

Article

Water-Based Supplementation Technology for Grazing Cattle in the Tropics: A Large-Scale Commercial Case Study

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Featured Application: This study demonstrates the potential of water-based nutrient injection technology to optimize supplementation strategies in extensive grazing systems.

Abstract: Water-based nutrient injection technology, widely used in sectors like viticulture, hydroponics, and intensive animal systems, has previously seen limited application in livestock production. Early mechanical dispensers for nutrients, such as non-protein nitrogen (NPN) and phosphorus (P), were prone to malfunction, leading to inconsistent dosing and potential livestock health risks. This contributed to skepticism and slow adoption among producers. However, recent technological advancements have renewed interest in water-based supplementation for grazing animals. This case study assessed the use of water injection technology to deliver nutrients and a methane-reducing compound to cattle on a commercial cattle station under extensive grazing conditions. A total of 120 steers [initial liveweight (LW) 322.5 ± 28.3 kg] were assigned to three groups: water only (Control), a water supplement containing nutrients such as nitrogen and phosphorus, known as uPRO GREEN[®] (Green), and uPRO GREEN[®] combined with Agolin Ruminant L[®] (Blue). The experiment lasted 90 days, during which LW was continuously monitored via a walk-over weighing system, and water disappearance was measured at the mob level. Methane emissions were forecasted using dry matter intake estimates based on observed animal growth rates. Additionally, 24 steers were equipped with on-animal sensors with GPS to monitor behavioral changes. The results indicate that despite the potential reduction in water intake (Control and Green: 948.1 and 973.5 L/d, respectively, versus 547.5 L/d for Blue), there were no negative effects on growth (mean average daily gain of 1.32 kg/d) or animal behaviors. The predicted methane emission of 209.04 g CH₄/head/day could potentially be reduced by 10–15% with the compound used in the current trial. These findings suggest that water-based supplementation can be used to optimize nutrient delivery and a methane-reducing compound without compromising cattle productivity in extensive grazing environments. In addition, the potential enteric methane mitigation presents an opportunity for livestock producers to generate additional



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revenue through carbon credits or to create new markets for beef with low greenhouse gas emissions when cattle consume methane-reducing compounds.

Keywords: animal nutrition; greenhouse gas; methane enteric; tropical C4 grass; water medication

1. Introduction

The global cattle population has been estimated at over 1.5 billion heads [1], with the largest concentrations found in India, Brazil, the United States of America (USA), China, and Ethiopia. Together, these five countries represent more than 40% of the total population, each with distinct production systems. Brazil, India, and Ethiopia primarily rely on extensive grazing systems, while the USA and China incorporate more intensive feedlot operations. Australia, ranked as the 14th largest cattle producer with approximately 24.3 million heads [1], represents a hybrid approach, with about half of the beef production coming from grain-fed systems [2]. However, in all of these regions, breeding herds (primarily the females) are typically maintained on grazing lands, where implementing the delivery of methane-reducing feed additives poses unique challenges due to remoteness and animal behavior [3].

The early adoption of water supplementation was hindered by cattle deaths from urea toxicity as pioneers initially used crude methods like urea dispensers or water injection with no safety mechanisms to stop delivery in case of equipment malfunction. Modern technology has resolved these challenges, resulting in commercially available systems with enhanced safety and precision [4]. Using cutting-edge water injection technology, Batley et al. [5] recently demonstrated that it is possible to deliver a methane-reducing compound to cattle fed tropical hay. In the latter study, the authors evaluated the efficacy of a rumen modifier, Agolin Ruminant L[®], as part of a technological approach for methane mitigation. In an in vitro study, the group also investigated other methane-reducing compounds that could potentially be integrated into this delivery method [6]. Amongst the various products, some are classified as rumen modifiers, which alter the rumen microbiota composition, and others are methane inhibitors, which disrupt metabolic pathways involved in enteric methane production [7].

The latter works [4–7] demonstrate significant potential to mitigate methane emissions from beef cattle in extensive grazing systems by integrating existing technologies. The primary challenge lies in making this approach both operational and reliable, ensuring the continuous and controlled delivery of methane-reducing compounds. In response to this challenge and the opportunity for emission reduction, water supplementation has emerged as an effective method to administer feed additives through drinking water while preserving natural animal behaviors [4].

To assess water injection as a new approach to deliver methane-reducing compounds via the livestock water supply, this large-scale case study was conducted under commercial conditions at a cattle station in northern Australia, where extensive grazing practices are standard. This study aimed to evaluate the impacts of this technological package on cattle grazing under extensive conditions. Measurements included animal performance, behavior (monitored via on-animal sensors), and water disappearance (reflecting the animals' water consumption). In addition, enteric methane mitigation was predicted based on assumptions regarding dry matter intake and the use of a water-soluble and stable additive previously tested in a more controlled environment. Estimating enteric methane emissions from ruminant animals has become increasingly important as policymakers rely on these predictions to develop regulations and strategies for mitigating greenhouse

gas emissions. The hypothesis tested whether water supplementation, with or without a methane-reducing compound, impacts beef cattle production outcomes in a commercial cattle station in northern Australia.

