



Final report

Exploring methane inhibitors supplemented through water to increase beef industry sustainability

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Abstract

The use of additives that inhibit enteric methane emissions from ruminants in extensive grazing conditions is limited by the ability to stabilise additives when exposed to extreme climatic conditions, animal-to-animal variation in supplement intake and the discontinuous supply into the rumen. The current project evaluated the potential to deliver additives via the drinking water to solve this challenge.

A synthetic stabilised bromoform-based additive ranked highest for ability to reduce methane emissions during *in vitro* fermentations with a tropical grass. Replicated *in vivo* studies conducted with yearling steers demonstrated no productivity benefits from the inclusion of Agolin or Rumin8 IVP in the water. Over, 70- (Rumin8 IVP) and 56- (Agolin Ruminant L) day experimental periods, methane production (g CH₄/day) was reduced by 28% (Rumin8 IVP) and 5% (Agolin), with neither feed additive having significant effects on methane yield (g CH₄/kg DM.day) or methane intensity (g CH₄/kg DM.day) when delivered via the drinking water. The reduction in methane production in response to Agolin was time-sensitive, with significant reductions in methane production and yield of 15% measured only during weeks 7 and 8 (g CH₄/day) and week 8 (g CH₄/kg DM.day) of the experimental period. Application of water delivery of methane reducing additives to cattle under grazing conditions failed to reduce methane emissions due to higher-than-normal rainfall resulting in low and inconsistent water, and methane reducing additive, intake.

The delivery of methane reducing additives via the drinking water requires further research to optimise dose rates, test more additives and to validate this strategy under a range of different climatic and production conditions.

Executive summary

Background

The project investigated the potential to deliver methane reducing additives to cattle in extensive grazing systems via the drinking water. The project measured the effects of the water delivery strategy on feed and water intake, liveweight gain and enteric methane emissions from cattle fed a tropical grass hay in pens or grazing tropical forages under commercial conditions. The results of the project are relevant to producers with extensive ruminant grazing systems, companies that are developing methane reducing additives and industry bodies and policy makers.

Objectives

The objectives of this project were to,

- Determine which methane suppressing compounds can be safely and successfully delivered via drinking water to cattle,
- Determine the reduction in methane emissions achieved from the delivery of methane suppression compounds in the drinking water, and
- Demonstrate the delivery of methane suppression compounds via the drinking water to cattle in northern Australia at a commercial scale.

Methodology

The project used a combination of laboratory, pen and grazing studies to meet the objectives,

- Laboratory studies determined the solubility and stability in water of a range of methane reducing compounds,
- Pen studies determined the feed and water intake, liveweight gain and enteric methane emissions from cattle consuming methane reducing additives via the drinking water, and
- Grazing studies were conducted to identify any challenges associated with the application of the water delivery strategy under commercial grazing conditions.

Results/key findings

The results demonstrate that a number of methane reducing additives are soluble and stable in water and/or water-based nutrient supplements, and reduce methane emissions from cattle when included in the drinking water and when tested under specific conditions (i.e., young castrated *Bos indicus* cattle, low quality forages, low liveweight gain or liveweight loss, short duration studies, combination with N and P water-based nutrient supplements, single or two doses at most). Importantly, different additives will require different time periods to exert their effects in the rumen (e.g., effects of Agolin were only apparent after 7 weeks of consumption via the drinking water and at a specific dose rate) and dose rates need to be optimised for water delivery due to differences in kinetics of solids and liquids entering and retained in the rumen. Challenges exist in extending the strategy to grazing conditions where rainfall events can reduce intake of drinking water and, hence, the methane reducing additive.

Benefits to industry

The project demonstrated that potential exists to deliver methane reducing additives to cattle via the drinking water. These preliminary findings would be of interest to companies developing methane reducing additives and to producers in extensive grazing systems that are looking to incorporate methane reducing additives into their beef businesses. The combination of methane reducing

additives with water-based nutrient-supplementation is likely to generate a production response but the economics of this require testing in the absence of any existing approved methodology for gaining carbon credits from such a delivery strategy.

Future research and recommendations

Based on the results of the project, future research is recommended to broaden the context of which water delivery of methane reducing additives are tested including,

- Longer-term studies to determine the persistence of effects on methane emissions,
- A wider range of seasonal conditions (e.g., rainfall, temperature and humidity),
- A wider range of animal classes (other ruminant species, animals in different ages and physiological status), and
- Optimisation of dose rates of methane reducing additives tested in the current project and other emerging additives that may be suitable for water delivery.
- Research that considers total cost of intervention for producers, including the dosing technology and supplements.

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1. Background

Australia is projected to have approximately 30.1 and 73.2 million cattle and sheep, respectively, by the end of 2025 (Meat & Livestock Australia, 2025a,b) contributing an estimated 7% to 10% of Australia's total greenhouse gas (GHG) emissions, with the red meat industry accounting for 44% of total national methane emissions in 2021 (Mayberry, 2024). These emissions are mainly from the enteric production of methane and have a direct impact as a GHG but also potentially result in reduced productivity (through energy loss). Reductions in energy lost as methane would potentially be available for microbial synthesis in the rumen, resulting in higher liveweight (LW) gain (LWG), directly affecting profitability. The Australian red meat sector aims to support Australia's net zero ambitions primarily by reducing enteric methane emissions through a combination of interventions that directly target the rumen and strategies that increase productivity and indirectly reduce lifetime methane emissions.

A range of feed additives have been demonstrated to directly suppress methane emissions from ruminants (e.g., *Asparagopsis taxiformis* [Roque et al., 2021; Cowley et al., 2024], Rumin8 investigational veterinary product [IVP; Gyeltshen et al., 2025], 3-nitrooxypropanol [3-NOP; Martínez-Fernández et al., 2018], nitrate [Tomkins et al., 2018], Agolin [Belanche et al., 2020], and tannins [Yang et al., 2017]) while other strategies (e.g., improved feed-base, high N and energy supplements, culling of unproductive animals) have an indirect effect on methane intensity through increased weaning rates and increased LWG (Berndt and Tomkins, 2013) per adult equivalent or per hectare of land. Methane reducing additives that directly suppress methane are more suited to inclusion in the diet in the intensive ruminant industries (dairy, feedlots) where controlled and sustained delivery to the rumen of individual animals is possible. However, the red meat sector relies on large extensive breeding and pasture-based backgrounding production systems in which the controlled delivery of additives via the feed is a challenge due to remoteness, environment, infrastructure and animal behaviour. The inclusion of these additives in supplements of extensively grazed ruminants is problematic due to the stability of additives when exposed to the environment for extended periods and the sporadic and variable intake of supplements by animals in extensive grazing scenarios. Strategies that indirectly reduce methane intensity are not always possible or economically feasible to implement in all production systems (e.g., rainfall distribution, soil type, costs, infrastructure). As a result, adoption of many of these technologies and strategies may be a challenge in extensive ruminant (cattle, sheep, goat) grazing systems across Australia.

Ruminants in all production systems have access to drinking water, often via artificial reticulation systems ultimately resulting in the delivery of water to animals via water troughs. Water supplementation to address specific nutrient deficiencies (i.e., N, P) in extensive grazing systems is not new and application is increasing with improvements in the delivery technology. Therefore, controlled delivery of methane reducing additives to grazing ruminants via drinking water using new dosing technologies may present an innovative method to reduce methane emissions from ruminants in extensive conditions at a scale not possible via the feed (i.e., supplement) or other delivery technologies (e.g., slow-releases boluses). Recently, the inclusion of 3-NOP (Bovaer®) in the drinking water available to sheep reduced methane yield by 35 to 41% relative to control in short-term pen studies, and this reduction was higher than that achieved when the equivalent dose of 3-NOP was supplied in the feed (27%; Rivelli et al., 2025).

