Final report

EAP – Lick blocks for methane mitigation and production in grazing cattle

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Abstract

This project reviewed and identified nutritional requirements and limitations of grazing beef cattle across different seasons and regions of Australia. Lick blocks (LB) emerged as a viable nutrient delivery method with the potential to add ingredients that reduce methane (CH4) emissions. Molasses lick blocks containing energy, minerals, protein, and anti-methanogenic ingredients were then evaluated both in vitro and in vivo. In vitro studies demonstrated a dose-response relationship, reducing CH4 production by up 90%. Subsequent in vivo studies with low and high-quality forages reduced CH4 production (g CH4/day) by 10.7 % ($P = 0.07$), CH4 yield (g CH4/ kg DMI) by 11.7% ($P < 0.05$) and CH4 intensity (g CH4/kg ADG) by 16.8% ($P = 0.06$) at an inclusion of 100 g/head/day of liquid LB into pelleted grain-based supplements compared to the control. In another trial offering the liquid product free-choice, cattle consumed 0.74 g/hd/d, resulting in a reduction of CH4 production of 7.5%, yield by 16.2%, and intensity ($P < 0.05$). The effect of methane mitigants added into the liquid LB on methane yield requires further confirmation as a 'molasses only' control was not implemented in the experimental design. Two subsequent in vivo trials using a solid LB in pens and grazing animals did not reduce methane emissions perhaps due to possible deactivation of the active ingredients during manufacturing and storage. Similarly further research is required with versions of the lick block not including methane mitigants to confirm this effect. Lick block products as a supplement for cattle can reduce CH4 and address nutritional deficiencies but further research is needed to maintain the anti-methanogenic properties after manufacturing.

Executive summary

Background

This project aimed to explore practical, effective, and cost-efficient strategies and technologies for beef cattle producers to lower methane emissions and improve productivity. This issue is particularly important in grazing regions where establishing pastures is not an option, and animals experience long dry spells with inadequate feed allowance and quality. This research will benefit grazing producers from different regions in Australia to provide supplementation in an effective manner to meet unique nutritional deficiencies. Molasses Lick Blocks (MLB) were investigated for their ability to provide methane inhibiting ingredients to cattle from varying backgrounds. The project was discontinued after results of the lickblock in controlled grazing conditions were found to be non-efficacious for methane suppression or performance.

Objectives

(1) Design feed block formulations to deliver nutritional support and CH_4 mitigation options to ruminant livestock under grazing focussing on northern Australian rangeland systems (e.g., P, N supplementation, rumen modifiers).
(2) Determine the efficacy of the feed block system to deliver precision doses of a range of anti-methanogenic products (as a test model system to deliver powders and liquids).
(3) Understand the dose-response relationships of anti-methanogenic blocks in vivo and under rangeland conditions.
(4) Design mathematic models (e.g.: equations) for new Emission Reduction methodologies (dose-response models) that simplify the requirements for measuring animal performance (e.g., live weight proxies and abatement response curves based on block consumption only).

Methodology

This project included the following activities:

(1) A literature review of nutritional deficiencies affecting beef cattle in different regions of Australia.
(2) An investigation into the development of lick blocks to fulfill this nutritional deficiency.

(3) Screening in vitro batch culture studies to ensure active ingredients can reduce CH_4 production.

(4) In vivo studies (4) assessing the effects of lick block supplements on CH_4 emissions and productivity, through 4 feeding trials in controlled environments.

Results/key findings

A literature review was completed and identified seasonal and region-specific deficiencies and limitations that could be overcome with lick block products including crude protein, energy and minerals in Northern Australia, and bloat in Southern Australia.

Several key ingredients such as minerals, veterinary products, anti-methanogenic ingredients, and other ingredients can be added to molasses lick blocks as a viable cost-effective option for industry adoption.

In vitro studies have shown a possible reduction in CH_4 of up to 90% and a dose of 4-5% of the incubation fluid optimising feed degradation, volatile fatty acid concentrations, and CH_4 reduction with both high- and low-quality forages. However, doses above this threshold could reduce feed degradation and digestibility.

The first in vivo study involved adding the liquid form of the product into grain-based pellets with 3 treatments of a target intake of 0, 100, and 200 g/hd/d of the liquid MLB formulation delivered via the GreenFeed system (15 animals per treatment). Results revealed a tendency of 10.7% reduction in CH_4 production (g/d), 11.7% in CH_4 yield (g CH4/ kg DMI; $P < 0.05$), and 16.8% in CH_4 intensity (g CH4/kg ADG; $P < 0.01$) compared to control with no differences between 100 and 200 g/hd/d. Dry matter intake (DMI) was not affected by treatment and average daily gain (ADG) was numerically improved by 10% with the MLB product at 100 g/d but no further benefits were observed with doses of up to 200 g/d of the liquid product. Individual animals that consumed more product had lower CH_4 production, yield and intensity, and faster growth rate ($P < 0.05$).

The second in vivo study, the lick block supplement free-choice in liquid form was mixed with pure molasses to control intake found an average product intake of 0.743 kg/d with an increase of 9% in total DMI, a 7.5% reduction in CH_4 production (g/d), 16.5% lower CH_4 yield, and lower CH_4 intensity, and greater BW compared to control ($P < 0.05$) with no effect in ADG and feed efficiency ($P \geq 0.05$). This second in vivo trial confirmed results of the first trial of the effectiveness of the product to reduce CH_4 emissions and slightly improving beef cattle total feed intake and performance. The effect of methane mitigants added into the liquid LB on methane yield requires further confirmation as a ‘molasses only’ control was not implemented in the experimental design.

The third in vivo trial used the final solid molasses lick block which was consumed at 0.60 kg/d and decreased hay and pellet intake, increased feed efficiency (gain to feed ratio), ADG, final BW, water intake, and CH_4 yield (g/MJ MEI) likely due to lower feed intake, but did not affect CH4 production (g/d) or intensity (g CH_4/kg ADG).

A grazing trial was also conducted in a cross over experimental design with 26 steers randomly assigned to 1 of 2 treatments (control or MLB) and 2 experimental periods of 56 days. MLB was delivered to allow for ad libitum consumption and resulted in an estimated consumption of 0.20 kg/hd/d. However, the MLB used in this trial did not significantly affect enteric CH4 emissions or performance traits of grazing beef cattle $P > 0.05$), in agreement with the pen trial.

The in vitro trials and the in vivo trials using the liquid form of the lick block reduced CH_4 production, yield, and intensity adding another technology for producers to reduce the environmental footprint of beef cattle production fed forage diets. A dose-dependent relationship was found between liquid block intake and ADG, CH_4 production, intensity and yield. However, treatments increasing the concentration in the pellet or in the liquid mix with pure molasses did not produce further decreased in methane outputs. Surprisingly, the manufactured solid lick block product did not replicate the effects observed with the liquid form suggesting that the manufacturing process or storage eliminated the anti-methanogenic properties of the liquid formulation, or the formulation has changed. Further research is required with versions of the lick block not including methane mitigants to confirm this effect.

Benefits to industry

The project has also developed a lick block product with anti-methanogenic products that can reduce CH_4 emissions and performance but needs further development to extend the anti-methanogenic properties in a commercially viable solid lick block supplement product. The project also identified key considerations for producers when using molasses lick blocks in grazing animals.

Future research and recommendations

There is a need to further investigate the effectiveness of delivery options of nutritional and sustainable supplementation for grazing cattle in different areas. Some deficiencies such as protein and energy in the dry season are severe and challenging to solve.

Molasses lick blocks are a viable palatable, desirable, and readily available tool that should be encouraged to be utilised as a means of delivering key nutrients to cattle effectively as a supplement. However, the manufacturing process involves treatments and ingredients that may inactivate key ingredients such those investigated in the present project to reduce methane emissions. Further research is recommended to improve the stability of the ingredients during the manufacturing and storage. Supporting the development of anti-methanogenic products that are safe to animals, ecosystem and food chain for use in cattle grazing systems across Australia are keys to the future of sustainable farming and food safety.

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1. Background

This project focused on practical, effective, and affordable methodologies and technologies available to beef cattle producers to allow them to reduce methane emissions. This is particularly lacking under grazing conditions and in the rangelands where sowing of pastures is not feasible, and animals go through long dry periods with low feed quality. This has a huge negative impact on both livestock production and the intensity of greenhouse gas emissions (GHGE), normally reflected in low weaning rates and slow growth rates. Lick blocks have the potential to supplement grazing animals in a practical, accurate and cost-effective way. Virtually, all Australian beef grazing systems can play an important role in achieving net zero emissions and assist the industry to achieve carbon neutrality by 2030. More than 10,000 tonnes of feed blocks were used in grazing systems in 2020. The incentives for producers to use lick blocks are (i) precision technologies to deliver combinations of nutrients and other products (mitigants, medicines) that is not feasible through conventional loose feeds; (ii) the ability to deliver any future product used to manage GHG and health; (iii) ease of management within feeding systems where intake of nutrients and mitigants can be determined simply through weighing of the block (a proposition that is almost impossible using loose feeds).

Most properties in the rangelands have weaning rates between 50 and 75% and mortality rates for young and adult animals above 5%, implying a substantial proportion of unproductive animals that emit methane and consume resources (feed, veterinary products, etc) with no production. Our recent modelling research indicates that feed supplementation of a beef herd with 3,000 breeding cows in northern Australia could see a reduction of 42% in the intensity of GHGE (or 6,954 tonnes CO_2e/year) while increasing by 83% the production of live weight (LW). This is achieved by improving weaning rates and weights, joining heifers at an earlier age, increasing sale weight of adult cattle, and reducing mortality. However, these results need to be confirmed with field and metabolism trials.

We contend that the structure of the research, development and adoption program requires all stages to assist producers to achieve carbon neutrality within their production systems and across the value chain. Producers need to start the processes of deployment of technologies that reduce GHG emissions and improve animal production. Once products are accepted by producers, deployment of direct abatement technologies within those products is simple and there will be no time lag between emissions abatement and adoption – a situation that is highly desirable to producers and policymakers alike. New policy instruments and methodologies naturally develop to quantify the extent of abatement and are communicated proactively across the industry. It is recognised that feed supplementation can increase productivity and reduce GHG emissions in the rangelands however this has not been clearly demonstrated and there is a lack of practical, economical, and effective feed supplementation technologies or strategies. Molasses lick blocks (MLB) containing feed additives have been shown to increase productivity and can be economically feasible (Imaz et al., 2019a,b). The consumption of the lick blocks is controlled by the hardness, which is controlled by the ingredient formulation and 'cooking' method. This is the main way to control the intake of nutrients and anti-methanogenic additives used in the blocks. The partner company Four Season Co /Agcotech Pty Ltd has been selling and designing lick block in the market for 25 years.

Although previous research has demonstrated the potential benefits of the blocks as a delivery method for reducing methane emissions (Ali et al., 2019; Hegarty, 2015; Callaghan et al., 2020), there is a need to evaluate the effectiveness in vivo and refine block formulations to increase productivity and mitigate methane emissions. The MLB balance the positive impact of providing energy, protein, minerals and vitamins on animal production with mitigation of methane emissions from rumen modifiers, specially formulated for breeder cows to increase weaning rate and live weight produced and reduce mortality in the rangelands. Furthermore, the blocks could also improve performance, feed efficiency, and reduce emissions in southern high-quality pastures. In addition, an associative effect between ingredients could drive a change in digestibility or fermentation profile (e.g., more propionate, less CH_4, and more H_2).

The MLB have proven their benefit to improve performance of cattle with traditional ingredients and this would reduce the intensity of methane emissions per unit of forage digested or per unit of meat produced. For example, MLB providing molasses, urea, P and other minerals, vitamins, and by-pass protein have demonstrated to improve growth rate and fertility which can be translated to heavier animals at sale and improved weaning rates. However, further reductions in methane could be realised by including anti-methanogenic additives or several products to the MLB. Importantly, MLB have already demonstrated the ability to successfully deliver a range of products in various forms including solid (powder, granules), aqueous solutions, and oil emulsification (FAO, 2007). Feed block engineering and technology is critical to achieve the optimal hardness to ensure the target consumption and deliver the products to the animal at precise quantity and with uniformity. Block consumption will determine the dose received of the different ingredients to produce the targeted methane abatement and effectiveness of the anti-methanogenic products. An important aspect of these mitigants when added to MLB is that these can be fed to animals with tailored composition for different regions, seasons, and production systems. For example, MLB containing the mitigant and urea can be used for animals grazing low quality forages and a similar MLB without urea can be used for animals grazing high quality pastures and even while receiving cereal grain supplementation.

2. Objectives

(1) Design feed block formulations to deliver nutritional support and CH_4 mitigation options to ruminant livestock under grazing focussing on northern Australian rangeland systems (e.g., P, N supplementation, rumen modifiers) was successfully completed.
(2) Determine the efficacy of the feed block system to deliver precision doses of a range of anti-methanogenic products (as a test model system to deliver powders and liquids) was successfully completed.
(3) Understand the dose-response relationships of anti-methanogenic blocks in vivo and under rangeland conditions was successfully completed.
(4) Design mathematic models for new Emission Reduction methodologies (dose-response models) that simplify the requirements for measuring animal performance (e.g., live weight proxies and abatement response curves based on block consumption only)

3. Methodology

3.1 Literature review

A literature review was conducted by reviewing recent and historic literature to understand the nutritional deficiencies and how these differ across Australian regions. This review also focuses on identifying a suitable way of providing supplementation to cattle in these regions using a feed block system. Key minerals, vitamins and supplements will be identified and assessed for their ability to be included in a feed block system.

3.2 In vitro trial 1. Product selection for a reduction of CH_4 emissions in cattle and dose response relationships

3.2.1 Animal ethics

The experimental protocols and use of animals have been approved by the Animal Ethics Committee of the University of Sydney for the in vitro trials (2023/2180).

3.2.2 Experimental procedure

The in vitro trial extracted rumen samples from 3 cannulated steers. This in vitro trial aimed to screen different products, determine the effective dose, and assess the effectiveness of lick block mixtures to use in subsequent vivo experiments on both low- and high-quality forages. This initial trial was done in vitro for screening of potential products to reduce CH_4 emissions. Four different products were received from 4 Season Pty for the evaluation (Figure 1).

Figure 1. Four candidate mixtures received from 4 Season Pty to evaluate in vitro gas production and fermentation profile.

For the in vitro trial, the incubations were done using a completely randomised design (CRD) with 3 incubation bottles (replicates) per treatment per run (Figure 2). Two incubation runs with 117 bottles/run were completed. Gas samples of the headspace were obtained to measure CH_4 concentration at 24 h after the inoculation. The remaining liquid was used to measure pH, and VFA, and the feed residue in the bag was used to estimate in vitro dry matter digestibility (IVDMD) at 24 h from the weight of the filter bags. VFA and CH_4 samples were analysed by GC. Mixtures 1, 2, and 4 were freeze-dried and added to the forage substrate as both replacing the forage or in addition to the 0.5 g of forage. In contrast, mixture 3 was not dried and used in its liquid form as a proportion v/v of incubation media (fluid + artificial saliva).

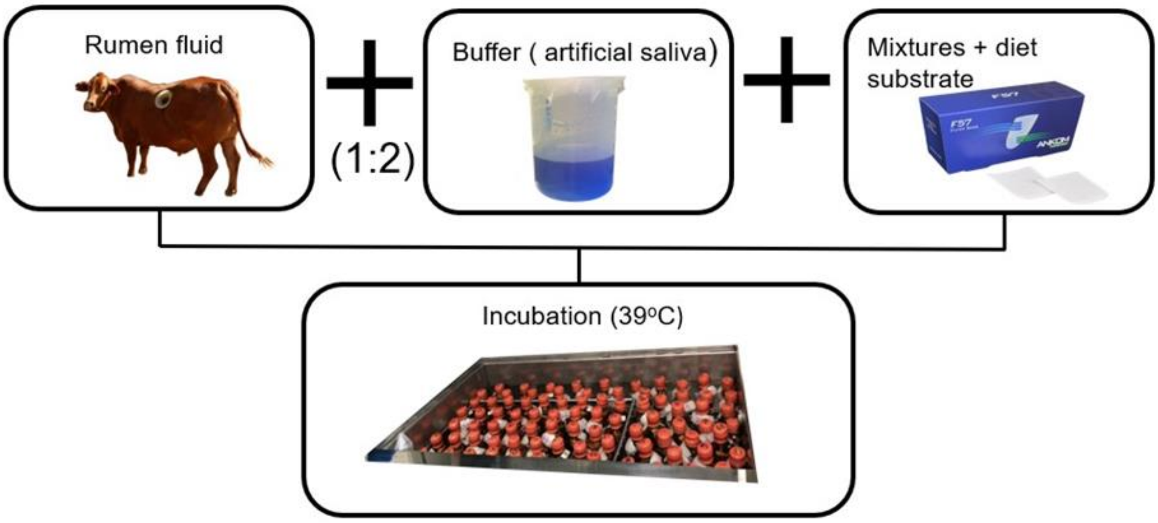


Figure 2. Workflow for the fermentation in vitro trials using lick block products.

3.2.3 Data analysis

The data was analysed as a CRD using Proc Mixed of SAS software (9.4 version, SAS institute Inc., Cary, NC)with fixed effects being Treat, Dose and Treat × Dose, whereas the random effects were run and run within treat. The incubation run was the experimental unit. The LSMEANS were estimated to compare treatment means and significance declared at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

3.3 In vitro trials 1, 2 and 3. Effects of an incremental dose of lick block mixture on CH_4 production added to both high- and low-quality feed

3.3.1 Animal ethics

The experimental protocols and use of animals have been approved by the Animal Ethics Committee of the University of Sydney for the in vitro trials (2023/2180).

3.3.2 Experimental procedure

In vitro fermentations were completed extracting the rumen fluid of two fistulated animals used as inoculum and artificial saliva, which had substrate added for the fermentation. The materials and methods are described in detail by O'Reilly et al. (2021). Briefly, 0.5 g of forage was incubated with 25 mL of rumen fluid and artificial saliva for 24 hours to measure total gas production, CH_4 production, in vitro dry matter digestibility (DMD), and volatile fatty acids (VFA) concentrations. Two contrasting diets were used as substrates: low-quality (tropical grass) and high-quality (lucerne) hays which were added to the fermentation bottles inside of weighed filter bags (Figure 2).

A derivative of Mixture 3 was received in May 2023, and it was formulated from) to manufacture the pellets for the dose-response in vivo trial. This mixture was evaluated for in vitro gas production in its liquid form and then in the pelleted form fed to animals. Mixture 5 was evaluated via in vitro trials using a completely randomised design (CRD) with 3 incubation bottles (replicates) (108 bottles/trial + 3 blanks). The chemical composition of both the liquid product and pellets are shown in Table 1.

The liquid form was evaluated at 0, 2, 4, and 6% v/v of the incubation media, with the substrate being a ratio of 80% hay (60% NDF) + 20% feedlot finishing diet. The liquid product (mixture) was added to the incubation liquid (not into the bag); thus, the product's digestibility cannot be measured although it contributes to total gas, CH_4 and VFA after fermentation.

The pellets for in vitro trial 3 and in-vivo trial 1 were manufactured to result in a target consumption of 0, 100, 200, and 400 g/head per day with 1.5 kg/head per day of the main pellet ingredient (66% rice bran and 34% wheat bran) and 500 mg of these were used as substrate for the incubations placed inside Ankom bags. Gas samples of the headspace were obtained to measure CH_4 concentration 24h after the inoculation. The pH, VFA concentrations, and in vitro dry matter digestibility (IVDMD) were also measured at 24 h. The CH_4 samples were analysed in duplicate for each replicate (bottle).

3.3.3 Data analysis

The data was analysed as a CRD using Proc Mixed of SAS 2023 with fixed effects being Dose. The incubation run was the experimental unit. The LSMEANS were estimated to compare treatment means and significance declared at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

3.4 In vivo Trial 1. Dose-response of supplement liquid lick block incorporated to pellets

3.4.1 Animal ethics

The experimental protocols and use of animals have been approved by the Animal Ethics Committee of the University of Sydney for the in vivo trials (2023/2293).

3.4.2 Animals

Forty-five 9-month-old Angus steers weighing (mean ± SD) 250 kg ± 36.6 kg were subjected to 110 days of an experiment, consisting of 19 days of training period to the facilities, 21 days pre-treatment period (before treatment) when all animals were fed a basal diet of hay and control pellets, followed by a 70-day treatment period (after treatment). However, the data from the training period were not analysed due to inconsistent visits of each animal to the electronic equipment. Also, the water station and Greenfeed units were installed without panels to train the animals for visiting both units during the training period. Each steer had been fitted with a unique radio-frequency identification (RFID) number tag. All steers were vaccinated against BVD virus, major clostridial diseases (Ultravac® 5 in 1, Zoetis Australia Pty Ltd., Rhodes, New South Wales, Australia), and BRD (Bovilis® MH + IBR; Intervet Australia Pty Ltd., East Bendigo, Victoria, Australia).

Animals were fed a basal low-quality forage diet of Rhodes grass for 19 days (training) and oaten hay for 21 days (before treatment) and a control pellet before switching to the treatment (LB) pellets with continued oaten hay as the basal diet, which lasted for 70 days (after treatment). Individual feed intake, live weight (LW), average daily gain (ADG), and CH_4 emissions were measured throughout both periods (before and after treatments). Control pellets were delivered via the

GreenFeed unit for all animals before the treatment diets were imposed. Upon and after treatment diets were imposed, the GreenFeed unit delivered the treated pellets for each treatment group as described below while all animals continued receiving the basal forage diet in the electronic feeders.

The 45 steers were randomly allocated to one of three treatment groups (TG; *n*=15). The average initial LW (± SEM) for each TG was 252 ± 9.6 (control; TG 0), 248 ± 10.3 (TG 100), and 252 ± 9.7 (TG 200) kg, respectively. Each group received different doses of LB supplement in the pellets to target a pellet product consumption of 0, 100 and 200 g/head/day, respectively. The three groups were split into two pens (40 m length × 20 m width). Pen 1 contained steers from the control group, while pen 2 contained steers from both TG 100 and TG 200. Each pen was equipped with electronic feeders (Intergado®, Betim, MG, Brazil) to measure individual feed intake, a water trough with two cattle walk-on weighing scales (Intergado®, Betim, MG, Brazil), and a GreenFeed® (GF) system with dual hoppers (C-Lock Inc., Rapid City, SD, USA) to deliver predefined amount of LB pellets and measure individual daily CH_4 production (MP; g CH_4/day). All steers received the same basal oaten hay and freshwater to allow for *ad libitum* consumption since the first day of the before treatment period.

3.4.3 Faecal and blood collection

All animals were mustered to a yard to obtain faecal and blood samples using low-stress handling techniques at the end of the trial. After 15 minutes in a yard, each animal was walked into a crush to collect the samples. Faecal grab samples were directly obtained by rectal stimulation with a circular movement of 2 fingers against the rectum wall. These samples will be used to analyse diet quality using faecal NIRS. Blood samples were collected in 10 mL using lithium heparin vacutainers (BD vacutainers; Multipoint Technologies® Pty Ltd, Balwyn, Victoria, Australia) from the tail through the coccygeal vein and immediately conserved in ice until processing and freezing for later analysis. The blood samples were centrifuged using SkyLine CM-6MT Swing Rotor Centrifuge (ELMI® Ltd, Vidzeme, Riga, Latvia) at 20,000 × g for 10 min. The plasma supernatant was pipetted off and stored at −80°C until the blood chemical analysis for ruminant health assessment.

3.4.4 Lick block supplement

Lick-block product (LB; 4 Season Company Pty Ltd., Crestmead, Queensland, Australia) in liquid form were mixed with the ingredients to manufacture control and LB pellets (4.4 mm diameter × 12 mm length) without adding the settling agent used for manufacturing the solid lick blocks. The pellet's ingredients composition was rice bran (66.7%) and canola meal (33.3%). To achieve the targeted intake of 0, 100, and 200 g LB/head per day in a pellet amount of 1.5 kg/head/day, 1,000 kg pellets were proportionally mixed with 0, 67, and 133 kg liquid LB resulting in 0, 6.28, and 11.74% of LB proportion in the LB pellets, respectively. These pellets were fed at 1.5 (0 g LB), 1.6 (100 g LB), and 1.7 (200 g LB) kg LB pellets/head/day. This allocation was designed to make the LB the only difference between treatment groups. The LB supplement in this project contains natural and approved products, including vegetable and essential oils.

3.4.5 Feed chemical composition

The chemical composition of the liquid LB, LB pellets and oaten hay provided to the cattle during the experimental periods is presented in Table 1. The oaten hay was used as the basal diet to represent a low-quality diet (7 MJ/kg DM) mimicking conditions of an extensive grazing environment in northern Australia. Although the pellets contained higher energy, the predetermined allocation of the pellets (1.5-1.7 kg/head/day) would provide a similar amount of energy (15.5-15.8 MJ/kg DM) for each steer across all TG (Table 1).

Table 1. Chemical composition of the treatment pellets and oaten hay used for the in vitro and in vivo trials.

	Unit	Liquid Lick-Block	Lick-block Pellets			Oaten hay
			0 g	100 g	200 g	
Dry Matter (DM)	%	38.4	91.3	92.4	90.3	92.0
Neutral Detergent Fibre (NDF)	% DM	NA	21.0	21.0	22.0	67.0
Acid Detergent Fibre (ADF)	% DM	NA	12.0	12.0	12.0	42.0
Crude Protein (CP)*	% DM	14.4	23.1	22.8	22.7	5.9
Crude Fat (Ether Extract)	% DM	25.3	15.7	15.5	15.3	-
Water Soluble Carbohydrates (WSC)	% DM	19.8	8.7	9.6	10.1	4.3
Dry Matter Digestibility (DMD)	% DM	NA	78.0	78.0	76.0	50.0
DOMD	% DM	NA	78.0	77.0	76.0	49.0
Inorganic Ash	% DM	9.4	9.4	9.5	9.6	7.0
Organic Matter (OM)	g/kg DM	906	906	905	904	930
Metabolisable Energy (ME)**	MJ/kg DM	15.5	15.8	15.8	15.5	7.0
Total Starch	% DM	NA	14.0	14.1	13.6	NA
Urea	g/100 g	2.79	<0.03	0.09	0.17	NA
Nitrate	mg/kg	29	13	42	46	NA
Aluminum	mg/kg	13	120	99	73	NA
Arsenic	mg/kg	<5	<5	<5	<5	NA
Boron	mg/kg	<4	10	11	10	NA
Calcium	%	0.66	0.30	0.37	0.44	NA
Cadmium	mg/kg	<0.2	<0.2	<0.2	<0.2	NA
Cobalt	mg/kg	0.52	0.20	0.21	0.24	NA
Chromium	mg/kg	0.23	0.67	0.62	0.54	NA
Copper	mg/kg	0.94	8.1	7.8	7.8	NA
Iron	mg/kg	55	200	160	150	NA
Potassium	%	0.70	1.6	1.6	1.7	NA
Magnesium	%	0.21	0.81	0.80	0.84	NA
Manganese	mg/kg	15	180	180	180	NA
Molybdenum	mg/kg	0.12	0.61	0.65	0.67	NA
Sodium	%	0.71	0.027	0.090	0.14	NA
Nickel	mg/kg	<0.7	0.91	0.88	1.0	NA
Phosphorus	%	0.031	1.9	1.8	1.9	NA
Lead	mg/kg	<2	<2	<2	<2	NA
Sulfur	%	0.12	0.34	0.33	0.34	NA
Selenium	mg/kg	<0.05	0.17	0.17	0.17	NA
Zinc	mg/kg	5.3	62	57	58	NA
Dietary Cation-Anion Difference *(calc)*	meq/kg	NA	200	200	220	NA
Chloride	%	NA	0.028	0.13	0.22	NA
Monensin	mg/kg	<2	NA	NA	NA	NA

3.4.6 The Greenfeed® system

Two GF units equipped with two hoppers were used (Figure 3). This system is typically configured to provide a small amount of pellets to entice the animals to visit the GF multiple times per day (C-Lock

Inc., 2023). Each GF was situated on each pen to provide one type of pellet for control and the two treatment pellets through the hoppers for 100 g and 200 g/head/day. The pellets were dropped into a tray in a semi-enclosed hood when the RFID tag of an animal was detected and registered. The visit frequency (visits/day), number of feed drops (cups/visit), and the interval between visits were adjusted to ensure that each steer received the targeted LB intake if willing to do so. Because the amount of pelleted LB per drop is 42 (control), 70 (100g), and 41 (200g) g/cup, the GF setting for LB provision per group was configured as follows:

0 g/head/day : frequency = 6 visits/day (120 min interval); feed drops/visit = 5 (40 s interval)

100 g/head/day: frequency = 4 visits/day (120 min interval); feed drops/visit = 6 (40 s interval)

200 g/head/day: frequency = 6 visits/day (120 min interval); feed drops/visit = 7 (40 s interval)

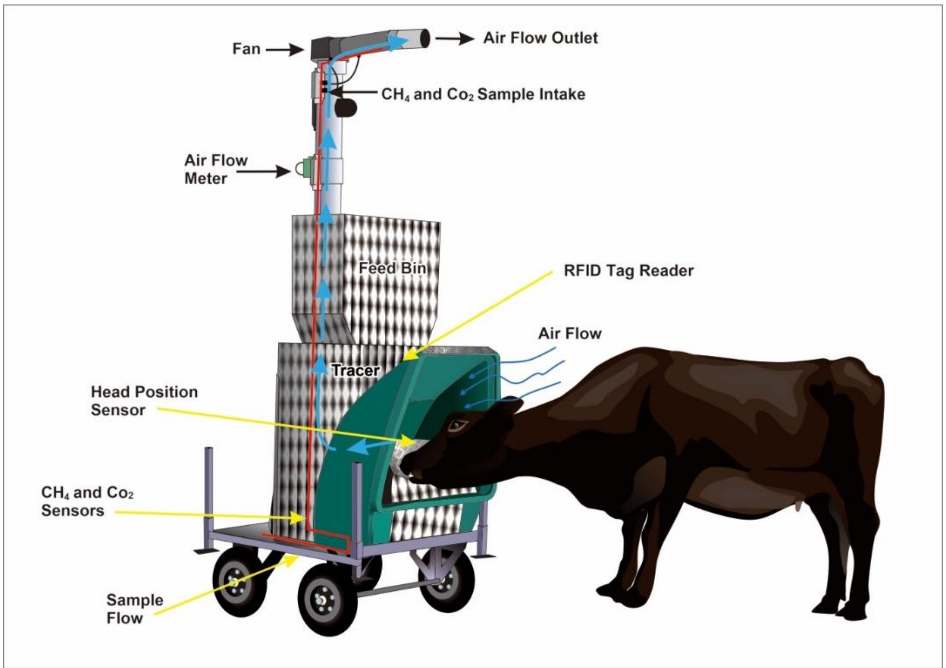


Figure 3. The layout of the GreenFeed® system

3.4.7 Quantification of CH_4 emissions

Individual CH_4 emissions measured by the GF system is expressed as daily CH_4 production (g CH_4/day). Visit duration and the number of records per individual are critical for CH_4 measurement because CH_4 is typically belched at 40 – 120 s intervals (Hammond et al., 2016). The GF operation commences when the steer places its head inside the shroud (Hammond et al., 2015). Following this, the proximity sensor in the shroud will monitor the head position of the animal during each visit, which will also be used to dismiss all measures where animals stepped out from the GF. Air is continuously drawn through the shroud and past the neck of the animal at a precisely measured rate, and the CH_4 concentration and propane are quantified in the exhaust air stream (Velazco et al., 2016). As the GF system provides multiple short-term breath measures, 30 measurements with a minimum of 3 min duration per visit are needed to achieve a minimal variance of MP rate per animal (Arthur et al., 2017). Data are logged and transmitted into the C-Lock Inc. data management system and can be downloaded through the C-Lock Inc. website interface (https://greenfeed.c-lockinc.com). The GF system is calibrated as per manufacturer protocol and gas recovery performed regularly.

3.4.8 Lick block hardness

The partner company (4 Season Pty Ltd.) provided a prototype of the LB. These blocks were tested in the laboratory and provided to a group of cattle on Pye Farm from October to December 2022. The hardness for the LB was 200 Psi at 14 kg/cm². Birbe et al. (2006) reported that molasses LB intake by cattle decreased from 3.3 kg/d when the block hardness was 4.4 kg/cm² to 0.9 kg/d when it was 6.9 kg/cm² and 0.1 kg/d when it was 9.6 kg/cm². Additionally, Zhu et al. (1991) recommended that the LB hardness should be < 1000 g/mm² (100 kg/cm²) to be considered edible for cattle. This prototype lick block was found to be too hard and result in low intake for cattle so the company manufactured a softer lick block to increase consumption in later trials.

In addition to the hardness, the density was measured to indicate how tightly a material is packed together (ρ; g/cm^3) which was calculated using the formula:

$$\rho = \frac{m}{v}$$

Where *m* is the mass of the LB (kg) and *v* is the volume of the LB (m³). The density of the LB is presented in Table 2.

Table 2. The density of prototype lick-blocks (LB) as a proxy of the LB hardness

Block	Mass (g)	Volume (cm³)	Density (g/cm³)
1	19,150	15,309	1.251
2	18,650	15,309	1.218
3	18,800	15,309	1.228
4	18,750	15,309	1.225
5	19,000	15,309	1.241
Mean	**18,870**	**15,309**	**1.233**

3.4.9 Data analysis

Data from the GF system, Intergado® electronic feeders and weighing system were statistically processed using R (R Core Team, 2023). The raw data was filtered from illogical values before analysis and averaged by date for each animal before (*n* = 21 days) and after (*n* = 70 days) the LB pellets were fed. There were three stages of analysis:

a. *Change in performance and emissions before compared to after the LB pellets were delivered*. Average growth rate (ADG; kg/day) and CH_4 production (g/day) during the before and after treatment periods were calculated for each animal, then analysed with mixed-effects linear regression models with treatment group (TG; *n* = 3), period (before and after), and their interaction assigned as fixed effects with animal EID set as random effect.

b. *Treatment effects during the LB pellet delivery period only*. The average of all variables throughout the 70-day LB pellet delivery period was calculated for each animal. The effect of treatment was assessed using the analysis of variance (ANOVA) with treatment as a fixed effect.
c. *Pearson correlation analysis during the treatment period only* was performed to analyse the association between GHG emissions, O_2 consumption, ADG, LW and intake of each individual animal. This analysis was done on a database containing the average for each animal of all variables measured through the 70-d LB pellet delivery period, same as in 2 above. During this period, average daily LB intake was calculated by multiplying the daily LB pellet intake measured by GreenFeed by the concentration of LB in the pellet according to treatment.

Differences were considered significant when $P \leq 0.05$, and tendencies were discussed at $0.05 \leq$ P-value ≤ 0.10.

3.5 In vivo trial 2. Effect of increasing liquid product dosage on methane emissions, feed intake, and performance from beef cattle

3.5.1 Animal ethics

The experimental protocols and use of animals have been approved by the Animal Ethics Committee of the University of Sydney (ARA No. 2023/2293).