2. Materials and Methods

The experiment was carried out at Wilburra Downs, a commercial station located in Richmond, QLD, Australia (coordinates: 143°14' E, 20°47' S; elevation: 220 m). A total of 120 yearling steers [322.5 ± 28.3 kg liveweight (LW)] from four genetic groups (i.e., Angus, Brahman, Charolais, and Senepol) were assigned based on initial LW to one of three treatments [Control (water with no supplements), Green (water with non-protein nitrogen and phosphorus supplements, uPRO GREEN® (DIT AgTech, Wilsonton, QLD, Australia)), and Blue (water with uPRO GREEN® and a methane-reducing compound)]. The steers grazed on 886 hectares of mixed pastures typical of the Central Queensland region, consisting of Mitchell grass (*Astrebla lappacea*), Flinders grass (*Iseilema* spp.), buffel grass (*Cenchrus ciliaris*), pigweed (*Portulaca oleracea*), and prickly acacia (*Vachellia nilotica*) for a total of 90 days. The paddock had a central fenced area containing three water troughs, each designated for one of the treatments.

The treatments (Table 1) were applied by delivering the supplements using injection technology (uDOSE™, DIT AgTech, Wilsonton, QLD, Australia) with three dosing units to measure water flow and inject supplements and an additive according to the treatment. The supplements were prepared at the beginning of the experiment, and the pods were refilled once halfway through the trial. The uDOSE™ system allows adjustments on delivery rates in accordance with the animal’s requirements. However, in the current trial, the delivery rate for supplemented treatments was fixed at 50 mL/20 L.

Table 1. Treatment mix and chemical composition of each water supplement evaluated during this case study.

Variable	Treatment		
Supplement Ingredient	Control	Green	Blue
Water (%)	100	50.00	49.80
uPRO GREEN® ¹ (%)	-	50.00	50.00
Agolin Ruminant L® ² (%)	-	0.00	0.20
Chemical composition (mg/L)			
Nitrogen	-	86,600	86,600
Phosphorus	-	85,500	85,500
Sulfur	-	12,000	12,000
Agolin Ruminant L®	-	-	5.00

¹ uPRO GREEN® is a DIT AgTech supplement composed of 17.32% nitrogen, 17.10% phosphorus, and 2.40% sulfur. ² Agolin Ruminant L® (Feedworks, Brisbane, QLD, Australia) composed of plant extracts, including coriander (*Coriandrum sativum*) seed oil (up to 10%), eugenol (up to 7%), geranyl acetate (up to 7%), and geraniol (up to 6%), and preservatives.

The same water source was used across all treatments. The water quality was evaluated at the beginning of the experiment (February 2024) and was found to have a pH of 8.95, EC (electrical conductivity) of 374 µS, TDS (total dissolved solids) of 266 ppm, salinity of 0.19 ppt, nitrogen concentration of 1.5 mg/L, and phosphorus concentration of 0.27 mg/L.

Water disappearance from troughs (consumption, evaporation losses, and rainfall) was evaluated at the mob level. A walk-over weighing (WoW, Tru-Test Remote WoW; Tru-Test® by Datamars Australia Pty Ltd., Banyo, QLD, Australia) system and an auto-drafter were used to identify and weigh animals, with the draft guiding them to specific water troughs within the square fenced area according to the treatment (Figure 1).

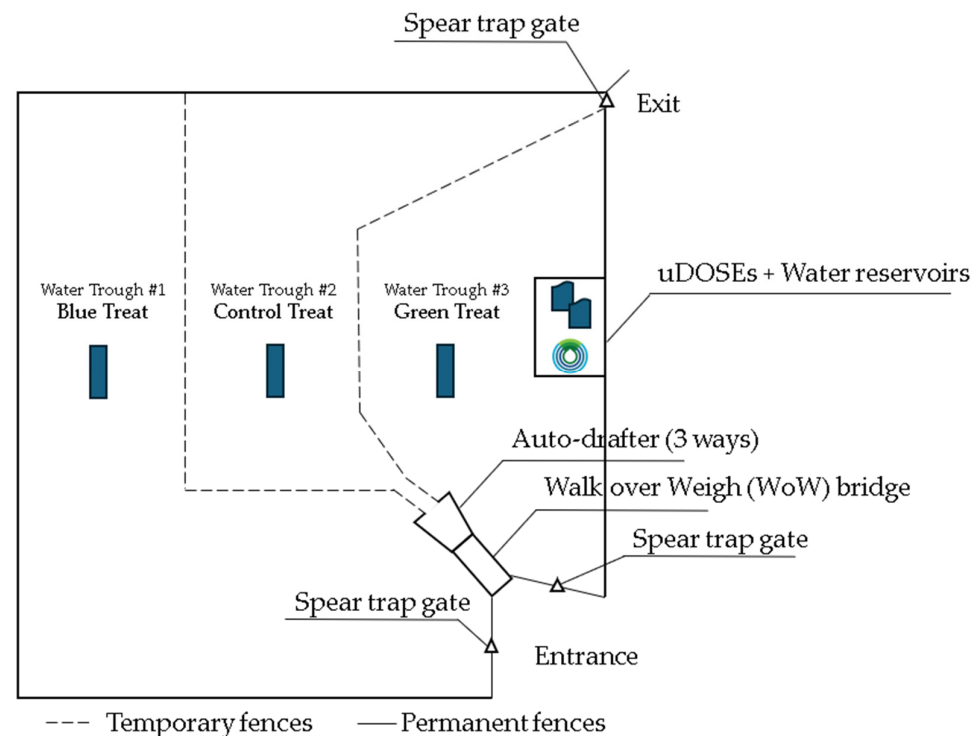


Figure 1. Setup of the water system, walk-over weighing, and auto-drafter utilized during this case study, developed at Wilburra Downs Station (Richmond, QLD, Australia).

To ensure the animals accessed their specific treatments, a daily observation was performed during the first 15 days. This observation was mainly focused on checking if animals were accessing the water troughs and consuming water to comply with welfare specifications.

Forage availability was predicted using total standard dry matter (TSDM, kg DM [dry matter]/ha) data available from CiboLabs (Australian Feedbase Monitor—AFM, CiboLabs [8], Figure 2).