The current project evaluated the potential to safely deliver a range of potential methane reducing additives to ruminants via the drinking water and quantify the effect of this form of delivery on the efficacy of these additives to suppress methane emissions. The project determined solubility and

stability of potential methane reducing additives in water, and the effect of some of these on dry matter (DM) and water intake, LWG, rumen fermentation characteristics and efficacy to reduce methane emissions from cattle when delivered via the drinking water. The results provide red meat producers with information to support decision making around the suitability of water delivery of methane reducing additives for their own livestock production enterprises.

2. Objectives

The objectives of this project were to,

- 2.1 Determine which methane suppressing compounds can be safely and successfully delivered via drinking water to cattle,
- 2.2 Determine the reduction in methane emissions achieved from the delivery of methane suppression compounds in the drinking water, and
- 2.3 Demonstrate the delivery of methane suppression compounds via the drinking water to cattle in northern Australia at a commercial scale.

3. Methodology

The report is prepared to provide a brief overview of the methodologies used, the key results, the relevance of the research findings to industry, and future research recommendations. Access to more detailed information on the experiments is available from published scientific papers (with URL links provided; Table 6). The report only describes deliverables associated with experimental studies.

3.1 Phase 1

***In vitro* testing of solubility, stability, digestibility and gas production of commercially available additives**

A review of literature identified 34 compounds or additives with confirmed or potential capacity to reduce enteric methane emissions from ruminants. Based on known chemical properties, 15 of these additives were then identified on their likelihood to be soluble and stable in water, indicative of their potential for water delivery to ruminants and were selected for laboratory analysis (Table 1).

Table 1. Additives selected to investigate their efficacy to reduce methane emissions when combined with water during *in vitro* fermentations.

Additive	Source	Comment
Bromoform (analytical grade)	Sigma-Aldrich Bayswater, VIC, AUS	Included as positive control
Rumin8 Investigational Veterinary Product	Rumin8 Pty Ltd, West Perth, WA, AUS	Synthetic, stabilised bromoform Two formulations investigated
Agolin (Ruminant L)	Alltech Technology, Nicholasville, KY, USA	Blend of essential oils Commercially available
Saponin	Sigma-Aldrich Bayswater, VIC, AUS	Derived from <i>Quillaja saponaria</i>
Polygain	TPM Keysborough, VIC, AUS	Polyphenols, flavonoids, organic acids derived from a sugarcane extract Commercially available
SilvaFeed	SilvaTeam, San Michele, MD, Italy	Quebracho tannins, saponins and polyphenols derived from <i>Schinopsis lorentzii / balansae</i> Commercially available
Beeocitrix	Beeotec; Santa Rita do Sapucaí, Brazil	Source of ricinoleic acid, polyprpolis Commercially available
Monensin, Sodium salt	Sigma-Aldrich	Commercially available as Rumensin

	Bayswater, VIC, AUS	
Tartaric acid	Sigma-Aldrich Bayswater, VIC, AUS	
Choline chloride	Sigma-Aldrich Bayswater, VIC, AUS	
Nitrates (magnesium, calcium, sodium, potassium; analytical grade)	Sigma-Aldrich Bayswater, VIC, AUS	
uPRO-Orange	DIT AgTech Toowoomba, QLD, AUS	Water soluble nutrient (N, P) supplement to which methane reducing additives could be combined Commercially available with existing adoption across northern Australia beef herds

Briefly, Fourier transform infrared (FTIR) spectroscopy was used to confirm the solubility and stability of these additives in water and/or a commercially available nutrient water-delivery supplement (uPRO-Orange) under temperature (4°C, 25°C and 45°C) and pH (5.5, 7.0, 8.5) extremes that may be expected under extensive grazing conditions. Effects of these additives on total gas and methane production and on dry matter (DM) and organic matter (OM) digestibility were determined when incubated with a low-quality forage (*Chloris gayana*; 52 g crude protein [CP]/kg DM) *in vitro*. Gas production was monitored in real-time (Ankom RF units; John Morris, Murarrie, QLD, Australia) and the methane concentration of the produced gas was determined using gas chromatography (6890 GC system; Agilent Technologies, California, USA).

Additional testing of efficacy of various additives (e.g., Agolin, Choline) on *in vitro* digestibility of dry matter (DMD), total gas production and methane concentration within gas produced during fermentations were conducted at varying doses alone and in combination with uPRO-Orange. For example, refer to Batley et al. (2024c; Table 6) for a detailed description of the Agolin *in vitro* experiment.

A detailed description of the materials and methods used is available in the publications of Batley et al. (2024a, 2024b; Table 6).

3.2 Phase 2

***In vivo* evaluation of the efficacy of a bromoform-based additive to reduce methane emissions from growing *Bos indicus* steers when delivered via the drinking water with and without a nitrogen-phosphorus supplement (Phase 2a)**

Rumin8 Investigational Veterinary Product (IVP) was identified in Phase 1 as the additive with the highest likelihood to reduce methane emissions from cattle when delivered via the drinking water. A 2 x 2 factorial experiment incorporating the basal solution (water or water + nitrogen-phosphorus supplement; uPRO-Orange) as the first factor and additive (no additive Rumin8 IVP additive or no additive) as the second factor. *Bos indicus* cross-bred steers (n = 28; 293 ± 24.5 kg LW [mean ± standard deviation]) were ranked and blocked on LW and randomly allocated to one of four treatments balanced for replicate (i.e., individual steer) and to adjacent individual pens (n = 4) within pen blocks (n = 7). The treatments were, 1. water-without Rumin8 IVP (Control; W), 2. water-with Rumin8 IVP (W+R8), 3. Water with uPRO-without Rumin8 IVP (W+uPRO), and 4. Water with uPRO-with Rumin8 IVP (W+uPRO+R8). Permeation tubes releasing sulphur hexafluoride (SF₆) gas were placed in the rumen of steers one week prior to the first gas collection. Steers had *ad libitum* access to a moderate-quality Rhodes hay (63 g CP/kg DM) and water-based treatments, delivered via a direct water injection technology (uDOSE; DIT AgTech) within their individual pens throughout the experiment.

The experiment involved a 28-day adaptation phase to the individual pens, uPRO delivery and the experimental protocols, followed by a 70-day measurement period. Hay intake was measured daily, LW was measured every 7-days, faecal output was measured over 7 consecutive days in the middle of the experiment, and breath samples were collected from steers over six consecutive days for methane and SF₆ analysis via cannisters under negative pressure attached to steers with harnesses. Rumen fluid and blood samples were collected after completion of the 70-day measurement period.

A detailed description of the materials and methods used is available (Batley et al. [unpublished]; Table 6).

***In vivo* evaluation of the efficacy of Agolin to reduce methane emissions from growing *Bos indicus* steers when delivered via the drinking water with a nitrogen-phosphorus supplement (Phase 2b)**

An additional pen experiment was incorporated into the project to determine the effects of a commercially available additive on enteric methane emissions from cattle when delivered via the drinking water. Despite its low efficacy in reducing the total gas production and the methane content of gas produced after *in vitro* fermentation in Phase 1, Agolin was selected for inclusion as the additive to be tested in this experiment. This decision was based on its commercial availability including its existing commercially available combination with existing nutrient supplements (uPRO-Blue; DIT Ag-tech), so any results would be immediately available for adoption by industry; particularly those producers that were already adopting nutrient supplementation via water delivery. It was also noted that for some additives tested in Phase 1 of the project, including Agolin, that *in vitro* testing may not be indicative of *in vivo* responses given the longer-term required for the modulation of the rumen microbiome mode of action to take effect.

Bos indicus cross-bred steers ($n = 24$; 264 ± 22.9 kg LW [mean \pm standard deviation]) were ranked and blocked on LW and randomly allocated to one of three treatments within each LW block. The treatments were, 1. uPRO-Orange added to water (uPRO), 2. uPRO-Orange with a low dose of Agolin (6 μ L/L) added to water (uPRO-LA), and 3. uPRO-Orange with a high dose of Agolin (24 μ L/L) added to water (uPRO-HA).