3.5.2 Animals and experimental procedures

Forty 12-month-old Angus steers weighing (mean ± SD) 279 kg ± 43.1 kg were subjected to a molasses lick block trial that lasted 56 days, including 7 days of baseline low-quality oaten hay and grain pellets as attractant to the GreenFeed unit. Each steer had been fitted with a unique radio-frequency identification (RFID) number tag. All steers were vaccinated against BVD virus, major clostridial diseases (Ultravac® 5 in 1, Zoetis Australia Pty Ltd., Rhodes, New South Wales, Australia), and BRD (Bovilis® MH + IBR; Intervet Australia Pty Ltd., East Bendigo, Victoria, Australia).

Animals were fed oaten hay in electronic feeders (Intergado®, Betim, MG, Brazil) and barley grain pellets in the GreenFeed unit (C-Lock Inc., Rapid City, SD, USA) to attract the animals for measurements of methane and other gases. Individual feed intake, live weight (LW), average daily gain (ADG), and CH_4 emissions were measured throughout the trial.

The 40 steers were randomly allocated to a control group with no supplement or a LB treatment group which received free choice of the liquid LB product delivered in electronic feeders. The LB product was mixed with pure molasses to increase and control intake. Oaten hay and freshwater were delivered to allow for *ad libitum* consumption throughout the trial.

The lick-block product (LB; 4 Season Company Pty Ltd., Crestmead, Queensland, Australia)was fed in the liquid form and mixed with pure molasses at 50, 60, 70 and 80% over a 56-d period to allow for animals to adapt to higher concentration of the product and determine the effect of increasing doses on performance and CH_4 production. The oaten hay was used as the basal diet to represent a low-quality diet (7 MJ/kg DM) mimicking conditions of an extensive grazing environment in northern Australia. Although the pellets contained higher energy, the predetermined allocation of the pellets

(1.5 kg/head/day) would provide a similar amount of energy (15.5-15.8 MJ/kg DM) for each steer across all TG. Table 3 presents the chemical composition of each feed ingredient.

The pellets weighed on average 42 g/cup and it was setup to allow animals to consume these through 6 visits/day (120 min interval/visit) and 5 feed drops/visit at 40 s intervals. As the GF system provides multiple short-term breath measures, 30 measurements with a minimum of 3 min duration per visit are needed to achieve a minimal variance of MP rate per animal (Arthur et al., 2017). The GF system was calibrated as per manufacturer protocol and gas recovery performed regularly.

Animals were allowed ad-libitum consumption of hay and liquid product, and the intake of pellets from the GreenFeed could not have reached the maximum allotment for each day. Such diet selection can affect the intake of nutrients (CP, WSC, NDF, and ME) and therefore the final concentration of each nutrient selected by the animals was estimated from the daily intake of each nutrient from each feedstuff (concentration of nutrient in each feed by the amount of that feed consumed). For example, daily metabolizable energy intake (MEI) was estimated by multiplying the ME concentration by the DMI of hay, liquid product, and pellets. A similar calculation was made to estimate NDF intake (NDFI), water soluble carbohydrate intake (WSCI), and crude protein intake (CPI). In addition, the sum of the daily intake of each nutrient from all feeds was used to estimate the final concentration of each nutrient in the final diet of the animals. Product intake was calculated multiplying the total liquid intake by the concentration of the product in the liquid mix, i.e. 50, 60, 70, or 80%. Total daily DMI was estimated as the sum of hay, pellet, and liquid product DM consumed for each animal.

Live weight and growth rate (ADG) were measured by the remote weighing system. The ADG was then used estimate feed conversion ratio (FCR) diving total DMI by ADG and gain to feed ratio (GF) diving ADG by total DMI. However, DMI may not be representative of the nutrients consumed in each treatment because a unit of DMI from pellets, LB and hay is very different. Therefore, the MEI to ADG ratio (MEIG) was calculated as total MEI divided ADG.

3.5.3 Data analysis

Data from the GF system, Intergado® electronic feeders and weighing system were statistically processed using R (R Core Team, 2023). The raw data was filtered from illogical values before analysis and then daily summary values were calculated for each variable. This database was then used to create two databases for independent analysis:

1. Data was averaged for each animal before (n = 7 days) and after (n = 56 days) the LB product was fed to study the change in performance and emissions before (baseline) compared to after (treatment) the LB product was fed. Thus, data was averaged for each animal across the baseline and the treatment period, independently of the mixing proportion of pure molasses and LB product. A mixed-effects linear regression model was used with treatment group, period (baseline and treatment), and their interaction assigned as fixed effects with animal EID as random effect. The treatment effect was sliced for each period when a significant treatment × period interaction existed.
2. Data was averaged for each animal during the baseline with no product (7 d), 50% product (14 d), 60% (7 d), 70% (7 d), and 80% (21 d) of LB product mixed with the remaining being pure

molasses. The treatment effect was sliced for each period when a significant treatment × period interaction existed.

Pearson correlation analysis was performed for each treatment period to analyse the association between GHG emissions, O_2 consumption, ADG, LW and intake of each individual animal. Differences were considered significant when $P \leq 0.05$, and tendencies were discussed at $0.05 \leq$ P-value ≤ 0.10.

Table 3. Chemical composition of the liquid lick-block (LB), LB pellets and oaten hay used for the in vivo study.

	Unit	Liquid Product 50%	Liquid product 60%	Liquid product 70%	Liquid product 80%	Liquid product 100%	Oaten hay	Pellets
Moisture	%	43.7	45.0	48.5	54.3	62.2	3.8	8.8
Neutral Detergent Fibre (NDF)	% DM	-	-	-	-	-	59.4	34.3
Acid Detergent Fibre (ADF)	% DM	-	-	-	-	-	34.9	12.3
Crude Protein (CP)*	% DM	4.4	4.1	4.1	4.3	4.3	9.7	14.7
Crude Fat (Ether Extract)	% DM	-	-	-	-	-	-	3.0
Water Soluble Carbohydrates (WSC)	% DM	0.5 %	32.8	31.9	30.4	-	9.4	5.6
Dry Matter Digestibility (DMD)	% DM	56.3	55.0	51.5	45.7	-	55.7	71.0
DOMD	% DM	-	-	-	-	-	53.1	70.3
Inorganic Ash	% DM	-	-	-	-	-	8.9	8.0
Organic Matter (OM)	g/kg DM	-	-	-	-	-	91.1	92.0
Metabolisable Energy (ME)**	MJ/kg DM	15.1	15.2	15.2	15.6	17.3	7.8	11.4
Total Starch	% DM	-	-	-	-	-	-	26.3
Urea	g/100 g	0.51	0.47	0.36	0.86	1.18	-	-

* = measured value from feed analysis using Dumas Combustion Method

** = ME value for a liquid block is measured using bomb calorimeter; others were estimated using the following formula:
ME = [(crude protein x 35) + (crude fat x 84.6) + (WSC x 35) kcal/kg] × 0.004184

3.6 In vivo trial 3. Performance and GHG emissions of cattle fed a solid lick block supplement

3.6.1 Animal Ethics

Experimental procedures and animal use had been approved by the Research Integrity & Ethics Administration of the University of Sydney (ARA 2023/2293).

3.6.2 Experimental design

Forty, 12–20-month-old Angus steers with an initial liveweight of (± SD) of 320.8 (± 40.2) kg were used. Each steer was fitted with a unique radio-frequency identification (RFID) number tag. All steers were vaccinated against BVD virus, major clostridial diseases (Ultravac® 5 in 1, Zoetis Australia Pty Ltd., Rhodes, New South Wales, Australia), and BRD (Bovilis® MH + IBR; Intervet Australia Pty Ltd., East Bendigo, Victoria, Australia)). Animals were randomly assigned to 1 of 2 treatment groups (control and MLB) in 2 × 2 crossover experimental design with 2 treatments and 2 experimental periods of 56 days each. An adaptation and a washout period of 14 days was used at the start of the trial and between experimental periods with no treatment, respectively.

Animals were fed ad libitum 50:50 oaten-vetch hay in electronic feeders (Intergado®, Betim, MG, Brazil). The control pen was be fitted with 4 electronic feed bins, 1 water trough + 2 weighing stations, and 1 GreenFeed unit (Figure 4). The equipment for the treatment pen was similar to the control pen, with the addition of 2 electronic feed bins for delivering the treatment feed (lick-block supplement). Animal attendance at electronic equipment was recorded continuously including body weight, growth rate, feed intake, GHG emissions (CH_4, CO_2, H_2, and O_2), and health.

Both groups were fed medium-quality hay and provided access to freshwater ad libitum and consumption was monitored on an individual animal basis using electronic feeders. Hay was provided to the four electronic feeders in each pen twice a day, providing 20-60kg of hay per load (chopped in a commercial mixer with 20% water added to reduce dustiness). Samples of the feed offered were taken weekly to measure DM, as well as samples of any hay refusal.

A lucerne pellet was utilised as an attractant to the Greenfeed system at a rate of 1kg/head. The average weight of the pellet was 30 g/drop, so 25 drops were allocated for each animal per day, configured as 6 visits 5 drops per visit.

The lick-block supplement (AgCoTech Pty Ltd./4 Season Company Pty Ltd., Crestmead, Queensland, Australia) was provided in two electronic feeders ad libitum during the experimental periods, allowing individual intake to be recorded. A total of 629.7 kg of lick block was added and average intake was calculated as 0.552 kg/head/day for period 1. During the washout period, the lick block was removed from the feeders, and the animals were switched pens as such group 1 was in the lick block pen and group 2 was in the control pen. During period 2 the lick block was offered ad libitum to group 1 via the electronic feeders (totalling 706.3 kg) and intake was estimated as 0.628 kg/head/day. A full composition of the feed is available in Table 4. The trace element composition of the Lick Block is found in Table 5.

In addition, the sum of the daily intake of each nutrient from all feeds was used to estimate the final concentration of each nutrient in the final diet selected by the animals. Total daily DMI was estimated as the sum of hay, pellet, and lick block DMI consumed for each animal multiplied by their respective DM content.

Live weight and growth rate (ADG) were measured by both the scales in the yards and the remote weighing system. The ADG measured by both yard and in-pen scales was then used to estimate feed conversion ratio (FCR) diving average DMI by ADG and gain to feed ratio (GF) diving ADG by average DMI. Liveweight was also recorded every 2 weeks at the central handling facilities to ensure accuracy and monitor weight gain.

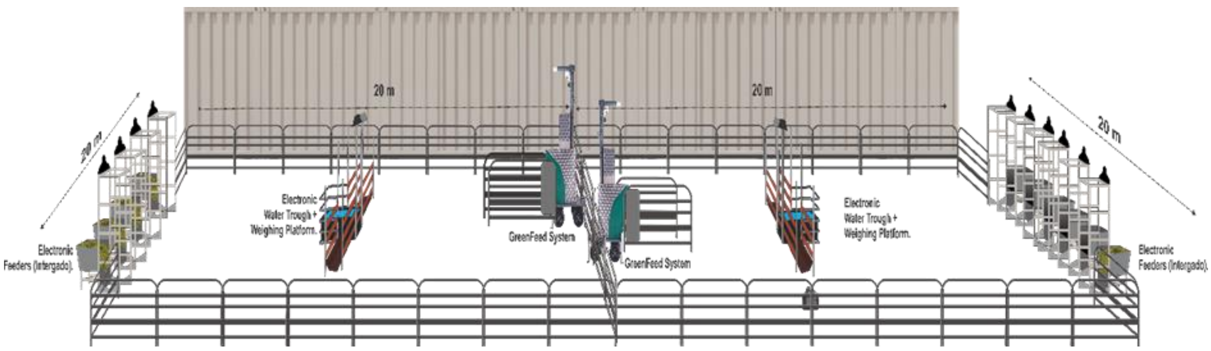


Figure 4. The layout of the experimental pens in the lick blocks trial 3.

Table 4. Chemical composition of the lick-block (LB), pellets and oaten hay used for the in vivo study.

	Unit	Oaten-Vetch hay	Pellets	Lick Block
Moisture	%	38.1	6.84	25.8
Neutral Detergent Fibre (NDF)	% DM	54.2	34.7	<10.0
Acid Detergent Fibre (ADF)	% DM	30.7	25.8	<4.0
Crude Protein (CP)	% DM	12.1	19.9*	5.5*
Crude Fat (Ether Extract)	% DM	-	1.9	2.4
Water Soluble Carbohydrates (WSC)	% DM	11.3	4.7	32
Dry Matter Digestibility (DMD)	% DM	62.2	64.6	69.7
DOMD	% DM	57.7	63.5	69.1
Inorganic Ash	% DM	11.5	10.4	35
Organic Matter (OM)	g/kg DM	88.5	89.6	65
Metabolisable Energy (ME)	MJ/kg DM	8.7	10.1	11

* = measured value from feed analysis using Dumas Combustion Method

Table 5. Trace elements composition of the Lick Block

Trace Elements	UNITS	Lick Block
Aluminium	mg/kg	87
Arsenic	mg/kg	<5
Boron	mg/kg	6.3
Calcium	%	3.6
Cadmium	mg/kg	<0.2
Cobalt	mg/kg	2.6
Chromium	mg/kg	1.6
Copper	mg/kg	2.1
Iron	mg/kg	190
Potassium	%	1.6
Magnesium	%	3.4
Manganese	mg/kg	73
Molybdenum	mg/kg	0.91
Sodium	%	2.6
Nickel	mg/kg	6.6
Phosphorus	%	1.5
Lead	mg/kg	<2
Sulphur	%	0.43
Selenium	mg/kg	0.29
Zinc	mg/kg	9

3.6.3 Data analysis

Data from the GF system, Intergado® electronic feeders and weighing system were statistically processed using SAS (Statistical Analysis System, 2021) and Excel (Microsoft Corporation, 2024). The raw data was filtered from illogical values before analysis as described by Imaz et al. (2021) and then daily summary values were calculated for each variable. Daily data was then averaged for each animal and experimental period 1 and 2. A mixed-effects linear regression model was used with treatment (MLB or Control), and Period (1 or 2) as fixed effects, and animal EID within Pen as random effect. Then the estimated marginal means for treatment groups with SEM were calculated from the model. Differences were considered significant when $P \leq 0.05$, and tendencies were discussed at $0.05 \leq$ P-value ≤ 0.10.

3.7 In vivo trial 4. Effect of lick block supplementation on the performance and GHG emissions of cattle in a grazing system

3.7.1 Animal Ethics

Experimental procedures and animal use had been approved by the Research Integrity & Ethics Administration of the University of Sydney (ARA 2023/2293).

3.7.2 Experimental Design

Twenty-six 8–15-month-old Angus, Charolais or mixed breed steers weighing (325.19 ± 48.09 kg/hd) were included in the grazing trial. Each steer was fitted with a unique radiofrequency identification (RFID) ear tag. All steers were vaccinated against BVD virus, major clostridial diseases (Ultravac® 5 in 1, Zoetis Australia Pty Ltd., Rhodes, New South Wales, Australia), and BRD (Bovilis® MH + IBR; Intervet Australia Pty Ltd., East Bendigo, Victoria, Australia)). Animals were randomly assigned to 1 of 2 treatment groups (control and MLB) in 2 × 2 crossover experimental design with 2 treatments and 2 experimental periods: (a) control group drafted to the right pen without access to molasses-lick block (MLB), but water; (b) treatment group drafted to the left pen with access to water and MLB placed in an automatic weighing station inside the yard (Optiweigh, Armidale, NSW; https://www.optiweigh.com.au/). This allowed the identification of any control gaining access to the MLB and to measure the time spent at the MLB.

An adaptation and a washout period of 14 days was used at the start of the trial and between experimental periods with no treatment, respectively. Animals were given a longer adjustment period to the paddocks at the start of the trial to learn the water sources and to use the technology for 32 days. The lick block was provided then for 56 days in the walk on weigh system to the treatment animals. The steers were then swapped into the opposite treatment group for the next period, with an adaptation phase of 21 days with no lick block but switched drafting. The second experimental period was 84-days long because there was remaining MLB that could be used. Two steers were removed from the trial due to repeated escaping the trial and no data was obtained post initial weights. Liveweight and growth rate (ADG) were measured by both the scales in the yard, as well as via the Walk over Weighing (WOW) system and using an Optiweigh walk on weighing system.

3.7.3 Nutrition

Animals had access to one of seven paddocks (ranging between 0.6 hectares to 1.6 hectares) at any given time with a similar feed base, with rotational grazing used as a main source of nutrition for these animals. Pasture samples were collected from the paddocks regularly and average composition across the paddocks is provided in Table 6. Lucerne hay was provided as an ad libitum feed source for the last 117 days as animals were not gaining weight and pasture growth was slow. This resulted in a total of 43 bales (500 kg) delivered every 2-3 days.

The lick-block supplement (AgCoTech Pty Ltd./4 Season Company Pty Ltd., Crestmead, Queensland, Australia) was provided to the treatment animals via the Optiweigh system and resulted in an estimated consumption of 0.22 kg/head per day for period 1 and 0.18 kg/head per day for period 2 when total intake was divided by the number of animals assigned to MLB. During period 1, 133.6 kg of lick block were provided and during period 2 a total of 208.76 kg was provided. This lick block was the same batch used in the in vivo pen trial 3 described in section 3.6 and information can be found in Table 5. Lucerne pellets were also delivered to each animal via the Greenfeed system and the chemical composition can be found in Table 4.

Table 6. Chemical composition of the forage available across the trial and lucerne hay provided in the grazing in vivo study.

	Unit	Lucerne Hay	Forage (Paddock)
Average Dry Matter (DM)	%	83.1	44.3
Neutral Detergent Fibre (NDF)	% DM	45.9	66.3
Acid Detergent Fibre (ADF)	% DM	32.1	38.7
Crude Protein (CP)	% DM	16.7	9.3
Inorganic Ash	% DM	10.7	10.0
Organic Matter (OM)	g/kg DM	89.3	90.0
Dry Matter Digestibility (DMD)	% DM	56.2	46.9
Dry organic matter digestibility (DOMD)	% DM	54.4	45.9
Metabolisable Energy (ME)	MJ/kg DM	8.0	6.3
Water Soluble Carbohydrates (WSC)	% DM	5.4	<4

3.7.4 Drafting System

A 3-way auto-drafter integrated with WOW system (Precision Pastoral Pty, Alice Springs, NT) operates using electric motors powered by a 12V battery charged by solar panels. The system was installed at the only water point as previously described by Imaz et al. (2021). No animals were assigned to the second (middle) direction. The system recorded animal's EID, date, time and LW every time an animal walked through the platform to access water or MLB, or both for the MLB group. A yard was built at a central location of the paddocks grazed in the experiment and subdivided into two equal-sized pens, each sharing a single water point. The only entry access was through the auto-drafter and at the end of each pen was a one-way spear gate for exiting, although animals could choose the exit via the auto-drafter which did not have spear gates. The auto-drafter was activated after 20 days of introducing the animals to the experimental area. The left pen yard utilised with the Optiweigh walk on weigh system that housed the MLB for the treatment, when provided. This system records the weight of the front feet of the animal on the platform while the animal consumes the MLBs and converts it full body weight with proprietary algorithms. The Optiweigh also recorded the time each animal spent standing on the platform.

3.7.5 Quantification of CH4 emissions

One Greenfeed unit (GF) with two hoppers was utilised in this study and operated on solar sourced battery power. The GF was placed before the entry to the auto-drafter and WOW system, so animals could access the GF without engaging with any part of the auto-drafter, WOW, Optiweigh or spear gates. The same lucerne pellets were provided in both hoppers with animals assigned to each to allow for an even consumption form both hoppers. A lucerne pellet was utilised as an attractant to the Greenfeed system at a rate of 1 kg/head. The average weight of the pellet was 30 g/drop, so 35 drops were allocated for each animal per day, configured as 7 visits 5 drops per visit. The pellets drop into the tray in a semi-enclosed hood when the RFID tag of the animal was detected and registered.

Individual CH4 emissions were measured by the GF and is expressed as daily CH4 production (g CH4/day) for each animal. The GF operation commences when the steer places its head inside the shroud (Hammond et al., 2015). Following this, the proximity sensor in the shroud will monitor the head position of the animal during each visit, which will also be used to dismiss all measures where animals stepped out from the GF. Air is continuously drawn through the shroud and past the neck of the animal at a precisely measured rate, and the CH4 concentration and propane are quantified in the exhaust air stream (Velazco et al., 2016). As the GF system provides multiple short-term breath measures, 30 measurements with a minimum of 3 min duration per visit are needed to achieve a minimal variance of MP rate per animal (Arthur et al., 2017). Data is logged and transmitted into the C-Lock Inc. data management system and can be downloaded through the C-Lock Inc. website interface (https://greenfeed.c-lockinc.com). The GF system is calibrated as per manufacturer protocol and gas recovery performed regularly.

3.7.6 Data Analysis

Data from the GF system, Optiweigh, WOW and manual liveweight measurements were statistically processed using SAS (Statistical Analysis System, 2021) and Excel (Microsoft Corporation, 2024).

The raw data was filtered to remove illogical values prior to analysis, as outlined by Imaz et al. (2021). Daily summary statistics were calculated for each variable, and then the daily data was averaged for each animal across experimental periods 1 and 2. A mixed-effects linear regression model was employed, incorporating treatment (MLB or Control) and period (1 or 2) as fixed effects, while accounting for animal EID within pen as a random effect. Estimated marginal means for the treatment groups, along with standard error of the mean (SEM), were derived from the model. Statistical significance was determined at $P \leq 0.05$, with tendencies noted for P-values between 0.05 and 0.10.

4. Results

4.1 Literature Review

The objectives of the present review are to:

1) Identify nutritional limitations for different regions and seasons in Australia,

2) Review mode of delivery of supplements focussed on lick blocks for grazing livestock production.

3) Explore opportunities to tailoring products to specific regions or producers to increase animal performance and reduce GHG emissions intensity. This will help to lay the basis for producers to enter Emissions Reduction Fund (ERF)herd management methodology agreements tailored for each region.

The objective of the present review is not to describe the nutritional requirements in detail or provide recommendations on supplementation levels of grazing animals because this has been widely studied and multiple reviews, fact sheets, textbooks, and online sources are available for the reader (e.g., State Departments, MLA, Freer et al., 2007; Lean et al., 2011). Instead, the objective is to identify the different nutritional limitations of different regions and different regions of Australia where MLB can play an important role.

It is important to note that supplementary feeding to grazing animals must consider current and target intake of nutrients, which depends on feed availability, diet selection, and substitution rate of supplements. There are very dynamic and changing interactions between these factors across seasons, regions, management strategies, and herd requirements, amongst others which adds to the complexity of the supplementation task. Therefore, a 'one bill fits all' approach cannot be recommended. For this reason, feed supplementation of grazing is often, and should perhaps be done, all year around in large parts of Australia (Dixon et al., 2020). However, multiple limitations exist to broad adoption of feed supplementation because of factors such as availability and distance of supplements, cost, knowledge, labour, practicality to deliver, controlling intake of supplement, or obtain uniform intake between animals in a herd, amongst others (Lean et al., 2011).

4.1.1 Nutritional Limitations for different regions and seasons

Supplements can be used to correct deficiencies, improve production, or improve feed efficiency. An important consideration is the fact that supplementation of grazing animals can result in substitution, complementation, or supplementation (Freer et al., 2007).

Substitution occurs when the dry matter intake (DMI) of the basal (pasture) diet is reduced by supplementation, which depend on the relative quantity and quality of the supplement and basal diet (roughage). Substitution rate of pasture by the new supplement increases with higher quality and quantity of the supplement available to the animal (Dixon and Stockdale, 1999). Substitution is not expected to occur often with MLB supplementation except if using 'soft' blocks that result in a high intake of nutrients such as starch, sugars, or vegetable oils which may also decrease fibre digestibility of the pasture.

Complementation is when the intake of the basal pasture diet increases, when additional supplementation is provided that corrects deficiencies, such as with MLB supplementation containing N, P or sulphur that restrict the growth of the microbial population in the rumen of low-quality forages. This occurs because the N supplied by MLB increases for microbial degradation of fibre in the rumen. In this case, there is a synergy between the basal pasture diet and the supplement, and the substitution rate is negative (Freer et al., 2007). However, there could be a point where further increases in supplement intake containing the deficient nutrient may not result in further increase of pasture intake and it may even reduce pasture intake because the nutrient is not in deficit anymore and may even become toxic for the rumen microbes or the animal as it may be the case with urea, oils or even starch. Finally, complementation may also occur when the supplement contains compounds that neutralise the negative effects of the basal diet such as with MLB containing polyethylene glycol which binds to tannins of plants and intake is increased.

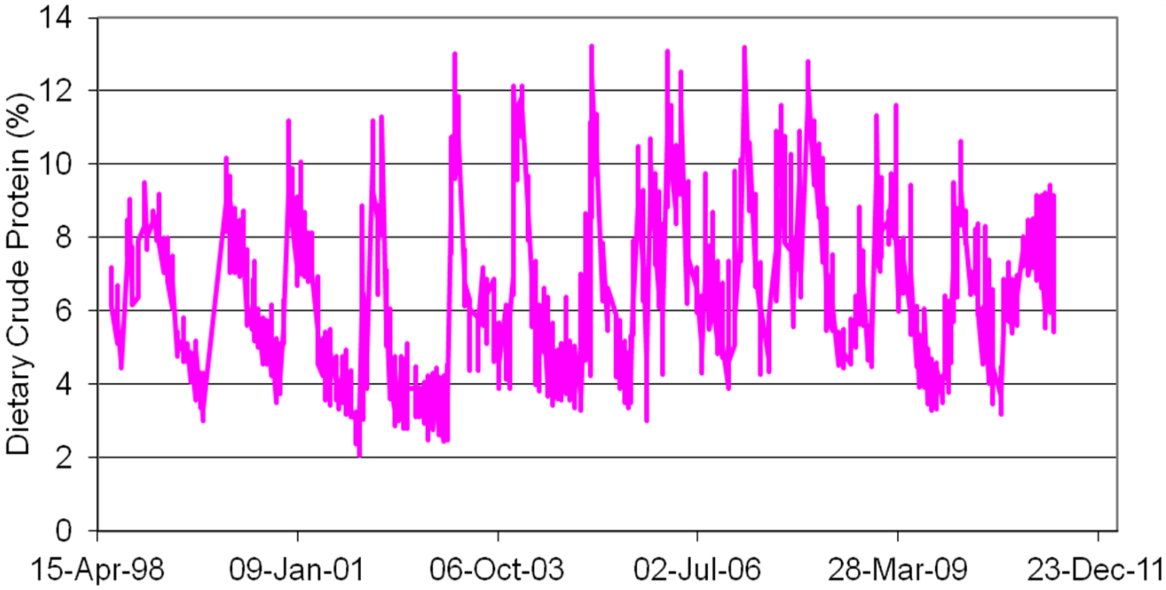
Supplementation results in no change of DMI of the basal pasture diet and the supplement adds on to the total DMI or nutrient/energy intake such as in the case of MLB containing by-pass protein such as cottonseed meal or energy from molasses of animals grazing good quality forages.

4.1.1.1 Northern Australia dry season

Marked deficiencies in crude protein (CP), energy, macro and micro mineral, and vitamins are experienced by cattle in northern Australia due particularly to the long dry season, in addition to poor soils and limited use of improved pastures and crops (Poppi and McLennan, 1995; Lean et al., 2011). Supplementary feed for rangeland cattle with lick blocks containing N (most often as urea) during the dry season and phosphorus (P) during the rainy season has been fundamental to successful cattle breeding in northern Australia. Supplementation has demonstrated increased weight gain, improved fertility, and reduced mortality in northern Australia (Cash Cow project). The nutritional composition, growth rates and cattle response to supplementation were reviewed by Lean et al. (2010).

Most pastures of the beef cattle regions in Australia go through periods of low CP and dry matter digestibility (DMD) depending on the length and severity of the environmental conditions that stop pasture growth. An example of the effect of weather on CP and DMD measured by faecal NIRS is provided in Figure 5 for pastures in Charters Towers (QLD) of the Wambiana grazing trial. The 'peaks' and 'troughs' of both measures of pasture quality are a result of pasture growth (green up) increasing CP and DMD during the wet season in summer, and pasture dry off during the dry season. The length and extent of these peaks and troughs will depend particularly on rainfall events although grazing management, plant species, and other factors also have an influence on these. Protein and energy supplementation are needed to maintain live weight (LW), reduce LW loss, or production.

A survey to industry stakeholders in northern Australia reported wide adoption of supplementation strategies (from 100 days to year around) and those nutritional deficiencies varied by region. However, all nutritional deficiencies that were important included N, energy, Ca, P, salt, Se, S, Co, Cu, I, and Mn (Lean et al., 2011). In addition, the ranking of perceived benefits of feed supplements from highest to lowest were non-protein N (NPN), microminerals, protein, energy, macrominerals, ionophores, antibiotics and Bambermycin. However, the authors stated that such nutrient deficiencies need to be validated.



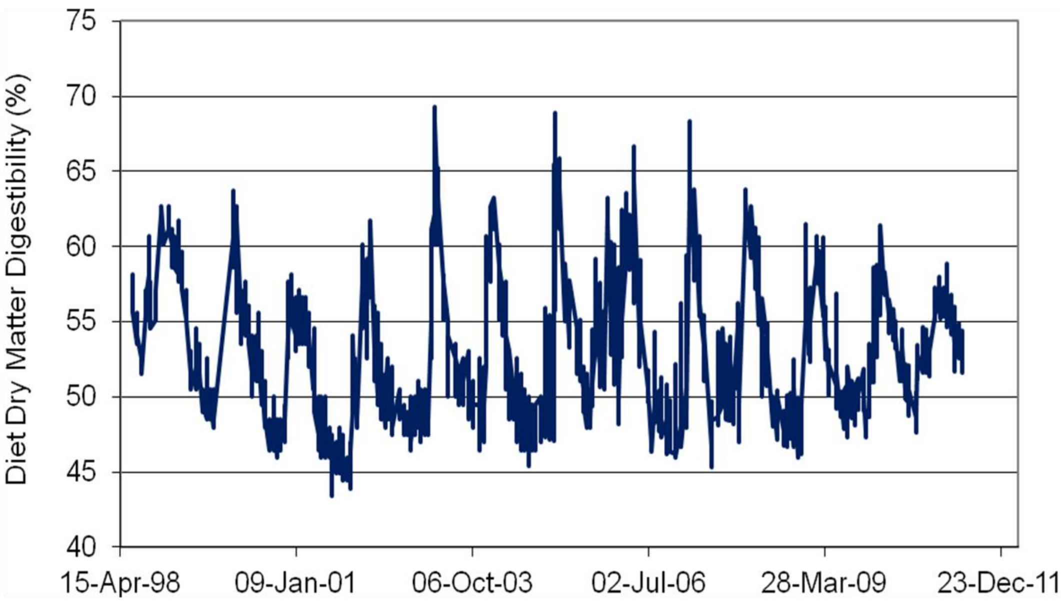
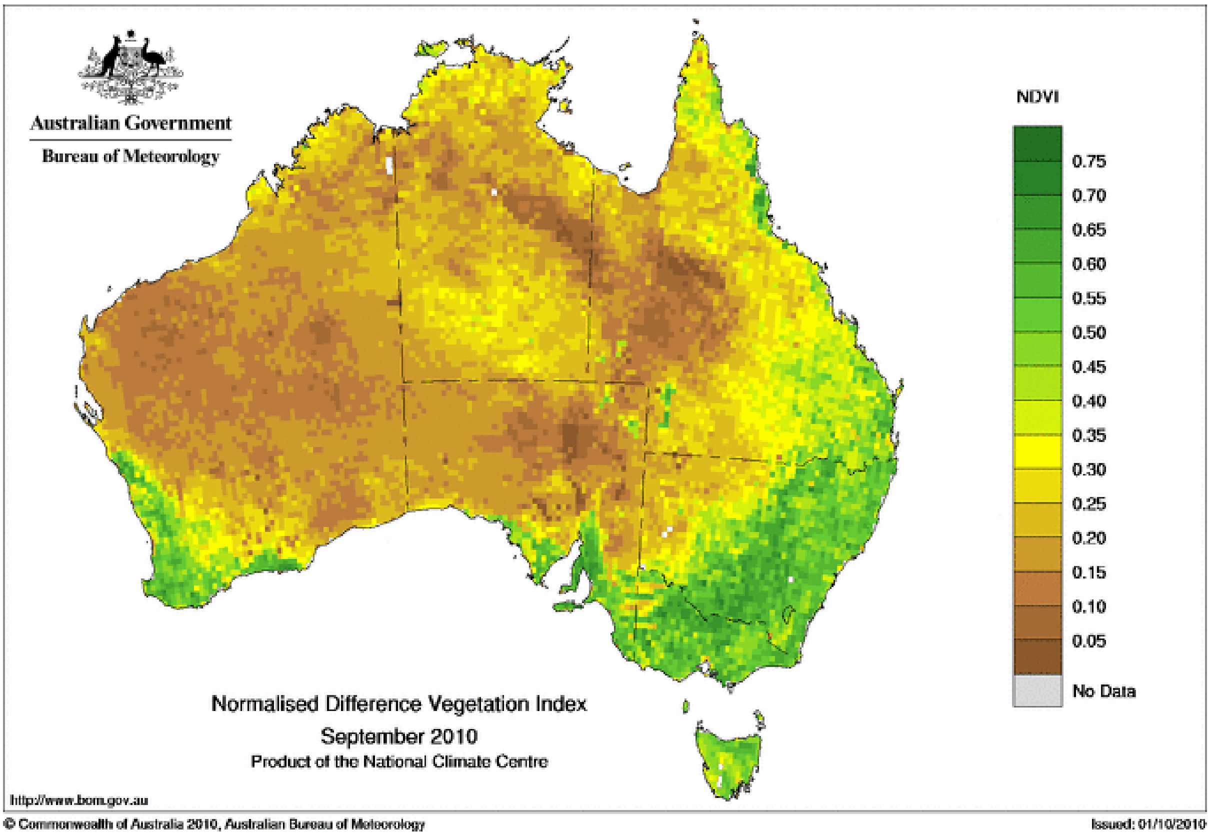


Figure. 5. Crude protein concentration and dry matter digestibility of native pastures throughout several years of the Wambiana grazing trial (QLD). Source: Hunt et al. (2007).

Green forage has higher CP and DMD and thus pasture quality is reflected through satellite vegetation indexes such as Normalised Difference Vegetation Index (NDVI or greenness index). Therefore, satellite imagery of NDVI values can give us an idea of the extent of these nutritional deficiencies for different regions and different seasons. Figure 6 shows examples to visualise regions with low-quality forage or bare ground (brown colours) for a relatively wet year (2010) and a dry year (2018). These maps demonstrate that CP and DMD (energy) are widespread nutritional limitations for cattle production, except for the high rainfall zones.