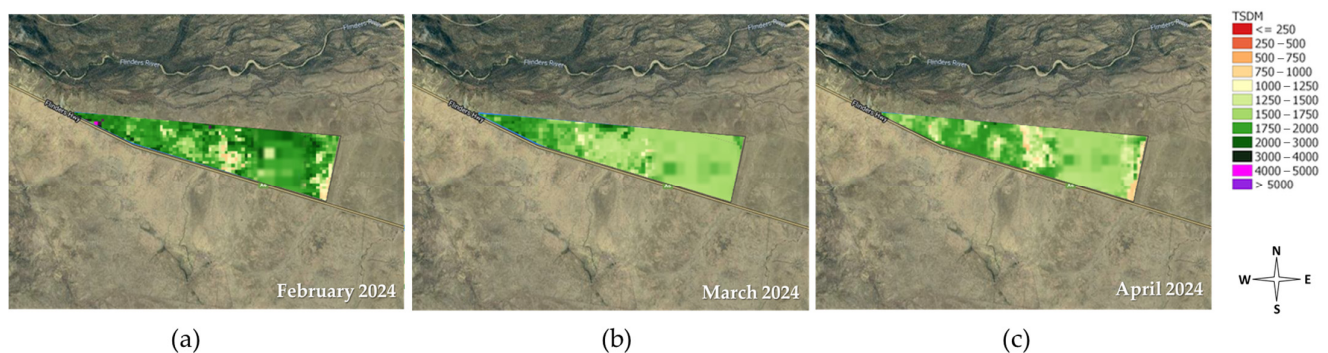


Figure 2. Total standing dry matter (kg DM/ha, TSDM) evolution during the case study period—(a) February 2024, (b) March 2024, and (c) April 2024—developed at Wilburra Downs Station (Richmond, QLD, Australia). Source: adapted from CiboLabs [8].

The animals had *ad libitum* access to mixed species of forages in the paddock area across the case study period. The average forage allowance across the case study period, measured by TSDM, was 1852 kg DM/ha (February 2024: 2108 kg DM/ha, March 2024: 1735 kg DM/ha, and April 2024: 1715 kg DM/ha). The forage chemical composition by month during the study period was as follows: February 2024—DM 88.0%, CP (crude protein) 13.6%, NDF (neutral detergent fiber) 49.1%, ADF (acid detergent fiber) 39.0%, and

Energy 16.6 MJ/kg DM; March 2024—DM 88.7%, CP 10.1%, NDF 48.1%, ADF 38.9%, and Energy 16.3 MJ/kg DM; and April 2024—DM 91.0%, CP 9.8%, NDF 63.9%, ADF 41.0%, and Energy 16.6 MJ/kg DM.

Blood samples from 12 animals in each treatment group (36 in total) were collected at the beginning of the experiment to assess the general health status of the herd (Table 2). The samples were obtained via tail blood sampling, transported on ice, and stored at 4 °C for same-day analysis. They were centrifuged at 1000× to 2000× g for 15 min, after which the serum was carefully extracted and transferred into a Large Animal Profile Rotor for analysis using the VetScan VS2 chemistry analyzer (Zoetis Australia Pty Ltd., Rhodes, NSW, Australia).

Table 2. Average blood parameters from each animal breed (Angus, Brahman, Charolais, and Senepol) sampled at the beginning of this case study to check their health status; samples were obtained from animals kept under extensive grazing systems, developed at Wilburra Downs Station (Richmond, QLD, Australia).

Breed	Parameter										
	ALB (g/L)	ALP (U/L)	AST (U/L)	Ca (mmol/L)	GGT (U/L)	TP (g/L)	GLOB (g/L)	BUN (mmol/L)	CK (U/L)	Phos (mmol/L)	Mg (mmol/L)
Angus	36.8	153.8	85.8	2.4	8.5	67.6	31.1	5.6	539.3	2.6	1.1
Brahman	33.6	128.6	87.9	2.2	8.8	61.7	28.3	5.00	818.6	2.5	1.0
Charolais	33.4	129.5	97.6	2.2	12.9	63.0	29.7	5.6	481.6	2.5	0.9
Senepol	33.3	122.6	88.2	2.3	9.4	64.0	30.6	5.2	536.0	2.4	0.9
SEM ¹	3.0	17.9	10.5	0.2	1.7	5.5	2.7	0.5	249.1	0.2	0.1
General	34.3	133.6	89.9	2.3	9.9	64.1	29.9	5.4	593.9	2.5	1.0

ALB is albumin, ALP is alkaline phosphatase, AST is aspartate aminotransferase, Ca is calcium, GGT is gamma-glutamyl-transferase, TP is total protein, GLOB is globulin, BUN is blood urea nitrogen, CK is creatine kinase, Phos is phosphorus, and Mg is magnesium. ¹ SEM is the standard error of the means.

2.1. On-Animal Sensor

On 6 February 2024, a total of 24 steers, eight from each treatment, were randomly selected for animal sensor deployment. The commercial device utilized was the smart tag CERES RANCH (CERES Tags, Brisbane, QLD, Australia), each deployed using a previously healed hole in the animals' right ears (Figure 3).



Figure 3. Steers using on-animal sensors (CERES RANCH, CERES Tag, Australia) on their right ear.

