block were weighed prior to feeding. Individual steer RFID numbers were scanned by a RFID scanner, recorded and each steer was implanted with a Synovex H (Oestradiol benzoate 20 mg, testosterone propionate 200 mg) HGP. The schedule of DOF reimplant liveweight measurement and HGP implantation sessions is shown in Appendix 10.7.

3.2.5.5 Measurement of Liveweight at Final Tag QA Check -DOF 140

At approximately 135.0 ± 1.06 SD DOF, the steers in the two project pens of each block – the Control treatment pen and the adjacent 3-NOP treatment pen were walked as separate mobs to a pair of adjacent dispatch pens. Thus, matched pairs of pens from each Block were moved from their respective home pens to a matched pair of dispatch pens in the same row. While in the dispatch pens, the steers were weighed (nonfasted unadjusted liveweight), Individual steer RFID numbers were scanned by a RFID scanner and recorded for a final tag quality assurance check and review of veterinary withholding periods at 139.8 + 0.58 SD DOF (final weight). The schedule of final measurement sessions for each block are shown in Appendix 10.7.

In combination with the final weight at 139.8 + 0.58 SD DOF an project exit liveweight was estimated for both treatments calculated from the feedlot's historic average dressing percentage of 54.3 for the same cattle type, feeding period and the project treatments hot carcass weight data values.

3.2.6 Diets and feeding

There were two different 3-NOP treatment supplement pellet formulations utilised during the project. The theoretical composition of each pellet is shown in Table 2. The first pellet supplement of a theoretical concentration of 90 mg 3-NOP/kg DM was batched and used from22nd June, 2022 (DOF 20) until the 16th of October, 2022. After this time, a higher 3-NOP concentration pellet of 110 mg/kg DMI was batched and utilised from 17th October, 2022 until completion of the project feeding period on the 6th of March, 2023.

There were a number of different composition rations fed throughout the project period. The theoretical composition of the project rations is shown in Table 3. These different formulations were required due to a change in ingredient availability, change in 3-NOP concentration of the supplement pellets and the inclusion of betaine in the rations to coincide with onset of the summer period. The control and 3-NOP finisher rations provided monensin at 25.0mg/kg DM.

Table 2. Theoretical composition of the 3-NOP treatment supplement pellets

Ingredient detail (As fed) **Ingredient name**	**3-NOP pellet 2% inclusion (kg)**
16/5/2022 concentration 90 mg 3-NOP/kg (DM basis) pellet	
Wheat - dry rolled	736.25
Millrun	195.00
Bovaer®10	34.75
Vegetable oil	30.00
Clay	4.00
Total	1,000.00
20/9/2022 higher concentration 110 mg 3-NOP/kg (DM basis) pellet	
Wheat - dry rolled	743.10
Millrun	180.00
Bovaer®10	42.90
Vegetable oil	30.00
Clay	4.00
Total	1,000.00

Table 3. Theoretical diet compositions

Ingredient detail (As fed) **Ingredient name**	**Starter R 1 (kg)**	**Intermediate 1 R 2 (kg)**	**Intermediate 2 R 3 (kg)**	**Control Finisher R 6 (kg)**	**3-NOP Treatment Finisher R·5 (kg)**
16/5/2022 rations	*3-NOP 90 mg/kg concentration supplement pellets*				
Barley - steam flaked	480.00	580.00	662.00	757.00	737.00
Barley hay	250.00	160.00	90.00		
Cottonseed high lint	100.00	100.00	110.00	120.00	120.00
Liquid starter supplement 2205	80.00	35.00			
Sorghum silage	60.00	60.00	60.00	55.00	55.00
Almond hulls	30.00	38.00	28.00		
Liquid finisher supplement		27.00	40.00	52.00	52.00
3NOP supplement pellet 2%					**20.00**
Vegetable oil			10.00	16.00	16.00
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
7/11/2022 rations	*3-NOP 110 mg/kg concentration supplement pellets*				
Barley - steam flaked	490.00	590.00	700.00	795.00	775.00
Barley hay	250.00	160.00	90.00		
Cottonseed high lint	100.00	100.00	100.00	77.00	77.00
Liquid starter supplement 2211	80.00	35.00			
Sorghum silage	60.00	60.00	60.00	60.00	60.00
Almond hulls	20.00	28.00			
Liquid Finisher Supplement 2211 2X		27.00	40.00	51.00	51.00
3NOP supplement pellet 2%					**20.00**
Vegetable oil			10.00	17.00	17.00
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Ingredient detail (As fed)					

Ingredient name	Starter R 1 (kg)	Intermediate 1 R 2 (kg)	Intermediate 2 R 3 (kg)	Control Finisher R 6 (kg)	3-NOP Treatment Finisher R·5 (kg)
14/12/2022 rations	*3-NOP 110 mg/kg concentration supplement pellets with betaine supplement inclusion for heat load periods*				
Barley - steam flaked	490.00	585.00	689.00	800.00	780.00
Barley hay	250.00	150.00	90.00		
Liquid starter supplement 2211	80.00	35.00			
Cottonseed high lint	100.00	95.00	91.00	77.00	77.00
Rhodes grass silage	60.00	60.00	60.00	50.00	50.00
Almond hulls	20.00	48.00	20.00		
Liquid finisher supplement 2212 1X - Betaine		27.00	40.00	53.00	53.00
3NOP 110mg supplement pellet 2%					**20.00**
Vegetable oil			10.00	20.00	20.00
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
24/01/23 rations	*3-NOP 110 mg/kg concentration supplement pellets with betaine inclusion for heat load periods and steam flaked wheat*				
Barley – steam flaked	490.00	598.00	570.00	526.00	516.00
Wheat – steam flaked			135.00	263.00	253.00
Barley hay	250.00	160.00	90.00		
Liquid starter supplement 2212 Betaine	80.00	35.00			
Cottonseed high lint	100.00	100.00	100.00	97.00	97.00
Rhodes grass silage	60.00	60.00	60.00	50.00	50.00
Almond hulls	20.00	20.00			
Liquid finisher supplement 2301 1X Betaine		27.00	40.00	53.00	53.00
3-NOP 110mg supplement pellet 2%					20.00
Vegetable Oil			5.00	11.00	11.00
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**

3.2.7 Feeding management

An accuracy calibration check was conducted of the feed mill batching scales, the liquid weigh bins, and the feed delivery truck scales prior to the commencement of the project in February 2022. Feedlot protocols indicate that all feedmill scales and feed delivery truck scales are calibrated annually and whenever scale technicians are onsite calibrating the main truck weighbridge. Feed delivery truck scales use ±5kg increments and weighbridge use ±50kg increments. Weekly scale accuracy tests were conducted of the feed delivery truck scales thereafter for duration of the project period.

3.2.7.1 Batch mixer evaluation

The batch mixer evaluation was undertaken using calcium as the marker from control diets manufactured in both stationary mixers (1 and 2). The purpose of the evaluation was to objectively assess the consistency of mixing by calculating a co-efficient of variation (CV%) based on ten samples of the control diet removed from multiple feed bunks from a single mixer. The target CV% was 10% or less.

Table 4. Mixing evaluation of Mixer's 1 and 2 using calcium (mg/kg DM) as the marker to assess effects of mixing time and loading order.

Sample	1	2	3	4	5	6	7	8	9	10	sd	mean	%CV	Mixing Time	Loading Order
Mixer1-1	6770	5940	7720	6590	6960	7550	6750	7020	6530	6550	556.62	6912.50	**8.05**	8min	Standard
Mixer2-1	7320	5350	6610	6610	5900	6590	5670	5830	7190	8300	896.13	6537.00	**13.71**	8min	Standard
Mixer1-2	7290	6150	6140	6070	6810	7300	6660	6000	6600	6550	477.59	6557.00	**7.28**	4min	OptionA
Mixer2-2	5870	5660	5560	6310	6490	7630	7190	6070	6830	6250	666.80	6386.00	**10.44**	4min	OptionA
Mixer1-3	5960	6450	7350	7580	7540	6820	7170	7170	6920	7090	498.78	7005.00	**7.12**	8min	OptionA
Mixer2-3	6630	5970	5800	5440	7700	6540	6990	6290	8250	7840	936.60	6745.00	**13.89**	8min	OptionA

In all cases Mixer 2 failed to achieve the target CV (see Table 4). Mixing efficiency was improved to a CV of 5.64% in December 2022 when Mixer 2 screws were re-aligned allowing a feed manufacturing protocol to return to a three-minute mixing time and standard ingredient loading order.

3.2.7.2 Feed analysis

The project rations fed daily were sampled by on site project personnel from each batch as it was delivered to the project pen feedbunks by the feed trucks. The rations were sampled, subsampled, and dried as per the project protocol shown in Appendix 10.8.

Ration samples after drying were sent to a laboratory on a monthly basis for chemical analysis. The analysis was conducted for the following ration chemical components using the diagnostic method listed.

- Determination of Moisture Content by Air Oven g/100g,
- Determination of Crude Protein by Combustion (N*6.25, g/100g),
- Determination of Crude Fats and Oils by Soxhlet (%w/w),
- Determination of Ash Content by Muffle Furnace (%w/w),
- Determination of Neutral Detergent Fibre by Refluxing(NDF) (%w/w),
- Determination of Acid Extractable Elements by ICPOES for Phosphorous (mg/kg).

Results from monthly ration analysis shown in Table 10 below and Appendix 10.8

3.2.7.3 Analysis of 3-NOP supplements and 3-NOP activity

The 3-NOP treatment supplement pellets were sampled regularly from a dedicated feedmill bulk commodity storage bay. There were a number of 3-NOP treatment supplement pellet batches manufactured by a proprietary feed mill in southern Queensland and road transported to the project feedlot. The sampling, drying, and packaging of the pellets was as per the project protocol shown in Appendix 10.8.

Following transport and storage of the processed pellet samples to the University of Queensland, Gatton Campus, the samples were airfreighted in batches to a laboratory in NZ, equipped to analyse for 3-NOP concentration. The analytical method used to chemically analyse the pellet 3-NOP concentration was Ultrasound assisted organic extraction and analysis by HPLC.

3.2.8 Daily cattle observations and removal of sick/debilitated cattle from treatment pens

Daily observations of all project steers for health and welfare purposes were conducted as per the feedlot's protocols. Steers pulled from any project home pen for any ailment were treated and managed as per the feedlots Animal Health protocol. Following treatment, steers were either returned to their home pen or remained in the hospital pens until their course of treatment was completed. On occasions a steer may have pulled again as it required retreatment for the same ailment or for an unrelated ailment. If a steer had been absent from its home pen for a period longer than 10 days, that steer was culled from the project and its data excluded. Any steer that had been hospitalised and was considered unlikely to recover during the remainder of that steer's study period; or was in a medication withholding period and likely to miss the exit for slaughter, such steers were culled from the project.

Management of Buller Syndrome.

Prior to the 22nd of November, 2022 any animal treated as a Buller was culled from the project and sent to a Buller cull pen. As of the 22nd of November, 2022, steers pulled from their home pen for the ailment 'Buller' were treated in the hospital with the appropriate course of treatment and were reassigned to a pen under the following assessment criteria:

- If the pulled steer was in the early stage of its feeding period and not likely to fall within a treatment course 'withhold period', the steer was allowed to recover in the hospital for two days before being returned to its home pen;
- If the pulled steer was in the later stage of its feeding period and did not require treatment it was allowed to recover in the hospital for two days before being returned to its home pen;
- If the pulled steer was in the later stage of its feeding period and required treatment that would cause the animal to be within a 'withhold period' at the nominated date of dispatch, the steer was culled from the project and sent to a dedicated buller cull pen;
- If a steer was pulled a number of times as a recurrent Buller, the steer was culled from the project and sent to a dedicated Buller cull pen.

3.2.9 Project pen maintenance.

Scraping of the manure from each pending respective home pen was conducted prior to each sequential Induction session and in addition, as the feedlot schedule required. Water trough cleaning was conducted prior to each sequential Induction session and then weekly for the duration of each project Lot's residency.

3.2.10 Exit procedures.

The exit schedule for the project blocks of steers to the collaborating abattoir is shown in Appendix 10.9.

At the scheduled date of exit from the feedlot (day before slaughter), the relevant lots of the project block were walked separately from their Dispatch pen at least 30 minutes prior to exit. The steers were walked to the feedlot trucking yards where they remained in separate yards until loading onto livestock transport trailers. Every project Exit-Livestock Transport Session involved two consignments of livestock transports, the first consignment consisting of multiple livestock transport units loaded and departed the feedlot in the early PM for the collaborating abattoir. These multiple livestock transport units upon returning to the feedlot's trucking yards in the later PM, loaded their second consignment of project steers from the same block and departed the feedlot for the collaborating feedlot. The two consignments of transported project steers consisted of equal proportions of steers from each project Lot, that were randomly allocated to livestock trailer compartments of each livestock transport unit.

All procedures carried out at exit were as per the feedlots' protocols and the project's protocol as shown in Appendix 10.10. The eligibility of steers for dispatch and slaughter was assessed as per the feedlot's welfare protocols.

Following arrival of the livestock transport trucks at the trucking yards, the project steers as they moved through the yards were drafted into smaller mobs for loading onto the livestock trailers. The number of steers in each smaller mob was dependent on the livestock trailer configuration.

The project exit-transport program involved 'B' triple and 'B' double multicombination livestock trailers, the actual configuration varied on occasions, but most common was the use of 2 'B triple' and 2 'B double' multicombination livestock trailers for each consignment on an Exit Day. Variations involved scenarios where the collaborating abattoir was operating on a reduced daily slaughter and required the project block to be slaughtered over 2 days instead of one day, therefore livestock transported over 2 days; or site works being carried out at the abattoir that precluded 'B triple' multi combination livestock trailers being used. The details of these variations were:

- Block 1: 1/11/22 – 'B doubles' only – due to site works at abattoir;
- Block 2: 8/11/22 –' B doubles' only - due to site works at abattoir;
- Block 13: 12-13/2/23 - Trucked over 2 days – Due to abattoir reduced daily kill.

At the actual time of loading the project steers onto their respective livestock transport unit, each steer's RFID number was scanned by a RFID scanner located on the ramp at the point of entry into the livestock trailer.

3.2.11 Transport to abattoir procedures.

The loaded livestock transport units were weighed over the feedlot's truck weighbridge and commenced their journey to the collaborating abattoir. Dependent on road conditions, traffic density and rest stops to check the welfare of the project steers, the transport time varied between 3 to 5 hours (averaging ~4 hours) over the project's experimental period. As stated in subsection 3.2.10 above, there were two consignments of project steers in the livestock transport units for each project Exit session. The same transport procedures and a similar duration was achieved for each consignment. The procedures are outlined in Appendix 10.10.

3.2.12 Lairage at abattoir.

The procedures followed at the time of arrival of the livestock transport units at the collaborating abattoir, unloading and placement in lairage are outlined in Appendix 10.10. and Appendix 10.11.

The time of arrival for each truck of each block consignment is shown in Appendix 10.12.

The procedures followed at arrival of each of the two consignments of project steers for each block were the same. The only difference in protocol was the different arrival times of the two consignments i.e., late PM of day of exit for Consignment 1 and early AM of the day of slaughter for Consignment 2.

All project steers upon unloading were placed in adequately sized lairage yards with access to water under a covered lairage yard facility. Upon receival at the collaborating abattoir lairage yards, all livestock handling and procedural aspects were under the control of abattoir personal. The procedures involved, randomised placement of project Lot groups, personnel involved, and approximate time scheduling are shown in Appendix 10.11.

For all exits, cattle arrived within required time to meet abattoir protocols prior to slaughter.

3.2.13 Procedures at slaughter.

The slaughter processing date schedule for each project block is shown in Appendix 10.9. All practices involving the project steers carried out during the lairage and slaughtering processes were as the collaborating abattoirs codes of practice and protocols associated with animal welfare.

For the slaughter of each project block steers', the timing of slaughter, preprocessing handling and washing preslaughter, processing at slaughter, measurements and assessments by the collaborating abattoir and the monitoring of the procedure and recording of each individual steer visual identification by project personnel are outlined in the project protocol shown in Appendix 10.11.

The measurements and assessments caried out by the abattoir staff are documented in subsection 3.3.2.

Over the duration of the project's slaughter periods (i.e., as for Phase 1 and Phase 2), there was one variation to the scheduled date of slaughtering. The Consignment group 2 of Project Block 1 had their slaughtering schedule changed from Day 1 of the abattoir period to Day 2, due to an abattoir computer management systems breakdown. As the design of the project Exit/Slaughter protocols were based on an exit/transport/slaughter program of both Lots within a block being similarly represented in each Consignment Group, the effects of the breakdown mentioned above and consequential delay of Consignment Group 2 slaughter, the effects on the project were not considered to be marked.

On the day following slaughtering of each block, chiller assessment of each individual carcass was carried out by the collaborating abattoirs accredited 'Grader's' approximately 18-21 hours post stunning. This assessment was undertaken in the abattoir's carcass side chillers after the carcass side chilling period of 18-21 hours. The procedures followed during the carcass side chilling period and chiller assessment are outlined in the project protocol shown in Appendix 10.11. Carcass side chiller temperature profiles were recorded by the collaborating abattoir, with a sample shown in Appendix 10.15.

3.3Measurements

3.3.1 Feedlot period

Service and calibration of the automated weather stations (AWS's)– Environdata Product DL3010 Weather Maestro 10Ch located near the feedlot office administration site, and a Environdata Weather Maestro 10Channel Data Logger product DL3110 AWS, adjacent to the feedlots project pens was carried out in August 2022.

An accuracy calibration of the Induction Processing Shed individual animal liveweight scales, the T-Row Yards, and Y-Row Dispatch Yard individual animal liveweight scales was conducted prior to the commencement of the project. Prior to each induction/allocation session, for every HGP re-implant session and every final tag check QA measurement session for every block, an accuracy check of each of these individual animal liveweight scales was conducted by placing two by 20kg weight blocks on the scale to achieve a total test weight of 500kg. During an accuracy check, if any liveweight scale was found to be inaccurate by ±3.0 kg, the liveweight scale was re-calibrated prior to use.

List of measurements recorded during the feedlot period:

- Weather data measurements were recorded every hour using an automated weather station (Environdata Weather Maestro 10Channel Data Logger product DL3110) adjacent to the feedlots project pens, these being:
  - Windspeed (WS; km/h);
  - Relative humidity (RH; %)
  - Air temperature (°C);
  - Black globe temperature in the sun (BG; °C);
  - Maximum wind gust (km/h);
  - Calculated current heat load index (HLI) (HLU);
  - Calculated current AHLU (HLU); and
  - Total daily rainfall was measured at the Environdata Product DL3010 Weather Maestro 10Ch AWS located next to the feedlot office administration site. The rainfall detection sensor failed and was inoperative for the period of June 2022 to August 2022. For this period, rainfall recordings from a nearby Bureau of Meteorology Automatic Weather Station were referenced for the project's purposes.
- Daily observation of steer health and welfare;
- Individual steer RFID numbers were scanned by a RFID scanner and recorded at every block induction/allocation session, every block's HGP reimplant session and every block's final tag QA check measurement session;
- Individual steer nonfasted (full) liveweight at each block Induction/allocation session (as per the schedule listed in Appendix 10.6);
- Individual steer nonfasted (full) unadjusted liveweight at each block's HGP reimplant session as per the schedule listed in Appendix 10.7);
- Individual steer nonfasted (full) unadjusted liveweight at each block's final tag QA check measurement session, this liveweight being the project's final nonfasted (unadjusted) individual liveweight;
- Assay of 3-NOP concentration in the supplement pellets and in the ration;
- For each day, the cumulative amounts of the following variables were calculated;
  - Pen feed intake (FI_t)
  - Pen dry matter intake (DMI_t)
  - Number of head present in the pen on that day (NHd_t)
  - Pellet intake (PI_t)
  - 3NOP intake ($NOPI_t$)
- For any required interval (Induction to 70DOF, 70DOF to 140DOF, Induction to 140DOF), the cumulative amounts of feed to the beginning of the interval, and the cumulative amounts at the end were determined. Subtracting the starting cumulative values from the ending cumulative values gives the total intake amounts for the interval. Dividing interval totals for FI, DMI, PI and $NOPI_t$ by the interval total NHd_t results in intakes on a per head per day basis. Thus, over any interval average daily FI, average daily DMI, average daily PI (all in kg/hd/d) & average daily NOPI (mg/hd/d) can be derived. Lastly, to express 3-NOP relative to DMI, the interval total NOPI is divided by the interval total DMI, with the result in mg 3-NOP/kg DMI.
- Daily pen feed intake on 'as fed' and 'dry matter' (DM) basis in kg/hd/d. Daily 'as fed' intake was defined as the total feed offered per pen per day on a wet basis less any discarded residue adjusted to a similar moisture content as the original feed offered. Dry matter pen feed intake was defined as the total feed offered per pen on a dry matter basis as determined from the dry matter of the daily ration samples less the dry weight of any discarded ration as determined by the dry matter of a residue sample;
- Nutritional analysis of the final finisher ration on a DM basis included:
  - Protein (N*6.25, g/100g);
  - Fat (%w/w);
  - Ash (%w/w);
  - Neutral Detergent Fibre (NDF) (%w/w);
  - Phosphorous (mg/kg);

- Starch (%w/w).

Results from monthly ration analysis shown in Appendix 10.8

3.3.2 At slaughter

The exit and slaughter of the treatment pens of steers commenced when the first of the project blocks reached on average 151.5 $\pm$0.89 SD DOF the designated end of each block's study period. Block 1 exited the feedlot on 1 November 2022 and was slaughtered at the collaborating abattoir on 2nd November 2022. The exit and slaughter of blocks continued in sequence up to and including 20th December 2022 when the collaborating abattoir entered its planned closure period. The return of the sequential exit and slaughter of project blocks schedule recommended with Block 9 on 19th January 2023 and continued up to 7th March 2023 when Block 16, the final block of project steers was slaughtered. The schedule of feedlot exits, and slaughter dates is shown in Appendix 10.9.

List of measurements at slaughter:

- Scanning of individual steer RFID number;
- Recording of individual steer visual identification number;
- Measurement of dressed carcass left and right hand side weights (kg);
- Overall hot standard carcass weight (HSCW) (kg);
- Assessment of dentition;
- Assessment of bruising score;
- Measurement of subcutaneous fat depth (mm) at the P8 site;
- Placement location of each carcass side in the postslaughter carcass chillers;

The offal of every project steer was inspected by the Abattoir's inspectors for lesions/faults/defects and possible condemnation as per Abattoir protocols.

3.3.2.1 At slaughter in the carcass chiller following 18-21 hours chilling at the 12th/13th rib quartering point on either the left or right hand carcass side.

List of data items recorded post chilling:

- AUS·MEAT chiller assessment parameters, these being:
  - Ossification score. A numerical score ranging from 100 to 590;
  - Eye muscle area (cm^2);
  - Meat colour of the ribeye (*m.longissimus.dorsi*) muscle at the 12th rib carcass side quartering point. The range of scores are 1A, 1B, 1C and 2 to 7;
  - Intermuscular fat colour at the 12th rib carcass side quartering point. A range of numerical scores from 0 to 9;
  - AUS·MEAT marbling score of the ribeye (*m.longissimus.dorsi*) muscle at the 12th rib carcass side quartering point. A range of numerical scores from 0 to 9.
- MSA chiller assessment parameters, these being:
  - MSA marbling score of the ribeye (*m.longissimus.dorsi*) muscle at the 12th rib carcass side quartering point. A numerical marbling score over a range of 100 to 1190 in increments of 10;
  - MSA Index. This index is a number value, from one (lowest) to 100 (highest), which represents the potential eating quality of a MSA compliant carcass, derived from a weighted average of a selection of cut by cook outcomes using measurement inputs managed by a producer.

3.4 Statistical analysis

A comprehensive description of the project statistical analysis in included as Appendix 10.13.

3.4.1 Data import and statistical methods conducted by project statistician.

Each of the animal performance data sheets contained columns that identified when key events such as induction, first day on feed, reimplant and final weighing were identified. Refer to Table 2 of Appendix 10.13.

Performance and Carcass data

After removal of culls, largely incomplete records and individual records of carcases that had been downgraded, the data set for performance comprised 7546 head and that for carcass characteristics consisted of 7468 head. The distribution across the blocks and pens is shown in Table 4. All pens had excellent representation, with numbers ranging from 210 up to 244.

There were seventy-eight (78) carcases removed that did not meet the collaborating abattoir's AR150 grade. The project animals had been fed for and targeted at the AR150 grade. This removed 39 animals from each of the CT and DT Lots (out of 3742 and 3726 carcases respectively). The 78 carcases that failed to make the desired AR150 grade specifications were downgraded to the abattoir's lower valued carcase grade of SM (69 carcases) due to dark meat colour or high pH values; or to the MSFYP grade (9 carcases).

The loss in carcase value due to downgrading of the 78 SM and MSFYP carcases was $3.214/kg HSCW, compared to the 7468 carcases that graded AR150.

Refer to Appendix 10.14 for tabulated detail of the downgraded carcases and the number downgraded by Block and treatment.

Table 4. Distribution of cell numbers across treatments and replicates (Blocks)

Block no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Treatment																
3-NOP	244	236	241	238	239	227	228	237	241	239	237	224	230	229	220	232
Control	238	235	240	242	240	227	238	235	238	238	233	210	234	219	225	234

For analysis, means were calculated for each of the 32 pens. Prior to this, some filtering of each variable was carried out, with observations more than 3.5 standard deviations from the initial pen mean removed.

The 32-pen means were analysed using a mixed model ANOVA, with means weighted by the number of observations used. As noted in 3.2.2 project data were split into two phases with Blocks 1 to 8 as Phase 1 and Blocks 9 to 16 as Phase 2. The mixed model estimated fixed effects of Phase, Treatment and Phase X Treatment. Random effects were estimated for 'Blocks within Phase'. Models were fitted using the *lmer* function from the *lme4* library (version 1.1-32). The key elements of the analyses were exported to Excel, including ANOVA summary information, expected marginal (least squares) means, standard errors, confidence intervals and differences. Although fitted as a factorial model we have focussed on treatment effects nested within each phase.

Processing of discrete carcass measurements is described in Appendix 10.13.

Dry Matter percentage data

Processing of the Dry Matter percentage (DM%) data is outlined in Appendix 10.13. Ultimately a lookup table was created giving the DM% for any ration on any date.

Feed Intake data

The daily feed intake calculation accounted for the animals that were in the pen on each day. Cattle that were removed due to buller, sickness or death were therefore not included in the days head number. Cattle that were returned to home pen from the hospital were accounted by change in head number.

The quantities of each ration supplied to each pen on each day were supplied in two Excel files. The relatively complex import and processing is described in Appendix 10.13. For each required interval, average daily feed intake (FI), average daily DMI, average daily pellet intake (PI) (all in kg/hd/d) and average daily NOPI (mg/hd/d) were derived.

3-NOP intake data

The method to determine 3-NOP concentration per kg of feed delivered required daily calculation of feed intake for each project pen rather than calculating pen feed intake by dividing total feed delivered to pen by total head days.

The amount of ration batch supplied was multiplied by the pellet inclusion rate (PIR_t) to give the (approximate) quantity of pellets supplied to the pen (PI_t).

The amount of 3-NOP supplied in pellets was calculated as: PI_t x $NOPconc_t$. The $NOPconc_t$ refers to the estimated 3-NOP concentration on any day. For each pellet batch, the laboratory measured concentrations of 3-NOP at start and end of storage were used to linearly interpolate the estimated 3-NOP concentration while that batch was in use.

Calculations of feed intake, daily pellet intake and average daily 3-NOP intake were carried out for:
- First DOF to Reimplant (DOF 70) – combined with ADG to give Feed Conversion ratios (FCR's);
- Reimplant (DOF 70) to Final (DOF 140) and Exit (DOF 150)– combined with ADG to give FCR;
- First DOF to Final (DOF 140) and Exit (DOF 150)– combined with ADG to give FCR;
- DOF 22 to DOF 49 (Period 2), representing the day in which the treatment pellet was included
- DOF 50 to DOF 77 (Period 3);
- DOF 78 to DOF 105 (Period 4);
- DOF 106 to DOF 133 (Period 5).

For the intervals corresponding to liveweight measurement, data for daily FI, DMI, feed conversion ratios for both 'As fed' and 'DM' basis were analysed using a similar linear mixed model to carcass and performance data. That is, with fixed effects for project phase, treatment, and their interaction, with block within phase as a random effect. In respect to earlier analysis, the feed intake analysis differs in that an unweighted model is used. Data for pellet and 3-NOP intake, as well as 3-NOP concentration was summarised across the 16 blocks.
A more complex model was used for the five 21- or 28-day DMI periods. Only FI and DMI can be analysed since weights are not available at that resolution. Periods were regarded as repeated measurements within each pen leading to fixed effects being estimated for Phase, Treatment, Feed Period, and all interactions. Block within Phase, and Pen within Block were specified as random effects.
Summary information from the analyses along with means, standard errors and comparisons were exported to Excel.

Analysis of Cull steer data

Data were assembled as cull steer counts per project pen out of the number of animals inducted per pen. Categories considered were Buller culls, Hospital culls, Project culls, Mortality and the combined number 'rejected' from the trial (Rejects). In addition, the number of animals that spent any time hospitalised was accounted for by removal of that head number from the home pen (Hospital pulls).

The categories and calculations were:

Morbidity (Hospital pulls) = [(every project steer pulled for a hospital ailment(only first visit counted))/(head inducted into the project)] * 100.

Mortality % = [(head dead from any cause)/(head inducted into the project)] * 100. Head dead included (Buller cull &Mortality) + (Hospital Cull & Mortality) + (Mortality) + (Project cull & Mortality).

Buller % = [(unique head culled due to buller syndrome)/(head inducted into the project)] * 100.

Hospital culls % = [(head culled from the hospital (included buller culls that had been hospitalised and then culled from the hospital))/(head inducted into the project)] * 100.

Project culls % = [(head culled from the project for reasons other than hospital or buller syndrome(missing animal, missed measurement session, missed HGP reimplant))/(head inducted into the project)] * 100.

Rejects % = [(total of all cull reasons)/(head inducted into the project)] * 100.

Some animals received multiple category descriptions. For example, an animal could be described as a buller and a mortality. However to simplify health data and avoid counting the one animal multiple times the following definitions were applied. Mortality takes precedence over Hospital, which takes precedence over Buller and Project. In the case of the example provided, animal would be classified as mortality only.

Data were analysed as a generalized linear model assuming a binomial distribution with the default logit link. As preliminary inspection showed little difference between treatments, a simple two-way (Block + Treatment) model was fitted. Analysis of deviance tables were produced for each variable analysed and the residual deviance tested for possible over-dispersion.

3.5 Modelling of methane emission reduction using Bovaer10®

The methodology used to evaluate the effect of 3-NOP utilisation on the carbon footprint of Australian feedlot cattle is outlined in detail in Appendix 10.17. 'Carbon Baseline Assessment on the effect of Bovaer® feed supplement on the carbon footprint of trial cattle in MLA Project P.PSH.1375'.

3.6 Estimation of the cost benefit of Bovaer®10 to feedlot operations

Sensitivity analysis was conducted across a range of 3-NOP costs ranging from 5 to 40 c/head/d.

Four scenarios to cover the cost of feeding 3-NOP were compared below:

- Breakeven carcass sales premium assuming no performance loss;
- Breakeven carcass sales premium assuming 2.6 kg HSCW loss/carcass with a 5-year average HSCW of $7.40 per kilogram;
- Breakeven carbon price for VERRA credits assuming no performance loss;
- Breakeven carbon price for VERRA credits assuming 2.6 kg HSCW loss/carcass.