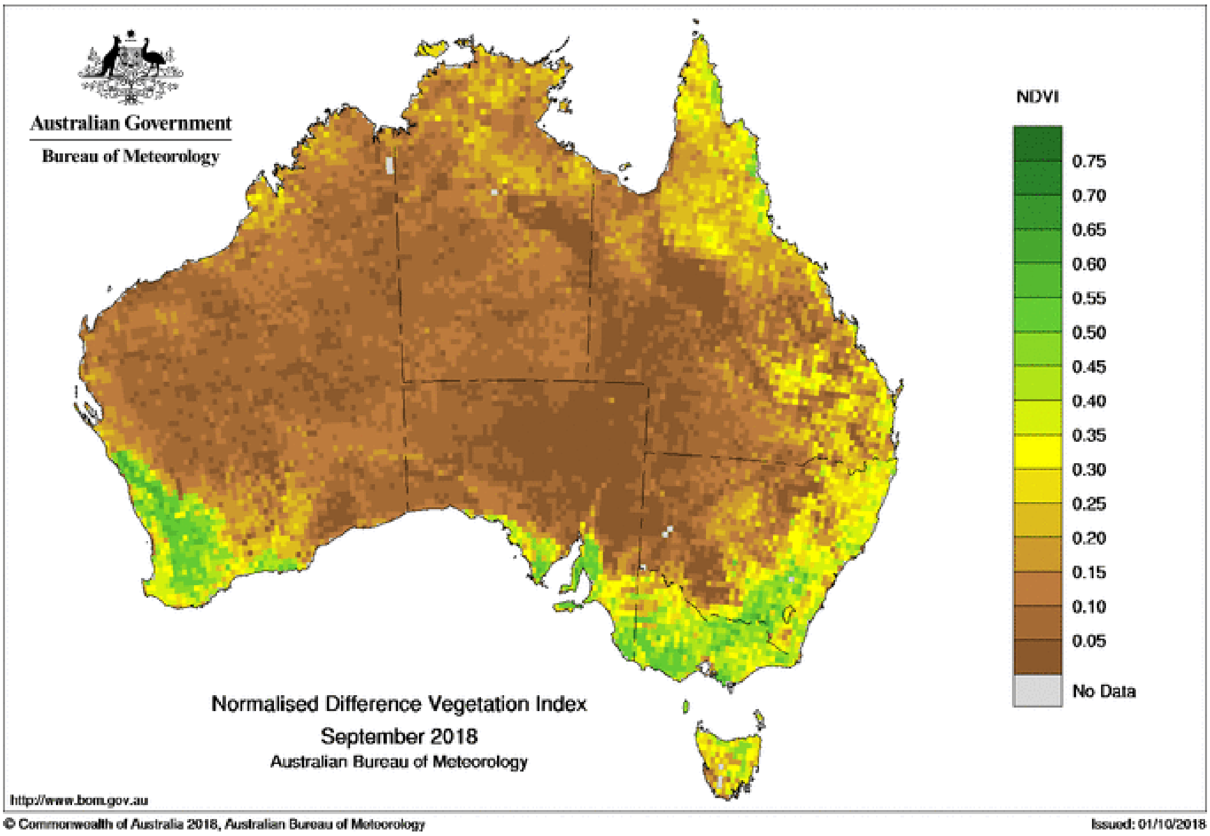


Figure 6. Normalised Difference Vegetation Index (NDVI) as an indicator of forage quality and quantity for two contrasting years (wet year in 2010; dry year in 2018).

As a result of the afore-mentioned changes in pasture quality, cattle live weight (LW) and daily LW changes (LWC) reflect both pasture quality and NDVI. An ongoing MLA project measured LW and daily LWC using remote weighing stations and Normalised Difference Vegetation Index (NDVI) near Kununurra (WA) for a period of 3 years. Figure 7 shows the close relationship between LW, daily LWC and NDVI. These results demonstrate the nutritional limitations of these animals during the dry seasons in northern Australia. Reducing the amount of live weight loss during these periods is critical to improve productivity (growth rate and weaning rates), reduce mortality, and reduce GHG emissions. Feedblock formulations for these scenarios should be evaluated.

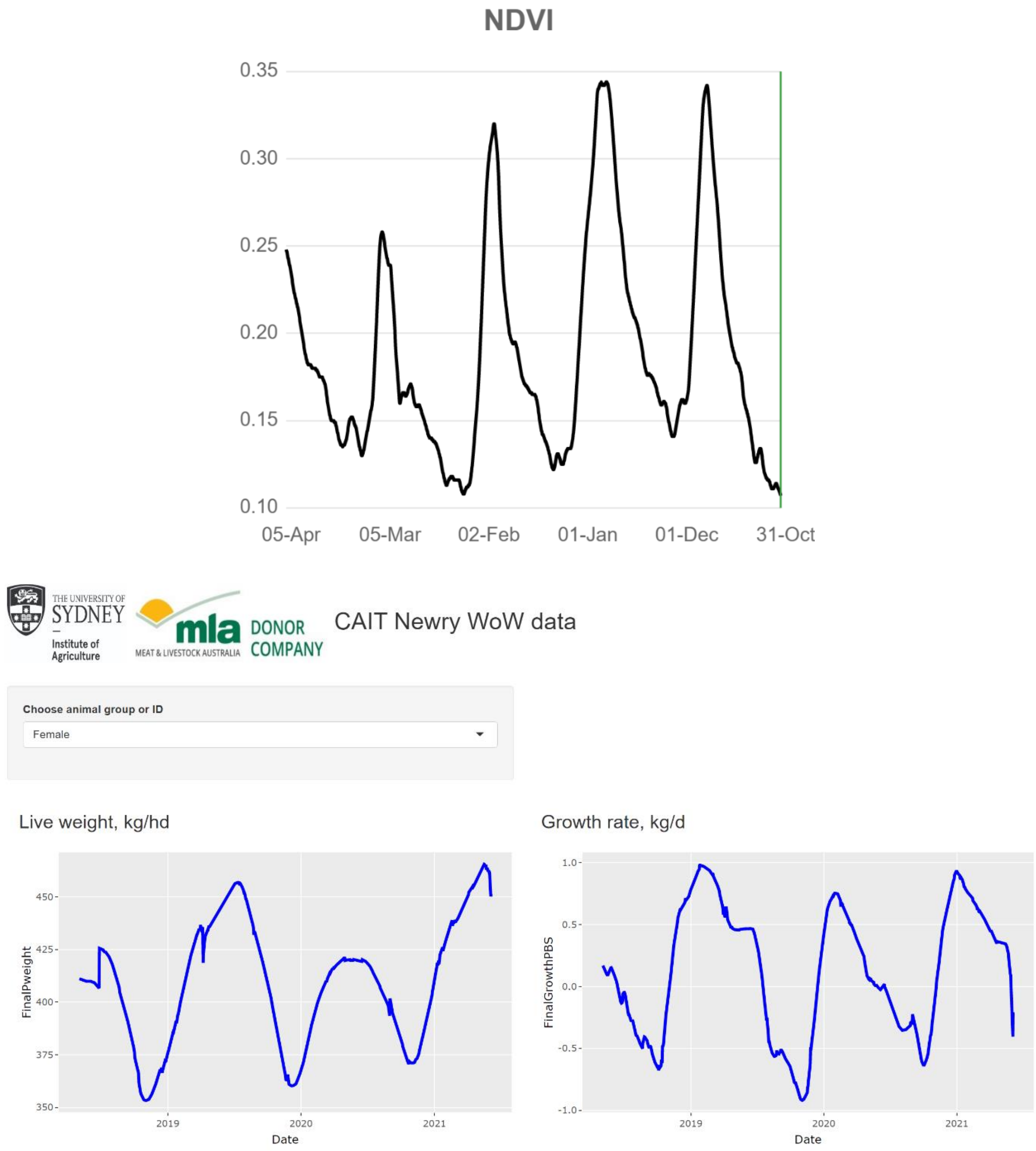


Figure 7. Satellite-derived Normalized Difference Vegetation Index (NDVI; top panel) and live weight and daily live weight change of breeder cows near Kununurra, WA.

4.1.1.1.1 Protein supplementation

During the dry season, the quality of tropical grasses decreases as the plants mature and fibre content increases (60-70% NDF). Native pastures and cereal crop stubbles often contain 4-6% CP when dry and thus, NPN and RDP supplementation are used to increase diet CP, DMD, feed intake and animal production. Urea is commonly supplemented to grazing cattle in northern Australia during the dry season and is the most cost-effective crude protein source for livestock. However, urea supplementation is mainly a strategy to reduce LW loss or maintain LW and improve survival rather than improve performance (Freer et al., 2007).

Compared to other livestock, however, ruminants are less efficient in terms of utilising nitrogen, which varies between 10 - 40 % (25% on average) (Calsamiglia et al., 2010), with 60 – 80% nitrogen derived from urea being excreted through urine, faeces, and scurf (Van Horn, 1996). High ammonia concentration in the rumen in the first hour after consuming urea is due to its rapid hydrolysis. The excess ammonia will flow to the bloodstream, transported to the liver, and converted to urea (Taylor-Edwards et al., 2009). Several forms of urea supplements exist in the market such as lick blocks, loose mixes, water medication, and molasses. The amount of urea consumed could be risky for cattle, and adequate mean to control urea intake should be followed to prevent intoxication, and potentially death.

Animals normally respond well to N supplementation when CP of the basal diet is below 9% of DM and ruminal fermentation is severely impaired below 7% CP (Freer et al., 2007), which normally occurs during the dry season for tropical and subtropical pastures. Urea is often mixed with molasses either in the liquid form or in MLB to provide readily fermentable carbohydrate for the rumen microbes to use the NH_3 although it also provides sulphur (S). Molasses lick blocks often contain urea in proportions of 0 to 30% plus other ingredients such as salt, P and microminerals. By-pass protein can also be added to molasses lick blocks to provide amino acids directly to the small intestine however consumption per day is low (an intake of 500 g/hd/d of MLB containing 15% cottonseed meal would provide 75 g/head per day).

4.1.1.1.2 Energy supplementation

Energy supplementation in northern Australia is a limiting factor to production particularly during the long dry season. It has been suggested that N may limit microbial protein synthesis if the ratio of DMD/CP exceeds 8-10:1, and animals then respond well to dietary rumen degradable protein (RDP) or NPN (Dixon et al., 2007). As the forage matures, this ratio increases and can reach values above 20:1 in the rangelands or cereal stubbles. In high quality pastures of southern Australia, the DMD/CP ratio if often below 5:1. However, energy supplementation may be beneficial to improve performance when DMD/CP is below 8-10:1 although energy may also be generated from the fermentation of amino acids in the rumen under this situation. Therefore, RDP has more beneficial effects on animal performance than NPN.

Ionophores (lasalocid and monensin) are often used in feed supplements and MLB in northern Australia to improve DMD and weight gain although research is limited for pasture-based systems to confidently determine the potential gains. Supplementation with these ionophores in northern Australia is made through molasses, urea, and cottonseed meal have increased growth rate by 130 to 270 g/d as reviewed by Lean et al. (2011).

4.1.1.2 Northern Australia wet season supplementation

Soils from northern Australia are deficient in phosphorus, and therefore pastures and the animals grazing on them (Figure 8). This reduces feed intake, growth rate and fertility of cattle with weaning rate of 60% being common. Cows mobilize P and energy to maintain lactation and they need to replenish this P and recover body condition when they are dry in preparation for the next lactation. Supplementing cows with P during lactation is necessary, however P supplementation during late pregnancy is another valid alternative (Dixon et al., 2020; Hegarty et al., 2021a)

Phosphorus (P) deficiency reduces feed intake, live weight gain, and reproductive performance of ruminants. Phosphorus deficiency is usually observed during the wet season (Judson & McFarlane, 1998) and it is widespread throughout northern Australia. P supplementation is not practiced often during the dry season because nitrogen (N) and energy are often the primary nutrients limiting responses to P supplementation when cattle are on dry pastures (Hegarty et al., 2021a). However, P deficiencies are so severe in many regions that year around P supplementation may be necessary such as the WA rangelands (Pilbara and Kimberley regions).

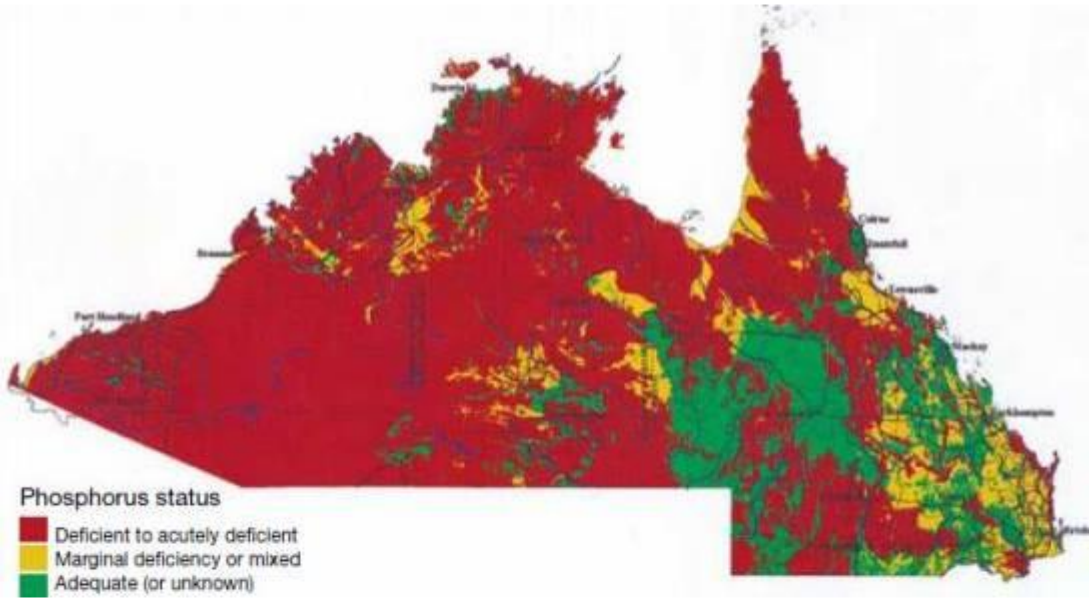


Figure 8. Phosphorus deficient regions of northern Australia.

4.1.1.3 Southern Australia supplementation during the pasture growing season

Many of the nutritional limitations of cattle in southern Australia are similar to those in northern Australia such as CP and energy during the feed gaps when pastures are dormant and dry. However, there are other nutritional limitations that are unique to southern Australia such as bloat from legume grazing,

4.1.1.3.1 Bloat

Bloat is the 7th most costly health issue of Australian beef production, estimated to cost over $75M per year (Shephard et al., 2022). Bloat is an over-distention of the reticulorumen due to gases from fermentation of feed forming a persistent foam with ruminal contents (primary or frothy bloat) or separated from the ruminal contents as free gas (secondary or free-gas bloat). Bloat occurs when the animal cannot eructate the gas from fermentation which continues being produced. Bloat can cause large number of deaths and it is most prevalent on animals grazing lush, young pastures in early spring particularly high in legumes including (lucerne, clover, or medics). Therefore, bloat is an issue particularly in southern Australia during the autumn, winter, and early spring. Bloat is also prevalent with plants that contain prussic acid, which produces rumen paralysis thereby leading to frosty bloat. Various strategies exist to prevent and reduce bloating in cattle including grazing management (e.g., avoid placing hungry animals in risky pastures), supplementation with roughage high in fibre (hay or straw), anti-bloat preparations for drenching or water addition, spraying pastures with oils, adding oils to drinking water, or monensin supplementation or ruminal pellets.

Various lick blocks are also available in the market with anti-bloating agents including Protect as molasses lick block from 4Season, BLOAT LIQ from Olsson's, and MegaMin Lush Legume Lick with anti-bloating agents, monensin, oils, monensin, anti-foaming agents, macro- and microminerals. Molafos Bloat plus Teric is a molasses and the anti-foaming agent Teric blend. Further information can be found on NSW-DPI fact sheep 'Bloat in cattle and sheep'.

Ionophores such as Monensin, Lasalocid, Salinomycin, shift the microbial fermentation towards greater propionate production, potentially reducing gaseous end-products of fermentation and subsequently the occurrence of bloat. In addition, these ionophores could also reduce methane emissions from cattle. Ionophores have also demonstrated increased growth rate in grazing cattle and feed efficiency (Freer et al., 2007).

4.1.1.3.2 Southern Australia supplementation during feed gaps

For Mediterranean pastures in southern Australia, CP, DMD and animal performance normally decreases during the summer, whereas this occurs during the winter in native pastures in non-Mediterranean weather such as the Tablelands. In addition, stubbles from cereal crops can also be grazed and have low CP and DMD similar to low quality pastures (DMD below 50% and CP below 5%). Protein supplementation of growing or lactating animals is often done on these pastures during such seasons and regions. Supplementation of CP to grazing animals can be done with NPN, rumen degradable proteins, or rumen undegradable (by-pass) protein (such as cottonseed meal). Supplementation with NPN and RDP provide a source of nitrogen for the rumen microorganisms to grow and produce microbial protein which the animal will then digest, whereas cottonseed provides amino acids for the animal to absorb in the lower gastrointestinal tract. However, animals can also obtain some energy from rumen degradable true protein, in contrast to NPN which is mainly provided to the microbes for protein synthesis and growth. As in northern Australia, rumen degradable protein (RDP) or non-protein nitrogen (NPN) such as urea are supplemented to promote ruminal microbial activity, which may increase feed intake and thus extra ME from the low-quality feed consumed (Freer et al., 2007) However, it is important to note the risk of urea toxicity and the potential energy expenditure of animals to recycle and eliminate urea from the body when it is consumed in large amounts.

4.1.1.4 Supplementation in the mulga country

Large parts of the semi-arid and arid regions of the Australian rangelands are dominated by plant species with high concentration of tannins such as mulga trees (Acacia spp., mainly A. aneura) which are primarily used for livestock production (Figure 9). Tannins have multiple effects on the ruminants including a reduction in enteric methane production, palatability, parasite load, CP digestibility, DMD, and feed intake, amongst others (Mantz et al., 2009; Aboagye and Beauchemin, 2019; Suybeng et al., 2020; Stifkens et al., 2022). Therefore, supplementation strategies to reduce these detrimental effects and improve CP digestibility of mulga are needed as stated by industry stakeholders via a survey (Lean et al., 2011).

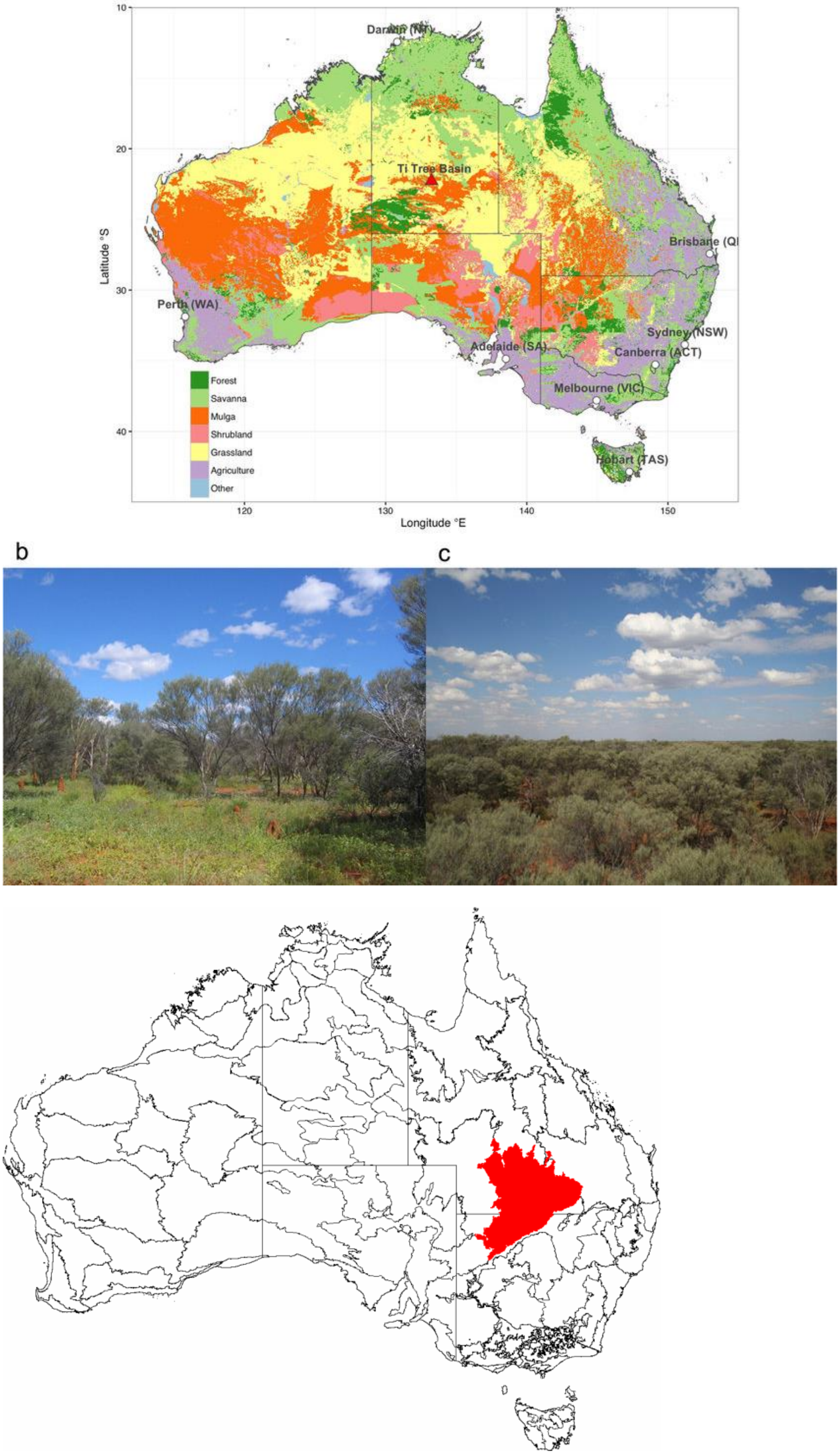


Figure 9. The mulga woodlands and shrublands of Australia (top panel) and Eastern Australia (bottom panel). Source: Eamus et al. (2016) and Wikipedia.

Mulga has a high CP content of 18.2% and low energy of 7.6 ME MJ/kg DM but the high concentration of tannins reduces both ruminal degradation and total tract digestibility of both CP and DM. Polyethylene glycol (PEG) binds to tannins to reduce their biological effects on the animals and the rumen microbes. The PEG has demonstrated in various in vitro and in vivo studies that can improve protein and diet digestibility of diets containing high tannin concentration (Frutos et al., 2004; Mantz et al., 2009; Xie et al., 2021). However, research by Leigo (2011) in central Australia did not find a positive effect of PEG supplementation in drinking water at 60 g/head/day (range 14 - 105 g/head/day) for 8 weeks or during daily drenching at 200 g of PEG/head per day. However, this trial was done with only 10 heifers and no energy supplementation was provided so the results may be hindered by the fact that CP may not has been the limiting factor and methane emissions have not been measured.

In contrast, supplementation of PEG with phosphate, urea, and sulphate of ammonia to steers in south-west Queensland reported a 32% increase in DMI (Strachan et al. 1988). Similarly, research in sheep has demonstrated improved DMI, LWG, and wool growth when mulga was supplemented with PEG (Pritchard et al., 1992; Miller et al., 1997). In vitro gas production trial adding PEG to diets with high tannins increased gas production and OMD (Canbolat et al., 2005), which suggests a neutralisation of the negative effects of tannins when binding to dietary protein and improve the nutritive value of tanniferous plants.

Interestingly, molasses lick blocks with added PEG (and no urea) to inactivate tannins increased the utilization of tannin-rich browses and trees in livestock (FAO, 2007; FAO, 2011). These authors also highlighted that addition of urea to MLB with PEG fed to animals consuming vegetation with high tannin content is not required because the main purpose is to increase the availability of protein from PEG competing with protein for binding.

4.1.1.5 Mineral supplementation

The most common mineral deficiencies in Australian grazing systems are phosphorus (P), selenium (Se), cobalt (Co), and copper (Cu) due to low soil availability. However, many other deficiencies are also common seasonally or in certain regions including calcium (Ca) and magnesium (Mg), and less often salt (NaCl). These minerals are therefore provided in various forms to livestock including injections, capsules, drenches, pellets, pour-ons, lose licks, and dry or molasses-based lick blocks. However, the present review focuses on prevention of mineral deficiencies through feed supplementation.

Minerals are divided into macrominerals, those required in large daily amounts and normally expressed as % of diet dry matter (DM), and micro or trace minerals required in small daily quantities expressed often in ppm. Macrominerals include phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Sulphur (S), sodium (Na), and chlorine (Cl). Microminerals include selenium (Se), cobalt (Co), copper (Cu), iron (Fe), zinc (Zn), iodine (I), and manganese (Mn). These minerals play important roles in animal physiology and deficiencies, or excesses can affect reproduction, growth rate, milk production, and survival of the animal (Judson & McFarlane, 1998).

4.1.1.5.1 Micromineral supplementation

Micronutrients are needed in small quantities and therefore often delivered with dry or molasses lick blocks to grazing animals. There is widespread micromineral deficiency across Australia although severe deficiencies occur in more localised regions and more than one micromineral can be deficient in a region (Figure 10).

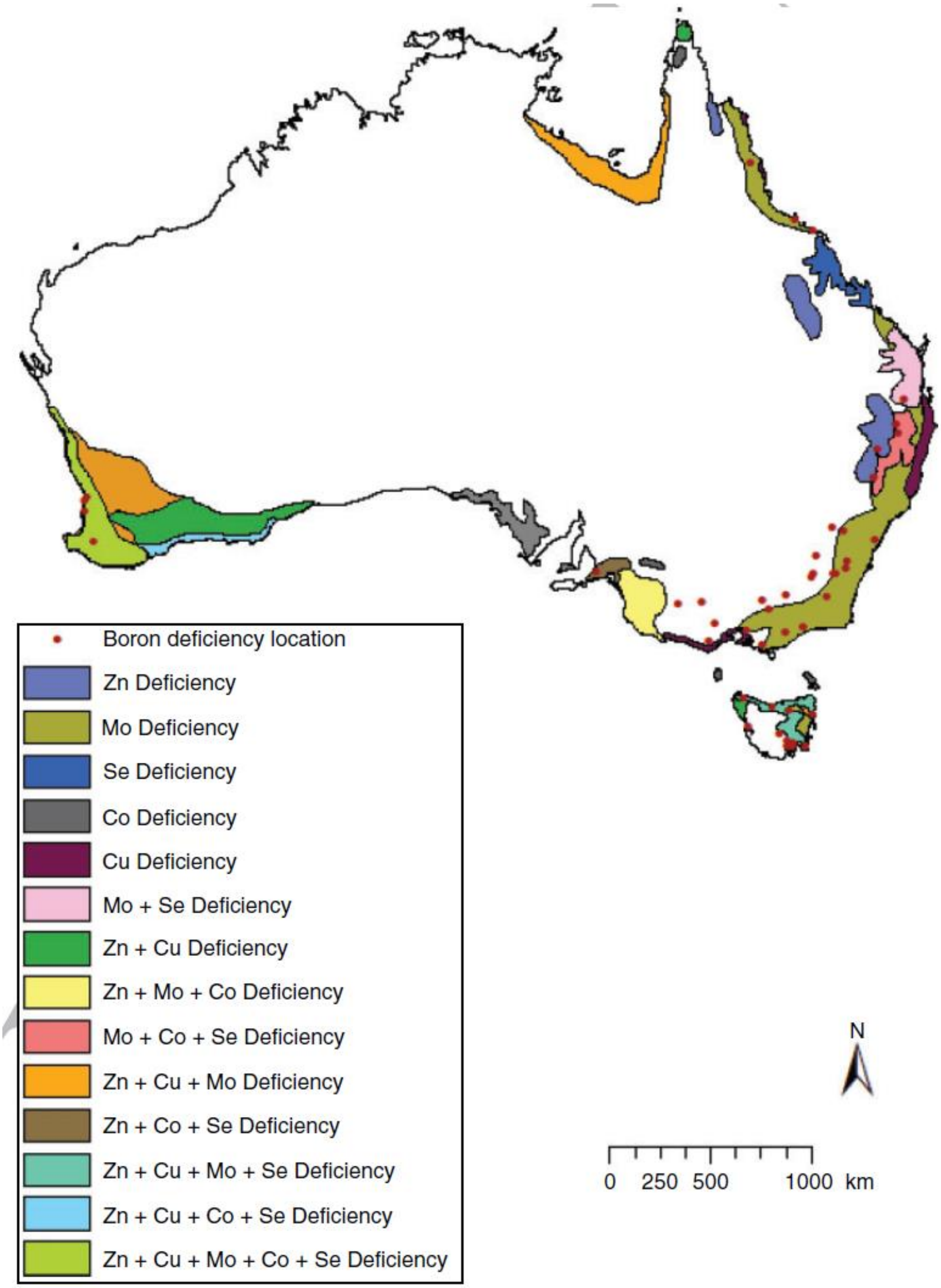


Figure 10. Inherent and potential micronutrient deficiencies in agricultural soils. From Brennan et al. (2019).

It is also important to note that mineral deficiencies in soils are partly a consequence pf soil pH which limits their availability for absorption by the plants (Figure 11).

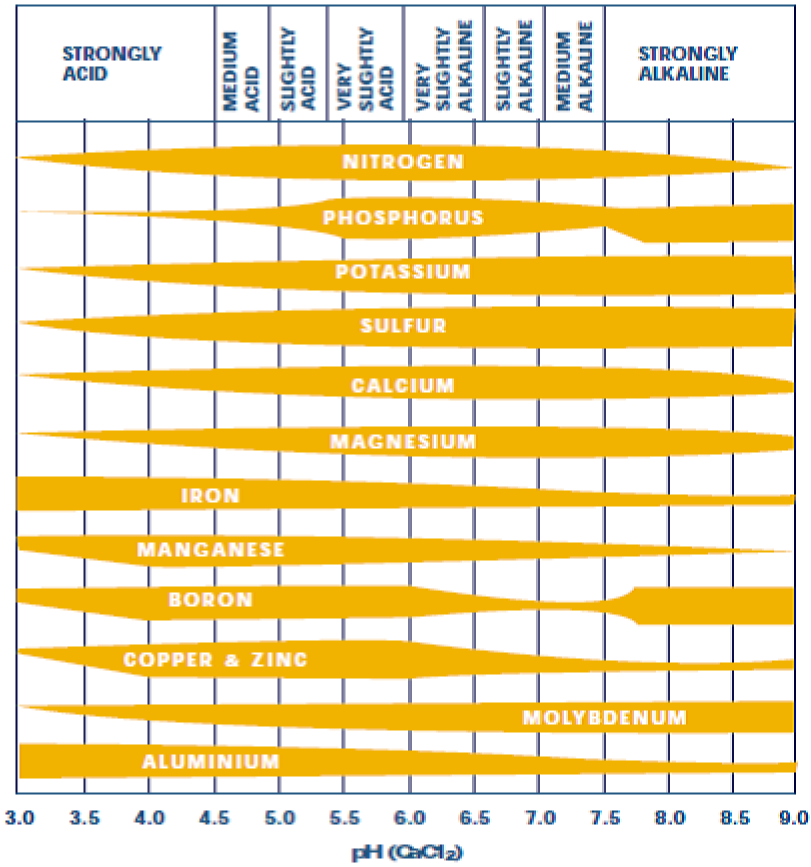


Figure 11. Effect of soil pH on the availability of plant nutrients. (Source: Understanding soil pH, NSW Agriculture)

A map of soil pH across Australia is also provided in Figure 12 because this can be used to determine regions where particular minerals may be limiting because of soil pH, and thus to formulate MLB tailored for those regions. However, research on micromineral deficiencies and the response of animals to supplementation in Australia is limited.

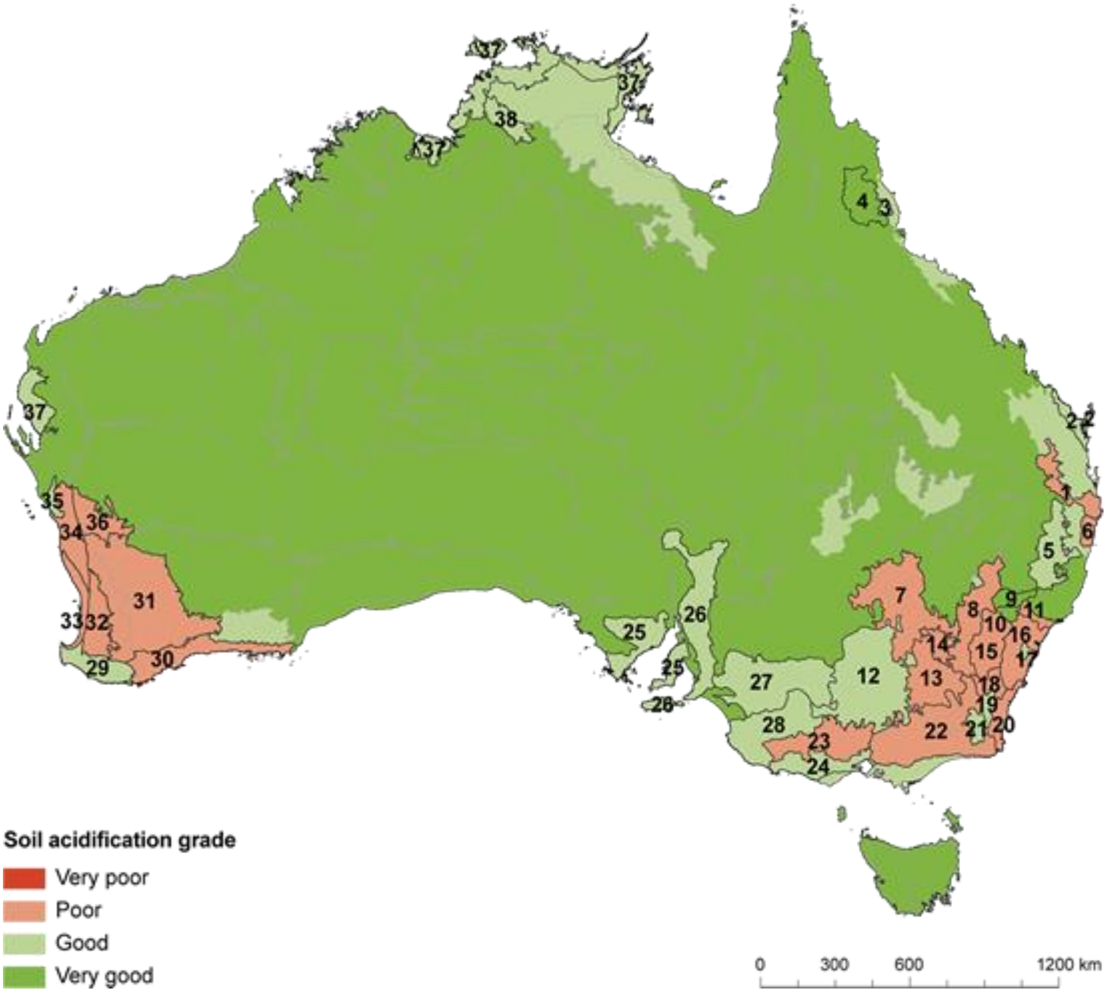


Figure 12. Soil acidification in Australia. Source:
http://www.environment.gov.au/science/soe/2011-report/5-land/2-state-and-trends/2-2-soil

4.1.1.5.1.1 Selenium

Selenium is an essential micromineral for ruminants and deficient in soils of all Australian states, particularly in regions of high rainfall (>500 mm/year) in southern Australia (Figure 13). Low soil Se concentration also affects the concentration of Se in the plants that grown on them including pastures, and subsequently the animals. Selenium works together with vitamin E to help preventing and repairing cell damage due to its antioxidant properties supports neutralising toxic substances produced by the cells during metabolism. Se also improves immune and thyroid function, particularly during periods of stress such as transport and marketing of cattle (Freer et al., 2007). Se also participates in the supply of oxygen to skeletal muscles and improves growth and fertility. Se is stored in the liver of cattle for a short period of time and therefore, it is required as a constant supplementation strategy in regions with deficient soils. For this reason, many products are available in the market including ruminal pellets, injections, and lick blocks to a lesser extent.