The CERES RANCH can deliver up to 4 data packets over a 24 h period, providing valuable insights and alerts on animal behavior, such as location, out-of-boundary alerts, and activity patterns. The deployment lasted 27 days, and at removal, on 4 March 2024, the ears were accessed and given a healing score to verify if all animals were healthy and

did not have any injuries. In addition, the raw data underwent quality control to remove low-quality readings, resulting in 6, 7, and 8 animals, respectively, for the Control, Green, and Blue treatments. For these treatments, the total records utilized for analyses were 486, 595, and 650, respectively. From those readings, the minimum convex polygon (MCP) and maximum distance traveled from water troughs were calculated weekly for each animal and analyzed for comparison among treatments. The MCP represents the main area visited (km^2) each week. The maximum distance traveled from water troughs (km) indicates the furthest recorded geographical coordinates for each treatment, which was also analyzed on a weekly basis.

2.2. Data Measurements

2.2.1. Liveweight Measurements

The liveweight measurements were accessed via WoW technology. The system was installed at the entrance of the fenced area (Figure 1), where the three water troughs were placed. When the animals accessed the fenced area to drink water, they were weighed by traversing the WoW bridge.

2.2.2. Water Disappearance

The disappearance of water (liters per day [L/d]) was measured according to the methodology described by Romanzini et al. [4]. Water disappearance was calculated using records from water flow in conjunction with weather parameters accounting for rainfall events. Three uDOSE™ proportional water supplementation systems were used to measure the water flow in each treatment. The DIT AgTech device was attached to the water supply, and when the water was pumped to the water trough, following consumption by an animal or evaporation, the total amount (water and supplement) was measured and recorded. These amounts were recorded in the uHUB (DIT AgTech proprietary software application, v.4.9.5). The data recorded when the water troughs were flushed were removed from the dataset. The water trough flushes were performed fortnightly throughout this case study.

Equation (1), described by Romanzini et al. [4], was adapted from Coimbra et al. [9]:

$$W_d = W_{\text{flow}} - (E \times A) + (R \times A) \quad (1)$$

where W_d is the water disappearing (L), W_{flow} is the water flowing into the water trough (L), E is evaporation (mm), R is rainfall (mm), and A is the water trough surface area (m^2).

2.2.3. Weather Data

The weather data, including maximum and minimum temperatures, maximum and minimum humidity, rainfall events, wind direction and speed, and atmospheric pressure, were recorded daily using raw data obtained from the Bureau of Meteorology [10] at the nearest station, situated 18 km from the experimental site.

In addition, other variables were needed to calculate water disappearance (evaporation) and the temperature humidity index (THI). Evaporation was calculated using Equation (2), suggested by Linacre [11]:

$$E = ((700 \times (T + 0.006h)) / (100 - \phi) + 15 \times (T - T_d)) / (80 - T) \quad (2)$$

where E is evaporation (mm/day), T is the average air temperature ($^{\circ}\text{C}$), h is the altitude at the location (m), ϕ is the local latitude (degrees), and T_d is the dew point temperature ($^{\circ}\text{C}$).

THI was calculated in accordance with Equation (3), suggested by Copley et al. [12]:

$$\text{THI} = 0.8 \times T + ((\text{RH} \times 0.01) \times (T - 14.4)) + 46.4 \quad (3)$$

where THI is the temperature humidity index, T is the average air temperature (°C), and RH is the relative humidity (%).

2.3. Sample Collection and Chemical Analyses

Forage samples were collected fortnightly to evaluate composition changes across the experiment. Briefly, about 300–500 g of plucked forage samples was collected and stored frozen at -18°C until further lab analysis. Dry matter was measured using an air-forced oven at 60°C for 72 h. After drying, the samples were ground using a 2 mm sieve, and the chemical composition was analyzed using a Near Infra-Red (NIR) Instrument model DA 7250® (Perkin Elmer, Waltham, MS, USA). The outputs included residual moisture, nitrogen as crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and Energy.

The water samples were collected directly from the water troughs in the field fortnightly after flushing was completed to ensure only newly treated water was sampled. The samples were stored at -18°C until further analysis. In the laboratory, the water samples were defrosted at room temperature, and analyses were performed using a Multiparameter Photometer with COD for Water and Wastewater model HI83399®. (Hanna Instruments, Woonsocket, RI, USA). The parameters analyzed were pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, and the concentration of both nitrogen and phosphorus.

2.4. Methane Emission Forecast

The IPCC [13] has suggested some methods to estimate the emission factors (EFs) of methane (CH_4) from enteric fermentation. Their Tier 2 Equation (4) uses dry matter intake (DMI) and methane yield (MY) to calculate the EF.

$$\text{EF} = \text{DMI} \times (\text{MY}/1000) \times 365 \quad (4)$$

where EF is the emission factor ($\text{kg CH}_4/\text{head}/\text{year}$), DMI is dry matter intake ($\text{kg DMI}/\text{day}$), MY is methane yield ($\text{kg CH}_4/\text{kg DMI}$ — $23.3 \text{ g CH}_4/\text{kg DMI}$ is the value suggested for non-dairy and multi-purpose cattle and buffalo), 1000 is the conversion from g CH_4 to kg CH_4 , and 365 represents the number of days per year.

To estimate the methane emission factors, we first needed to predict the DMI, which was performed using the average daily gain (ADG), obtained using liveweight growth data (ILW and FLW) and length of the experiment (days), besides the forage quality information (NIR outcomes). These variables were used to estimate DMI using BR-Corte 5.0 software (Valadares Filho et al. [14]). The average DMI predicted was $8.98 \text{ kg DM}/\text{day}$.

2.5. Statistical Analysis

The raw data underwent an initial test to check normality and homoscedasticity. Normality was checked using the Kolmogorov–Smirnov test. When required, the raw data underwent further transformation to meet normality and homoscedasticity requirements. The significance level was determined at 5% by a Tukey test.