VERRA is a non-profit corporation which manages a voluntary carbon market program called the Verified Carbon Standard (VCS) program. The VCS is the most widely used greenhouse gas (GHG) auditing program. Carbon mitigating projects undergo an independent assessment process. Once verified, the project is eligible to use Verified Carbon Units (VCU's). One VCU is equivalent to 1 metric tonne of carbon dioxide reduced or removed from the atmosphere. Project VCU's can be monetised in the carbon market.

The steps required to initiate a VCS program with the usage of Bovaer® 10 with VERRA include:

1. Account opened in VERRA's registry;
2. Draft submitted for project design document (PDD) for pipeline listing and a 30-day public consultation at VERRA's registry. Project design following required program rules;
3. PDD finalised and with project's public comment period completed, PDD submitted for validation (with an accredited auditor from VERRA's supplied list);
4. Once validated, project provided a positive statement and report is submitted to VERRA for review and approval for registration;
5. Project status updated as registered and validated, and can now issue VCU's;
6. Project start date is when project begins generating GHG emission reductions. Projects shall complete validation within two years of the project start date. That means project can start 2 years before validation (steps 1-5);
7. Reductions require monitoring and described in a monitoring report. Monitoring report completed for defined monitoring period. Report submitted for verification with auditor ;
8. When project monitoring report approved by auditor, VERRA submits the project verification approval;
9. Once Verra approves the project's verification request, project is issued VCUs;

Steps 7-9 need to be repeated for each monitoring period which is generally for the life of the project. Monitoring periods cannot overlap.

VERRA projects can run for 7 or 10 year periods. The 7 year period is a minimum requirement. Projects can be renewed as many times as required after each 7 year cycle. Projects run for 10 years and cannot be renewed.

4. Results and discussion

4.1 Weather conditions

Rainfall was recorded at the feedlot over the project period (Figure 2) apart from June, July, and August 2022 when the rainfall recording sensor faulted. Due to the incomplete rainfall dataset available for the feedlot, data recorded from a nearby Bureau of Meteorology site AWS is also presented in Figure 2 Based on 22-year rainfall means from the nearby BOM site, rainfall was above average at that site for June, August, September, and October 2022. At the feedlot it was speculated that rainfall means would have been above average for June , August, September, and October, 2022 also.

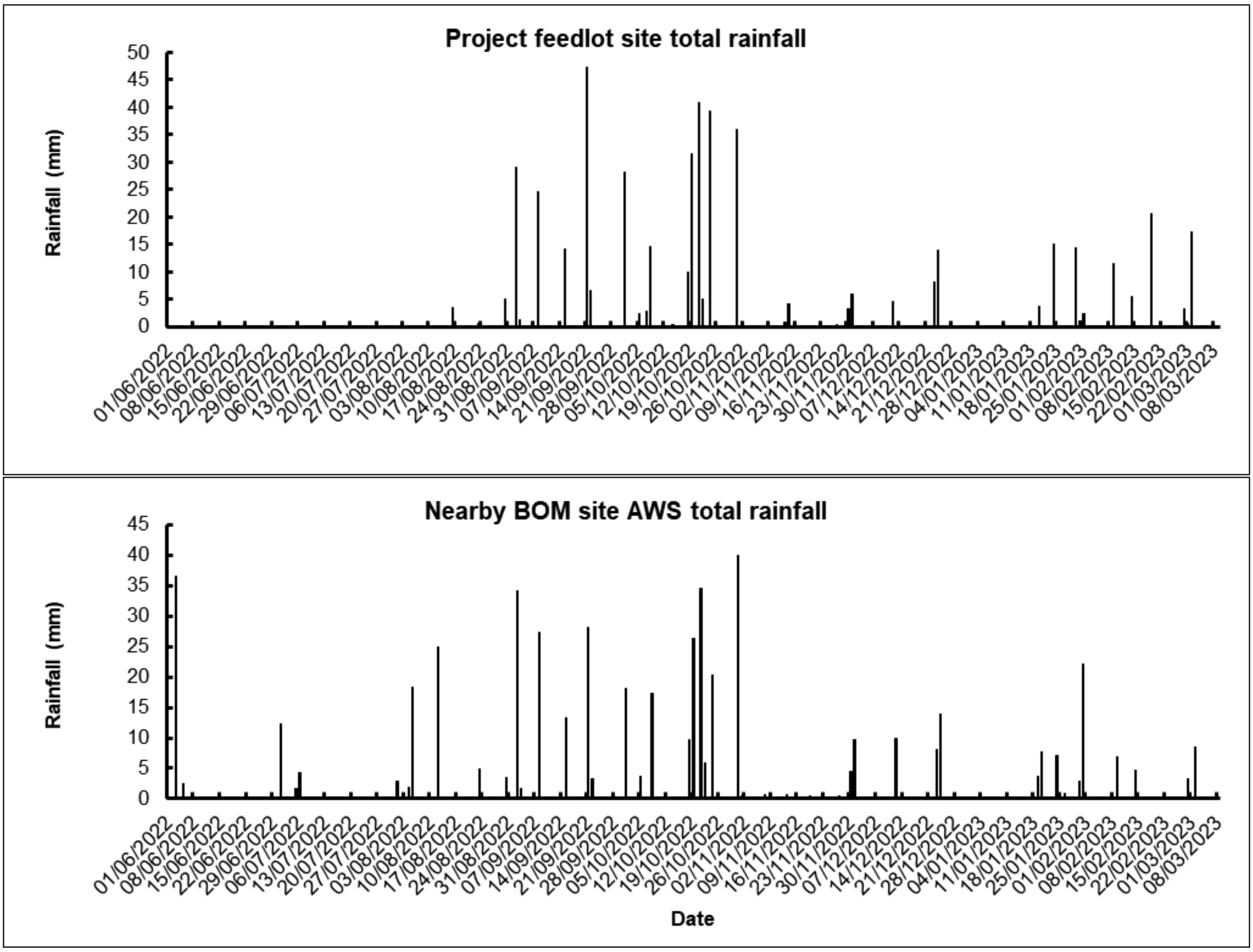


Figure 2. Rainfall recorded (excluding June, July, and August 2022) at the project feedlot and rainfall recorded at the nearby Bureau of Meteorology Station Number: 041230 AWS over the project period.

Mean and maximum windspeed and minimum, mean, and maximum relative humidity was recorded at an onsite AWS adjacent to the project feedlot pens over the study period (Figure 3).

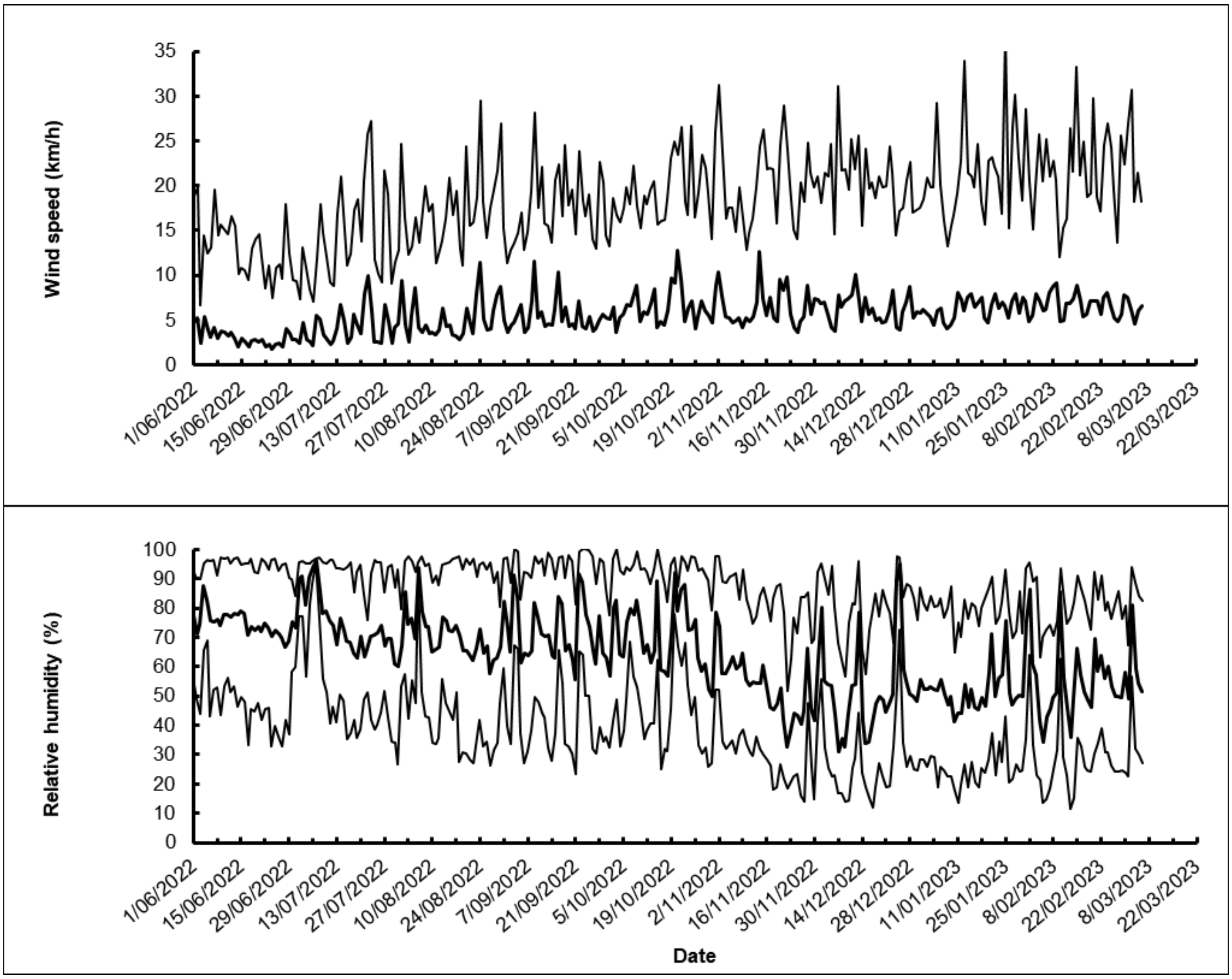


Figure 3. Mean and maximum windspeed, minimum, mean, and maximum relative humidity recorded adjacent to the project feedlot pens over the study period.

Windspeeds were always above zero, were routinely above 20 km/h with maximums over 30 km/h recorded during January, February, and March, 2023. Relative humidity values were in general, above 20% over the June to October 2022 period, but were lowest, though still above 10% during the January to March 2023 period. The highest humidity values recorded coincided with the June to October 2022 period which was also the period when above average rainfalls were recorded.

Air temperature and Black Globe temperature was recorded at an onsite AWS adjacent to the project feedlot pens over the study period (Figure 4).

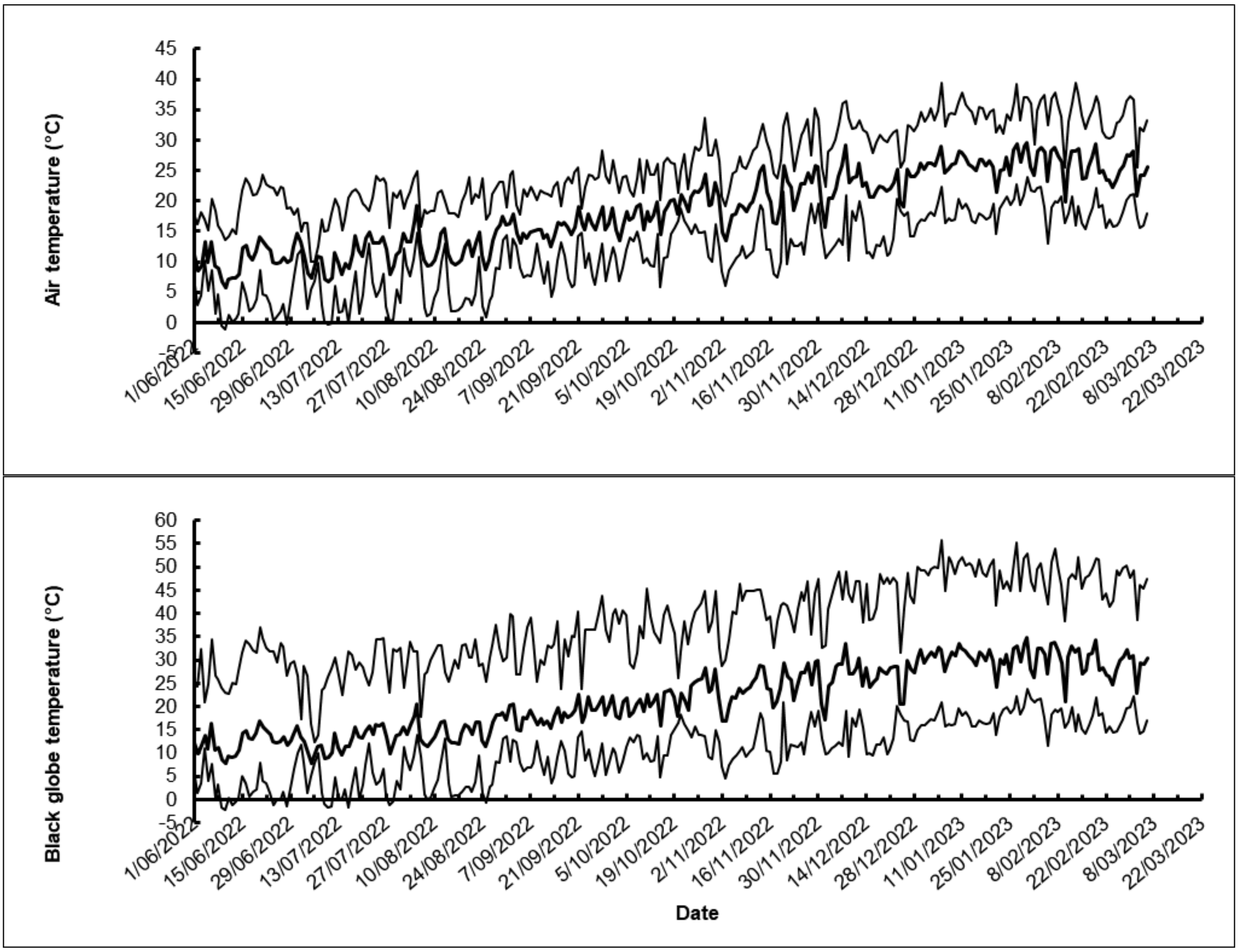


Figure 4. Minimum, mean, and maximum air temperature, black globe temperature recorded adjacent to the project feedlot pens over the study period.

With the project commencing in June 2022, temperature values increased over time as shown in Figure 4. The lowest minimum temperatures were recorded over the June to September, 2022 period with negative values recorded in June 2022. In contrast and as expected, consistent maximum temperature values were recorded during the January to March 2023 summer period. There were a number of hot days over 35°C air temperature during February and March, 2023. The highest minimum air temperature recorded was 22.8°C on 27th January, 2023. As stated in the project methodology the study period was separated into two experimental phases based on Blocks 1-8 (study period of 1st June 2022 to 19th December 2022) and Blocks 9-16 (study period of 17th August 2022 to 6th March 2023). The maximum daily air and black globe temperature plots are in concert to a greater extent with the study periods of Phase 1 and Phase 2. That is and as expected, minimum temperatures were recorded during Phase 1 and maximum temperatures recorded during Phase 2.

The mean and maximum Heat Load Index (HLI) calculated for the study period is shown in Figure 5.

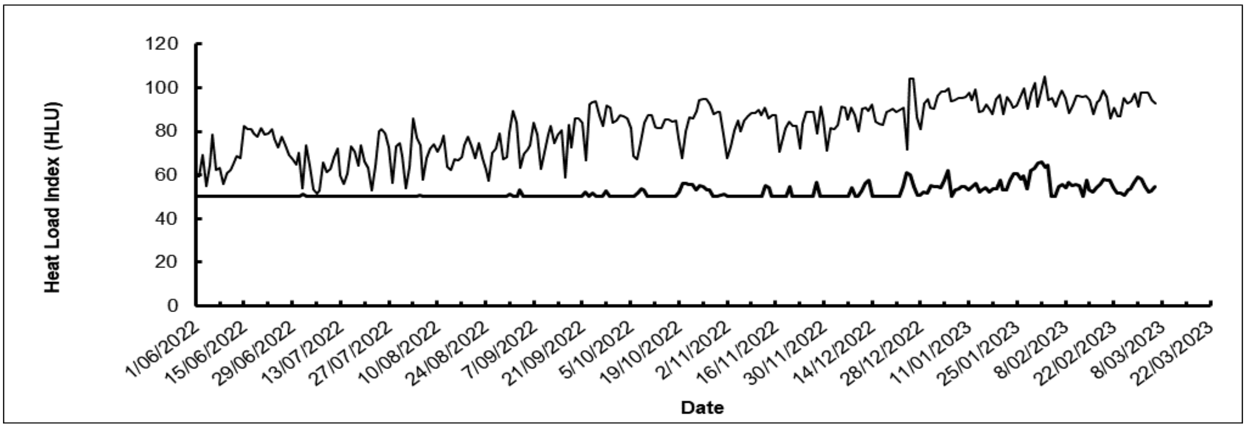


Figure 5. Mean, and maximum Heat Load Index calculated from AWS weather data recorded adjacent to the project feedlot pens during the study period.

As expected, the HLI increased over time during the study period with the highest values recorded from late December 2022 onward. There were 116 days with a maximum HLI > 86, 79 days with HLI > 90, 33 days with HLI > 95 and 4 days when HLI > 100. The maximum HLI recorded was 103.9 on 26th December, 2022. No cases of heat stress were reported during the study as all pens had feedlot shade available and Betaine had been incorporated into the rations from 14th December 2022 to coincide with the summer heat load period.

Betaine (trimethylglycine) is a natural product isolated from sugar beet. It possesses characteristics which enhance metabolic function and resilience of animals exposed to heat load conditions (Gaughan et al., 2005). One of its functions is acting as a methyl donor (Eckland et al., 2005). This potentially has implications for methane production, specifically in relation to the second most energetically efficient methane pathway which relies on methyl amines and methanol known as the methylotrophic pathway (Henderson et al., 2015). However, as all methane pathways require the function of the specific methanogen enzyme, methyl co-enzyme M reductase (Chen et al., 2020) which the active component of Bovaer10®, 3-NOP directly inhibits, it is unlikely that betaine would have impacted the treatment response.

Minimum HLI values were not calculated during this project. Possibly contributing to an absence of reported heat stress, was the number of minimum air temperatures recorded during the night over this heat load period that were less than 20°C, apart from 15 nights, thus ensuring there was sufficient nighttime cooling for the cattle to dissipate accumulated body heat back to the environment.

As reported above there is a suggested association between maximum air and black globe temperature and the project phases, it is not surprising that there appears an association between HLI and the project phases also. This is not unexpected as black globe temperature is a significant variable in the calculation of the HLI. The highest number of maximum HLI values were reported during Phase 2, with the lowest number of maximum HLI values being reported during Phase 1.

The accumulated heat load units (AHLU) calculated during the project period are presented in Figure 6.

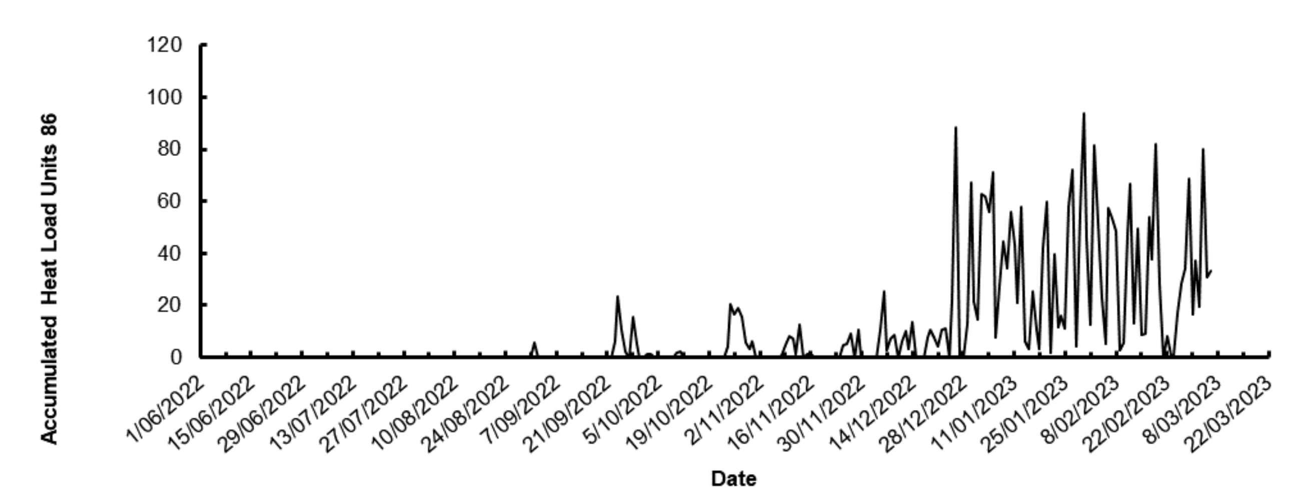


Figure 6. Study period Accumulated Heat Load Units calculated from AWS weather data recorded adjacent to the project feedlot pens.

The AHLU index records the number of hours over a day or days when the HLI is above the threshold value of 86. Above this threshold, cattle will gain body heat resulting in a positive heat load balance. Heat Load events (AHLU>50) occurred during December 2022, January and February 2023 which of course coincided with the Phase 2 study period. There were 22 days with AHLU >50 units. While there were 22 days considered as heat load events, as stated previously there were no reported cases of heat stress in any of the project animals. The maximum AHLU was reported on the 30th of January 2023. The trend in AHLU units is not surprising as black globe temperature is a significant variable in the calculation of the HLI and the AHLU index is directly related to the HLI.

4.2 Feed analysis

4.2.1 Bovaer® 10 pellet.

A 2% inclusion (as-fed) wheat-based pellet was used to enhance effective and consistent distribution of 3-NOP within the 20-tonne capacity mixing and delivery equipment. The ingredient and nutrient composition of the Bovaer® 10 pellet is described in Table 5.

Table 5. Formulated composition of the two versions of the 3-NOP pellet

	Version 1 **June to Oct. 2022** **Deliver 90mg/kg DM**	**Version 2** **Oct. 2022 to study end** **Deliver 110mg/kg DM**
Ingredient %, as fed		
Wheat – hammermilled	73.63	74.00
Millrun	19.50	17.46
Bovear10®	3.48	4.29
Vegetable oil	3.00	3.00
Bentonite	0.40	1.25
Total	100.00	100.00
Nutrient composition, DM basis		
Dry matter %	86.38	85.75
Crude protein %	11.96	11.67
Fat %	5.47	5.11
3-NOP mg/kg	3922.8	4962.4

Previous research has used different methods of adding 3-NOP to total mixed rations including direct addition in its concentrate form (Vyas et al., 2016) or via liquid carriers including a molasses/water blend prepared fresh daily (Martinez-Fernandez et al., 2018) and water via micromachines (Alemu et al. 2021a). Most feedlots in Australia currently utilise molasses-based suspension supplements, however no published research has occurred on the stability of 3-NOP for this supplementation method over typical periods of retention in feedlot storage tanks (weeks to months). Given these factors and the currently marketed concentration of 100,000mg/kg, 3-NOP is most practically incorporated into total mixed rations in Australia via mineral concentrate (Almeida et al. 2023) or pelleted with a cereal carrier.

Pellets were sampled twice from each delivery received; once on receival and again at the end of the storage period. These samples were analysed for 3-NOP activity and used to calculate losses of activity associated with pelleting and storage compared with the theoretical 3-NOP concentrations of Table 5. The changes in activity concentration are described in Table 6.

Table 6. 3-NOP activity concentration losses in pellets (As received basis).

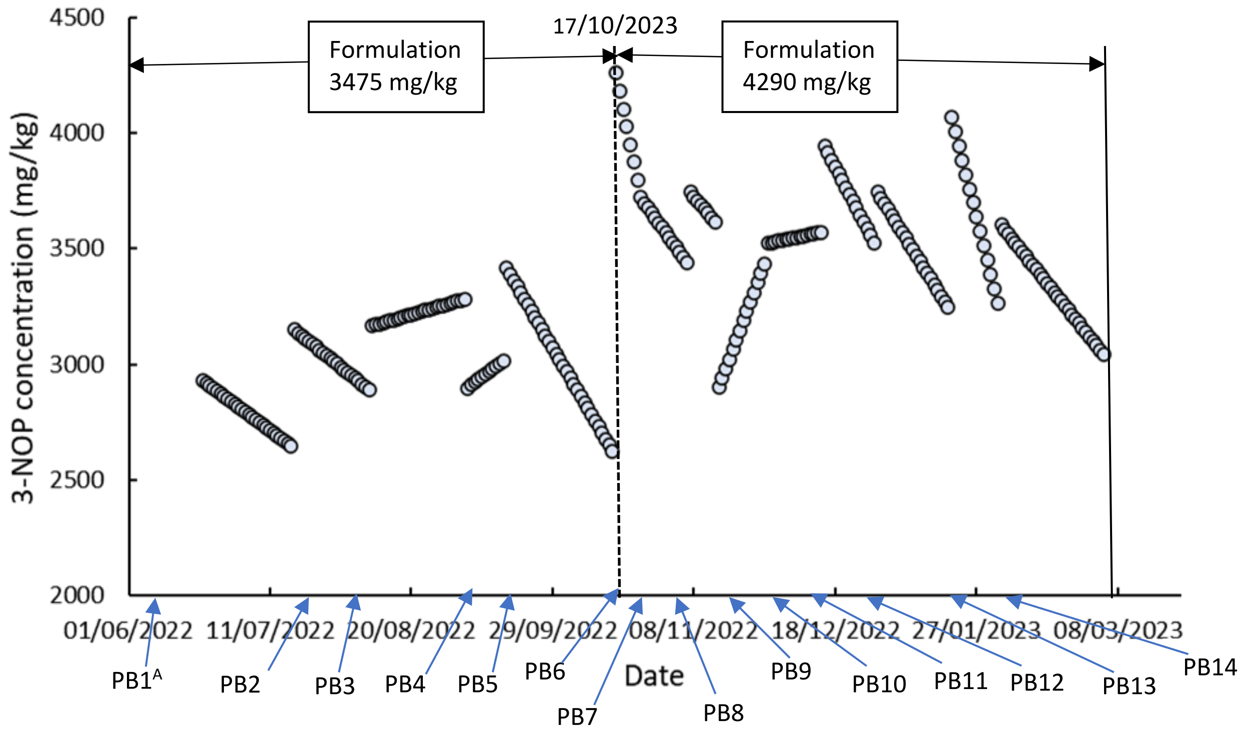
Pellet sampling date	Pellet batch no.	No. storage days	Loss due to pelleting (%)[A]	Loss due to storage (%)	Overall loss (%)
8/06/2022	1		-11.1		
18/07/2022	1	40		-13.0	-24.1
18/07/2022	2		-9.4		
09/08/2022	2	22		-7.8	-17.2
09/08/2022	3		-8.9		
05/09/2022	3	27		3.5	-5.4
05/09/2022	4		-16.6		
16/09/2022	4	11		3.7	-12.9
16/09/2022	5		-1.8		
17/10/2022	5	31		-23.5	-25.2
17/10/2022	6		-0.7		
25/10/2022	6	8		-14.3	-15.1
25/10/2022	7		-13.8		
07/11/2022	7	13		-6.5	-20.3
07/11/2022	8		-12.8		
15/11/2022	8	8		-3.4	-16.1
15/11/2022	9		-32.4		
29/11/2022	9	14		13.4	-19.0
29/11/2022	10		-17.9		
14/12/2022	10	15		1.1	-16.8
15/12/2022	11		-8.1		
30/12/2022	11	15		-10.4	-18.5
30/12/2022	12		-12.7		
20/01/2023	12	21		-12.2	-25.0
20/01/2023	13		-5.2		
3/02/2023	13	14		-20.2	-25.3
3/02/2023	14		-16.0		
2/03/2023	14	27		-12.2	-28.2
Mean			**-12.0**	**-7.3**	**-19.2**

[A]Losses arithmetically calculated as difference between dates in 'As received pellet sample' concentrations (mg/kg)

The co-efficient of variation for 3-NOP concentration in the received pellets of version 1 (batch 1 to 5, June to October 2022) was 5.58%. In contrast, the co-efficient of variation for 3-NOP concentration in the received pellets of version 2 (batch 6 to 14, October 2022 to study end) was 11.48%. The version 2 CV is greater than the desired maximum manufacturing CV of 10%. It is not clear what caused the increase in CV between version 1 and 2 pellet formulations.

A second pellet formulation was required due to 3-NOP activity loss associated with pellet manufacture. Analysis of initial pellet samples (up to batch 5) showed an average 3-NOP activity loss of 9.6%. This increased to an average loss of 13.3% for the second pellet version. The overall 3-NOP activity loss associated with pelleting was 12.0% as described in Table 6. Bampidis et al. (2021) reported a 11% loss of 3-NOP activity after pelleting at 80°C. In this study, pellets were manufactured at night at 60°C and composed of ingredients such as wheat to promote pellet integrity and vegetable oil (Table 5) to reduce the heat from friction as the meal is extruded through the pellet die. Despite modifying the pelleting process and increasing the 3-NOP concentration, 3-NOP activity loss remained, indicating the volatile nature of 3-NOP and its susceptibility to degradation, particularly when stored in a commercial feedlot facility.

The concentration of 3-NOP within the pellet is described in Figure 7. The second pellet formulation with the higher 3-NOP concentration can be seen starting from the 6th sampling period. The loss of 3-NOP can be seen in most sampling periods (1, 2, 5 to 8, 11 to 14). The rate and extent of loss varies across sampling periods and appears to increase from sampling period 5 compared to the first 4 sampling periods. Factors influencing extent of loss may relate to both variation in pellet manufacture and sampling methodology. On four sampling periods (3, 4, 9, 10), 3-NOP concentration increases. This effect is likely related to changes in pellet dry matter. As pellet dry matter decreases, 3-NOP concentration will decrease. Changes in pellet dry matter can occur following manufacture and from rainfall events during storage.



[A]Pellet batch 1 start date = PB1, Pellet batch 2 start date = PB2, etc. to PB14.

Figure 7. Change in as-fed 3-NOP concentration (As-Fed basis) at manufacture and storage of the pellet for each sampling period.

Pellet storage duration at the feedlot also resulted in 3-NOP activity loss. Pellets were stored in a walk-through ingredient bay (no back wall) within the commodity shed. Although pellets were protected from direct sunlight and rainfall including covering with a plastic sheet, they were exposed to changes in air temperature and humidity. On limited occasions, pellets were exposed to moisture associated with rainfall events and were observed to lose pellet integrity completely. There was no relationship between storage period and 3-NOP loss. The average storage loss for the entire sampling period was 19.2%. Bampidis et al. (2021) reported a 16.7% loss of 3-NOP when pellets were stored for three months at 50% relative humidity and 25°C air temperature. This highlights that 3-NOP needs to be provided in a more stable and robust form for commercial feedlot use.

The target feeding rate of 3-NOP was 100mg/kg DM ration. The mean 3-NOP intake data which includes the activity losses associated with manufacture and storage is presented in three formats including treatment, block and weigh period (Table 7), days on feed (DOF) periods (Table 8) and by phase (Table 9). All tables present 3-NOP intake from day 22, the day in which the 3-NOP pellet was first included in the finisher ration. The overall average 3-NOP intake for the feeding period (DOF 22 to exit) was 86.278 + 3.699 SD mg/kg DM ration.