The experiment involved a 28-day period to adapt steers to the auto-feeders (Smartfeed Pro, C-Lock, USA) followed by a 56-day measurement period. Steers had *ad libitum* access to a low-quality Rhodes grass hay (51 g CP/kg DM) and water-based treatments throughout the experimental period, with all data available from the C-Lock portal. Water treatments were delivered by direct water injection technology. Feed and water were available *ad libitum* from the auto-feeders which controlled individual steer access to allocated feeders via radio-frequency identification (RFID) ear-tags which also allowed for the measurement of intake of hay and water supplement (uPRO-Orange) by individual steers (i.e., the steer remained the replicate, despite retained within a group setting) with the steers maintained as a single mob throughout the experiment.

Hay and water-based treatment intake were measured daily, LW was measured every 7-days and methane emissions were measured by providing steers with free-access to a single greenfeed emissions measurement (GEM) unit for the duration of the experimental period. Five visitations were allowed per steer per day, with a minimum of 4-hours between visits, and a commercial pellet was used as an attractant to the GEM unit with 4 pellet drops of 35 g/drop for each visitation (140 h/visitation). Rumen fluid and blood samples were collected after completion of the 56-day measurement period.

A detailed description of the materials and methods used is available in the publication of Batley et al. 2024c; Table 6).

3.3 Phase 3

***In vivo* evaluation of the efficacy of Agolin to reduce methane emissions from growing *Bos indicus* steers when delivered via the drinking water with a nitrogen-phosphorus supplement under field conditions**

The experiment was conducted at Belmont Research Station in central Queensland, Australia from March to May 2024. Yearling bulls ($n = 44$; 379.0 ± 6.3 kg LW, mean \pm standard deviation) were allocated to receive either control (nitrogen and phosphorus; uPRO-Orange) or treatment (uPRO-Orange plus Agolin) supplements delivered via drinking water troughs located in separate but adjacent water yards in the experimental paddocks (total area of 14 hectares). The bulls grazed Rhodes grass pastures as a single mob and were drafted to their allocated water treatments via an auto-drafter located at entry to two adjacent water yards. Water intake of bulls was estimated from flow rate meters attached to each trough divided by the number of water yard visits/day by the bulls allocated to respective treatment troughs. The LWG and methane emissions were measured from bulls that were successfully drafted into the correct treatment paddock more than 70% of visits and successfully accessed the Greenfeed unit deployed to measure methane emissions. Only a small number of bulls met these criteria for inclusion in the statistical analysis.

A detailed description of the materials and methods used is available in the publication in preparation of Castro et al.[unpublished]; Table 6).

3.4 Phase 4

Demonstration of technologies under commercial conditions

Wilburra Downs, Station Richmond, QLD was selected as the demonstration and evaluation site with the work conducted between 06-February and 06-May-2024 (90-days). Angus, Brahman, Charolais and Senepol ($n = 30$ /breed) steers ($n = 120$ in total) grazed a single 886-hectare paddock containing Mitchell grass (*Astrebla* spp.), Flinders grass (*Iseilema* spp.), buffel grass (*Cenchrus ciliaris*), pigweed (*Portulaca oleracea*), and prickly acacia (*Vachellia nilotica*) for a total of 90-days.

The steers were ranked and blocked on LW within breed and randomly allocated to one of three experimental treatments. The treatments were, 1. Water alone (Control), 2. Water with uPRO-Green (a wet season N and P supplement; DIT AgTech [Green]) and 3. Water with uPRO-Green with Agolin (Blue). Treatments were delivered to three independent water troughs with dedicated direct water-injection technology that dosed the three experimental treatment additives (water only for Control) into the same source of water at a rate of 50 mL/ 20 L of water. Water troughs were confined to three separate, but adjacent water yards and steers accessed their allocated treatments based on a RFID reader attached to a three-way auto-drafter.

A walk-over-weighing system recorded change in steer LW over time, RFID readers recorded visitation to water yards, flow rate meters on water troughs monitored group level water intakes and CERES Ranch (CERES Tags, Brisbane, QLD, Australia) ear tags (deployed 8 steers/treatment) monitored grazing behaviour of steers over a period of 27-days. Blood samples were collected from 12 steers selected at random prior to the commencement of the experiment. No additional blood, rumen or faecal samples were collected, and measurements of enteric methane emissions were not conducted at any stage during the experiment.

4. Results and discussion

4.1 Phase 1

In vitro testing of solubility, stability, digestibility and gas production of commercially available additives

Additives tested were ranked on their effects on DM digestibility (DMD), total gas production and proportion of methane within gas production relative to control when combined with water with individual assay rankings aggregated to generate a final ranking list of the additives tested. Across all *in vitro* assays, the analytical grade bromoform and the second Rumin8 IVP were the highest ranked additives (Table 2). A selection of these additives (choline chloride, tartaric acid, sodium nitrate, Agolin, Silvafeed and Rumin8 IVP) were then incubated as above in the presence of uPRO-Orange rather than water alone, with Rumin8 IVP again the highest ranked additive.

Table 2. Individual and cumulative (overall) ranking¹ of additives for effects on *in vitro* dry matter digestibility (IVDMD), total gas production (total gas) and methane (CH₄) as a proportion of total gas produced to control fermentations of a medium quality hay without additives.

Additive	IVDMD	Total gas production	CH ₄ : total gas production	Overall
Bromoform	11	14	16	41
Rumin8 IVP ² (2)	10	13	15	38
Tartaric acid	16	8	9	33
Beeocitrix	15	6	10	31
Potassium nitrate	12	12	7	31
Monensin	8	10	11	29
Rumin8 IVP (1)	3	11	14	28
Sodium nitrate	7	9	8	24
Agolin	13	3	6	22
uPRO-Orange	14	5	3	22
Silvafeed	9	7	5	21
Saponin	6	2	12	20
Choline chloride	5	1	13	19
Magnesium nitrate	1	16	1	19
Calcium nitrate	2	15	2	18
Polygain	4	4	4	12

¹Higher ranking equates to highest IVDMD, lowest total gas production and lowest methane content as a proportion of total gas production; ²Rumin8 Investigational Veterinary Product (IVP) is a synthetic stabilised bromoform-based additive with two formulations tested.

The additives analysed in the *in vitro* assays were evaluated for solubility and stability under simulated water trough conditions with analysis based on stability of absorbance peaks using FTIR. Beeocitrix and tartaric acid could not be measured at the target dose rates recommended to reduce methane emissions. All other additives were observed to remain stable across the simulated water trough conditions (temperature and pH ranges). However, it is likely that changes in concentration of additive occurred through evaporative losses of solvent and solute under these conditions. Changes in absorbance values across treatment and time indicated that Agolin, Polygain and saponin maintained a consistent concentration across all treatments, choline chloride may have precipitated slightly, SilvaFeed may have become more soluble at higher temperatures while Rumin8 IVP was highly variable.

The inclusion of most additives (with the exception of Beeocitrix) with water (pH 7) made the final concentration of the solution more acidic, and this was exacerbated by the addition of uPRO-Orange

and the additives to acidic water (pH 5.5), with many solutions less than pH 3. It is unknown what effect the acidity of these solutions may have on palatability of drinking water and water intake of ruminants, and this warrants further investigation.

Additional *in vitro* experiments determined the effect of increasing doses of Agolin and choline chloride on DMD, gas production and the proportion of methane within the gas produced. The DMD of low-quality tropical grass hay was unaffected by the inclusion of Agolin in the *in vitro* fermentations (Table 3). Total gas and methane production were variable but largely unresponsive to increasing doses of Agolin combined with uPRO-Orange when included in Rhodes grass fermentations. However, methane production, as a proportion of total gas production, was reduced at increased rates of Agolin when combined with uPRO-Orange (i.e., 4.5% and 6.0%). Importantly, the *in vitro* DMD, total gas production and proportion of methane in gas produced were unaffected by the inclusion of Agolin with water alone in the absence of the nutrient supplement.