A Se deficiency in calves has been associated with white muscle disease, also known as 'subacute enzootic muscular dystrophy' and it has been associated with ‘weaner ill thrift’. Deficiency in older cattle can produce cystic ovaries, early and late embryo death, mastitis and retained placenta.

Clover pastures and rapidly growing pastures have lower concentration of Se, and sandy, acidic basalt or granite soils are usually low in trace minerals (Judson and McFarlane, 1998).
An ongoing trial near Kununurra (WA) did not find Se deficiency in breeder cows in the middle or end of the dry season.

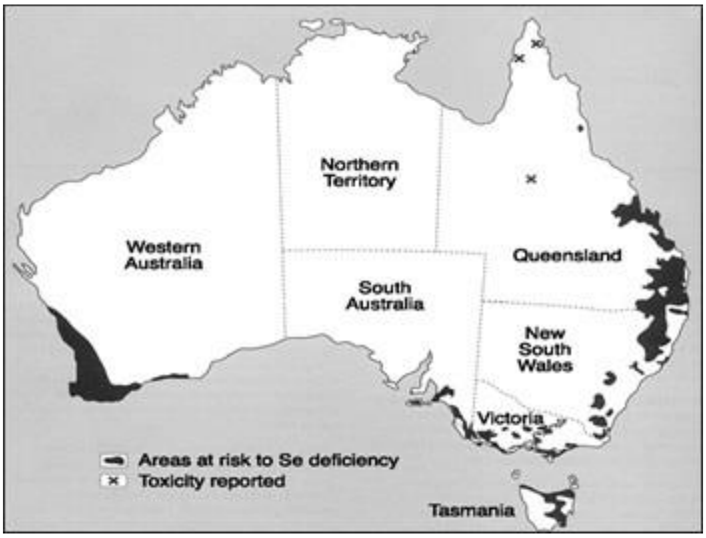


Figure 13. Selenium deficient areas in Australia (from Judson and Reuter, 1999 Soil Analysis: An Interpretation Manual).

4.1.1.5.1.2 Cobalt

Cobalt (Co) deficiency is observed in Western Australia and South Australia and in restricted areas of Tasmania and Victoria. Specially in Coastal calcareous sands, soils of high manganese oxide and lush grass-dominant pastures (Brennan et al., 2019). Rumen microorganisms need Co for the synthesis of B12. Insufficient vitamin B12 may affect fertility and growth rate by reducing appetite and cause white liver disease or anaemia (Judson & McFarlane, 1998). Australian zones with Co deficiency were presented in the figure above but it is also important to note that subclinical deficiency of Co (and perhaps other minerals) may be much more widespread than shown in the map. These deficiencies may go undetected and result in suboptimal animal performance due to reduced feed intake and body condition.

Co is normally supplemented together with the rest of the trace minerals (pre-mix) as lose lick, dry lick, or MLB however intraruminal slow-release pellets (or boluses) are also used in weaners and B_{12} injections in young animals (Judson et al., 2002). The boluses can also contain additional minerals such as Cu and Se, and last for 3 to 6 months.

4.1.1.5.1.3 Zinc

Zinc (Zn) deficiency is associated with reproductive malfunction in ruminants, particularly in the male by disrupting spermatogenesis, whereas in female, supplemental Zn improved conception rates by 23% (Hidiroglou, 1979). Zn deficiency is found in Western Australia in dry mature pastures (Judson & McFarlane, 1998). A deficiency in Zn may reduce growth and fertility rates and milk production, a loss of appetite and skeletal disorders, and impair testicular development in ruminant (Brennan et al., 2019). Previous research showed that Zn supplementation at a concentration of 250 ppm might reduce possibility of urea toxicity and improve the efficiency of rumen fermentation (Arelovich et al., 2000)

The annual loss of Zn from an intensively grazed system represents about one-third to one-half of the amount of Zn fertilizer rate applied in agriculture systems in Western Australia. The set stocking rate grazing system is the most used system for dryland pastures in WA and should be appropriately supplied with Zn for >15 years after applying 1 kg Zn/ha or more if pastures are covered with P fertilizer enriched with Zn (Brennan et al., 2019). An ongoing trial near Kununurra (WA) did not find Zn deficiency in breeder cows in the middle or end of the dry season.

4.1.1.5.1.4 Copper

Cu is also an essential trace mineral deficient in some Australian regions, and its absorption in cattle can be affected by an excess of S and Mo (Freer et al., 2007). Pasture concentration of 100-300 mg/kg DM for cattle have been suggested as appropriate (Suttle, 1999). Cu is less available in lush green feed in winter and spring in southern Australia, and after the summer rainfall in northern Australia. Blood concentration of Cu and reserves in liver normally follow these trends. A deficiency is often reflected by the loss of hair pigmentation, reduced growth rate, fragility of the long bones, diarrhoea, and anaemia (Underwood 1977). Deficiencies of Cu have been reported in western Victoria and Gippsland due to high Mo and S, coastal regions of South Australia, WA, QLD, and NSW due to low soil Cu availability. Prolonged consumption of *Heliotropium echium* (e.g., Paterson's curse), or Senecio (e.g., ragwort) spp. which contain pyrrolizidine alkaloids has also been associated with chronic Cu toxicity in parts of New South Wales and Victoria. Supplementation is the best way to correct deficiencies, but injections are also successful and practical.

An ongoing project with breeders near Kununurra (WA) found 60% of cows with copper deficiency in blood at the end of the dry season. This is likely to contributed to reduced animal performance, reduced fertility and compromised immune functions, and reduced coat quality. In calves, the effects of copper deficiencies are more pronounced including ill thrift, anaemia and sudden death. However, no copper deficiencies were found in the same animals at the end of the wet season which suggests that Cu stores depletion occur throughout the dry season but animals replenish Cu during the wet season. However, Cu concentration in blood at the end of the wet season was lower in wet compared to dry cows, which was attributed to the loss of minerals in lactating cows. This highlight the importance of mineral supplementation for lactating cows but further research is required to test for copper after supplementation.

4.1.1.5.1.5 Iodine

Iodine is essential for the thyroid hormones in humans and animals, thyroxine (T4) and triiodothyronine (T3). Iodine supplementation to pregnant ewes improved birth weight, growth rate and survival of lambs in Australia, suggesting that iodine may be a limiting factor in livestock production (Knights et al. 1979; Ellis and Coverdale 1982; Hosking et al. 1986). Sporadic lamb and kid losses due to iodine deficiencies have been observed in NSW, VIC and TAS (Freer et al., 2007).

Supplementation of iodine is normally done through iodised salt licks, potassium iodide, and calcium iodide. Drenches and injections are also available for ewes in late pregnancy although the extent of adoption is unknown.

4.1.1.5.1.6 Manganese

Mn is part of several enzymes and critical for skeletal development and reproductive function such as oestrus in females. Diets with Mn concentration below 20 mg/kg DM has been suggested as the lower limit below which deficiencies may occur. However, optimum dietary content for performance has been suggested at up to 120 mg Mn/kg DM (Freer et al., 2007). Manganese (Mn) deficiency in cattle can be identified by excessive tongue activities (Karatzias et al., 1995). It is associated with reproductive failure in female by depressing conception rates (Hidiroglou, 1979).

4.1.1.5.2 Macromineral supplementation

Pastures often contain mineral imbalances in different regions and seasons which are affected by a multitude of factors including fertilisation, soil mineral composition, weather, and management practices, amongst others. This section presents a summary of limitations observed in Australia and the main function of macrominerals.

4.1.1.5.2.1 Phosphorus

The concentration of P in cattle diets is recommended to be above 2.7 g P/kg DM and soils with less than 4 ppm are considered acutely deficient, and 4-8 ppm are considered deficient. Soil P availability is shown in Figure 14 to demonstrate that deficiencies are severe across vast regions of Australia. The impact of P deficiency on beef cattle production may be more widespread than where supplementation efforts are being directed at present.

An ongoing project near Kununurra (WA) found 18% of cows with P deficiency at the end of the wet season (April), 36 % of cows at the start of the dry season (June) and 0% of cows deficient at the end of the dry season (October) without P supplementation in the dry season. In a subsequent year, 26% of cows were P deficient at the end of the wet season and it was lower in wet than dry cows, despite P supplementation during the wet in both years. In addition, pregnant cows had higher P concentration compared to empty cows, which demonstrates the effect of P on conception. These results demonstrates that P supplementation may not always be efficient when provided through lose lick at libitum and the higher requirements for lactating cows. However, removal of P supplementation resulted in widespread P deficiencies in 92% of the cows. Some potential factors to consider include the type of P ingredients and absorption, method of delivery, and cattle attendance. Phosphorus deficiencies are associated with reduced appetite and feed intake, reduced grazing and energy and protein intake, reduced weaning rates, poor body condition and increased mortality in both adults and calves.

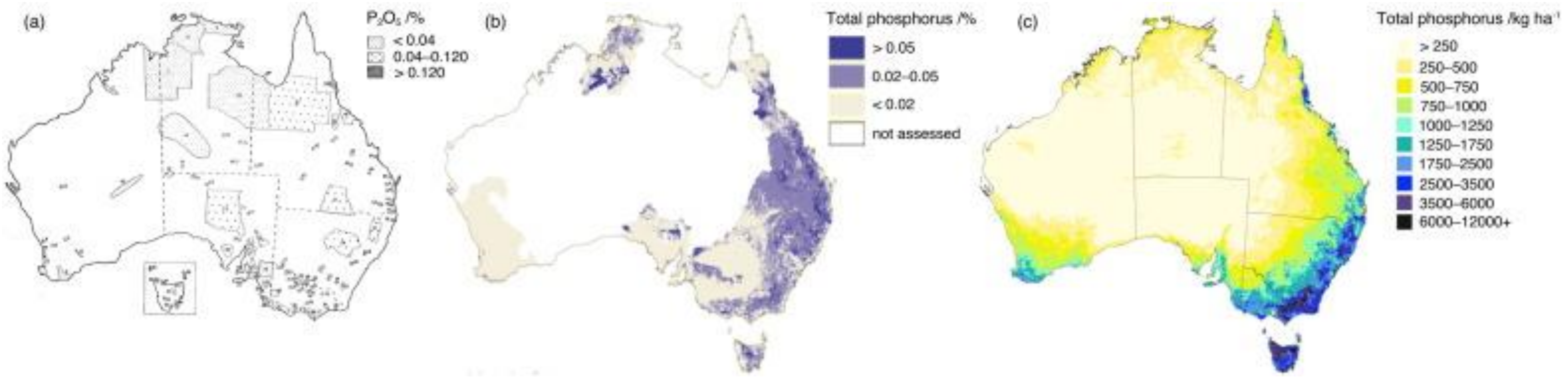


Figure 14. Soil phosphorus concentration and availability in Australia.

4.1.1.5.2.2 Salt supplementation

Salt (refer to the main ions Na, Cl and K) is involved in the regulation of osmotic pressure and acid-base equilibrium in animals. Salt is recommended to be supplemented all year for grazing livestock to have ad libitum access in most regions of southern Australia. Insufficient sodium in cattle is common in areas of south-eastern Australia, especially on pastures fertilised with K (Harris et al.1986).

Salt has frequently been used as a vehicle to supply trace elements (e.g., copper, cobalt, and selenium etc.) to grazing cattle because it controls intake. However, marked variation in salt consumption between animals in a group suggests that salt offered free choice may have serious shortcomings as a vehicle for supplying trace elements. Salt is also typically provided to regulate the intake of a highly palatable supplement by grazing ruminants (Morris et al., 1980). However, salt used to limit intake also showed negative impacts on animal performance with large between and within-animal intake variability (White et al., 2019).

Forages generally contain adequate concentrations of Na, Cl and K, and often an excess of K is more common than a deficiency which can affect Mg metabolism (Freer et al., 2007). However, positive responses to salt supplementation have been reported (Freer et al., 2007) including improved growth rate of steers in northern Australia (Winter and McLean 1988), beef cows and calves on native pastures of the Darling Downs (Kaiser, 1975), calves grazing kikuyu in Wollongbar (NSW), and steers fed *Setaria sphacelata* hay (Little, 1987). These ions may be also important during periods of hot weather.

4.1.1.5.2.3 Calcium supplementation

Hypocalcaemia (a.k.a. milk fever or periparturient paresis) is a result of an imbalance in the diet of lactating cows during winter and spring, particularly during the first few days after calving. Reduced growth rate in livestock fed grain and little roughage during drought has also been associated with Ca deficiency in south-eastern Australia (Judson & McFarlane, 1998).

Hypocalcaemia in cows occurs after calving when Ca requirements increase sharply and suddenly, and primarily in rapidly growing pastures with high water, protein, and potassium contents. Therefore, Ca supplementation during late pregnancy of cattle consuming low-Ca diets has been recommended (McLachlan 2004). However, most pastures have adequate concentrations of both calcium and magnesium, and therefore supplementation is not required, except during drought feeding with cereal grain that are low in Ca.

Supplementary Ca of grazing animals may be beneficial when the concentration in pastures is below 0.25% of DM although its low cost makes it a good insurance against potential Ca deficiencies, especially in lactating cattle (Langlands et al., 1967).

4.1.1.5.2.4 Magnesium

Hypomagnesemia, a.k.a. grass tetany, is a common nutritional deficiency in different seasons and regions across Australia (both tropical and temperate regions). However, Mg deficiency is more prevalent in lactating cows in grass pastures or cereal crops of south-eastern Australia during late autumn and winter. Higher risk is also present in green pastures and cereal crops high in N (>40 /kg DM) and low in Mg, Ca, and Na (less than 2.0, 3.0 and 1.5 g/kg DM), and K concentration above 30 g/kg DM (Mayland and Grunes 1979; Caple and West 1992). Furthermore, high K concentration could also result in metabolic alkalosis and increase the risk of hypocalcaemia (Judson & McFarlane, 1998).

Mg is an essential mineral required for many physiological functions including muscle contraction, nerve conduction, and adrenaline release (Mayland, 1988). Therefore, grass tetany produces a nervous disorder reflected through incoordination, stiffness, staggers, over-alertness, and aggressiveness. Stress of the animals also reduces feed intake, contributing to low body condition, low performance in growing cattle, calving difficulties, reduced milk production of lactating animals, and can be the major cause of cattle mortality. Mg is not stored in the body of cattle in a readily available form and therefore a daily supply is required.

Late autumn or winter pasture is short, when pastures are rapidly growing and young, are the most prevalent times of the year because of weather stress reduces Mg uptake by plants in periods of low photosynthesis. Predisposing factors are:

- Age and physiological status (older cows with young in peak lactation are at higher risk)
- High potassium in soils
- Weather including wind, rain, and exposure/sudden lowering of temperature
- Grass-dominant pastures or young cereal crops
- Soil acidity (such as those in southern Australia; Figure 12)

Daily supply of Mg is required and therefore, Mg supplementation is required when pastures do not contain sufficient concentration. The grass tetany index or tetany ratio has been developed as an indicator of the risk of grass tetany, which is calculated as the concentration of potassium/ (calcium + magnesium) (Kemp, & t Hart, 1957). Potassium is part of this index because high concentrations of this mineral can impair Mg absorption in the rumen. However, this index has not been associated with dark cutters in a recent study by Loudon et al. (2021).

It has also been demonstrated that animals not experiencing clinical signs of grass tetany are also benefitted by Mg supplementation potentially because it reduces stress in period after weaning, marketing, and transport (Loudon et al., 2018 and 2021). Because of this, Mg supplementation has demonstrated to reduce dark cutters in cattle off green pastures in southern Australia, thereby improving meat quality (Loudon et al., 2018 and 2021). The latter research showed that pasture with MG concentrations greater than 0.24% reduced the risk of dark cutting by 26% whereas feed supplementation with hay or silage during the last 7 days prior to slaughter reduced dark cutting by 25%.

Magnesium is often provided as magnesium oxide or Mg sulphate salt in lick blocks to animals lush and rapidly growing grass pastures and cereal crops in the autumn, winter, and early spring. Several products are in the market as

- lose lick (MegaMin extra magnesium from AgSolutions),
- dry lick (8.5% pure magnesium Econo Mag from Olsson's with a target intake of 50-100 g /head/ per day),
- molasses lick blocks (MAGPLUS from 4Season with 10% Mg; Beefmaster from Olsson's with 11% Mg and essential trace minerals),
- liquid molasses (MaxPro and Organic Pro from Performance Feeds to be fed at a maximum of 2 kg/head per day)
- other liquid formulations for addition to water (Yellow Cap High Magnesium for milk fever and grass tetany)
- MgO salt to add to pelleted rations

A recent study reported that canola-based pellets with added MgO or Mg sulphate to provide 10 g/d of Mg had palatability issues and therefore the second part of the study added molasses to the pellets to increase pellet intake and reduce dark cutters (Loudon et al., 2021). Supplementation for cattle is recommended at 60-100 g/head per day of MgO during critical periods and deficiency risk, which is often recommended together with lime (calcium carbonate) and salt on equal proportions. These periods are at calving and peak lactation for older cows, autumn/winter/early spring for lush pastures.

4.1.1.5.2.5 Sulphur

Sulphur (S) is particularly important for amino acids and thus required for the synthesis of protein, particularly by the rumen microorganisms. Therefore, dietary requirements are often expressed as N:S ratio. Kandylis (1984) stated that to optimise utilisation of nitrogen in the rumen, it is important to consider the nitrogen (N): sulphur (S) ratio. The optimum ratio for sheep diets is 10-13.5 :1 and for beef cattle is 13.5-15: 1. Deficiency of S could reduce feed intake, microbial protein synthesis, and OMD. Molasses contains a high concentration of S. There is widespread of S deficiency across Australian soils and supplementation has demonstrated to improve animal performance (Minson, 1982b). However, it has been suggested by the NRC (1996) that S intake from all sources should be below 0.4% of DMI to avoid over-production of H2S in the rumen, absorption into the blood stream, and signs of cerebro-cortical necrosis (CCN).

4.1.1.6 Vitamins

Vitamin B, C, and K seem less critical for grazing cattle nutrition because they are synthesized by the rumen microbes and are abundant in forages (Freer et al., 2007) so these will not be described in the present review. However, the effects of these vitamins in pre-ruminant calves in regions with prolonged dry seasons or during drought in Australia still unknown but they may be required for young calves. The present review focuses on fat-soluble vitamins A, D and E because these are not synthesized by rumen microorganisms and have been reported as potential causes of lost animal performance, reproduction, and mortality in cattle.

Vitamin A is found in green forages mainly as β-carotenes (carotenoids are precursors) and stored in the liver to be used during periods of low vitamin A intake. Vitamin A is important for various physiologically distinct functions such as vision, reproduction (resulting in retained placenta, stillbirth, and absorption), bone growth, epithelial tissues, and the immune response (Bendich 1989; NSW DPI, Primefact 1697, 2019). Early research reported vitamin A deficiency was not a problem in cattle in Queensland particularly in periods of long drought (Gartner and Alexander 1966). However, rams and bulls have shown seminal degradation and infertility after periods consuming diets low in vitamin A (Gunn et al., 1942). Calves weaned from drought-affected dams have also shown vitamin A deficiency (Parker et al. 2017). In addition, recent research by our group demonstrated marked deficiencies of vitamin A in breeder cows near Kununurra (WA) in cows at the end of the dry season with 72 and 42% of the cows deficient in vitamin A in two different years (mortality project). Therefore, vitamin A deficiency seems to a limiting nutrient in cattle during drought or regions with long dry seasons when hepatic stores can be depleted, such as in the rangelands of Australia. Vitamin A can be administered via injections (usually together with vitamin E and D), drenches, or supplements for feed or water. Supplementation is recommended for pregnant and lactating cows, and calves born in the drought. However, vitamin A can be degraded by light, heat, humidity, and when mixed with other feed, which could limit the potential for supplementation in feed. Multiple commercial products exist containing vitamins and minerals pre-mixes in multiple forms including lose licks and pellets (e.g., Byrumen from International Health Products for a target intake of 50 g/d in breeders).

Vitamin D is also frequently supplemented to cattle in Australia, which is critical for normal skeletal development (together with calcium and phosphorus) and immune function, but it has also been linked to earlier resumption of oestrus post-partum in cattle and improve Ca and P metabolism after calving (Freer et al., 2007). Vitamin D precursors are also stored in the liver as is the case for vitamin A and the conversion from cholecalciferol to vitamin D3 occurs in the skin by solar ultraviolet radiation (Smith and Wright 1984; Littledike and Goff 1987). Supplementation of rangeland cattle with vitamin D has been proposed as a mechanism to improve both Ca and P absorption and utilisation (McGrath et al., 2013).

Vitamin E protects the cell membrane from the formation of peroxides which can cause damage from free radicals. Vitamin E complements the enzyme GSH-Px (as Se does) to prevent unregulated oxidation and cell damage, and therefore plays a role in health and immune function. Vitamin E is found green forage and measured as α-tocopherol. Therefore, it has been postulated that vitamin E deficiency may occur in rangeland cattle through the dry season. An ongoing project near Kununurra (WA) found vitamin A deficiency not a concern with only 2% of the animals showing deficiency at the end of the dry season.

The most common vitamin supplemented for grazing cattle are vitamin A, D and E which often come formulated in pellets or lick blocks together urea, macro (Ca and P) and microminerals, by-pass protein, ionophores such as monensin or lasalocid, and oils. A current MLA project in sheep seem to demonstrate an improvement in lamb survival with the supplementation of a vitamin and mineral premix in sheep.

4.1.2 Development of a feed block system to deliver precise doses on an anti-methanogenic product

Molasses lick blocks (MLB) are extensively used as a supplementation strategy of livestock across the world, so much that the Food and Agriculture Organisation of the United Nations has been supporting this technology for many decades and published a comprehensive compilation of scientific literature on this technology titled 'FEED SUPPLEMENTATION BLOCKS - Urea-molasses multi-nutrient blocks: simple and effective feed supplement technology for ruminant agriculture' to demonstrate the improvement in animal production that can be achieved (FAO, 2007).

These technologies have been designed and tested with strong market success using therapeutics to manage parasite load, non-steroidal anti-inflammatory drugs for pain management, and tailored nutrition. The product is technology readiness level 9 (TRL-9) being already deployed in industry with advanced maturity during the acquisition phase of a program. The ingredients used are available from various industries and at a competitive price, and the system is ready for full scale deployment.

Molasses lick blocks are formulated to provide energy, protein, and minerals for ruminants to improve production, although they have become a means of delivering other nutrients, minerals, or therapeutic substances to improve animal performance (FAO, 2007; FAO, 2011). Therefore, MLB are an appropriate test model system to deliver powders and liquids to grazing ruminants. A recent review by Zhao et al. (2022) defined lick blocks as "a solidified mixture of molasses, urea, minerals, filler, coagulant, and binder that is supplemented to livestock mainly in relatively extensive rearing systems."

Apart from rectifying nutrient deficiencies in the basal (forage) diet, feedblocks can be incorporated with medicaments to treat parasites such as fenbendazole (Garossino et al., 2005) and rumen modifiers such as monensin (McLennan et al., 2012). They can also act as long-term preservation of agricultural by-products (Salem & Nefzaoui, 2003). Lick blocks are also easy to transport and an excellent vehicle to deliver supplements for grazing cattle (Makkar, 2007).

Molasses lick-blocks have multiple advantages as follow:

1. Simple manufacturing
2. Easy to transport
3. Easy of storage
4. Easy of delivery: no special containers are needed because these can be fed on the ground.
5. Low capital cost
6. High palatability

7. Potential to deliver a wide range of nutrients and additives including macro and micro minerals, rumen modifiers and other veterinary products

Some of the limitations include:

1. Delivery during the wet season may be difficult
2. Cost can be high compared to other protein or energy sources (seeds and meals)

Lean et al. (2011) summarised the advantages and disadvantages of MLB compared to other supplements and highlighted the high proportion of non-feeders and losses during the wet season. However, intake variability between animals depends on the number of blocks delivered for a given herd or the number of animals per MLB, and the containers. Imaz et al. (2019) found a large variability in MLB intake between animals and 11% non-feeders. However, it is important to note that there was only one electronic feeder with pneumatic gates to control access for 26 animals, which required animals to learn how to access the MLB. A different MLB delivery system such as ground delivery and a reduction of the number of animals per MLB may reduce this variability in MLB intake. The possibility of 'just delivering them on the ground' can reduce the effect of social dominance structures within a herd which typically leads to uneven intake of supplements (Bowman and Sowell, 1997). In addition, the survey carried out by Lean et al. (2011) to experts in animal production and nutrition (research and advisory personnel) of northern Australia suggested that loose mixes and blocks were top ranked according to adoption, feasibility, and cost-effectiveness.

Lick blocks are of three main types, although variations also exist:

1) Salt, or mineral or dry lick blocks (SLB) where the carrier is mainly salt or various clays.
2) Molasses or multi-nutrient lick blocks (MLB) where the carrier is sugar cane molasses and can also contain protein, starch, or other nutrients
3) Complete feed blocks (CFB) which incorporate fibre and by-products from the agroindustry including straw, rice hulls, and grains, amongst others (FAO, 2009; FAO, 2011).

Eggington et al. (1990) performed a wet season supplementation trial in the NT of Australia and reported losses were much lower in MLB (only 7%) compared to SLB (33%) during the wet season, and that all animals consumed some supplement as determined using radioisotope techniques.

4.1.2.1 Feedblock formulation and design

MLB Molasses lick blocks are formulated to deliver a range of products and nutritional support for grazing cattle, increasing the palatability of other ingredients that are less palatable, controlling intake, providing energy, and sulphur. A review on rumen modifiers as feed additives, some of them with potential applications in MLB, for the grazing northern pastoral industry were presented by Lean et al. (2011). Advancement in adoption of new technologies since then have been low and the reader is referred to that review for further details.

Molasses lick blocks are used to supplement grazing livestock across the world and normally sold in blocks of 20 to 750 kg (Mordenti et al., 2021). The blocks maintain their structure after being manufactured and resist atmospheric conditions, maintaining their organoleptic and nutritive properties. The rage MLB products available target their composition to different regions, seasons, characteristics of the pasture, nutritional limitations, livestock species and class (e.g., cows or weaners), and production systems. The MLBs are consumed by cattle at a rate of 0.1 to 2.5 kg/head per and recommended to feed out one block for every 5-10 cattle to avoid competition and reduce shy feeders (Eggington et al., 1990). Animals may consume large amounts of MLB during the first few days after the blocks are introduced and it is believed this will replenish the nutrients that were deficient for the animal so that MLB intake decreases thereafter. Recent research has demonstrated the ability of grazing livestock to regulate MLB according to the quality and quantity of pasture available (Imaz et al., 2019 and 2021).

4.1.2.2 Ingredients of blocks

A variety of ingredients can be used in the formulation of the blocks such as molasses, urea, wheat bran, rice bran, protein meals (soybean, cottonseed, olive, sunflower), grain seeds (barley, fava bean), unconventional feeds (Moringa oleifera leaf, citrus pulp, grape marc, distiller's dried grains), cement or quicklime as settling or hardening agents, salt, vitamins, and minerals. Examples of block formulations are presented in Table 7.

As previously mentioned, the main components from a nutritional point of view are:

1. Energy sources
2. Protein sources
    a. NPN
    b. Rumen degradable protein (RDP)
    c. Rumen undegradable protein (RUP)
    d. Rumen protected or essential amino acids
3. Minerals
4. Fibre
5. Other additives
    a. Rumen modifiers for feed efficiency (e.g., ionophores)
    b. Methane mitigants
    c. Enzymes
    d. Veterinary drugs
    e. Growth promotants
    f. Chemical reagents
    g. Flavors
    h. Preservatives

Table 7. Example ingredients used in lick blocks for ruminant animals.

Ingredients	Amount (g/100 g DM)			
	Cattle[1]	Buffalo[2]	Sheep[3]	Goat[4]
Molasses	45	42	45	38
Urea	8	5.3	15	10
Binder/hardener	8	8.5	6	10
Preserver (NaCl)	1	4.2	1	1
Dicalcium Phosphate ($CaHPO_4$)	1	1	3	-
Magnesium Oxide (MgO)	-	-	6	
Minerals	1	1	1	1
Cottonseed meal	18	8.5	-	-
Corn gluten feed (20%)	18	-	-	-
Rice Bran	-	21	23	-
Wheat Bran	-	8.5	-	40
Total (g)	100	100	100	100

Sources: 1 Mirza et al. (2002); 2 Mirza et al. (2004); 3 Sudana and Leng (1986); 4 Singh et al. (1999)

4.1.2.2.1 Molasses

Sugar cane molasses is one of the main ingredients normally used in MLB manufacturing in Australia because it is readily available, it acts as a binder itself, provides energy, and increases palatability. Other syrups and molasses can also be used such as sugar beet molasses (Mordenti et al., 2021). Interestingly, molasses has also been replaced by waste from mulberry fruit or dates in other countries (FAO, 2007; FAO, 2011). Molasses contains 45-51% sugars (sucrose, glucose, and fructose), 72-79% DM, 4-10% CP, and 1-2% ether extract (EE; Table 8). Sugar cane molasses contain lower concentration of CP and total sugars compared to sugar beet molasses. Most of the CP in molasses is in the form of nitrates with the remainder as free amino acids. The amino acid betaine has important biological value as a methyl donor (0.5–0.7%) which can contribute to reducing fatty liver problems in dairy cows (Mitchel et al., 1979). The greater the sugar content of molasses, the higher the Brix value, and better hardening to control intake. Molasses provides readily available and rumen fermentable sugars for the rumen microbes to increase their growth if N is also available.

Table 8. Chemical composition of molasses used for molasses lick blocks fed to livestock. Adapted from Mordenti et al. (2021).

		Sugar Cane Molasses	
	Sugar Beet Molasses	<475 g/kg	>475 g/kg
Density, kg/L	1.39	-	1.39
DM, g/kg	754-787	724	721
Ash, g/kg DM	90-127	112	91-146
Crude protein, g/kg DM	98-142	51	41-55
Crude Fat, g/kg DM	2	1	1
Nitrogen-free Extract, g/kg DM	597	554	582
Total sugars, g/kg DM	512-634	454	488-641
Non-starch polysaccharides, g/kg DM	100	120	115
NPN, g/kg	59.3	61.8	-

NPN: non-protein Nitrogen

The mineral composition of ash in molasses (8–9% of DM) are dominated by Na, Cl sulphates in an inorganic form (Table 9).

Table 9. Mineral composition of sugar beet and cane molasses (adapted from Mordanti et al., 2021).

	Sugar Beet Molasses	Sugar Cane Molasses
Ca, g/kg DM	0.7-1.2	6.8-9.2
P, g/kg DM	0.3-0.5	0.7
Mg, g/kg DM	0.1-0.3	2.7-4.0
K, g/kg DM	41.0-51.2	28.8-51.0
Na, g/kg DM	6.9-7.2	1.0-2.4
Cl, g/kg DM	4.3	18.5-21.7
S total, g/kg DM	5.6	-
S inorganic, g/kg DM	-	8.2
S organic, g/kg DM	0.1	0.1
Fe, mg/kg DM	22-154	173
Mn, mg/kg DM	19-38	19-74
Zn, mg/kg DM	13-22	9-18
Cu, mg/kg DM	7-17	6
Mo, mg/kg DM	0.2	0.5
I, mg/kg DM	0.3	0.9
Co, mg/kg DM	0.6	-

Molasses contains a low proportion of fatty acids but some of them are of high nutritional value and considered essential fatty acids (Table 10).

Table 10. Fatty acid composition of sugar beet and cane molasses (adapted from Mordenti et al., 2021).

Fatty Acid (g/100 g FAME)	Sugar beet Molasses	Sugarcane Molasses
C 8:0	1.66	0.32
C 10:0	1.08	0.03
C 12:0	7.92	0.16
C 14:0	4.77	0.44
C 15:0	0.12	0.29
C 16:0	17.48	24.39
C 16:1 cis9	0.18	0.24
C 17:0	0.20	0.21
C 18:0	10.80	4.56
C 18:1 cis9	22.85	19.96
C 18:1 cis11	0.52	0.91
C 18:2 trans9, 12	0.17	0.15
C 18:2 cis9, 12	29.98	39.20
C 18:3 (n-3)	1.43	7.07
C 21:0	0.12	0.26
C 20:2 (n-6)	--	0.05
C 22:0	0.39	0.47
C 20:3 (n-6)	--	0.06
C 20:3 (n-3)	0.14	--
C 22:2	--	0.46
C 20:5 (n-3)	--	0.07
C 24:0	0.19	0.69
Others FA	7.78	4.04
SFA	44.74	31.83
UFA	55.26	68.17
PUFA	31.55	47.07
MUFA	23.72	21.11
PUFA (n-6)	29.98	39.47
PUFA (n-3)	1.57	7.14
n-6/n-3	19.15	5.53

FA: fatty acid; FAME: fatty acid methyl ester; SFA: saturated fatty acid; UFA: unsaturated fatty acid; PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid.

4.1.2.2.2 Oils and fats

Addition of oils and fats in animal diet is considered one of the top 5 feeding strategies to mitigate methane emissions in livestock (Arndt et al 2021). Vegetable oils that effectively decreased daily CH4 emissions were: (1) coconut oil (-28%, -20 to -35%), (2) canola oil (-22%, -12 to -32%), (3) linseed oil (-22%, -14 to -29%), and (4) sunflower oil (-17%, -9 to -24%) (Arndt et al. 2021).

Factors such as the amount of supplementation, the source of oils and fats and associated fatty acids profile, and the form in which the lipid is administered (i.e., refined oil or oilseeds) can result in highly variable responses (Meale et al. 2012).