Tables 7,8 and 9 collectively highlight that the target 3-NOP intake of 100mg/kg DM was not achieved consistently across the study period, but increased and approached the daily intake target when the version 2 pellet (table 5) was introduced on 17/10/2022 (outlined in Appendix 10.1). The phase 1 blocks (1 to 8) were fed the version 2 pellet for less than a third of their time on feed. In contrast, phase 2 blocks (9 to 16) received the version 2 pellet from 50 to 90% of the time on feed (Appendix 10.1). Table 7 and table 9 shows the increasing 3-NOP intake associated with blocks fed the version 2 pellet for a greater proportion of their feeding time.

Table 7. Calculated 3-NOP intake (mg/kg DM) for treatment blocks over key project periods.

Block	**Lot**	**DOF 22 to DOF 70[A] 3-NOP (mg/kg DM)**	**DOF 70 to DOF 140 3-NOP (mg/kg DM)**	**DOF 22 to DOF 140 3-NOP (mg/kg DM)**
1	22 CT23	75.27	80.44	78.40
2	22 CT24	76.00	83.27	80.44
3	22 CT25	77.15	84.73	81.70
4	22 CT26	79.17	86.17	83.35
5	22 CT27	80.04	85.39	83.18
6	22 CT28	78.90	85.78	82.93
7	22 CT29	80.56	85.53	83.49
8	22 CT30	81.14	86.59	84.29
9	22 CT34	83.33	90.92	87.73
10	22 CT35	85.85	90.78	88.68
11	22 CT36	87.15	89.53	88.58
12	22 CT37	87.79	90.43	89.34
13	22 CT38	89.95	91.26	90.72
14	22 CT39	92.03	92.22	92.14
15	22 CT40	91.04	92.10	91.66
16	22 CT41	89.20	91.34	90.45
Overall				
Mean		83.41	87.90	86.07
SD		5.61	3.60	4.32
SEM		1.40	0.90	1.08
Minimum		75.27	80.44	78.40
Maximum		92.03	92.22	92.14

[A]3-NOP was fed to all treatment Lots (pens) in the diet from DOF 22 onward.

The inability to achieve the target 3-NOP intake of 100 mg kg DM for phase 1 blocks was a combination of the initial lower 90mg 3-NOP/kg DM pellet until 17/10/2022 and the loss of activity associated with pellet manufacture and storage.

Additional 3-NOP loss has been reported with feed delivery and time held in the feed bunk (Alemu et al., 2021). In this study, potential sources of additional 3-NOP loss include exposure to heat generated by steam flaked grain during feed batching, and rainfall degrading pellets within delivered feed. Temperature of the delivered ration was assessed across 63 samples across 7 days (13th to 19th January, 2023) using a Wurth digital temperature gun at a ration bunk depth of 20cm. Readings occurred within 30 minutes after delivery. Sampling provided a mean temperature of 51.5 $\pm$0.30°C. With pellet integrity intact, this temperature level is not sufficient to cause further 3-NOP loss (Bampidis et al. 2021). However, as observed with activity loss associated with pelleting and storage, exposure to rainfall and structural loss of the pellet could further contribute to a decrease in 3-NOP concentration. The actual loss of 3-NOP within delivered feed is unknown as mixed ration samples from 3-NOP treatment bunks were not sampled or assessed.

A slight increase of 3-NOP intake was recorded over different feeding periods (Table 8) associated with use of the version 2 pellet.

Table 8. Calculated 3-NOP intake (mg/kg DM) for days on feed (DOF) periods.

	3-NOP intake (mg/kg DM)			
DOF	**22 to 49**	**50 to 77**	**78 to 105**	**106 to 133**
Mean	82.26	85.09	86.33	89.71
SD	7.17	5.54	5.92	4.55
SEM	1.79	1.39	1.48	1.14
Minimum	72.66	78.64	77.91	78.98
Maximum	96.56	94.26	95.02	94.86

The comparison in calculated mean 3-NOP intakes between the two project phases are shown in Table 9. Given the design of the project where blocks were inducted and allocated over two distinct periods of time (Phases), most of the blocks in Phase 2 were fed the higher formulated concentration 110 mg 3-NOP/kg DM pellet. Consequently, the calculated mean 3-NOP intakes for the 22 to DOF 70, 70 to DOF 140 and 22 to 140 DOF (overall) were higher in Phase 2.

Table 9. Calculated 3-NOP intake (mg/kg DM) by phase over key project periods.

	3-NOP intake (mg/kg DM)		
Phase 1	**DOF 22 to DOF 70[A]**	**DOF 70 to DOF 140**	**DOF 22 to DOF 140**
Mean	78.53	84.74	82.22
SD	2.16	2.01	1.95
SEM	0.76	0.71	0.69
Minimum	75.27	80.44	78.40
Maximum	81.14	86.59	84.29
Phase 2			
Mean	88.29	91.07	89.91
SD	2.86	0.88	1.57
SEM	1.01	0.31	0.56
Minimum	83.33	89.53	87.73
Maximum	92.03	92.22	92.14

[A]3-NOP was fed to all treatment Lots (pens) in the diet from DOF 22 onward.

4.2.2 Treatment rations.

As described in subsection 3.2.6 Diets and feeding, there were four ration changes during the study period. Changes were required due to ingredient availability, a change to 3-NOP pellet formulation concentration and inclusion of betaine to coincide with the summer feeding period.

Ration samples of the control and 3-NOP treatment finishers were collected monthly for nutrient analysis (Appendix 10.8). Nutrient values from collected finisher samples were comparative to theoretical values as described in Table 10.

Table 10. Comparison of formulated (FM) treatment ration nutrient values with analytical (AL) nutrient values (DM basis)

	3-NOP treatment finisher diets							
	16/05/22		**07/11/22**		**14/12/22**		**24/01/23**	
	FM	**AL**	**FM**	**AL**	**FM**	**AL**	**FM**	**AL**
Dry matter %	76.8	76.9	77.8	77.3	77.3	77.8	77.4	79.3
Crude protein %	13.8	14.4	13.6	13.0	13.5	12.2	14.3	14.4
Fat %	6.5	6.7	6.3	6.3	6.0	5.8	5.3	5.6
Ash %		4.7		4.5		4.3		4.3
NDF %	22.5	21.9	23.1	17.8	21.0	18.0	20.4	19.1
P %	3.7	3.7	3.7	3.6				
	Control treatment finisher diets							
	FM	**AL**	**FM**	**AL**	**FM**	**AL**	**FM**	**AL**
Dry matter %	76.6	76.2	77.8	77.9	77.3	76.9	77.2	79.3
Crude protein %	13.7	13.9	13.6	13.1	13.4	12.3	14.3	14.1
Fat %	6.4	6.0	6.3	5.9	6.0	6.0	5.3	5.6
Ash %		4.5		4.4		4.0		4.4
NDF %	22.5	22.0	23.1	18.9	21.0	17.5	20.4	20.0
P %	3.6	3.9	3.6	3.5				

A characteristic of the treatment diets was the 5 to 6% dietary fat (DM basis) derived from the addition of vegetable oil and oil contained in whole cottonseed. Zhang et al., 2021 showed that 3-NOP (200mg/kg DM) and 5% canola oil had an independent effect on reducing methane production. Fat addition has shown to interfere with methanogen function and protozoa activity (Beauchemin et al., 2008; Zhang et al., 2021) Although methane production was not assessed in this project, dietary fat levels were sufficient to reduce methane production further than using 3-NOP alone.

Treatment diets were formulated to achieve the same energy levels throughout the study period. On a dry matter basis, energy levels included 13.82 MJ/kg ME, 1.48 Mcal/kg NEg and 2.15 Mcal/kg NEm.

4.3 Animal performance

4.3.1 Dry matter feed intake.

There was no difference in dry matter intake (DMI) for the main effect of treatment (P>0.05) for the weigh periods as indicated in Table 11. DMI's were higher in Phase 1 over both the DOF 70 to DOF 140 period (P<0.0001) and the Induction to DOF 140 period (P<0.001). A treatment x phase interaction (P < 0.01) however was present for DMI from DOF 70 to DOF 140.

Analysis of treatment within phase means reported a 2.1% decrease in DMI (0.3 kg/d) for cattle fed 3-NOP during Phase 1, but no effect in Phase 2. Dry matter intake of cattle during the latter half of Phase 1 was on average 1.36 kg/d greater than Phase 2 (*P* < 0.01). Phase 1 also coincided with rainfall events. Further investigation is required to determine if the decreases in feed intake for the 3-NOP treatment during this phase is the result of wet weather and/or high dry matter feed intakes. Overall average daily DMI from DOF 22 to Exit for Control and 3-NOP was 12.749 + 0.532SD and 12.622 + 0.407kg/hd/d respectively. Average daily DMI from 22DOF to exit for Control and 3-NOP was 13.162 + 0.722SD and 13.024 + 0.572SD kg/hd/d.

Previous research has reported 3-NOP supplementation can decrease feedlot finisher feed intake when provided at 100 to 200mg/kg DM (Vyas et al, 2016). A number of small pen finisher studies however, reported no effect (Pedrini et al. 2023; Araujo et al. 2023) or variable effects of 3-NOP on DMI (Alemu et al. 2021). Further research is required under large pen replicated studies such as those conducted under this study to appropriately determine the effects of dietary 3-NOP concentrations on feed intake. One possible theory on effects of 3-NOP on feed intake may relate to volatile fatty acid production and/or their ratios in the methane inhibited rumen. Alemu et al. (2021) reported 4.9 fold increases in rumen hydrogen concentrations associated with feeding 3-NOP at 100mg/kg DM. Ungerfeld et al. (2022) hypothesized that inhibition of methanogenesis results in an decreased acetate:propionate ratio which may have effects on satiety and feed intake.

Table 11. Effect of treatment, project phase and the treatment within phase effect on steer dry matter feed intake.

	Dry matter feed intake (kg/hd/d)		
	Induction (I) to DOF 70 (reimplant)	**DOF 70 (reimplant) to DOF 140[C] (tag check)**	**I to DOF 140 (tag check)**
Treatment			
Control	12.23	13.37	12.80
3-NOP	12.15	13.28	12.71
SE[A]	0.09	0.12	0.08
p-value[B]	0.3936	0.1687	0.2265
Phase			
1	12.20	14.01	13.09
2	12.19	12.65	12.42
SE	0.10	0.16	0.11
p-value	0.9726	0.0000****	0.0005***
Treatment x Phase			
Overall p-value	0.2587	0.0080**	0.5422
Control – Phase 1	12.18	14.16	13.15
3-NOP – Phase 1	12.21	13.86	13.03
SE	0.12	0.17	0.12
p-value	0.8366	0.0063**	0.2027
Control – Phase 2	12.29	12.59	12.44
3-NOP – Phase 2	12.09	12.70	12.39
SE	0.12	0.17	0.12
p-value	0.1677	0.2658	0.6576

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[c] Tag check liveweight conducted on average at 139 days on feed. Dispatch occurred on average at 151.5 days on feed.

4.3.2 Dry matter intake data by period

Analysis of feed intake by period (Table 12) reported a 2.91% decrease in DMI for cattle fed 3-NOP between DOF 50 to DOF 77 (P<0.05) while there was no difference between the treatments for the remaining feeding periods (P>0.05). Finisher period DMI was in general greater(P<0.0001) in Phase 1 than Phase 2 as previously discussed. In contrast to Table 11, no treatment x phase interactions were reported.

Table 12. Effect of treatment, project phase and the treatment within phase effect on steer dry matter intake over specific periods.

	Dry matter feed intake (kg/hd/d)				
	Induction to DOF 21	**DOF 22 to DOF 49**	**DOF 50 to DOF 77d**	**DOF 78 to DOF 105**	**DOF 106 to DOF 133**
Treatment					
Control	10.20	12.70	13.70	13.60	13.20
3-NOP	10.20	12.80	13.30	13.60	13.20
SE[A]	0.14	0.14	0.14	0.14	0.14
p-value[B]	0.8063	0.6339	0.0420*	0.7730	0.8890
Phase					
1	9.58	12.81	14.02	14.29	13.95
2	10.82	12.66	12.94	12.96	12.51
SE	0.15	0.15	0.15	0.15	0.15
p-value	0.0000****	0.4752	0.0000****	0.0000****	0.0000****
Treatment x Phase					
Overall p-value	0.9737	0.3176	0.9929	0.2050	0.4113
Control – Phase 1	9.60	12.68	14.20	14.43	14.01
3-NOP – Phase 1	9.55	12.94	13.83	14.15	13.89
SE	0.19	0.19	0.19	0.19	0.19
p-value	0.8441	0.2972	0.1468	0.2713	0.6293
Control – Phase 2	10.84	12.71	13.12	12.87	12.43
3-NOP – Phase 2	10.80	12.61	12.76	13.05	12.60
SE	0.19	0.19	0.19	0.19	0.19
p-value	0.8807	0.7107	0.1503	0.4875	0.4967

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001

4.3.3 Live animal performance

Similar to DMI, there was no effect of 3-NOP on body weight or average daily gain (ADG) to reimplant (DOF 70). Treatment differences were recorded in the live weight at final tag check (DOF 140) (P<0.001) and I to DOF 140 average daily gain (P<0.001) as shown in Table 13. The numerical difference was 5.2 kg and 0.031 kg/hd/d respectively.

The project phase influenced final tag check (DOF 140) liveweight (P<0.001) with Phase 1 recording higher values; influenced ADG for the I to DOF 70 reimplant (P<0.01), reimplant to final tag check (P<0.05) and overall period (P<0.001) (Table 13). As described in subsection 4.3.1, the greater DMI recorded in Phase 1 has been reflected in the greater final tag check (DOF 140) liveweight and daily gain levels for all weigh periods of that phase. A trend for a treatment x phase interaction for overall ADG (P = 0.0968) was detected.
Analysis of within phase means reported a 1.89% decrease in ADG for Phase 1 for cattle fed 3-NOP (P < 0.001), however no statistical difference (P > 0.05) was observed in Phase 2 for both ADG and DOF 140 liveweight. The decreases in feed intake in the latter period of Phase 1 for cattle fed 3-NOP may have contributed to the lower ADG observed.

Table 13. Effect of treatment, project phase and the treatment within phase effect on steer liveweight performance session.

	Parameter					
				Average daily gain (kg/hd/d)		
	Induction (I) liveweight (kg)	**DOF 70 Reimplant liveweight (kg)**	**DOF 140 Tag check liveweight(kg)[C]**	**I-DOF 70**	**DOF 70 to DOF 140**	**I to DOF 140**
Treatment						
Control	452.1	636.9	774.2	2.612	1.953	2.287
3-NOP	451.5	634.4	769.0	2.584	1.925	2.256
SE[A]	2.44	4.08	3.95	0.063	0.053	0.033
p-value[B]	0.3477	0.2832	0.0002***	0.3032	0.3253	0.0001***
Phase						
1	446.6	642.0	786.8	2.761	2.069	2.416
2	456.9	629.3	756.4	2.435	1.808	2.127
SE	3.42	5.53	5.50	0.087	0.073	0.047
p-value	0.0341*	0.1047	0.0001***	0.0083**	0.0109*	0.0000****
Treatment x Phase						
Overall p-value	0.8593	0.9778	0.1826	0.9436	0.2761	0.0968*
Control – Phase 1	446.9	643.3	790.4	2.776	2.100	2.439
3-NOP – Phase 1	446.4	640.7	783.2	2.746	2.039	2.393
SE	3.45	5.76	5.58	0.089	0.075	0.047
p-value	0.5898	0.4336	0.0002***	0.4335	0.1317	0.0000****
Control – Phase 2	457.3	630.5	758.1	2.448	1.806	2.135
3-NOP – Phase 2	456.6	628.1	754.8	2.423	1.811	2.118
SE	3.45	5.78	5.58	0.089	0.075	0.047
p-value	0.4309	0.4624	0.0965	0.5006	0.9167	0.1254

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[C]Final tag check liveweight conducted on average at 140 days on feed. Dispatch occurred on average at 151.5 days on feed.

4.3.4 Feed conversion

There was no difference in feed conversion ratio (FCR) ratio between the treatments (P>0.05) for the measurement periods as shown in Table 14.

Project phase influenced feed to gain ratio from I to DOF 70 reimplant (P<0.05) and the overall period (P<0.01) with Phase 1 recording lower values (Table 14). This result reflects the higher dry matter intakes, increased energy intake in excess of maintenance requirements and higher average daily gains recorded in Phase 1. As discussed previously, the project phase effect was associated with different environmental conditions. Phase 1 was characterised by cool and wet conditions and Phase 2 by hot and dry conditions.

There were no significant differences (P>0.05) recorded between treatments in either phase.

Differences in feed efficiency were related to the environmental differences experienced by cattle during Phase 1 and 2. Australian summer feeding conditions are known to depress dry matter intake and performance of feedlot cattle (Gaughan et al., 2008).

The effects of 3-NOP on average daily gain in this study were variable, either equivalent to DOF 70 reimplant on all cattle, and in the latter half of the feeding period either slightly negative or equivalent for Phase 1 and 2, respectively. Additionally, feed conversion did not differ, and hence 3-NOP at the current dosage in this study did not improve feedlot cost of gain. This contrasts with a common assumption associated with methane mitigation that the energy loss from methane production (Johnson and Johnson, 1995) is re-directed into productive fermentation pathways (Ungerfeld., 2015), increasing animal production.

Table 14. Effect of treatment, project phase and the treatment within phase effect on steer feed conversion ratio (dry matter feed intake: average daily gain)

	Feed conversion ratio (FCR)		
	Induction (I) to DOF 70 (reimplant)	**DOF 70 (reimplant) to DOF 140[C] (final tag check[c])**	**I to DOF 140 (final tag check)**
Treatment			
Control	4.75	6.93	5.62
3-NOP	4.75	6.96	5.66
SE[A]	0.11	0.17	0.06
p-value[B]	0.9554	0.8224	0.2810
Phase			
1	4.43	6.78	5.43
2	5.06	7.10	5.85
SE	0.16	0.22	0.08
p-value	0.0132*	0.3310	0.0021**
Treatment x Phase			
Overall p-value	0.1792	0.9907	0.6867
Control – Phase 1	4.40	6.77	5.40
3-NOP – Phase 1	4.47	6.80	5.45
SE	0.16	0.24	0.08
p-value	0.3791	0.8803	0.2966
Control – Phase 2	5.09	7.09	5.84
3-NOP – Phase 2	5.03	7.12	5.86
SE	0.16	0.24	0.08
p-value	0.4217	0.8674	0.6237

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[C]Final tag check liveweight conducted on average at 140 days on feed. Dispatch occurred on average at 151.5 days on feed.

Feedlot performance data analysed from DOF 0 to exit (average day 151.5) is included in Appendix 10.16, but overall trends in feedlot cattle performance are consistent with the findings presented above.

Currently, global research focus is shifting to understanding metabolic hydrogen flows in the methane inhibited rumen (Pereira et al., 2022; Ungerfeld, 2022). The hydrogen utilising pathways to capture available hydrogen and prevent accumulation does not have sufficient capacity when methane production is inhibited. Enhancing the capacity of these already existing and natural pathways may provide an efficient and cost-effective method of avoiding ruminal hydrogen accumulation and associated effects on animal performance and carcass characteristics. Possible methodologies for dissolved dihydrogen capture may include decreasing the dose rate of 3-NOP to decrease hydrogen load and maximise opportunities for hydrogen capture in alternative sink pathways (Almeida et al. 2023), increase VFA production (Ungerfeld, 2015), supplementation of unsaturated fat sources (Zhang et al. 2021) and the possibility of hydrogen utilising bacteria establishing and competing with methanogens (Ungerfeld et al., 2015; Pereira et al., 2022; Nollet et al. 1997; Van et al. 1998; Lopez et al. 1999;; Karekar and Ahring, 2023) when the methanogens' activity is inhibited by the presence of 3-NOP.

Reducing 3-NOP feeding level may provide a possible potential for the rumen to effectively dispose of increasing hydrogen availability with methane inhibition. Almeida et al. (2023) reported excellent efficacy of lower doses of 3-NOP in finisher diets, with methane yields decreased by 65.5 to 87.6 % for 50 and 100 mg/kg DM 3-NOP, respectively, in diets with 6.66% DM ether extract.

The dietary addition of unsaturated vegetable oils can also influence hydrogen utilization pathways. Zhang et al. (2021) fed 200 mg/kg DM 3-NOP in barley-silage based growing diets with or without the addition of 5% DM dietary canola oil (to diets with 3.24% basal ether extract). The addition of unsaturated vegetable oil in 3-NOP diets decreased hydrogen emission yields by 36.8%, however follow up microbiome analysis by Gruninger et al. (2022) of the Zhang et al. (2021) rumen samples reported a decrease in the concentration of fibre digesting bacteria and protozoa. Continued research on the interaction between fat and 3-NOP is required given finisher diets typically have lower roughage concentrations that those examined by Zhang al. 2021.

Acetogens are naturally occurring rumen bacteria which can utilise hydrogen to produce acetate (Ungerfeld, 2015). However, when methanogens are present, acetogens cannot rely on this pathway as they cannot compete for hydrogen at low concentrations (Lopez et al., 1999; Ungerfeld, 2015; Pereira et al., 2022) while the acetate pathway is less energetically efficient compared to methane (Cottle et al., 2011). However, when methanogens are inhibited, rumen hydrogen concentration increases which enables greater acetogen activity and capture of hydrogen. Additional possibilities include sourcing acetogens from other species such as kangaroos (Kareker & Ahring, 2023). Promisingly, several studies have demonstrated that when 2-bromoethanesulfonic acid (BES; which inhibits the same enzyme as 3-NOP involved in the last step of methanogenesis) is fed in combination with reductive acetogen strains, then acetate production, a major energy source for beef cattle increases in the rumen. However, further research and development on culture, stabilisation and retention of these strictly anaerobic bacteria is required to enable their successful commercialisation.

4.4 Animal health

There was no effect (all P>0.05) of treatment on any health parameters for the duration of the study as shown in Table 15. Previous feeding studies have not reported a health risk associated with use of 3-NOP. Characteristics of the 3-NOP compound which reduce adverse effects to the rumen environment and animal health include its specific mode of action affecting only methanogens through the binding of the methyl co-enzyme M reductase enzyme (Duin et al., 2018) and no reported adverse effect on common rumen bacteria (Duin et al., 2018) or protozoa (Haisan et al., 2014). The 3-NOP compound undergoes rapid degradation within the rumen with nearly 50% loss 15 hours post feeding and complete degradation within 15 hours after feeding (Duin et al., 2016) as described by Figure 1. Pathways of degradation include metabolism either in the liver or washed out as part of rumen turn-over or degraded within the rumen to nitrate, nitrite and eventually 1,3-propanediol (Duin et al., 2016; Alemu et al., 2021).

Table 15. Health status categories of steers over the project period by treatment; presented as numbers of steers, and percentages of total inducted steers per category. Chi-square analysis, 1 df.

Parameter	Control	3-NOP	Total	P-value[A]
Steers inducted (n)	3997	4000	7997	
Morbidity (Hospital pulls)[B] (n)	445	419	864	
(%)	11.13	10.48	10.80	0.3395
Mortalities (n)	38	35	73	
(%)	0.95	0.88	0.91	0.7212
Hospital culls (n)	70	69	139	
(%)	1.75	1.73	1.74	0.9279
Buller culls (n)	87	85	172	
(%)	2.18	2.13	2.15	0.8726
Project culls (n)	36	30	66	
(%)	0.90	0.75	0.83	0.4531
Rejects (n)	231	219	450	
(%)	5.78	5.48	5.63	0.5519

[A]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001

[B]A comprehensive definition of each cull category is included in Section 3.4.1, Analysis of cull steer data.

4.5 Carcass characteristics

4.5.1 Slaughter data

The assessment of detention at slaughter indicated the project steers were youthful in nature with 51.8% having no permanent incisors, 42.6% with 2 permanent incisors, 5.2% with 4 permanent incisors and 0.4% with 6 permanent incisors.

The Control treatment carcasses were heavier (P<0.01) than the 3-NOP treatment carcasses, the difference being 2.6 kg (Table 16).

The carcasses from Phase 1 were heavier (P<0.0001) at slaughter than the carcasses from Phase 2 (Table 16). The numerical differences were 17.8 kg HSCW. A trend of a treatment x phase interaction was present for hot standard carcass weight (HSCW; $P = 0.0674$).

Analysis of within phase means observed a 4.2 kg decrease in HSCW ($P < 0.001$) for cattle fed 3-NOP in Phase 1, but no difference in HSCW between the treatments in Phase 2. These differences in HSCW, were similar to the effects on overall feeding period ADG mentioned above.

Table 16. Effect of treatment, project phase and the treatment within phase effect on steer carcass attributes.

	HSCW (kg/carcass)
Treatment	
Control	438.5
3-NOP	435.9
SE[A]	1.65
p-value[B]	0.0016**
Phase	
1	446.1
2	428.3
SE	2.26
p-value	0.0000****
Treatment x Phase	
Overall p-value	0.0676
Control – Phase 1	448.2
3-NOP – Phase 1	444.0
SE	2.33
p-value	0.0003***
Control – Phase 2	428.8
3-NOP – Phase 2	427.8
SE	2.33
p-value	0.4119

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001

Given the slightly negative (Phase 1) or neutral (Phase 2) effects of 3-NOP on HSCW in this study, further research is required to understand energetics in the methane inhibited rumen, or if wet weather and/or high feed intakes in Phase 1 (and corresponding high intake of 3-NOP), influenced carcass weight accretion of cattle fed 3-NOP.

4.5.2 Carcass fatness

There was no difference between the treatments (P>0.05) in any of the carcass fatness parameters as shown in Table 17.

Project phase influenced steer carcass subcutaneous fat depth at both the P8 site and the rib site with Phase 1 carcasses having greater subcutaneous fat depth at the P8 site (P<0.0001), but lesser fat depth at the rib site (P<0.01).. Marbling score was not affected by project phase (P>0.05). The AUS·MEAT Marbling system provides an indication of the amount of marbling fat at the rib eye muscle assessment site, while the MSA marbling system provides an additional indication of the marbling fat distribution, and the size of the marbling fat deposits in the rib eye muscle at the assessment site.

The Control treatment carcasses recorded a greater fat depth at the P8 site (P<0.05) in Phase 1. There were no differences between treatments in either phase in the balance of the remaining carcass fatness parameters (P>0.05). Treatment differences in **AUS·MEAT** marbling score approached significance (P>0.05) in Phase 1.

Table 17. Effect of treatment, project phase and the treatment within phase effect on steer carcass attributes – carcass fatness

	P8 fat depth (hot) (mm)	**Rib fat depth (cold) (mm)**	**AUS·MEAT[C] marbling score**	**MSA-US[D] marbling score**
<u>Treatment</u>				
Control	15.3	9.9	1.95	431.0
3-NOP	15.1	9.8	1.91	425.6
SE[A]	0.4	0.3	0.03	3.0
p-value[B]	0.1292	0.6298	0.1721	0.0825
<u>Phase</u>				
1	17.0	9.1	1.93	428.3
2	13.3	10.5	1.93	428.2
SE	0.5	0.4	0.03	3.6
p-value	0.0000****	0.0088**	0.9307	0.9897
<u>Treatment x Phase</u>				
Overall p-value	0.2313	0.2789	0.1998	0.4377
Control – Phase 1	17.2	9.3	1.96	432.3
3-NOP – Phase 1	16.8	8.9	1.90	424.3
SE	0.5	0.4	0.04	4.2
p-value	0.0490*	0.2525	0.0537	0.0712
Control – Phase 2	13.4	10.4	1.93	429.7
3-NOP – Phase 2	13.3	10.6	1.93	426.8
SE	0.5	0.4	0.04	4.3
p-value	0.8514	0.6511	0.9883	0.5099

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[C]AUS·MEAT marbling score – score over a range of 0 to 9.
[D]MSA-US marbling score – numerical marbling score over a range of 100 to 1190 in increments of 10 units.

4.5.3 Chiller assessment data

The carcass side chillers used for the post slaughter chilling period are monitored routinely for numerous ‘within’ chiller temperature profiles and random carcass side internal muscle temperature by the collaborating abattoir. This routine protocol was followed for all the project slaughter occasions. An example of the collaborating abattoir’s carcass side chiller temperature cycle from Block 11 is shown in Appendix 10.15.

Measurement of eye muscle area, carcass maturity and carcass quality attributes that were assessed following carcass side chilling are shown in Table 18.

The magnitude of the ossification scores indicates the youthful nature of the project carcasses. Ossification is a measure of the physiological maturity of an animal. The lower the score, the better the eating quality outcome.

For the project steer population, the subjective meat colour score data suggests meat of a desirable bright cherry red colour; the subjective intermuscular fat colour data suggests a desirable white coloured fat; and the low pH values indicating a potentially good eating quality product.

There were no differences between the treatments (Table 18) in any of the measured/assessed post chilling parameters (all P>0.05). Treatment differences approach significance for eye muscle area (P=0.0697) and MSA Index (P=0.0673).

Project phase influenced steer carcass intermuscular fat colour scores (P<0.01) at the rib eye muscle assessment site with Phase 2 carcasses recording a higher score than Phase 1 carcasses (Table 18). Although significantly different, this relates in commercial relevance to the Phase 1 carcasses having only a marginally lighter intermuscular fat colour. There was no difference in any of the other the measured/assessed post chilling parameters (P>0.05) between the project phases, however phase differences approach significance (P=0.0671) for meat colour score.

A trend for a treatment x phase interaction (*P* = 0.0828) was observed for MSA Index.

Analysis of within phase means reported the Control treatment recorded a higher MSA Index (P<0.01) than the 3-NOP treatment in Phase 1 (Table 18), the numerical increase in magnitude being 0.3 which is unlikely to have influenced potential eating quality of the Control treatment carcasses. The MSA index represents the potential eating quality of a MSA compliant carcass. Overall, the result of this study indicates average MSA Index scores across the project's steer population.

Table 18. Effect of treatment, project phase and the treatment within phase effect on steer carcass attributes - Chiller assessment

	Eye muscle area (cm^2)	**Meat[C] Colour score**	**pH_{18}[D]**	**Intermuscular[E] fat colour score**	**Ossification[F] score**	**MSA Index[G]**
Treatment						
Control	81.8	0.73	5.52	0.24	202.3	56.5
3-NOP	80.8	0.71	5.52	0.25	201.4	56.4
SE[A]	0.5	0.02	0.01	0.03	0.6	0.1
p-value[B]	0.0697	0.1553	0.2040	0.8019	0.0797	0.0673
Phase						
1	81.4	0.75	5.53	0.18	201.1	56.6
2	81.2	0.69	5.51	0.32	202.6	56.4
SE	0.6	0.02	0.01	0.03	0.8	0.1
p-value	0.7504	0.0671	0.1170	0.0020**	0.1918	0.2702
Treatment x Phase						
Overall p-value	0.8619	0.3363	0.7761	0.6356	0.7365	0.0828
Control – Phase 1	81.9	0.76	5.53	0.17	201.5	56.7
3-NOP – Phase 1	80.9	0.75	5.53	0.19	200.7	56.4
SE	0.7	0.03	0.01	0.04	0.9	0.1
p-value	0.2438	0.7617	0.4852	0.6007	0.3157	0.0085**
Control – Phase 2	81.7	0.71	5.51	0.32	203.2	56.4
3-NOP – Phase 2	80.6	0.67	5.51	0.31	202.1	56.4
SE	0.7	0.03	0.01	0.04	0.9	0.1
p-value	0.1622	0.0897	0.2730	0.8698	0.1411	0.9785

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[C]Meat colour score was coded as either 1B and 1C (roughly 27%) to equal 0, or 2 and 3 (roughly 73%) to equal 1.