Table 3. The effect of Agolin alone and in combination with a water based nutrient supplement (uPRO-Orange) on *in vitro* dry matter (DM) digestibility (IVDMD), total gas production and the proportion of methane (CH₄) within the gas^{1,2}.

Treatment	IVDMD, %	Total gas production, mL/g DM	CH ₄ production, mL/g DM	CH ₄ : total gas production ⁴
Control ³	42.7 ± 1.5	59.9 ± 2.4 ^b	6.4 ± 0.4 ^{ab}	10.8 ± 0.3 ^{ab}
Agolin	41.9 ± 0.8	60.4 ± 1.1 ^b	6.3 ± 0.7 ^{ab}	10.5 ± 0.1 ^{ab}
uPRO-Orange + 0% Agolin	42.0 ± 0.5	55.8 ± 1.7 ^b	6.3 ± 0.2 ^{ab}	11.3 ± 0.03 ^a
uPRO-Orange + 3.0% Agolin	40.7 ± 0.9	67.7 ± 1.7 ^{ab}	7.6 ± 0.3 ^a	11.2 ± 0.1 ^a
uPRO-Orange + 4.5% Agolin	43.5 ± 1.8	75.0 ± 0.3 ^a	7.3 ± 0.02 ^a	9.8 ± 0.05 ^b
uPRO-Orange + 6.0% Agolin	42.8 ± 1.6	66.8 ± 6.0 ^{ab}	5.4 ± 0.6 ^b	8.1 ± 0.3 ^c
<i>P</i> -value	0.74	0.006	0.003	< 0.001

¹Reproduced from Batley et al. (2024c); ²Values are least-square means ± standard error of the mean with different superscripts within a column indicating a significant difference between means at $P < 0.05$; ³Control is fermentation of Rhodes grass hay alone without uPRO-Orange supplement or Agolin; ⁴Methane (CH₄) as a proportion of total gas produced.

The addition of choline chloride to water increased total gas production, the concentration and proportion of methane in the gas produced *in vitro* compared to fermentations without choline chloride included. Total gas production and the concentration of methane in the gas increased in response to increasing choline chloride concentration (i.e., 100, 200 and 400 mM) but the proportion of methane in the total gas produced was similar at the three concentrations of choline chloride tested. These results were consistent when incubated with both low- (Rhodes grass) and high- (lucerne) quality forage substrates.

The project developed a method for screening additives for their solubility and stability in water and other solvents (i.e., water containing concentrated nutrients as a nutrient supplement for ruminants). While the methodology allowed for the ranking of additives, it is noted that the short-term (48-h) *in vitro* fermentations used may not be appropriate to investigate some additives (e.g., Agolin) that take a longer time (weeks not hours) to modify the rumen microbial population for effects on methane emissions to be evident.

Rumin8 IVP, a synthetic stabilised bromoform based additive, was identified as the additive with the highest potential to reduce methane emissions from the additives tested when included in the drinking water. At the time of experimentation, 3-NOP was not included in the assays as no water soluble and stable forms were available for testing. However, recent research (Rivelli et al., 2025) demonstrated that 3-NOP delivered in drinking water was able to reduce enteric methane emissions

from sheep in pens over a short duration, which would suggest a soluble and stabilised form of this additive is now available and this should be investigated for water delivery in the future.

4.2 Phase 2

***In vivo* evaluation of the efficacy of a bromoform-based additive to reduce methane emissions from growing *Bos indicus* steers when delivered via the drinking water with and without a nitrogen-phosphorus supplement (Phase 2a)**

The intake of urea (7.9 and 10 g urea/steer.day, W+uPRO and W+uPRO+R8, respectively) and the bromoform via Rumin8 IVP (204 and 231 mg bromoform/steer.day, W+R8 and W+uPRO+R8, respectively) when supplied in the drinking water were below the pre-experiment targets (19.5 g urea/steer.day and 300 mg bromoform/steer.day). The pH of drinking water was lower in drinking water that contained uPRO-Orange with (3.7; W+uPRO+R) or without (3.8; W+uPRO) Rumin8 IVP compared to water alone (7.6; W) or water with Rumin8 IVP (7.5; W+R). The DMD of the diet was unaffected by treatment ($55 \pm 2\%$).

Intake of a low-quality tropical grass hay was unaffected by the inclusion of uPRO-Orange and/or Rumin8 IVP in the drinking water (

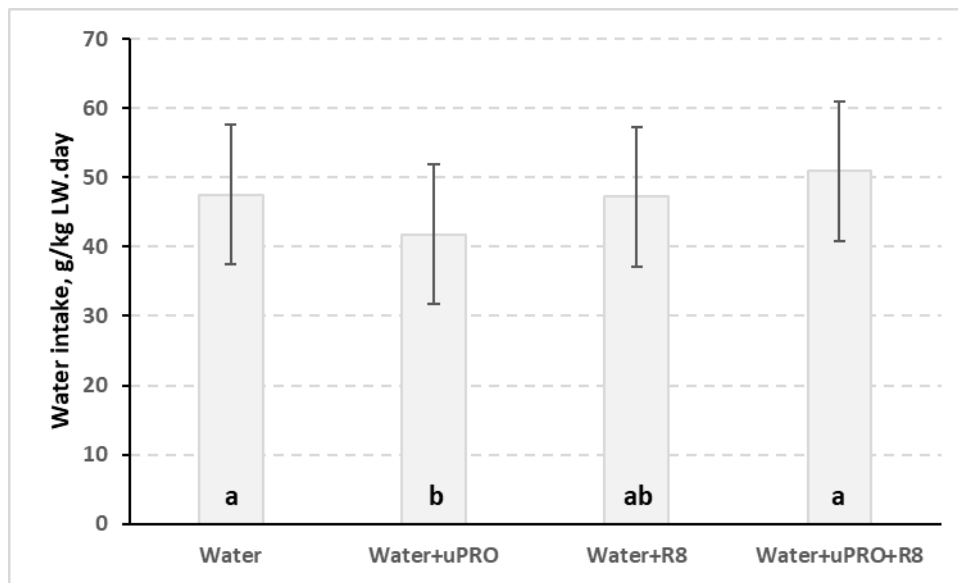
Table 4). Water intake was higher for steers consuming drinking water containing Rumin8 IVP compared to steers consuming drinking water without Rumin8 IVP, with a significant interaction between the inclusion of uPRO-Orange and Rumin8 IVP on water intake observed (Figure 1). In the absence of the uPRO-Orange in the drinking water, steers lost LW whilst the inclusion of uPRO-Orange alleviated this LW loss and resulted in a modest LWG, as would be expected for a urea-based solid-form supplement in the dry season.

Methane production (g CH₄/day) was lower for steers consuming drinking water containing Rumin8 IVP compared to steers consuming drinking water without Rumin8 IVP. While the inclusion of uPRO-Orange in the drinking water had no effect on methane production, a significant interaction between the inclusion of Rumin8 IVP and uPRO-Orange in the drinking water was observed (Figure 1). Methane yield was unaffected by treatment, largely due to the lack of treatment effect on intake of the low-quality tropical grass hay used in the experiment. The concentration of VFA and the molar proportion of individual VFA in the rumen fluid were unaffected by treatment, as were the concentration of ammonia-N (21.9 mg/L) and the pH (7.0) of rumen fluid. The concentration of urea-N (1.6 mmol/L) and inorganic-P (2.07 mmol/L) in plasma was also unaffected by treatment, as were all other analytes measured.

