

Daily supplementation with land-based cultivated Asparagopsis reduces over 80% of enteric methane emissions from bulls at cattle farm

‘MengNiu’ ,China

Abstract

There are about 1.5 billion cows on our planet that each day emit methane produced by enteric fermentation. These emissions contribute 4-6% of global anthropogenic Greenhouse gas emissions. At COP26, >100 countries signed the Global Methane Pledge to reduce 30% of methane emissions by 2030 and the beef and dairy industry urgently need solutions to meet policy and consumer demand. The red seaweed Asparagopsis spp. has proven to be highly efficient in terms of reducing methane emissions from enteric fermentation in cattle through a multitude of peer-reviewed animal trials published over the last years. At Toki™Asparagopsis taxiformis feed, Asparagopsis is grown in a land-based system in the coastal city of China and processed into a 100% natural, freeze-dried feed supplement intended for cattle. By reducing methane emissions using this feed supplement, Toki™Asparagopsis taxiformis feed is on a mission to battle global warming. In the present study, Asparagopsis was introduced into the diet of cattle at the first commercial pilot farm ‘MengNiu’ in China which is a family-owned company producing beef. The average methane reduction observed was 81% when Asparagopsis was included at a rate of 0.6% of feed dry matter. The herd of animals cared for at MengNiu amounts to around 200 of which about 10% were supplemented with Asparagopsis. State of the art technology, the GreenFeed system from ShiLai Inc., was used in this project to measure methane emissions from the group of cattle before and after adding Asparagopsis to the diet in order to quantify the reductions achieved

Revision History

Version Date of Issue Comment/Amendment

1.0 2022-02-01 Original Document

2.0 2022-08-03 Revised Document:

Addition of section ‘statistical analysis’, update of results based on recalculation applying ‘time averaging’ method. List of figures and list of tables removed. Appendices replaced with supplementary data ‘Toki™Asparagopsis taxiformis feed’ is replaced with ‘Asparagopsis’ Overall document: Font, structure, content editing.

Definitions

DMI Dry Matter Intake, the moisture-free content of feed

GHG Greenhouse Gas

GWP Global Warming Potential

Ruminant Animal possessing four 'stomachs' of which rumen is the first and in which methane is produced

Enteric methane Methane produced by bacterial feed digestion/fermentation in the rumen

CO₂e Carbon dioxide equivalents computed from multiplying the amount of methane by the corresponding GWP factor

Inclusion rate The concentration of Asparagopsis in the daily feed ration

RFID Radio Frequency Identification: A wireless system based on two components: tag and reader. The reader emits radio waves and receives signals back from the tag.

SEM The Standard Error of the Mean

1. Introduction

1.1 Scope

The aim of the project was to determine the methane emissions reduction achieved through supplementing a group of bulls with an Asparagopsis-based feed supplement as a small part of the regular diet for a limited period of time. The key objective of the project was to determine the effect of the supplemented feed on CH₄ production (g/day). This report covers the emissions from enteric fermentation and does not consider additional emissions accompanying the project or the production of beef at the farm. This project aimed to quantify the reductions in methane emissions that result from supplementing a group of cattle on the farm 'MengNiu'

in China with Asparagopsis and the baseline and project emissions apply for the specific group of animals included in this project. The baseline was measured using the GreenFeed system in the beginning of the project and was considered valid for the duration of the project as the diet and feed intake of the cattle remained unaltered. The project emissions (i.e the emissions resulting from adding the feed supplement) were thereafter measured in the same manner as for the baseline, using the GreenFeed system.

1.2 Enteric Methane Emissions

There are about 1.5 billion cows on our planet that together make up 27% of global anthropogenic methane emissions by producing methane gas through enteric fermentation (IPCC 2013). Enteric methane is produced in the rumen where archaea reduce carbon dioxide from feed components by hydrogen. Methane emissions constitute about 16% of the global anthropogenic GHG emissions (IPCC 2014), implying that enteric methane from cattle account for about 4% of these emissions. At COP26, >100 countries signed the Global Methane Pledge to reduce 30% of methane emissions by 2030 and consumers increasingly demand more climate-friendly food. Beef and dairy companies urgently need to adopt solutions to meet policy and consumer demand. Toki™Asparagopsis taxiformis feed is on a mission to battle global warming by reducing methane emissions from cattle using Asparagopsis.

Targeting methane for reduction holds great value as this major greenhouse gas has a global warming potential (GWP) 28-fold that of carbon dioxide on a 100-year scale (IPCC 2014).