Oils and fats supplementation not only helps mitigate CH4 emissions, oils and fats provide twice as much energy than protein and/or carbohydrates (9 kcal/g vs. 4 kcal/g, respectively) to the animal. Also, fats and oils improve feed palatability. Addition of fats and oils to the MLB may increase the palatability of the diet, promote feed intake, and decrease methane emissions.

4.1.2.2.3 Urea

Urea is one of the most widely used ingredients of MLB as a NPN source because of its high CP value and low cost per unit of N. Commercially available MLB contain up to 30% urea however it is recommended to maintain urea below 15% to avoid urea toxicity in hungry animals, and ensure animals have plentiful low-quality forage available. MLB with high concentration of urea should be harder to control intake. Urea is rapidly converted to NH_3 in the rumen and thus available for the rumen microbes to use in cell growth. However, this readily available N also requires readily available energy (e.g., from molasses) to increase microbial protein yield, fibre degradation, intake, and performance of cattle consuming low quality diets (Loest et al., 2001; Toppo et al., 1997; Schiere et al., 1989; Bandla Srinivas and Gupta, 1997; Windsor et al., 2020). The readily available energy provided by molasses in MLB for the rumen microbes is one of the main advantages compared to lose lick blocks that do not contain urea. It is important to note that studies where MLB intake or the concentration urea were high for low-quality diets seemed to show greater effect on intake and production (Cherdthong et al., 2014; Bandla Srinivas and Gupta (1997). However, the use of a urea-calcium sulphate mixture can help reducing the rate of NH_3 formation in the rumen and reduce the risk of poisoning and urea can also be added as rumen-protected forms such as polymer-, lipid- or formaldehyde-coated forms (Cherdthong et al., 2014).

4.1.2.2.4 True protein sources

Both RUP and RDP can be added to MLB with the former increasing the flow of amino acids for absorption in the lower gastrointestinal whereas RDP is consumed by the rumen microbes, which then supply microbial protein. Cottonseed meal is the most widely used by-pass protein in Australia however other readily available protein meals could also be used such as canola protein meal or even oil seeds. In some cases, protein supply by the blocks can reach up to 30% of the total daily protein requirements by the animals (FAO, 2007).

4.1.2.2.5 Minerals

Various minerals are often added to the MLB formulation depending on the region, season and animal class that are targeted. These may include sodium, phosphorus, calcium, potassium, chloride, copper, and selenium, amongst others. Sodium bicarbonate is a rumen pH buffer that can also be added if the animals are at risk of ruminal acidosis such as that during drought feeding. Salt is often added to provide the minerals but, in some cases, it can also be used to control MLB intake if the blocks are not too soft. Microminerals pre-mixes are also often added to MLB in Australia. Minerals is one of the most common nutrients added to MLB and these include P, Cu, Zn, amongst others to improve performance and improve reproduction in cattle (FAO, 2007). Mineral can also come from hardeners added to the block and this can be a substantial amount.

4.1.2.2.6 Veterinary products

Various studies have demonstrated the ability of medicated MLB with fenbendazole to reduce parasite load in cattle and sheep which reduced faecal egg count of worms by 98% at 14 days after being fed (Fishpool et al., 2012; Junkuszew et al., 2015). Our partner company 4Season has also successfully used MLB with fenbendazole to control *Toxocara vitulorum* and *Fasciola gigantica* in cattle and buffalo in Laos and Vietnam (Olmo et al., 2020). Non-steroidal anti-inflammatory drugs such as meloxicam has also been added to MLB to reduce pain and inflammation after husbandry procedures such as castration by our partner company. The potential to use other veterinary products to improve production of grazing beef cattle is enormous.

4.1.2.2.7 Essential oils

Essential oils from 10 plant species containing anti-parasitic properties have also been added to lick blocks as a drug free method to reduce coccidiosis in lambs (Junkuszew et al. (2015).

4.1.2.2.8 Vitamins and microminerals

Most of the lick blocks sold in Australia contain trace minerals either because of addition of mineral premixes or because of the coagulants and binders. In addition to microminerals, vitamins can also be added to lick blocks such as those used in the NT by Eggington et al. (1990), which contained vitamin A dry season feeding and NPN. Vitamin premixes are commonly used in Australia and elsewhere (de Evan et al. 2020; Travieso et al., 2022; Molina-Alcaide et al., 2014).

4.1.2.2.9 Commercial products

There are hundreds of commercial products available in the Australian market to cater for different regions, seasons, animal classes and production systems as previously mentioned. We have extracted the tonnage sales data from our partner company for the fiscal year 2021/2022 and then calculated the proportion of the total annual sales of each of the 21 most sold lick block products they have in the market (Figure 15).

The data revealed that feedblock products where the main objective was to deliver minerals (36% of all sales) had the highest sales by volume, followed by urea (28.6%), protein (27.5%), and energy (24.9%). Interestingly, 14 out of 21 products were tailored to delivering minerals either for particular animal classes (calving cows) or with high concentration of a particular mineral (e.g., Ca, Mg, S, trace minerals, or Iodine). Interestingly, lick blocks to combat bloat represented 4.4% of the total sales. These sales data demonstrated the wide range of lick block products catering for different regions and production systems, offering a great opportunity to include methane mitigation products.

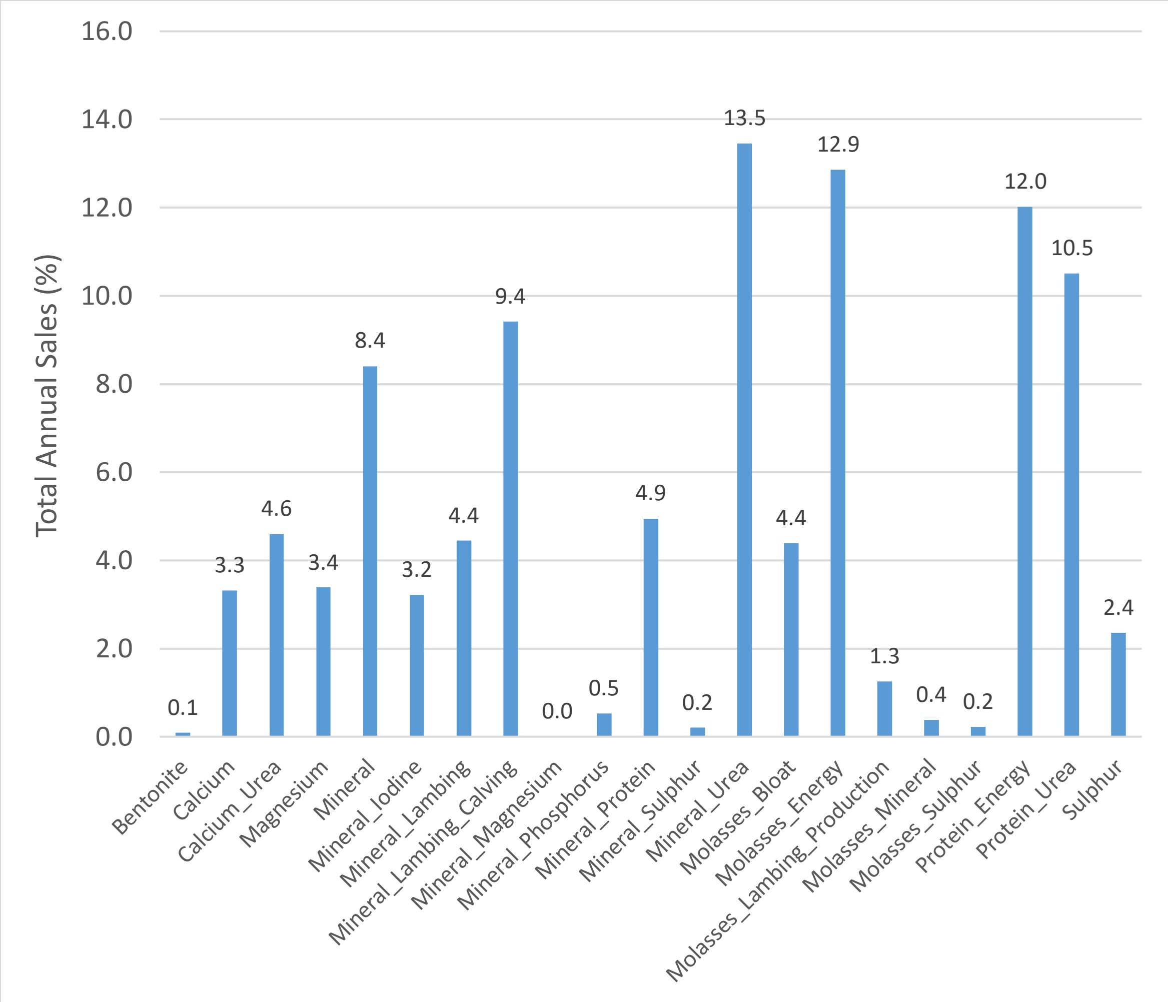


Figure 15. Proportion of total sales (in tonnage) of each of the top 21 lick block products for the 2021/2022 fiscal year across Australia from one of the major companies. Protein_Urea products comprise various products with different concentration of urea.

Total sales in each state as a proportion of the national sales is shown in Figure 16. The data shows that sales are dominated by NSW and QLD in second place but no sales for the Northern Territory. These results suggest that data should be interpreted with caution because it is only from one company and may be biased by the proportion of the market share in different regions. However, these results show the level of product development and provides an indication the huge market available for methane mitigation.

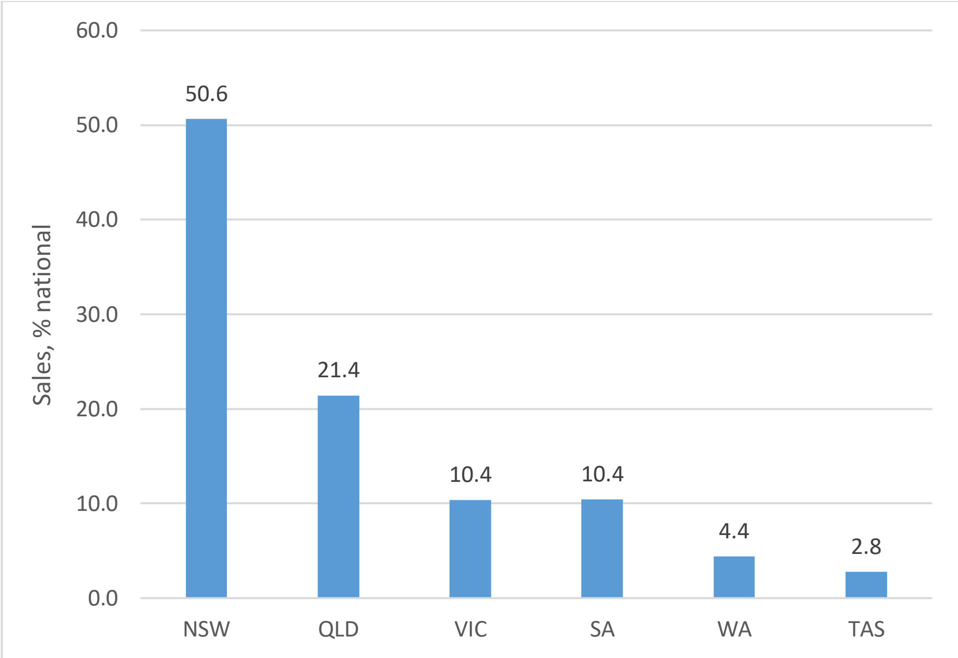


Figure 16. Proportion of total lick block sales (in tonnage) of each state on the national sales for the 2021/2022 fiscal year.

In addition to the sales by product or state, the data was used to calculate the proportion of sales by volume for each product type as a percentage of total sales in each state (Figure 17). It was found that NSW consumed all types of products although the largest proportion of sales were for minerals at calving and protein-energy blocks. Victoria had a similar distribution of sales to NSW but the largest proportion of sales in QLD were from lick blocks containing urea. The largest proportion of blocks for bloat were sold in NSW (Figure 17).

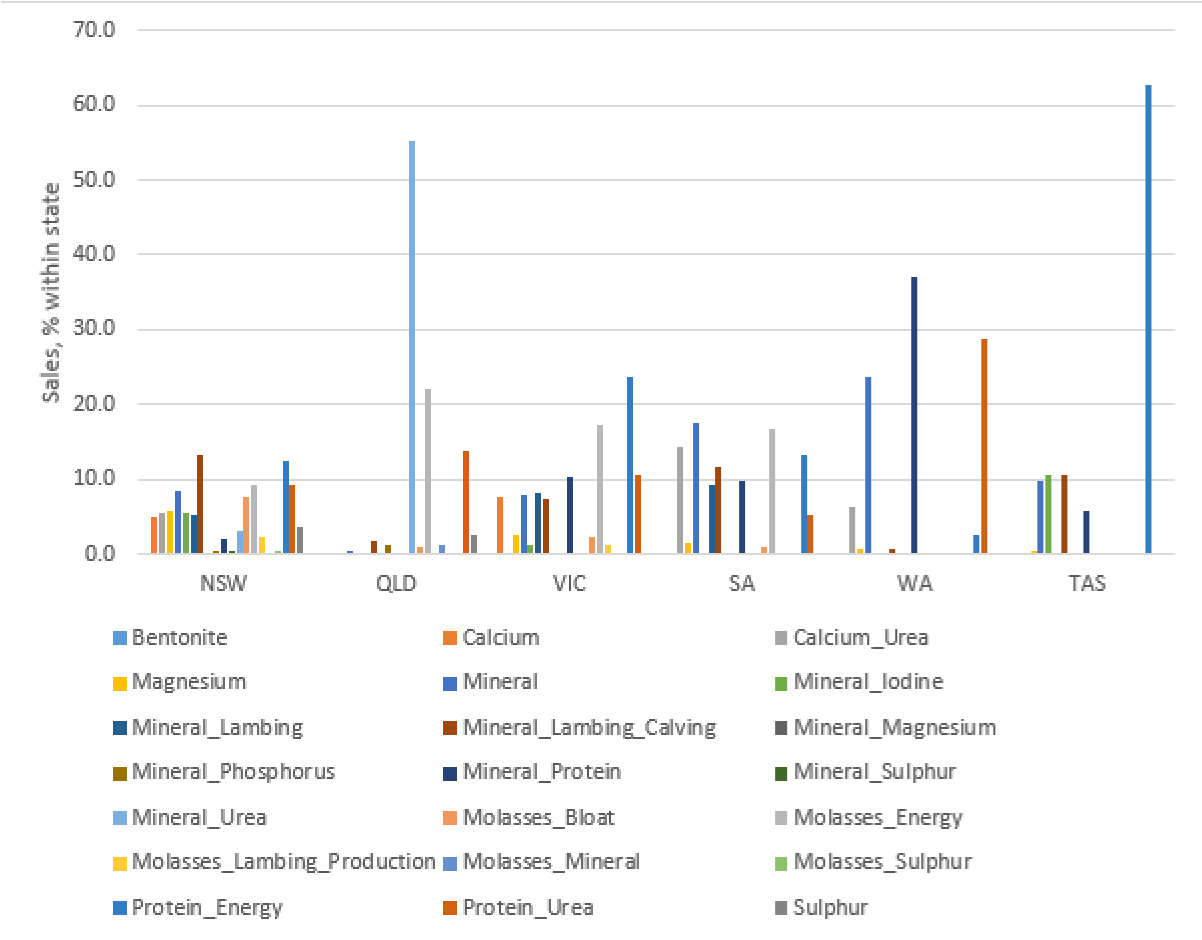


Figure 17. Proportion of total lick block sales by volume of each product as the total sales in each state for the 2021/2022 fiscal year.

Examples of other lick block types from another two companies are presented in Table 11 and 12. It is evident from the table below that more information about the block products would be helpful for the customers.

Table 11. Commercial lick block products and composition from Company 2.

Type	Pasture	Animal class	Molasses (%)	Urea	P	K	Ca	Sulphur	Mg	Salt	Se (mg)	Vitam	Bypass protein	Mn	Zeolite	Ferrous Iron	Iron	Iodine	Cobalt	Cooper	Zinc	Total prot Eq	Other
Molasses-Mineral Biochar	All pasture conditions	All	45.1	0	1.07		7	0	4.5	12	9.02		5.9		2.5			60.8 mg	60.1 mg	600 mg	1040 mg		Biochar 5%
Sulphur-Salt-external parasites	Northern Australian	All	4					12		80													Allicin 0.3%
Molasses -Mg-Bypass protein	Green pasture	Late Preg or Lactating	59.77		0.11		0.21		11.1	0.32													MgO 18.9% Cotton seed meal
Bentonite-Salt-Protein	Green Pastures	All	7					2.2		31			25									9	33% Bentonite
Molasses-Mineral -Surfactant	Bloat	All	67.5		0.6		2.3	0.1	2.7	3.2			2.8									1	10% Alcohol Ethoxylate
Calcium-Salt	Fed grain	All	6		0.6		13.7	0.025	200 mg	54.5	26		3.5			200 mg	975 mg					1	
Calcium-Salt-Urea	Fed grain	All	5	10	1.2		6.5	1	200 mg	52	26		15			1350	650					35	
Salt-Microminerals	Copper and cobalt deficiency	All	4.5		0.44		11.5	0.83		58.7	26								0.4	3			
Molasses-10 Urea-Minerals	Dry and fibrous feeds	All	57.6	10	1.7		5.5		3.5	5	32		4		3	500 mg		150 mg	150 mg	100 mg		30.8	

Type	Pasture	Animal class	Molasses (%)	Urea	P	K	Ca	Sulphur	Mg	Salt	Se (mg)	Vitam	Bypass protein	Mn	Zeolite	Ferrous Iron	Iron	Iodine	Cobalt	Cooper	Zinc	Total prot Eq	Other
Molasses-20 Urea-Minerals	Dry and fibrous feeds	All	37.9	20	1.7		5.5		3.5	5	32		4		3	500 mg	150		150	100		58.9	3.65 MJ of ME/kg
Calcium-P-S-Salt	Mineral deficient	Pregnant/ lactating or grain fed livestock	6		2		17	2		16			3.5		15							1	
P-Ca-S-Mg-Micro-Bypass Protein-Vitamin	All	All	6		3	330 mg	10	3	3.5	39	65 mg	A,D,E	10	500 mg		1350 mg		1400	400 mg	1000 mg	500 mg		Boron 10 mg
Molasses-P-Ca-Mg-Bypass	Mineral deficiencies Pastures	Pre-calving	45		1.7		2.2		9	4			5										Glycerol 10%
Urea-P-Ca-Salt-Vitamin-Bypass-Micromin	Dry and fibrous feeds	All	6.3	11	2		6	3	1	25	45 mg	A,D,E	10	160 mg	2.5	1000 mg	250 mg		200 mg	250 mg	450 mg		Boron 10 mg
Salt-S	Dry and fibrous feeds also when offering high urea	All	4					16		75					5								

Table 12. Commercial lick block products and composition from Company 3.

Market	Salt or molasses	Ingredient	Pasture	Animal class	Urea	P	Ca	Sulphur	Mg	Salt	Se (mg)	Iodine (mg)	Cobalt (mg)	Cooper (mg)	Zinc (mg)	Crude Fat	Total prot Eq	CP (%)	Eq CP (%)	Crude Fibre (%)
Phosphorus deficient regions	Molasses	High P	Grazing phosphorus deficient pastures during the wet season	Breeder, Growing		8	10	0.8	4	5	2.5	30	30	300	500	0.1	1.2	1.2		5
Weaner, growing and breeding cattle	Molasses	Urea and P	All	Breeder, Growing and weaning	10	5	6		3.5	8	2.5	30	30	300	500	0.1	30	2	28	5
Weaner, growing and breeding cattle	Molasses	Urea, vegetable protein meal, macro and trace minerals	All	Breeder, Growing and weaning	10	2.5	6	1	3	10	2.5	30	30	300	500	0.1	30	4.5	25.5	5
Weaner, growing, and breeding cattle grazing on dry pasture.	Salt	High P, macro and trace minerals	Mature low-quality Pasture	Breeder, Growing and weaning	30	3.5	7	2		28	2.5	30	30	300	500	0.1	86	1	85	5
Cattle grazing on pasture	Salt	High P, macro and trace minerals	Mature low-quality Pasture	Breeder, Growing and weaning	30	3.5	7	2		28	2.5	30	30	300	500	0.1	86	1	85	5
P for pregnant cows and replacement heifers. All year growing and breeding cattle	Molasses	15% urea, 5% P	All	Breeder, Growing and weaning	15	5	6	1	5	5	2.5	30	30	300	500	0.1	45	2	43	5

4.1.2.2.10 Block manufacturing and quality

There are two main methods for the manufacturing of MLB often called '**hot method**' where temperature is used after the ingredients are mixed to further reduce water content and solidify the blocks, or 'cold methods' where hardeners, binders and/or pressure are used to replace temperature. The most common method for salt mineral blocks is pressure. Cooking increases the binding action of heated molasses, but it may not be required depending on the type and proportion of hardeners and binders. In the cold method for mineral blocks, the ingredients are mixed, poured into moulds and pressed, and normally use coagulants and binders for MLB, which are not always needed for SLB. The absence of heating reduces the manufacturing cost and equipment required. In addition, the cooking method requires long moulding/settling times, and the blocks can also loose texture and result in unstable quality, sometimes 'melting' in hot weather if the coagulants and binder are inappropriate. Cooking and pressing methods can also be combined.

From a manufacturing point of view, the main ingredients are:

a. Fillers

Fillers can be added in various forms and proportions (18 to 95%) to provide structural support to the block and nutrients depending on the target animals. Various types of fillers can be used such as wheat or rice bran, protein meals and grain seeds, barley flour, olive cake, fava bean flour, and distiller's dried grains. Cottonseed meal is a filler widely used in Australia which also adds by-pass protein. Fillers can also affect hardness and intake of the blocks, with higher amounts resulting in softer blocks and higher intake.

b. Coagulants (solidifying agents)

Coagulants (or curing agents) are included to increase the hardness and control intake of the blocks and include di-ammonium phosphate (DAP), calcium oxide, magnesium oxide, calcium hydroxide, quicklime, and cement at a rate of 5 to 20% with higher amounts increasing the hardness of the block. In addition to silica, cement can also provide minerals in the form of oxides of aluminium, magnesium, sulphur, iron, and potassium.

c. Binders

Bentonite and other clay minerals are added to bind the ingredients because they have large surface area, and cation exchange and adsorption capacity. Sodium and calcium bentonites, and vegetable oils are the most widely used in Australia due to availability, no toxicity, and low cost, and are included at 5-30%. Bentonite also provide macro and microminerals and can also absorb mycotoxins. The blocks can be hardened without the use of pressure at high concentration of the binder.

d. Packaging

After manufacturing, the blocks can be packaged in polyethylene bags or cardboard boxes to avoid losses due to fungal growth, insects, rats, and birds in regions with high humidity.

e. Delivery to animals

Delivery to animals can be done on feeders or troughs, or just placed on the ground. The blocks can also be hanged for the animals to lick and thus avoid dirt or disintegration during the wet season.

Temperature

The temperature reached during the cooking of the MLB in the hot or casting method can have an influence on the biological activity of the ingredients and additives that are used. For example, enzymes could lose their biological activity at high temperatures, and it has been demonstrated that

the stability of phytase and xylanase was not affected at 60 ºC but phytase lost activity at 100 ºC (Ainscough et al. [95]). Other enzymes such as cellulolytic enzymes or microbes that need to maintain their activity to reproduce in the rumen such as yeast culture or direct fed microbials need further research and likely a combination of low temperature and cold pressing.

Lick block quality

Block quality is determined by its hardness (kg/m2), density (g/cm3), chemical analysis, and subjective traits including ‘shelf life’, surface roughness, crack size, colour, and smell. Hardness can be assessed using a penetrometer. Hardness is determined by pressing pressure [4], salt ratio [65], type, ratio of binder to coagulant [58], curing time, and the proportion of the bulk ingredients and fillers [76]. Hardness and density are also correlated with the densest blocks also being hardest. The hardest blocks are obtained using the combination of hot and cold methods of manufacturing (41.4 kg/cm2) whereas the hot method only gives the softest blocks (2-5 kg/cm2). The nutritive value of LB can be determined by chemical analysis, in vitro rumen fermentation and animal feeding studies.

4.1.2.2.11 Block intake by cattle

Measuring block intake of individual animals can be achieved using electronic feeders weighing the blocks or measuring the time animals spend licking the block (via RFID tags or similar) because a close relationship exists between these which showed an R2 between 0.80 and 0.96 under different pastures (Imaz et al., 2019 and 2020).

Our previous research by Imaz et al. (2020a,b) has shown that animals can regulate MLB consumption depending on the quality and quantity of forage available, which is linked to seasons and regions amongst other factors. Tailoring products to a specific region or producer need can increase animal performance and reduce GHG emissions intensity and lay the basis for producers to enter into ERF herd management methodology agreements tailored for each region.

The MLB intake rate per animals varies markedly depending on the factors highlighted below. However, urea MLB normally results in 500 to 800 g/head per day in cattle in most countries. MLB is normally harder in Australia compared to other countries and intake is between 200-300 g/head per day. It would be important to evaluate softer blocks to increase consumption in Australia (using 10% urea) because we could not find literature with high intakes as often used in other countries. The main factors controlling intake of MLB (and most supplements) are a combination and interaction between the following:

a. Hardness

The hardness of the blocks is critical to regulate intake and it is normally achieved using settling agents and different feed ingredients. Blocks should be hard enough to allow for the slow release of nutrients, particularly molasses and urea, which increases the efficiency of the blocks. Hardness depends on the amount and type of hardener incorporated, the cooking method and length, the pressure (in salt blocks), and the rate of cooling (hot molasses blocks).

As the block hardness declined from hard to partial and loose, Ortolani (1999) found that daily mineral intake of cattle was significantly increased from 27, 45, and 60 g/head/d, respectively ($P < 0.05$). Zhu et al. (1991) conducted a trial by feeding cattle with a three-tiered density of blocks (< 450, 500-1000, and > 1100 g/mm3). These latter authors found a negative relationship between block hardness and daily intake of cattle, which were 1.7, 0.4, and 0.1 kg/day in phase I, and 2.0, 1.5, and 1.1 kg/day in phase II. It was then advised that the maximum lick-block hardness for cattle to be considered edible is 1000 g/mm3. Herrera et al. (2009) reported that MLB intake by cattle decreased from 3.3 kg/d when the block hardness was 4.4 kg/cm2 to 0.9 kg/d when it was 6.9 kg/cm2, and 0.1 Kg/d when it was 9.6 kg/cm2.

b. Palatability

Some ingredients such as molasses and/or oils and fats may increase palatability of the blocks whereas others such as high salt content may produce aversion and reduce intake. Thus, block intake can be controlled not only by the hardness of the block but also by balancing the amount of ingredients that may increase consumption and those that may reduce it. An experiment in housed cattle by Weber et al. (1992) found that intake variability was greater in salt-block compared to softer-protein blocks, indicating that protein block was more palatable than the salt-block.

c. Ingredient composition

Animals increase the intake of feeds that provide nutrients needed to meet their requirements or improve nutrient/energy intake, performance, fitness, reproduction, or welfare. For example, animals may select blocks with high salt when Na is limiting in their diet or to balance the calcium-phosphorus ratio (Chládek and Zapletal, 2007).

In addition to proving key nutrients that are often limiting production or deficient, MLB could also replace concentrates as demonstrated with lick blocks using various agro-industrial by-products by De Evan et al. (2020 and 2022) where mango and avocado waste by-products replaced 50% of the TMR without impact on intake and DMD and milk yield. Similarly, MLB based on tomato or cucumber waste, and olive cake were used to replace 50% of concentrate in goats (Molina-Alcaide et al., 2010; Ainscough et al., 2018).

The intake of supplement blocks is primarily affected by the limiting nutrient in the animal's main diet and the nutrient content level in the block. For example, increasing fermentable energy intake by increasing molasses inclusion rate in supplement blocks (25, 50, and 75%) significantly affected the level of block intake from 5.8 to 11.7 and 15.7 g/kg BW per day, respectively ($P < 0.05$). However, the different amounts of molasses did not alter the total DMI of steers ($P > 0.05$) (Tuyen et al., 2014), but Ciriaco et al. (2015) demonstrated that the energy provided by molasses could also stimulate rumen microbial activity for carbohydrate digestion that leads to increased DMI and LWG. An increase in the efficiency of microbial CP synthesis from 132 to 138 g/kg DOM when the level of molasses was lifted from 50 to 75% (molasses mix and chaffed pangola grass (*Digitaria eriantha*) hay (w/w); $P < 0.01$) was indicated by Tuyen et al. (2014). On low protein diets, an increase in protein concentration enhances the efficiency of microbial protein supply and utilisation of forages (Bowen et al., 2016). Sudana and Leng (1986) offered either UMMB or UMMB + cottonseed meals (CP=36%) to sheep fed wheat straw-based diets. They found that the inclusion of cottonseed meal reduced UMMB intake by 12%, identifying that nutrient supplements differ in their effectiveness as feeding attractants (Nolan et al., 1974),

d. Main (base) diet availability and quality

Animals may consume lesser MLB when forage quantity and quality available to them is high (Imaz et al., 2019 and 2020). Beef cattle consumed more lick blocks during the winter, but intake declined as the high-quality pasture started to grow in the spring (Aubel et al., 2011). Similarly, Eggington et al. (1990) reported that the intake of Uramol lick blocks increased throughout the wet season as native pasture quality decreased.

The intake of a mineral supplement by grazing cattle in the late spring to summer was higher than in the fall season, where the forage biomass was maintained (Manzano et al., 2012). The increase in supplement intake in the summer (dry) season could be attributed to the lower forage quality, especially lower CP and digestibility, compared to the wet season (Leng, 1990). Hence, in a situation where ruminants are grazed on high-quality forages, the inclination of animals to ingest supplemental feeds is reduced. Also, voluntary DMI may be limited by high forage consumption that elicits aversion of ruminants to supplements (Dado & Allen, 1996).

e. Animal characteristics

Multiple factors such as body condition and body size, physiological status, production stage and nutrient requirements also affect MLB similar to what occurs with daily DMI or the intake of other supplements. Heavier animals consume more lick blocks than lighter animals suggesting that LW is the most important factor determining individual intake (Eggington et al., 1990). However, other factors such as animal class or physiological status also affects consumption. Lactating beef cows in the NT consumed 64% more wet-season supplement than non-lactating cows with a wide variation of 10 to 835 g/head/day for lactating cows although lick block intake was not correlated to pregnancy rate or growth rate (Eggington et al., 1990).

f. Location of the lick blocks within a paddock in grazing cattle

The MLB have demonstrated that can be used to manipulate animal distribution within a paddock through their strategic location. Animals concentrate around the water points during the dry season and avoid walking over approximately 2 km from them. This results in overgrazing around the water points and underutilisation of forage further from water. Bailey et al. (2001 and 2008) strategically placed MLB in various locations in hilly terrain of extensive grazing cattle to successfully manipulate their distribution. These studies that cows grazed at higher elevations and further away from water points and that MLB was more effective than mineral blocks. This could be a particularly important technology to improve grazing distribution, stocking rate and live produced per hectare in Australian rangelands. Bailey et al. (2008) suggested that a low-moisture block supplement effectively attracts cattle to graze in high elevations away from the water source.

4.1.3 Tailored feedblocks to be developed for different rangeland environments that would have the added benefit of rumen modifiers.

There is a lack of practical, effective, and affordable methodologies and technologies available to beef cattle producers to allow them to reduce methane emissions. This is particularly lacking under grazing conditions and in the rangelands where sowing of pastures is not feasible, and animals go through long dry periods with low feed quality. This has a huge negative impact on both livestock production and the intensity of greenhouse gas emissions (GHGE), normally reflected in low weaning rates and slow growth rates. Lick blocks have the potential to supplement grazing animals in a practical, accurate and cost-effective way. Virtually all Australian beef grazing systems are affected by GHGE and therefore achieving net zero emissions from this sector of the red meat supply chain will assist the industry to achieve carbon neutrality by 2030.

More than 10,000 tonnes of feed blocks were used in grazing systems in 2020. The incentives for producers to use lick blocks are (i) precision technologies to deliver combinations of nutrients and other products (mitigants, medicines) that is not feasible through conventional loose feeds; (ii) the ability to deliver any future product used to manage GHG and health; (iii) ease of management within feeding systems where intake of nutrients and mitigants can be determined simply through weighing of the block (a proposition that is almost impossible using loose feeds); (iv) reduced non-target species intake of feeds (e.g. native wildlife do not consume feed blocks but will consume loose feeds).

We contend that the structure of the research, development and adoption program requires all stages to assist producers to achieve carbon neutrality within their production systems and across the value chain. Producers need to start the processes of deployment of technologies that reduce GHG emissions and improve animal production. Once products are accepted by producers, deployment of direct abatement technologies within those products is simple and there will be no time lag between emissions abatement and adoption – a situation that is highly desirable to producers and policymakers alike. New policy instruments and methodologies naturally develop within this project to quantify the extent of abatement and are communicated proactively across the industry.

It is recognised that feed supplementation can increase productivity and reduce GHG emissions in the rangelands however this has not been clearly demonstrated and there is a lack of practical, economical, and effective feed supplementation technologies or strategies. Molasses lick blocks (MLB) containing feed additives with anti-methanogenic activity increased productivity and can be economically feasible (Imaz et al., 2019a,b). The research team of the present proposal has confirmed the ability of MLB to change the microbial fermentation and to reduce methane emissions in vitro (reports available upon request). We have used proprietary block formulations based on readily available additives, but new additives could also be included in the blocks if available (e.g., 3NOP). The current formulation of the blocks contains well known anti-methanogenic compounds that reduce methane production in the rumen including condensed tannins (Jayanegara et al., 2011; Norris et al., 2021), vegetable oils (Fiorentini et al., 2014; Vargas et al., 2020), ionophores (e.g., monensin; Odongo et al., 2007), and tea saponins (Liu et al., 2019). Importantly, these feed ingredients may have additive effects because the mechanisms of action differ. For example, monensin reduces Gram + bacteria which shifts the microbial fermentation towards more propionate and less acetate and methane, at least in vitro. Unsaturated fatty acids compete with methanogens for hydrogen and may also affect protozoa which have a positive relationship with methanogens. Condensed tannins either directly inhibit methanogens or indirectly target protozoa and form complexes with proteins and carbohydrates that can potentially decrease CH4 production. Tea saponins reduce protozoa numbers and thus methane emissions. Furthermore, none of these feed additives are routinely used in feed supplements for grazing animals where urea-based and P supplementation is the norm for northern Australia and cereal grains in southern Australia. This ensures the additivity of the proposed ingredients to be recognised both as a new practice immediately and beyond the increase in productivity in ERF methods (future methods beyond productive increases). The novel 3-NOP has also demonstrated its anti-methanogenic properties in cattle and there is interest to include this in a block formulation which could be based on molasses or dry licks. The option to include this at a later stage are 1) To include the additive into the blocks of the already planned trials, 2) to do a contract variation including new trials.