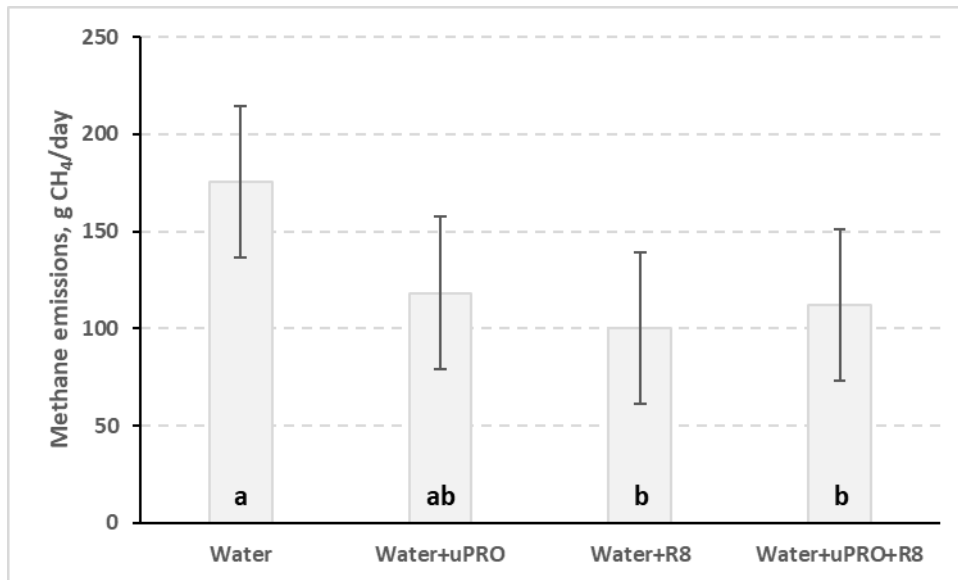
Table 4. Feed dry matter (DM) and water (imbibed) intake, liveweight (LW) gain (LWG) and methane (CH₄) emissions estimates for *Bos indicus* steers fed a low-quality Rhodes grass hay with or without a nutrient supplement (uPRO) and with or without a methane reducing additive (Rumin8 Investigational Product) included in the drinking water.

Parameter	Nutrient supplement ²		Additive ³		Pooled SEM ⁴	P-value		
	None	uPRO	None	Rumin8		Supp	Additive	Interaction ⁵
Feed intake, g DM/kg day	4.0	4.4	4.2	4.2	0.63	0.09	0.92	0.23
Feed intake, g DM/kg LW.day	13.6	14.6	14.2	13.9	1.86	0.08	0.76	0.59
Water intake, L/day ⁶	13.9	13.8	13.2	14.5	3.03	0.87	0.004	< 0.01
Water intake, g/kg LW.day ⁶	47.4	46.4	44.7	49.0	1.01	0.55	0.005	0.002
LWG, kg/day	-0.06	0.05	-0.008	-0.003	0.73	0.02	0.92	0.7
CH ₄ production, g/day	137	115	147	106	39.14	0.37	0.01	0.02
CH ₄ yield, g/kg DM intake ⁷	34.4	29.7	36.1	28.0	11.12	0.42	0.08	0.25
CH ₄ intensity, g/kg LWG ⁸	N/D	23,000	N/D	N/D

¹Values are least-square means; ²First factor is the nutrient supplement (Supp) of either water alone (None) or water with uPRO-Orange (uPRO; concentrated nitrogen-phosphorus supplement; DIT Ag-Tech); ³Second factor is the exclusion (None) or inclusion (Rumin8 Investigational Veterinary Product [IVP]) of a methane reducing additive in the drinking water; ⁴standard error of the mean; ⁵Treatment means of interaction terms are presented elsewhere if $P < 0.05$; ⁶water imbibed from drinking troughs; ⁷calculated using feed intake during the methane measurement period only; ⁸methane intensity could not be determined (N/D) for steers with negative LWG and was unrealistic for steers with low LWG.



A.



B.

Figure 1. The effect of the interaction between a nutrient supplement (uPRO; uPRO-Orange) and a methane reducing additive (R8; Rumin8 Investigational Veterinary Product) in the drinking water on water intake (A.) and methane emissions (B.) of steers consuming a low-quality tropical grass hay. Lower-case letters on columns within charts indicate significant differences between treatment means ($P < 0.05$).

***In vivo* evaluation of the efficacy of Agolin to reduce methane emissions from growing *Bos indicus* steers when delivered via the drinking water with a nitrogen-phosphorus supplement (Phase 2b)**

Feed (6.4 kg/day) and water with uPRO-Orange (N, P) supplement (17.5 kg/day) intake, and LWG (0.55 kg/day) of steers were unaffected by the inclusion of Agolin in the water with nutrient supplement (Batley et al. 2024c). Rumen fermentation characteristics and plasma biochemistry parameters were all unaffected by the inclusion of Agolin in the water with uPRO-Orange (N, P) supplement and were within the expected range for this class of cattle fed this hay. Over the entire experimental period, the inclusion of Agolin in the water with nutrient supplement had no effect on methane yield (22.6 g

CH₄/kg DM intake) or methane intensity (262 g CH₄/kg LWG). Over the entire experimental period, the inclusion Agolin at 6 μ L/L in the water with nutrient supplement resulted in a significant (5%) reduction in methane emissions (131 g CH₄/day) compared to steers consuming the water with nutrient supplement without Agolin (138 g CH₄/day) or with Agolin included at 24 μ L/L (143 g CH₄/day). However, this finding was driven by the significantly lower (~15%) methane emissions from steers consuming Agolin at the lower concentration compared to steers consuming no Agolin or Agolin at the higher concentration in weeks 7 and 8 of the experiment only (Figure 2). Similarly, methane yield was significantly lower (~15%) from steers consuming Agolin included in the water with nutrient supplement at the lower concentration compared to steers consuming no Agolin or Agolin at the higher concentration, in week 8 of delivery only.

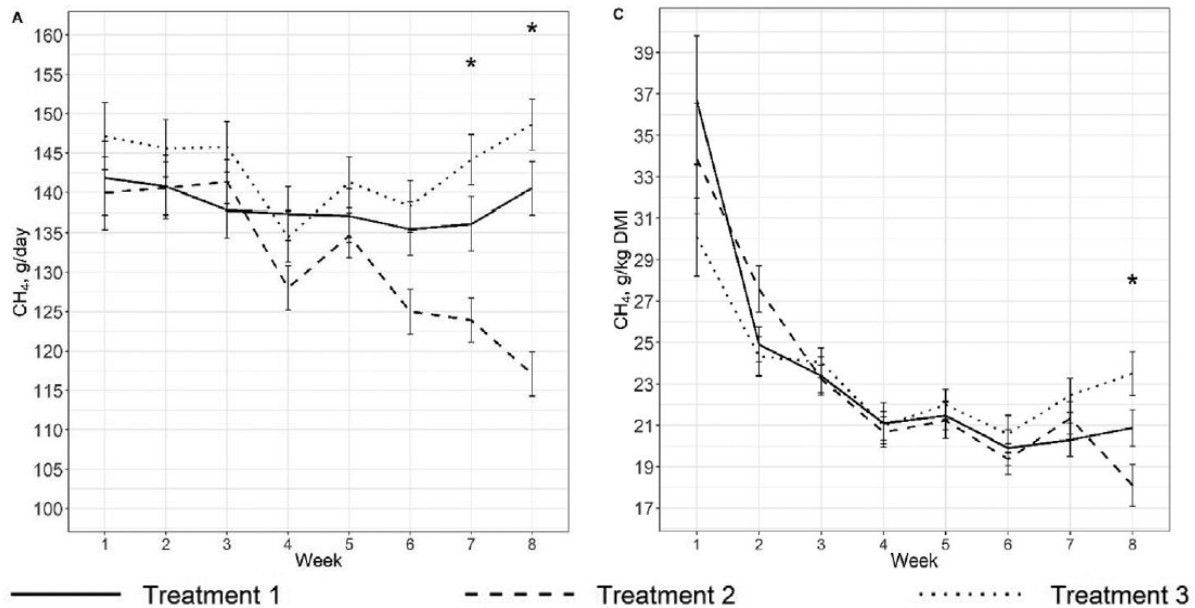


Figure 2. Methane emissions (A) and methane yield (C) of steers consuming water containing uPRO-Orange N, P supplement) alone or with low (6 μ L/L) or high (24 μ L/L) of Agolin. *indicates a significant difference between treatments at each week of the experiment.