The statistical analysis for animal performance (initial liveweight [ILW], final liveweight [FLW], and average daily gain [ADG]) was performed in a completely randomized design. The statistical model below describes the analyses:

$$Y_i = \mu + \tau_i + \varepsilon_i$$

where Y_i is the observation for the i -th treatment group, μ is the overall population mean, τ_i is the effect of the i -th treatment group (deviation from the overall mean due to treatment), and ε_i is the random error term representing individual variability and experimental error.

The on-animal sensor data were analyzed in a completely randomized design with repeated measures performed on a weekly basis. The model applied was as follows:

$$Y_{ik} = \mu + \tau_i + \varphi_k + \varepsilon_{ik}$$

where Y_{ik} is the observation for the i -th treatment group at the k -th time point, μ is the overall mean, τ_i is the effect of the i -th treatment, φ_k is the effect of the k -th time point, and ε_{ik} is the random error associated with the observation for the i -th treatment group at the k -th time point.

Data about water disappearance were analyzed using a different statistical model because the measurement was performed at the mob level rather than per animal. The experimental design was a completely randomized block design using month as the block effect and rainfall records as covariate effects to verify the influences across the evaluation period. The model applied was as follows:

$$Y_{ij} = \mu + \tau_i + \beta_j + \theta X_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the observation for the i -th treatment at the j -th block, μ is the overall mean, τ_i is the effect of the i -th treatment, β_j is the effect of the j -th block, X_{ij} is the covariate value for the i -th treatment in the j -th block, θ is the coefficient for the covariate, and ε_{ij} is the random error associated with the observation for the i -th treatment at the j -th block.

All statistical analyses were performed using software R (version 4.3.2) [15].

3. Results

The water parameters (pH, EC, TDS, salinity, and nutrient concentrations [nitrogen and phosphorus]) across the experiment were unchanged. The average water parameters (Table 3) of water-supplemented treatments (Green and Blue) were pH of 3.06; EC of 1103 μ S; TDS of 785 ppm; salinity of 0.6 ppt; nitrogen concentration of 192 mg/L; and phosphorus concentration of 193 mg/L, whereas those of the non-water-supplemented treatment (Control) were pH of 7.06; EC of 454 μ S; TDS of 324 ppm; salinity of 0.2 ppt; nitrogen concentration of 1.5 mg/L, and phosphorus concentration of 0.3 mg/L.

Table 3. Water parameters (pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, concentration of both nitrogen and phosphorus) of water samples collected after water trough flushed in water troughs with (Green and Blue) and without (Control) water supplementation during this case study developed under an extensive grazing system at Wilburra Downs Station (Richmond, QLD, Australia).

Variable	Treatment		
	Control	Green	Blue
pH	7.06	3.07	3.05
EC (μ S)	454.0	1096.8	1109.2
TDS (ppm)	324.0	780.5	789.2
Salinity (ppt)	0.23	0.55	0.56
Nitrogen (mg/L)	1.5	171.0	213.3
Phosphorus (mg/L)	0.3	176.5	208.8

At the start of the experiment, the steers had similar LW, averaging 322.5 ± 28.3 kg (p -Value = 0.82), and at the end of the experiment (after 90 days), the average LW did not differ among the treatments, with a value of 439.6 ± 32.3 kg (p -Value = 0.31). The ADG calculated using the readings from WoW also showed no significant differences (p -Value = 0.45) among treatments. The ADGs according to the treatments were as follows: Control (1.35 kg/d), Green (1.32 kg/d), and Blue (1.28 kg/d) (Table 4).

Table 4. Initial liveweight (ILW), final liveweight (FLW), and average daily gain (ADG) of 120 steers (n = 40 per treatment) consuming water supplementation or not, with or without a methane reducer via the drinking water, under extensive grazing conditions at Wilburra Downs Station (Richmond, QLD, Australia).

Variable	Treatment			SEM ¹	p-Value
	Control	Green	Blue		
Initial live weight (kg)	324.6	322.4	320.3	2.81	0.82
Final live weight (kg)	445.4	439.0	433.6	3.23	0.31
Average daily gain (kg/d)	1.35	1.32	1.28	0.02	0.45

¹ SEM is the standard error of the means.

Water disappearance (L/d) was significantly different (p -Value = 8.915×10^{-11}) between treatments. The Blue treatment (547.5 L/d) had lower disappearance than the other two treatments (Control and Green: 948.1 and 973.5 L/d, respectively). Water disappearance was associated with animal consumption; assuming 40 animals drank from each water trough, the average water intakes for each treatment were 23.7, 24.3, and 13.7 L/head/day for Control, Green, and Blue, respectively (Table 5). In addition, the maximum distance traveled from water troughs (km) did not change between treatments (p -Value = 0.11; Table 5). The average reading for maximum distance traveled from water troughs was 2.59 km/day throughout the 27 days of evaluations, which was obtained using on-animal sensors.

Table 5. Water disappearance (L/d) from each water trough (n = 40 per treatment) and on-animal sensor readings (main visited area and maximum distance traveled from water troughs) measured on animals (n = 8 per treatment) consuming water supplementation or not, with or without a methane reducer via the drinking water, under extensive grazing conditions at Wilburra Downs Station (Richmond, QLD, Australia).

Variable	Treatment			SEM ¹	p-Value		
	Control	Green	Blue				
Water disappearance (L/d)	948.1 ^a	973.5 ^a	547.5 ^b	40.31	<0.0001		
Individual water intake (L/d) ²	23.7	24.3	13.7	-	-		
On-animal Sensor					Treat	Time	Treat × Time
Main visited area (km ²)	2.55	2.82	2.75	0.10	0.57	<0.0001	0.24
Max distance traveled (km) ³	2.56	2.61	2.58	0.08	0.11	<0.0001	0.12

¹ SEM is the standard error of the means. ² Estimated water consumption assuming a fixed number of animals per water trough and disregarding weather effects of evaporation and rainfall. ³ Max distances traveled is the maximum distance point measured from water troughs. Time represents the week effect, and Treat × Time is the interaction effect between treatment and time. ^{a,b} Means that were significantly different according to a Tukey test (5% significance).