[D]pH_{18} =pH measured at 18 hours post slaughter
[E]Fat colour score data were recoded as colour=0 (roughly 75%) or 1 otherwise.
[F]Ossification score. A numerical score ranging from 100 to 590 in increments of 10.
[G]MSA Index. This index is a numerical value, from one (lowest) to 100 (highest).

Within this study and the operational and environmental characteristics of the feedlot, we can at least conclude no positive productivity, carcass trait or efficiency gains were observed due to the inclusion of Bovaer® 10.

4.6 Modelling of methane emission reduction using Bovaer10®

The effect of 3-NOP utilisation on the carbon footprint of Australian feedlot cattle is reported in detail in Appendix 10.17. 'Carbon Baseline Assessment on the effect of Bovaer® feed supplement on the carbon footprint of trial cattle in MLA Project P.PSH.1375'.

4.7 Estimation of the cost benefit of Bovaer10® to feedlot operations

An estimate of the cost benefit of feeding Bovaer10® to feedlot operations is outlined under the following three scenarios.

4.7.1 Cost of feeding Bovear®10

A Sensitivity analysis was conducted across a range of 3-NOP costs ranging from 5 to 40 c/head/d.

Four scenarios to cover the cost of feeding 3-NOP were compared below:

- Breakeven carcass sales premium assuming no performance loss;
- Breakeven carcass sales premium assuming 2.6 kg HSCW loss/carcass with a 5-year average HSCW of $7.40 per kilogram;
- Breakeven carbon price for VERRA credits assuming no performance loss;
- Breakeven carbon price for VERRA credits assuming 4.2 kg HSCW loss/carcass.

4.7.2 Carcass value required to offset cost of Bovear10®

If value is derived from carcass weight premiums, breakeven prices per kg of HSCW of $0.017, $0.034, $0.069, $0.103, and $0.138 are required at 5, 10, 20, 30 c and 40 c/hd/d Bovaer®10 cost respectively assuming no performance loss at a 5-year average carcass price of $7.40/kg. If a 2.6 kg HCSW loss/carcass was factored, breakeven premiums increase to $0.061, $0.079, $0.113, $0.148, and $0.183/kg respectively.

4.7.3 Assess the ability to monetise emissions reduction using the internationally recognised carbon trading scheme - VERRA.

VERRA is a not for profit corporation which manages a voluntary carbon market program called the Verified Carbon Standard (VCS) program. The VCS is the most widely used greenhouse gas (GHG) auditing program.

The example VERRA project for Bovaer10® is described in Table 19. The project analysis assesses the cost to set up the project, including annual costs and costs that cover the term of the project, as well as the return from selling the VCU's. Based on a 150day feeding program, the cost per head to set up and run VERRA is $4.57. Assuming Bovear10® reduces methane production by 80%, carbon dioxide equivalent emissions is reduced by 336 kg per head.

In this study, using the VERRA example as the cost basis and level of carbon dioxide emission reductions shown in Table 19, the breakeven carbon prices of $36, $59, $104, $148, and $193/t were required at Bovaer®10 cost of 5, 10, 20, 30 and 40 c/hd/d, respectively assuming no performance loss. If a 2.6 kg HSCW loss/carcass at a 5 year average hot-carcass weight of $7.40/kg is factored, breakeven carbon prices increase to $93, $116, $161, $206 and $251/t respectively.

The Australian feedlot industry has greater opportunity to cover the cost of using Bovaer®10 through a carcass premium rather than via improved production efficiency (daily gain, feed efficiency) or carbon value.

Table 19. Monetised emission reduction using VERRA for the 4000 head of Bovaer10® fed cattle.

Cost component	**Frequency**	**Cost $**	**Cost $/head/150days**
Feedlot costs:			
Feedlot administration		$10,000	$1.04
Verification	Annual	$20,000	$2.08
Auditor		$ 2,000	$0.21
Reporting		$ 8,000	$0.83
VERRA project costs:			
Set-up		$20,000	$0.04
Development and submission		$40,000	$0.09
Validation	Project Term	$20,000	$0.04
Monitoring		$100,000	$0.21
Project Fee		$10,000	$0.02
Total			**$4.57**
International carbon value $/$_eCO_2$ t		$40.00	
Feedlot production:			
Grain-fed daily methane production g/hd/day		100	
Days on feed		150	
Grain-fed methane production kg/hd/150days		15	
Bovaer10® methane mitigation %		80%	
Adjusted methane production kg/hd/150days		3	
Methane $_eCO_2$ constant		28	
$_eCO_2$ kg/hd		**336**	

5. Conclusion

Conclusions from P.PSH.1375 Effect of Bovaer on performance, health, carcass characteristics and carbon footprint of Australian feedlot cattle include:

- The active ingredient of Bovaer®, 3-nitroxypropanol (3-NOP) lacked stability when incorporated into a cereal-based pellet formulation used in this project. Sampling after pelleting and storage resulted in variable losses of 3-NOP, reducing the consistency of the concentration at which 3-NOP was supplied;
- This requires investment into developing a more robust method of incorporation into rations, promoting effective and consistent active levels when included in commercial manufacturing feedlot diet equipment. Bovaer® 10 modification must enable addition via stable liquid formats and/or micro-doser, removing the requirement for an additional loading activity into a batch mixer or daily management;
- Bovaer®10 methane mitigation potential was not measured in this project, however based on previously published MLA research (Almedia et al. 2023) and 3-NOP recoveries from supplemental pellets, methane could have been reduced by 66 to 80% in the Bovaer10® treated animals;
- This project provided further evidence that 3-NOP had no effect on the animal health of the project animals in line with the findings of other studies;
- Additional research is required to determine the most effective and profitable inclusion rate, given a lack of performance response in this project. Further research is required at lower dose levels (such as 50 to 75 mg/kg DM) as recent Brazilian research has reported gain responses in cattle fed 75 mg/kg DM 3-NOP vs. 100 mg/kg DM (Perini et al. 2023).
- The response to feeding 3-NOP at the rates in this project was different in the two weather season periods identified and monitored during the project period;
- To improve the effect of Bovaer® on feedlot production but still achieve sufficient methane mitigation, Bovaer® may benefit from being fed in conjunction with a hydrogen utilising compound or probiotic i.e., supplementing cattle with live cultures that can either enhance uptake of hydrogen into fermentation end products such as propionate, lactate, or acetate (acetogen);
- In the absence of a performance benefit, sensitivity analysis to the Bovaer® 10 feed additive cost for both international carbon trading accounts (VERRA) and carcase weight premiums has been reported in this study.
- For wide adoption of Bovaer10® within commercial feedlot operations, a return on investment to feeding the additive will need to be present either through a productivity benefit, carcass weight premium or carbon credit benefit (either singular or in combination).
- Bovaer10® reduces feedlot Scope 1 and 2 carbon emissions by approximately 50%, but emission reduction is only 5% when including carbon emissions of cattle purchased by the feedlot (Scope 3). The challenge is mitigating the carbon emissions associated with cattle purchased from grazing systems for feedlot production, and future research should focus on this area;

5.1 Key findings

- The study provided further evidence that 3-NOP had no effect on the animal health of the project animals.
- The project highlighted responses to the feeding of Bovaer®10 at a recommended formulated rate of 100 mg 3-NOP/kg DM/d of ration consumed differed within the two project phases of the experimental period;

- The two phases while only separated by a 4 week interval provided data on the responses to Bovaer®10 in a southern Queensland commercial feedlot during a winter-spring-early summer period which provided cool and wet conditions known as Phase 1 ; and during a late spring-full summer period which provided hot and dry conditions known as Phase 2.
- A statistically significant treatment x phase interaction was reported for dry matter intake ($P < 0.01$) between reimplant (average 70 DOF) and final tag check (average 140 DOF), which translated to a trend for a treatment x phase interaction for overall HSCW ($P = 0.068$). A treatment within phase analysis was conducted to examine the interactions.
- The treatment within phase data analysis clearly outlined that:
  - There was no effect of Bovaer®10 on animal performance to DOF 70 HGP re-implant.
  - The inclusion of Bovaer®10 resulting in an estimated mean consumption of 82.22 mg 3-NOP/kg DM for the overall feeding period during Phase 1, did not affect dry matter intake yet decreased ADG by 1.9% ($P<0.0001$), decreased carcass weight by 4.2 kg ($P < 0.01$), reduced subcutaneous fat at the P8 site ($P<0.05$) (but did not affect subcutaneous rib fat) and reduced MSA index ($P<0.01$). However, a 2.1% decrease ($P<0.01$) in dry matter intake of cattle in the latter half of the feeding period of this phase. This may have contributed to the lower ADG reported;
  - The inclusion of Bovaer®10 resulting in an estimated mean consumption of 89.91 mg 3-NOP/kg DM for the overall feeding period during Phase 2 had no effect on dry matter intake, animal performance their carcass characteristics, value, and quality.
- Further research is required to understand if decrease in carcass weight in Phase 1 was the result of Bovaer®10 inclusion or other factors including inclusion rate, wet weather and/or the high magnitude of dry matter feed intakes observed.
- Including Bovaer®10 in its current composition within a cereal based pellet does not avoid degradation and loss of the active ingredient, 3-nitrooxypropanol;
- This project demonstrated that Bovaer®10 lacked stability in the supplemental pellet utilized in its delivery for this project. Bovaer®10 had active losses of 12.0% with pelleting and 7.3% during storage, and alternative supplementation mechanisms will need to be identified. Future research to understand energy pathways in the methane inhibited rumen such as hydrogen utilization will be important to develop mechanisms to improve cattle performance, given the absence of performance benefits in this study.
- Using efficacy values from recently completed MLA research study (Almeida et al. 2023) Bovaer®10 reduces feedlot Scope 1 and 2 carbon emissions by more than 50%, but when including Scope 3 (carbon associated with purchased cattle), reduction falls to 5%;
- If value is derived from carcass weight premiums breakeven prices of $0.017, $0.034, $0.069, $0.103, and $0.138/kg are required at 5, 10, 20, 30 c and 40 c/hd/d Bovaer®10 cost, respectively assuming no performance loss at a 5-year average $7.40 carcass price ($/kg). If a 2.6 kg HCSW loss was factored, breakeven premiums increase to $0.061, $0.079, $0.113, $0.148 and $0.183/kgrespectively.
- Bovaer®10 as an intervention strategy is accepted by international carbon trading systems such as VERRA which offers feedlots the opportunity of monetising the reduction of methane. In our study breakeven carbon prices of $36, $59, $194, $148 and $193/t were required at Bovaer®10 cost of 5, 10, 20, 30 and 40 c/hd/d, respectively assuming no performance loss. If a 2.6 kg HSCW loss at a 5 year average hot-carcass weight of $7.40/kg is factored, breakeven carbon prices increase to $93, $116, $161, $206 and $251/t respectively.

5.2 Benefits to industry

With the Australian red meat industry seeking a carbon neutral supply chain by 2030, feed additives which mitigate methane provide an efficient tool to meeting this objective. Research has shown that Bovaer®10 effectively mitigates methane of cattle on feedlot diets by more than 80%.

This project has generated a large-scale commercial dataset, to inform Australian lot feeders and consulting nutritionists on the effect of Bovaer®10 on feed additive stability, performance, health, carcass characteristics and carbon footprint of Australian feedlot cattle.

6. Future research and recommendations

The findings from this project identifies the following further research and recommendations:

- The carrier matrix for Bovaer® 10 and its delivery mechanism requires further research to avoid activity loss associated with commercial feedlot ration manufacture, delivery, and tenure in the feedbunk; The inherent volatility of 3-NOP increases its loss under pelleting and storage as demonstrated in this research project. Further research into the manufacture and presentation of Bovaer®10 to enable inclusion into existing feed additive delivery systems such as micro dosing equipment where it can be supplemented fresh daily. Incorporation of Bovaer® into stable liquid forms should also be explored.

- Reassess Bovaer®10 inclusion levels to minimise reduction in feed intake, animal performance and carcass weight observed during the latter half of Phase 1 in this research project. Further performance trials should occur at reduced dose levels of 50 to 75 mg/kg DM, as initial MLA research (Almeida et al. 2023) reports excellent efficacy of methane yield suppression at these levels (65.5 to 80.1%). The initial respiratory calorimeter studies of Almeida et al (2023) were generally too small to identify 3-NOP dose effects on animal production.

- Conduct further studies on the utilisation of Bovaer®10 in feedlot diets during different seasonal periods, market categories and levels of feed intake. Further research on effect of 3-NOP on palatability through diet preference tests with feed exposed to simulated rainfall is required. If wet weather is proven to influence diet palatability this could simply be managed by withdrawing 3-NOP during rain events.

- Identify existing or novel feed additives and probiotics which capture rumen hydrogen but are not contraindicated to Bovaer®10 and its efficacy or compromising of animal performance.

7. Project staff

The following personnel were involved in the project and are acknowledged for the input and support:
Ms. Danielle Shirley, NH Foods Australia, Texas, Qld.
Mr Mariano Parra Munoz, The University of Queensland, Gatton, Qld.
Ms. Brianna Meacle, NH Foods Australia, Texas, Qld.
Ms. Kirralee Melinz, The University of Queensland, Gatton, Qld.
Mr Joe Williams, Queensland Animal Science Precinct, The University of Queensland, Gatton, Qld
Dr Stephen Bonner, Integrated Animal Production, Toowoomba Qld.
Mr Allan Lisle, The University of Queensland, Gatton, Qld.
Dr John Doyle, Integrated Animal Production, Toowoomba Qld.
Dr Robert Lawrence, Integrated Animal Production, Tamworth, NSW.
Mr Ian Loxton, Yeppoon, Qld.

8. Acknowledgements

We thankfully acknowledge the support and assistance of NH Foods Australia; Mr Tony Fitzgerald, NH Foods Australia, Texas, Qld; Staff and personal of NH Foods Australia, Texas, Qld; Staff and personnel of Oakey Beef Exports, NH Foods Australia, Oakey, Qld; Staff of Integrated Animal Production, Toowoomba, Qld; Dr John Gaughan and Dr Sarah Meale, The University of Queensland, Gatton, Qld; Mr. Glen Whitton Riverina Feeds, Warwick, Qld and technical representatives from DSM Nutritional Products. Australia.

9. References

ALEMU, A.W., SHRECK, A.L., BOOKER, C.W., MCGINN, S.M., PEKRUL, L.K.D., KINDERMANN, M. & BEAUCHEMIN, K.A. 2021a. Use of 3-Nitrooxypropanol in a commercial feedlot to decrease enteric methane emissions from cattle fed a corn-based finishing diet. Journal of Animal Science, 99, 1-13.

ALEMU, A.W., PEKRUL, L.K.D., SHRECK, A.L., BOOKER, C.W., McGINN, S.M., KINDERMANN, M. & BEAUCHEMIN, K.A. (2021b). 3-Nitrooxypropanol decreased enteric methane production from growing beef cattle in a commercial feedlot: Implications for sustainable beef cattle production. Frontiers in Animal Science, 2, 1-12.

ALMEIDA, A.K., COWLEY, F., McMENIMAN, J.P., KARAGIANNIS, A., TAMASSIA, L.F.M., McGRATH, J.J. & HEGARTY, R.S. (2023). Effect of 3-nitrooxypropanol on enteric methane emissions of feedlot cattle fed a tempered barley-based diet with canola oil. Journal of Animal Science. 101, 1-10.

ARAUJO, T.L.R., RABELO, C.H.S., CARDOSO, A.S., CARVALHO, V.V., ACEDO, T.S., TAMASSIA, L.F.M., VASCONCELOS, G.S.F.M., DUVAL, S.M., KINDERMANN, M., GOUVEA, V.N., FERNANDES, M.H.M.R. & REIS, R.A. (2023). Feeding 3-nitrooxypropanol reduces methane emissions by feedlot cattle on tropical conditions. Journal of Animal Science, 101, 1-11.

BAMPIDIS, V., AZIMONTI, G., BASTOS, M.D., CHRISTENSIN, H., DUSEMUND, B., DURJAVA, M.F., KOUBA, M., LOPEZ-ALONSO, M., PUENTE, S.L., MACRON, F., MAYO, B., PECHOVA, A., PETKOVA, M., RAMOS, M. F., SANZ, Y., VILLA, S.E., WOUSTERSIN, R., AUILINA, G., BORIES, G., BRANTOM, P.G. GROPP, J., SVENSSON, K., TOSTI, L., ANGUITA, M., GALOBART, J., MANINI, P., TARRES-CALL, l, J. & PIZZO, F. 2021. Safety and efficiency of a feed additive consisting of 3-nitroxypropanol (Bovaer®10) for ruminants for milk production and reproduction. European Food Safety Authority Journal, 19, 6905-6940. doi: 10.2903/j.efsa.2021.6905

BEAUCHEMIN, K.A., KREUZER, M., O'MARA, F. & MCALLISTER, T.A. 2008. Nutritional management for enteric methane abatement: A review. Australian Journal of Experimental Agriculture, 48, 21-27.

BEAUCHEMIN, K.A. & MCGINN, S.M. 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. Journal of Animal Science, 84, 1489-1496.

CHEN, H., GAN. Q., & CHENGUANG, F. 2020. Methyl-coenzyme M reductase and its post-translational modifications. Frontiers in Microbiology, 11, 1-7.

COTTLE, D.J., NOLAN, J.V. & WIDERMANN, S.G. 2011. Ruminant enteric methane mitigation: A review. Animal Production Science, 51, 491-514.

DUIN, E.C., WAGNER, T., SHMA, S., PRAKASH, D., CRONIN, B., YANEZ-RUIZ, D.R., DUVAL, S., RUMBELII, R., STEMMLER, R.T., THAUER, R.K. & KINDERMANN, M. 2016. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitroxypropanol. Proceedings of the national academy of science, 113, 6172-6177.

EKLUND, M., BAUER, E., WAMATU, J. & MOSENTHIN, R. 2005. Potential nutritional and physiological functions of betaine in livestock. Nutrition Research Reviews, 18, 31-48.

GAUGHAN, J.B., VAN BARNEVELD, R. & CADOGAN, D. 2005. Effect of Bos Koolus on dry matter intake, rectal temperature and respiration rate of grain fed steers exposed to hot conditions. Journal of Animal Science, (Suppl 1) 83, 369.

GAUGHAN, J.B., MADER, T.L., HOLT, S.M. & LISLE, A. 2008. A new heat load index for feedlot cattle. Journal of Animal Science, 86, 226-234.

GRUNINGER, R.J., ZHANG, X.M., SMITH, M.L., KUNG, L., VYAS, D., MCGINN, S.M., KINDERMANN, M., WANG, M., TAN, Z.L. & BEAUCHEMIN, K.A. 2022. Application of 3-nitrooxypropanol and canola oil to mitigate enteric methane emissions of beef cattle results in distinctly different effects on the rumen microbial community. Animal Microbiome (2022) 4:35 https://doi.org/10.1186/s42523-022-00179-8

HAISAN, J., SUN., Y., GUAN, L.L., BEAUCHEMIN, K.A., IWAASA, A., DUVAL, S., BARREDA, D.R. & OBA, M. 2014. The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation. Journal of Dairy Science, 97, 3110-3119

HENDERSON, G., COX, F., GANESH, S., JONKER, A., YOUNG, W. & JANSSEN, P.H. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Science Report, 5, 14567.

HUANG, R., ROMERO MARQUES, P., BELACHE, A., UNGERFELD, E.M., YANEZ-RUIZ, D., POPVA, M. & MORAGAVI, D.P. 2022. Synergistic effects of natural hydrogen acceptors and a methanogenesis inhibitor on in vitro rumen fermentation. GGAA, Florida, USA, June 5 to 9.

JEYANATHAN, J., MARTIN, C. & MORGAVI, D.P. 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: A review. Animal, 8, 250-261.

JOHNSON, K.A. & JOHNSON, D.E. 1995. Methane emissions from cattle. Journal of Animal Science, 73, 2483-2492.

KAREKAR, S.C. & AHRING, B.K. 2023. Reducing methane production from rumen cultures by bioaugmentation with homoacetogenic bacteria. Biocatalysis and Agricultural Biotechnology, 47, 102526-102536.

LE VAN, T.D., ROBINSON, J.A., RALPH, J., GREENING, R.C., SMOLENSKI, W.J., LEEDLE, J.A.Z. & SCHAEFER, D.M. 1998. Assessment of reductive acetogenesis with indigenous ruminal bacterium populations and *Aceitomaculum ruminsis*. Applied and Environmental Microbiology, 64, 3429-3436.

LEE, C., ARAUJO, R.C., KOENIG, K.M. AND BEAUCHEMIN, K.A. 2015. Effects of feed consumption rate of beef cattle offered a diet supplemented with nitrate ad libitum or restrictively on potential toxicity of nitrate. Journal of Animal Science, 93, 4956-4966.

LOPEZ, S., MCINTOSH, F.M., WALLACE, R.J., & NEWBOLD, C.J. 1999. Effect of adding acetogenic bacteria on methane production by mixed rumen microorganisms. Animal Feed Science & Technology, 78, 1-9.

MARTINEZ-FERANDEZ, G., DUVAL, S., SCHIRRA, H.J., DENMAN, S.E. & MCSWEENEY, C.S. 2018. 3-NOP vs halogenated compound: Methane production, ruminal fermentation and microbial community response in forage fed cattle. Frontiers in Microbiology, 9, 1-13.

NOLLET, L., DEMEYER, D.I. & VERSTRAETE, W. (1997). Effect of 2-bromoethanesulfonic acid and Peptostreptococcus productus ATCC 35244
addition on stimulation of reductive acetogenesis in the ruminal ecosystem by
selective inhibition of methanogenesis. Applied Environmental Microbiology, 63, 194–200.

PEDRINI, C.A., MACHADO, F.S., NIEHUES, M.B., PERDIGAO, A., VALERIO de CARVLHO, V., ACEDO, T.S.S., KINDERMANN, M., TAMASSIA, L., WALKER, N. & GANDRA, J. (2023). 3-Nitrooxypropanol dosages to reduce methane emissions by feedlot beef cattle. Journal of Animal Science, 101 (Suppl. S3), 556.

PEREIRA, A.M., DAPKERVICUIS, M. & BORBA, A. 2022. Alternative pathways for hydrogen sink originated from the rumen fermentation of carbohydrates: Which microorganisms are involved in lowering methane emissions. Animal Microbiome, 4, 5-17.

UNGERFELD, E.M. 2015. Shifts in metabolic hydrogen sinks in the methanogenesis inhibited ruminal fermentation: A meta-analysis. Frontier Microbiology, 6, 1-17.

UNGERFELD, E.M., BEAUCHEMIN, E.M. & MUNOZ, C. 2022. Current perspectives on achieving pronounced enteric methane mitigation from ruminant production. Frontiers in Animal Science, 2, 1-16.

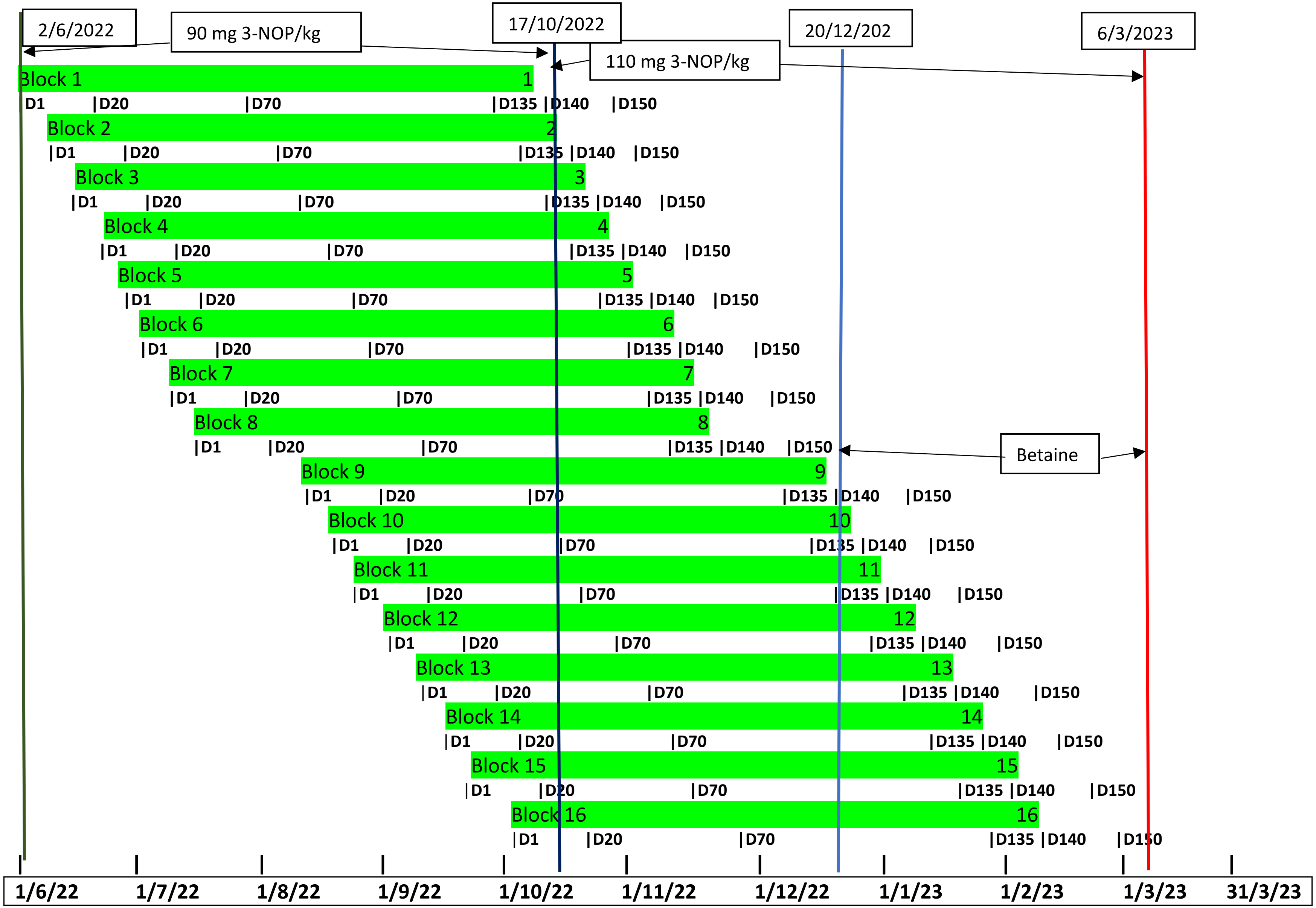
VYAS, D., MCGINN, S.M., DUVAL, S.M., KINDERMANN, M. & BEAUCHEMIN, K.A. 2016. Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle. Journal of animal science, 94, 2024-2034.

VYAS, D., ALEMU, A.W., MCGINN, S.M., DUVAL, S.M., KINDERMANN, M. & BEAUCHEMIN, K.A. 2018. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high-forage and high-grain diets. Journal of Animal Science, 96, 2923-2938.

ZHANG, X.M., SMITH, M.L., GRUNINGER, R.J., KUNG, L., VYAS, D., MCGINN, S.M., KINDERMANN, M., WANG, M., TAN, Z, L. & BEAUCHEMIN, K.A. 2021. Combined effects of 3-nitrooxypropanol and canola oil supplementation on methane emissions, rumen fermentation and biohydrogenation and total tract digestibility in beef cattle. Journal of Animal Science, 99: 1-10

10 Appendix

10.1 Project timeline schema



10.2 Project feedlot pen layout

'New side' feedlot pens

Location of feedlot project treatment Home Pens and Dispatch Pens

3-NOP Project selected study pens' specifications						**Aspect**	East	
Pen	**Feed bunk length (m)**	**Water trough length (m)**	**Trough to feedbunk (m)**	**Shade area (m^2)**	**Trough to gate distance (m)**	**Pen depth (m)**	**Pen area (m^2)**	**Elevation (m)**
O04	60	5	33	680	21	59	3540	305
O05	60	5	33	680	21	59	3540	
O06	60	5	33	680	21	59	3540	
O07	60	5	33	680	21	59	3540	
P04	60	5	33	680	24	62	3720	303
P05	60	5	33	680	24	62	3720	
P06	60	5	33	680	24	62	3720	
P07	60	5	33	680	24	62	3720	
Q04	60	5	33	680	21	59	3540	300
Q05	60	5	33	680	21	59	3540	
Q06	60	5	33	680	21	59	3540	
Q07	60	5	33	680	21	59	3540	
R04	60	5	33	680	21	59	3540	299
R05	60	5	33	680	21	59	3540	
R06	60	5	33	680	21	59	3540	
R07	60	5	33	680	21	59	3540	
S04	60	5	33	680	21	59	3540	298
S05	60	5	33	680	21	59	3540	
S06	60	5	33	680	21	59	3540	
S07	60	5	33	680	21	59	3540	
U03	60	5	42	680	15	62	3720	296
U04	60	5	42	680	15	62	3720	
U05	60	5	42	680	15	62	3720	
U06	60	5	42	680	15	62	3720	
V03	60	5	39	680	15	59	3540	294
V04	60	5	39	680	15	59	3540	
V05	60	5	39	680	15	59	3540	
V06	60	5	39	680	15	59	3540	
W03	60	5	39	680	15	59	3540	290
W04	60	5	39	680	15	59	3540	
W05	60	5	39	680	15	59	3540	
W06	60	5	39	680	15	59	3540	

10.3 Project steer induction and processing protocol

Bovaer (3-NOP) Project animal induction and processing protocol

This protocol will be used to induct and process steers for the Bovaer (3-NOP) project weekly for 16 weeks to produce the 16 project treatment replications required to meet the project design.

Project design

There are two project treatments:

- Control = standard feedlot diet with 0 mg 3-NOP/kg DM
- 3-NOP = standard feedlot diet with 100 mg 3-NOP/kg DM

The project is based on a randomised block design.
There will be 16 project treatment replications.

Allocation of steers to project blocks

The allocation of the project steers for a randomised block design will be based on the following procedure.

Around 700 suitable steers arrive at the feedlot on a weekly basis. Once 700 suitable steers are available, they will be processed as per feedlot routine (Project feedlot Processing Cattle (Receivals) Protocol) into the 'Reserve' (100% Black Angus genotype) cattle class. During processing, 500 suitable steers will be identified and allocated to one of the two treatments and put into their treatment pens. Thus, there will be one 'block' allocation per week which will be repeated weekly for 16 weeks. The selection of each steer for a block will be a fully randomised procedure i.e., the steers suitability determined on its liveweight and other criteria (as below) as it moves through the race, scales box and into the Veterinary crush/chute. For e.g., the first 3 steers in the race could be randomly allocated to, Project Group 1 (or I1) or Project Group 2 (or I2) or Group 3 Outliers (Out of Spec., or I3).