The results of these two experiments demonstrate that both Rumin8 IVP (a bromoform-based additive) and Agolin can reduce methane emissions (g CH₄/day) at specific times and under specific conditions when included in the drinking water of growing steers. However, no effects on methane yield or intensity were measured in response to Agolin in the water and could not be measured in response to Rumin8 IVP given the cattle lost LW in this experiment when fed low-quality tropical grass hay. Across the entire experimental period, the 5% reduction in methane production in response to the lower inclusion rate of Agolin in the water with nutrient supplement was less than that described in the meta-analysis of methane emissions from dairy cows reported by Belanche et al. (2020). This may be attributed to the 4-week period required for modulation of the rumen microbial population, a lower efficacy from water delivery (potentially due to bypass of water from the rumen) or interactions with the nutrient supplement. The lack of an effect of the higher concentration of Agolin in the drinking water on methane emissions was unexpected and can not be explained without further research. The 28% reduction in methane emissions for steers consuming Rumin8 IVP (whether alone or in combination with a nutrient supplement) in the drinking water is less than that reported for feed-based consumption of Rumin8 IVP (Gyeltshen et al., 2025) or for *Asparagopsis* (Cowley et al., 2024), which contains a natural form of the same active ingredient (i.e., bromoform). One potential reason for the lower response to Rumin8 IVP delivered via the drinking water in this experiment may include

the lower than targeted dose of bromoform entering the rumen and the likely immediate bypass of water (Café and Poppi 1994) and, hence, bromoform from the rumen which together may have resulted in approximately 50% of the target bromoform dose entering and remaining in the rumen relative to that achieved through provision via the feed. The lack of an effect of additive on methane yield and methane intensity may potentially be attributed to low quality of the basal diets used (tropical grass hay) which resulted in low DM intake and low LWG measured. The tropical grass hay was selected to represent feed typical of the protracted dry season across most of northern Australia. In the absence of a large step-change in nutrient content and availability required to induce biologically important changes in metabolisable energy (and crude protein) intake and LWG, it is difficult to see significant changes in methane yield or intensity from ruminants consuming low quality forages representative of the dry season in northern Australia.

Importantly, both experiments fed growing steers a low-quality roughage hay as the main feed source, with single sources of drinking water in a small, covered area resulting in lower water requirements than animals that might be grazing under extensive grazing conditions. This may have resulted in a lower but more consistent water intake and, hence, methane reducing additive intake than under grazing conditions where alternative water sources (e.g., surface water after rain, pasture water content) may influence intake from water from troughs. It is noted that to date most studies, including the studies described in the current report, investigating the effect of inclusion of methane reducing additives in the drinking water have used dose rates similar to those used for feed delivery (on either a DM intake, water intake or LW basis). Different efficacy may be expected between feed and water delivery due to a portion of water imbibed (19% to 25% for forage and concentrate respectively; Café, Poppi, 1994) immediately bypassing the rumen, differences in rumen kinetics for liquid and feeds within the rumen, and requirements to adjust the inclusion rate of an additive in the water in response to changing water intake due to changing seasonal conditions, changing animal LW and/or physiological status (lactation v dry cows) and changing water requirements.

4.3 Phase 3

***In vivo* evaluation of the efficacy of Agolin to reduce methane emissions from growing *Bos indicus* steers when delivered via the drinking water with a nitrogen-phosphorus supplement under field conditions**

Higher than normal rainfall (~280 mm) during the experiment with a large number of rain days resulted in low visitations of bulls to the water troughs to consume water. It was assumed that the bulls met a significant portion of their water requirements from surface water and pasture water, resulting in low and inconsistent intake of water, and hence additive, from the water troughs. Consumption from the water trough was significantly lower for bulls consuming the water-based supplement (uPRO-Orange) containing Agolin compared to those consuming water with uPRO-Orange without Agolin (12.5 v 14.7 L/bull.day). The inclusion of Agolin in the water-based supplement had no significant effect on LWG (0.31 ± 0.03 kg/day), methane production (108 ± 3.91 g CH₄/day) or methane intensity ($340.9 \text{ g} \pm 26.1$ CH₄/kg LWG) of bulls. Given the lower-than-expected water intake and heavier animals than in experiment 2b, the actual intake of Agolin was approximately half of the target Agolin intake than that achieved in experiment 2b. These findings demonstrate the challenges and, likely, limitations of water delivery of methane reducing additives during wet seasons or in association with any high rainfall events. The low and inconsistent intake of additives via the water is particularly problematic for additives that require a continuous supply to the rumen to be effective (3-NOP, bromoforms), or those additives that take a long-time to modulate their effect within the rumen

(Agolin). Further research is required to establish the effect of the discontinuous supply of additives into the rumen via the drinking water on enteric methane emissions from ruminants.

4.4 Phase 4

The baseline blood sample analysis indicated no nutrient deficiencies in the unsupplemented steers at the commencement of the experiment. There were no differences in LWG or grazing behaviour of steers in response to the provision of the nutrient supplement (1.32 kg/day) or the nutrient supplement with Agolin via the drinking water (1.28 kg/day) compared to unsupplemented steers (1.35 kg/day). Given that there were no expected nutrient deficiencies in the paddock or cattle during the wet season, as indicated by the baseline blood biochemistry analysis, the lack of a response to the water delivered nutrient supplement was not unexpected. Steers consuming water containing the Agolin combined with the nutrient supplement had much lower mob level intake than steers consuming water alone or water with the nutrient supplement alone. Similar to the observations in Phase 3, alternative water sources (surface water, pasture water content) may have provided more palatable water sources for this group. Interventions that reduce water intake in extensive rangelands environments where temperatures can be extreme, shade can be limited, and few (alternative) water points exist (in the dry season) raise significant concern for animal welfare. Water test results indicated the inclusion of the nutrient supplement in the drinking water had a major influence on pH, electrical conductivity, total dissolved solids and salinity of drinking water, as well as the concentration of the supplemented nutrients, although there was little change in the response to the inclusion of Agolin (Green v Blue) (Table 5). As such, further research is recommended on the effect of the inclusion of any additive to the drinking water, on water intake of animals.

Table 5. Description of water (Control) and water with nutrient (P, N) supplement with (Blue) and without (Green) Agolin during the case study implemented under an extensive grazing system at Wilburra Downs Station.

Parameter	Control	Green	Blue
pH	7.06	3.07	3.05
Electrical conductivity, μ S	454	1097	1109
Total dissolved solids, ppm	324	781	789
Salinity, ppt	0.23	0.55	0.56
Nitrogen, mg/L	1.5	171	213
Phosphorus, mg/L	0.3	177	209

No conclusions can be drawn on the potential of the inclusion of Agolin with a nutrient supplement delivered via the drinking water on methane emission from this study as neither methane emissions nor rumen fermentation characteristics were measured.

4.5 Publications

A minimum of four scientific publications derived from project generated data and/or activities were to be drafted from this project. Three scientific manuscripts have now been published with two additional publications in preparation (Table 6).

Table 6. Manuscripts published and in preparation using project derived data and/or describing project activities.