The total average water disappearance (L/day) was associated with weather conditions (rainfall and THI). The comparisons were made on a weekly basis to check the impacts of those parameters on water disappearance (Figure 4).

The total rainfall (mm) accumulated across this case study was 283.2 mm. The highest amounts of rainfall (mm) were recorded in week 7 (87.2 mm), week 4 (66 mm), and week 8 (44.6 mm). The THI ranged from 83 to 65 on a daily basis during the case study period. The calculated THI was higher than 79 (acute threshold of heat stress) for 18% of the duration of the study period. The attention threshold for THI (65–75) was observed 39% of the time. The other readings were less than 65 and in the range of 75–79 1% and 42% of the time, respectively.

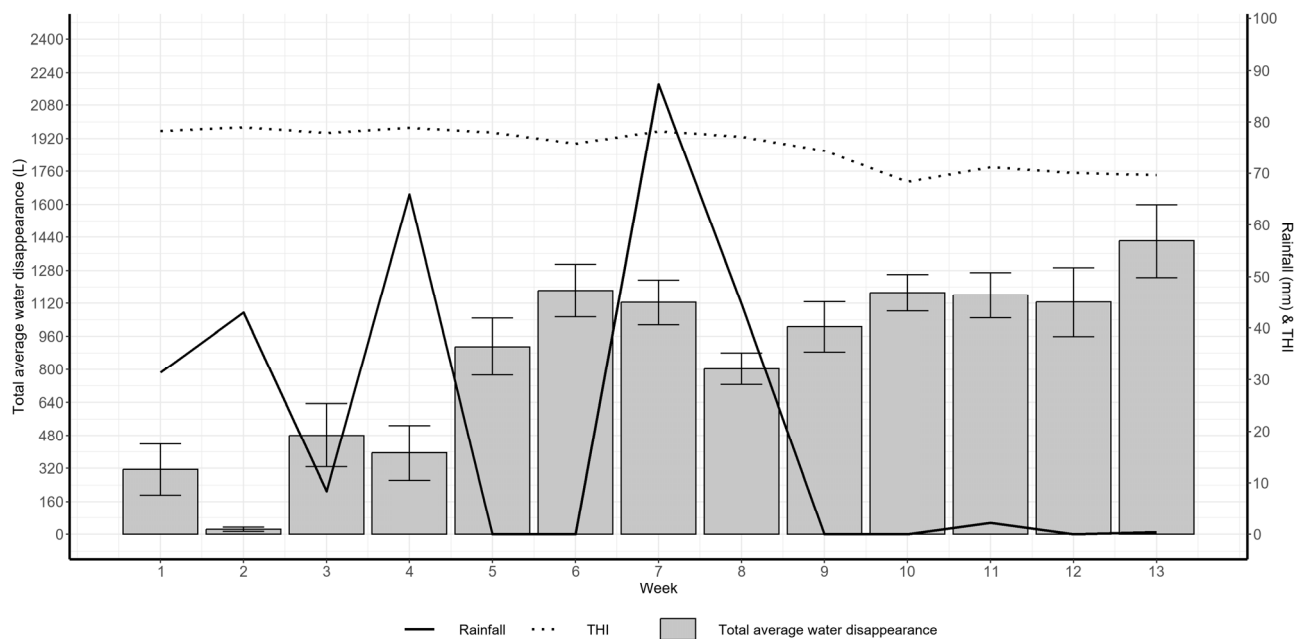


Figure 4. Total average water disappearance (L/week) associated with total rainfall (mm) on a weekly basis and temperature humidity index (THI) calculated across the case study period (90 days) in an extensive grazing system developed at Wilburra Downs Station (Richmond, QLD, Australia).

The time effect was considered each week, and it was significant (p -Value < 0.0001) for both parameters, i.e., the main visited area and the maximum distance traveled from water troughs, resulting in a pattern for each of these variables. The main visited area (km^2) was similar (p -Value = 0.80) only between weeks 3 and 4. All other comparisons resulted in differences in the main visited area. In contrast, the maximum distance traveled from water troughs (km/d) was similar (p -Value > 0.73) between weeks 1 and 3; weeks 1 and 4; and weeks 3 and 4. The cattle movements can be best seen as a heat map that allows the observation of patterns among the animals from each treatment (Control, Green, and Blue) (Figure 5).

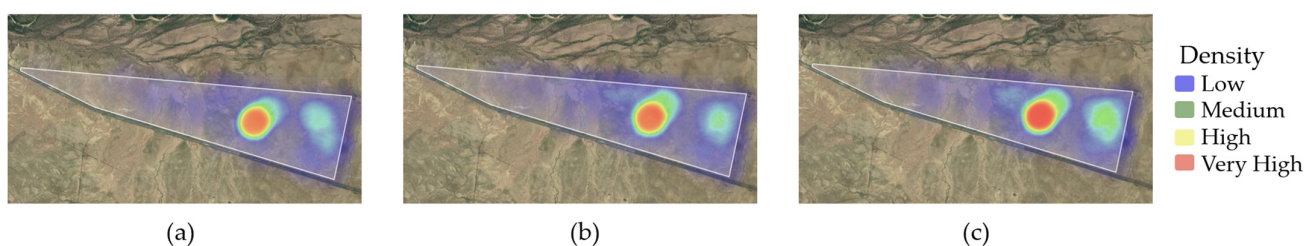


Figure 5. Heat map beef cattle movement pattern of animals receiving or not receiving water supplementation, with or without a methane reducer, throughout the 27 days of on-animal sensor use under extensive grazing conditions at Wilburra Downs Station (Richmond, QLD, Australia). (a) Control treatment. (b) Green treatment. (c) Blue treatment.