The procedure to be followed at each project induction, processing and allocation is:

- All steers will be individually identified, inducted, and processed;
- The project steer liveweight range is 400-500 kg:
- Individual liveweights recorded;
- Individual steers implanted with Revalor S (28mg oestradiol 17β, 140mg of trenbolone acetate);
- Any outlier steers in respect to liveweight, genotype, age, temperament, or health status will be identified and removed from the allocation;
- As an individual steer leaves the Veterinary crush/chute following routine practices (Project feedlot Processing Near-Side (Left Side) and Processing Off-Side (Right Side) Protocols)), ;the steer can be drafted into one of 3 yards – Group 1(I1), Group 2(I2) or Group 3(I3) Outliers (Out of Spec) based on its project group allocation;
- From this drafting, there will be three groups of steers in 3 separate yards – Group 1 of ~250 project steers (I1), Group 2 of ~250 project steers (I2) and Group 3 of 200 Outlier (Out of Spec.) steers (I3);
- Groups 1 and 2 of 250 steers each are then randomly allocated to one each of the two treatment diets as per the table below:

Week	Week commencing	Induction group[1]	Treatment code	Treatment name	Full project treatment code[2]
1	30/5/2022	I1	T1	T1-Control	3-NOPBLOCK1T1
1	30/5/2022	I2	T2	T2-3-NOP	3-NOPBLOCK1T2
2	06/6/2022	I1	T1	T1-Control	3-NOPBLOCK2T1
2	06/6/2022	I2	T2	T2-3-NOP	3-NOPBLOCK2T2
3	13/6/2022	I1	T1	T1-Control	3-NOPBLOCK3T1
3	13/6/2022	I2	T2	T2-3-NOP	3-NOPBLOCK3T2
4	13/6/2022	I1	T2	T2-3-NOP	3-NOPBLOCK4T2
4	13/6/2022	I2	T1	T1-Control	3-NOPBLOCK4T1
5	20/6/2022	I1	T1	T1-Control	3-NOPBLOCK5T1
5	20/6/2022	I2	T2	T2-3-NOP	3-NOPBLOCK5T2
6	20/6/2022	I1	T2	T2-3-NOP	3-NOPBLOCK6T2
6	20/6/2022	I2	T1	T1-Control	3-NOPBLOCK6T1
7	27/6/2022	I1	T2	T2-3-NOP	3-NOPBLOCK7T2
7	27/6/2022	I2	T1	T1-Control	3-NOPBLOCK7T1
8	27/6/2022	I1	T1	T1-Control	3-NOPBLOCK8T1
8	27/6/2022	I2	T2	T2-3-NOP	3-NOPBLOCK8T2
9	04/7/2022	I1	T2	T2-3-NOP	3-NOPBLOCK9T2
9	04/7/2022	I2	T1	T1-Control	3-NOPBLOCK9T1
10	04/7/2022	I1	T2	T2-3-NOP	3-NOPBLOCK10T2
10	04/7/2022	I2	T1	T1-Control	3-NOPBLOCK10T1
11	11/7/2022	I1	T1	T1-Control	3-NOPBLOCK11T1
11	11/7/2022	I2	T2	T2-3-NOP	3-NOPBLOCK11T2
12	11/7/2022	I1	T2	T2-3-NOP	3-NOPBLOCK12T2
12	11/7/2022	I2	T1	T1-Control	3-NOPBLOCK12T1
13	18/7/2022	I1	T1	T1-Control	3-NOPBLOCK13T1
13	18/7/2022	I2	T2	T2-3-NOP	3-NOPBLOCK13T2
14	18/7/2022	I1	T2	T2-3-NOP	3-NOPBLOCK14T2
14	18/7/2022	I2	T1	T1-Control	3-NOPBLOCK14T1
15	15/8/2022	I1	T1	T1-Control	3-NOPBLOCK15T1
15	15/8/2022	I2	T2	T2-3-NOP	3-NOPBLOCK15T2
16	15/8/2022	I1	T2	T2-3-NOP	3-NOPBLOCK16T2
16	15/8/2022	I2	T1	T1-Control	3-NOPBLOCK16T1

[1]Note that the 2 induction groups I1 and I2 of each week's induction processing for scientific statistical purposes are referred to as a Block. For e.g. I1 and I2 groups of Week 1 are also known as Block 1. There will be 16 Blocks in the project, 1 Block for each 16 weekly Induction Processing.
[2]At each project Induction processing or immediately after, the Full project treatment code needs to be entered into StockaID in the relevant field against each individual animal record.

- The 3rd Group (I3) of 200 Outlier (Out of Spec.) steers. This group of 200 unallocated steers will not be used in the project and will be directed to a different feedlot feeding program.

This procedure is repeated weekly for another 15 weeks to produce the 16 project treatment replications as per the project design.

Once the Induction groups are allocated to the project treatments, the project treatments Control and 3-NOP at each induction processing are randomly allocated to the project feedlot pens as per the procedure of the Bovaer (3-NOP) Project allocation of cattle blocks pens protocol.

10.4 Allocation of project steers to pens.

Bovaer (3-NOP) Project allocation of cattle blocks pens protocol

This protocol will be used to randomly allocate Groups 1 and 2 of 250 steers each resulting from a weekly processing to one of the 2 treatment diets and to their respective feedlot pens.

Project design

There are two project treatments:

- Control = standard feedlot diet with 0 mg 3-NOP/kg DM
- 3-NOP = standard feedlot diet with 100 mg 3-NOP/kg DM
  3-NOP was added to diet via a pellet included at 2% inclusion

Allocation of treatments to pens.

Immediately following the weekly project steer Induction processing, each Group 1(I1) and 2(I2) of 250 steers each will be randomly allocated to a set of 2 adjacent feedlot pens – Group 1(I1) in one pen; Group 2(I2) in a separate but adjacent pen in the same pen row.

The schedule of project rows/pens to be used for each of the 16-week project cattle inductions are:

Week	Week commencing	Row/Pen	Treatment code	Treatment name
1	30/5/2022	O4	T2	T2-3-NOP
1	30/5/2022	O5	T1	T1-Control
2	06/6/2022	O6	T1	T1-Control
2	06/6/2022	O7	T2	T2-3-NOP
3	13/6/2022	P4	T2	T2-3-NOP
3	13/6/2022	P5	T1	T1-Control
4	13/6/2022	P6	T2	T2-3-NOP
4	13/6/2022	P7	T1	T1-Control
5	20/6/2022	Q4	T1	T1-Control
5	20/6/2022	Q5	T2	T2-3-NOP
6	20/6/2022	Q6	T1	T1-Control
6	20/6/2022	Q7	T2	T2-3-NOP
7	27/6/2022	R4	T2	T2-3-NOP
7	27/6/2022	R5	T1	T1-Control
8	27/6/2022	R6	T2	T2-3-NOP
8	27/6/2022	R7	T1	T1-Control
9	04/7/2022	S4	T1	T1-Control
9	04/7/2022	S5	T2	T2-3-NOP
10	04/7/2022	S6	T1	T1-Control
10	04/7/2022	S7	T2	T2-3-NOP
11	11/7/2022	U3	T1	T1-Control
11	11/7/2022	U4	T2	T2-3-NOP
12	11/7/2022	U5	T2	T2-3-NOP
12	11/7/2022	U6	T1	T1-Control
13	18/7/2022	V3	T2	T2-3-NOP
13	18/7/2022	V4	T1	T1-Control
14	18/7/2022	V5	T2	T2-3-NOP
14	18/7/2022	V6	T1	T1-Control
15	15/8/2022	W3	T2	T2-3-NOP
15	15/8/2022	W4	T1	T1-Control
16	15/8/2022	W5	T1	T1-Control
16	15/8/2022	W6	T2	T2-3-NOP

Following the completion of this procedure, the project Treatment groups are taken to their feedlot treatment pens.

This allocation procedure is repeated weekly for another 15 weeks to generate the 16 blocks of 2 treatments and assign them to their respective feedlot treatment pens as per the project design.

10.5 Induction liveweights

Lot no.	Block	Treatment	No.	Average induction liveweight (kg)
22 DT23	1	DT-Control	250	450.9
22 DT24	2	DT-Control	250	455.1
22 DT25	3	DT-Control	249	441.5
22 DT26	4	DT-Control	250	461.2
22 DT27	5	DT-Control	250	457.1
22 DT28	6	DT-Control	250	444.5
22 DT29	7	DT-Control	250	427.9
22 DT30	8	DT-Control	250	437.0
22 DT34	9	DT-Control	250	442.6
22 DT35	10	DT-Control	250	467.9
22 DT36	11	DT-Control	250	457.9
22 DT37	12	DT-Control	250	457.3
22 DT38	13	DT-Control	250	458.2
22 DT39	14	DT-Control	250	458.2
22 DT40	15	DT-Control	250	450.2
22 DT41	16	DT-Control	248	464.7
22 CT23	1	CT-NOP	251	450.4
22 CT24	2	CT-NOP	250	454.1
22 CT25	3	CT-NOP	250	439.5
22 CT26	4	CT-NOP	250	463.8
22 CT27	5	CT-NOP	250	454.0
22 CT28	6	CT-NOP	251	445.4
22 CT29	7	CT-NOP	250	427.7
22 CT30	8	CT-NOP	249	437.7
22 CT34	9	CT-NOP	250	443.7
22 CT35	10	CT-NOP	250	463.8
22 CT36	11	CT-NOP	249	461.1
22 CT37	12	CT-NOP	250	457.5
22 CT38	13	CT-NOP	249	454.0
22 CT39	14	CT-NOP	250	456.0
22 CT40	15	CT-NOP	251	452.0
22 CT41	16	CT-NOP	250	463.5

10.6 Schedule of project induction/allocation sessions including liveweight measurement

Phase no.	Block no.	Induction/allocation Date of commencement	Induction/allocation Date of completion	First DOF	Lot no.
1	1	1/06/2022	2/06/2022	3/06/2022	22 CT23
1	1	1/06/2022	2/06/2022	3/06/2022	22 DT23
1	2	8/06/2022	9/06/2022	10/06/2022	22 DT24
1	2	8/06/2022	9/06/2022	10/06/2022	22 CT24
1	3	14/06/2022	16/06/2022	17/06/2022	22 CT25
1	3	14/06/2022	16/06/2022	17/06/2022	22 DT25
1	4	21/06/2022	23/06/2022	24/06/2022	22 CT26
1	4	21/06/2022	23/06/2022	24/06/2022	22 DT26
1	5	28/06/2022	29/06/2022	30/06/2023	22 DT27
1	5	28/06/2022	29/06/2022	30/06/2023	22 CT27
1	6	5/07/2022	6/07/2022	7/07/2022	22 DT28
1	6	5/07/2022	6/07/2022	7/07/2022	22 CT28
1	7	12/07/2022	14/07/2022	15/07/2022	22 CT29
1	7	12/07/2022	14/07/2022	15/07/2022	22 DT29
1	8	19/07/2022	20/07/2022	21/07/2022	22 CT30
1	8	19/07/2022	20/07/2022	21/07/2022	22 DT30
2	9	17/08/2022	19/08/2022	20/08/2022	22 DT34
2	9	17/08/2022	19/08/2022	20/08/2022	22 CT34
2	10	22/08/2022	24/08/2022	25/08/2022	22 DT35
2	10	22/08/2022	24/08/2022	25/08/2022	22 CT35
2	11	30/08/2022	1/09/2022	2/09/2022	22 DT36
2	11	30/08/2022	1/09/2022	2/09/2022	22 CT36
2	12	5/09/2022	7/09/2022	8/09/2022	22 CT37
2	12	5/09/2022	7/09/2022	8/09/2022	22 DT37
2	13	12/09/2022	15/09/2022	16/09/2022	22 CT38
2	13	12/09/2022	15/09/2022	16/09/2022	22 DT38
2	14	20/09/2022	23/09/2022	24/09/2022	22 CT39
2	14	20/09/2022	23/09/2022	24/09/2022	22 DT39
2	15	27/09/2022	29/09/2022	30/09/2022	22 CT40
2	15	27/09/2022	29/09/2022	30/09/2022	22 DT40
2	16	4/10/2022	7/10/2022	8/10/2022	22 DT41
2	16	4/10/2022	7/10/2022	8/10/2022	22 CT41

10.7 Schedule of project animal measurement sessions, post induction

Phase no.	Block no.	Liveweight at HGP reimplantation	Liveweight at Final session	Lot no.
1	1	11/8/2022	20/10/2022	22 CT23
1	1	11/8/2022	20/10/2022	22 DT23
1	2	18/8/2022	27/10/2022	22 DT24
1	2	18/8/2022	27/10/2022	22 CT24
1	3	25/8/2022	3/11/2022	22 CT25
1	3	25/8/2022	3/11/2022	22 DT25
1	4	1/9/2022	10/11/2022	22 CT26
1	4	1/9/2022	10/11/2022	22 DT26
1	5	7/9/2022	16/11/2022	22 DT27
1	5	7/9/2022	16/11/2022	22 CT27
1	6	14/9/2022	23/11/2022	22 DT28
1	6	14/9/2022	23/11/2022	22 CT28
1	7	22/9/2022	1/12/2022	22 CT29
1	7	22/9/2022	1/12/2022	22 DT29
1	8	28/9/2022	7/12/2022	22 CT30
1	8	28/9/2022	7/12/2022	22 DT30
2	9	28/10/2022	5/01/2023	22 DT34
2	9	28/10/2022	5/01/2023	22 CT34
2	10	1/11/2022	10/01/2023	22 DT35
2	10	1/11/2022	10/01/2023	22 CT35
2	11	9/11/2022	19/01/2023	22 DT36
2	11	9/11/2022	19/01/2023	22 CT36
2	12	16/11/2022	25/01/2023	22 CT37
2	12	16/11/2022	25/01/2023	22 DT37
2	13	24/11/2022	2/02/2023	22 CT38
2	13	24/11/2022	2/02/2023	22 DT38
2	14	2/12/2022	10/02/2023	22 CT39
2	14	2/12/2022	10/02/2023	22 DT39
2	15	8/12/2022	16/02/2023	22 CT40
2	15	8/12/2022	16/02/2023	22 DT40
2	16	14/12/2022	22/02/2023	22 DT41
2	16	14/12/2022	22/02/2023	22 CT41

10.8 Project supplement pellet and ration sampling protocol

FEED SAMPLING PROTOCOL – 3-NOP PROJECT **v3**

Sampling Objectives

1. Representative – Samples collected are of sufficient size to ensure they represent quantity available;
2. Random – Increase sampling number or frequency to minimize bias. For example, remove number of smaller samples (aliquots) to create one larger composite sample;
3. Relative – Composite sample size and/or frequency relative to quantity loaded and/or consumed. For example, when taking a ration sample, sample size reflects what animal may consume, at least 10kg.

A list of the treatment pens is included at the end of this protocol.

Sampling Protocol – 3-NOP Pellets

Frequency

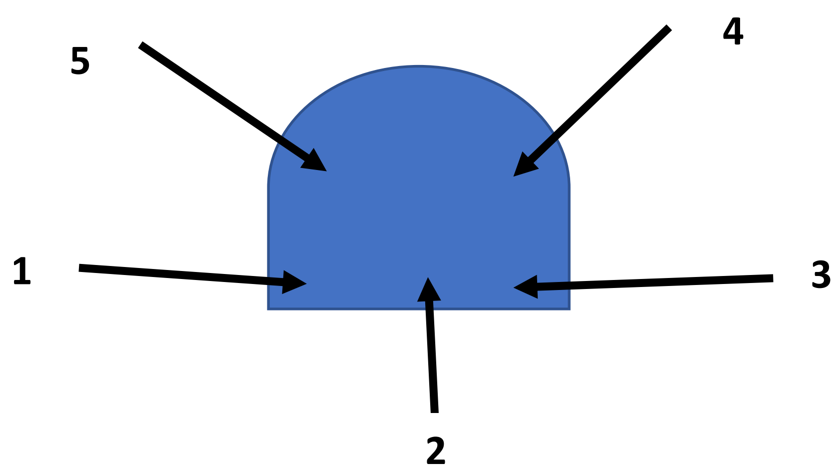
3-NOP pellets are sampled twice for each delivery.
Samples taken at commodity shed directly from bay.
First sample collected after unloading (START, S).
Second sample collected before receiving next new delivery (END, E).

Objective

Identify changes to 3-NOP activity associated with manufacture of pellet (S sample) and associated with storage period at commodity shed (E sample). For all subsequent deliveries after the first load require sampling of the pellets remaining & pellets of the new load

3-NOP Pellets Sample Protocol – Start

a) Collect 10L bucket from mill & grain spear;
b) To sample new delivery use grain spear at different locations to collect 5 samples as described in figure below (3 from base, 2 from centre of stockpile);



c) Empty each spear into bucket;
d) Mix the contents of bucket by hand.
e) Transfer pellets from bucket to:
   i) Fill five (5) 250ml bottles (3-NOP activity assessment at DSM NZ);
   ii) One (1) sample for dry matter in oven (60ºC for 48hours);
   iii) One (1) sample stored in zip lock bag for durability testing at Riverina Stockfeeds.

Label bottles with:
- Date;
- Feedlot name;
- 3-NOP Pellets;
- Sample identification:
    - 1st delivery S11, S12, S13, S14, S15;
    - 2nd delivery S21, S22, S23, S24, S25.
    Repeat identification for all end samples.
- Transfer bottles to freezer.

Label zip lock bag for durability with:
- Date;
- Feedlot name;
- 3-NOP Pellet Durability;
- Sample identification:
    - New (Start).
- Transfer zip lock bag to freezer.

3NOP Pellets Sample Protocol - End

a) If sufficient quantity remains, follow Sample Protocol – Start;
   For smaller quantities, use the following instructions.
b) Collect 10L bucket from mill, broom and grain shovel;
c) As required, sweep remaining pellets into a pile;
d) Using grain shovel, transfer 5 small scoops from into bucket;
e) Mix the contents of bucket by hand;
f) Transfer handfuls of pellets from bucket to fill five (5) 250ml bottles.
g) Label bottle with:
   - Date;
   - Feedlot name;
   - 3-NOP Pellets;
   - Sample identification:
       - Last of 1st delivery E11, E12, E13, E14, E15;

    - Last of 2nd delivery E21, E22, E23, E24, E25.
      Repeat identification for all end samples.
  - Transfer bottles to freezer.

h) Label zip lock bag for durability with:
  - Date;
  - Feedlot name;
  - 3-NOP Pellet Durability;
  - Sample identification:
    - Remaining (End).
  - Transfer zip lock bag to freezer.

Transfer to UQ
Retain all samples in freezer.
For 3-NOP samples in bottles, pack samples in esky with sufficient cold bricks to remove as much air space as possible.
Secure lid. Avoid direct sunlight and avoid opening lid while in transit.
Return to freezer at UQ on arrival.

Sampling Protocol – Ration Samples for Dry Matter Testing
Frequency
Ration samples (#5 for 3-NOP and #6 for Control) require collection every day for dry matter (DM) assessment.

Ration #5 (3-NOP treatment) sampled on a batch basis (to account for variation in manufacture between batches/mixers)
Ration #6 (Control) sampled randomly (large quantities of #6 ration manufactured daily), but with equivalent number of samples as Ration #5.

Starter ration samples (#1, #2 and #3) collected twice weekly.

On any one day, the following samples will be dried for DM determination:
  - 1 #5 3-NOP sample from each batch;
  - 1 #6 Control sample of equivalent sample number of #5 ration;
  - 1 sample of either #1, #2 or #3 rations when fed;

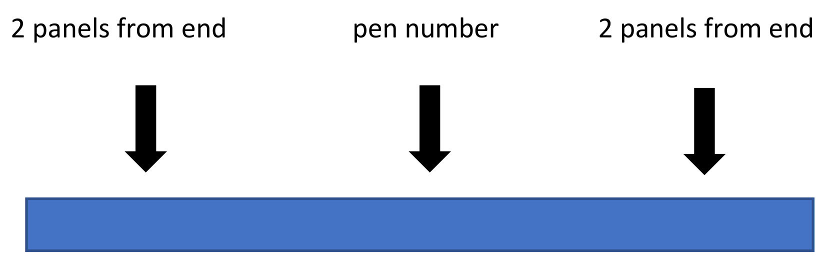
Samples will be dried in oven at 100°C for 18 hours.

Objective

Rations have inherent variation due to ingredient loading accuracy, variation in feedstuff particle size, ingredient moisture content, mixing efficacy and mixing time.
Ration sampling identifies variation in dry matter of treatment diets during feeding period.
Project will use dry matter values to accurately calculate actual 3NOP intake level (g/head/day, g/kg DM).

Ration Sampling Protocol

a) Collect 2 10L buckets from mill (1 bucket for 3-NOP ration, 1 bucket for Control ration). Additional buckets may be required as number of treatment pens increase;
b) Check Treatment Allocation to Pen List to ensure 3-NOP & Control rations are sampled from the correct pens;
c) As soon after delivery of ration to bunk as possible, remove three (3) aliquots (full hand grabs) from each treatment bunk from locations described by following figure. Arrows represent sampling locations.



d) Place aliquots in appropriate treatment bucket;
e) Mix contents in bucket gently by hand;
f) Place lid on buckets;
g) Return buckets to oven for dry matter assessment.

Ration Dry Matter Assessment Using Drying Oven

a) Oven is maintained at 100^{o}C;
b) From each treatment bucket, remove a full hand grab equivalent (250 to 500g);
c) Select a numbered tray, record number and tare weight;
d) Place hand grab sample in tray. Ensure sample is evenly spread within tray. Record treatment, tray number and weight;
e) Place trays, two (2) 3-NOP samples & two (2) control samples in oven for 18hours
f) Enter tray number, treatment and tray weights (tare, with sample) in Ration DM Record spreadsheet on Mill laptop;
g) After drying period, remove and weigh trays. Record tray number and weight
h) Enter dry weights in Ration DM Record spreadsheet;

i) Spreadsheet will calculate ration DM using the following calculations:
   - Wet sample weight = Wet sample weight – Tray weight;
   - Dry sample weight = Dry sample weight – Tray weight;
   - Moisture weight = Wet sample weight – Dry sample weight;
   - Moisture % = Moisture weight / Wet sample weight x 100;
   - Dry matter % = 100 – Moisture %.

Sampling Protocol – Ration Samples for Mixing Efficacy, Nutrient Analysis & 3-NOP Assessment

Frequency

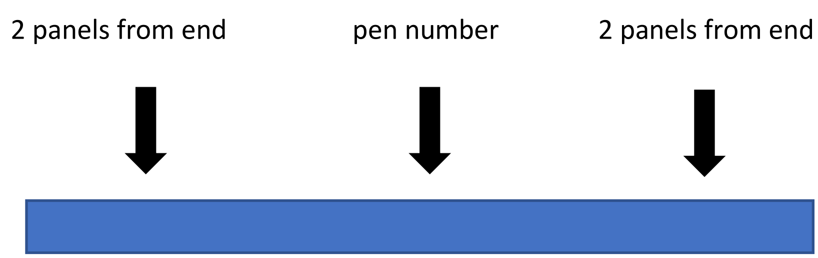
Ration samples (3-NOP and Control) require collection for mixing efficacy, nutrient analysis and 3NOP assessment weekly.

Objective

Ration treatment samples collected as a weekly record throughout project. Weekly collection will reflect changes to ration composition due to ingredient availability. Samples will be used to review mixing efficacy (consistency of mixing) as well as nutrient analysis (Dry matter, Crude protein, NDF, Fat & Ash) and assessment of 3-NOP activity within the ration to further determine 3-NOP intake level.

Ration Sampling Protocol

j) Collect 2 10L buckets from mill (1 bucket for 3-NOP ration, 1 bucket for Control ration). Additional buckets may be required as number of treatment pens increase;
k) Check Treatment Allocation to Pen List to ensure 3-NOP & Control rations are sampled from the correct pens;
l) As soon after delivery of ration to bunk as possible, remove three (3) aliquots (full hand grabs) from each treatment bunk from locations described by following figure. Arrows represent sampling locations;



m) Place aliquots in appropriate treatment bucket;
n) Mix contents in bucket gently by hand;
o) Place lid on buckets;
p) From bucket remove grab sample and place in sample bags supplied by weighbridge;
q) Label bags with Date, Feedlot name, Treatment (Control or 3-NOP) & Week;

r) At determined time, remove grab samples from 3-NOP bucket and transfer to 10 1.2Litre HDPE bottles;
s) Place all samples in freezer until time for transfer to UQ.

Treatment allocation to pen list

Row/Pen	Treatment code	Treatment name
O4	T2	T2-3-NOP
O5	T1	T1-Control
O6	T1	T1-Control
O7	T2	T2-3-NOP
P4	T2	T2-3-NOP
P5	T1	T1-Control
P6	T2	T2-3-NOP
P7	T1	T1-Control
Q4	T1	T1-Control
Q5	T2	T2-3-NOP
Q6	T1	T1-Control
Q7	T2	T2-3-NOP
R4	T2	T2-3-NOP
R5	T1	T1-Control
R6	T2	T2-3-NOP
R7	T1	T1-Control
S4	T1	T1-Control
S5	T2	T2-3-NOP
S6	T1	T1-Control
S7	T2	T2-3-NOP
U3	T1	T1-Control
U4	T2	T2-3-NOP
U5	T2	T2-3-NOP
U6	T1	T1-Control
V3	T2	T2-3-NOP
V4	T1	T1-Control
V5	T2	T2-3-NOP
V6	T1	T1-Control
W3	T2	T2-3-NOP
W4	T1	T1-Control
W5	T1	T1-Control
W6	T2	T2-3-NOP

Project treatment ration analysis for June to July 2022, August to September 2022 and October 2022 to March 2023 provided.

Symbio LABORATORIES

CERTIFICATE OF ANALYSIS

Symbio LABORATORIES *Proudly* AUSTRALIAN

ABN: 82 079 645 015

Certificate Number	B1190800-G [R00]	Page	1/1
Client	Whyalla Beef Pty Ltd	Registering Laboratory	Brisbane
Contact	John Doyle	Contact	Customer Service Team
Address	PO Box 101 Texas QLD 4385	Address	52 Brandl Street, Eight Mile Plains, QLD 4113
		Email	admin@symbiolabs.com.au
Telephone	07 4650 9177	Telephone	1300 703 166
Order Number	---	Date Samples Received	26/08/2022
Job Description	Stockfeed	Date Analysis Commenced	26/08/2022
Client Job Reference	---	Issue Date	01/09/2022
No. of Samples Registered	4 | Sampler: Customer	Receipt Temperature (ºC)	16
Priority	Normal	Storage Temperature (ºC)	20

NATA

Accreditation No: 2455
Accredited for compliance with ISO/IEC 17025 - Testing

This report supersedes any previous revision with this reference. This document must not be reproduced, except in full. If samples were provided by the customer, results apply only to the samples 'as received' and responsibility for representative sampling rests with the customer. Results are reported on as 'as is' basis unless otherwise indicated in the 'Report Comments' section. Measurement Uncertainty is available upon request. If the laboratory was authorised to conduct testing on samples received outside of the specified conditions, all test results may be impacted. Details of samples received outside of the specified conditions are mentioned in the sample description section of this test report.

Definitions

| <: Less Than | >: Greater Than | RP: Result Pending | ~: Estimated | MPN: Most Probable Number | CFU: Colony Forming Units | —: Not Received/Not Requested | | ^ Subcontracted Analysis| NA:Not Applicable| [NT]:Not Tested| LOR:Limit of Reporting| TBA:To Be Advised| ND:Not Detected| * Test not covered by NATA scope of accreditation | # Result derived from a calculation and includes results equal to or greater than the LOR | IH: Inconsistent results possibly caused by sample homogeneity

Authorised By

Name	Position	Accreditation Category
Hongmei Kuang	Chemistry Laboratory Manager, Brisbane	Environmental and Food Chemistry

Sample Information - *Client/Sampler Supplied*

Sample ID	Sample Description	Remarks
B1190800-G/62	8334 Ration 6 June	
B1190800-G/63	8335 Ration 5 June	
B1190800-G/64	8336 Ration 6 July	
B1190800-G/65	8337 Ration 5 July	

Analytical Results

Compound/Analyte	Method	LOR	Units	B1190800-G/62	B1190800-G/63	B1190800-G/64	B1190800-G/65
Protein	CF003.1 - Determination of Crude Protein by Combustion	0.1	%w/w	14.0	14.8	15.0	14.6
Fat	CF004.1 - Determination of Crude Fats and Oils by Soxhlet	0.1	%w/w	3.3	4.0	4.4	4.1
Moisture	CF005.1 - Determination of Moisture Content by Air Oven	0.1	%w/w	22.5	21.7	23.5	23.7
Ash	CF007 - Determination of Ash Content by Muffle Furnace	0.1	%w/w	4.5	4.5	4.9	4.5
Neutral Detergent Fibre	CF038.3 - Determination of Neutral Detergent Fibre by Refluxing	0.1	%w/w	22.2	20.1	21.2	21.6

Analysis Location

All in-house analysis was completed by Symbio Laboratories - Brisbane.

Report Comments

Protein calculation factor is 6.25 unless otherwise stated.

Client	Integrated Animal Production	Job Description	Stockfeed
Certificate Number	B1205096 [R00]	Order Number	---
Page	2/2	Received Date	05/10/2022

Symbio LABORATORIES Proudly AUSTRALIAN

Analytical Results

		Client Sample Description	8434 Ration 6 - August	8435 Ration 6 - September	8436 Ration 5 - August	8437 Ration 5 - September
		Client Sampling date/time	---	---	---	---
Compound/Analyte	LOR	Units	B1205096/1	B1205096/2	B1205096/3	B1205096/4
			Results	Results	Results	Results
Chemistry Food General Tests						
CF003.1 - Determination of Crude Protein by Combustion						
Protein	0.1	%w/w	13.8	14.1	14.4	14.8
CF004.1 - Determination of Crude Fats and Oils by Soxhlet						
Fat	0.1	%w/w	6.3	5.6	6.0	6.3
CF005.1 - Determination of Moisture Content by Air Oven						
Moisture	0.1	%w/w	22.8	23.2	22.2	23.2
CF007 - Determination of Ash Content by Muffle Furnace						
Ash	0.1	%w/w	4.8	4.2	4.8	4.6
CF031.1 - Starch by Glucoamylase Digestion						
Starch*	0.1	%w/w	44.4	45.8	43.2	41.3
Chem General AES Food						
ESI02 - Determination of Acid Extractable Elements by ICPOES						
Phosphorus (P)	2.5	mg/kg	3860	3470	3740	3640
CF038.3 NDF						
CF038.3 - Determination of Neutral Detergent Fibre by Refluxing						
Neutral Detergent Fibre	0.1	%w/w	21.8	22.3	21.1	21.9

Analysis Location

All in-house analysis was completed by Symbio Laboratories - Brisbane.

Report Comments

Protein calculation factor is 6.25 unless otherwise stated.