Manuscript No.	Phase (Milestone) of project	Publication	Status
1	Phase 1 (Milestone 3)	Batley RJ, Romanzini EP, Johnson JB, de Souza WL, Naiker M, Trotter MG, Quigley SP, de Souza Congio GF, Costa DFA. 2024a. Rapid screening of methane-reducing compounds for deployment in livestock drinking water using <i>in vitro</i> and FTIR-ATR analyses. <i>Methane</i> 3 , 533-560. https://doi.org/10.3390/methane3040030	Published
2	Phase 1 (Milestone 3)	Batley RJ, Romanzini EP, Johnson JB, de Souza WL, Naiker M, Trotter MG, Quigley SP, de Souza Congio GF, Costa DFA. 2024b. Rapid screening of methane-reducing compounds for deployment via water with a commercial livestock supplement using <i>in vitro</i> and FTIR-ATR analyses. <i>Methane</i> 3 , 437-455. https://doi.org/10.3390/methane3030025	Published
3	Phase 2 (Milestone 4)	Batley RJ, Romanzini EP, da Silva KD, de Souza WL, Quigley SP, Harper KJ, Trotter MG, Bernardes PA, Naiker M, Costa DFA. 2024c. The essential oil blend Agolin Ruminant L reduces methane production <i>in vitro</i> and <i>in vivo</i> when included in the drinking water of cattle. <i>Journal of Animal Science</i> 102 . doi: 10.1093/jas/skae315	Published
4	Phase 2 (Milestone 4)	Batley RJ, Romanzini EP, Bajagai YS, Bernardes PA, Trotter MG, Williams TM, Naiker M, Jacques S, Alvarez-Hess PS, Gianvecchio SB, Mercadante MEZ, Pereira LGR, Quigley SP, DFA Costa. Responses of growing <i>Bos indicus</i> crossbred steers to a bromoform methane inhibitor and non-protein nitrogen commercial livestock supplement delivered via drinking water alongside a moderate-quality Rhodes grass basal diet	Draft (preliminary) Target journal; <i>Animal Feed Science and Technology</i> (Appendix 9.1)
5	Phase 3 (Milestone 3)	Castro MAO, Romanzini EP, Bernardes PA, Williams TM, Batley RJ, De Souza WL, Bates AL, Ferreira EM, Reis RA, Quigley SP, Trotter MG, Costa DFA. Methane emissions, water intake and liveweight gain of young entire male cattle consuming a blend of essential oils via the drinking water under grazing conditions	Draft (advanced) Target journal; <i>Animal Feed Science and Technology</i> (Appendix 9.1)
6	Phases 1 and 2	Batley RJ. Unpublished PhD Thesis. <i>Exploring methane inhibitors supplemented through water to increase beef industry sustainability</i> . CQUniversity, QLD, Australia.	In preparation for submission Dec-2025. The thesis will include Batley first author publications in addition to unpublished data.

4.6 Engagement and extension activities

Mainstream media, field days and conferences were used to disseminate project progress and outcomes (Table 7).

Table 7. Dissemination activities undertaken within the project.

Dissemination method	Date	Title	Source
Online article	Oct-2024	Study shows promise using methane reducing feed additives through water	https://beefcentral.com/carbon/study-shows-promise-in-using-methane-reducing-feed-additives-through-water
Online article	May-2024	Research provides methane reducing solution for extensive grazing systems	https://www.cqu.edu.au/news/1143094/research-provides-methane-reducing-solution-for-extensive-grazing-systems
Online article	Oct-2023	Grazing systems research provides methane reducing solution	https://www.cqu.edu.au/research/impact/grazing-systems-research-provides-methane-reducing-solution
Online article	Nov-2022	First methane-inhibiting compound for delivery through water medication	https://www.beefcentral.com/production/first-methane-inhibiting-compound-for-delivery-through-water-medication/
Online article	May-2024	Advance Queensland alumni steer the way at Beef 2024	https://advance.qld.gov.au/innovation-in-queensland/innovation-stories/advance-queensland-alumni-steer-way-beef2024
Online article	Nov-2021	Research to focus on delivery of methane-inhibiting supplements to paddock cattle	https://www.beefcentral.com/production/research-to-focus-on-delivery-of-methane-inhibiting-supplements-to-paddock-cattle/
Online article	May-2024	Methane-reducing compounds in water supply reduces emissions by 15pc	https://www.queenslandcountrylife.com.au/story/8632072/methane-reducing-compounds-in-water-reduces-cattle-emissions/
Online article	Oct-2024	Is water delivery the answer to reducing methane emissions in Australian Livestock?	https://ditagtech.com.au/is-water-delivery-the-answer-to-reducing-methane-emissions-in-australian-livestock/
Online article	Aug-2023	CQU researchers working to reduce methane emissions through water soluble supplementation	https://www.queenslandcountrylife.com.au/story/8318124/backpack-wearing-cattle-how-methane-is-being-measured-in-beef/
Online article	Dec-2022	Ground breaking trial to reduce methane emitted from cows	https://www.couriermail.com.au/subscribe/news/1/?sourceCode=CMWEB_WRE170_a_GGL&dest=https%3A%2F%2Fwww.couriermail.com.au%2Fnews%2Fqueensland%2Frockhampton%2Fcquuniversity-rockhampton-to-host-world-first-methane-reduction-trial%2Fnews-story%2F6d597cba6b311dfa4004472df5aac244&memtype=anonymous&mode=premium&v21=GROUPA-Segment-2-NOSCORE
Radio	May-2024	N/A	ABC Radio
Radio	May-2022	N/A	ABC Radio
Television	June-2022	N/A	Chanel 7 News – Capricornia
Site tour (CQIRP)	May-2024	CQIRP Researchers quick research overview – Water methane mitigation (60 attendees)	Beef Australia, 2024
Site tour (CQIRP)	May-2024	DIT AgTech tours, 100 producers attended	Beef Australia, 2024
Field day (Richmond)	Jun-2024	20 producers engaged	Richmond races, 2024
Field day (Belmont)	Aug-2023	Methane mitigation research, 120 producers	Belmont Research Station Field Day
Site visits (Belmont)	May-2024	DIT AgTech tours, 100 producers attended	Beef Australia, 2024

Workshop	Oct-2024	Exploring methane inhibitors administered through water to increase beef industry sustainability	Australian Association of Ruminant Nutrition (AARN) conference Batley, oral presentation
Workshop	Apr-2025	Exploring methane inhibitors administered through water to increase beef industry sustainability	The 1st Northern Australia Universities Alliance Postgraduate Research Conference Batley, oral presentation
Workshop	Dec-2022	P.PSH.1378 Exploring methane inhibitors administered through water to increase beef industry sustainability	MLA organised, CN30 EAP research update
Workshop	May-2023	P.PSH.1378 Exploring methane inhibitors administered through water to increase beef industry sustainability	MLA organised, CN30 EAP research update
Workshop	Nov-2023	P.PSH.1378 Exploring methane inhibitors administered through water to increase beef industry sustainability	MLA organised, CN30 EAP research update
Workshop	Sep-2023	Are there methane mitigation strategies for extensive grazing systems?	AgForce webinar
Workshop	Aug-2022	Translating practical research into farm productivity	Longreach GroWQ Innovation Expo
Workshop	Aug-2023	Delivery of methane inhibitors via the water supply	Longreach GroWQ Innovation Expo
Workshop	Oct-2022	Water medication technology and potential role in methane mitigation	Clermont BeefUp Forum MLA
Project Advisory Group meeting	Sep-2022	P.PSH.1378 Exploring methane inhibitors administered through water to increase beef industry sustainability	Zoom meeting
Project Advisory Group meeting	May-2023	P.PSH.1378 Exploring methane inhibitors administered through water to increase beef industry sustainability	Zoom meeting
Project Advisory Group meeting	Dec-2023	P.PSH.1378 Exploring methane inhibitors administered through water to increase beef industry sustainability	Zoom meeting

5. Conclusions

Water delivery of additives that potentially reduce methane emissions remains a potential option to address issues of inconsistent intake of additives when provided in supplements and other carriers under extensive grazing systems. Whilst methane reductions were measured when additives were delivered in (drinking) water *in vitro* and in specific situations *in vivo* under more controlled experimental conditions, significant challenges were faced when deployment was extended to grazing situations when seasonal conditions resulted in low and inconsistent intake of both water available in troughs and, hence, the additive itself. Further research is required to determine optimum dose rates of a wider range of additives supplied in the drinking water to what were tested in the current project, and across a wider range of animal classes (e.g., age, physiological state, production targets), climatic conditions (e.g., rainfall events, temperature extremes), production systems (e.g., distance between water points, frequency of drinking events) and in the absence of nutrient-supplements (i.e., water-carrier alone). In addition, technologies to better estimate individual animal water and, hence, additive intake under grazing conditions may assist producers who wish to use these technologies for carbon accounting schemes in the future.