The consumption of the lick blocks is controlled by the hardness, which is controlled by the ingredient formulation and 'cooking' method. This is the main way to control the intake of nutrients and anti-methanogenic additives used in the blocks. Our partner company Four Season co /Agcotech has been selling and designing lick block in the market for 25 years.

Although previous research has demonstrated the effects of the blocks on methane emissions, there is a need to evaluate the effectiveness in vivo and refine block formulations to increase productivity and mitigate methane emissions. The MLB balance the positive impact of providing energy and/or protein on animal production with mitigation of methane emissions from rumen modifiers, specially formulated for breeder cows to increase weaning rate and live weight produced and reduce mortality in the rangelands. Furthermore, the blocks could also improve performance, feed efficiency, and reduce emissions in southern high-quality pastures. In addition, an associative effect between ingredients could drive a change in digestibility or fermentation profile (e.g., more propionate, less CH4, and more H2).

Most properties in the rangelands have weaning rates between 50 and 75% and mortality rates for young and adult animals above 5%, implying a substantial proportion of unproductive animals that emit methane and consume resources (feed, veterinary products, etc) with no production. Our recent modelling research indicates that feed supplementation of a beef herd with 3,000 breeding cows in northern Australia could see a reduction of 42% in the intensity of GHGE (or 6,954 tonnes CO2e/year) while increasing by 83% the production of live weight (LW). This is achieved by improving weaning rates and weights, joining heifers at an earlier age, increasing sale weight of adult cattle, and reducing mortality. However, these results need to be confirmed with field and metabolism trials.

This project focuses on the deployment of new feedblock products that have a proprietary technology designed to deliver precise rates of rumen modifiers, therapeutics, and the future pipeline of mitigation technologies such as red algae and 3NOP. These technologies have been designed and tested with strong market success using therapeutics to manage parasite load, pain management and tailored nutrition. The product is technology readiness level 9 (TRL-9) being already deployed in industry with advanced maturity of the technology during the acquisition phase of a program. The ingredients used are available for various industries and at a competitive price and the system ready for full scale deployment.

The MLB have proven their benefit to improve performance of cattle with traditional ingredients and this would reduce the intensity of methane emissions per unit of forage digested or per unit of meat produced. For example, MLB providing molasses, urea, P and other minerals, vitamins, and by-pass protein have demonstrated to improve growth rate and fertility which is be translated to heavier animals at sale and improved weaning rates. However, further reductions in methane could be realised by including anti-methanogenic one or several products to the MLB. Importantly, MLB have already demonstrated the ability to successfully deliver a range of products in various forms including solid (powder, granules), aqueous solutions, and oil emulsification (FAO, 2007).

Feed block engineering and technology is critical to achieve the optimal hardness to ensure the target consumption and deliver the products to the animal at precise quantity and with uniformity. Block consumption will determine the dose received of the different ingredients to produce the targeted methane abatement and effectiveness of the anti-methanogenic products.

Potential feed additives that can be added to MLB can be ranked based on their efficacy in mitigating methane in ruminants as follows: 1) 3NOP, 2) *Asparagopsis*, 3) nitrate, 4) essential oils, 5) saponin, 6) tannins, 7) monensin, 8) microalgae, 9) biochar, 10) direct-fed bacterial, and 11) direct-fed fungal (Hegarty et al., 2021b). However, no research has been done with the addition of these additives to MLB (except for the replacement of urea by nitrates) and therefore, their effectiveness to reduce emissions is unknown. It may be possible that not all these compounds may be incorporated into MLB supplement for grazing cattle due to several reasons including preservation of their biological activity and chemical stability during the manufacturing process.

An important aspect of these mitigants when added to MLB is that these can be fed to animals with tailored composition for different regions, seasons, and production systems. For example, MLB containing the mitigant and urea can be used for animals grazing low quality forages and a similar MLB without urea can be used for animals grazing high quality pastures and even while receiving cereal grain supplementation. This later MLB can also contain surfactants if the animals are grazing lucerne, Ca and Mg if the animals are at risk of hypocalcaemia or hypomagnesemia, and so on.

The potential methane-suppressants that can be incorporated into lick-blocks are listed below.

1. 3-Nitrooxypropanol (3NOP)

The 3NOP is potential to be provided in feed block for grazing ruminants. However, its efficacy would be dependent upon the level of intake by individuals. Some efforts have already been made to prolong rumen mitigation (e.g., with 3-NOP and cyclodextrin protected haloforms). Preserving stable volatility of additives within the feed has also been investigated. However, current evidence for effective delivery of methane-mitigant feed additives is insufficient for enabling their use in substantive mitigation in grazing and mixed farming sectors in any country.

2. *Asparagopsis*

Asparagopsis (red algae) has been known as a potential feed additive for inhibiting enteric CH4 formation in ruminants and thus contribute to GHG emissions reduction. Research suggests that low concentrations of 0.5% or less of bromoform compounds in the diet can lower CH4 emissions by up to 90% without deleterious effects on feed intake. Further research is required to develop methods for minimising the decrease in bromoform activity when incorporated into feeds. Research is also required to show that the supplement can be fed to rangeland animals through lick-blocks or other methods to prove applicability for mitigating CH4 emissions from rangeland cattle. If such applications are successful, *Asparagopsis* supplementation could be used across the whole of the Australian ruminant population (Black et al., 2021)

3. Nitrates

Nitrate is readily included in diets or lick-blocks as either calcium nitrate or ammonium nitrate as a full or partial replacement for urea. Integrity of lick-blocks is sometimes reduced because a greater proportion of the block is from the nitrate compound than from urea. The cost of nitrate compounds is higher than an equivalent amount of nitrogen from urea. The major concern with feeding nitrate for reducing methane emissions is the risk of nitrite poisoning, which is particularly dangerous when animals are subjected to exercise (Callaghan et al., 2014).

An experiment by Benu et al. (2021) reported that lick blocks incorporating calcium nitrate decahydrate was effective to mitigate CH4 emissions during the wet season (September – October) [200 ± 2.8 g/day (urea) vs (156 ± 2.7 g/day (nitrate)] but not during the dry season (June – July). In this trial, however, statistical tests were not conducted on daily CH4 emissions because they were conducted at a herd level and lacked replication. As the design was not allowed to quantify CH4 at an individual level, intake variability was likely to affect the CH4 yield (kg/DMI). Use of nitrate for alleviate CH4 emissions had long been investigated in sheep (Li et al., 2012) and cattle (Benu et al. 2015). According to Hegarty et al. (2021b), nitrate is the third most effective additive after 3NOP and *Asparagopsis* and can safely deliver 10% or more mitigation when consumed. However, a substantial risk of toxicity was the main issue of using nitrate as a feed supplement for cattle (Lee & Beauchemin, 2014). Also, Nolan et al. (2016) accentuated that inclusion of nitrate could potentially reduce intake by lowering the mastication rates.

4. Plant bioactive compounds (saponin, tannins, essential oils)

Laboratory studies suggest that the bioactive compounds could be included in diets at concentrations of 25-50 g/kg feed and decrease CH4 emissions, when offered as supplements or in lick-blocks. Although the cost of the bioactive is difficult to assess, it is likely that these compounds could be manufactured commercially. However, for their efficacy to be assessed, dose response experiments are required to quantify their effects on CH4 over the longer term in ruminants (Black et al, 2021; Almeida et al., 2021).

Plant saponin reduce protozoal numbers, which can improve the microbial growth and protein synthesis, and supply post-ruminally. However, further research into these compounds appears unnecessary as the extraction procedures would be expensive and other supplements such as *Asparagopsis* species and 3-NOP (Bovaer®) are already proven enteric methane mitigants and are in commercial production.

Tannins have been successfully added to MLB to reduce internal parasites by Wan Zahari et al. (2009) and Knox (2009). This demonstrates that tannins conserve their biological activity after the manufacturing process, and therefore, could also be added to reduce methane emissions. Similarly, grape mark has also been used in MLB as well as multiple leaves of taniferous plants as un-conventional by-products (FAO, 2007).

Agolin is a blend of essential oils that has demonstrated to reduce methane emissions by approximately 10% when added to diets at 1 g/hd/d and fed for over 4 weeks (Belanche et al., 2020). Agolin is working towards the certification of carbon credit or carbon reduction methods for the livestock industries. However, most of the research has been done with dairy cattle and research with beef cattle is lacking.

5. Ionophores (monensin and lasalosid)

Monensin decreases the population of gram-positive bacteria and protozoa by increasing permeability of cell membranes to ions. It also decreases the availability of H2 for methanogenesis and alters fermentation from acetate to propionate (McGinn et al., 2004). A study by Guan et al. (2006) reported that monensin could decrease CH_4 emission of cattle by 30% and 27% in the first two and four weeks of treatment, respectively. Reduction of CH4 was mainly because of the decrease in ciliate protozoa population by 82.5% and 76.8% (Ushida & Jouany, 1996; Guan et al., 2006). A meta-analysis in beef and dairy cattle (Appuhamy et al., 2013) summarized that CH_4 production in cattle was reduced by 5% due to the depressed DMI. Incorporating monensin with the block has been commonplace to mitigate the CH4 emission in grazing cattle, particularly in Australia. Both monensin and lasalosid (which has a similar model of action) are widely used as feed additives in MLB but the second has the advantage of being less likely to cause toxicity to monogastric animals and therefore it is often the preferred option for blocks that are placed unattended in the paddock.

6. Vegetable oils

Lipids (oils) are well known for their ability to reduce methane emissions in cattle and the federal government has an approved methodology under the Emissions Reduction Fund to earn carbon credit by feeding fat to dairy cows. Fiorentini et al. (2014) found a 30% reduction in methane emissions (g/kg DMI and g/d) in beef steers fed palm oil, linseed oil, and whole soybean meal (5% of DM) compared to a control without fat or a control with rumen protected fat whereas ADG was not affected. A meta-analysis demonstrated that vegetable oils reduce feed intake, fibre digestibility and methanogenesis by unsaturated fatty acids.

4.2 In vitro trial 1. Product selection for a reduction of CH4 emissions in cattle and dose response relationships

Results from the first in vitro trial evaluating all four products received from 4 Seasons Pty are shown in Table 13. Treatments affected all in vitro fermentation parameters, with mixture 3 used in its liquid form being the most effective to reduce CH_4 production by over 90% at 6% v/v dose compared to the control ($P < 0.05$). However, 6% of the product also resulted in a large reduction of VFA concentration and a slight reduction in IVDMD ($P < 0.05$). These results suggest that fermentation is negatively affected at high doses of mixture 3, shutting down fermentation, which is not desirable in mitigation products. Nevertheless, the lowest dose of mixture 3 seemed to enhance fermentation due to greater total VFA concentration and gas production. A dose of 4% v/v seems to be a good compromise because it reduces CH_4 production without negatively affecting fermentation, as suggested by no differences in gas production and VFA against the control. The molar proportion of acetate was drastically reduced with high doses of mixture 3 whereas molar proportion of propionate and butyrate were increased (P < 0.01). The concentration of VFA shown in Figure 18 suggests that the production of all VFA is drastically reduced at 6% v/v of mixture 3 but at 4% v/v, propionate is reduced, and butyrate is increased. However, interpreting VFA should be done with caution because mixture 3 product added to the liquid phase also contributes to VFA as it was added at 0.0, 0.5, 1.0, and 1.5 mL for 0, 2, 4, and 6% v/v, respectively. Similarly, the interpretation of gas production and IVDMD should also be done cautiously because the mixture added to the liquid media also contributes to gas and VFA production, whereas the IVDMD was only measured for the feed inside the bags.

Mixture 1 and 4 only increased IVDMD as the proportion of mixture in the substrate increased, which could be likely due to the replacement of less digestible feed by the mixtures containing soluble sugars and minerals or having greater digestibility. However, these rations did not affect any other fermentation parameter.

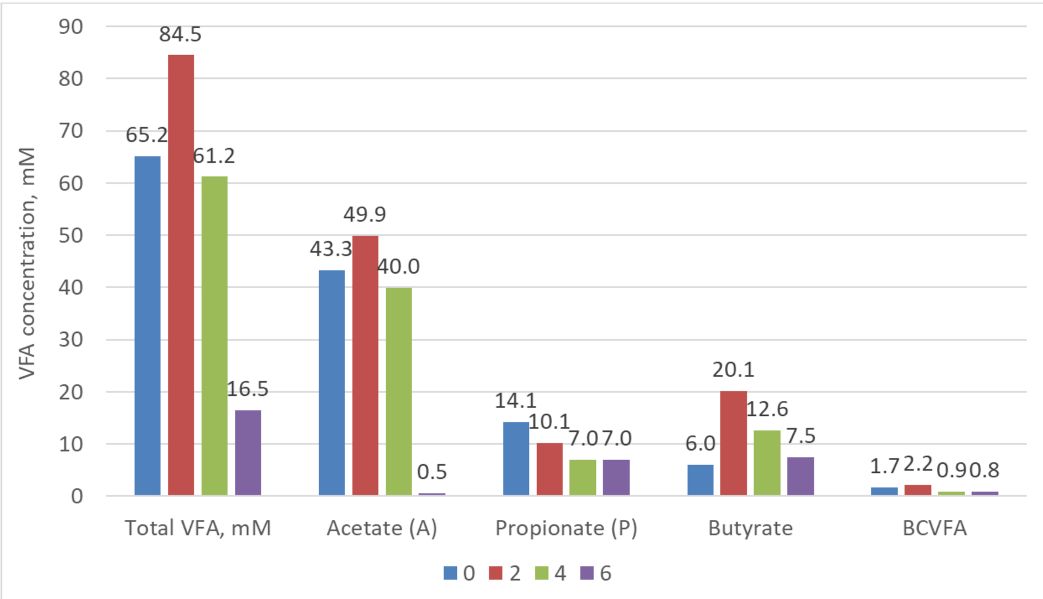


Figure 18. Volatile fatty acid concentration during in vitro incubations of mixture 3 in its liquid form

Table 13. In vitro gas production and fermentation parameters of 4 different products submitted by 4 Season Pty. to evaluate their effectiveness on CH4 production using lucerne as the substrate.

	Control	**Mixture 1**					**Mixture 2**					**Mixture 3**			**Mixture 4**			**SEM**	***P*-value**
	0%	**1%**	**5%**	**10%**	**20%**	**40%**	**1%**	**5%**	**10%**	**20%**	**40%**	**2%**	**4%**	**6%**	**10%**	**20%**	**40%**		
Gas, mL/ g DM	85.0	82.0	85.8	87.3	84.4	78.8	91.8	93.5	98	105.7†	117.0*	131.8*	102.4	76.5	91.7	92.4	96.1	8.42	<0.01
CH4, %	14.8	15.1	15.4	15.5	13.2	12.7	15.3	14.4	14.7	15.2	14.6	15.6	5.6*	1.2*	15.2	14.8	14.3	1.4	<0.01
CH4, mL/g DM	12.7	12.4	13.3	13.7	11.1	10	14.2	13.5	14.4	16.1	17.2	20.7*	6.8*	0.92*	13.9	13.7	13.8	2.25	<0.01
pH	6.89	6.89	6.88	6.84	6.86	6.9	6.86	6.81	6.74	6.69†	6.54*	6.48*	5.84*	5.00*	6.79	6.81	6.70†	0.05	<0.01
Total VFA, mM	66.4	68.9	66.9	65.3	67.3	58	72.8	69	67.9	75.7	88.2	84.5	61.2	16.5*	69.8	79.9	75.3	6.14	<0.01
% of total VFA																			
Acetate (A)	65.2	64.8	64.5	65	65.4	66.5	65.5	63.5	64.5	64.6	62.7	59	65.3	3.2*	67.6	65.8	64.9	2.01	<0.01
Propionate (P)	21.3	21.4	20.6	20.4	20.6	19.3	21.3	21.4	22	21.5	22.8	12.0*	11.5*	42.5*	20.5	20	20.9	2.15	<0.01
Butyrate	9.1	9.4	9.3	9.8	9.6	9.6	8.7	9.6	9.7	10.1	10.4	23.8*	20.6*	45.6*	8.2	10.5	10.7	1.87	<0.01
BCVFA	2.53	2.51	3.1	2.75	2.48	2.65	2.56	3.04	2.2	2.19	2.24	2.63	1.49	5.06*	2.18	2.12	2.03	0.707	<0.01
Ratio A:P	3.06	3.03	3.14	3.19	3.17	3.44	3.08	2.97	2.93	3	2.75	4.92	5.68	0.08	3.3	3.28	3.1	1.1	N/A
IVDMD, %	51.7	51.4	54.1*	54.4*	57.7*	63.9*	51.4	53.7	56.6*	59.7*	66.9*	49.5†	48.9*	44.7*	57.0*	60.2*	69.3*	0.72	<0.01

Mixtures 1 and 2 replaced substrate on weight basis (500 mg substrate; w/w) at 0, 1, 5, 10, 20 and 40%.

Mixture 3 was added as a percentage of rumen inoculum (25 mL media; v/v) at 0, 2, 4 and 6%.

Mixture 4 replaced substrate on a weight basis (500 mg substrate; w/w) at 0, 10, 20 and 40%.

CH_4=methane; IVDMD=in vitro dry matter digestibility; VFA=volatile fatty acids; BCVFA=branched chain fatty acids; SEM=standard error of the means.

* Means differences ($P \le 0.05$) from control treatment, † means tendency from control treatment ($P \le 0.10$

4.3 In vitro trials 1, 2 and 3. Effects of an incremental dose of lick block mixture added to both a high- and low-quality feed on CH_4 production

The second in vitro trial assessed the effect of mixture 3 (now labelled as mixture 5 because it was a new batch) in its liquid form on rumen fermentation with high- (Table 14) and low- (Table 15) quality forages as substrate. Most fermentation parameters had a significant effect of treatments and treatment × dose interaction. In agreement with previous results, mixture 5 reduced CH_4 production at 5% v/v compared to control and the effect was greatest at 10% for both high- and low-quality forages ($P < 0.01$). However, the reduction in CH_4 with the addition of the LB was up to 10-fold, albeit the reduction in IVDMD was approximately 20%. As previously discussed, gas production and VFA concentration should be interpreted cautiously because these fermentation products come from both the substrate feed incubated inside the bags where IVDMD is measured, and the liquid product added to the liquid media. The latter also contributes to VFA and gas production but not to IVDMD. The pH values contradict the total VFA concentration because the mixture has an acidic pH (4.8). In an in vivo situation, lower pH could be caused by higher total VFA concentration due to increased intake or rumen degradable products. In this in vitro trial, however, the intake is considered equal amongst treatments so that it would have no impact on total VFA.

In agreement with Mixture 3 evaluated in trial 1, Mixture 3 at a dose of 6% (v/v) decreased CH_4 production by 61.4% ($P \leq 0.01$) and CH_4 concentration in the gas by 65.7% compared to the control (Table 16). However, it is important to highlight that a dose of 6% is very high cattle and it may not be economically viable because the amount of the mixture 3 to be consumed to achieve similar dose in the rumen would need to be 1.8L per animal per day (300 kg BW with a 10% volume of BW = 30L of rumen volume).

Dose of liquid Mixture 5 in the in vitro trial 3 with the product incorporated to pellets did not affect total gas or CH_4 production ($P > 0.10$; Table 17). A sample of 500 mg pellet was used as a substrate for the incubations, which resulted in a dose of 58.8 mL of the Mixture 5 product. The surprising lack of effect of the lick block product on rumen fermentation using the trial pellets may be explained by a much lower dose compared to the high dose in previous in vitro trials and thus, in vitro trials should not be compared with in vivo trials. Provided these results in vitro, no effects of the mixture 5 were expected for the in vivo trials. The product was added to the ingredients used to manufacture the pellets, and no freeze-drying occurred before manufacturing. Therefore, the effects of mixture 5 observed in the previous in vitro studies where the product was added in its liquid form at high concentrations (up to 1.5 mL per bottle) were not observed with the pellets in Table 17. The main reason for the lack of effects may be the much lower concentration of the product resulting from the pellets (up to 0.1 mL per bottle if assumed the density of the product is the same as water).

Table 14. The effects of an incremental dose of lick block mixture on CH_4 production, dry mater digestibility, and rumen profile of cattle in vitro on high-quality forage

	Dose (%)				SEM	*P*-value
	0	1	5	10		
Gas, mL/g DM	80.9c	113.0a	93.5b	79.5c	3.71	<0.01
CH_4, %	12.99a	13.81a	4.11b	1.33c	0.76	<0.01
CH_4, mL/g DM	10.5b	15.7a	3.98c	1.1d	0.85	<0.01
IVDMD, %	41.2a	38.9a	32.1b	29.5b	2.16	0.03
Total VFA, mM	67.7a	80.3a	47.8b	30.7c	6.41	<0.01
pH	6.84a	6.58b	5.32c	4.65d	0.05	<0.01

DM=dry matter; VFA=volatile fatty acids; SEM=pooled standard error of means

Table 15. The effects of an incremental dose of lick block mixture on CH_4 production, dry mater digestibility, and rumen profile of cattle in vitro on low-quality forage

	Dose (%)				SEM	*P*-value
	0	1	5	10		
Gas, mL/g DM	57.4c	85.0b	94.6a	83.5b	4.72	<0.01
CH_4, %	9.02b	11.10a	4.49c	1.83d	0.63	<0.01
CH_4, mL/g DM	5.2b	9.4a	4.6b	1.6c	0.75	<0.01
DMD, %	23.0a	23.3a	14.3b	12.9b	2.51	0.02
Total VFA, mM	50.4a	59.3a	36.5b	19.2c	6.81	0.02
pH	7.07a	6.76b	5.36c	4.69d	0.07	<0.01

DM=dry matter; VFA=volatile fatty acids; SEM=pooled standard error of means

Table 16. In vitro gas production and fermentation profile of a liquid supplement added at 0, 2, 4, and 6% v/v to the incubation buffer and 80:20 forage to concentrate ration used as substrate. These pellets were latter used in an in vivo trial to measure metabolic gas production in steers.

	Mixture 5, % v/v				SEM	*P*-value		
	0%	2%	4%	6%		Treat	L	Q
Gas, mL/g DM	100.8	133.7*	141.0*	113.1	5.14	0.03	0.14	<0.01
CH_4, %	12.58	13.97	11.18	4.32*	0.584	<0.01	<0.01	<0.01
CH_4, mL/g DM	12.7	18.7*	15.8	4.9*	1.3	0.01	0.01	<0.01
pH	6.25	6.15	5.95	5.33	0.027	<0.01	<0.01	<0.01

Table 17. In vitro gas production and fermentation profile of pellets containing liquid lick block supplement calculated to consume 0, 100, 200, 300, and 400 g/head/d plus 1,500 g/head/d of rice and wheat bran delivered through the GreenFeed units for cattle diets.

	Pellet 0	Pellet 100	Pellet 200	Pellet 400	SEM	*P*-value
Gas, mL/g DM	118.4	118.6	125.3	122.1	5.65	0.32
CH4, %	15.0	14.4	14.9	14.4	0.34	0.43
CH4, mL/g DM	16.75	16.98	17.5	17.7	1.16	0.88
pH	6.09	6.28	6.24	6.27	0.084	0.38

SEM=pooled standard error of means

4.4 In vivo trial 1. Dose-response of liquid lick block product incorporated to pellets

The lick block product mixture 5 received from 4 Seasons Pty to perform the dose-response in the in vitro trial was also evaluated in vivo in its liquid form into trial pellets. The analysis of DMD and VFA was analysed and presented above.

4.4.1 Change from baseline to treatment period

The treatment × period interaction was significant for growth rate (kg/d; $P < 0.01$). Steers fed the 100 and 200 g/head/day of LB showed a larger increase in ADG after the LB was fed compared to those in the control group with 0 g/head/day of LB (Figure 19; $P < 0.01$).

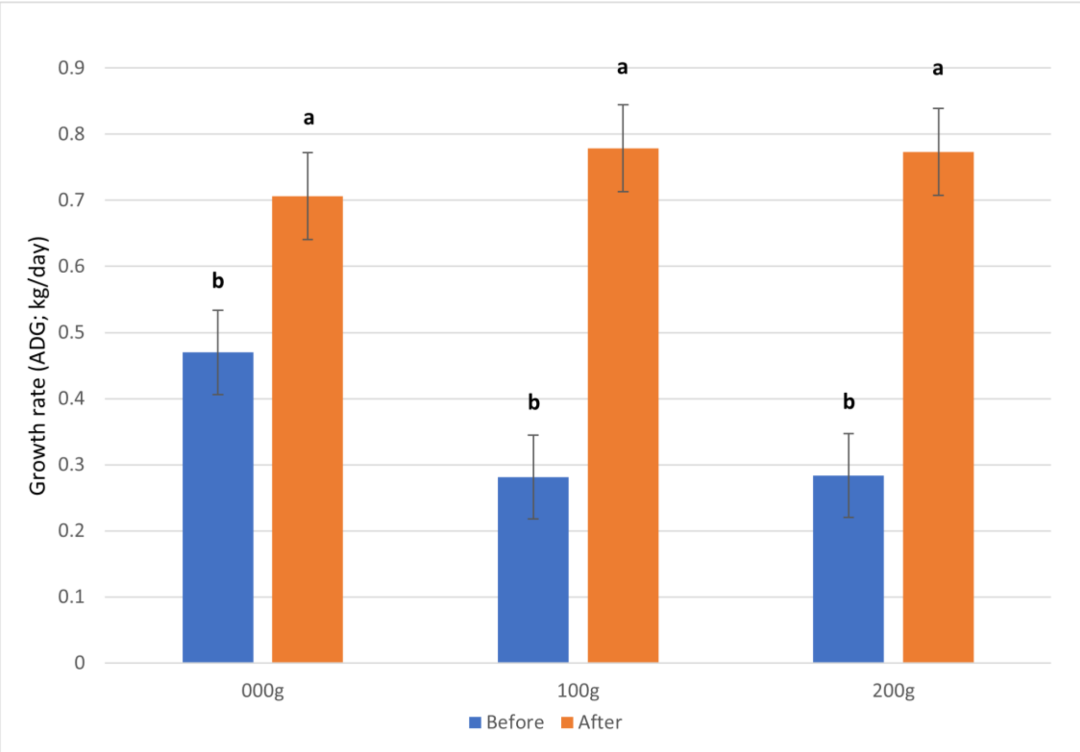


Figure 19. Average daily gain (ADG) of beef steers receiving 1 kg/d of pellets with liquid lick block (LB) formulated to target an intake of 0, 100, and 200 g LB/head per day before and after being offered LB pellets. [a, b] means with different superscripts within treatment differ ($P < 0.05$).

The change in CH_4 production of beef steers before and after LB treatment is depicted in Figure 20. The treatment × period interaction was significant for CH_4 production (g/day; $P < 0.01$). For the control animals, there was no difference in CH_4 emissions before and after feeding the LB ($P > 0.10$).

However, CH_4 emissions were lower after compared to before (baseline) feeding the LB product for both 100 and 200 g/head/day ($P < 0.01$).

Steers fed 100 g/head/day emitted 13.1% lower CH_4 after the LB was provided compared to when they were fed the pellets without the LB ($P < 0.01$). However, CH_4 production from steers offered 200 g LB/head per day was reduced by only 10.2% when the LB was offered compared to before the LB was fed ($P < 0.01$).

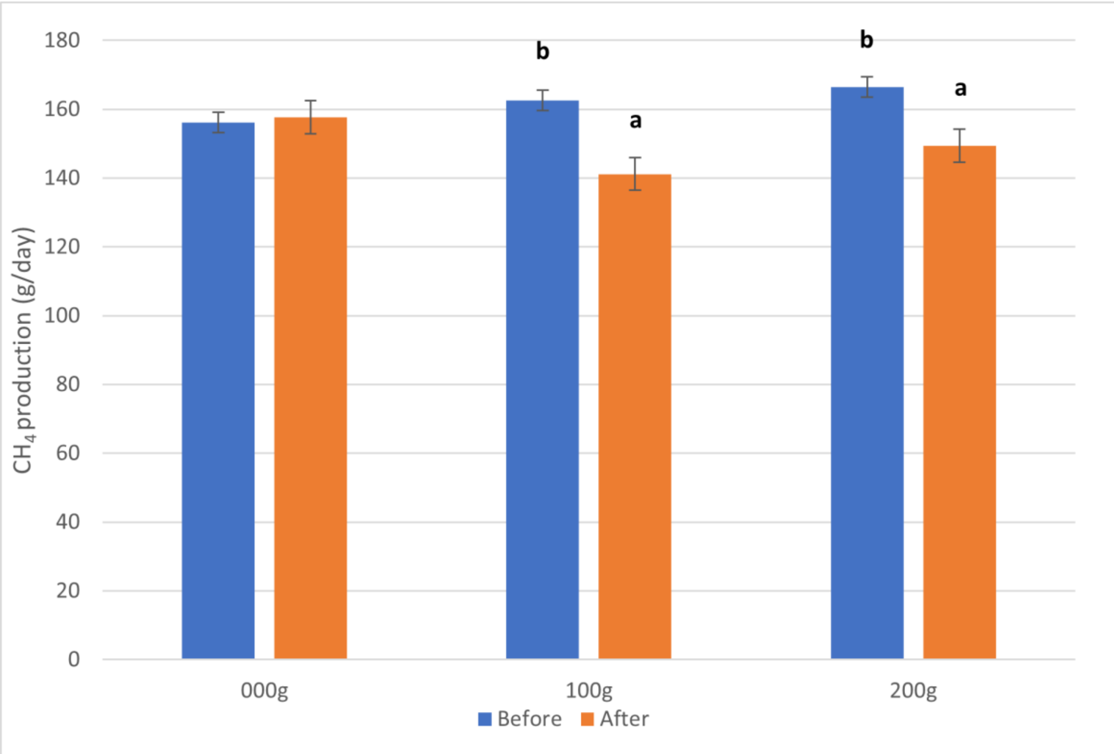


Figure 20. Methane production (g/day) of beef steers with a target lick block product (LB) intake of 0, 100, and 200 g LB/head per day) before and after consuming the pellets. [a, b] means with different superscripts within treatment differ ($P < 0.05$).

4.4.2 Effect of lick-block (LB) supplementation on the performance and CH_4 emissions of beef cattle during the LB pellet delivery period only

Both total and hay DMI during the LB pellets delivery period were not affected by LB supplementation (Table 18; $P > 0.05$). Pellet DMI was not affected by treatment, and none of the treatments reached the targeted LB pellet intake (Table 18). However, the estimated LB intake (pellet intake x LB content of the pellet) was close, albeit lower, to the target (Table 18). Therefore, the effects on CH_4 production need to be interpreted in this context. Daily water intake increased linearly by up to 11.4 L/day (39%) with the incremental dose of LB supplementation. The increased water consumption with increasing doses of the LB may be due to the product's greater sodium concentration, stickiness, or astringent flavour, leading animals to drink more water to maintain a high LB pellet intake (Lopez et al., 2021). Steers in both 100 and 200 g/head/day had 11.3% greater

ADG compared to control steers, albeit the difference was not statistically significant (Table 18; P = 0.35).

Methane production (g/day) of beef steers fed 100 g/head/day LB into pelleted grain-based supplements tended to be lower from the control group given no LB with their pellets (Table 18; P = 0.07). However, there was no significant difference between the control and 200 g/head/day of LB on CH_4 production (P > 0.05). When total DMI was considered (CH_4 yield; g CH_4/kg DMI), steers fed 100 g/head/day LB had 11.7% lower CH_4 yield compared to the control groups fed the control 0 g/head/day (Table 18; P < 0.01). However, there was no difference between steers treated with 200 g/head/d compared to the steers given the control 0 g/head/day treatment. Moreover, there was no difference between steers consuming 100 and 200 g/head/day LB treatments (P > 0.05; Table 18). Methane intensity (g CH_4/kg ADG) was not different between treatment groups, although it showed a tendency for steers fed 100 and 200 g/head/day to have lower intensity compared to the control (P = 0.06) (log-transformed). The back-transformation of CH_4 intensity to g CH_4/kg ADG showed a reduction of 16.8 (100 g/head/day) and 14.9% (200 g/head/day) in the intensity of emissions (Table 18). To put this in context, for an animal putting on 200 kg LW without and with LB supplement generates 44.8 and 37.4 kg CH_4, respectively, which translates to a reduction of 7.53 kg CH_4/head or 211 kg CO_2-e/head.

Table 18. Performance and greenhouse gases (GHG) emissions of growing beef steers after being offered lick-block (LB) pellets for 10 weeks of the experiment

	Treatment Group (g LB/head per day)			SEM	*P*-value
	0 g	100 g	200 g		
Total DMI, kg/day	6.93	6.90	7.01	0.272	0.96
Hay DMI, kg/day	5.74	5.60	5.85	0.251	0.78
Pellet DMI, g/day	1,199	1,289	1,154	58.83	0.28
Target pellet intake, g/d	1,500	1,600	1,700	-	-
Block intake, g/day	0.00^{a}	92.3^{b}	172.5^{c}	4.99	<0.01
ADG, kg/day	0.71	0.78	0.78	0.04	0.35
CH_4 production, g/day	159	142	149	5.10	0.07
CH_4 yield, g/kg DMI	24.0^{a}	21.2^{b}	22.2^{ab}	0.55	<0.01
CH_4 intensity, log (g/kg ADG)	2.35	2.27	2.28	0.03	0.06
CH_4 intensity, g/kg ADG	223.9	186.2	190.5	1.07	0.06
CO_2 production, g/day	4492	4424	4479	146	0.94
H_2 production, g/day	0.217	0.236	0.225	0.01	0.42
O_2 consumption, g/day	3258	3449	3484	119	0.35
Water intake, L/day	28.9^{a}	37.0^{bc}	40.3^{cd}	2.31	<0.01

LB=lick-block; DMI=dry matter intake; ADG=average daily gain.

$^{a, b}$ means sharing the same superscript do not differ (P > 0.05).

4.4.3 Correlations among performance, intake, and GHG emissions of beef cattle after the LB pellet delivery period only

The correlation matrix among performance, intake and emissions is shown in Figure 21. There were strong correlations between CH_4 (r = 0.90), CO_2 production (0.88), O_2 consumption (r = 0.75), total DMI (r = 0.74) and LW (r = 0.90) (Figure 21; P < 0.01). These results demonstrate that the data collected show results as expected from previous literature (Bai et al., 2014; Charmley et al., 2015; Renand et al., 2019). Interestingly, the estimated LB intake (g/day), treated as a continuous variable depending on how much pellets each animal consumed and the concentration of LB in the pellet, showed a negative correlation with CH_4 yield and a positive correlation with ADG, water intake, and O_2 consumption (Figure 21; P < 0.05). These results are important because they demonstrate that the product effectively reduces CH_4 in a dose-dependent manner.