Client	Integrated Animal Production	Job Description	Stockfeed
Certificate Number	B1283830 [R00]	Order Number	---
Page	2/3	Received Date	15/03/2023

Symbio LABORATORIES Proudly AUSTRALIAN

Analytical Results

		Client Sample Description	8540 Ration 5 - Feb	8541 Ration 6 - Feb	8542 Ration 5 - Jan	8543 Ration 6 - Jan	8544 Ration 5 - Dec
		Client Sampling date/time	---	---	---	---	---
Compound/Analyte	LOR	Units	B1283830/1	B1283830/2	B1283830/3	B1283830/4	B1283830/5
			Results	Results	Results	Results	Results
Chemistry General Tests BNE							
CF003.1 - Determination of Crude Protein by Combustion							
Protein	0.1	g/100g	14.4	14.1	13.2	13.5	12.7
CF004.1 - Determination of Crude Fats and Oils by Soxhlet							
Fat	0.1	%w/w	5.6	5.6	6.2	7.0	5.8
CF005.1 - Determination of Moisture Content by Air Oven							
Moisture	0.1	g/100g	20.7	20.7	20.9	21.7	22.2
CF007 - Determination of Ash Content by Muffle Furnace							
Ash	0.1	g/100g	4.3	4.4	4.5	5.1	4.3
CF038.3 - Determination of Neutral Detergent Fibre by Refluxing							
Neutral Detergent Fibre	0.1	%w/w	18.5	17.9	19.8	22.1	18.0

Client	Integrated Animal Production	Job Description	Stockfeed		Symbio LABORATORIES Proudly AUSTRALIAN
Certificate Number	B1283830 [R00]	Order Number	---		
Page	3/3	Received Date	15/03/2023		

Analytical Results

Compound/Analyte	LOR	Units	8545 Ration 6 - Dec	8546 Ration 5 - Nov	8547 Ration 6 - Nov	8548 Ration 5 - Oct	8549 Ration 6 - Oct
		Client Sample Description	8545 Ration 6 - Dec	8546 Ration 5 - Nov	8547 Ration 6 - Nov	8548 Ration 5 - Oct	8549 Ration 6 - Oct
		Client Sampling date/time	---	---	---	---	---
			B1283830/6	B1283830/7	B1283830/8	B1283830/9	B1283830/10
			Results	Results	Results	Results	Results
Chemistry General Tests BNE							
CF003.1 - Determination of Crude Protein by Combustion							
Protein	0.1	g/100g	12.3	13.0	13.1	14.0	13.7
CF004.1 - Determination of Crude Fats and Oils by Soxhlet							
Fat	0.1	%w/w	6.0	6.3	5.9	7.9	6.0
CF005.1 - Determination of Moisture Content by Air Oven							
Moisture	0.1	g/100g	23.1	22.7	22.1	23.8	25.3
CF007 - Determination of Ash Content by Muffle Furnace							
Ash	0.1	g/100g	4.0	4.5	4.4	4.7	4.4
CF038.3 - Determination of Neutral Detergent Fibre by Refluxing							
Neutral Detergent Fibre	0.1	%w/w	17.5	17.8	18.9	22.8	21.9

Analysis Location

All in-house analysis was completed by Symbio Laboratories - Brisbane.

Report Comments

Protein calculation factor is 6.25 unless otherwise stated.

Results are reported on dry weight basis.

10.9 Schedule of project exit and slaughter dates

Phase no.	Block no.	Feedlot exit and slaughter schedule Date/s of feedlot exit	 Date/s of slaughter	Lot no.
1	1	1/11/2022	2-3/11/2022	22 CT23
1	1	1/11/2022	2-3/11/2022	22 DT23
1	2	8/11/2022	9/11/2022	22 DT24
1	2	8/11/2022	9/11/2022	22 CT24
1	3	15/11/2022	16/11/2022	22 CT25
1	3	15/11/2022	16/11/2022	22 DT25
1	4	22/11/2022	23/11/2022	22 CT26
1	4	22/11/2022	23/11/2022	22 DT26
1	5	28/11/2022	29/11/2022	22 DT27
1	5	28/11/2022	29/11/2022	22 CT27
1	6	5/12/2022	6/12/2022	22 DT28
1	6	5/12/2022	6/12/2022	22 CT28
1	7	13/12/2022	14/12/2022	22 CT29
1	7	13/12/2022	14/12/2022	22 DT29
1	8	19/12/2022	20/12/2022	22 CT30
1	8	19/12/2022	20/12/2022	22 DT30
2	9	18/01/2023	19/01/2023	22 DT34
2	9	18/01/2023	19/01/2023	22 CT34
2	10	22/01/2023	23/01/2023	22 DT35
2	10	22/01/2023	23/01/2023	22 CT35
2	11	30/01/2023	31/01/2023	22 DT36
2	11	30/01/2023	31/01/2023	22 CT36
2	12	6/02/2023	7/02/2023	22 CT37
2	12	6/02/2023	7/02/2023	22 DT37
2	13	12-13/02/2023	13-14/02/2023	22 CT38
2	13	12-13/02/2023	13-14/02/2023	22 DT38
2	14	19/02/2023	20/02/2023	22 CT39
2	14	19/02/2023	20/02/2023	22 DT39
2	15	27/02/2023	28/02/2023	22 CT40
2	15	27/02/2023	28/02/2023	22 DT40
2	16	6/03/2023	7/03/2023	22 DT41
2	16	6/03/2023	7/03/2023	22 CT41

10.10 Livestock trucking loading, transporting, and unloading protocol.

Bovaer (3-NOP) Project livestock trucking loading, transporting, and unloading protocol.

This protocol will be followed to load and transport project steers at dispatch in livestock trailers from the Project Feedlot to Oakey Abattoir, Oakey, and the unloading of the project steers at Oakey into holding/lairage pens.

On the day of dispatch (1 day prior to scheduled processing at Oakey Abattoir), the project pens of steers from the assigned block for dispatch are walked in the AM from their Starter row pens to the trucking yards, where they will be still held in their treatment groups until loading.

The steers will be loaded onto 'B' triple and 'B' double multicombination livestock trailers as per the standard procedures of the WI-0251 Penning up Cattle for Dispatch protocol. One of the project students will attend each project loading to monitor and record relevant notes.

The first consignment of the project steers (~250 steers) will be loaded in the early PM. Half (½) of T1 treatment steers from a block will be loaded across 1 multicombination 'B' triple and 1 'B' double livestock trailers. Half (½) of T2 treatment steers from a block will be loaded across a second 1 multicombination 'B' triple and 1 'B' double livestock trailers. This comprises Consignment 1 of the project steers for transport to Oakey Abattoir.

Data to be recorded at the loading of each consignment group:
- Registration number of each project livestock transport prime mover;
- Actual time of loading of each livestock transport;
- Departure time of each livestock transport.

Once loaded, at around 2:30 PM, the 2 multicombination 'B' triple and 2 'B' double livestock trailers will convoy to Oakey Abattoir to arrive in the early evening, at around 6:30 PM. At Oakey Abattoir, the 2 multicombination 'B' triple and 2 'B' double livestock trailers will be unloaded in sequence. One of the project team members will attend each project unloading to monitor and record relevant notes The T1 steers will be placed into one lairage holding pen (or into more than one pen due to numbers of steers) while all T2 steers will be placed into a separate lairage holding pen/s. Treatment groups of steers to always remain in their respective groups during lairage and are not to be boxed at any time

The 2 multicombination 'B' triple and 2 'B' double livestock trailers return to the Project feedlot by mid evening (around 8:30 PM) of the Dispatch Day, for loading of the balance of steers from the project block as per the procedure above for the first consignment of steers.

Once loaded, this second consignment of project steers (~250 steers) in the 2 multicombination 'B' triple and 2 'B' double livestock trailers will convoy to Oakey Abattoir to arrive in the early morning of the following day (Day 1 of processing). At Oakey Abattoir, the 2 multicombination 'B' triple and 2 'B' double livestock trailers will be unloaded in sequence. The T1 steers will be placed into one lairage holding pen (or into more than one pen due to numbers of steers) while all T2 steers will be placed into a separate lairage holding pen/s.

Data to be recorded at the unloading of each consignment group:

- Registration number of each project livestock transport prime mover;
- Arrival time of each livestock transport
- Actual time of unloading of each livestock transport;
- Lairage pen number of each group of 125 project steers.

With arrival and unloading of the second consignment of project steers, the procedure for the dispatch of a project block has been completed.

At Oakey Abattoir, each dispatch of a project block will result in a total of minimum 4 lairage pens being used:

- 1 pen/s ~ 125 T1 steers from first consignment;
- 1 pen/s ~ 125 T2 steers from first consignment;
- 1 pen/s ~ 125 T1 steers from second consignment;
- 1 pen/s ~ 125 T2 steers from second consignment.

The groups of steers will always remain in their respective groups during lairage and are not to be boxed at any time.

10.11 Abattoir lairage, slaughter, sampling, and chiller protocol

Bovaer (3-NOP) Project Abattoir lairage, slaughter, sampling, and chiller protocol

The project studies the effect of Bovaer (3-NOP), an anti-methanogen supplement on the performance, health, carcase characteristics and carbon footprint of Australian feedlot cattle.

The project commenced on June 1, 2022 when the first block of 500 project steers were inducted into the Project feedlot. There will be a total of 16 blocks of 500 steers inducted into the project between June 1 and October 4, 2022.

The steers will be slaughtered in project blocks of 500 steers each between November 2, 2022 and March 3, 2023. There will be a total of 16 blocks of 500 steers consigned over this period. Each block of 500 steers will be slaughtered on the same day, preferably on a working weekday.

A summary of the proposed timetable for delivery and processing for each project block is:

- Day 1 – day of dispatch. Each block to be transported as two consignments of 250 steers. Consignment 1 to arrive in the late PM and consignment 2 to arrive in the early AM of the following day;
- Day 2 – day of slaughter. Second consignment of project steers arrive at Oakey Abattoir in early AM. Unloaded and enter lairage;
    - At end of the lairage period for first consignment, 250 steers slaughtered as one group at approximately 7:00 AM. Measurements taken, and bodies enter chiller/s for 24-hour period;
    - At end of lairage period for second consignment, 250 steers slaughtered as one group at approximately 11:00 AM. Measurements taken, and bodies enter chiller/s for 24-hour period;
- Day 3 – day after slaughter. Chiller assessment and MSA grading conducted within chiller/marshalling area, for each consignment in sequence.

The purpose of this protocol is to outline the slaughter and chiller procedures and measurements required to meet the project's objectives.

This protocol will be followed for each project block of steers for the lairage period, the slaughter procedure and chiller assessment at Oakey Abattoir, Oakey.

Project steer arrival at Oakey Abattoir – Day 1-2

At dispatch of each project block, steers will be loaded, transported from Project feedlot to Oakey Abattoir, Oakey and unloaded into holding/lairage pens as per the Bovaer (3-NOP) Project livestock trucking loading, transporting, and unloading protocol. There will be two consignments of project steers (~250 steers in each consignment) per block comprising 2 separate convoys of 2 multicombination 'B' triple and 2 'B' double livestock trailers each, with the first consignment to arrive mid evening and the second consignment, to arrive early AM of the following day, the actual day of slaughter.

Lairage – Day 1-2

At arrival at Oakey Abattoir, each convoy of the 2 multicombination 'B' triple and 2 'B' double livestock trailers will unload in sequence. From each consignment, the T1 steers will be placed into one lairage holding pen/s (or into more than one pen due to numbers of steers) while all T2 steers will be placed into a separate lairage holding pen/s. One project person will attend each project unloading to monitor and record relevant notes.

At Oakey Abattoir, each dispatch of a project block will result in a total of minimum 4 lairage pens being used:

- 1 pen/s ~ 125 T1 steers from first consignment;
- 1 pen/s ~ 125 T2 steers from first consignment;
- 1 pen/s ~ 125 T1 steers from second consignment;
- 1 pen/s ~ 125 T2 steers from second consignment.

The groups of steers will always remain in their respective groups during lairage and are not to be boxed at any time. The graphic at the end of this protocol will assist Oakey personnel in making a quick identification of the steer groups:

After the lairage time requirement is reached:

- The groups of 125 steers are moved to smaller pens that will hold 40 steers each. Therefore, a group of 125 steers will be reduced in a random assignment basis to 3 smaller groups of approximately 40 steers each. The resulting block of 3 pens is still a discrete group and will not be boxed with any other project group;
- Steers are washed as per abattoir protocols, the amount of washing dependent on the magnitude of mud and dags;
- The groups of steers will always remain in their respective groups in the kill pens and are not to be boxed at any time.

Day 2- Slaughter timetable:
Considerations:

- Day shift kills throughout the project slaughter period;
- Kills to be scheduled on weekdays, such that carcase sides from each project block are chilled for 24 hours, not 48 or 72 hours as for e.g., over a weekend;
- Final nomination of kill time for each group to be based on kill rate/chain speed at the time of respective block slaughters;
- No split kills within groups;
- The 1st group kill to be scheduled before, and to be completed before smoko! No project bodies hanging on rail over smoko;
- The 2nd group kill to be scheduled before, and to be completed before lunch, or commenced immediately after lunch.

On the slaughter floor:

- Carcase sides are to be hung as per standard abattoir procedures;
- Data to be collected and procedures to be carried out are:
    - Animal identification and slaughter sequence, and recording of any salient notes; specifically, Project feedlot visual ID tag number, Oakey body number, Slaughter floor chain

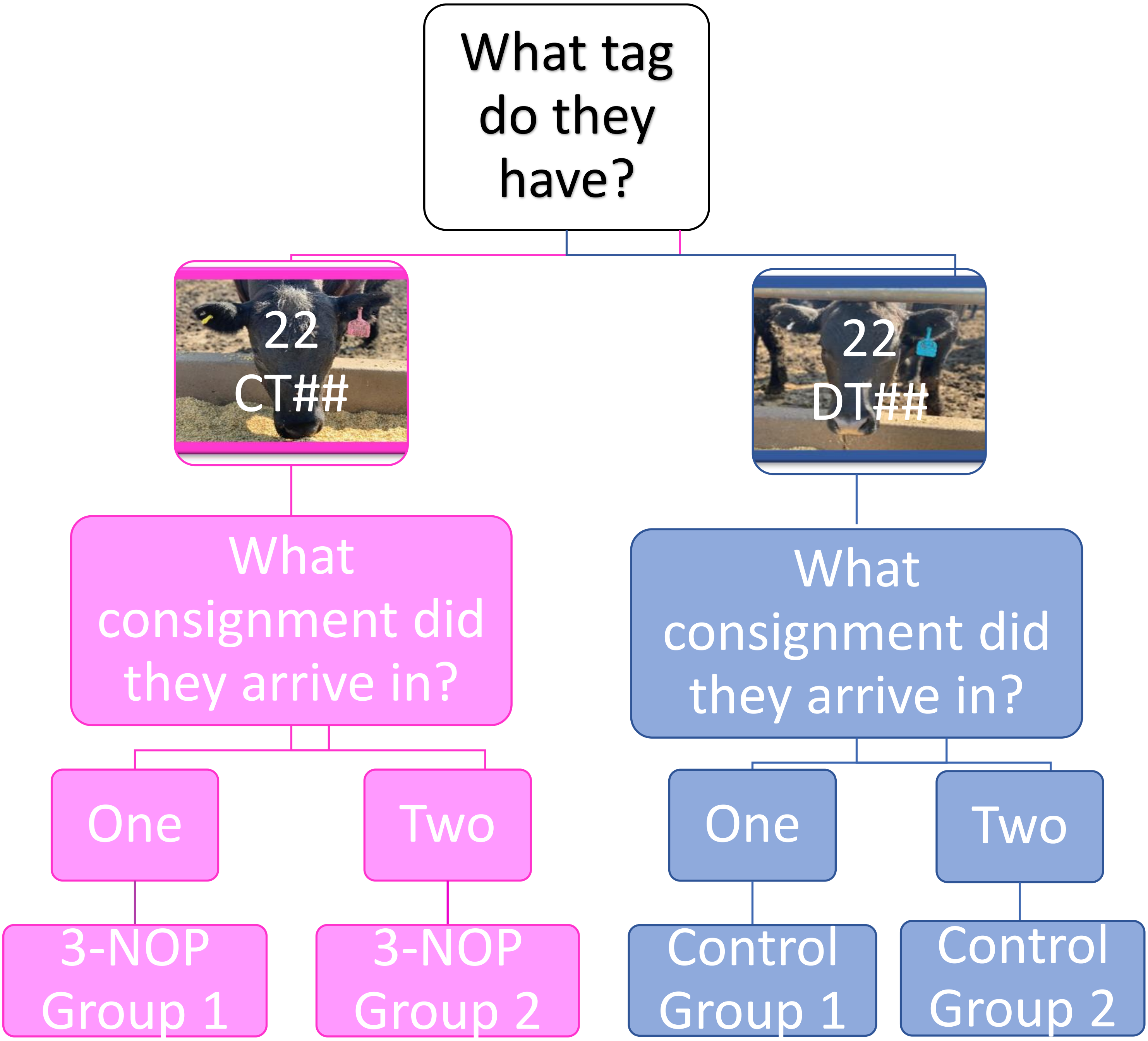
start and stop time for every stop-start occasion during kill of each Lot (i.e., each Oakey Kill Lot) and reason for stop e.g., Lappo, scheduled break, lunch, breakdown etc.– 1 project person;
- Inspection/assessment of each liver and lung for lesions and number of condemns;
- Collection of AUS-MEAT standard carcase feedback data by Oakey Abattoir's normal protocol.

Day 3 Chiller assessment:
All Chiller assessment and MSA grading to be conducted after 24-hour chilling as per Oakey Abattoir protocol and MSA's grading protocol.

Data requested of Oakey Abattoir staff at each kill of project cattle:

- Stock arrival and Lairage climatic conditions;
- Temperature of wash water;
- Documentation of all electrical inputs on the slaughter floor - low voltage electrical stimulator, immobiliser, and hide puller;
- AUS-MEAT standard carcase feedback and other relevant Oakey Abattoir data??
- Lesions, faults, condemn status, each liver and lung.
- Time of entry of each group into carcase side chillers;
- Chiller Room temperature cycles and temperature-time profile for chilling period??
- Cold carcase side weights if available.

Project Personal:
Robert Lawrence OR Stephen Bonner


What tag do they have?
22
CT##
22
DT##
What consignment did they arrive in?
What consignment did they arrive in?
One
Two
One
Two
3-NOP Group 1
3-NOP Group 2
Control Group 1
Control Group 2

10.12 Arrival time of each multi combination livestock unit at the collaborating abattoir for the project period

Block number	Arrival date	Arrival time	Truck number
1	1/11/2022	17:45	1
	1/11/2022	18:40	2
	1/11/2022	18:45	3
	1/11/2022	19:30	4
	1/11/2022	20:00	5
	1/11/2022	20:00	6
	2/11/2022	01:40	1
	2/11/2022	02:00	2
	2/11/2022	02:30	3
	2/11/2022	02:35	4
	2/11/2022	02:38	5
2	8/11/2022	18:00	1
	8/11/2022	18:45	2
	8/11/2022	18:20	3
	8/11/2022	17:38	4
	8/11/2022	20:40	5
	8/11/2022	22:45	1
	8/11/2022	23:45	2
	9/11/2022	00:30	3
	9/11/2022	00:45	4
	9/11/2022	01:06	5
3	15/11/2022	15:45	1
	15/11/2022	17:30	2
	15/11/2022	18:05	3
	15/11/2022	19:20	4
	15/11/2022	23:30	1
	15/11/2022	23:50	2
	16/11/2022	00:32	3
	16/11/2022	02:36	4
4	22/11/2022	17:00	1
	22/11/2022	17:50	2
	22/11/2022	18:20	3
	22/11/2022	18:30	4
	23/11/2022	00:02	1
	23/11/2022	00:30	2
	23/11/2022	00:50	3
	23/11/2022	01:00	4
5	28/11/2022	18:50	1
	28/11/2022	19:00	2
	28/11/2022	19:25	3
	28/11/2022	19:30	4
	29/11/2022	01:00	1
	29/11/2022	01:20	2
	29/11/2022	01:39	3
	29/11/2022	02:23	4

Block number	Arrival date	Arrival time	Truck number
6	05/12/2022	18:50	1
	05/12/2022	19:00	2
	05/12/2022	19:22	3
	05/12/2022	19:29	4
	05/12/2022	21:10	5
	06/12/2022	00:30	1
	06/12/2022	01:15	2
	06/12/2022	01:30	3
	06/12/2022	01:58	4
7	13/12/2022	19:00	1
	13/12/2022	19:30	2
	13/12/2022	20:00	3
	13/12/2022	20:19	4
	14/12/2022	01:30	1
	14/12/2022	02:05	2
	14/12/2022	02:45	3
	14/12/2022	04:00	4
8	19/12/2022	18:55	1
	19/12/2022	19:05	2
	19/12/2022	19:36	3
	19/12/2022	20:00	4
	19/12/2022	20:25	5
	20/12/2022	01:10	1
	20/12/2022	01:20	2
	20/12/2022	01:55	3
	20/12/2022	02:10	4
9	18/01/2023	18:50	1
	18/01/2023	19:00	2
	18/01/2023	19:30	3
	18/01/2023	20:00	4
	18/01/2023	20:10	5
	19/01/2023	01:10	1
	19/01/2023	01:45	2
	19/01/2023	02:40	3
	19/01/2023	02:45	4
10	22/01/2023	18:49	1
	22/01/2023	19:15	2
	22/01/2023	19:45	3
	22/01/2023	19:50	4
	22/01/2023	20:15	5
	23/01/2023	01:30	1
	23/01/2023	02:40	2
	23/01/2023	03:00	3
	23/01/2023	03:25	4
	23/01/2023	NA	5
11	30/01/2023	19:00	1
	30/01/2023	19:20	2
	30/01/2023	19:31	3
	30/01/2023	21:15	4
	31/01/2023	01:45	1
	31/01/2023	02:00	2

Block number	Arrival date	Arrival time	Truck number
	31/01/2023	02:25	3
	31/01/2023	NA	4
	31/01/2023	NA	5
12	6/02/2023	19:05	1
	6/02/2023	19:30	2
	6/02/2023	19:57	3
	6/02/2023	NA	4
	7/02/2023	01:35	1
	7/02/2023	01:40	2
	7/02/2023	02:14	3
	7/02/2023	NA	4
13 – Monday Kill	12/02/2023	19:00	1
	12/02/2023	19:05	2
	12/02/2023	19:35	3
	13/02/2023	00:35	1
	13/02/2023	01:20	2
	13/02/2023	02:15	3
13-Tuesday Kill	13/02/2023	19:25	1
	13/02/2023	19:48	2
	13/02/2023	20:00	3
	13/02/2023	NA	4
	14/02/2023	00:25	1
	14/02/2023	01:40	2
	14/02/2023	02:10	3
14	19/02/2023	18:50	1
	19/02/2023	19:25	2
	19/02/2023	20:30	3
	19/02/2023	20:55	4
	19/02/2023	21:40	5
	20/02/2023	01:05	1
	20/02/2023	01:50	2
	20/02/2023	03:10	3
	20/02/2023	03:50	4
15	27/02/2023	19:00	1
	27/02/2023	19:10	2
	27/02/2023	19:15	3
	27/02/2023	20:35	4
	28/02/2023	00:50	1
	28/02/2023	01:50	2
	28/02/2023	02:20	3
	28/02/2023	03:15	4
16	6/03/2023	19:00	1
	6/03/2023	19:00	2
	6/03/2023	19:30	3
	6/03/2023	19:30	4
	7/03/2023	00:40	1
	7/03/2023	01:15	2
	7/03/2023	01:25	3
	7/03/2023	02:15	4

10.13 Data import and statistical methods conducted by project statistician.

This appendix summarises the complete steps taken to import the raw data into the 'R' statistical analysis program from Excel workbooks, as well as the subsequent methods used within R to analyse the data.

Import of performance data

Raw data were supplied in two Excel workbooks. Data for each Project feedlot Lot (e.g., CT23, DT40 etc) was held in a separate worksheet. To simplify the import process, some sheets were renamed so that all names followed the convention "Block***BB***Lot22 ***LLLL*** Performance", where BB is 01, 02, … 16 and LLLL is the Project feedlot Lot CT23, DT23, … DT41. Enforcing a constant string length simplified the assignment of block and treatment details to each Lot as it was imported.

Secondly, summary calculations embedded in the sheets were removed. An extra column (AM) was created to indicate the Cull/Remove status of each record, with 0 meaning retain for analysis and 1 allowing simple removal of culls. Columns A to AM of the worksheets were read, culls deleted, and columns assigned simple names. Columns were created for Block and Treatment as well as a pointer to the original location of each data record.

Import of performance data was managed by a custom R function which was able to select which parts of each worksheet were required, as well as renaming existing columns and creating new ones.

Import of carcase data

Data were supplied in 16 different Excel workbooks – one for each block. Carcase data for each Project feedlot lot were held in separate worksheets. Again, some manual editing was required. All data sheets were named "Summary 22 ***LLLL***" where LLLL is the Project feedlot Lot as for performance data above.

Summary calculations at the bottom of each sheet were removed and an extra column (AH) created to indicate the Cull/Remove status of each record, with 0 meaning retain for analysis and 1 allowing simple removal of culls. As for the performance data there was no direct modification of any of the data held in the files. Columns A to AH of the worksheets were read, culls removed, and columns assigned simple names. Columns were created for Block and Treatment as well as a pointer to the original location of each data record.

Import of carcase data was managed by a custom R function which was able to select which columns of each worksheet were required, as well as renaming existing columns and creating new ones.

Analysis of Performance and Carcase data

After removal of culls, largely incomplete records and individual records of carcases that had been downgraded, the data set for performance comprised 7546 head and that for carcass characteristics consisted of 7468 head. The distribution across the blocks and pens is shown in Table 4. All pens had excellent representation, with numbers ranging from 210 up to 244.

There were seventy-eight (78) carcases removed that did not meet the collaborating abattoir's AR150 grade. The project animals had been fed for and targeted at the AR150 grade. This removed 39 animals from each of the CT and DT Lots (out of 3742 and 3726carcases respectively). The 78 carcases that failed to make the desired AR150 grade specifications were downgraded to the abattoir's lower valued carcase grade of SM (69 carcases) due to dark meat colour or high pH values; or to the MSFYP grade (9 carcases).

The loss in carcase value due to downgrading of the 78 SM and MSFYP carcases was $3.21/kg HSCW, compared to the 7468 carcases that graded AR150.

Refer to Appendix 10.14 for tabulated detail of the downgraded carcases and the number downgraded by Block and treatment.

Table 1. Distribution of cell numbers across treatments and replicates (Blocks)

Block no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Treatment																
3-NOP	244	236	241	238	239	227	228	237	241	239	237	224	230	229	220	232
Control	238	235	240	242	240	227	238	235	238	238	233	210	234	219	225	234

A copy of this data was exported to Excel. A further copy was saved in R binary format to avoid having to repeat the complex import process.

For analysis, means were calculated for each of the 32 pens. Prior to this, some filtering of each variable was carried out using a one-way ANOVA as a tool to screen individual observations, with observations more than 3.5 standard deviations from the initial pen mean removed. Fitting a model with 32 'pens' allowed assessment of how much individual animals vary from that cohort's mean (A cohort defined as a particular block-treatment combination or feedlot Lot).

A 'standardised residual' of 3.5 was used as a cut-off within each Lot. Cases within each variable that are outside 3.5 SDs from the cohort mean were set to missing before calculating a mean and the number of observations for each cohort. Table 1 shows the 'best case' numbers where no trimming takes place.

All means and counts used in the final analyses were exported to Excel for convenience. Variables were identified by having either "_mean" or "_n" appended to the variable name. Binary versions of the files were created to avoid re-running the screening process.

The 32-pen means were then analysed using a mixed model ANOVA, with means weighted by the number of observations used. Project data were split into two phases with Blocks 1 to 8 as Phase 1 and Blocks 9 to 16 as Phase 2. The mixed model estimated fixed effects of Phase, Treatment and Phase X Treatment. The logic being that negative effects in phase 1 and minimal differences in phase 2 might be seen. Random effects are estimated for 'Blocks within Phase'.

All data processing and analysis took place using R version 4.3.0 running in the RStudio environment version 2023.03.01. Mixed models were fitted using the *lmer* function from the *lme4* library (version 1.1-32). The key elements of the analyses were exported to Excel, including ANOVA summary information, expected marginal (least squares) means, standard errors, confidence intervals and difference.

Several of the carcase measurements were discrete categories (dentition, bruising etc.). Many of these contained quite sparse information and were simply summarised in tables. Others such as the two marbling scores and the ossification score were managed as continuous variable with mean scores calculated for each Lot. The two variables that required more complex processing were meat colour and fat colour. Since the analysis only used AR150 grade animals all colours recorded were regarded as acceptable to some degree (Meat colour: 1B, 1C, 2, 3) (Fat colour: 0, 1, 2, 3). For Fat Colour, data were recoded as colour=0 (roughly 75%) or '1' for non-zero fat colour. Meat colour was split between 1B and 1C (roughly 27%) versus colours 2 and 3 (roughly 73%). This proportion was calculated for each Lot and analysed using the same mixed model formulation as other variables. Because the proportions being analysed were all between 0.2 and 0.8 and based on more than 200 animals per Lot, there was no advantage in pursuing more complex logistic regression models.

Import of Dry Matter percentage data

The Dry Matter percentage (DM%) data for most of the rations used was supplied in an Excel file containing information on ration numbers 1, 2, 5, 6 and 8 at varying times. It also contained dry matter percentages for the NOP supplement pellet. Further information on ration numbers 3 and 7 was available in a 'Ration history report' from the feedlot. For all starter rations used (numbers 1,2,3,7,8) the average DM% from available records was used. For the primary rations (numbers5 & 6), a *loess* (Locally Estimated Scatterplot Smoothing) trend was fitted to each ration using the R *loess* function. The daily estimated smooth trend was saved to create a 'lookup value' for any trial day.

The construction of daily DM% estimates across the whole project required complex intervention and editing of the Excel source at an unexpected level. As such, the data file was modified with several new data sheets that trace the development of a dry matter lookup. The final lookup table was imported into R and allowed the estimated DM% of each ration to be added to each day a ration was used.

Key dates

Each of the animal performance data sheets contained columns that identified when key events such as induction, first day on feed, reimplant and final weighing were identified (Table **2**.

Table 2. Key event dates for analysis of feed intake periods.

Block number	1st day on feed	HGP re-implantation	Final session
1	2/06/2022	11/08/2022	20/10/2022
2	9/06/2022	18/08/2022	27/10/2022
3	16/06/2022	25/08/2022	3/11/2022
4	23/06/2022	1/09/2022	10/11/2022
5	29/06/2022	7/09/2022	16/11/2022
6	6/07/2022	14/09/2022	23/11/2022
7	14/07/2022	22/09/2022	1/12/2022
8	20/07/2022	28/09/2022	7/12/2022
9	19/08/2022	28/10/2022	5/01/2023
10	24/08/2022	1/11/2022	10/01/2023
11	1/09/2022	9/11/2022	19/01/2023
12	7/09/2022	16/11/2022	25/01/2023
13	15/09/2022	24/11/2022	2/02/2023
14	23/09/2022	2/12/2022	10/02/2023
15	29/09/2022	8/12/2022	16/02/2023
16	7/10/2022	14/12/2022	22/02/2023

These dates were imported into R as a separate table for use with Feed Intake data.

Import of Feed Intake data

The quantity of each ration supplied to each pen on each day were supplied in two Excel files. Data for the Control pens had a relatively simple structure, while the data for the Treated animals was much more complex. Prior to data import, it was noted that due to a feedlot software problem, no data were available for November 13th 2022. This missing day impacted Blocks 5 onward.

Control treatment data

Data were held in a single sheet identifying the Date, Block, Lot, Pen, and Ration number of each record. To simplify data import this sheet was copied, some extraneous information removed, and 'Total amount fed' calculated for each record. This allowed for residual quantities on arrival and exit of pens. At this point an estimated amount of Ration 6 (Control treatment Finisher ration) supplied on November 13th was calculated by averaging the amounts supplied on the 12th and 14th and manually added to the Excel file. These records are highlighted in the data sheet. Import of control data was straight forward, with Treatment and Lot identification and coded to ensure consistency with earlier analyses.

3-NOP Treatment data

Data were held in two separate sheets – one containing starter rations and the other with the 3-NOP Finisher Ration 5. Starter consumption was simply structured, similar to the Control information above. These data were imported from Excel without having to modify the worksheet contents.