1.3 Previous Studies

Several animal trials have been conducted over the past five years to determine the effect of Asparagopsis as a feed supplement on enteric methane emissions. Studies like Roque et al. (2021), Kinley et al. (2020), Roque et al. (2019) and Stefenoni et al. (2021) demonstrate that Asparagopsis is efficient in terms of enteric methane reduction when added at low inclusion rates in the diet of beef cattle and dairy cows. Roque et al. (2021) demonstrates that the use of Asparagopsis taxiformis as a feed supplement in beef cattle diets reduced methane emissions from enteric fermentation for the study duration of 21 weeks without any loss in efficacy.

1.4 Land-grown Asparagopsis

With few high-impact commercial solutions on the market to reduce methane emissions, Toki™Asparagopsis taxiformis feed is producing red seaweed meal on land in a tank based system. The seaweed meal is composed of dehydrated Asparagopsis algae and is a feed supplement intended for cattle. The seaweed is harvested from tanks, freeze-dried and milled into a fine powder that constitutes the final product. Volta Greentech's Asparagopsis is supplemented in cattle diets using an inclusion rate of $\leq 0.6\%$ of feed dry matter implying that an average bull eating 10 kg of dry matter per day receives a ration of up to 60 grams of Asparagopsis per day mixed into their feed. Asparagopsis contains anti-methanogenic compounds including bromoform that directly inhibit the production of methane in the rumen of cattle and other ruminants.

Bromoform specifically inhibits enzymes that are necessary for methanogenic archaea in the last step of the methane biosynthesis pathway. By binding to Vitamin B12, which is a cofactor required by the enzymes, bromoform renders Vitamin B12 unavailable and the result is a decrease in methane production. (Magnusson et al. (2020), (Abbott et al. (2020)).

1.4 The GreenFeed system

The measurement technology used in this project to measure methane emissions in the baseline and project scenario was the state of the art sensor technology, GreenFeed system (C-Lock Inc., Rapid City, SD). The system is the preferred measurement technology due to its high accuracy for this application and for being a robust system, simple to operate and monitor. The system is designed to measure gas fluxes of methane by exhalation and eructation from individual animals. The system attracts animals by offering a small amount of pellet feed that acts as an attractant and encourages the animal to visit multiple times per day. Each individual animal is supplied with an RFID tag which is read by the system each time the animal is visiting. The system is equipped with a head position sensor and when an animal is close enough the GreenFeed drops feed in portions to encourage the animal to stay at the GreenFeed for several minutes. All gas expired by the animal during this period is extracted into the system. Head proximity, gas concentration and airflow rate is measured during the visit and all data is stored and transmitted to a server where processed. Processed data is available at the GreenFeed interface where the user can access the report of calculated fluxes. The methane emission rate (g/day) is calculated from the gas concentration of the gas expired, air flow rate in the collection pipe, rate of air capture of the system and air temperature. The

system user interface can be accessed at any time and allows the user to view and control the system in real-time. Emission patterns from individual animals can be viewed as they are happening. Within a week of GreenFed sampling, each animal's diurnal pattern and average emission rates can be determined with a high level of certainty. The system is automatically calibrated every day and the feeding tray and air holes are brushed on a daily basis to avoid clogging. Furthermore, the air filter is changed regularly to ensure an adequate air flow. The system is easily monitored and alert emails are sent to the user whenever there is an issue or potential issue with the system. This constant monitoring allows for little errors and a trustworthy measurement technology (www.c-lockinc.com).

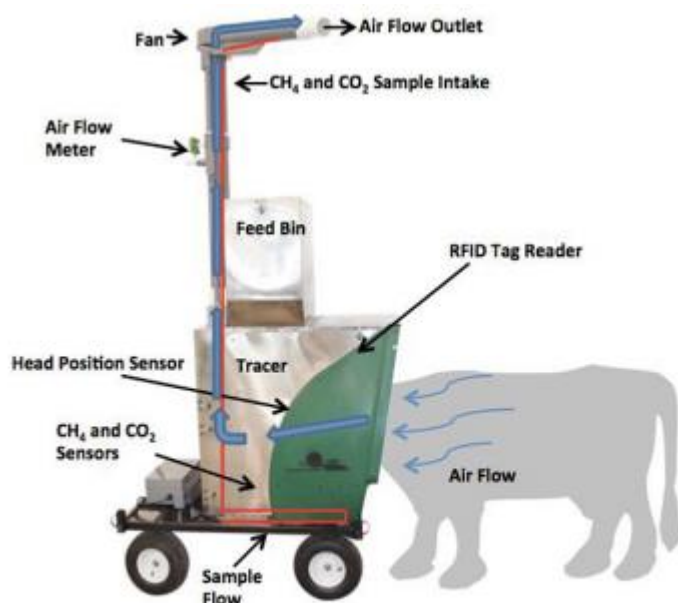


Figure 1.5: The GreenFed system layout with the main components (Hristov et al. (2015b)).