5.1 Key findings

The key findings from this project include,

1. A range of additives with purported methane reducing properties were found to be soluble and stable in water with beneficial impacts on digestibility, total gas production and the proportion of methane within produced gases when fermented with a low-quality tropical grass substrate *in vitro*. A synthetic and stabilised bromoform-based additive (Rumin8 IVP) had the greatest efficacy on reducing methane production within these *in vitro* experiments.
2. Agolin and Rumin8 IVP both reduced methane emissions from young steers fed a low-quality tropical grass hay when delivered via the drinking water (with or without a nutrient-supplement) but had no effect on production, or methane yield or intensity. The results are very specific to the experimental context and, in the case of Agolin, also demonstrated time-specific sensitivity.
3. Challenges were encountered in extending the water delivery strategy to commercial conditions where rainfall events resulted in low intake of water and, hence, the additive, due to a larger portion of water requirements met from surface and pasture water which reduced visitations to artificial water sources.

5.2 Benefits to industry

While the approach to deliver methane reducing additives via the drinking water has potential to reduce methane emissions from the large number of ruminants grazing in extensive production systems, further research is required. There were no significant productivity benefits observed from the inclusion of methane reducing additives in the drinking water under the conditions tested within the current project. However, a productivity benefit was observed in response to the inclusion of a dry season nutrient supplement in the drinking water of cattle fed a low crude protein content hay representative of dry season pastures in northern Australia.

The likely early adopters of such technology are those producers that already have direct water injection technology installed to provide nutrient supplements. As such, these producers will likely derive a production benefit from by combining methane reducing additives with the nutrient supplements delivered via the water, but any production benefit is attributed to the nutrient supplement rather than the methane reducing additive. This productivity gain may offset some of the additional costs associated with the methane reducing additive. However, with the lack of an approved feed or water additive methodology and restrictions to the herd productivity methodology in Australia, it may not be possible for the approach to be profitable at this time. If methodologies are approved that allow producers to claim carbon credit units from both methane reductions and

increased productivity from the combination of methane reducing additives and nutrient supplements, then such strategies may become financially viable for producers.

Regardless, additional research is required to establish the efficacy of the approach at optimised additive inclusion rates, with different additives, different classes and species of animals, under different additive carriers and across seasonal conditions where rainfall events can significantly impact water intake and water consumption patterns.

6. Future research and recommendations

Whilst the project demonstrated that methane reducing additives can reduce methane emissions from growing cattle when delivered via the drinking water combined with a nutrient supplement it is difficult to extrapolate these results generated under very specific conditions to the diverse potential applications of the technology. Therefore, additional research is required to generate the information required by producers to make informed decision on the adoption of such technologies within specific production systems and environments.

1. Both *in vitro* and *in vivo* experiments demonstrated a significant reduction in water pH when additives were added to water, either alone or in combination with nutrient (uPRO-Orange, uPRO-Blue) supplements. Given the importance of the provision of palatable drinking water to ruminants, particularly in extensive conditions, research is required to *determine the effects of additives and/or nutrient supplements on water palatability and water intake*.
2. The majority of experiments conducted within the current project used methane reducing additives in the drinking water when combined with a commercially available nutrient supplement (uPRO-Orange, Green or Blue) with the justification that early adopters of any water delivered additive are likely to be those producers that are already providing nutrient supplements via the drinking water. Nevertheless, future adoption may also be of interest to producers that do not require or are reluctant to provide nutrient supplements via the drinking water or operate in areas where nutrient deficiencies can not be addressed through water supplementation. As such, research is required to *determine the efficacy of additives when delivered in drinking water alone*.
3. The project focussed on the *in vivo* testing of Agolin, with some preliminary testing of Rumin8 IVP, included in the drinking water at a limited number of inclusion rates. Additional research is required to *determine the optimum level of inclusion of both the tested (e.g., Agolin) and emerging (e.g., 3-NOP) additives through full dose response studies* incorporating at least five levels of inclusion (doses) and three to five animal replicates per inclusion rate. Future research in pen studies should be conducted for a minimum of 70-days after the animals have been adapted to the additive under investigation and it has started to exert its effect on methane emissions, regardless of the mode of action. The 70-day minimum measurement period is for productivity measures (liveweight gain, feed and water intake) and to allow longer term measurements of the persistence of effects on methane production.
4. Both experiments implemented under commercial conditions were conducted in the wet season and on farms that were not deficient in phosphorus (a major nutrient deficiency in the wet season across much of northern Australia). As such, the experiments were compromised by rainfall events that resulted in low and inconsistent water and, hence additive, intake and no productivity response would be expected to a P supplement (regardless of form of delivery). Given the unlikely suitability of water delivery of additives during the wet season, future research *should focus on the inclusion of additives in the drinking water during the dry season* when more consistent water intake from troughs might be expected. This research should *include consideration of methane yield and methane intensity under dry season conditions* when pasture quality declines resulted in lower DM intake and lower LWG.
5. It is unknown what the effect of variable water and, hence, additive intake via the drinking water in response to rainfall events may have on the persistence of the efficacy of the different additives on methane emissions. Research is required to *determine the persistence of the*

effect of additives on methane emissions when they are removed and reintroduced to the rumen over different durations.

6. The experiments conducted within the current experiment solely used growing male steers or bulls. Future research *should incorporate other ruminant species (sheep, goats) that have different water requirements and different water drinking behaviour to cattle.* Similarly, research *should determine responses in cattle of different physiological states (pre-weaned calves, lactating v dry cows) where water requirements may be different.*
7. There is little available information on rumen water kinetics. Research should *determine the bypass, mixing, utilisation and retention of additives within the rumen when delivered in drinking water.*
8. If the delivery of additives to pre-ruminant animals is proven to result in persistent reductions in methane emissions later in life (e.g., 3-NOP, Meale et al., 2021) then research on *the use of water delivery of additives to pre-ruminant animals from a young age may be warranted* where it is assumed young ruminants may commence consuming water from troughs from approximately 2 weeks of age.
9. If low and inconsistent drinking water and, hence additive, intake is a limitation to the effectiveness of the delivery of additives via the drinking water future research could *examine the potential use of combinations of additives with different modes of functionality and persistence of effects on methane emissions.* Combinations of mitigants that elicit a more immediate response through inhibition of methanogenesis with mitigants that take a longer period to elicit a response through modulation of the rumen environment may maintain methane emissions in the event of inconsistent water intake.
10. Any future research (some of which may be described above) could include examination of changes in the rumen microbiome in response to the delivery of additives via the drinking water.
11. A standard method needs to be developed to express methane intensity from animals undergoing LW loss. This may not require additional research and may just require consultation with experts in the field. Most work to date focusses on animals in intensive, high productivity systems where LW loss is not an issue. This contrasts with extensive production systems, especially in the seasonal dry tropic tropics of northern Australia, where low productivity (e.g., LW loss) can occur during the dry season.
12. The absorption, distribution, metabolism and excretion of methane reducing additives when delivered via the drinking water may be different to when delivered via the feed. As such, it is recommended that future investigations on the delivery of existing and emerging additives in the drinking water include measurements and/or sample collection that will facilitate regulatory approvals for use in this manner.

7. References

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9. Appendix

9.1 Scientific manuscripts in preparation using project derived data

Publication anticipated mid-2026.