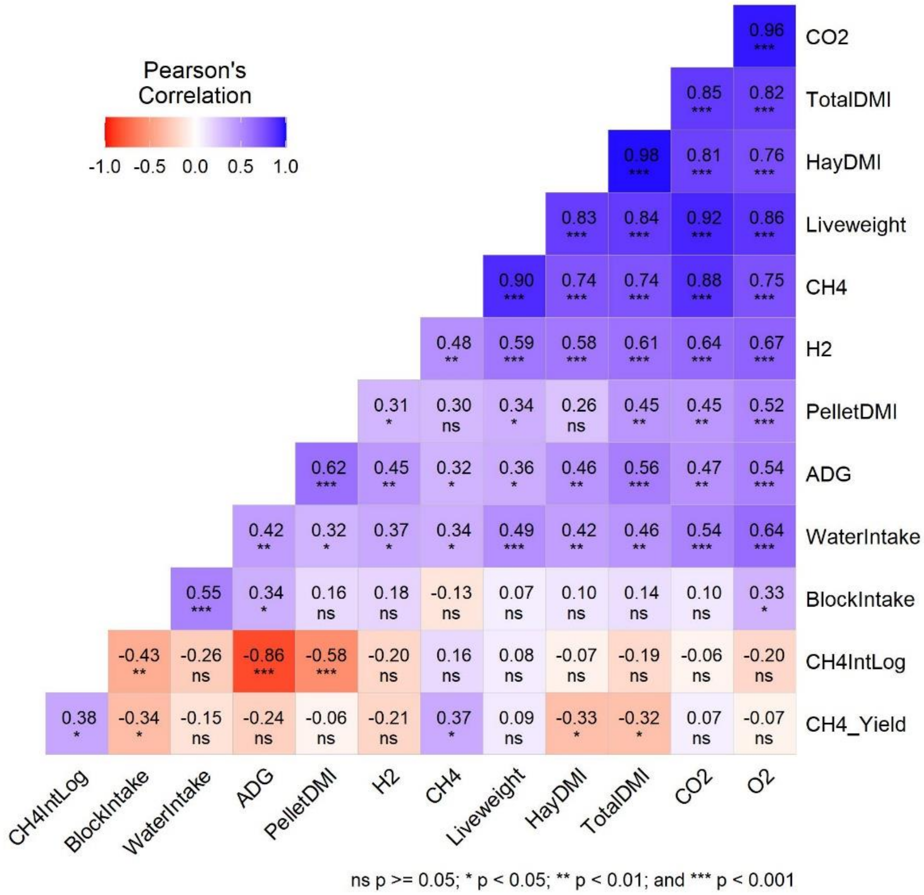


Figure 21. Correlation matrix of performance and greenhouse gas (GHG) emissions of beef steers offered lick-block (LB) pellets during 10 weeks of the experiment.

An incremental dose of LB supplement (0, 100, and 200 g LB/head per day) did not linearly decrease CH_4 production (159, 142, and 149 g CH_4/day) in the model with treatment as a factor. However, there was a linear decrease in CH_4 yield and ADG when LB intake was treated as a linear variable depending on the LB intake of each animal using Pearson correlation analysis. This may be caused by LB intake being different between animals within a treatment and it suggested that the response to LB depends on the consumption of it.

The results from the first in vivo trial indicated that the LB supplement tended to reduce CH_4 production (g CH_4/day) from steers by 10.7 % ($P = 0.07$), CH_4 yield (g CH_4/ kg DMI) by 11.7% ($P < 0.05$) and CH_4 intensity (g CH_4/kg ADG) by 16.8% ($P = 0.06$) compared to the control with no LB product. To put this in context, for an animal putting on 200 kg LW, this translates to 619 kg CO2e/head reduction in emissions. The dose rate of active ingredients was low but reflected best practices in animal welfare, economics, and manufacturing safety. Further work may consider a greater concentration of active ingredients in the feed supplements. There were significant manufacturing difficulties with adding LB to achieve doses above 200 g/head/day, resulting in a soft pellet. This situation results in product instability. Further work on manufacturing and palletisation should be undertaken before greater rates of addition of the active ingredients can be delivered as a dry pellet product. These comments do not preclude a higher rate of addition via a feed block supplement and work is being undertaken to manufacture a feed block with high concentration of active ingredients.

4.5 In vivo trial 2. Performance and GHG emissions of cattle fed a liquid lick block supplement

Results are presented in two sections for each of the statistical analysis performed, i.e. 1) baseline vs treatment period with the former being the period before the liquid product was delivered to the steers and the latter averaging all data during the treatment period independently of the concentration of the product in the liquid mixture, and 2) with each experimental period being the concentration of liquid product in the molasses liquid mix with baseline, 50, 60, 70, and 80% of the product and the remainder molasses.

4.5.1 Change from baseline to treatment period

Intake and performance of steers during the baseline and treatment period is presented in Table 19. All variables showed a significant Period x Treatment interaction ($P \leq 0.05$) except feed conversion ratio (FCR; $P = 0.19$) and a tendency for ME concentration in the final diet and MEIG ratio ($P < 0.10$).

The average intake of the product was 0.374 ± 0.0116 kg DM/d. This intake is nearly double the amount of product added to the pellets in the previous trial. Hay and pellet DMI were similar amongst treatments during the treatment period ($P > 0.05$) which suggests that the liquid product offered did not have a substitution effect on hay and pellet intake. In contrast, total DMI, MEI, WSCI, and CPI were similar between treatments during the baseline period, but LB steers had higher intakes during the treatment period ($P < 0.05$). Similarly, water consumption was similar between treatments during the baseline period ($P > 0.05$), and it was greater during the treatment for LB compared to the control steers ($P < 0.05$).

Surprisingly, ADG was greater in the LB compared to the control steers ($P < 0.05$) in the baseline period but no differences between treatments were observed during the treatment period (Table 19). Steers in the LB treatment were 4 kg/head heavier compared to control steers during the treatment period on average. Caution to interpretation of body weight data should be taken given the experiment was designed to detect differences in methane production and yield and not differences in cattle performance. Further replication of pens would be required to confirm performance differences.

Table 20 shows feeding behaviour of the steers showing steers spent 19.2 min/d consuming the liquid product which was consumed at an eating rate of 61.4 g/min. Surprisingly, steers in the control group spent shorter time eating hay at approximately 2-fold faster than LB steers. It is possible that this effect was due to higher competition for feed access in the control animals compared to the LB steers due to lower total feed available because it is known that competition for feed increases eating rate (Gonzalez et al., 2012). Similarly, LB steers showed higher drinking frequency compared to control steers ($P < 0.05$) reflecting higher water consumption (Table 20).

Table 19. Intake and performance of steers fed hay, pellets in the GreenFeed unit, and a molasses lick product with anti-methanogenic ingredients during the baseline period with no product and the treatment periods.

	Baseline period		Treat period			P-value		
Variable	Control	LB	Control	LB	StdErr	Period	Treat	Per × Treat
Liquid intake, kg FM/d	0.000	0.000	0.000y	1.094x	0.0346	<0.001	<0.001	<0.001
Product intake, kg FM/d	0.000	0.000	0.000y	0.743x	0.0232	<0.001	<0.001	<0.001
Liquid intake, kg DM/d	0.000	0.000	0.000y	0.558x	0.0177	<0.001	<0.001	<0.001
Product intake, kg DM/d	0.000	0.000	0.000y	0.374x	0.0116	<0.001	<0.001	<0.001
Hay DMI, kg/d	5.83x	5.47y	6.28	6.39	0.2176	<0.001	0.664	0.054
Pellet DMI, kg/d	0.86y	0.91x	0.86	0.85	0.0062	<0.001	0.972	0.041
Total DMI, kg/d	6.694	6.378	7.146y	7.797x	0.2194	<0.001	0.090	<0.001
Total DMI, g DM/kg $BW^{.75}$	88.02	84.30	89.70y	97.68x	1.7664	<0.001	0.315	<0.001
Total DMI, %BW	2.082	1.997	2.090y	2.273x	0.0422	<0.001	0.337	<0.001
Hay ME Intake, MJ/d	40.81x	38.3y	43.98	44.74	1.5233	<0.001	0.000	<0.001
Liquid MEI, MJ/d	0.000	0.000	0.000y	8.533x	0.2703	<0.001	0.000	<0.001
Pellet MEI, MJ/d	10.03x	10.5y	10.01	9.83	0.0723	<0.001	0.103	<0.001
Total MEI, MJ/d	50.84	48.82	53.99y	63.11x	1.5638	<0.001	0.000	<0.001
Diet CP, %DM	0.071	0.072	0.072x	0.068y	0.0005	<0.001	0.306	<0.001
Hay CP intake, kg/d	0.344x	0.32y	0.371	0.377	0.0128	<0.001	0.664	0.054
Liquid CPI, kg/d	0.000	0.000	0.000y	0.046x	0.0015	<0.001	0.000	<0.001
Pellet CPI, kg/d	0.126y	0.12x	0.126	0.124	0.0009	<0.001	0.043	<0.001
Total CPI, kg/d	0.470	0.455	0.497y	0.547x	0.0131	<0.001	0.000	<0.001
Diet DMD, %DM	0.720	0.720	0.720y	0.741x	0.0007	<0.001	0.563	<0.001
Diet ME, MJ/kd DM	7.615	7.669	7.668y	8.174x	0.0370	<0.001	0.683	0.071
Total NDF intake, kg/d	4.226x	4.00y	4.529	4.596	0.1460	<0.001	<0.001	<0.001
Liquid WSC intake, kg/d	0.000	0.000	0.000y	0.318x	0.0101	<0.001	<0.001	<0.001
Total WSCI, kg/d	0.303	0.291	0.323y	0.644x	0.0140	<0.001	<0.001	<0.001
Water consumption, L/d	35.81	39.69	33.22y	59.97x	2.1812	<0.001	0.003	<0.001
ADG, kg/d	1.903y	2.66x	0.405	0.429	0.1776	<0.001	0.004	<0.001
BW, kg	323.5	321.8	339.8y	343.7x	9.9662	<0.001	0.025	0.022
Gain: Feed ratio, kg/kg	0.288y	0.41x	0.051	0.054	0.0510	<0.001	0.019	0.024
Feed: Gain, kg/kg	2.96	2.66	-35.63	22.74	21.867	0.674	0.193	0.187
MEI: Gain, MJ/kg ADG	22.5	20.4	-274.8	187.9	168.97	0.134	0.828	0.082

^{x,y} within a period, means with different superscript differ (P < 0.05).

The smart ear tags showed that LB steers spent longer time eating and ruminating, and shorter time not active compared to the control steers during the treatment period (P < 0.05), reflecting their higher DMI. Unexpectedly, LB steers had higher ear temperature compared to the control steers during the baseline period (P < 0.05) but no differences were found between treatments during the treatment period (P > 0.05; Table 20).

Table 20. Feeding and drinking behaviour of steers fed hay, pellets in the GreenFeed unit, and a molasses lick (LB) product with anti-methanogenic ingredients during the baseline period with no product and the treatment periods.

	Baseline period		Treatment period			P-value		
Variable	Control	LB	Control	LB	StdErr	Period	Treat	Treat x Period
Electronic feeders								
Hay feeding time, min/d	181.7	194.8	86.5y	144.2x	6.5	<0.001	<0.001	<0.001
LB feeding time, min/d	0.00	0.00	0.00y	19.30x	0.702	<0.001	<0.001	<0.001
Total feeding time, min/d	181.7	194.8	86.5y	163.5x	6.48	<0.001	<0.001	<0.001
Hay eating rate, g/min	49.89	43.57	134.14x	76.00y	7.254	<0.001	<0.001	<0.001
LB eating rate, g/min	-	-	-	61.44	4.456	-	-	-
Hay visit size, kg FM	0.114y	0.160x	0.150	0.161	0.0130	0.040	0.097	0.049
LB visit size, kg FM	0.000	0.000	0.000y	0.066x	0.0020	<0.001	<0.001	<0.001
Hay visit Size, kg DM	0.076y	0.105x	0.099	0.106	0.0086	0.040	0.097	0.049
LB visit Size, kg DM	0.000	0.000	0.000y	0.034x	0.0010	<0.001	<0.001	<0.001
Hay Visit Length, min	2.38	3.69	1.44y	2.42x	0.23	<0.001	<0.001	<0.001
LB Visit Length, min	0.00	0.00	0.00y	1.22x	0.04	<0.001	<0.001	<0.001
Hay Visit frequency, #/d	85.2x	59.5y	77.2	76.6	4.44	<0.001	<0.001	<0.001
LB Visit frequency, #/d	0.00	0.00	0.00y	16.80x	0.543	<0.001	0.054	0.691
Total visit frequency, #/d	85.2	59.5	77.2y	93.4x	4.56	0.146	0.021	<0.001
Weighing stations								
Drinking frequency, #/d	3.93	4.75	3.33y	5.33x	0.248	0.942	0.062	0.053
Smart ear tags								
Eating time, min/d	152	137	132y	156x	11.8	0.942	0.751	<0.001
Ruminating, min/d	491	471	359y	388x	17.8	<0.001	0.844	0.008
High Active, min/d	160	140	312	291	10.1	<0.001	0.135	0.932
Active, min/d	199y	242x	221	233	20.3	0.316	0.330	0.018
Not Active, min/d	430	456	390x	371y	11.7	<0.001	0.815	0.005
Temperature, ºC	26.68y	27.74x	29.09	29.69	0.305	<0.001	0.057	0.039

$^{x, y}$ within a period, means with different superscript differ (P < 0.05).

Results from gas exchange measured by the GreenFeed system are shown in Table 21. No differences between treatments (P > 0.05) were observed during the treatment period on the number of cups consumed daily but the LB steers had greater cup consumption during the baseline period (P < 0.05). However, LB steers had higher visit frequency and length (P < 0.05) compared to the control steers during the treatment.

Importantly, LB steers had approximately 10% lower CH4 production compared to control steers (P < 0.05) which was reflected in lower intensity and yield of CH4 production (P < 0.05; Table 21). It is important to note that methane intensity numbers in Table 21 are affected by some animals that have very low weight gains during the treatment period.

Table 21. Gas exchange of steers fed a liquid molasses product with anti-methanogenic ingredients during the baseline period (no product) and the treatment periods.

	Baseline period		Treatment period			P-value		
Variable	Control	LB	Control	LB	StdErr	Period	Treat	Treat x Period
Cup consumption, #/d	24.24y	26.00x	24.19	24.26	0.407	<0.001	0.104	<0.001
GF visit length, sec	272	295	227y	277x	6.2	<0.001	0.723	0.059
GF visit frequency, #/d	5.15	5.42	4.87y	5.72x	0.231	<0.001	0.402	<0.001
CH4, g/d	150.4	148.4	211.7x	195.9y	5.46	<0.001	0.231	0.009
CO2, g/d	4,100	4,235	5,099	5,224	113.0	<0.001	0.403	0.908
H2, g/d	0.144x	0.121y	0.345	0.359	0.0110	<0.001	0.004	<0.001
O2, g/d	3,042	3,266	3,776y	4,026x	87.2	<0.001	0.239	0.008
CH4 intensity, g/kg ADG	108.9	52.2	700.0	269.7	190.22	0.037	0.204	0.329
CH4 intensity, log(g/kg ADG)	4.360x	3.915y	5.811x	5.527y	0.1398	<0.001	0.004	<0.001
CH4 Yield, g/kg DMI	22.51	23.30	30.09x	25.22y	0.632	<0.001	0.008	<0.001
Log CH4 Yield, log(g/kg DMI)	3.108	3.143	3.396x	3.225y	0.0238	<0.001	0.101	<0.001
CH4 Yield, g/MJ MEI	2.959	3.041	3.967x	3.110y	0.0772	<0.001	<0.001	<0.001

$^{x, y}$ within a period, means with different superscript differ (P < 0.05).

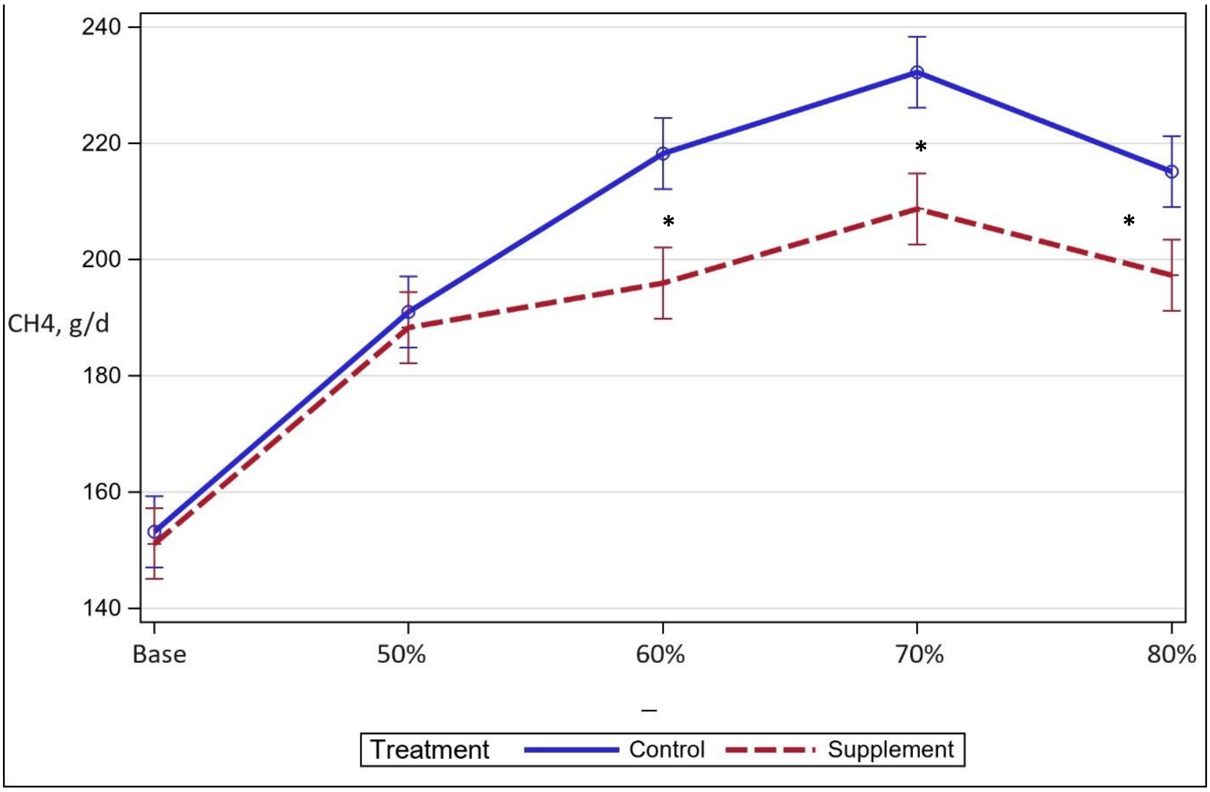
4.5.2 Increasing the proportion of product in the liquid molasses mix

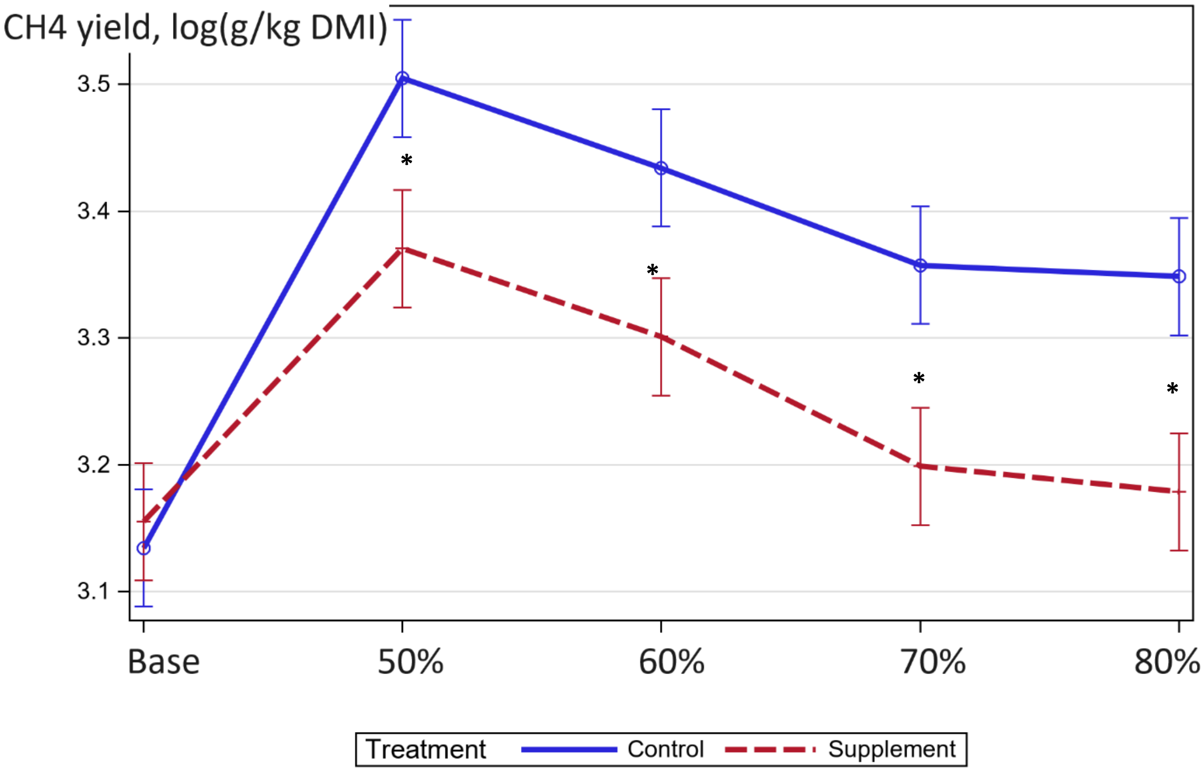
It is important to note that results of this section should be interpreted with caution because some periods were only 7 days duration and thus the results may not be accurate to draw firm conclusions. In addition, the effect of methane mitigants added into the liquid LB on methane yield requires further confirmation as a 'molasses only' control was not implemented in the experimental design.

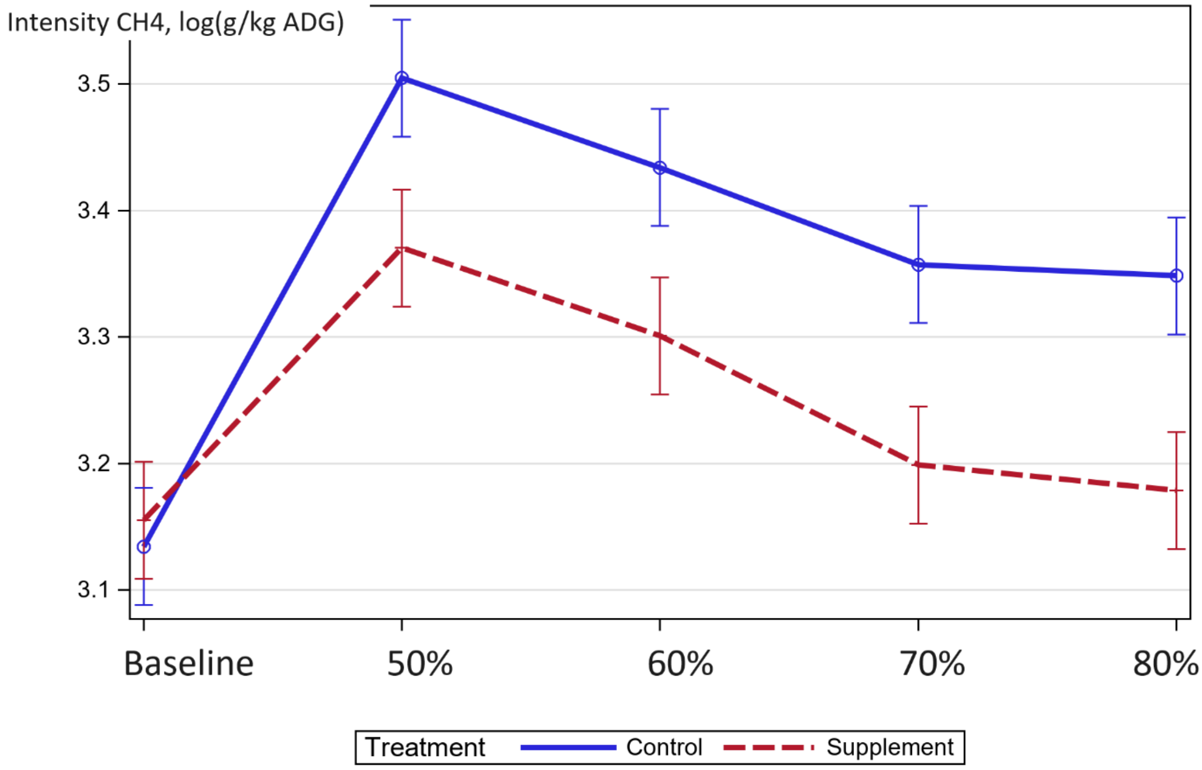
Figure 22 shows gas exchange, intake, and body weight of steers throughout the trial split by periods with increasing concentration of LB product in the liquid mix. Daily CH4 production was similar amongst treatments in the baseline and during the period with 50% product mix (P > 0.05) but it was greater for control animals at 60% and above of the product in the molasses mix (P < 0.05). This either suggests that the concentration of the active ingredients reaches the threshold to produce significant reduction of CH4 production at 60% and that no clear benefit is observed beyond this. Alternatively, these results may also suggest that the active ingredients may require a period longer than 2 weeks to change the microbial rumen population to see a significant reduction in CH4 production.

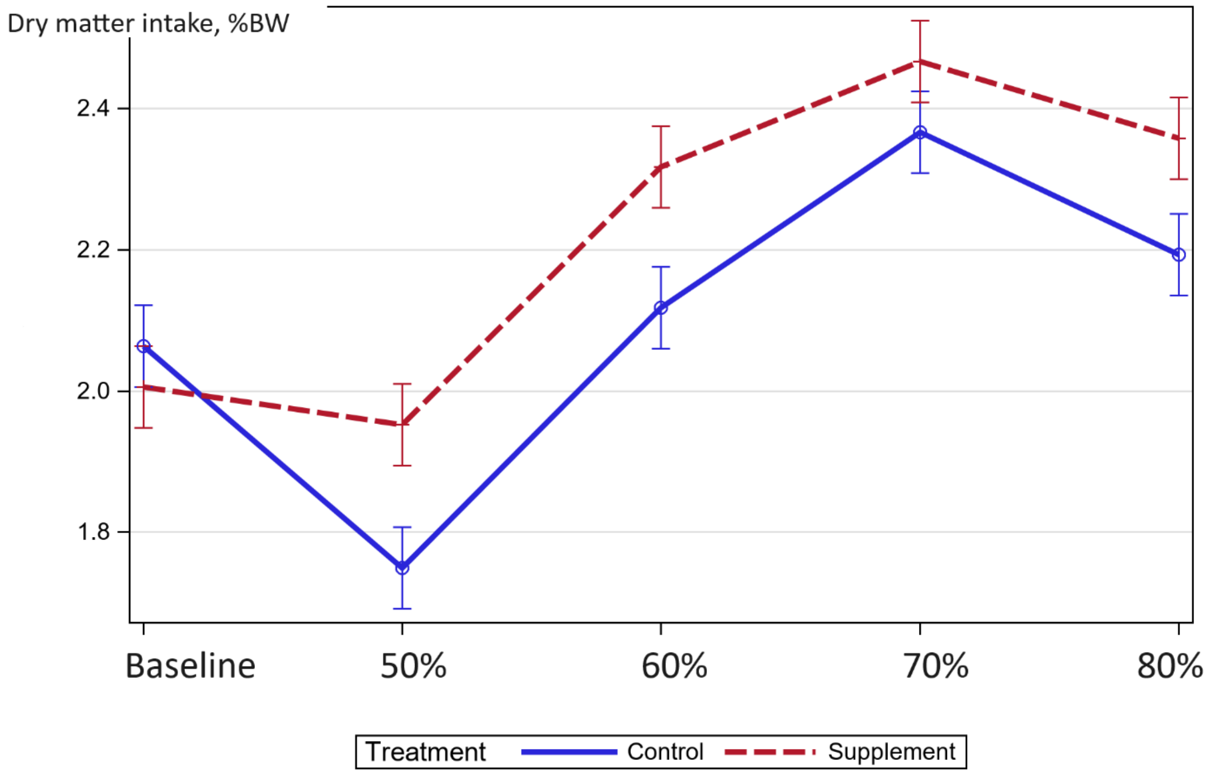
Similar results were also observed in the intensity of CH4 production per kg of ADG although control animals had greater intensity of CH4 production compared to LB steers from 50% of product in the molasses mix (P < 0.05). Therefore, the effects on intensity of CH4 product seem to be observed earlier than those in daily CH4 production.

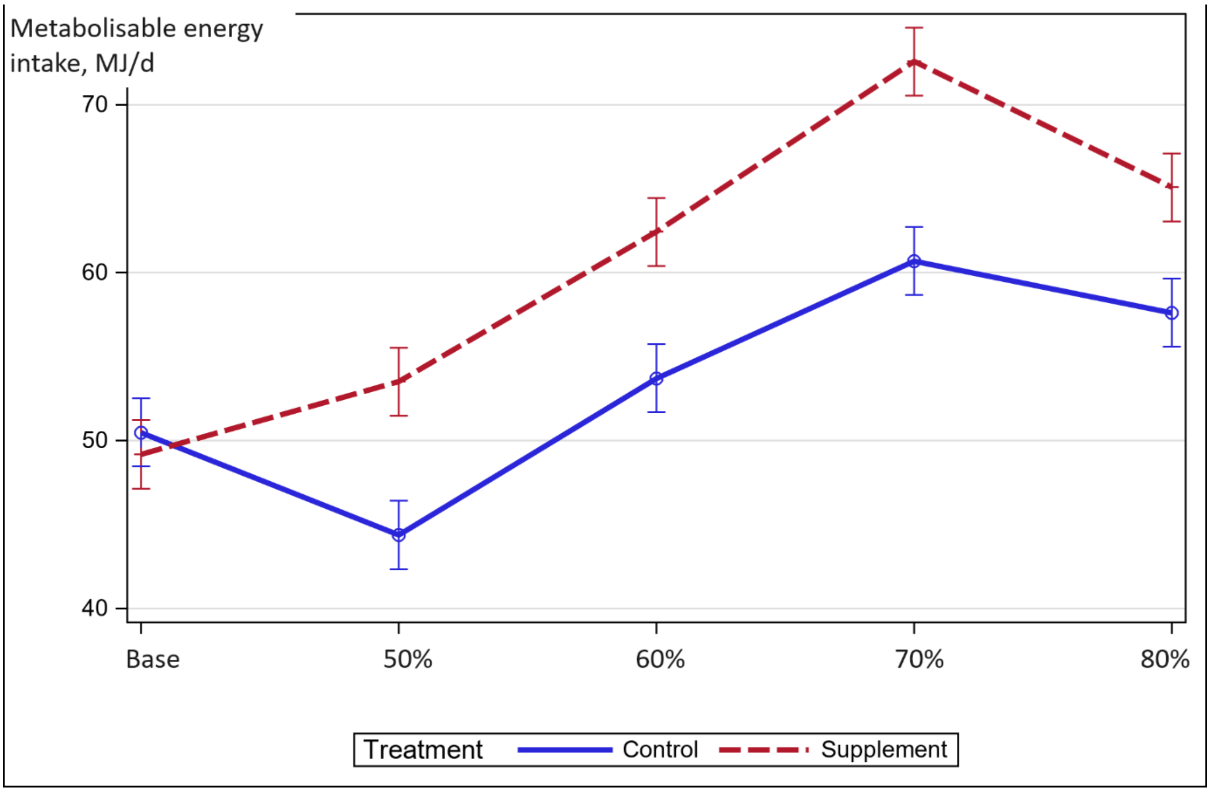
Both total DMI intake and MEI was no different between treatments during the baseline period (P > 0.05) but LB steers showed greater intake compared to control steers (P < 0.05) after the liquid product was fed with these differences between treatments being similar independently of the concentration of the product in the molasses mix.