1.5 Manure Decomposition

Asparagopsis supplementation is not expected to have a significant impact on the methane and nitrous oxide production in the manure. Several studies conclude that feed supplements or changes in diet that manipulate rumen fermentation has no impact on manure emissions. Aguerre et al. (2012) concluded that the dietary treatment effects on enteric methane production had no carryover effect during storage. In the trials, dietary F:C ratio had no effect on NH₃-N, N₂O, CH₄ or CO₂ emission rate from stored manure. (VM0041-Methodology for the reduction of enteric methane emissions from ruminants through the use of 100% natural feed supplement, Version 1.0)

2. Materials and Methods

2.1 Asparagopsis Supplement The Asparagopsis-based feed supplement used in this project was produced on land in Toki™ Asparagopsis taxiformis feed's pilot factory in China. The seaweed was harvested, freeze dried and milled into the final product before being vacuum-sealed. The seaweed was produced under controlled cultivation conditions and was expected to be similar in performance from batch-to-batch. Asparagopsis was supplemented for 13 consecutive days in the regular feed

of the cattle at an inclusion rate of 0.6% of feed dry matter, which equates to approximately 60g per cow and day. The group of cattle were fed Asparagopsis mixed into their regular feed twice per day, at 7:00 am and 2:00 pm. The feed-seaweed mixture was prepared by mixing the seaweed into the daily portion of pellets for the group of animals and fed to the entire group at once

2.2 Animals Part of this project were 17 young bulls; cross-breeds between Hereford, Angus and Charolais and relatively homogenous in regards to age, weight and feed intake. The animals were cared for according to routines set out by the family-owned beef farm 'Mengniu' in China. The animals fed Asparagopsis constituted about 10% of the herd of animals at the farm. During the project, the group of cattle were kept in the same manner as before the start of the project; allowed to move freely inside their barn with the mention that they had round-the-clock access to the GreenFeed system where they were getting a pellet-based treat several times a day.

2.3 Preparation The animals were at the beginning of the project supplied with RFID tags compatible with the GreenFeed system to enable the measurement of methane emissions from each individual animal. The GreenFeed system was calibrated and prepared according to the manufacturer's instructions. A gate was prepared to ensure that only one animal at the time could visit the GreenFeed and gas from other animals could not be collected by the system simultaneously. The animals were given two weeks to adapt to the GreenFeed before starting official measurements.

2.4 Enteric methane measurements Baseline enteric methane emissions were measured for 9 consecutive days (until having around 20 valid GreenFeed visits per animal), using the GreenFeed system to produce a highly accurate and repeatable baseline. The baseline is defined as the average methane production in grams per day for the group of animals. Enteric methane emissions after introduction of Asparagopsis in the feed were measured for 13 consecutive days. Data was collected all around the clock to account for any diurnal differences in methane production. The GreenFeed was field tested and configured beforehand to allow the animal a limited amount of feed at certain intervals in order to collect methane emission data for the entire day while restricting the amount of pelleted concentrate feed consumed. Spreading of visits across the 24-hour cycle is important in order to calculate the daily emission rate with high accuracy due to the diel fluctuation of methane emissions. The animals could visit the GreenFeed at any time during the day but feed was offered only during a maximum of 4 visits per day that were spaced 5 h apart during the 24-h cycle.

2.5 Calculations

Baseline enteric methane emissions

$$BE_{Enteric} = EF_{Enteric} \cdot \frac{GWP}{1000} \quad (1)$$

Where,

$BE_{Enteric}$ = Baseline enteric CH₄ emissions during the feeding period (tCO₂e)

$EF_{Enteric}$ = Enteric CH₄ emission factor for the animal group during the feeding period (kg CH₄)

GWP = Global Warming Potential of methane (tCO₂/tCH₄)

1000 = kg per one metric tonne

The enteric methane emission factor was calculated by performing direct on-site measurements to determine the methane production for the animal group per day.

$$EF_{Enteric} = EF_{Production} \cdot N \cdot Days \quad (2)$$

Where,

$EF_{Enteric}$ = Enteric CH₄ emission factor for the animal group during the feeding period (kg CH₄)

$EF_{Production}$ = Enteric CH₄ emission production factor for the animal group (kg CH₄ head⁻¹ day⁻¹)

N = Number of head in the animal group during the feeding period (head)