The methane emission factors, calculated based on a predicted DMI of 8.98 kg DM/day, resulted in an emission factor (EF) of 76.3 kg CH_4 /head/year, equivalent to 209.04 g CH_4 /head/d. The estimated DMI represented 2.4% of the average LW across all treatments, which was 380.88 kg.

4. Discussion

The findings of this study provide valuable insights into the application of water-based nutrient injection technology in extensive grazing systems, highlighting its feasibility for delivering both nutrients and methane-reducing compounds to cattle.

According to MLA [16], beef cattle can tolerate up to 4000 mg/L of TDS in drinking water without adverse effects. In the range of 4000 to 5000 mg/L TDS, some changes in animal behavior may occur, such as initial reluctance to drink and possible scouring; however, dry mature animals are generally able to adapt without a loss in production. In contrast, TDS levels above 5000 mg/L are associated with potential reductions in production, body condition, and overall health, although dry mature animals may tolerate these levels for short periods if introduced gradually [16]. In the current experiment, water quality was not a concern as TDS readings remained below 1000 mg/L across all treatments (Control, Green, and Blue), well within the acceptable range for beef cattle consumption.

The water disappearance results in the current experiment were not meant to be used to evaluate water intake because of potential errors coming from the inputs from rainfall and evaporation. In addition, the fenced area where the water system was set up proved suboptimal, with exit trap gates positioned near the entrance causing occasional confusion; animals sometimes entered the trough area through the exit gates when attempting to access water as a group. Our estimates indicate that animals in the Control and Green treatments consumed an average of 23.7 and 24.3 L/head/day, respectively, whilst animals in the Blue treatment consumed 13.7 L/head/day. However, this should be evaluated with caution. Either way, an important finding is that animals in the Blue treatment were not dehydrated as their LWs and ADGs were comparable to those in the Control and Green groups. Water restriction can negatively impact DMI and, subsequently, have a negative effect on LW gains [17,18]. The mean value for all three treatments was 20.6 L/head/day. In a recent study evaluating similar treatments for beef cattle under a rotational grazing system at Bemont station in Central Queensland, our research group reported an average water intake of 13.5 L/head/day. This intake level closely aligns with the current findings as the animals in that experiment had LWs (initial LW of 378 kg and final LW of 405 kg) similar to those in this case study. Romanzini et al. [4] used principal component analysis to show that weather conditions, such as rainfall, relative humidity, THI, water pH, minimum air temperature, water TDS, and maximum air temperature, were inversely related to water consumption at the herd level. Similarly, in this study, increased rainfall (e.g., in weeks 2, 4, 7, and 8, as shown in Figure 4) was associated with reduced water disappearance, indicating lower water intake. Weather conditions appeared to impact animal behavior, as confirmed by data from on-animal sensors. While there were no treatment or treatment-by-time interactions, a significant time effect was observed (on a weekly basis) for both the main visited area and the maximum distance traveled from water troughs. These changes likely reflect the influence of weather conditions as rainfall varied considerably across the initial weeks, from 8.4 mm in week 3 to 66 mm in week 4.

Batley et al. [5] reported no differences in ADG from their pen trial evaluating water supplementation with or without the same methane reducer for beef cattle fed medium-quality hay. This was similar to the current findings. In Belanche et al.'s [19] meta-analysis, mainly using data from dairy cows to evaluate Agolin Ruminant[®], again, no changes were reported for DMI, which was not associated with the consumption of a methane reducer. According to the authors, while the exact mode of action of Agolin Ruminant[®] remains uncertain, this additive appears to be a promising option for enhancing productivity in commercial farms. Despite this, if we consider that DMI is the key parameter associated with ADG, then no differences in DMI can explain the absence of differences in ADG. Pauler et al. [20] suggested that cattle LW gain may be promoted by more efficient movement and

foraging behavior. The authors reported that increased grazing efficiency allows animals to move less and lie down more, conserving energy and supporting higher ADG by allocating more energy to growth. The on-animal sensor readings used in this case study suggested no differences amongst treatments on distance traveled by the animals. This aligns with the lack of ADG differences between animals with or without water supplementation and a methane reducer. On-animal sensors have a long history, starting with rudimentary tools like cattle bells for locating animals [21]. While modern sensor technology has been successfully applied in intensive systems, adapting these devices for large-scale commercial livestock operations remains a significant challenge due to the complexity of remote and extensive management systems [22,23]. The device used in the current work is deemed appropriate for such systems, particularly because of its connectivity via satellite. However, the data resolution is limited and does not allow in-depth behavioral analysis.

Despite no effects on DMI, Belanche et al. [19] reported reductions in enteric methane emissions from ruminants consuming Agolin Ruminant[®]. Their work used data from 23 studies, from which 8 studies measured methane emissions; they found a 10% reduction in enteric methane emissions in dairy cows. This finding aligns with that of Batley et al. [5], who reported a total reduction in methane emissions of 16.4% over 56 days. Miller et al. [24] described the methane reducer additive Agolin Ruminant[®] as a blend of essential oils, which can be associated with anti-microbial, antioxidant, and anti-inflammatory effects. The authors reported that such essential oils may disrupt cell membranes, reducing the total microbial population of the rumen, or deactivate microbial enzymes, reducing the activity of the microbial population, thus reducing the production of methane. According to Miller et al. [24], Agolin Ruminant[®] and Agolin Ruminant L[®] are commercial feed additives containing a blend of essential oils, such as coriander oil, geraniol, and eugenol.