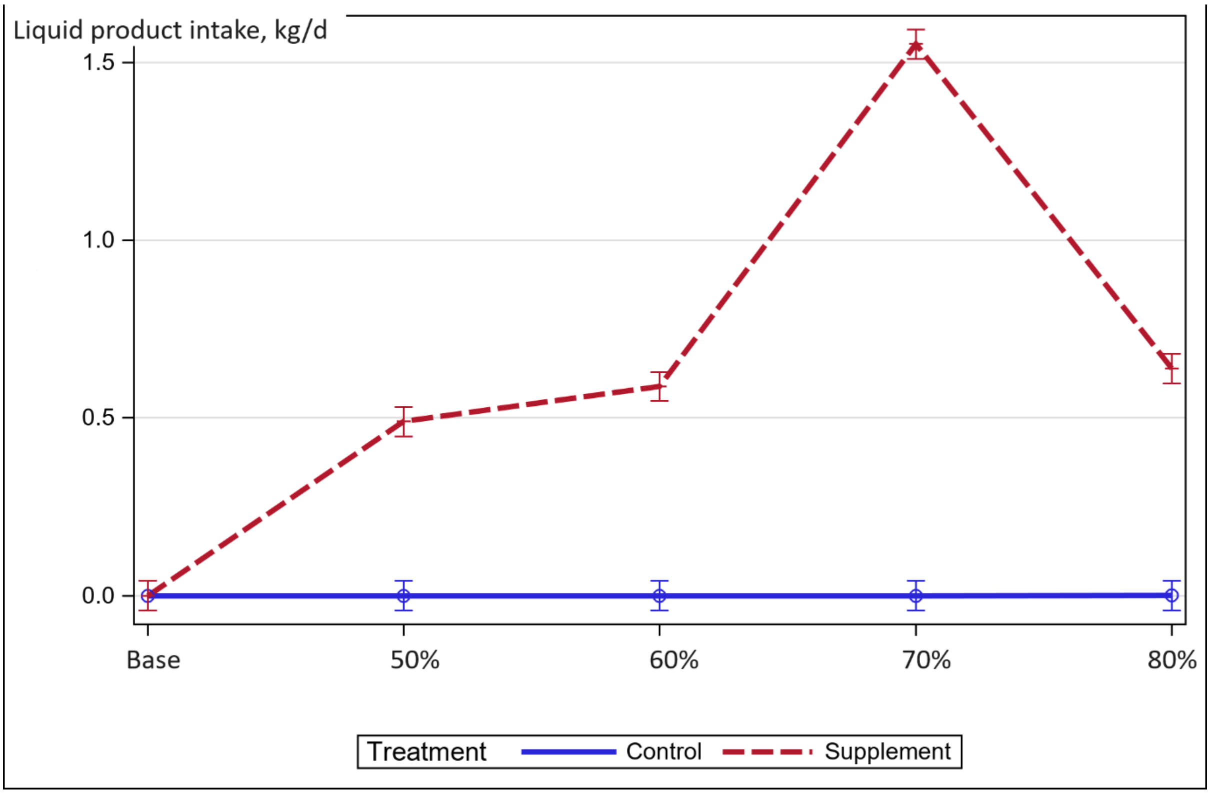

CH4, g/d
240
220
200
180
160
140
Base
50%
60%
70%
80%
*
Treatment
Control
Supplement


CH4 yield, log(g/kg DMI)
3.5
3.4
3.3
3.2
3.1
Base
50%
60%
70%
80%
*
Treatment
Control
Supplement


Intensity CH4, log(g/kg ADG)
3.5
3.4
3.3
3.2
3.1
Baseline
50%
60%
70%
80%
Treatment
Control
Supplement


Dry matter intake, %BW
2.4
2.2
2.0
1.8
Baseline
50%
60%
70%
80%
Treatment
Control
Supplement


Metabolisable energy intake, MJ/d
70
60
50
40
Base
50%
60%
70%
80%
Treatment
Control
Supplement


Liquid product intake, kg/d
1.5
1.0
0.5
0.0
Base
50%
60%
70%
80%
Treatment
Control
Supplement

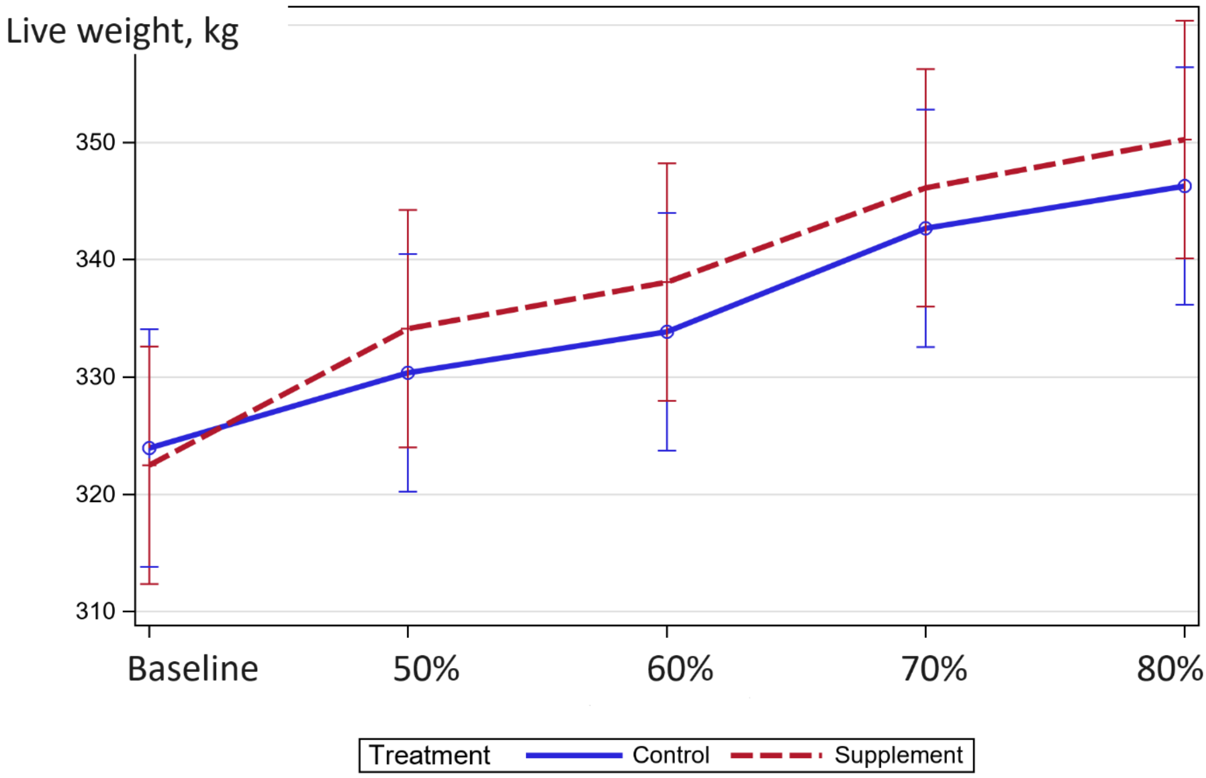


Figure 22. Gas exchange, performance, and intake of steers fed a liquid molasses product with anti-methanogenic ingredients during the baseline (no product), and periods with 50, 60, 70, and 80% period with no product and the treatment periods. * P < 0.05 within period.

These results demonstrate that the molasses product offered free choice and ad libitum also reduces CH4 production at doses higher than 50% mixed with pure molasses with slight improvement in animal performance and live weight and no perceived negative impacts on animal health. However, the current trial was of short duration (56 d) and studies with longer feeding periods are required. No extra benefit on CH4 emissions and performance are apparent even after the high product intake reported in the present trial.

4.6 In vivo trial 3. Effect of solid lick block supplementation on methane emissions from beef cattle in pens

Results are presented in Table 22, 21 and 22 to showcase differences because of treatment compared to the control.

Yard weighing showed no difference between treatments in initial BW ($P > 0.05$) but final BW and ADG were greater in MLB compared to control animals ($P < 0.05$; Table 22). In contrast, BW and ADG of the steers measured by the in-pen weighing stations was not significantly affected by treatments ($P > 0.05$). The disagreement between results from both methods of weighing animals may be due to several factors including rumen fill with the in-pen technologies measuring full BW whereas the yard weighing is shrunk BW and the much greater number of measurements compared to yard weighing.

Interestingly, steers in the MLB treatment had lower pellet DMI ($P < 0.05$), which was partly counterbalanced by higher MLB intake compared to control steers ($P < 0.05$). This resulted in total DMI being similar between treatments ($P > 0.05$) except when DMI was expressed with data from yard weighing as g/kg $BW^{0.75}$ and %BW which was lower in MLB compared to control steers ($P < 0.05$; Table 22).

MLB steers had a higher Gain: Feed ratio than the Control animals ($P < 0.05$) but this was not significant for the inverse Feed: Gain or MEI: Gain ratios ($P > 0.05$).

Total ME intake was not significantly different between treatments, largely due to the inclusion of the Lick Block to the diet which provided 4.89 ME intake (MJ/d) counterbalancing lower pellet ME intake in the MLB compared to the control steers ($P < 0.05$). The quality of the total diet of steers considering their selection of the different feedstuffs resulted in MLB steers selecting a diet with higher DMD and WSC, and lower CP and NDF ($P < 0.05$) but similar estimated ME concentration ($P > 0.05$; Table 22). These results highlight the importance of understanding diet selection changes when multiple feedstuffs are offered to forage-fed animals, and the consequences these could have on nutrient intake, ruminal degradation and fermentation, and GHGE.

The average intake of the lick block was 0.528 FM/d, during Period 1 and 0.634 (FM/d) in Period 2, this is similar to what was estimated to be fed by raw delivery. This MLB intake showcases the animals' preference for the Lick Block as no aversion was shown and the ad libitum delivery ensured it was an animal's free choice to consume the Lick Block. Of note, there was no significant difference between the Lick Block DMI (kg/d) between the two Periods in lick block intake.

Water consumption estimated from the changing BW while the animal stands on the scale to drink water was 55% higher in the MLB steers compared to the control ($P < 0.05$) and these results agree with earlier studies in the present project. This is likely due to the Lick Blocks' sweetness and stickiness, inducing the need for higher water intake, where lick blocks have been shown to increase water consumption (Salem et al., 2007). MLB are recommended from suppliers to be provided near water sources.

Table 22 Intake and performance of steers fed hay, pellets in the GreenFeed unit, and a molasses Lick Block with anti-methanogenic ingredients.

	Treatment			P-value	
	Control	MLB	SEM	Treatment	Period
Initial BW, kg	408.3	412.1	8.73	0.142	<0.001
Final BW, kg	467.0	479.6	10.21	<0.001	<0.001
ADG, kg/d	1.04	1.20	0.053	0.018	<0.001
WoW Mean body weight, kg	469.1	466.7	10.19	0.634	<0.001
WoW ADG, kg/d	0.99	1.00	0.039	0.808	0.004
Total DMI, g/kg $BW^{0.75}$/d	98.89	92.27	1.872	0.003	0.004
Total DMI, %BW/d	2.17	2.01	0.037	0.001	0.104
WoW Total DMI, g/kg $BW^{0.75}$/d	93.69	89.50	2.167	0.203	0.284
WoW Total DMI, %BW/d	2.02	1.93	0.035	0.161	0.895
Total DMI, kg/d	9.37	9.04	0.297	0.436	0.001
Hay DMI, kg/d	8.40	7.92	0.288	0.244	0.009
Lick block DMI, kg/d	0.00	0.44	0.015	<0.001	0.154
Lick block intake, kg FM/d	0.00	0.60	0.021	<0.001	0.142
Pellet DMI, kg/d	0.97	0.68	0.041	<0.001	<0.001
Feed: Gain, kg/kg	8.89	7.78	0.907	0.392	0.994
Gain: Feed, kg/kg	0.109	0.135	0.0053	<0.001	0.009
Metab Energy Intake/kg ADG	76.85	67.74	7.667	0.407	0.640
WoW Feed: Gain, kg/kg	10.55	9.76	0.720	0.465	0.013
WoW Gain: Feed, kg/kg	0.105	0.119	0.0040	0.053	<0.001
WoW Metab Energy Intake/kg ADG	91.18	85.87	5.774	0.518	0.001
Water consumption, L/d	55.18	87.38	2.919	<0.001	0.722
Diet DMD, % DM	62.06	62.23	0.051	0.019	<0.001
Diet ME conc, MJ/kg DM	8.72	8.75	0.020	0.302	<0.001
Total ME Intake, MJ/d	81.23	79.04	2.343	0.591	<0.001
Hay ME Intake, MJ/d	70.65	66.95	2.439	0.286	<0.001
Lick block ME Intake, MJ/d	0.00	4.89	0.169	<0.001	0.142
Pellet ME Intake, MJ/d	10.30	7.17	0.431	<0.001	<0.001
Diet CP, %DM	14.97	13.99	0.084	<0.001	0.548
Total CP Intake, kg/d	1.37	1.27	0.038	0.004	<0.001
Hay CP Intake, kg/d	1.15	1.08	0.037	0.239	0.033
Lick block CP Intake, kg/d	0.00	0.03	0.001	<0.001	0.142
Pellet CP Intake, kg/d	0.22	0.15	0.009	<0.001	<0.001
Total NDFI, kg/d	4.74	4.39	0.142	0.004	0.001
Hay NDFI, kg/d	4.72	4.39	0.153	0.124	0.062
Lick block NDFI, kg/d	0.00	0.02	0.001	<0.001	0.142
Pellet NDFI, kg/d	0.29	0.20	0.012	<0.001	<0.001
Total WSCI, kg/d	0.82	0.96	0.026	<0.001	<0.001
Hay WSCI, kg/d	0.77	0.72	0.029	0.262	<0.001
Lick block WSCI, kg/d	0.00	0.19	0.007	<0.001	0.142
Pellet WSCI, kg/d	0.08	0.06	0.004	<0.001	<0.001

Table 23 shows the behaviour of the steers, showing steers spent 13.68 min/d consuming the Lick Block with no significant difference between periods. This indicates that there was a consistent behaviour observed of cattle choosing to eat the Lick Block, even when forage was available ad libitum, and animals were growing throughout the trial. The animals consumed the lick block at an eating rate of 67.94 g DM/min (92 ± 23.7 g FM/min) but it is important to note that visual observation showed that the steers were able to chew entire pieces of the blocks separated from the block or the remaining of an entire block.

Animals in the control treatment spent shorter time drinking and attended the water trough less frequently compared to the MLB steers ($p < 0.001$; Table 23) in agreement with their estimated lower water intake. These results suggest greater need of water availability in the paddock when lick blocks are offered but it is unknown whether lesser water availability would reduce MLB intake or not.

The control steers spent similar daily time on hay feeding ($P > 0.05$) but ate it at a slower rate and less frequently compared to the MLB steers, who spent more time in the feeders containing the MLB ($P < 0.05$; Table 23). However, no significant differences were found between treatments in the visit length and size for hay.

The Smart ear tags showed that ear temperature was higher ($P < 0.05$) in the MLB than in the control steers which is similar to the trend observed in the previous trial where the lick block products was in the liquid form. Unexpectedly, the MLB steers spent more time ruminating compared to the control steers ($P < 0.05$) despite their lower hay intake but reasons for these findings are unknown because it would suggest more rumination time per kg of NDF and hay DMI. Finally, the MLB steers spent shorter time Not Active than the control steers ($P < 0.05$) likely because of longer time spent ruminating.

Table 23. Feeding and drinking behaviour of steers fed hay, pellets in the GreenFeed unit, and a Lick Block with anti-methanogenic ingredients.

	Treatment			P-value	
	Control	MLB	SEM	Treatment	Period
Trough scale					
Drinking time, sec/d	26.31	39.97	2.024	<0.001	<0.001
Drinking frequency, #/d	2.97	3.80	0.142	<0.001	<0.001
Smart ear tags					
Ear temperature, C	27.47	28.06	0.249	0.119	<0.001
Eating, min/d	212.33	225.75	12.989	0.088	<0.001
Ruminating, min/d	734.29	802.91	30.076	0.092	<0.001
High Active, min/d	317.76	329.33	11.473	0.487	0.969
Active, min/d	454.92	423.23	26.193	0.419	0.696
Not Active, min/d	982.10	947.24	21.184	0.008	0.002
Electronic feeders					
Total Feeding time, min/d	115.75	126.97	3.967	<0.001	<0.001
Hay Feeding time, min/d	112.94	112.84	3.987	0.974	<0.001
Lick blocks Feeding, min/d	0.00	13.68	0.505	<0.001	0.248
Hay Eating rate, d DM/min	87.05	77.58	5.639	0.001	<0.001
Lick blocks Eating rate, g DM/min	-	67.94	17.479	-	-
Hay Visit Length, min/visit	5.91	6.29	0.659	0.479	0.898
Lick block Visit Length, min/visit	0.00	3.15	0.084	<0.001	0.990
Hay Visit Size, kg DM/visit	0.42	0.42	0.045	0.930	0.001
LB Visit Size, kg DM/visit	0.00	0.10	0.003	<0.001	0.930
Hay visit frequency, #/d	30.11	26.65	2.277	0.299	0.437
LB visit frequency, #/d	0.00	4.04	0.155	<0.001	0.298

Results from the GreenFeed system are shown in Table 24. The MLB group had fewer visits to the GreenFeed but of longer visit duration compared to the control group ($P < 0.05$). This resulted in similar total daily time with good GreenFeed data (1178 vs 1144 sec/d; data not shown).

Gas fluxes revealed higher CH_4 and CO_2 concentration in the MLB compared to the control group ($P < 0.05$; Table 24). However, daily methane production (g/d) showed no significant difference between treatments whereas H_2 production was lower and O_2 consumption higher for the MLB compared to the control steers ($P < 0.05$). When evaluating efficiency measures, MLB steers had 6.4% lower CH4 intensity compared to control steers ($P = 0.026$) but the MLB group showed higher CH4 yield ($P < 0.05$; Table 24). These results are surprising and in contrast with all previous trials described in the present project (both in vitro and in vivo). One potential explanation for higher CH_4 yield in MLB animals is their lower feed intake leading to the feed staying for longer in the rumen exposed to ruminal degradation. Another potential explanation is diet selection because the MLB animals selected a diet with higher estimated DMD and WSC which are expected to yield higher CH_4 due their higher ruminal degradation by microbes. Finally, another potential explanation for the lack of repeatability of the results on CH_4 compared to earlier studies is that the active ingredients (essential oils) may have been 'deactivated' during the manufacturing process due to evaporation during cooking or fixation to other particles including the settling agent. These are two processes that did not occurred in the liquid form used in previous studies and highlight the importance of protecting these ingredients during the manufacturing process. However, further research needs to be done to understand the reasons for these findings such as analysing the active ingredients in the blocks or performing in vitro studies with the blocks. This may include in the future including 'positive controls' versions of the block that do not contain methane mitigants. The research team has stored samples of the MLB for further analysis, but this has not been budgeted in the present project. The concentration of active ingredients in the block has not been measured and thus it is difficult to draw firm conclusions.

Table 24. Gas exchange of steers fed a molasses lick block (MLB) with anti-methanogenic ingredients in trial 3 in pens.

	Treatment			P-value	
	Control	MLB	SEM	Treatment	Period
GF Visit frequency, #/d	3.86	3.55	0.099	0.035	<0.001
Visit Duration, sec/visit	305.1	322.4	4.58	<0.001	0.139
CH4 concentration, ppm	121.4	137.4	2.68	<0.001	0.315
CO2 concentration, ppm	1151	1343	25.9	<0.001	<0.001
CH4, g/d	277.6	279.4	5.74	0.790	0.865
CO2, g/d	7147	7431	149.7	0.196	0.039
H2, g/d	0.80	0.61	0.033	0.001	<0.001
O2, g/d	5026	5719	113.7	<0.001	0.837
CH4 Intensity, g/kg ADG	61.96	59.43	0.969	0.069	<0.001
CH4 Intensity, log(g/kg ADG)	4.12	4.08	0.016	0.100	<0.001
WoW CH4 Intensity, g/kg ADG	320.0	295.5	23.99	0.452	0.405
WoW CH4 Intensity, log(g/kg ADG)	5.69	5.66	0.026	0.721	0.039
CH4 Yield, g/kg DMI	30.06	32.42	0.731	0.024	<0.001
CH4 Yield, g/kg MEI	3.48	3.75	0.086	0.029	<0.001

This experiment showcased animals will consume a Lick Block supplement, even when provided with other food sources ad libitum. This consumption also occurs in tandem with an increase in water consumption and should be considered when designing delivery strategies on grazing conditions, especially in northern Australia where water sources are scarce. Animals fed this Lick Block had reduced intake and improved Gain:Feed ratio compared to the control animals which could lower the cost production from improved feed efficiency. However, this study did not replicate the reduction in CH_4 emissions from earlier studies, suggesting an alteration of the during product manufacturing is needed before further studies are conducted. The lick block supplements were received from the company in February 2024 and delivery to animals started in April 2024 until the end of the trial. The LB were stored for the entire duration of the trial in a shipping container at ambient temperature in their original packaging before being provided to the animals in the feeders. The lick block for the entire trial was delivered together upon hay delivery when needed (2-3 times per week) and as such the lick block in Period 2 had a longer time since development which may have contributed to a possible deterioration of the product.

4.7 In vivo trial 4. Effect of solid lick block supplementation on methane emissions from grazing beef cattle

In grazing trial 4, there was no difference in initial or final BW, or ADG between treatments either measured in the yards or using the WoW data ($P > 0.05$; Table 25). This is contrast to the pen study where animals offered the MLB grew faster using the yard weighing data for the calculation of ADG.

The total MLB intake for period 1 was 133.36 kg, which would result in an intake of 0.22 kg/head per day if that is divided by the 11 animals in the MLB treatment group across 56 days. The total MLB intake for period 2 was 208.76 kg, which would result in an intake of 0.18 kg/head per day if that is divided by the 14 animals in the MLB treatment group across 85 days.

Table 25. Body weight and performance from grazing beef cattle offered a molasses lick block (MLB) supplement free choice.

	Treatment			P-value	
	Control	MLB	SE	Treatment	Period
Initial BW, kg	374.7	373.7	11.97	0.955	0.250
Final BW, kg	413.9	414.4	13.58	0.977	<0.001
ADG, kg/d	0.40	0.42	0.045	0.697	<0.001
WoW average BW, kg	387.6	394.2	12.54	0.718	0.020
WoW ADG, kg/d	0.49	0.51	0.049	0.779	<0.001

Table 25 shows the behaviour of animals measured with the smart ear tags, attendance to the WoW system, and to the Optiweigh. There was no difference between treatments in any of the behaviour variables or ear temperature measured with the smart tags and steers spent approximately 12.2 ± 0.46 h/d grazing and eating hay, and 9.5 ± 0.64 hr/d ruminating. This suggests that feed intake may have been similar between treatments and that no detrimental effects of the MLB were observed.

Steers in the MLB treatment spent 5-fold greater time at the Optiweigh station compared to control steers ($P < 0.001$; Table 26). On average, 8% of the control animals were identified at the lick blocks whereas 45% of the MLB steers were identified by the Optiweigh throughout the trial ($P < 0.001$). This suggests that the auto-drafter was effective, but some animals found a way to access the lick blocks. The impact of these results on animal performance and emissions is unknown but likely to have had a minimal effect because significant differences in time at the Optiweigh was found with no differences on ADG.

Table 26. Behaviour, time at the Optiweigh delivering a molasses lick block (MLB), and use of a walk-over-weighing with auto-drafter (WoW) to separate treatment animals of grazing steers.

	Treatment			P-value	
	Control	MLB	SE	Treatment	Period
Eating time, min/d	721.3	747.9	27.50	0.502	0.000
Ruminating time, min/d	559.7	578.4	38.43	0.735	0.001
Ear Temperature, C	24.59	25.08	0.604	0.574	0.228
Not Active time, min/d	711.4	605.6	65.09	0.261	0.589
Active time, min/d	307.8	324.9	35.26	0.735	0.182
High Active time, min/d	230.5	258.8	13.45	0.147	0.032
Optiweigh time, sec/d	47.1	258.5	20.37	<0.001	1.000
Optiweigh attendance, % animals	8.12	44.87	2.25	<0.001	0.021
WoW attendance, #/an/d	1.43	1.52	0.117	0.585	0.003

Table 27 show the gas exchange and other variables measured by the GreenFeed unit. Attendance, and time and pellet intake from the GreenFeed unit was similar between treatments ($P < 0.05$). Similarly, there was no difference between treatments in any of the GHG emission variables measured in g/d or intensity ($P > 0.05$). These results agree with observations in the previous pen trial 3 except for the lack of difference in CH4 concentration and intensity in the current trial. This trial was done concurrently with the pen trial and using the same batch of MLB. It is important to note that this trial was performed in an open paddock with the GreenFeed situated without shelter or protection other than the panels on the side the GreenFeed. Previous research elsewhere has demonstrated that wind speed in grazing situations can affect the measurements and reduce the accuracy.

Table 27. Gas exchange of grazing steers fed a molasses lick block (MLB) with anti-methanogenic ingredients in trial 4.

	Treatment			P-value	
	Control	Lick blocks	SE	Treatment	Period
Total GF Visits, #/an	117.9	124.0	11.96	0.721	<0.001
GF Visits, #/d/an	1.61	1.65	0.156	0.875	0.001
Visit duration, sec/visit	283	284	7.1	0.901	0.043
Cup delivered, #/hd/d	12.67	12.71	1.160	0.979	0.002
Pellet DMI, kg/d	0.49	0.47	0.045	0.826	0.002
CH4 conc., ppm	123.9	128.6	2.57	0.217	0.800
CO2 conc., ppm	1080	1116	23.8	0.305	0.424
CH4, g/d	250.4	261.1	5.24	0.164	<0.001
CH4 Intensity, (g/kg ADG+1)	201.4	194.7	8.85	0.599	<0.001
CH4 Intensity, log(g/kg ADG+1)	5.26	5.24	0.040	0.829	<0.001
WoW CH4 Intensity, g/kg ADG	210.0	-29.8	330.89	0.616	0.409
WoW CH4 Intensity, log(g/kg ADG+1)	5.61	5.57	0.076	0.759	<0.001
CO2, g/d	5947	6168	131.5	0.248	<0.001
H2, g/d	0.35	0.36	0.016	0.479	<0.001
O2, g/d	4002	4146	94.0	0.291	<0.001

Table 28 shows a heatmap of the results from the nonparametric Spearman rank correlation analysis between variables averaged across the trial for each animal and period. A similar analysis using parametric Pearson correlation analysis and diving the analysis by periods yielded very similar results (data not shown). Daily CH4 emissions was positively correlated with both BW and ADG (P < 0.001) as expected because heavier and faster growing animals are expected to consume more feed and produce more methane. However, daily CH4 production was not associated with time spent at the Optiweigh where the lick blocks were delivered (P > 0.05). Daily CH4 production was strongly and positively correlated with both CH4 and CO2 concentration (P < 0.05).

The intensity of CH4 production (g/kg ADG) showed a strong negative correlation with ADG as expected (P < 0.05). Time at the Optiweigh where the MLB was delivered was not associated with production or intensity of CH4 emissions (P > 0.05). These results suggest that lick block intake was not correlated to CH4 emissions or ADG in the current study because time at the lick blocks has a very close association with lick block intake (Imaz et al., 2021a and b), even if some control animals gained access to the lick blocks and identified by the Optiweigh.

Table 28. Spearman rank correlation between performance and greenhouse emissions of grazing beef steers offered a molasses lick block using an auto-drafter to separate animals in different treatment yards with one yard containing an Optiweigh with the lick blocks. Correlation coefficients are above and P-values below the diagonal.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Initial BW		0.86	0.14	-0.15	0.08	0.04	-0.26	0.69	0.85	0.65	0.22	0.69	0.28
2. Final BW	***		0.59	0.00	0.26	0.40	-0.35	0.62	0.77	0.88	-0.24	0.90	0.60
3. ADG	0.340	***		0.23	0.32	0.71	-0.24	0.14	0.18	0.63	-0.88	0.66	0.69
4. Time Optiweigh	0.318	0.998	0.118		0.20	0.18	-0.07	0.07	-0.04	0.15	-0.22	0.08	0.19
5. Attendance WoW	0.604	0.075	0.029	0.153		0.71	-0.48	-0.04	0.06	0.38	-0.20	0.40	0.47
6. GF Visits	0.788	0.005	***	0.195	***		-0.13	0.00	0.06	0.49	-0.61	0.48	0.57
7. Visit duration	0.073	0.016	0.106	0.663	0.001	0.378		-0.08	-0.19	-0.37	0.10	-0.40	0.25
8. CH4 ppm	***	***	0.348	0.626	0.783	0.986	0.614		0.82	0.70	0.26	0.59	0.25
9. CO2 ppm	***	***	0.230	0.792	0.680	0.665	0.211	***		0.69	0.18	0.77	0.41
10. CH4, g/d	***	***	***	0.317	0.009	***	0.010	***	***		-0.25	0.93	0.70
11. Log CH4 Intensity	0.128	0.099	***	0.129	0.174	***	0.493	0.083	0.238	0.095		-0.32	0.48
12. CO2	***	***	***	0.611	0.006	0.001	0.005	***	***	***	0.031		0.76
13. H2	0.059	***	***	0.191	0.001	***	0.092	0.091	0.004	***	0.001	***	

*** $P < 0.001$

Results of the present grazing study allow us to conclude that the MLB used in the present trial does not affect enteric CH4 emissions or performance of grazing beef cattle in agreement with the pen trial. As previously discussed, it is likely that the manufacturing process or the storage of the lick blocks, or both, has eliminated the effectiveness of the active ingredients to reduce CH4 production in the rumen of grazing beef steers. The lick block for this trial was received in February 2024 and it was used in trial 3 and 4 until the trials were completed in September 2024, stored in the same manner as in vivo trial 3 inside shipping containers in their original packaging.

5. Conclusion

Through this project nutritional limitations for different regions and seasons in Australia have been identified and proposed strategies to deal with the varying conditions. In particular in Northern Australia, there are marked deficiencies in Crude Protein, energy, minerals and vitamins in the dry season, and P in the wet season but arguably all year around. Pasture quality fluctuates vastly with seasonal changes, and this affects the nutritional status of grazing cattle. In contrast, Southern Australia has different issues including Bloat that occurs on lush pastures and excess CP or inorganic N, and mineral imbalances particularly after recent rainfall. Implementing supplementation practices based on seasonal pasture quality can help to maintain the production and more importantly the health of cattle, especially during times of severe drought. A literature review on different modes of delivery of supplements for grazing producers was completed and identified the potential for specific solutions to different nutritional deficiencies.

Supplementation can occur via substitution and complementation; both techniques are valuable tools for different environments. Understanding how different types of supplementation effects cattle can guide the development of targeted supplementation strategies that will maximise nutrient utilisation and beef cattle productivity, whilst minimising wastage, and reducing the intensity of GHGE.

The primary mineral deficiencies outlined by this project included phosphorus, selenium, cobalt and copper due to low availability from the soil, as well as calcium (milk fever) and magnesium (grass tetany). Micronutrient deficiencies are widespread and often a result of soil pH. Seasonal shifts can also cause deficiencies in other minerals including occasionally sodium. Regions and graziers need targeted strategies of fulfilling these nutrient gaps.

A proposed solution was the supplementation of animals using a lick blocks. Typically lick blocks are of three main types, salt or mineral blocks, molasses or multi nutrient lick blocks and finally complete feed blocks. For further research, this project chose to utilise lick blocks, specifically a molasses lick block was chosen for further research due to its ease of storage, transportation, low cost, simple manufacturing, high palatability, industry adoption, and the ease of delivering a range of nutrients or additives in an easy method involving no special containers.

Molasses lick blocks are designed to deliver energy, protein and minerals while also allowing the incorporation of other minerals, urea, true protein sources, veterinary products, essential oils and vitamins.

5.1 Key findings

The present project has resulted in the successful completion of five trials including 2 in vitro trials and 3 in vivo trials. This project has also resulted in a thorough review of the differing Australian landscape and clearly identified a need for supplementation in some areas of Australia. In particular, the grazing animals are most in need of tailored resources for use in their conditions.

This project explored tailoring a molasses lick block for grazing cattle to increase animal performance whilst reducing GHG emissions intensity. The project aimed to lay the basis for producers to understand how changes they can make help to improve the productivity of their cattle but also potentially assist with entering carbon markets and other programs.

The in vitro experiments identified the trade-off needed when attempting to reduce methane production, as there can be negative impacts on the volatile fatty acids and dry matter digestibility if doses are too high. The project identified a mixture that consistently resulted in a reduction of CH4 across both low- and high-quality pastures. Limitations of this dose included potentially not being an economically viable option as cattle would have to have too high consumption, for a 300-kg animal this would be approximately 1.8 L per animal per day.

A following in vivo experiment was performed and identified a dose optimisation of 100 g/head/day because higher doses did not result in further improvements. This resulted in a reduction of 7.66% CH4 g/d along with reduction on CH4 intensity (g CH4/kg DMI) and yield (g CH4/kg DMI). This experiment was the first to observe an increase in water consumption with the supplement. This research also showcased a numerical increase in average daily gain of 100 g/d, highlighting the potential to be used as a methane reduction and a performance improvement.

The second in vivo study aimed to assess the effect of offering the lick block product in its liquid form and mixed with pure molasses to control intake. The product liquid product was consumed at 0.74 kg FM/hd/d. Daily methane (CH4) production was similar across treatments during the baseline and with a 50% product-pure molasses mix, but control animals produced significantly more CH4 at 60% and above in the molasses mix. This indicates that the active ingredients may effectively reduce CH4 at higher concentrations. Compared to the control group, CH4 production of LB steers decreased by approximately 10% ($P < 0.05$), as it was the case for CH4 yield and intensity of CH4 ($P < 0.05$). In addition, the product increased DMI, MEI, and BW by 4 kg/hd. This second trial confirmed that the LB product is effective in reducing CH4 emissions fed in its liquid form and free choice. The effect of methane mitigants added into the liquid LB on methane yield requires further confirmation as a 'molasses only' control was not implemented in the experimental design.

The third in vivo trial utilised the lick block supplement but in the form of a commercial Lick Block, with hardness regulated to result in high intake. This study involved a crossover design enabling for two research periods to be observed and recorded. The MLB consumption was 0.6 kg/head per day and there was no reduction in CH4 as the earlier studies. Animals did perform well on the lick block with no negative effects although it reduced hay DMI compared to control steers. This may have resulted in greater CH4 yield (g/MJ MEI) in animals fed the lick block but this was not translated to greater CH4 (g/d). It is suggested that the inconsistent results with earlier in vitro and in vivo trials using the liquid form of the lick block product is due to a loss of the activity of the ingredients used during the manufacturing process (evaporation, destruction, or inactivation). Of note the behaviour of the animals was able to be observed through electrical feeders and watering systems. These identified animals visited the lick block often throughout the day for short durations but also there was a 55 % increase in water consumption when in the treatment with the Lick Block. Water availability should be considered when using lick block products similar to those of the present study.

5.2 Benefits to industry

The project identified nutritional deficiencies across different Australian regions and seasons, allowing producers to implement targeted supplementation strategies. By addressing these deficiencies, particularly during dry periods, producers can enhance cattle health and overall productivity. Understanding the impact of seasonal pasture quality allows producers to adapt their supplementation practices, ensuring that cattle receive adequate nutrition year-round, which is especially crucial during periods of drought or after heavy rainfall. The project explored the potential of MLB to deliver a range of nutrients effectively, making it easier for producers to manage the nutritional needs of their cattle without complex logistics or special equipment.

MLBs are at a high readiness level for adoption within the red meat industry. Emphasising the cost-effectiveness and feasibility of MLB will be beneficial to wider adoption of lick blocks among livestock producers. MLBs represent a critical tool in livestock nutrition, providing an effective means to enhance animal health and productivity. Optimising their use through improved delivery and understanding of intake dynamics can further enhance their benefits in grazing systems.

Implementing MLB as a supplementation strategy will be a viable strategy but will need to be optimised for maximum returns. Providing a MLB near a water source is a preferred strategy due to the increased water consumption when consuming lick block if no toxicity risks are involved (e.g. high urea content). This project has shown animals will choose to eat a lick block even when other feed sources are available to allow for ad libitum consumption, indicating the preference for consumption. The current project has identified key intake behaviours related to MLB that should be delved into further to optimise adoption by industry and ensure cattle use the systems that are put in place.

Producers needing to supplement cattle will benefit from the information from this project by having a larger understanding of the common nutritional deficiencies in their location. They will also benefit from knowing MLBs are a viable solution to use with supplements added into its formulation, for delivery of those supplements. Methane inhibitors can be successfully added to the diet of cattle and will be consumed by cattle by free choice, but further research in the MLB form is needed. Overall, the findings from this project provide actionable insights that can lead to improved cattle health, increased profitability for producers, and a more sustainable red meat industry.

6. Future research and recommendations

Addressing mineral deficiencies in Australian grazing systems through tailored supplementation is critical for enhancing livestock health, reproductive performance, overall productivity, and reducing GHG emissions. Ongoing research and region-specific strategies will further improve outcomes for the grazing industry. MLBs represent an opportunity for the provision of crucial nutrients to offset the deficiency that may be observed across Australia. Understanding feeding behaviours can inform management practices to reduce competition and optimize feeding strategies, particularly in mixed feeding scenarios.

Incorporating lick-blocks into diets presents a viable strategy for reducing greenhouse gas emissions in beef production while enhancing growth performance, aligning with sustainability goals in livestock agriculture. However, further research is required to improve the stability of the biological action of ingredients and products during the manufacturing process. The reduction in methane emissions associated with MLB supplementation underscores its potential role in promoting more sustainable beef production practices. This project faced the limitation of providing cattle with a sufficient dose of anti-methanogenic product that would reduce methane but also still be palatable and a desired choice for consumption.

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