$Days$ = Number of days in the feeding period (d)

Enteric methane emissions with *Asparagopsis* supplementation

$$PE_{Enteric} = EF_{Enteric} \cdot (1 - ERF_{Enteric}) \cdot \left(\frac{GWP}{1000}\right) \quad (3)$$

Where,

$PE_{Enteric}$ = Enteric CH₄ emissions when supplementation with *Asparagopsis* during the feeding period (tCO₂e)

$EF_{Enteric}$ = Enteric CH₄ emission factor for the animal group during the feeding period as determined in equation 2 (kg CH₄)

$ERF_{Enteric}$ = Enteric CH₄ emission reduction factor. Average percentage reduction in enteric CH₄ due to *Asparagopsis* during the feeding period (dimensionless)

GWP = Global Warming Potential of methane ($\text{tCO}_2/\text{tCH}_4$)

The enteric CH_4 emission reduction factor for the animal group was calculated by performing direct on-site measurements to determine the methane production for the animal group per day while consuming *Asparagopsis* during the feeding period.

$$ERF_{\text{Enteric}} = \frac{EF_{\text{Enteric}} - (PE \cdot N \cdot \text{Days})}{EF_{\text{Enteric}}} \cdot 100 \quad (4)$$

Where,

ERF_{Enteric} = Enteric CH_4 emission reduction factor for the animal group (dimensionless)

EF_{Enteric} = Enteric CH_4 emission factor for the animal group determined by equation 2 (kg CH_4)

PE = Enteric CH_4 production factor for the animal group when supplementation with *Asparagopsis* during the feeding period ($\text{kg CH}_4 \text{ head}^{-1} \text{ day}^{-1}$)

N = Number of head in the animal group during the feeding period (head)

Days = Number of days in the feeding period (d)

Enteric CH_4 Emission reductions

$$ER_{\text{Enteric}} = BE_{\text{Enteric}} - PE_{\text{Enteric}} \quad (5)$$

Where,

ER_{Enteric} = Enteric CH_4 emission reductions due to supplementation with *Asparagopsis* during the feeding period (tCO_2e)

BE_{Enteric} = Total baseline enteric CH_4 emissions during the feeding period (tCO_2e)

2.6 Data Analysis

Visit flux data output from the GreenFeed system includes animal identification, start and end time of visit, duration, airflow, CO_2 and CH_4 emissions. Gas fluxes are output as a rate in grams per day. Unvalid data is automatically removed from the generated workbook. At the end of the trial, the collected instrument data was reviewed by the GreenFeed supplier, C-lock Inc, as a final data review.

The visit fluxes were aggregated and averaged over a given period. Instead of arithmetic averaging in which the sum of gas fluxes are divided by the number of visits, the CH_4

emissions were calculated by considering the timing of the visit as described in published studies. This approach reduces the potential bias that can occur if the animals visit the GreenFeed more often during certain time intervals where the CH₄ emission rate is lower or higher. For each individual animal and period, the visit data were aggregated and averaged in six 4h blocks representing the time of the day; 0000-0400, 0400-0800, 0800-1200, 1200-1600, 1600-2000 and 2000-2400. Only data from animals with ≥ 10 valid visits spread in at least five of the six blocks within the baseline and each feeding period were used in the statistical analysis to represent the diurnal pattern of the group as CH₄ emissions fluctuate during the 24h cycle. The block averages were averaged for each animal and period.

The average CH₄ emission rate per animal was used in the subsequent statistical analysis to determine the CH₄ emission rate ($g\ CH_4\ animal^{-1}\ day^{-1}$) for the group during the periods of baseline and *Asparagopsis* supplementation. A paired t-test was conducted to determine the difference in enteric methane emissions between baseline and supplementation with *Asparagopsis*.

MS Excel and XLSTAT 2022.2.1 add-in were used for calculations and statistical analysis.

3. Results

3.1 Enteric methane emissions

All 17 animals fulfilled the requirement of ≥ 10 visits spread over at least five of the six 4-h blocks for the baseline and *Asparagopsis* supplementation periods. When *Asparagopsis* was supplemented on a daily basis for 13 days, CH₄ production (g/day) decreased ($p < 0.0001$) by 81% relative to baseline. [Table 3.1.1](#) presents the daily average enteric CH₄ production for baseline and *Asparagopsis* supplementation. [Figure 3.1.1](#) is a graphical presentation of the daily average enteric CH₄ production during *Asparagopsis* supplementation period relative to baseline enteric CH₄ production.

Table 3.1.1 Computed CH₄ production per treatment with SEM and computed p-value for the difference between Baseline and *Asparagopsis* supplementation.