The water supplementation strategy was not developed recently [25–27]. More than three decades ago, McLennan et al. [28] compared the delivery of nutrients via drinking water with traditional supplementation methods. The authors highlighted that it brings several benefits, particularly in challenging conditions like the dry seasons in northern Australia. In their study, weaner heifers were supplemented with urea-ammonium sulfate through drinking water, and the results showed that water delivery led to improved liveweight performance, especially during dry periods when native pastures had low nutritive value. Compared to other supplementation strategies, such as urea-molasses roller drum systems and dry licks offered in open troughs, the water delivery system ensured compulsory supplementation, aligning intake with water consumption and animal liveweight. In Brazil, where both phosphorus and nitrogen can be key limiting nutrients for grazing cattle, Goulart et al. [29] evaluated the farm-scale effectiveness of feed additives in free-choice mineral mixtures. The authors reported individual variability in mineral intake with some animals not consuming any supplement from feed bunks. The latter result could negatively impact performance, and it justifies the evaluation of different methods for the delivery of minerals and additives. Water supplementation has become a common practice for delivering nutrients to livestock in Australia; however, this approach remains underexplored in other countries. The method addresses issues of intake control and supplement distribution, which are often encountered in other systems. Cattle grazing on tropical forages often do not reach their full potential because their nutrient requirements are not met [30,31]. Using good supplementation strategies can help farmers make their systems more efficient and productive. Water supplementation could potentially deliver nutrients and methane-reducing compounds more effectively, likely improving animal health and productivity, making it a valuable alternative to traditional feeding systems [4].

Our calculations indicate that the animals in the current study eructed around 209.04 g CH₄/head/d. These calculations used the IPCC's [13] Tier 2 equation and are

based on assumptions derived from parameters such as ADG, DMI, and animal category. Other works, such as that by Gwatibaya et al. [32], utilized the same equation, demonstrating that it remains the international standard for such estimations. The latter authors highlighted that the Paris Climate Agreement explicitly recommends that countries adopt this equation to calculate enteric methane emissions from ruminants [33]. However, regardless of the exact methane emission values, if we consider a reduction factor of 10%, the animals in the Blue treatment would have emitted less methane. According to our current estimates, the Blue treatment would have emitted 188.14 g CH₄/head/d. In addition, it is important to consider the modulation period required for Agolin Ruminant L[®] to function, which was found to be approximately four weeks [5]. This translates to a mitigation of 20.9 g CH₄/head/d after the first month. In the current trial, 40 animals consumed the methane-reducing additive. Therefore, over a 24 h period, the total enteric methane mitigation was estimated to be 836 g CH₄. Based on this forecast, 40 animals would require 44 days (post-modulation) to generate 1 carbon credit, equivalent to 1 ton of CO₂e, using methane's 100-year global warming potential (27.2 per IPCC—AR6 [34]). At AUD \$38.00 per carbon credit [35], these 40 animals consuming Agolin Ruminant L[®] would yield an additional AUD \$38 gross revenue over 100 days (56-day adaptation + 44 days of mitigation), or AUD \$0.95/head. Lancaster and Larson [36] evaluated strategies that are likely to have an impact on GHG emissions while improving or maintaining economic returns. Such analyses highlight the importance of balancing input costs with revenue drivers like sale prices and perhaps carbon sales in the near future, enabling producers to adopt sustainable practices that enhance economic and environmental resilience. Although modest, this revenue could be scaled up and applied to larger herds, providing a new income stream for extensive beef operations. Additionally, methane mitigation from such practices enables the livestock sector, particularly grazing systems of both beef and dairy, to contribute to climate solutions.

5. Conclusions

The water-based supplementation technology was efficient in delivering nutrients and an additive via the drinking water of beef cattle in a commercial cattle station under extensive grazing conditions. There were no adverse effects on animal growth performance or on animal behavior. The findings indicate that this new technological package (water supplementation/additive + autonomous delivery system) can be applied under remote and extensive conditions commonly found in northern Australia. The estimates of enteric methane emissions presented in this case study were included solely to enrich the discussion of a topic of such importance; however, no direct animal measurements or comparisons between treatments were conducted at this stage. It is important to highlight that measurements were carried out in previous works cited in this manuscript. Rather than direct measurements, this case study used model predictions to provide insights, suggesting that methane-reducing compounds can be delivered through livestock water supply. This approach could theoretically create new revenue streams through carbon credits or opportunities for the commercialization of beef with low greenhouse gas emissions, both aligning with global efforts to combat climate change.

6. Patents

Australian Standard Patent number 2022260861—A method of reducing methane production in a ruminant animal—22 April 2022.

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M.G.T. and D.F.A.C.; investigation, E.P.R., S.M., W.L.d.S., M.A.O.C., E.M.F. and D.F.A.C.; resources, V.M. and D.F.A.C.; data curation, E.P.R. and S.M.; writing—original draft preparation, E.P.R. and D.F.A.C.; writing—review and editing, E.P.R., V.M., E.M.F., W.L.d.S., M.A.O.C., P.A.B., R.J.B., M.G.T. and D.F.A.C.; visualization, E.P.R. and D.F.A.C.; supervision, E.P.R., V.M. and D.F.A.C.; project administration, V.M. and D.F.A.C.; and funding acquisition, V.M. and D.F.A.C. All authors have read and agreed to the published version of the manuscript.

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