Data for the 3-NOP Finisher Ration 5 were complicated by information on the quantity of pellets supplied with each batch of ration (the pellet inclusion rate PIR_t). A 3-NOP Finisher pen could be supplied with quantities from several different ration mixes on the one day. Similarly, a particular batch of ration could be supplied to several pens on the one day. The Ration 5 data sheet was copied, some extraneous information removed, and 'Total amount fed' (Fi_t) calculated for each record. This allowed for residual quantities on arrival and exit of pens. At this point an estimated amount of Ration 5 supplied on November 13th was calculated by averaging the amounts supplied on the 12th and 14th and manually added to the Excel file. An estimated pellet inclusion was calculated in a similar fashion from the days on either side. These records are highlighted in the data sheet. Once modifications had been carried out, data were imported into R.

The next step required alignment of the Starter ration intakes and the Ration 5 intakes by adding zero pellet inclusion to the starter values. The amount of ration batch supplied was multiplied by the PIR_t to give the (approximate) quantity of pellets supplied to the pen (PI_t).

The amount of 3-NOP supplied in pellets was calculated as: PI_t x $NOPconc_t$. The $NOPconc_t$ refers to the estimated 3-NOP concentration on any day. For each pellet batch, the laboratory measured concentrations of 3-NOP at start and end of storage were used to linearly interpolate the estimated 3-NOP concentration while that batch was in use. These estimates formed a look-up table for deriving 3-NOP intake parameters. Combined starter and ration 5 data were sorted by date and ration number within each pen, and the result merged with the control data to form a single feed intake file with 5296 records.

Processing of feed intake data

The combined feed intake data was merged with information on DM% and key dates as noted above. The dry matter intake (DMI) for each ration supply event was calculated by multiplying the amount supplied by the proportion of DM. At this point the key quantities for each day are the feed intake (FI_t), the dry matter intake (DMI_t), the number of head present in the pen on that day (NHd_t), the pellet intake (PI_t) and the NOP intake ($NOPI_t$). For each pen the cumulative amounts for each of these variables over their time in the feedlot was calculated. The NHd calculation was modified to account for multiple deliveries on the same day.

For any required interval, the cumulative amounts to the beginning of the interval, and the cumulative amounts at the end were determined. Subtracting the starting cumulative values from the ending cumulative values gives the total intake amounts for the interval. Dividing interval totals for FI, DMI, PI and $NOPI_t$ by the interval total NHd_t results in intakes on a per head per day basis. Actual number of days in the calculations are not required because it is cancelled out i.e., DMI over 28 days divided by NHd_t over 28 days.

Thus, over any interval average daily FI, average daily DMI, average daily PI (all in kg/hd/d) & average daily NOPI (mg/hd/d) can be derived. Lastly, to express 3-NOP relative to DMI, the interval total NOPI is divided by the interval total DMI, with the result in mg 3-NOP/kg DMI.
This calculation was carried out for:

- First DOF to Reimplant (DOF 70) – combined with ADG to give Feed Conversion ratios.
- Reimplant (DOF 70) to Final (DOF 140) – combined with ADG to give Feed Conversion ratios.
- First DOF to Final (DOF 140) – combined with ADG to give Feed to Gain ratios.
- First DOF to DOF 21 – mostly (but not exclusively) Starter ration (Period 1)
- DOF 22 to DOF 49 (Period 2)
- DOF 50 to DOF 77 (Period 3)
- DOF 78 to DOF 105 (Period 4)
- DOF 106 to DOF 133 (Period 5)

The last 4 intervals are each 28 days long, and the final interval corresponds (roughly) to when animals were moved from their home pens to their dispatch pens. At this point onward (DOF 133 to DOF 150) intake records were considered less reliable.

To avoid unnecessary recalculation of any of the interval data above were save in binary format as well as exporting to Excel.

Analysis of Feed Intake data
For the key intervals corresponding to liveweight measurement, data for daily FI, DMI, Feed conversion ratios for both 'As fed' and 'DM' basis were analysed using a similar linear mixed model to carcase and performance data. That is, with fixed effects for project phase, treatment and their interaction, with block within phase as a random effect. In respect to earlier analysis, the feed intake analysis differs in that no weighting is involved since there are single measurements for each pen.

A more complex model was used for the five 21- or 28-day DMI periods. Only FI and DMI can be analysed since weight is not available at that resolution. The periods were regarded as repeated measurements within each pen leading to fixed effects being estimated for Phase, Treatment, Feed Period, and all interactions. Block within Phase, and Pen within Block were specified as random effects. A Treatment x Phase component was estimated for each feeding period. Summary information from the analyses along with means, standard errors and comparisons were exported to Excel.

Analysis of Cull steer data
Data were assembled as counts per project pen out of the number of animals inducted per pen. Categories considered were Buller culls, Hospital culls, Project culls, Mortality and the combined number 'rejected' from the trial. In addition, the number of animals that spent any time hospitalised was derived (Hospital pulls (Morbidity)).

The categories and calculations were:

Morbidity (Hospital pulls) = [(every project steer pulled for a hospital ailment(only first visit counted))/(head inducted into the project)] * 100.

Mortality % = [(head dead from any cause)/(head inducted into the project)] * 100. Head dead included (Buller cull &Mortality) + (Hospital Cull & Mortality) + (Mortality) + (Project cull & Mortality).

Buller % = [(unique head culled due to buller syndrome)/(head inducted into the project)] * 100.

Hospital culls % = [(head culled from the hospital (included buller culls that had been hospitalised and then culled from the hospital))/(head inducted into the project)] * 100.

Project culls % = [(head culled from the project for reasons other than hospital or buller syndrome(missing animal, missed measurement session, missed HGP reimplant))/(head inducted into the project)] * 100.

Rejects % = [(total of all cull reasons)/(head inducted into the project)] * 100.

Some animals received multiple category descriptions. For example, an animal could be described as a buller and a mortality. However to simplify health data and avoid counting the one animal multiple times the following definitions were applied. Mortality takes precedence over Hospital, which takes precedence over Buller and Project. In the case of the example provided, animal would be classified as mortality only.

Data were analysed as a generalized linear model assuming a binomial distribution with the default logit link. As preliminary inspection showed little difference between treatments, a simple two-way (Block + Treatment) model was fitted. Analysis of deviance tables were produced for each variable analysed and the residual deviance tested for possible over-dispersion.

10.14 Tabulated parameters of downgraded project carcases

Table 1. Parameters of carcases graded SM.

Block	Treatment	RFID	Visual.tag	Kill.date	Kill.lot	Kill.Body	Grade	HSCW (kg)	Meat.colour	pH.level
1	DT	982 123747887414	2658279	2/11/2022	43740	233	SM	421.0	4	5.52
1	DT	982 123754951607	2658471	2/11/2022	43740	219	SM	473.0	4	5.57
4	CT	951 000316700348	2670000	23/11/2022	43890	34	SM	456.5	5	5.94
4	CT	982 000029747526	2670024	23/11/2022	43892	325	SM	465.0	5	5.89
4	DT	982 123760531490	2659716	23/11/2022	43893	487	SM	457.0	5	5.96
6	CT	982 123530570332	2670697	6/12/2022	43972	210	SM	481.0	4	5.76
6	CT	982 123746226562	2670708	6/12/2022	43972	127	SM	448.5	4	5.77
6	CT	982 123750868925	2670756	6/12/2022	43972	200	SM	431.0	5	5.91
6	CT	982 123740659525	2670788	6/12/2022	43974	431	SM	442.0	5	5.83
6	CT	942 000039013109	2670800	6/12/2022	43972	142	SM	422.5	6	6.2
6	CT	982 123760505292	2670809	6/12/2022	43972	211	SM	419.5	5	5.9
6	CT	942 000036288922	2670907	6/12/2022	43972	209	SM	414.0	6	5.94
6	DT	942 000035856931	2660798	6/12/2022	43973	297	SM	479.0	4	5.77
6	DT	942 000035856061	2660809	6/12/2022	43971	6	SM	493.0	6	6.45
6	DT	942 000040018222	2660811	6/12/2022	43971	99	SM	464.0	5	5.95
6	DT	982 123745874631	2660886	6/12/2022	43971	54	SM	460.5	6	6.05
7	CT	982 123758865797	2671230	14/12/2022	44022	109	SM	445.0	4	5.77
7	CT	982 123742701933	2671367	14/12/2022	44022	115	SM	468.5	6	6.02
8	DT	982 123753258207	2661298	20/12/2022	44073	206	SM	416.0	7	6.83
8	DT	982 123755760795	2661340	20/12/2022	44073	205	SM	456.0	7	6.81
8	DT	942 000039311322	2661411	20/12/2022	44075	409	SM	414.0	5	5.88
8	DT	951 000322415536	2661423	20/12/2022	44073	197	SM	440.0	7	6.76
9	CT	982 123743411156	2673811	19/01/2023	44160	68	SM	405.0	4	5.87
10	CT	982 123711377445	2674389	23/01/2023	44176	132	SM	426.0	6	6.43
10	DT	982 123768693635	2664071	23/01/2023	44175	16	SM	336.5	6	6.43
11	CT	951 000322072816	2674775	31/01/2023	44204	440	SM	419.5	7	6.69

Block	Treatment	RFID	Visual.tag	Kill.date	Kill.lot	Kill.Body	Grade	HSCW (kg)	Meat.colour	pH.level
11	CT	982 123775742871	2674777	31/01/2023	44202	179	SM	394.0	6	6.43
11	CT	982 123775742571	2674803	31/01/2023	44202	187	SM	417.0	6	6.37
11	CT	951 000316669899	2674826	31/01/2023	44202	207	SM	502.0	6	6.35
11	CT	942 000039392669	2674855	31/01/2023	44202	198	SM	427.5	7	6.57
11	CT	982 123723862843	2674874	31/01/2023	44202	175	SM	384.0	6	6.37
11	DT	982 123752966920	2664788	31/01/2023	44201	87	SM	409.0	6	5.98
11	DT	951 010001870781	2664819	31/01/2023	44201	28	SM	373.0	5	5.92
12	CT	982 123740442341	2736329	7/02/2023	44252	286	SM	402.5	5	5.76
12	CT	982 123759856332	2736392	7/02/2023	44250	80	SM	410.0	5	5.79
12	DT	951 000320556183	2664653	7/02/2023	44251	204	SM	386.0	5	5.82
12	DT	982 123768694550	2664929	7/02/2023	44251	233	SM	364.0	3	5.64
12	DT	982 123775144612	2664942	7/02/2023	44253	385	SM	388.0	5	5.9
12	DT	951 010001547081	2664988	7/02/2023	44253	394	SM	422.5	6	6.19
12	DT	982 123740288687	2665016	7/02/2023	44253	446	SM	347.0	7	6.3
12	DT	951 000319588887	2665098	7/02/2023	44251	189	SM	397.0	5	5.98
12	DT	982 123742282775	2665103	7/02/2023	44253	445	SM	308.0	3	5.52
13	CT	982 123763636001	2736931	14/02/2023	44319	115	SM	418.5	7	6.59
13	DT	982 123738666138	2696476	13/02/2023	44311	326	SM	296.5	7	6.62
13	DT	942 000035919186	2696519	14/02/2023	44320	181	SM	393.0	5	5.94
13	DT	951 010001850232	2696594	14/02/2023	44320	118	SM	437.0	6	6.39
14	CT	942 000037973454	2737436	20/02/2023	44349	12	SM	397.0	7	6.68
14	CT	982 123757787419	2737461	20/02/2023	44351	338	SM	378.5	7	7.01
14	CT	982 123752747965	2737479	20/02/2023	44349	71	SM	382.0	7	7.06
14	CT	982 123765513078	2737496	20/02/2023	44349	106	SM	408.5	5	5.76
14	DT	982 123779791064	2696952	20/02/2023	44350	189	SM	410.5	5	5.89
14	DT	982 123778123327	2696956	20/02/2023	44350	196	SM	358.5	6	6.38
14	DT	982 123757031993	2696974	20/02/2023	44350	201	SM	415.5	6	6.16
14	DT	982 123760051497	2697002	20/02/2023	44350	218	SM	350.0	4	5.82
14	DT	942 000042308465	2697090	20/02/2023	44350	231	SM	407.5	7	6.87
14	DT	937 000000772486	2697101	20/02/2023	44350	233	SM	434.0	4	5.81

Block	Treatment	RFID	Visual.tag	Kill.date	Kill.lot	Kill.Body	Grade	HSCW (kg)	Meat.colour	pH.level
14	DT	942 000038087473	2697112	20/02/2023	44350	209	SM	370.0	5	6.03
14	DT	951 000320399585	2697144	20/02/2023	44350	220	SM	442.0	4	5.78
14	DT	982 123779669162	2697190	20/02/2023	44350	170	SM	401.0	7	7.14
15	CT	982 123757275496	2737628	28/02/2023	44406	45	SM	378.5	5	6.08
15	CT	942 000033037590	2737629	28/02/2023	44406	28	SM	364.0	6	6.09
15	CT	982 123773878045	2737639	28/02/2023	44408	281	SM	420.0	5	5.91
15	CT	951 000321312350	2737674	28/02/2023	44406	72	SM	437.5	6	6.18
15	DT	982 123757624757	2697284	28/02/2023	44407	255	SM	479.5	7	6.68
15	DT	982 123776459533	2697384	28/02/2023	44407	145	SM	432.5	5	5.75
16	CT	900 093001989884	2738088	7/03/2023	44457	220	SM	349.0	6	6.34
16	CT	982 123771946170	2738097	7/03/2023	44459	432	SM	379.0	6	6.33
16	CT	982 123771945908	2738108	7/03/2023	44459	431	SM	375.5	6	6.28
16	CT	982 123771945928	2738111	7/03/2023	44457	165	SM	389.5	7	6.88

Table 2. Parameters of carcases graded MSFYP or MSF

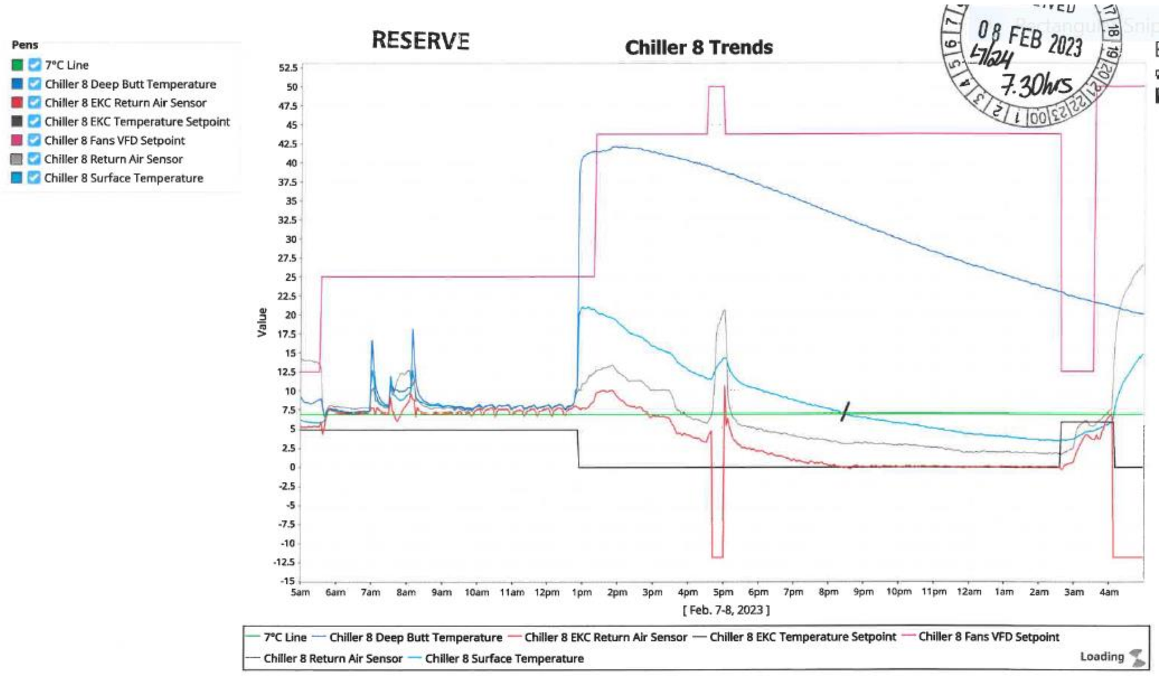
Block	Treatment	RFID	Visual.tag	Kill.date	Kill.lot	Kill.Body	Grade	HSCW (kg)	Meat.colour	pH.level
6	DT	951 000301423002	2660741	6/12/2022	43973	307	MSFYP	422.5	2	5.49
7	DT	982 123721261505	2661008	14/12/2022	44025	485	MSFYP	376.0	2	5.52
7	DT	982 123760981961	2661124	14/12/2022	44023	254	MSFYP	439.5	1C	5.63
8	CT	982 123775742627	2671525	20/12/2022	44074	291	MSFYP	447.5	2	5.51
9	CT	942 000038765150	2673689	19/01/2023	44162	438	MSF	409.0	3	5.53
10	DT	942 000040943789	2664042	23/01/2023	44175	33	MSFYP	395.5	1C	5.46
12	CT	982 123768456393	2736369	7/02/2023	44250	15	MSFYP	387.5	1C	5.52
14	CT	982 123764091362	2737328	20/02/2023	44351	326	MSFYP	457.5	1C	5.47
15	CT	982 123759316658	2737640	28/02/2023	44406	50	MSFYP	350.0	1C	5.47

Table 3. Number of downgraded carcases by block and treatment

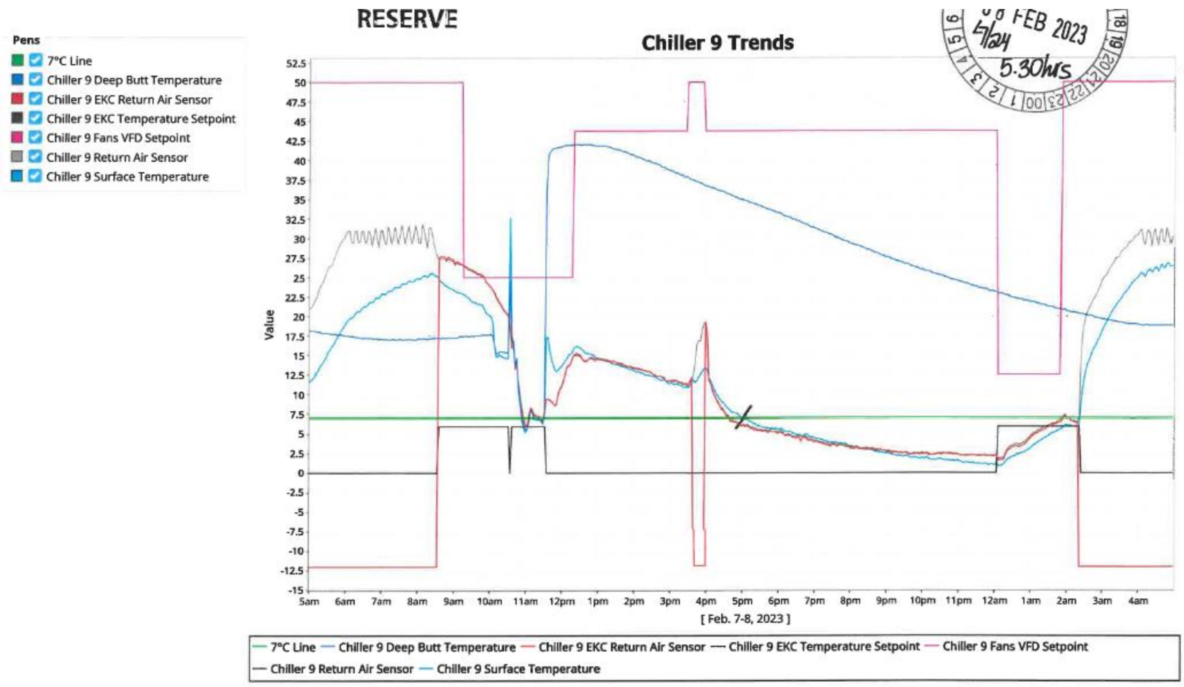
Block	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Treatment																
3-NOP	0	0	0	2	0	7	2	1	2	1	6	3	1	5	5	4
Control	2	0	0	1	0	5	2	4	0	2	2	7	3	9	2	0

10.15 Collaborating abattoir carcase side chiller temperature profiles

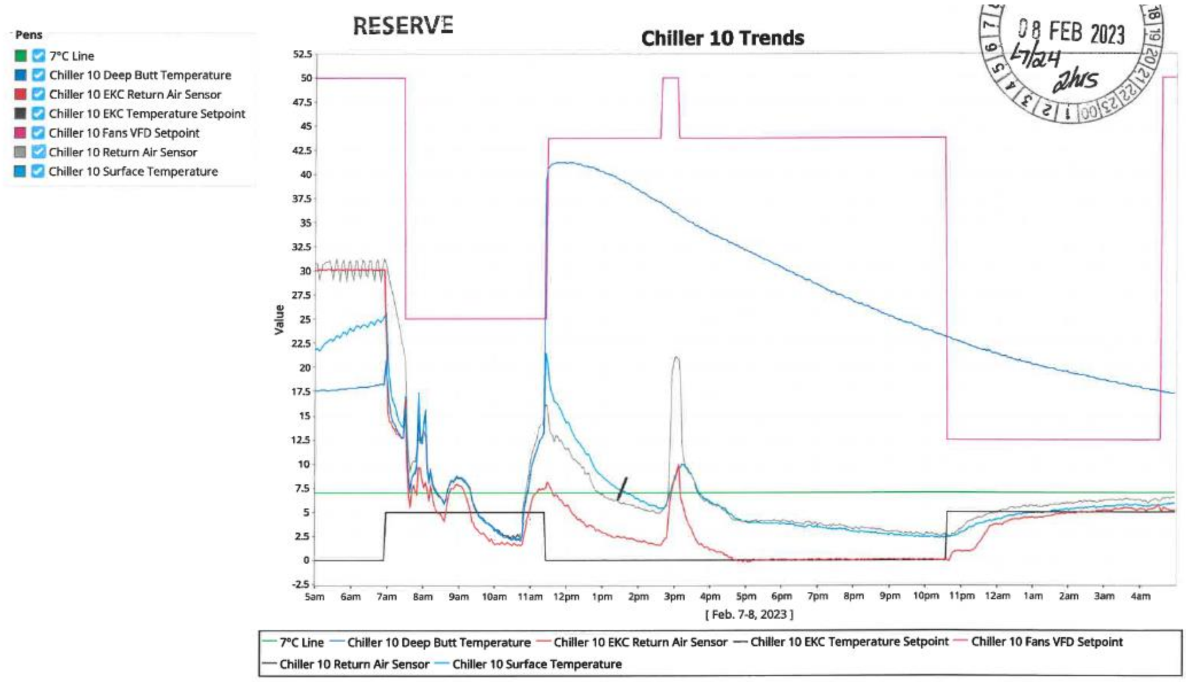
Block 11: 7/2/2023 Carcase side chiller temperature profiles 3NOP Project



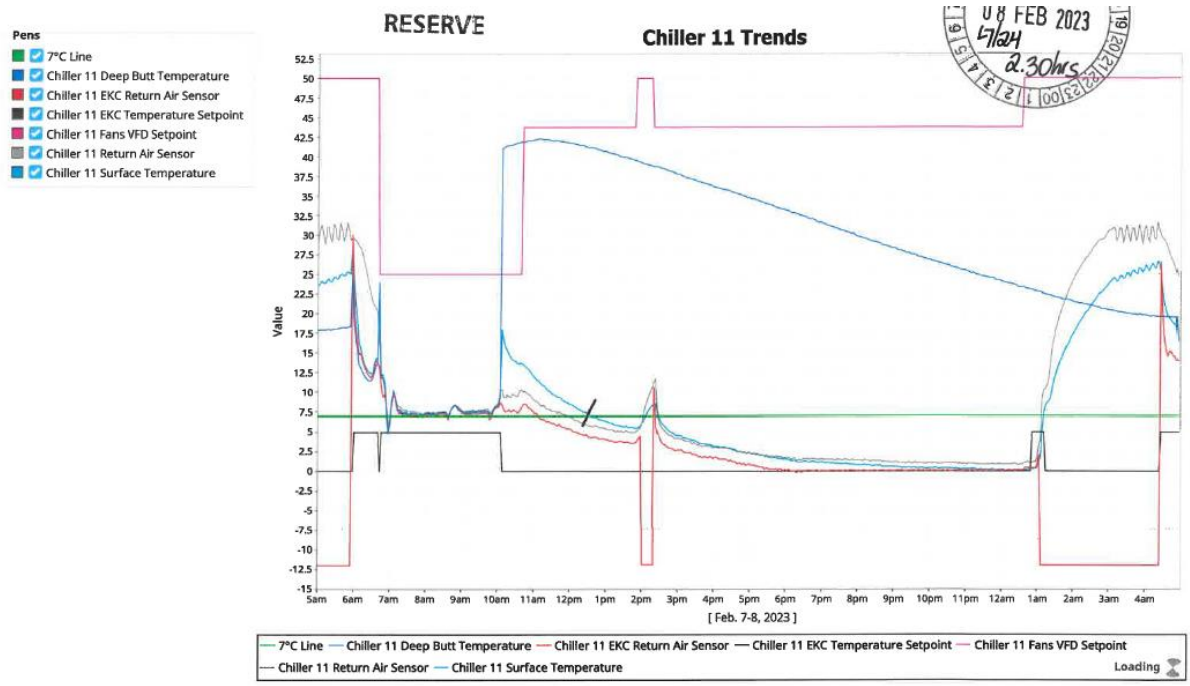
	MIN	MAX	AVE		MIN	MAX	AVE		MIN	MAX	AVE
7°C Line	7	7	7	Chiller 8 EKC Temperature Set...	0	6	2.03	Chiller 8 Surface Temperatu...	3.5	31.9	8.71
Chiller 8 Deep Butt Tempera...	6.9	42.2	24.09	Chiller 8 Fans VFD Setpoint	1.6	26.5	7.11				
Chiller 8 EKC Return Air Sensor	-12	10.9	3.32	Chiller 8 Return Air Sensor	12.5	50	36.05				



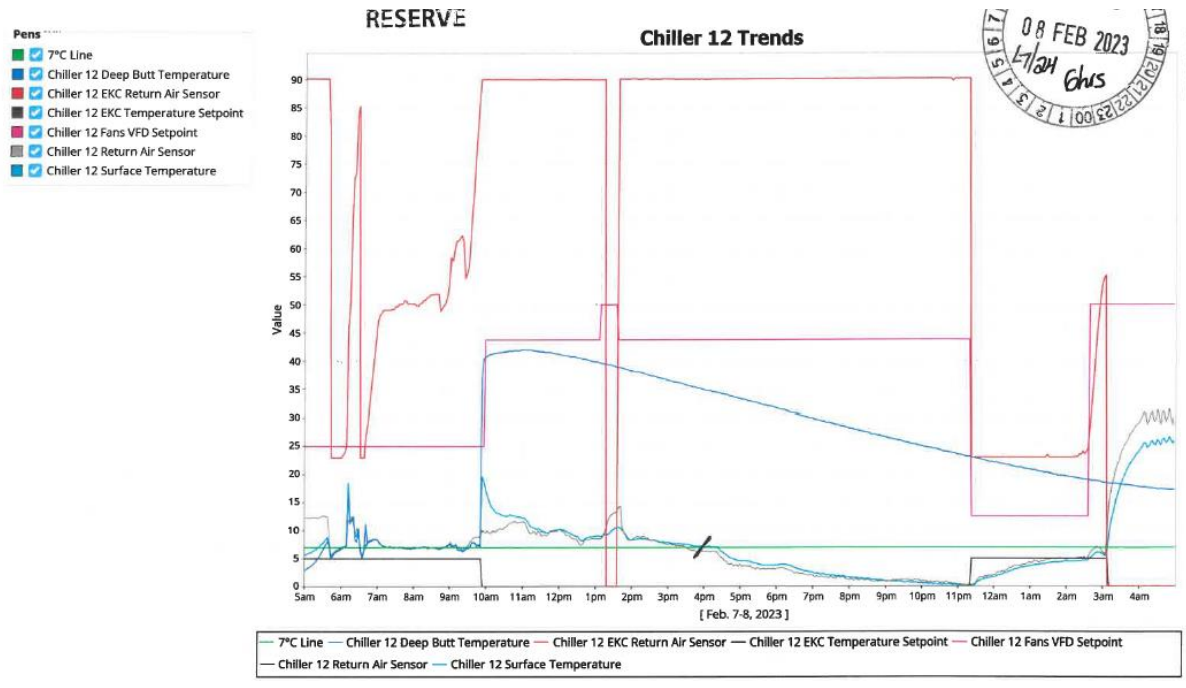
	MIN	MAX	AVE		MIN	MAX	AVE		MIN	MAX	AVE
7°C Line	7	7	7	Chiller 9 EKC Temperature Set...	0	6	1.32	Chiller 9 Surface Temperatu...	0.8	38	11.72
Chiller 9 Deep Butt Tempera...	5.4	42	25.92	Chiller 9 Fans VFD Setpoint	1.7	31.8	14.03				
Chiller 9 EKC Return Air Sensor	-12	27.8	3.21	Chiller 9 Return Air Sensor	12.5	50	41.11				



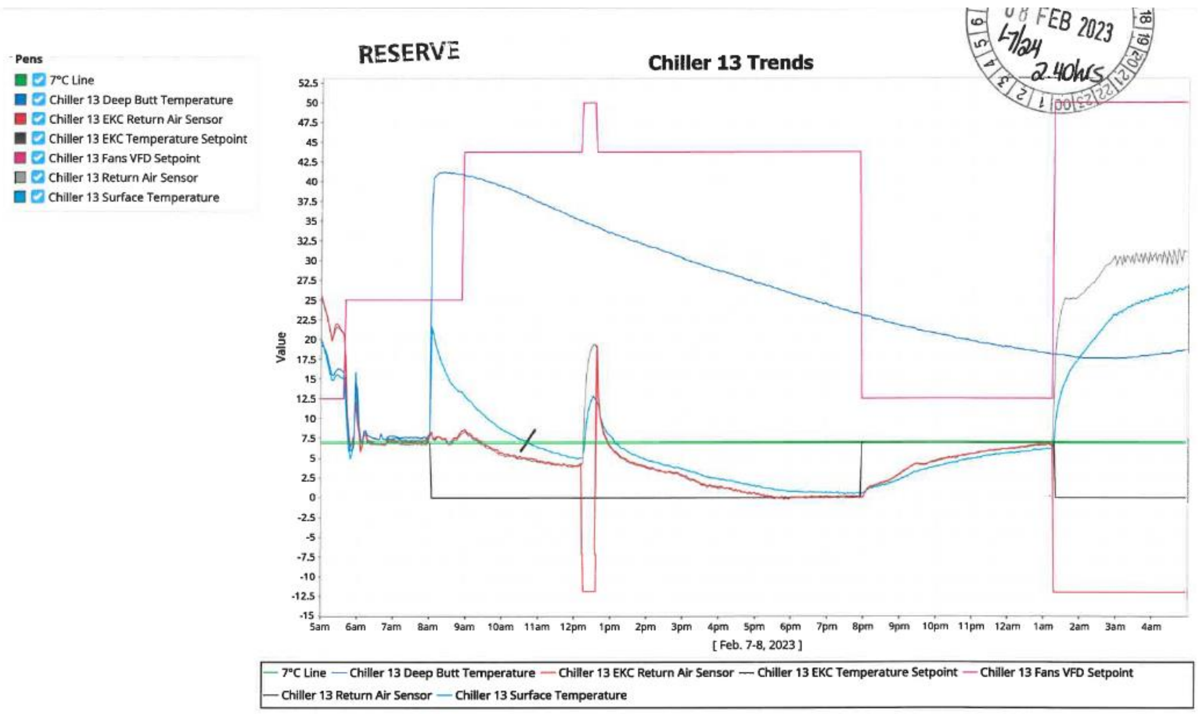
	MIN	MAX	AVE		MIN	MAX	AVE		MIN	MAX	AVE
7°C Line	7	7	7	Chiller 10 EKC Temperature Se...	0	5	2.25	Chiller 10 Surface Temperat...	2	37.3	7.5
Chiller 10 Deep Butt Temper...	1.9	41.3	23.29	Chiller 10 Fans VFD Setpoint	2.2	31.4	8.46				
Chiller 10 EKC Return Air Sensor	-0.2!	30.2:	5.49	Chiller 10 Return Air Sensor	12.5	50	33.69				



	MIN	MAX	AVE		MIN	MAX	AVE		MIN	MAX	AVE
7°C Line	7	7	7	Chiller 11 EKC Temperature Se...	0	5	1.04	Chiller 11 Surface Temperat...	0.1	29.4	8.16
Chiller 11 Deep Butt Temper...	4.8	42.3	26.54	Chiller 11 Fans VFD Setpoint	0.8	31.7	9.53				
Chiller 11 EKC Return Air Sensor	-12	30.1!	0.77	Chiller 11 Return Air Sensor	25	50	42.33				



	MIN	MAX	AVE		MIN	MAX	AVE		MIN	MAX	AVE
7°C Line	7	7	7	Chiller 12 EKC Temperature Se...	0	5	1.82	Chiller 12 Surface Temperat...	0.1	34.1	7.02
Chiller 12 Deep Butt Temper...	2.7	42	24.9	Chiller 12 Fans VFD Setpoint	0.1	31.6	7.37				
Chiller 12 EKC Return Air Sensor	-5	90.2	64.78	Chiller 12 Return Air Sensor	12.5	50	36.32				



	MIN	MAX	AVE		MIN	MAX	AVE		MIN	MAX	AVE
7°C Line	7	7	7	Chiller 13 EKC Temperature Se...	0	7	2.47	Chiller 13 Surface Temperat...	0.4	34.3	8.21
Chiller 13 Deep Butt Temper...	5.8	41.2	24.51	Chiller 13 Fans VFD Setpoint	-0.3	31.5	8.64				
Chiller 13 EKC Return Air Sensor	-12	26.3	2.09	Chiller 13 Return Air Sensor	12.5	50	34.42				

10.16 Feedlot performance of experimental treatments from DOF 0 to exit

Table 1. Effect of treatment, project phase and the treatment within phase effect on steer liveweight performance for derived exit weight.