Treatment ^a	CH ₄ (g/day)	SEM	Diff to Baseline CH ₄ (g/day) [95%CI]	p-value ^b (Two-tailed)
<i>Baseline</i>	165.1	6.3		
<i>0.6% DM^c Asparagopsis</i>	31.0	9.0	134.0 [108.6; 159.4]	<0.0001

^a Number of animals (n) = 17
^b Significance level $\alpha = 0.05$
^c Days on *Asparagopsis* = 13

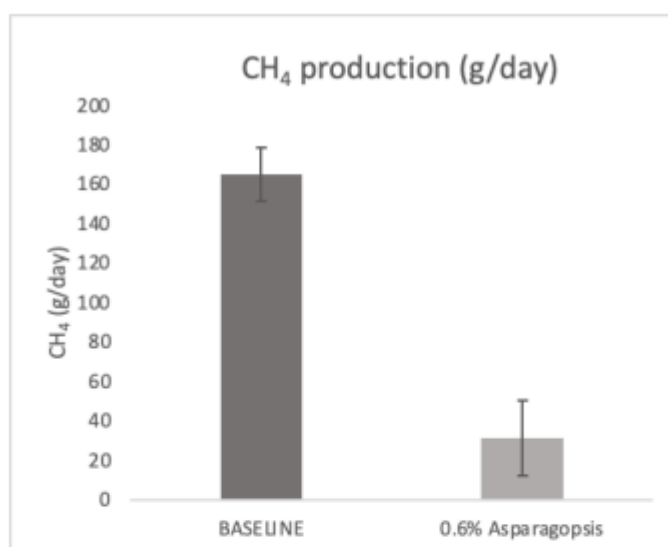


Figure 3.1.1 Average CH₄ production (g/day) ± 95% CI for baseline and *Asparagopsis* supplementation (daily inclusion 0.6% of feed DM).

Quantification of enteric methane reduction

The reductions in enteric methane emissions were quantified for the 13 day period of *Asparagopsis* supplementation. The quantified reductions are presented in tonne (t) CO₂e in [Table 3.1.2](#).

Table 3.1.2 Quantified enteric methane reductions (tCO₂ equivalents) for the *Asparagopsis* supplementation period.

	Days on <i>Asparagopsis</i>	Average enteric methane emissions reduction ^a	Enteric methane emission reductions (tCO ₂ e)
<i>Asparagopsis</i> supplementation on period	13	81%	0.83

^a Based on the amount of measured CH₄, i.e through breath. CH₄ emissions from flatulence are not accounted for.

4. Discussion

4.1 Enteric methane emissions A 81% reduction in enteric methane emissions was observed when *Asparagopsis* was included at a rate of 0.6% of feed DM in the daily feed ration of 17 growing bulls for a duration of 13 days. Future trials using Toki™ *Asparagopsis taxiformis* feed's land-grown *Asparagopsis* as a feed supplement to mitigate methane emissions will be conducted to determine long-term enteric methane reductions. Trials will also be aimed to optimise the inclusion rate of *Asparagopsis* in the feed and determine the efficacy in different breeds and diet compositions.

4.2 Net methane and GHG reductions The scope of this project did not include

emissions in addition to enteric methane that resulted from the project activities, i.e production- and transport emissions. Although the net emissions reductions achieved per unit of Asparagopsis is not concluded in this project, it can be hypothesised based on the results. Supplementing ruminant diets with Asparagopsis to reduce enteric methane emissions is a viable methane mitigation-strategy given that there is a net reduction in GHG emissions. An explorative LCA performed by has been conducted for the large-scale terrestrial production of seaweed in Toki™Asparagopsis taxiformis feed's pilot factory where the emissions per kg of Asparagopsis were estimated to be up to 9.2kg CO₂e. This estimation was performed based on hypothetical values and represents a worst case scenario. The emissions from transport of Asparagopsis are estimated to have a low contribution to the overall emissions. Even when transported long distances within China, as was the case in this project where the product was shipped 360 km from the site of production to the farm in China, the transport emissions were estimated to be low, resulting in 0.1 kg CO₂e 3 per kg of Asparagopsis. In the present study, when Asparagopsis was included in the daily feed ration at an average rate of 0.6% of feed DM, 1 kg of Asparagopsis resulted in an enteric methane reduction of 62 kg CO₂e, implying that after subtraction of the production and transport emissions, the emission reductions per kg of Asparagopsis was approximately 53 kg CO₂e. This is a clear indication that Asparagopsis is a viable option for mitigating enteric methane emissions.