	Parameter			Average daily gain (kg/hd/d)	
	Induction (I) liveweight (kg)	**DOF 70 Reimplant liveweight (kg)**	**DOF 151.5 Derived Exit liveweight (kg)[c]**	**DOF 70 to DOF 151.5**	**I to DOF 151.5**
Treatment					
Control	452.1	636.9	807.2	2.079	2.323
3-NOP	451.5	634.4	802.4	2.055	2.300
SE[A]	2.44	4.08	1.47	0.024	0.008
p-value[B]	0.3477	0.2832	0.0012**	0.3289	0.0015**
Phase					
1	446.6	642.0	821.5	2.189	2.454
2	456.9	629.3	788.0	1.945	2.173
SE	3.42	5.53	5.86	0.085	0.047
p-value	0.0341*	0.1047	0.0000***	0.0042**	0.0000****
Treatment x Phase					
Overall p-value	0.8593	0.9778	0.0617	0.1332	0.0033**
Control – Phase 1	446.9	643.3	825.4	2.220	2.478
3-NOP – Phase 1	446.4	640.7	817.7	2.157	2.431
SE	3.45	5.76	2.07	0.0034	0.0012
p-value	0.5898	0.4336	0.0002***	0.0695	0.0001****
Control – Phase 2	457.3	630.5	788.9	1.938	2.177
3-NOP – Phase 2	456.6	628.1	787.2	1.953	2.170
SE	3.45	5.78	2.09	0.0035	0.012
p-value	0.4309	0.4624	0.3984	0.6547	0.5658

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[C]Derived exit calculated from hot standard carcass weight and average dressing percentage of 54.3%. Dispatch occurred on average at 151.5 days on feed.

Table 2. Effect of treatment, project phase and the treatment within phase effect on steer dry matter feed intake.

	Dry matter feed intake (kg/hd/d)		
	Induction (I) to DOF 70 (reimplant)	**DOF 70 (reimplant) to DOF 151.5[C] (exit)**	**I to DOF 151.5 (exit)**
Treatment			
Control	12.23	13.20	12.75
3-NOP	12.15	13.03	12.62
SE[A]	0.09	0.06	0.06
p-value[B]	0.3936	0.0182*	0.0657
Phase			
1	12.20	13.78	13.04
2	12.19	13.60	12.33
SE	0.10	0.20	0.14
p-value	0.9726	0.0000****	0.0002***
Treatment x Phase			
Overall p-value	0.2587	0.0089**	0.4581
Control – Phase 1	12.18	13.97	13.13
3-NOP – Phase 1	12.21	13.60	12.95
SE	0.12	0.09	0.09
p-value	0.8366	0.0012**	0.0713
Control – Phase 2	12.29	12.44	12.37
3-NOP – Phase 2	12.09	12.46	12.29
SE	0.12	0.02	0.09
p-value	0.1677	0.8022	0.3978

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[c] Exit occurred on average at 151.5 days on feed.

Table 3. Effect of treatment, project phase and the treatment within phase effect on steer feed conversion ratio (dry matter feed intake: average daily gain)

	Feed conversion ratio (FCR)		
	Induction (I) to DOF 70 (reimplant)	**DOF 70 (reimplant) to DOF 151.5[C] (exit)**	**I to DOF 151.5 (exit)**
Treatment			
Control	4.75	6.40	5.50
3-NOP	4.75	6.37	5.50
SE[A]	0.11	0.08	0.02
p-value[B]	0.9554	0.6994	0.8497
Phase			
1	4.43	6.30	5.32
2	5.06	6.47	5.68
SE	0.16	0.25	0.09
p-value	0.0132*	0.5373	0.0008***
Treatment x Phase			
Overall p-value	0.1792	0.6483	0.3797
Control – Phase 1	4.40	6.30	5.30
3-NOP – Phase 1	4.47	6.31	5.33
SE	0.16	0.11	0.04
p-value	0.3791	0.9601	0.4496
Control – Phase 2	5.09	6.50	5.69
3-NOP – Phase 2	5.03	6.43	5.67
SE	0.16	0.11	0.04
p-value	0.4217	0.5527	0.6215

[A]SE – standard error of the mean
[B]P-value. Levels of significance; *when P<0.05, ** when P<0.01, *** when P<0.001 and **** when P<0.0001
[C]Exit occurred on average at 151.5 days on feed.

10.17 Carbon Baseline Assessment on the effect of Bovaer® feed supplement on the carbon footprint of trial cattle in MLA Project P.PSH.1375

Final report

Carbon Baseline Assessment on the effect of Bovaer® feed supplement on the carbon footprint of trial cattle in MLA Project P.PSH.1375

Project code: P.PSH.1375
Prepared by: Dr Stephen Wiedemann, Riley O'Shannessy and Jordan Peach
Integrity Ag

Date published: 29/08/2023.

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

This is an MLA Donor Company funded project.
Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Integrated Animal Production (IAP) was contracted by Meat and Livestock Australia (MLA) to assess the effect of Bovaer® (feed supplement) on the performance, health, carcase characteristics and carbon footprint of Australian feedlot cattle. Integrity Ag (IA) was contracted by IAP to assess the impact of feeding Bovaer® (a feed supplement designed to reduce the methane emission in cattle) on the carbon footprint of treatment (Bovaer®) and control (non-Bovaer®) cattle groups. A total of 32 experimental groups were assessed, comprising 16 treatment and 16 control. Each group comprised approx. 240 head of beef cattle (majority angus breed) and were fed for approx. 150 days in a commercial feedlot setting.

A carbon footprint and gate-to-gate emission intensity analysis was conducted to examine the impacts of the trial on performance. The study applied methods consistent with the industry carbon manual (Wiedemann and Longworth, 2021) with revisions to the enteric methane prediction for feedlot cattle and calculated reductions of enteric methane as a result of feeding Bovaer.

Based on analysis of the emissions data of the feeding groups, the emission intensity during the feedlot stage of production was 1.04 kg $CO_{2\text{-}e}$/kg LWG^{-1} for the treatment (Bovaer®) groups, and 2.30 kg $CO_{2\text{-}e}$/kg LWG^{-1} for the Control (non-Bovaer®) groups (an emission reduction of 56%).

The product carbon footprint at turnoff was 8.94 kg $CO_{2\text{-}e}$/kg LW $sold^{-1}$ for the treatment groups and 9.42 kg $CO_{2\text{-}e}$/kg LW^{-1} sold for the control cattle (an emission reduction of 5.3%).

The product carbon footprint at post-processing was estimated at 22.31 kg $CO_{2\text{-}e}$/kg Boxed $Beef^{-1}$ for the treatment cattle, and 23.37 kg/$CO_{2\text{-}e}$ boxed $beef^{-1}$ for the control cattle (an emission reduction of 4.5%).

These findings provide the Australian feedlot industry with a better understanding of the impact of feeding Bovaer® within a commercial feedlot setting.

Executive summary

Background

Integrated Animal Production (IAP) was contracted by Meat and Livestock Australia (MLA) to complete a project to assess the effect of Bovaer® (DSM Nutritional Products), a feed supplement designed to reduce enteric methane, on the performance, health, carcase characteristics and carbon footprint of Australian feedlot cattle. Integrity Ag (IA) was responsible for the project component relating to assessing the product carbon footprint.

This research will inform the Australian feedlot industry of the potential emission reduction potential and cost of abatement of feeding Bovaer® at commercial scale.

Objectives

IA component of the project included to determine the effect of feeding Bovaer® within feedlot rations on the product carbon footprint.

All objectives listed above were achieved.

Methodology

The Bovaer® feeding trial (completed by IAP) consisted of 32 pens of cattle on feed for 150 days in 16 paired feeding groups (the feedlot). Within each of the 16 pairs, 1 pen received Bovaer® within their finisher ration (treatment) and 1 pen did not receive Bovaer® (control). Each pen held 240 head of cattle. IAP provided summarised feedlot results to Integrity Ag (IA), which were used to complete the carbon baseline assessment of the feedlot and to assess the effect of Bovaer® feed supplement on the carbon footprint of the cattle feedlot trial.

The carbon footprint and gate-to-gate emission intensity analysis applied methods consistent with the industry carbon manual (Wiedemann and Longworth, 2021) with revisions to the enteric methane prediction for feedlot cattle and calculated reductions of enteric methane as a result of feeding Bovaer.

Results/key findings

The assessment found:

- Product carbon footprint for cattle from birth to feedlot exit was 8.94 and 9.42 kg CO_2-e/kg LW^{-1} sold for the treatment and control cattle, respectively.
- Product carbon footprint for cattle in feedlot stage only (Scope 1 and 2 emission) was 1.04 and 2.30 kg CO_2-e/kg LWG^{-1} for the treatment and control cattle, respectively.
- Product carbon footprint for boxed beef post-processing (Scope 1, 2 and 3 emission) was 22.31 and 23.37 kg $CO_{2\text{-e}}$/kg Boxed $Beef^{-1}$ for the treatment and control cattle, respectively.

Benefits to industry

This trial concluded that Bovaer® was effective in reducing the largest direct emission source (enteric methane), within an Australian feedlot. This improves the carbon account and product carbon footprint (emission intensity) of a feedlot enterprise. Provided the cost is low enough to make feeding Bovaer® commercially viable, this provides a key strategy to help the feedlot industry reduce business emissions and reduce the carbon footprint of grain finished beef.

Future research and recommendations

Further feedlot trials at commercial scale involving a larger trial size would provide important knowledge on the emission reduction potential and the cost of abatement of feeding Bovaer® to cattle at various days on feed. Feeding during backgrounding, and in long-fed cattle would be beneficial to examine the impact on the carbon footprint for finished beef.

Table of contents

1. Background

Integrated Animal Production was contracted by Meat and Livestock Australia to assess the effect of Bovaer® (feed supplement) on the performance, health, carcase characteristics and carbon footprint of Australian feedlot cattle. Integrity Ag was contracted by Integrated Animal Production to assess the impact of feeding Bovaer® on the carbon footprint of treatment (Bovaer®) and control (non-Bovaer®) cattle groups. Experiment groups were fed for 150 days in a commercial feedlot setting and were primarily composed of Angus cattle.

Bovaer®, also known as 3-nitrooxypropanol (3-NOP), is a synthetic product developed and manufactured by DSM. It is currently designed to be administered to livestock as a feed additive and has been found to reduce enteric methane emissions from ruminants. However, no study to date has examined the carbon footprint of Australian cattle fed Bovaer in the finishing phase. This study was established to examine productivity and GHG impacts from Bovaer feeding. The present report is focused on the carbon footprint.

2. Objectives

The Integrity Ag component of the project included to determine the effect of feeding Bovaer® within feedlot rations on the product carbon footprint.

3. Methodology

This study reported the product carbon footprint per kilogram of:

- Beef (liveweight) sold from the feedlot, birth to feedlot exit.
- Beef (liveweight) produced within the feedlot.
- Beef (boxed) following primary processing.

This was reported for both treatment and control groups.

3.1 GHG calculation methods

3.1.1 Emission boundaries

The product's carbon footprint assessed all impacts up to and including the primary processing (cradle-to-box).

For the purpose of the enterprise carbon account, emissions were disaggregated into scope 1, 2 and 3 sources according to the GHG Protocol (Ranganathan et al. 2004). These emission sources are described as follows:

Scope 1: "Direct GHG emissions occur from sources owned or controlled by the company".
Scope 2: "Accounts for GHG emissions from the generation of purchased electricity consumed by the company."
Scope 3: "Are a consequence of the activities of the company but occur from sources not owned or controlled by the company. Some examples of Scope 3 activities are extraction and production of purchased materials; transportation of purchased fuels; and use of sold products and services." These can be further broken down into two sources:

Upstream emissions: from pre-farm sources such as the production of purchased grain, manufacturing of chemicals, purchased livestock emissions and the burning of fossil fuels, including the extraction, production and transport of fuel and electricity. These sources were included in the present analysis.
Downstream emissions: are post-farm emissions associated with the processing of cattle, including emissions from transportation, meat processing and distribution. These sources were included up until post-processing in the present analysis.

3.2 Scope 1 & 2 emission calculation methods

Climate change impacts were modelled as the amount of greenhouse gas emissions (GHG) throughout the supply chain. Emission estimation approaches followed the published work of (Wiedemann et al. 2017) for feedlots and (Wiedemann et al. 2016) for grazing operations, with modifications made to reflect revisions to the National Inventory Report (NIR) and to report using the business accounting framework (scope 1, 2, 3 emissions, see sections 12.2.1, 12.2.2 and 12.3). The impacts were then converted to carbon dioxide equivalent units ($CO_{2\text{-}e}$) using 100-year global warming potentials (GWP100) of 28 for methane (CH_4) and 265 for nitrous oxide (N_2O) (IPCC 2014).

3.2.1 Scope 1 livestock emissions

Livestock emissions, including manure management emissions and enteric emissions from feedlot cattle, were determined using methods reported in the NIR (Commonwealth of Australia 2021a) except for enteric methane, where the more recent methods of the IPCC (2019) were applied, which was in-line with baseline research in Australian feedlot cattle (Almeida et al. 2023). This used a factor of 10 g CH_4/kg DMI^{-1} for high grain rations, and 13.6 g CH_4/kg DMI^{-1} for other rations.

Data was collected from the site regarding animal attributes (weight on entry and exit to feed pen, age, origin and feed period), feed intake (kg/hd/day), dietary crude protein (% of dry matter) and dry matter (% of moisture of feed stuffs supplied). This information was collected and collated by the feedlot site and project participants. The Integrity Ag Feedlot Footprint model was used to calculate emissions.

3.2.2 Feedlot services and feed milling

Energy use associated with the feedlot operations, including electricity and coal for the feed mill, was included using records of purchased electricity. Emissions from diesel and petrol were included based on records maintained by the feedlot.

Scope 1 and 2 fuel and electricity emissions were calculated from methods in the National Greenhouse Accounts Factors (Commonwealth of Australia 2021b).

3.3 Scope 3 emission calculation methods

Attributional life cycle assessment (aLCA) datasets were used to determine scope 3 emissions, consistent with international standards (ISO 2006). Scope 3 fuel and electricity emissions were determined using the National Greenhouse Accounts Factors (Commonwealth of Australia 2021b). Other factors associated with inputs utilised Life cycle inventory (LCI) data sourced from the Australian Unit Process LCI Library (Life Cycle Strategies 2015), published literature and the IA database. The LCA modelling was conducted using SimaPro v9.4 (Pré-Consultants 2022). Specific details for major scope 3 emission sources are detailed in sections 4.3.1, 4.3.2 and 4.3.3.

3.3.1 Feed

Feed consumed within the feedlot are a Scope 3 emission source. Feed impacts were determined by collecting commodity purchase data from the feed mill and ration details (dry matter %, ingredient composition and inclusion, protein %). Feed sources were assumed to be local for cereal grains. Environmental impacts from feed grains and commodities were derived from databases (AustLCI) and IA custom databases.

3.3.2 Livestock emissions

Emissions from feeder cattle were included using the IA VCF (Verified Carbon Footprint) model, which applied methods published by IA (Wiedemann et al. 2016) to determine emissions from feeder cattle based on breeder location. Emissions included livestock emissions (enteric methane and manure) and purchased inputs. Total emissions were determined based on the weight of cattle at induction.
For this analysis, as these cattle had been purchased by the feedlot operator, their pre-feedlot emissions are allocated into the Scope 3 feeder cattle emissions.

3.3.3 Other emission sources

Staff travel to and from work in non-company vehicles was included in assessing Scope 3 GHG emissions. This inclusion was done using a factor of 0.40 kg CO_2 /km of car transport (AusLCI 2020). Downstream scope 3 emissions, such as those from meat processing, were modelled separately, using data provided in AMPC and MLA's Environmental Performance Review: Red Meat Processing Sector 2015 (Ridoutt et al. 2015).

3.4 Bovaer® feeding trial

Bovaer® (DSM Nutritional Products Pty Ltd, Australia) is a feed supplement designed to reduced methane emissions from ruminant animals, in this case feedlot cattle.

The Bovaer® feeding trial layout included the placement of 32 pens of cattle placed on feed in 16 paired feeding groups. Within each of the 16 pairs, 1 pen received Bovaer® within their finisher ration (treatment pen) and 1 pen did not receive Bovaer® (control pen). Each pen held 240 head of cattle. Other ration ingredients, cattle attributes, feeding programs and environmental conditions were maintained as similar as possible across and between the treatment/control feeding groups. Integrated Animal Production provided Integrity Ag with animal and feeding data aggregated by pen group. This included treatment group number, Bovaer® inclusion status, head number in, days on feed, head number exit, daily weight gain, and feed consumption. There was no individual animal data provided for animals from the feeding trial.

The aggregated data for the 32 trial pens provided by IAP was exclusive to animals that fed with and processed with their allocated pen. Culls, deaths, rejects were not included. This aggregated data was used unamended by Integrity Ag.

The pens of treatment cattle received Bovaer® from feedlot induction to feedlot exit. The Bovaer® was mixed within their routine delivery of feed rations. Mean dose rate for Bovaer® was 83.98 mg/kg of dry matter feed intake . The Bovaer® was supplied in pellet form, and manufactured with a nutritional formulation that provides the same net energy for gain (NEg) concentration as the steam-flaked grain it replaces in the finished ration.

3.4.1 Emission reduction

Emission reduction (abatement) calculation methods when using anti-methanogenic supplements such as Bovaer® have been generally defined in Australia by the Livestock Emission Framework (Wiedemann and Longworth 2021; DEECCW 2023). The mitigation rate applied (Table 1) is based on a recent publication from the University of New England (Almeida et al. 2023), on feedlot diets equivalent to a commercial Australian feedlot diet.

Table 1. Methane mitigation rates of Bovaer®, when fed to feedlot cattle on a high-grain diet, at different inclusion rates.

Bovaer® dose (mg 3-NOP $kgDMI^{-1}$)	Reduction in enteric methane
50	66%
75	80%
100	85%
125	88%

4. Results

4.1 Product carbon footprint

The Bovaer® feeding trial assessed the impact of feeding this supplement on the emission estimates. Emission mitigation was assessed by entering the aggregated pen 'close outs' into Integrity Ag emission analysis calculators. Emission abatement rates (refer Table 1) were applied to the treatment groups based on the rate of Bovaer® in the formulated rations. No emission abatement was applied to the control groups.

Product carbon footprint is a suitable means for comparing the emissions generated within and between production systems. Within this assessment, the product carbon footprint was calculated for:

- Supply chain emission intensity, being liveweight sold from the feedlot, birth to feedlot exit (section 4.1.1).
- Feedlot emission intensity, being liveweight produced within the feedlot stage (section 4.1.2).
- Boxed beef emission intensity, being boxed beef post primary processing, from birth to boxed beef (section 4.1.3).

4.1.1 Supply chain emission intensity (carbon footprint)

Scope 1, 2 and upstream scope 3 emissions are reported in kg CO_{2-e}/ kg of LW sold to illustrate the emissions across the full life of the animal up to the point of exit from the feedlot. Here the emission intensity is expressed relative to LW sold. It includes pre-feedlot emissions from purchased cattle, grain, fuel, and transport.

The carbon footprint of cattle owned by the feedlot operator (scope 1, 2 and 3) was 8.94 kg CO_{2-e}/kg LW^{-1} sold for treatment group cattle and 9.42 kg CO_{2-e}/kg LW^{-1} sold for control group cattle (Table 2). Feeder cattle emissions were the largest contributor to the emission profile, with 85% and 79% contribution from treatment and control groups, respectively. This is because a larger proportion of an animal's life occurs prior to the feedlot. The breeding herd are also attributed to the feeder animal, so the emissions over the animal's life are much higher prior to the feedlot than in the feedlot.

Table 2. Carbon footprint (kg CO_{2-e} kg/LW^{-1} sold) for cattle in trial. A hotspot analysis indicates high (red), medium (yellow to orange) and low (green) emission sources.

Class	Control	Treatment
Scope 1		
Feedlot enteric methane	0.68	0.15
Feedlot manure direct nitrous oxide	0.15	0.15
Feedlot manure methane	0.05	0.05
Feedlot services	0.02	0.02
Feedmilling & feed production	0.01	0.01
Scope 2		
Feedlot services	0.04	0.04
Feedmilling & feed production	0.00	0.00
Scope 3		
Feeder cattle emissions	7.52	7.58
Feedlot services	0.01	0.01
Feedmilling & feed production	0.88	0.87
Manure indirect nitrous oxide	0.03	0.03
Transport	0.02	0.02
Carbon footprint (kg CO_2-e/kg LW^{-1} sold)[A]	**9.42**	**8.94**

[A] *Excludes custom-fed cattle and cattle fed at external feedlots.*

4.1.2 Feedlot emission intensity – Scope 1 & 2

For the feedlot only component of the supply chain, the scope 1 and 2 emission intensity were 1.04 and 2.30 kg $CO_{2\text{-}e}$ kg/LWG^{-1} for the treatment and control cattle respectively (see Table 3). Emission rates for enteric methane were calculated to be 2.0 and 10.0 g CH_4 /kg DMI^{-1} for the treatment cattle and control cattle respectively, based on the emission reduction with Bovaer.

With reduction in feedlot enteric methane from 1.64 to 0.37 kg $CO_{2\text{-}e}$ kg LWG^{-1} of cattle fed Bovaer®, the next highest area of emission contribution was feedlot manure direct from nitrous oxide (see Table 3). Australian research has shown this emission source may vary from >1% of excreted N, to <0.01%, and the inventory applies a factor of 0.5% (Wiedemann and Longworth 2020). This large range suggests there is more potential to reduce these emissions, either via mitigation, or revision of the inventory to better reflect Australian conditions.

Table 3. Scope 1 & 2 emission intensity (kg $CO_{2\text{-}e}$ kg LWG^{-1}) for cattle in trial. A hotspot analysis indicates high (red), medium (yellow to orange) and low (green) emission sources.

Class	Control	Treatment cattle
Scope 1		
Feedlot enteric methane	1.64	0.37
Feedlot manure direct nitrous oxide	0.37	0.36
Feedlot manure methane	0.13	0.13
Feedlot services	0.05	0.05
Feedmilling & feed production	0.03	0.03
Scope 2		
Feedlot services	0.09	0.09
Feedmilling & feed production	0.00	0.00
Emission intensity (kg CO_2-e kg LWG^{-1})^A	**2.30**	**1.04**

^{A} *Excludes custom-fed cattle and cattle fed at external feedlots.*

4.1.3 Boxed beef emission intensity

Boxed beef emission intensity (product footprint) was calculated based on the emissions from birth to post primary processing. This is reported in kg $CO_{2\text{-}e}$/kg^{-1} of boxed beef to illustrate the emissions across the full life of the animal through to post processing and up to point of dispatch from the abattoir. This calculation was based on an estimated retail meat yield of 70%. The carbon footprint of boxed beef was estimated at 22.31 kg $CO_{2\text{-}e}$/kg^{-1} Boxed Beef sold for treatment cattle and 23.37 kg $CO_{2\text{-}e}$/kg^{-1} Boxed Beef sold for control groups.

5. Conclusion

The treatment cattle fed Bovaer® had a scope 1 and 2 emission intensity of 1.04 kg $CO_{2\text{-}e}$/kg LWG^{-1}, control cattle had a scope 1 and 2 emission intensity of 2.30 kg $CO_{2\text{-}e}$/kg LWG^{-1}. This resulted in 56% lower scope 1 and 2 emissions per kilogram of gain for treatment (Bovaer® fed) cattle compared to the control group.

The supply chain carbon footprint was 8.94 kg $CO_{2\text{-}e}$/kg LW^{-1} sold for treatment cattle, and 9.42 kg $CO_{2\text{-}e}$/kg LW^{-1} sold for control group cattle, showing a 5.4% reduction in carbon footprint for processed cattle fed Bovaer during the feeding period.

Widespread adoption of supplements (e.g., Bovaer®) within the next 3-4 years would potentially make nitrous oxide from manure the primary scope 1 emission source at feedlots. Under current calculation methods, this estimation for feedlot cattle may be higher than expected, prompting the need for further research.

The trial concluded that Bovaer® was effective in reducing the largest direct emission source (enteric methane) at the feedlot. Primary considerations for implementation of Bovaer® supplementation would be determined by how each feedlot entity assesses the importance of emission reduction, and how the cost of supplementation is funded and allocated.

5.1 Key findings

Uncertainty remains around the final calculation methods and abatement that will be used for Bovaer® for generating carbon credits. The widely accepted method used within this research calculated abatement by reducing baseline emissions by a stated percentage, based on Bovaer® feeding rate. However, the Livestock Emission Framework also proposes a method which predicts abatement from the amount of Bovaer® fed, without considering baseline emissions and this method may yield different abatement outcomes in feeding classes with different baseline emissions. Further research and method development is required to finalise the preferred method for generating ACCUs in Australia, and this will determine the ultimate cost recovery achievable via carbon markets.

Costs may also be recovered through market means, where premiums are achieved, or partnerships are formed with customers to offset additional costs from supplement feeding.

5.2 Benefits to industry

This trial concluded that Bovaer® was effective in reducing the largest direct emission source (enteric methane) at the feedlot. Provided it can be included on cost effective terms, this is a key strategy to help the feedlot industry reduce business emissions and further reduce the carbon footprint of grain finished beef.

6. Future research and recommendations

Further research into animal performance on Bovaer® should be undertaken to determine if the cost of abatement of feeding the supplement can be reduced if the supplement is found to improve performance. Further research would also be beneficial to extend the use of Bovaer into the backgrounding phase to increase the proportion of the animal's life, further reducing the overall product carbon footprint. It is unclear if the efficacy of Bovaer in long-fed cattle will be as high as mid-fed cattle (as in the current trial) and further research would be valuable to understand the emission reduction potential from these feeding classes.

Further research into the impact of Bovaer® inclusion rates on the emission reduction results to determine the optimal inclusion rates for cost of inclusion and emission reduction.

7. References

Almeida AK, Cowley F, McMeniman JP, Karagiannis A, Walker N, Tamassia LFM, McGrath JJ, Hegarty RS (2023) Effect of 3-nitrooxypropanol on enteric methane emissions of feedlot cattle fed with a tempered barley-based diet with canola oil. Journal of Animal Science 101, 237. doi:10.1093/jas/skad237.

AusLCI (2020) The Australian Life Cycle Inventory Database Initiative - Agriculture Datasets. http://www.auslci.com.au/index.php/datasets/Agriculture.

Commonwealth of Australia (2021a) National Inventory Report 2019 Volume 1. Australian Government, Department of Industry, Energy and Resources, (Canberra, Australia)

Commonwealth of Australia (2021b) National Greenhouse Accounts Factors. Australian Government, Department of the Environment and Energy, (Canberra, ACT)

DEECCW (2023) Livestock Emissions Framework for Feed Technologies. https://www.dcceew.gov.au/sites/default/files/documents/livestock-emissions-framework-feed-technologies-factsheet.pdf.

IPCC (2014) 'Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.' (RK Pachauri and LA Meyer, Eds.). (IPCC: Geneva) https://www.ipcc.ch/assessment-report/ar5/.

ISO (2006) ISO 14044:2006 - Environmental Management - Life Cycle Assessment - Requirements and Guidelines. International Organisation for Standardisation (ISO), (Geneva, Switzerland)

Life Cycle Strategies (2015) Australasian LCI database 2015. Life Cycle Strategies Pty Ltd, (Melbourne, Australia)

Pré-Consultants (2022) SimaPro 9.4 Software. (Amersfoort, Netherlands)

Ranganathan J, Corbier L, Bhatia P, Schmitz S, Oren K, Dawson B, Spannagle M, McMahon M, Boileau P, Frederick R, Vanderborght B, Thomson F, Kitamura K, Woo C, Phankida N, Miner R, Segalen L, Koch J, Bhattacharjee S, Cummis C, Breidenich C, Eaton R, Gillenwater M, Marache M, Acosta R, Camobreco V, Cook E (2004) The greenhouse gas protocol: A corporate accounting and reporting standard. World Business Council for Sustainable Development & World Resources Institute, Geneva,

Ridoutt B, Sanguansri P, Alexander D (2015) Environmental Performance Review : Red Meat Processing Sector 2015. Australian Meat Processor Corporation (AMPC),

https://www.ampc.com.au/uploads/cgblog/id23/Environmental-Performance-Review-Red-Meat-Processing-Sector-2015.pdf.

Wiedemann S, Davis R, McGahan E, Murphy C, Redding M (2017) Resource use and greenhouse gas emissions from grain-finishing beef cattle in seven Australian feedlots: a life cycle assessment. Animal Production Science 57, 1149. doi:10.1071/AN15454.

Wiedemann S, Longworth E (2020) B.FLT.5012 - Review of nitrous oxide emission factors used in the National Inventory Report for estimating GHG from feedlots. Meat and Livestock Australia Limited, (North Sydney, NSW)

Wiedemann S, Longworth E (2021) Pathways to carbon neutrality for Australian feedlots. Meat & Livestock Australia (MLA), (North Sydney, NSW)

Wiedemann S, McGahan E, Murphy C, Yan M-J (2016) Resource use and environmental impacts from beef production in eastern Australia investigated using life cycle assessment. Animal Production Science 56, 882–894. doi:10.1071/AN14687.